estimated for each individual on the diet questionnaire that was reported to be consumed. Odds ratios (OR) and 95% confidence intervals (CI) were computed using unconditional logistic regression. The each nutrients intake amounts were categorized by quartiles based on the distribution among controls.

Results: Intakes of total fat, saturated fat, monounsaturated fat, trans-fat and cholesterol were positively associated with the risk of RCC; the ORs for the highest versus the lowest quartile were 1.67 (95% CI, 1.21-2.32), 1.53 (95% CI, 1.14-2.05) and 1.46 (95% CI, 1.05-1.97), 1.31 (95% CI, 1.04-1.65) and 1.48 (95% CI, 1.16-1.89), respectively. The positive association was apparently stronger in women, overweight or obese, and never smokers. An increased risk was also observed with increasing intake of sucrose. High fiber intake was inversely associated with RCC risk, the OR for the highest versus the lowest quartile were 0.69 (95% CI, 0.53-0.92). No association was found with intake of total protein and polyunsaturated fat, n-3 and n-6 polyunsaturated fatty acids and total carbohydrates.

Conclusion: Findings suggested that nutrition may play a role in the risk of RCC. A diet low in fat and rich in fiber could favourably affect the risk of RCC.

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Gene expression analysis of formalin-fixed, paraffin-embedded breast cancer tissues using the multiplex branched DNA assay

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Formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens represent the largest tissue archive where also the patient's clinical record is available. There is a growing interest to use RNA from FFPE tumor specimens to perform gene expression analyses to predict prognosis and response to treatment. The branched DNA (bDNA) assay measures mRNA directly from crude cell lysates and thus avoids variations introduced by RNA isolation, reverse transcription and amplification procedures. A modified version of the bDNA assay quantifies RNA directly from FFPE tissues specimens. The multiplex bDNA assay combines the bDNA assay with the xMAP (multi-analyte profiling) beads allowing simultaneous quantification of multiple RNA targets. The aim of the study was to investigate the molecular classification of breast cancer samples by quantifying the expression of selected genes directly from FFPE tissue, using the multiplex bDNA technology.

We used the 1.0 multiplex bDNA assay to measure the gene expression of 69 genes directly from 20 FFPE breast cancer samples. The genes were chosen from the list of genes able to discriminate between the 5 breast cancer subgroups (Sørlie et al Proc Natl Acad Sci , 2001). The genes were divided into 3 panels with PPIB, RPL19 and RPS3 as housekeeper genes. All the five breast cancer subgroups were represented in the FFPE samples. We also analyzed isolated total-RNA from fresh frozen tissue from 9 of the 20 samples.

The comparison between total RNA and FFPE on the bDNA technology showed that 42% of the genes had correlation >0.5. Hierarchical clustering of the FFPE samples based on the 69 genes was able to divide the samples reasonably well into their subgroups. Most of the luminal A samples clustered together, 2/3 samples for both basal-like and ERBB2+ samples clustered together in a main subcluster, and all luminal B and normal-like samples clustered together in a main subcluster. Hierarchical clustering of the FFPE samples, using only the genes with a correlation >0.6 between FFPE and total-RNA, showed that all the samples within the subgroups ERBB2+, normal-like and basal-like clustered together in a main subcluster, 5/8 luminal A samples clustered together and 3/4 luminal B samples clustered together in a main subcluster.

We conclude that the bDNA technology is able to quantify the expression of genes directly from FFPE tissues, and shows potential use in classifying breast cancer samples into their respective subgroups.

176 Poster

Genetic polymorphisms in the promoters of IL-6 and IL-10 in CIN and cervical cancer patients

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Introduction. Cell-mediated immunity is important in controlling both HPV infection and HPV –associated carcinogenesis. It was suggested that the cytokine response to HPV infection may potentially affect the disease process. Single nucleotide polymorphisms (SNP) in the promoters of IL-10 and IL-6 genes have been associated with different cytokine production and susceptibility to a number of diseases. The aim of this case-control study was to compare the IL-10 (-1082 G→A) and IL-6 (-174 G→C) polymorphisms in patients with cervical intraepithelial neoplasia (CIN) or cancer (CC) and the healthy controls. We would like to assess whether these polymorphisms increase the risk of cervical cancer in Russian patients.

Methods. Genomic DNA was isolated from the paraffin-embedded tissue from 130 CC patients and 45 patients with CIN I-III. The control DNA was extracted from peripheral blood from 144 females without any cancer, autoimmune or infectious diseases. Polymorphisms of IL-10 and IL-6 were studied in RFLP-PCR and the allele-specific PCR respectively. The Fisher's exact test was used to calculate statistical significance.

Results. We observed the increase of IL-10(-1082AA) low-secretor genotype frequency in CC patients versus control (p=0.012) and versus CIN patients (p=0.036). CC patients demonstrated the significant decrease of the high-secretor genotype IL-6 (-174CG) compared to the control (p=0.038).

Conclusions. These data suggest that the genetically determined ability to produce the different levels of IL-10 and IL-6 cytokines may be associated with cervical carcinogenesis.

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Cancer mortality in patients with schizophrenia - 11-year cohort study

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Schizophrenia is associated with a rate of premature mortality 2 to 3 fold higher than in the general population. The role of cancer in this excess mortality remains unclear, previous incidence or mortality studies having found contradictory results.

The authors initiated in 1993 a large prospective study in a cohort of 3470 patients with schizophrenia to determine mortality rates and specific causes of death. Standardized mortality ratios were calculated, adjusting for age and sex relative to a representative sample of the French general population.

During the eleven years follow-up, 476 (14%) patients died, corresponding to a mortality rate near 4-fold higher than in the general population. Cancer was the second cause of mortality (n=74), with a global SMR of 1.5 (95% confidence interval [95% CI], 1.2-1.9). For all cancers, the SMRs were 1.4 (NS) in men and 1.9 (95% CI, 1.4-2.8) in women. In men, lung cancer was the most frequent localization (n=23, 50%), with a SMR of 2.2 (95% CI, 1.6-3.3). In women, breast cancer was the most frequent localization (n=11, 39%), with a SMR of 2.8 (95% CI, 1.6-4.9). There were two significant baseline predictors of death by lung cancer in the final logistic regression model: duration of smoking and age ≥ 38 years old.

The results of the current study demonstrate an increased risk of mortality by cancer in patients with schizophrenia, especially in women for breast cancer and in men, for lung cancer. These results seem to be consistent with the lack of medical care in schizophrenia.

178 Poster

Polymorphisms in Fibroblast growth factor receptor 2(FGFR2) and susceptibility to breast cancer in Chinese women

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Background: FGFR2 belongs to the FGFR family which plays an important role in cell growth, invasiveness, motility and angiogenesis. Studies showed that FGF and FGFR expression is ER dependent and significantly correlated with an antiapoptotic role in human breast cancer. Recently, several single nucleotide polymorphisms (SNPs) of FGFR2 were identified as novel breast cancer susceptibility loci by whole genome association studies. In this study, we test the hypothesis that polymorphisms of FGFR2 may interact with estrogen related factors to contribute to breast cancer susceptibility in Chinese women.

Materials and methods: we genotyped three FGFR2 polymorphisms (rs2981582, rs1219648 and rs2420946) in a case-control study of 1,049 breast cancer patients and 1,073 cancer-free controls by using the SNPstream 12-plex genotyping platform.

Results: We found that the three SNPs were all associated with significantly increased breast cancer risk in a dose-dependent manner. Jointly, compared with subjects carrying '0-2 risk loci', the '3 risk loci' carriers had a 1.36-fold increased risk of breast cancer (adjusted OR =

1.36, 95% CI = 1.13-1.62, P = 0.001). In stratified analyses, this combined effect was more evident among premenopausal women, ER and/or PR positive women, and subjects with an older age at first live birth. Furthermore, there is a significant additive interaction between risk genotypes and menopausal status (P for multiplication interaction/additive interaction: 0.083/0.037).

Conclusions: These findings showed that genetic variants in FGFR2 may contribute to the susceptibility of breast cancer in Chinese women, possibly through mediating estrogen/progesterone related pathways.

179 Poster

Detection of novel biomarkers by plasma proteomic profiling of oesophageal cancer mouse xenografts using three human cell lines in response to chemotherapy

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Background - Oesophageal cancer is the 9th most common malignancy worldwide with poor survival rates and an increasing incidence in recent years. Use of neoadjuvant chemotherapy in locally advanced cancer prior to surgery has been shown to improve outcomes, but the response to therapy is variable. Hence, the effective use of chemotherapy could be greatly improved by the availability of biomarkers that predict response to therapy. The purpose of this study was to identify candidate biomarkers in mouse xenograft models of oesophageal cancer. Materials and methods - OE19, OE21 and OE33 xenografts were established in SCID immune-deficient mice and tumour growth rates recorded. A clinical dose of epirubicin, cisplatin or 5-fluorouracil was administered to xenografts/or controls, by once weekly peritoneal injection for up to 3 weeks. Plasma collected from treated and untreated xenografts/ controls was analysed by SELDI-TOF MS using Ciphergen CM10 (weak cationic) and Q10 (strong anionic) protein chips. Panels of markers were identified that distinguish between treatment groups using pattern recognition software and class prediction tested using k-nearest neighbours and support vector machine algorithms. Samples containing statistically significant markers were fractionated on anion exchange spin columns and peaks of interest identified by MS/MS analysis of SDS-PAGE gel pieces.

Results - Tumour growth was suppressed in treated compared with untreated xenografts. Protein peaks (m/z) were identified that differed significantly ($p<0.05$) between the treatment groups for each cell line with each drug. Peaks were identified that were both common and unique to each cell line and drug combination. Biomarker panels could correctly identify xenograft versus control or predict drug treatment in 95% or 70% respectively of a test set (n=20) using the support vector machine. Two statistically significant peaks (m/z 28303 and 29162) were identified by MS/MS as apolipoprotein A1.

Conclusion - These experiments have established a response to chemotherapy in oesophageal xenografts by proteomic profiling of plasma. Biomarker panels have been identified that can accurately distinguish xenografts from controls or between treatment groups. Identification of the proteins in these peaks is in progress, with one protein identified to date. A follow-up clinical study is being established.

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Genotypes and haplotypes in the insulin-like growth factors, their receptors and binding proteins in relation to plasma metabolic levels and mammographic density

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Increased mammographic density is one of the strongest independent risk factors for breast cancer. It is believed that one third of breast cancers are derived from breasts with more than 50% density. Variation in breast density may be due to lifestyle factors such as alcohol intake and diet as well as polymorphisms in genes involved in steroid hormone biosynthesis, metabolism and signalling genes.

Exposure to endogenous and exogenous steroid hormones and growth factors has been linked to increased mammographic density and breast cancer risk. Insulin-like growth factor 1 (IGF1) is one such growht factor. IGF1 is a mitogen in various cell types, and predicted to be involved in the development of several human cancers, including breast cancer. Furthermore the circulating levels of IGF1 are strongly associated with breast density. The levels of IGF1 are related to age and menopause status, young women tend to have higher levels of IGF than postmenonpausal women. In this study we have looked at the involvment of genetic polymorphisms in the IGF genes in postmenopausal women, and their influence on mammographic density and ciculating levels of IGF, the binding protein IGFBP3, and their ratio.

Samples from 964 postmenopausal women were genotyped on ABI 7900HT. The statistical analyses of the genotypes were perfomed by the use of SAS/GENETICS™ (SAS 9.1.3) and PROC HAPLOTYPE.

The haplotype analysis yielded six haploblocks within the genes IGF1, IGF2, IGF1R, IGF2R, IGFals and IGFBP3. Of the six haploblocks, four had significant associations to the parameters IGF level, IGFBP3 level and mammographic density. In IGF1 one haplotype variant is associated with mammographic density. Within the IGF2 gene one haplotype variant is associated with the level of both IGF1 and IGFBP3. The IGF2 receptor had two haplotype variants associated to the levels of IGF1. Both variants of the IGFBP3 haplotype are associated with the level of the IGFBP3 level and indicate cis regulation.

These result shows that polymorhisms within the IGF gene itself and related genes have an impact on IGF1 and IGFBP3 levels and are associated with mammographic density in postmenopausal women.

POSTER SESSION

Carcinogenesis

181 Poster

The effect of p53 on the DNA repair enzyme Thymine-DNA Glycosylase

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Esophageal cancer is a highly frequent and fatal malignancy presenting a remarkable geographical variation in its incidence worldwide. A characteristic of high incidence areas is the large proportion of double mutations at CpG sites of TP53 gene. The relevance to the cancer problem comes from the possibility that this cancer may be associated with acquisition of a defect in DNA repair - specifically in the DNA repair enzyme Thymine-DNA-Glycosylase (TDG) which is responsible for this repair - following an initial mutation in TP53, according to the concept of a “mutator phenotype”. The aim of this work is to analyse the effect of p53 on the expression and activity of TDG. TP53 was inhibited by using small interfering (si) RNA in TE-1 (esophageal cell line wich presents a temperature-sensitive mutation in TP53 resulting in inactive protein when cultured at 37°C and active at 32°C); increase of p53 levels was induced by transfecting an expression vector in TE-13 (a p53-deficient esophageal cell line); DNA damage was achieved by treating MN1 and MDD2 (derived from MCF-7 breast cancer cell line. MDD2 expresses an inhibitory peptide that blocks p53 oligomerization and MN1 is transfected with the same peptide without the coding sequence) with doxorubicin. Levels of TDG mRNA and protein expression were analysed by Quantitative - RT PCR and Western blotting, respectively. Moreover, DNA damage was induced in TE-1 by treatment with Methyl methanesulfonate (MMS). Analysis of expression and subcelular localization of TDG followed the treatment with MMS in TE-1 and doxorubicin in MN1 and MDD2 and were accessed by Confocal Microscopy. In TE-1 cells, a decrease of 80% was detected in cells kept at 37°C compared to cells at 32°C. Treatment with p53 siRNA decreased as the TDG mRNA levels by 45% at 32°C when compared to control. The transfection of 1,0 g of p53 expression vector in TE-13 increased (4,6-fold) the levels of TDG mRNA. TDG protein levels showed the same pattern with an induction following transfection. Doxorubicin treatment resulted in 2,2 - fold induction in MN1 TDG mRNA levels but not in MDD2 cells. Surprisingly, TDG was detected in the cytoplasm of TE-1 cultured at 37 ºC and 32°C. After the treatment with MMS, TDG was found into the nucleus of the samples treated and kept at 32°C, but not at 37°C. MN1 and MDD2 showed a similar result, presenting TDG in the cytoplasm and its migration into the nucleus after the treatment with doxorubicin in MN1 samples, but not for MDD2. The results suggest a role of active p53 on the expression and in the migration of TDG from cytoplasm to the nucleus and in its activity. These results provide evidence that wild-type p53 may regulate TDG activity, and that this property is lost after functional inactivation of p53 in cancer cells.