PIK3CD is strongly expressed in blood and we can show by realtime RT-PCR (TaqMan) and multiplex fragment analysis that this alternative spliced variant comprises on average 45% of all PIK3CD transcripts in this tissue. A panel (20) of normal tissues was tested but no other showed high expression of this alternative PIK3CD. Intriguingly we can show that this alternatively spliced variant of PIK3CD is also common in various human primary tumors commonly displaying 1p-deletions: advanced stage neuroblastoma (56% of all transcripts; 29 stage 4 samples tested); colorectal cancer (48%; 4 samples) and ovarian cancer (86%; 3 samples). By using a TaqMan-assay specifically detecting the alternatively spliced variant of PIK3CD compared to wild-type splicing of intron 5 we have been able to show significant ($p = 0.0001$) higher amounts of alternative spliced product in aggressive neuroblastoma tumors (patient died of disease) (63% splice variant) compared to neuroblastoma tumors from patients that was cured from disease (35%); 72 tumor samples were used in this study.
Conclusions: We speculate that this variant could have a regulatory function in the PI3-pathway, considering that the p85-binding domain is intact, leading to a possible binding of p85 without resulting in a functional PI3-kinase complex. The fact that this alternative product of PIK3CD is common in tumor cells is intriguing, implicating involvement in tumor biology.

511 POSTER

Tissue microarray immunohistochemical profiling of metastatic colorectal cancer

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Background: The aim of this study was to classify hepatic metastasis of colorectal cancer (HMCRC) based on tumor progression associated proteins assessed by immunohistochemistry (IHC) on Tissue Microarray (TMA).
Materials and Methods: In order to evaluate the expression of different proteins, a TMA block was constructed from 51 HMCRC patient biopsies, including 1-mm diameter tissue cores from each paraffin block. Tumor biopsies were histologically classified into encapsulated (presenting a fibrotic matrix between liver tissue and the tumor front) or invasive (with tumor cells penetrating the hepatic sinusoids). IHC on TMA slides was applied to analyse proliferation (ErbB2 and ki-67), angiogenesis (CD31 and a-actin), inflammation (IL-18Ra and VEGFR2), epithelial origin (CEA and EpCam), adhesion (CDH-1) and fibrosis (COL1A) markers. To evaluate their expression level arbitrary values between 0 and 3 were used: 0, non stained; 1. weak non-homogeneous staining; 2. weak homogeneous staining; 3. strong staining. Statistical analysis was based on non-parametric tests (Wilcoxon Signed Ranks Test, Kruskal Wallis, Mann-Whitney Test and Spearman Test), using SPSS v13 and a significance level of $p < 0.05$.
Results: In liver metastasis biopsy pairs including the central region of the metastatic tumor and its corresponding invasive front, ErbB2 and CDH-1 were found to be overexpressed in the invasive front, while VEGFR2, was only overexpressed in the central region of the metastasis. The morphological features studied showed a strong correlation with some proteins, such as the overexpression of VEGFR2 and IL18Ra in the invasive metastasis and the overexpression of CEA and CDH-1 in encapsulated tumors. Statistical analysis revealed a positive correlation of Ki-67 with S100A6 and SMA with CD31. Inversely, an opposite correlation was observed between IL18Ra and COL1A1 positivity. Finally, a strong positive correlation was observed for the staining intensity of ErbB2 and CDH-1 in colorectal cancer liver metastasis, being both negatively correlate to CD31 staining level.
Conclusions: This study shows that IHC-TMA can be reliably used to analyse multiple markers in large patient sets simultaneously. Protein expression level and distribution is related to tumor morphological features and microenviromental factors. To confirm the prognostic value of the described observations, clinical data regarding local recurrences and overall survival are being currently collected.

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Molecular profile of acquired docetaxel resistance in breast cancer cells

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Introduction: Docetaxel is one of the most active agents used in the treatment of breast cancer. However, tumours may be resistant, or develop resistance, to docetaxel during treatment. The mechanisms of resistance to docetaxel, whether innate or acquired, are poorly understood. The purpose of this study was to investigate the genetic pathways involved in docetaxel resistance using a unique model of docetaxel resistance, which we have developed in breast cancer cells.
Methods: We made two human breast cancer cell lines, MCF-7 and MDA-MB-231, resistant to docetaxel by exposure to increasing docetaxel concentrations. The resultant sublines were able to withstand 30 μM of docetaxel. Alterations of gene expression were determined using Affymetrix Genechip cDNA microarrays, and subsequently validated by RT-PCR and western analysis.
Results: After firstly selecting out gene changes that were common between both sets of sensitive cell lines and their resistant sublines (>2 fold), further normalisation and statistical filtering ($p < 0.01$) identified 124 probe-sets that were commonly changed in both resistant cell lines. Further statistical analyses were carried out on the gene list using ANOVA (assuming unequal variances) and the Benjamin-Hobbson false discovery rate was applied as a multiple correction factor with a significance level of $p < 0.01$. This identified a 14 probe-set, encoding 10 genes (including p-glycoprotein), which were significantly associated with resistance to docetaxel. These genes are currently being validated at the mRNA and protein level.
Conclusions: These changes, therefore, may represent common mechanisms of resistance in breast cancer cells. In addition, this is the first description, using microarray analysis, to identify the genetic pathways involved in the evolution of acquired resistance to docetaxel in a cell line model of resistance.

513 POSTER

Characteristic and outcome of young breast cancer patients with and without BRCA1 mutations

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Purpose: To investigate the clinical characteristic and outcomes of younger (<50 years old) breast cancer patients with BRCA1 mutation in comparison to patients without this germline mutation.
Methods and Materials: This is an ongoing study and patients will be enrolled till end of 2008. Till now we followed 480 breast cancer patients who were diagnosed before age 50 and were asked to provide a blood sample for BRCA1 mutation screening (5382insC, 300T/G, 185delAG, and 4153delA). We compared contralateral breast cancer and ovarian cancer incidence, disease free, metastases free, and overall survival, between BRCA1 mutation carriers and non-carriers.
Results: BRCA1 mutations were detected in 74 breast cancer patients; the remaining 406 women did not carry the mutation. BRCA1 related tumours showed higher grade, more frequent negative oestrogen, progesterone, HER2 receptor status. Patients with BRCA1 mutation had a higher incidence of bilateral breast and ovarian cancer. Multivariate Cox analysis for DFS (local-regional and distant failure) showed that node ratio >13%, tumour diameter, age >44 years and BRCA1 mutation negative patients significantly decreased DFS.
Conclusions: This data suggest that BRCA1 mutation carriers have better DFS and MFS compared to sporadic tumours. There is variability within BRCA1 mutation carriers with respect to lymph nodes metastases, DFS, and distant metastases.

514 POSTER

Immunodetection and cytogenetic characterization of disseminated tumor cells applied to the clinical management of patients with solid tumors

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Aim: The aim of the present study was to show that detection and characterization disseminated tumor cells in peripheral blood can be applied to the clinical management of patients with solid carcinomas.
Methods: A double gradient of Ficoll allowed the separation of mononuclear cells, granulocytes and epithelial origin cells from total peripheral blood. Then, for the inmunomagnetic selection of the epithelial cells

magnetic particles-labeled with antibodies against epithelial markers (cytokeratin 7/8 or EpCAM) were used. Finally the disseminated tumor cells are detected using anti-cytokeratin 7/8/18/19 antibodies followed by microscopic visualization. Once detected, cells were citogenetically characterized by means of FISH, to detect described chromosomic aberrations for each tumor type using commercially avilable kits (Vysis®).
Results: The described methodology was able to detect epithelial cells disseminated in the blood of aproximately 50% of the patients, with cell numbers ranging from 1 to 16 cells, in 6−10ml of peripheral blood, and with an exceptional case of 100 disseminated cells in a prostate cancer patient. The neoplasic nature of the identified cells was verified through cytogenetic characterization, evidencing that both metastatic colorectal carcinoma and bone metastatic breast carcinoma cells show amplification of the ZNF217 (20q13) gene, which is implicated in the development and progression of the cancer. For disseminated prostate carcinoma cells ProVysión panel containing probes for LPL (8p22) and c-myc (8q24), demonstrated that these genes' loss and gain respectively characterised these patients' tumors. In patients bearing bone metastatic from lung cancer, LaVysion kit identified in the disseminated tumor cells amplifications in c-myc (8q24), and EGFR (7p12), whose amplification predicts good responses to Gefitinib (Iressa) or Erlotinib (Tarceva), and the 5p15 region. Finally, UroVysion kit, for the detection of aneuploidies in chromosomes 3, 7, and 17 and loss of the 9p21in disseminated urothelial carcinoma of the bladder cancer in blood.
Conclusions: The optimised methodology allows the detection and phenotipical and genotipical characterization of disseminated tumor cells in routine peripheral blood samples, offering additional information to empirically evaluate clinical prognosis and select the most efficient chemotherapy treatment, and providing a new tool for the post-surgical monitorisation of patients with solid tumors.

515 POSTER

Zoledronic Acid (ZOL) treatment may improve survival in patients with lung cancer and high baseline N-telopeptide levels: a multivariate Cox regression analysis

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Introduction: In recent analyses, high N-telopeptide (NTX) levels were reported to be an indicator of poor prognosis in patients (pts) with bone metastases. ZOL can reduce NTX levels in this setting, and exploratory analyses have suggested that ZOL treatment correlates with improved survival in pts with NSCLC and high baseline NTX. Therefore, we conducted a multivariate analysis of baseline variables and treatment to examine their correlation with survival outcomes in pts with NSCLC and high baseline NTX levels in a placebo (PLA)-controlled, randomized clinical trial of ZOL.
Material and Methods: Pts with solid tumors and bone metastases were randomized to either ZOL or PLA for up to 21 months. Survival was assessed in the subset of NSCLC pts who had high baseline NTX levels (⩾64 nmol/mmol). The effects of >20 baseline variables and treatment group on survival were evaluated in univariate and multivariate Cox regression analyses, and significant covariates ($P < 0.05$) were included in a reduced model. The relative risk (RR) of death and associated 95% confidence interval (CI) were calculated for each.

Variable	RR	95% CI	P
ZOL vs PLA	0.565	0.381, 0.840	0.0047
Narcotics (Y/N)	1.757	1.110, 2.780	0.0161
Impaired PS (Y/N)	1.941	1.158, 3.255	0.0119
[Leu] (% incr v med)	0.977	0.960, 0.995	0.0112

Results: Among NSCLC pts, the association between ZOL treatment and survival was significantly different for pts with high v low NTX ($P = 0.020$), with RR = 1.34 ($P = 0.205$) and RR = 0.67 ($P = 0.034$) for the normal and high NTX pts, respectively. Pts with NSCLC and high baseline NTX levels (n = 144; 65% men, 35% women) had a median age of 64 yrs, ~76% had experienced ?1 SRE, ~80% required narcotic medication, 15% had some impairment of performance status (PS) and most pts had lymphopenia (median, 14% lymphocytes [Leu]). Among these pts, variables that correlated significantly with survival outcomes included treatment group, FACT-G score, race, narcotic use, PS, and [Leu]. After a full multivariate analysis, 4 significant covariates emerged for the reduced model (Table).
Conclusions: This multivariate analysis determined the following variables to independently correlate with improved survival in NSCLC pts with high baseline NTX: no narcotic use, no impairment of PS, higher lymphocyte count, and ZOL treatment. This retrospective analysis suggests that ZOL treatment is an independent variable for improved survival compared with PLA in pts with NSCLC and high NTX levels, warranting further study in prospective trials.

516 POSTER

Cap43/NDRG1 is a molecular marker of angiogenesis and prognosis in cervical adenocarcinoma

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Background: Cap43/NDRG1 is a nickel- and calcium-inducible gene that has been recognized to play a significant role in metastasis and invasion, as well as in the primary growth of malignant tumors, possibly through its ability to induce differentiation. The majority of studies until now have suggested a negative correlation between Cap43/NDRG1 expression and cancer progression. However, this plausible role of Cap43/NDRG1 in preventing cancer progression has been shown to depend on the tissue of origin and the tumor type. The aim of this study was to investigate the association between Cap43/NDRG1 expression and angiogenesis (microvessel density) and other clinicopathological factor in cervical adenocarcinoma.
Methods: A retrospective review was conducted of the records of 100 women with FIGO clinical stage I-II cervical adenocarcinoma who underwent surgery. We evaluated Cap43/NDRG1 and CD34 expression in the resected specimens by immunohistochemistry.
Results: A significant association was found between the expression level of Cap43/NDRG1 in the tumor specimen and the microvessel density, histologic grade of the tumor, tumor diameter, stromal invasion, lymph vascular space invasion and lymph node metastasis. Kaplan-Meier plots demonstrated a clear influence Cap43/NDRG1 expression on the survival time. The median overall survival time was 54.1 months in patients with tumors showing low Cap43/NDRG1 expression, as compared with only 36.4 months in patients with tumors showing high Cap43/NDRG1 expression (log-rank test; $p = 0.0018$).
Conclusions: These results suggest that increased expression of Cap43/NDRG1 may be associated with angiogenesis and might be a poor prognostic factor in patients with cervical adenocarcinoma.

517 POSTER

Comparison of allelic polymorphisms of insulin receptor substrate-1 and leptin receptor in breast and endometrial carcinomas

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Background: Obesity and diabetes mellitus are among cornerstone endocrine risk factors for several malignancies. These two pathologies, however, are unequally associated with endometrial cancer (EC) and breast cancer (BC), with the prevalence of former in obese and diabetic population (Calle et al., 2003). The cause for such difference is not currently known, and it seems reasonable to assume that the explanation is in polygenic nature of the two malignancies. The aim of the present study was to evaluate the distribution of polymorphic genetic variants of insulin receptor substrate-1 (IRS1 Gly972Arg) and leptin receptor (LepR Lys109Arg and Gln223Arg) in BC patients in comparison to EC patients. Polymorphisms mentioned above are considered to be associated with higher incidence of diabetes or with excessive body weight (Salopuro et al., 2005) as well as with risk of BC (Slattery et al., 2006; Snoussi et al., 2006); similar investigations in relation to EC have not been performed.
Methods: The study included 407 females (average age around 60 years): 105 healthy women, 192 patients with EC and 110 with BC. Additionally, we included a separate group of those who underwent glucose oral loading test (n = 80) in the study. Genomic DNA was extracted from peripheral blood leukocytes. Genotyping of IRS1 and LepR polymorphisms was performed by allele-specific real time PCR.