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.

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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.

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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