we examined the changes in methylation patterns during ductal breast cancer progression from atypical ductal hyperplasia to in situ and invasive carcinoma.
Materials and Methods: Paired samples of synchronous pre invasive lesions (Atypical Ductal Hyperplasia and/or Ductal Carcinoma in situ) and invasive ductal breast carcinoma from 31 patients, together with isolated lesions from additional 24 patients were analyzed. In total 96 preinvasive lesions and invasive tumour samples and 20 normal breast tissues were analyzed by Quantitative Methylation Specific PCR (QMSP) on a panel of 9 gene promoters (ESR1, APC, CDH1, CTNNB1, GSTPI, THBS1, MGMT, TMS1 and TIMP3).
Results: Of the nine genes tested APC, CDH1, and CTNNB1 showed an increase in frequency of methylation and increased methylation levels in primary breast cancer when compared with normal breast tissues. The analysis of the syncronous paired breast lesions demonstrated also an increase in methylation frequency and level for APC, CDH1, and CTNNB1 genes during progression. By establishing an empiric cutoff value, we were able to distinguish among pre-invasive and invasive lesions. Syncronous methylation of APC, CDH1, and CTNNB1 was associated only with invasive lesions, whereas simultaneous methylation of APC and CDH1 or APC and CTNNB1 were more frequent in ductal carcinoma in situ and invasive carcinoma.
Conclusions: Our data point to direct involvement of APC, CDH1, and CTNNB1 CpG island promoter methylation in the early stages of breast cancer progression, and suggest that these molecular alterations might be involved in the transition to an invasive phenotype.

318 POSTER

Treatment response following combined capecitabine, oxaliplatin and radiation therapy monitored by diffusion weighted magnetic resonance imaging (DW-MRI) in a xenograft model

D.R. Olsen, T. Seierstad, S. Folkvord, K. Røe, K. Flatmark, A. Hansen Ree. *The Norwegian Radiumhospital, Institute for Cancer Research, Oslo, Norway*

Background: Individualized cancer treatment requires prediction and early monitoring of response to therapy, and advanced MR techniques have evolved to promising tools for non-invasive response monitoring. Diffusion weighted magnetic resonance imaging (DW-MRI) provides in particular information about the microenvironment in tumour tissues and may thus be used in early monitoring of treatment response. Treatment induced necrosis and microvasculature damage will affect the apparent diffusion coefficient (ADC) measured DW-MRI.
Several studies indicate that combined capecitabine, oxaliplatin and radiation therapy is an effective treatment of locally advanced rectal cancers. It has also been demonstrated that capecitabine and oxaliplatin both possess radiosensitizing properties.
The aim of this study is to investigate whether the changes in ADC can predict tumor response following fractionated chemo-irradiation.
Materials and Methods: Bilateral HT29 xenografts on the rear flank of athymic mice were treated with capecitabine (359 mg/kg/day) alone and fractionated irradiation (2 Gy x) or combined capecitabine and oxaliplatin (10 mg/kg) and fractionated irradiation. One group was kept as control. DW-MR images were acquired prior to therapy and weekly for the 9 following weeks, and ADC values were calculated. Pre-treatment and changes in ADC were compared with tumour regrowth delay.
Results: Increased ADC values were seen in all treated tumours, except those receiving capecitabine alone ($p = 0.06$), 11 days after onset of therapy ($p < 0.05$). This increase in ADC values correlated strongly with tumor regrowth delay ($r = 0.92$, $p < 0.01$). Five days after completion of therapy the ADC values returned to pre-treatment values. No correlation, however, were seen between pre-treatment ADC values and tumour regrowth delay following therapy.
Conclusions: Early changes in tumour ADC values correlated strongly with tumour regrowth delay, indicating that ADC measured by DW-MRI may be used for early monitoring of treatment response to chemo-irradiation. However, pre-treatment ADC values did not predict the response to fractionated chemo-irradiation of individual tumours.

319 POSTER

EphA2 mediates the angiogenetic response of irradiated human lung adenocarcinoma cells

E. Fokas[1], F. Rose[1], J. Juricko[1], B. Eul[2], J. Haenze[2], R. Engenhart-Cabillic[1], H. An[1]. *[1]Philipps University, Radiotherapy and Radiation Oncology, Marburg, Germany; [2]Giessen University Hospital, Internal Medicine, Giessen, Germany*

Background: The Eph family of receptor tyrosine kinases (RTKs) and their ligands, ephrins, are dysregulated in different types of cancer and play an important role in tumor angiogenesis, vascular remodelling and metastasis. However, the influence of irradiation (IR) on this family of RTKs remains unknown. We hypothesised that radiotherapy induces different members in lung cancer and through this way it transmits a pro-angiogenic stimulus to its associated vasculature.
Materials: A549 cells and endothelial cells (HUVECs) were irradiated using different doses and expression of EphA2, EphrinA1, EphB4 and EphrinB2 in vitro was assessed at various time points using Real-Time PCR, Immunofluorescence and Western Blot. The expression of EphA2 receptor was analysed in irradiated A549 tumor xenografts in vivo. The proliferation rate of A549 upon irradiation and simultaneous EphA2 blockade was assessed by the WST-1 method. The invasion ability of HUVECs was studied using IR A549 cells co-cultured with endothelial cells in which EphA2 was previously blocked using a soluble EphA2-Fc receptor.
Results: IR promoted transcriptional activation of EphA2 and its ligand EphrinA1 but not EphB4 or EphrinB2 in A549 cells in vitro while none of these members analysed was induced in IR endothelial cells. EphA2 protein expression was significantly upregulated both in vitro and in vivo, in comparison to the unirradiated control group. There was no difference observed in the viability of A549 cells after irradiation and EphA2 blockade as compared to the EphA2 wild type group. IR of A549 cells and immediate co-culture with HUVECs increased endothelial cell migration, which was inhibited by a soluble EphA2-receptor chimera.
Conclusions: To our best knowledge, this is the first demonstration to show how IR affects different members of the Eph/ Ephrins both in vitro and in vivo. Our results suggest that irradiation of lung cancer cells can activate the vascular compartment through induction of EphA2 receptor, promoting in this way endothelial cell invasion. At the same time, they rationalise the use of EphA2 blocking agents in combination with radiotherapy in lung cancer treatment.

320 POSTER

Evidence for a tumor suppressor locus distal to Tp53 – a study in experimental endometrial adenocarcinoma

A. Behboudi[1], C. Nordlander[2], K. Klinga-Levan[3], S. Karlsson[3]. *[1]Göteborg University Inst. of Biomedicine, Clinical Genetics, Gothenburg, Sweden; [2]Göteborg University Inst. of Cell and Molecular Biology, Genetics, Gothenburg, Sweden; [3]Skövde University School of Life Sciences, Biomedicine, Skövde, Sweden*

Recently, we showed that in the BDII rat model for human endometrial adenocarcinoma (EAC), rat chromosome 10 (RNO10) is frequently involved in chromosomal aberrations. In the present study, we investigated the association between RNO10 deletions, allelic imbalance (AI) at RNO10q24 and Tp53 mutation in 27 rat EAC tumors. We detected chromosomal breakage accompanied by loss of proximal and/or gain of distal parts of RNO10 in approximately 2/3 of the tumors. This finding is suggestive of a tumor suppressor activity encoded from the proximal RNO10. Given the fact that Tp53 is located at RNO10q24-q25, we then performed Tp53 mutation analysis. However, we could not find a strong correlation between AI/deletions at RNO10q24 and Tp53 mutation. Instead, the observed patterns for AI, chromosomal breaks and deletions suggest that major selection was directed against a region located close to, but distal of Tp53. In different human malignancies a similar situation of AI at chromosome band 17p13.3 (HSA17p13.3) unassociated with TP53 mutation has been observed. Although RNO10 is largely homologous to HSA17, the conservation with respect to gene order among them is not extensive. We utilized publicly available draft DNA sequences to study intra-chromosomal rearrangement during the divergence between HSA17 and RNO10. Using reciprocal comparison of rat and human genome data, we could substantially narrow down the candidate tumor suppressor region in rat from a chromosomal segment of about 3 Mb to 0.5 Mb in size. There are 16 known and three predicted genes located in this region. Using real-time RT-PCR, we examined expression patterns of all 19 genes in a panel of 31 rat EAC, seven pre-malignant and three non-EAC tumor cell cultures. Three genes were singled out as potential candidate tumor suppressor genes. We plan to subject these three genes to promoter methylation tests, sequence polymorphisms, mutation analyses and functional assays. Results of this study will provide scientific groundwork for identification of the putative tumor suppressor gene(s) at HSA17p13.3 in human tumors.