Results: MGMT methylation promoter was observed in 42% of cases. Besides, we have also detected methylation of MLH3 (61%), MLH1 (43%), MSH2 (43%), MSH3 (39%), MSH6 (46%), and PMS2 (36%). The cases of unmethylated MGMT (UM) promoter had also a lower methylation in mismatch repair genes, being MLH1 methylation the most frequent. CGH showed that genomic changes were higher in UM and the number of deletion regions was higher. The 3q and 8q gains on chromosome regions were observed in cases of UM, and 9p losses was the most frequent in MGMT methylated (ME) cases. Amplifications of EFGR were detected in 18% of ME cases and overexpression of P53 in 36%. Moreover, in ME cases the expression of MLH1, MSH2, HDAC1, HDAC2, HDAC3 and PGFA proteins were higher than in UM. The median overall survival time for ME was 398 days vs. 378 days for UM. The median progression free survival was higher in ME than in UM cases (7 vs. 5 months). 72% of the ME cases showed complete or partial radiotherapy response versus 54% of the UM cases.
Conclusion: These data showed the evidence that methylation status of specific genes may contribute to the subclassificaction biological of high grade gliomas.

161 Chemotherapy-induced gastrointestinal disorders: alterations of epithelial ion transport and barrier function

M.R. Hoda[1], H. Heynemann[1], P. Fornara[1]. [1]*Martin-Luther University Halle/Wittenberg, Urology, Haale/Saale, Germany*

Background: Chemotherapy-induced gastrointestinal disorders are dose-limiting and costly side effects of cancer therapy. The mechanisms of intestinal damage are still unclear, and thus no definitive prophylaxis or treatment exists. In addition to structural changes, functional changes in intestinal epithelial absorptive and secretory functions may occur. We investigated whether chloride secretory response could contribute to methotrexate-induced diarrhea in a rat model.
Methods: Sprague Dawley rats were injected intraperitoneally with 40 mg/kg methotrexate (MTX) or PBS (control), and monitored over a period of 15 days for body weight and symptoms of diarrhea. Groups of animals were sacrificed each day and segments of distal colon were removed. After stripping of seromuscular layers, the mucosae were mounted in modified *Ussing Chambers* (aperture = 0.6 cm^2). Net ion transport was measured as changes in short circuit current (ΔIsc, in $\mu A/cm^2$) under basal conditions or following stimulation of chloride secretion with carbachol (CCh) or forskolin (FSK).
Results: Diarrhea occurred clinically in 72.3% of MTX injected rats (n = 62), with maximum severity score (4) after 3 days, resolving by day 6 post injection. During the acute diarrhea (day 3−5), basal tissue conductance of distal colon was significantly higher, compared to controls (MTX-treated = 38 ± 5.2; control = 23.8 ± 4.9 mS:cm^2, $p < 0.05$). MTX-treated distal colon also had a higher basal Isc than controls (93 ± 7.4 vs. $59.3\pm6.5\,\mu A/cm^2$, $p < 0.05$). Further, in MTX-treated rats, secretory responses to the Ca^{2+}-dependent agonist, charbachol (CCh; 200 μM), were potentiated 2-fold in the distal colon mucosa at 3−4 days when compared to controls (ΔIsc; 148.6 ± 8.9 vs. $63.3\pm9.8\,\mu A/cm^2$; $p < 0.01$). MTX also potentiated cAMP-dependent Cl^- secretion 3−4 days after treatment (forskolin; FSK 20 μM; ΔIsc; 73.2 ± 8.8 vs. $41.8\pm4.8\,\mu A/cm^2$; $p < 0.05$). MTX-induced Cl^- transport abnormalities gradually resolved thereafter.
Conclusion: The data presented here demonstrate that a secretory component with higher Cl^- secretion in distal colon likely contributes to the complex pathophysiology of chemotherapy-induced enterocolitis.

162 Gastric Adenocarcinomas.: methylation and deletions of DNA mismatch repair in tumoural cells and normal gastric mucosa cells

J.M. Gonzalez Valero[1], M. Sancho[2], J.F. Perez-Fontan[2], O. Bengoechea[2], R. Benito Sanchez[3], J.M. Hernández-Rivas[3], R. Gonzalez-Sarmiento[4], M. Sanchez-Martín[4], J.L. Garcia[1]. [1]*IESCYL, Hopsital Universitario de Salamanca, Salamanca, Spain,* [2]*Hopsital Universitario de Salamanca, Departmente of Pathology, Salamanca, Spain,* [3]*Hopsital Universitario de Salamanca, Center for Cancer Research, Salamanca, Spain,* [4]*Hopsital Universitario de Salamanca, Deparment of Medicine, Salamanca, Spain*

Background: It is now well accepted that the tumour and its microenvironment have a bidirectional relationship at multiple levels to elicit carcinogenesis, invasion and progression. Gastric adenocarcionamas may also be associated with deficiencies of DNA mismatch repair. Therefore, genomic loss or promoter methylation of mismatch repair genes could contribute to carcinogenesis.
Material and Methods: We have checked the methylation status of CpG islands from six MMR genes (MLH1, MSH2, MHSH6, MSH3, MLH3, PMS2 and for the MGMT promoter in a 39 gastric adenocarcioma cancer (ADC) samples and 30 normal gastric mucosa of gastric cancer patients. In order to achieve this study we have used the methylation-specfic multiplex ligation-dependent probe amplification (MS-MLPA) assay.
Result: The methylation status in ADC samples showed that MSH3 (64%), MSH6, and MLH1 (54%) were the most affected genes. Genomic deletions affecting to MSH2, MSH6 and MGMT genes were detected in 80% of ADCs. Importantly, in gastric normal tissues from these patients we can detect methylation on these genes: PMS2 (55%), and MSH2, MLH1, MSH3 and MGMT (52%). In Normal gastric mucosa we detected deletions on MSH3 (93%) and MLH1 (72%) genes. Regards to histology, enteric type showed losses of MSH6 and MGMT in all cases and methylation of MSH3 in 77%. In 38% of patients with enteric type and 24% with diffuse type showed the same profile of methylation in the tumoural samples vs normal gastric mucosa.
Conclusion: The accumulated of genomic changes in DNA mismatch repair and epigenetic alteractions in gastrointestinal cancer in tumoural cells such as microenvironment could be associated with status and progression of patients with these tumour.

163 Epigenetic target genes in malignant peripheral nerve sheath tumours identified as surrogate prognostic biomarkers

S. Aske Danielsen[1], G.E. Lind[1], K. Sundby Hall[2], S. Smeland[2], E. van den Berg[3], F. Mertens[4], M. Kolberg[1], R.A. Lothe[1]. [1]*Oslo University Hospital The Norwegian Radium Hospital, Department of Cancer Prevention and Centre for Cancer Biomedicine, Oslo, Norway,* [2]*Oslo University Hospital The Norwegian Radium Hospital, Division of Surgery and Cancer Medicine, Oslo, Norway,* [3]*University Hospital of Groningen, Department of Medical Genetics, Groningen, The Netherlands,* [4]*Lund University Hospital, Department of Clinical Genetics, Lund, Sweden*

Background: Malignant peripheral nerve sheath tumour (MPNST) is a highly aggressive malignancy that arises from neural crest-derived cells. Half of all MPNSTs are sporadic cases whereas the other half arises in individuals with the autosomal dominant genetic disorder neurofibromatosis type 1 (NF1). Epigenetic changes, in particular aberrant DNA methylation, are recognized to be at least as common as genetic changes in cancer, but only a limited number of methylation targets are identified in MPNSTs.
Materials and Methods: In the present study, twelve genes were analyzed by methylation-specific polymerase-chain reaction (MSP) in a series of 49 MPNSTs from patients with (n = 28) and without (n = 21) NF1.
Results: Four genes, *CRABP1, HOXA9, HOXB5,* and *SCGB3A1* were identified as novel targets for methylation in MPNST with frequencies ranging from 16 to 52%. In addition, we confirmed methylation of *RASSF1A*, although at a higher frequency than reported by Kawaguchi and co-workers (Modern Pathology, 2005). In univariate analysis, methylation of *CRABP1, RASSF1A* and *HOXA9* were associated with poor disease specific survival. *RASSF1A* is thought to be a tumour suppressor gene involved in a wide range of cellular activities and is frequently impaired in human tumours. When the patients were stratified according to NF1 status, methylation of *RASSF1A* was strongly associated with disease outcome in NF1 patients ($P = 0.009$), which was not seen for the patients with sporadic disease ($P = 0.854$). The mean survival for the NF-1 patients with methylation (n = 12) was 31 months, compared to a mean survival of 85 months for NF1-patients with unmethylated *RASSF1A* (n = 12).
Conclusion: In this study four targets for promoter hypermethylation novel to MPNST were identified. Two of these, in addition to *RASSF1A*, may be used as surrogate markers for survival. The outcome for MPNST patients is debated in regard to neurofibromatosis type one disease status. Here we have identified a molecular marker, methylation of *RASSF1A*, with strong prognostic value only among NF1 patients with MPNST.

164 Characterisation of the NEIL1 knockout mouse phenotype

A. Carter[1], W.M. Md Saad[1], A. Dumax-Vorzet[1], A.C. Povey[1], R.H. Elder[2]. [1]*University of Manchester, Centre for Occupational and Environmental Health, Manchester, United Kingdom,* [2]*University of Salford, School Environment and Life Sciences, Salford, United Kingdom*

Background: NEIL1 is a DNA glycosylase that removes a variety of oxidized bases and other DNA damage from single- and double-stranded DNA. As NEIL1 interacts with both single- and double-stranded DNA, excises a wide range of lesions and its expression is co-ordinated with the cell cycle, a role at DNA replication forks has been proposed for this enzyme. NEIL1 is also present in the mitochondrion and the persistence of oxidised DNA damage in this organelle has been proposed as one reason for the sporadic obese phenotype reported for NEIL1 knockout mice. In order to better characterise the biological role played by NEIL1 a new NEIL1 knockout has been created.
Material and Methods: The NEIL1 knockout was generated by the deletion of 101 bases, coding for 33 amino acids, in the helix 2-turn helix DNA binding region of the protein. The genotype has been confirmed by PCR and phenotype by reverse transcriptase PCR and western blotting. Animal weights were monitored over the course of 12 months. Previously it has been observed that the disruption of other base excision repair proteins has had a protective effect against organ damage due to inflammation, and thus in order to gauge the levels of neutrophil infiltration in mouse tissues a myeloperoxidase assay was performed.
Results: The NEIL1 knockout mice are viable and fertile and outwardly indistinguishable from wildtype litter-mates. However, from 5 months of age

the mean weight of NEIL1 males was significantly less than that of their wildtype counterparts ($p = 0.03$). In contrast, from 7 months onwards the NEIL1 knockout females weighed significantly more than the wildtypes ($p = 0.05$). Initial data from the myeloperoxidase assay suggests that whilst there is no difference in basal levels, there is significantly less neutrophil activity in the liver, heart and gut tissue of knockout animals when treated with 20 mg/kg lipopolysaccharide ($p = 0.05$).

Conclusions: The NEIL1 knockout mice show no obvious phenotypic change other than the weight differences described. No evidence of an obese phenotype was observed. The NEIL1 protein may, however, act in concert with other DNA repair proteins as a regulator of the immune system. In addition to the knockout mice, murine embryonic fibroblasts have been generated and together they should enable us to probe the biochemical and biological role of NEIL1 in genomic stability and the inflammatory process.

165 Array-based approach for early tumour detection in the biliary tract

K. Andresen[1], K.M. Boberg[2], M. Eknæs[1], H. Honne[1], E. Schrumpf[2], R.A. Lothe[1], G. Lind[1]. *[1]Department of Cancer Prevention, Institute for Cancer Research and Centre for Cancer Biomedicine Oslo University Hospital, Oslo, Norway, [2]Norwegian PSC Research Center, Medical Department Oslo University Hospital, Oslo, Norway*

Background and Aim: DNA methylation has been shown to play an important role in early tumourigenesis. So far, few DNA methylation target genes have been identified in hepatopancreatobiliary (HPB) cancers. Both cholangiocarcinomas and pancreatic carcinomas have a poor prognosis due to late clinical presentation. Cholangiocarcinomas in particular may be difficult to diagnose. In this study we aimed to identify novel methylated gene targets using HPB cancer cell lines.

Methods: Gene expression profiles of cholangiocarcinoma cell lines (n = 6) were analyzed before and after treatment with a combination of 5-aza-2′deoxycytidine and trichostatin A. CpG island containing genes upregulated after drug treatment in cell lines and simultaneously downregulated in cholangiocarcinomas compared with normal tissue, were selected for further analysis. Expression profiles of primary cholangiocarcinomas were acquired from published data sets [1,2]. The methylation status of these candidates was analyzed by methylation-specific polymerase chain reaction (MSP) in HPB cancer cell lines (n = 24).

Results: Fifty-seven candidate genes displayed increased expression in cholangiocarcinoma cancer cell lines and decreased expression in primary cholangiocarcinomas. Forty-one of these targets contained a CpG island in the promoter region and were subjected to DNA promoter methylation analyses. So far, twenty-one genes are analyzed in all cell lines. Four shared no methylation, others were methylated in one or few tumour types, and six genes including *SFRP1* and *ZSCAN18* were methylated across several HPB cancer cell lines.

Conclusions: By using a genome-wide approach we have identified several epigenetically regulated genes in HPB cancer cell lines. These target genes will be submitted for methylation studies in tumour and normal tissue as well as biliary brush cytology specimens in a hunt for early biomarkers.

Reference(s)

[1] Miller, G., et al., Genome wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. Journal of Experimental & Clinical Cancer Research 2009; 28: 62−74.
[2] Obama, K., et al., Genome-wide analysis of gene expression in human intrahepatic cholangiocarcinoma. Hepatology 2005; 41: 1339–1348.

166 Withdrawn

167 MicroRNA-34 family in triple-negative/basal-like breast cancer

J. Navratil[1], M. Svoboda[1], O. Slaby[1], P. Fabian[2], R. Vyzula[1]. *[1]Masaryk Memorial Cancer Institute, Department of Comprehensive Cancer Care, Brno, Czech Republic, [2]Masaryk Memorial Cancer Institute, Department of Diagnostic and Experimental Pathology, Brno, Czech Republic*

Background: Breast cancer is the most common malignancy of the woman after skin malignancies. “Basal-like” carcinoma is one of the most aggressive molecular subtype of breast cancer characterized by triple-negative (ER-, PR-, Her2-) and “basal-cell” phenotype and is associated with high grade, poor prognosis, and younger patient age. Adverse clinical outcome of these patients is also associated with frequent incidence of BRCA1 and p53 mutations. MicroRNAs have potential to post-transcriptionally regulate even one third of human genes, among them also significant number of important oncogenes, tumour suppressor genes and genes connected with invasion, dissemination and chemoresistance of tumours are involved. MicroRNA-34 family is under direct transcriptional control of p53 and seems to function as tumour suppressor. Mutations of p53 can induce decrease of microRNA-34 family and consequently apoptosis rate.

Material and Methods: In our study we examined expression levels of microRNA-34 family in 41 specimens of “basal-like” carcinoma by use of Real-Time PCR. Invasive breast carcinomas were immunohistochemically analysed for oestrogen receptors (ER), progesterone receptors (PR), cytokeratin 5/6 (CK5/6), epidermal growth factor receptors (EGFR), Ki67, p53 and vimentin. Tumours were considered to have basal-like phenotype if they were ER negative and HER2 negative, but positive for CK5/6 and/or EGFR and/or vimentin.

Results: Expression levels of miR-34b and miR-34c were markedly lower than those of miR-34a. We identified significantly higher levels of miR-34a in primary tumours disseminated to regional lymph nodes ($p = 0.0209$). Further, we observed increase of miR-34b levels in patients with significantly shorther overvall survival ($p = 0.05$). We did not proved association of microRNA-34 and grade, BRCA1 or clinical stage of breast cancer.

Conclusion: Our results suggest potential significance of miR-34a in invasiveness and dissemination of “basal-like” carcinoma and usage of miR-34b in diagnostic and predictive oncology of this aggressive molecular subtype of breast cancer.

Supported by grants: Internal Grant Agency of Czech Ministry of Health (IGA MZ CR) NS10357.

168 Grp78 activity is associated with Androgen Receptor status and upregulated in Hormone-Refractory prostate cancer

S. Tan[1], I. Ahmad[1], L. Singh[1], C. Nixon[1], J. Edwards[2], H. Leung[1]. *[1]Cancer Research UK Beatson Labs, Urology, Glasgow, United Kingdom, [2]University of Glasgow, Division of Cancer Studies, Glasgow, United Kingdom*

Background: Prostate Carcinoma (PC) is the most commonly diagnosed cancer of men in the developed world and relapse of PC following androgen deprivation remains a major clinical problem. Better prognostic biomarkers may facilitate treatment stratification and improve patient outcome. The aim of our study is to investigate if Grp78 expression in prostate cancer is associated with clinic-pathologic parameters including survival and the development of castrate resistance.

Materials and Methods: Immunohistochemical analysis was performed on formalin-fixed, paraffin embedded tissue microarrays containing PC specimens. 259 primary PC samples along with 36 matched pairs of hormone naïve and castrate resistant prostate cancer (CRPC) samples were studied for Grp78 expression.

Results: Using the weighted Histoscore method graded by independent observers, upregulated Grp78 expression was found to be associated with prostate carcinogenesis. Immunohistochemical expression of Grp78 in malignant tissue (n = 164) was significantly higher than benign tissue (n = 23) ($p = 0.000$). CRPC specimens also contained a significantly greater Grp78 staining than their matched hormone naïve specimens ($p = 0.028$). A higher Grp78 stain was significantly associated with 39 samples expressing androgen receptor positivity in the nucleus ($p = 0.010$). A Kaplan-Meier Survival analysis for androgen receptor positive tumours revealed a greater median survival time of 8.011 years in samples with low Grp78 stain as compared to 4.506 years in samples with high Grp78 stain ($p = 0.049$). Grp78 did not have any correlation with the degree of metastasis in patients ($p = 0.724$). It also did not have any influence on the biochemical relapse rate ($p = 0.501$) and time to death ($p = 0.653$) in the transition of hormone-naïve to castrate-resistance.

Conclusion: Grp78 expression is significantly associated with androgen receptor status and is upregulated in CRPC. It may play a key role in prostate carcinogenesis and further investigations are warranted to validate its use as a prognostic marker.

169 Reverse phase protein arrays: a powerful tool for cancer proteomics

L. De Koning[1], B. Marty-Prouvost[1], T. Dubois[1]. *[1]Institut Curie, Department of Translational Research, Paris, France*

Tumourigenesis implies major changes in cell signaling pathways involved in cell adhesion, proliferation and apoptosis. Activation of these pathways largely depends on post-translational modifications, such as phosphorylation, and thus cannot be analyzed at the mRNA level in transcriptome profiling. Therefore, high-throughput analysis at the protein level seems an absolute requirement to get a better insight in tumour biology. Yet, the technology required for such proteomics approaches has become available only very recently.

At the Translational Research Department of the Institut Curie (France), we developed a platform specialized in Reverse Phase Protein Arrays (RPPA). This highly quantitative technique consists of depositing in an automated manner very small amounts (1 ng) of cell- or tissue lysates onto microscope slides covered with nitrocellulose. Proteins of interest are subsequently detected using specific antibodies, directed against either modified (e.g. phosphorylated) or total protein pools. In this manner, up to 3000 samples can be analyzed simultaneously for the presence and the activation status of selected targets.