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