157 Hedgehog-Gli signaling pathway interactions in various proliferative human tumours

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The Hh-Gli signaling pathway has received increasing attention as a crucial regulator of not only embryonic organogenesis but also as an oncogenic pathway implicated in diverse human tumours.
The aim of our research was to study interactions of the Hh-Gli pathway with cell cycle progression through combinatorial strategy: blocking and activating the pathway in primary cultures of epithelial human tumours. In parallel we used corresponding cell line for in vitro studies addressed to Hh-Gli signaling.
We showed synergistic effects of tumour progression and cell cycle upregulation in two very frequent skin tumours. We found higher expression of p16 and Ptch in melanomas and basocelular carcinomas of the skin. Pathway components were associated with clinical and pathological features.
In two major categories of squamous cell carcinomas: oral and oropharyngeal, we found Ptch1 expression correlated with p16 and Survivin expression. Survivin, inhibitor of apoptosis, active in the G2/M phase of the cell cycle, is also involved in embryonic development, and usually is inactive in adult tissues. Its expression is high in most cancers, and related to increased recurrence rate and resistance to radiotherapy and chemotherapy. Therefore, our finding that Ptch1 is correlated with survivin expression suggest association of the Hh-Gli signaling pathway with cell survival through inhibition of apoptosis.

158 Carbon-11 methionine as an imaging biomarker for hepatocellular carcinoma differentiation and proliferation potential

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As ^{11}C-methionine (MET) is one of the commonly used tracers in positron emission tomography (PET) for oncological imaging, its use in hepatocellular carcinomas (HCC) has never been addressed. This study is to explore the possible role of MET-PET in HCC to reflect their biological behaviours such as differentiation and prolferation potential.
Material and Methods: Totally 26 patients with primary HCC were assessed with MET-PET. The MET avidity within HCC lesions were measured and compared with normal liver parenchyma and their ratios were calculated for each lesions. Also the MET avidity was correlated with their corresponding patho-histological features in 15 cases. Three cell lines of HCC, including HepG2, Hep3B and HA59T were treated with different concentration of all-trans retinoic acid (ATRA) and trichostatin acid (TSA), respectively, since ATRA has been known to induce apoptosis in HCC and TSA could render inhibitory effects on HCC growth. The *in vitro* MET assay was performed to measure the kinetics of MET uptake in ATRA or TSA-treated HCC at various time points. Their cell growth, clonogenic potential and survival was determined accordingly and used to correlate with their pertinent kinetic MET uptake.
Results: In general, primary HCC displayed diminished MET avidity as compared to their surrounding liver parenchyma and the decrement of MET uptake was associated with poor differentiation of HCC, i.e. the more decreased MET uptake, the more poor-differentiated of HCC. However, there appeared to be of no significant relationship with their mitotic figures or cell density within lesions. The *in vitro* study revealed that a significantly reduced MET uptake in both ATRA and TSA-treated HCCs in a dose-dependent manner. The reduction varied from 18−65% in different cell lines at the 24 hours after treatment as compared to the control group. The extent of reduced MET uptake correlated with the inhibition of cell growth and suppressed survival of HCC. However, a delayed wash-out of MET from HCC was noticed, particularly in HepG2 and Hep3B cells in presence of ATRA or high dose of TSA. More than 50% of lengthened half-lives of MET within HCC were reached by high dose of ATRA or TSA treatment.
Conclusions: The MET avidity within HCC lesion as measured by PET can be a parameter to indicate their differentiation as poor-differentiated HCC were prone to have reduced MET avidity. Further large-scale study is justified. However, the use of MET uptake to monitor certain systemic treatment for HCC requires further definition and mutiple time-point measurement may be required to clarify the kinetics of MET accumulation in HCC after treatment.

159 Selection and clinical relevance of monoallelic and biallelic TP53 defects in chronic lymphocytic leukemia

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Background: Chronic lymphocytic leukemia (CLL) is a frequent malignant disease with a highly variable clinical course. *TP53* defects have a well defined role in both prognostication and the prediction of treatment response in CLL patients. A presence of *TP53* locus deletion (17p) is used in a clinical setting as the most important negative prognostic marker. Although mutations in *TP53* gene are also connected with poor prognosis, their role was not reliably documented until very recently. We studied the association of mutations with deletions, their preferential acquirement, and assessed their impact on patients' prognosis and *in vitro* response to chemotherapy.
Materials and Methods: 17p deletions were examined using interphase FISH and *TP53* mutations were detected by functional analysis in yeast (FASAY). Cell viability after *in vitro* treatment was tested using the metabolic WST-1 assay, and induction of p53-downstream target genes was studied by real-time PCR.
Results: We examined 400 CLL patients and found 70 patients with *TP53* defect. As expected, complete inactivation of *TP53* gene through mutation of one, and deletion of the other allele was the most common type of abnormality (42 patients). However, a relatively large group of patients presented with a sole mutation; 20 patients harbored single mutations and 5 patients had two or even more mutations. On the contrary, separate deletion was detected in only 3 cases. Patients with monoalellic defects manifested a significantly reduced survival, almost comparable with patients exhibiting inactivation of both *TP53* alleles. Cells with both biallelic and monoallelic abnormalities showed significantly increased resistance to treatment by a purine analogue fludarabine. Induction of p53 downstream target genes CDKN1A, PUMA and BAX was intermediate after treatment in the cells with monoallelic defects in comparison with the biallelic defects and wt cells. Furthermore, 132 patients with originally intact *TP53* gene were examined consecutively in order to identify *TP53* defects early in their development. We observed the occurrence of a novel abnormality in 12 previously treated patients. All of them acquired mutations accompanied in 9 cases with deletion.
Conclusions: We showed that sole mutations are quite frequent in CLL patients, while separate deletions are rare. Monoallelic defects result in the significantly reduced survival and poor response to chemotherapy. We suggest that selection by therapy may play an important role in the clonal evolution of *TP53* defects.
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160 High-grade gliomas: epigenetic and genetic analysis

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Background: The DNA mismatch repair (MMR) system plays a prominent role in maintaining genomic integrity by mediating the activation of cell cycle checkpoints and apoptosis. Hypermethylation of the O6-methylguanine-DNAmethyltransferase (MGMT) gene, a predictive marker of sensitivity to alkylating agents, has been recently associated with improved outcome in glioblastoma (GBM). The aim of present study is to check the methylation status of MGMT promoter in high grade gliomas and its correlation with epigenomic and genomic changes of MMR associated genes.
Material and Methods: 66 samples of high grade gliomas (43 glioblastoma multiforme and 13 anaplastic astrocytomas) were studied by MS-MLPA, is a semi-quantitative method for methylation profiling studies.
We have checked the methylation status of CpG islands from six MMR genes (MLH1, MSH2, MHSH6, MSH3, MLH3, PMS2 and for the MGMT promoter. Besides, we have analyzed the amplification of EFGR gene, the mutations of TP53 gene and the genomic changes in the bulk tumours by comparative genomic hybridization (CGH). We also analysed the expression of TP53, MLH1, MSH2, HDAC1, HDAC2 and HDAC and PGFA proteins using a tissue array assay. All cases were analyzed at diagnosis.

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

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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

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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

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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

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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