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.