Results: HDAC 1−3 were expressed in the nuclei of cancer cells and normal tissue, with statistically significant higher expression in tumour cells compared to corresponding normal hepatocytes (HDAC 1: $p = 0.032$, HDACs 2−3 and Ki-67: $p < 0.001$). HDAC 7 expression was detected in the nuclei of endothelial cells from cancerous and normal tissue, without any significant difference between the two. HDAC IRS scores correlated significantly ($p < 0.001$) with each other and with Ki-67 expression in tumour tissue. HDAC 7 expression did not correlate with the other HDACs or with Ki-67. In addition, HDAC 1−3 and Ki-67 expression correlated significantly ($p < 0.001$) with tumour grade. Patient groups stratified for high and low HDAC 1 expression differed significantly regarding fatty degeneration of the hepatocytes, resection weight/volume and intrahepatic blood vessel invasion. HDAC 2 low and high expression groups differed significantly in their mean AFP serum levels, with the high HDAC 2 group showing lower AFP levels ($p = 0.001$).
Conclusion: The expression of the HDAC 1, 2 and 3 isoenzymes is correlated with tumour grading and proliferation and as well as with clinicopathological factors such as resection weight, blood vessel invasion and AFP levels. HDAC expression could thus be used as a new marker for the therapy of HCC with HDAC inhibitors.

724 Expression of Met in metastatic liver tumour from colorectal cancer

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Background: Liver metastasis is one of the most critical factors to estimate the prognosis of patients with colorectal cancer (CRC). Hepatectomy is the curative treatment, but hepatocyte growth factor (HGF) and its receptor (c-Met) related signal pathway are principal factors in the proliferation and progression of CRC, indicating that metastasis is adversely affected by hepatectomy. To evaluate the significance of surgical treatment, the present study was planned.
Methods: We operated on 94 patients with CRC (including 24 liver metastasis cases) at Gifu University Hospital (2002–2004) and the outcomes were studied. Expression of c-Met in the primary cancer and liver metastatic sites was evaluated by immunohistochemistry and western blot analysis. Experiments were also conducted on a mouse metastasis model and a CT26 murine CRC cell line.
Results: In the clinical study, liver metastasis was detected at significant levels ($p = 0.0316$) in the high c-Met expression group. The c-Met expression in liver metastatic sites was lower than in the primary sites in 87% of liver metastatic cases. In the *in vitro* study using the CT26 and mouse model, cell proliferation was promoted significantly by HGF. According to western blot analyses, the c-Met/ERK-related cyclin-dependent pathway was activated significantly by HGF. In the *in vivo* study using the mouse model, the expression of c-Met protein in the liver tumour on day 14 was significantly lower than in culture cells according to WB ($p = 0.033$) and was reduced in a time-dependent manner. Nevertheless, the c-Met expression level was found to have a significant inverse correlation to tumour weight ($p < 0.001$, $|R| = 0.856$). In IHC examination, the peripheral lesion of the tumour mass or the invasive intraluminal lesion had a higher expression of c-Met than the central lesion. In contrast, c-Met mRNA in the liver of day14 tumours was higher than in culture cells. In the examination for the effect of hepatectomy and c-Met expression, despite an increase in serum HGF by a factor of 1.35 in 12 hours in the ELISA assay, the growth of residual liver tumours was not significantly different between 30% hepatectomy group.
Conclusion: In the liver metastatic sites, c-Met is down-regulated. The elevation of the HGF serum level that was associated with surgery might not affect the proliferation of residual liver tumours through the HGF/c-Met signal pathway.

725 Structure and antiproliferation relationship of melatonin and its analogs

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Background: Melatonin is a hormone with neurotransmitter modulatory activity and was reported to possess anticancer activity via multiple mechanisms involving many pathological events. Melatonin prevents free radical damage to normal cells and limits oxidative damage to DNA due to its role as inducer of antioxidants and itself a weak preventive antioxidant. To improve the anticancer activity of melatonin, eight novel melatonin analogs were designed and synthesized. Their structures contained *N*-substituted indole nucleus with different electronic functional groups. The melatonin derivatives were explored for their structure and anticancer activity relationship.
Methods: The *N*-substitution melatonin analogs were synthesized by the esterification reaction of melatonin with various acid chlorides; acetic anhydride, bulky group (benzoyl chloride, napthoyl chloride), donating group (2-, 3-, 4-methoxy benzoyl chloride) and withdrawing group (4-Br, 4-NO_2 benzoyl chloride). The antiproliferation at 24 hr exposure was evaluated in leukemia cells (U937, Jurkat and MOLT-4) and hepatocarcinoma cells (HepG2) by using Neutral red assay.
Results: Moderate antiproliferation (20−35%) of 2 mM melatonin was observed in all cancer cell lines. Interestingly, the withdrawing group substitution exerted stronger antiproliferation (>70%) in all cancer cell lines than the bulky group and donating group substitution at 1 mM concentration, respectively. The napthoyl substitution showed 100% antiproliferation in Jurkat cells at 1 mM concentration. The distinctive antiproliferating effects of the withdrawing group and the bulky group substitution were found in the Jurkat and HepG2 cells.
Conclusion: The electronic effect played important role for antiproliferating activity of the melatonin analogs. Further increase in size of the N-substitution resulted in an increase in antiproliferating activity. This information could be useful for further development of melatonin analog as anticancer agent.

726 Hypermethylation of MGMT and RARbeta correlates with lymph node metastasis in laryngeal cancer patients

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Unlike genetic changes, epigenetic aberrations characteristic for larynx cancer have not been extensively studied despite the fact that they may possibly provide new diagnostic markers. So far, *p16, CDH1, MLH1* or *DAPK* were identified as genes frequently hypermethylated in this type of cancer.
The aim of this study was to assess the methylation status of three genes: *MGMT, RARbeta* and *GSTP1* in clinical samples of larynx cancer and corresponding microscopically normal mucosa from sites distant to the tumour. The study group consisted of 41 patients (35 men and 6 women) with T3 or T4 laryngeal cancer, with 12 patients showing lymph nodes metastasis (>N0). All patients underwent total laryngectomy. DNA isolated from surgical samples of the cancer tissue and normal mucosa from pharynx and trachea was bisulfite converted using the EZ DNA Methylation Kit (ZymoResearch) and promoter methylation status was assessed with methylation-specific PCR.
We found frequent methylation of promoter regions of both *MGMT* (54%) and *RARbeta* (59%) but almost a complete lack of methylation of *GSTP1* (4.9%) in DNA derived from tumour samples. Gene hypermethylation in tumour tissue was frequently accompanied by hypermethylation in normal tissue from trachea and pharynx. Methylation of *RARbeta* concurrently in tumour and pharynx or tumour and trachea was observed in 34.1% or 42.5% cases, respectively, while for *MGMT* the values were 34.1% or 37.5% cases, respectively. Gene methylation in trachea or pharynx was rarely observed in the absence of gene methylation in the tumour (2−7%). Hypermethylation of *MGMT* in cancer cells was positively correlated with lymph node metastasis ($P = 0.015$). On the other hand, negative correlation was observed between *RARbeta* methylation and lymph node metastasis ($P = 0.036$).
The data obtained are in agreement with the field cancerization model for oral cancers. Both high alcohol consumption and smoking are environmental factors which lead to aberrant DNA methylation and most larynx cancer patients are heavy smokers and/or consume high amounts of alcohol. It cannot be ruled out that these methylation changes occur early in carcinogenesis and affect many cells (thus frequent methylation in trachea or pharynx samples), of which only some acquire other changes, which finally lead to tumour formation. The results of our study allow to conclude that hypermethylation of *MGMT* and *RARbeta* is a marker of laryngeal cancers. Moreover, *MGMT* hypermethylation can be considered as a molecular predictor of lymph node metastasis.

727 Integrin-linked kinase promotes hepatocellular carcinoma oncogenesis

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Background: Integrin-linked kinase (ILK) was first discovered as an integrin binding protein. It localizes to focal adhesions and facilitates actin polymerization. Accumulating evidences suggest that ILK is a putative oncogene. ILK was over-expressed in various malignancies and its aberrant activation influenced a wide range of cellular functions. In this study, we aimed to elucidate the role of ILK in hepatocarcinogenesis and its clinical significance by assessing ILK expression in human hepatocellular carcinoma (HCC) tissues and functionally characterizing ILK in HCC cell models.
Material and Methods: Expression level of ILK in HCC cell lines was examined by Western blotting, while ILK expression in clinical samples was determined by quantitative PCR. ILK knock-down stable clones were

established in BEL7402 and HLE using a lentiviral-based short-hairpin knock-down approach. For each cell line, two stable ILK knock-down (shILK) clones and one stable non-target control (shCTL) were established. To functionally characterize ILK in HCC, the knock-down stable clones were subjected to various functional assays including cell proliferation assay, soft agar colony formation assay, cell migration assay, wound-healing assay and cell invasion assay. *In vivo* tumourigenicity of BEL7402 ILK knock-down stable clones was assessed by subcutaneous injection of the cells into nude mice.
Results: Western blotting revealed a higher ILK protein expression in HCC cell lines than in normal liver cell line. In the physiological context, qPCR analysis showed that ILK was over-expressed in 36.9% (21/57) of HCC tissues when compared to the corresponding non-tumourous livers. The overall ILK expression level was significantly higher in tumourous tissues ($P = 0.005$), with a stepwise increase of expression along tumour stage. Functional characterization of ILK in HCC using the two ILK stable knock-down cell lines showed a reduction in the rate of cell proliferation, migration, invasion and anchorage-independent growth. Knock-down of ILK in BEL7402 also suppressed tumour formation in nude mice, thus decreasing the *in vivo* tumourigenicity of HCC cells. To probe the underlying mechanism, AKT activity was evaluated in the shILK clones. Western blotting analysis showed a decrease in phospho-AKT(Ser473) level upon ILK silencing.
Conclusion: Our study suggests that ILK plays a role in the progression of HCC via the activation of the PKB/AKT pathway.

728 The TGFbeta co-receptor endoglin modulates the expression and transforming potential of H-Ras

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Background: Endoglin is a transforming growth factor-β (TGF-β) co-receptor that acts as a suppressor of malignancy during mouse skin carcinogenesis. H-Ras plays a crucial role in this model, by modulating tumour initiation and progression. We have studied the effects of endoglin on the expression of H-Ras in transformed keratinocytes.
Material and Methods: Epidermal mouse cell lines derived from chemically-induced skin carcinomas were used. H-Ras expression and promoter regulation was determined by RT-PCR, Western-blot and reporter assays. MAPK pathway was studied using specific antibodies and phospho-antibodies in western-blot assays. Foci formation assays were performed in mouse NIH3T3 fibroblasts transfected with $H\text{-}Ras^{Q61K}$ or $H\text{-}Ras^{G12V}$.
Results: TGF-β_1 increases the expression of H-Ras. The TGF-β_1-induced *H-Ras* promoter transactivation was Smad-independent, however it is necessary the activation of the TGF-b type I receptor ALK5 and the Ras-mitogen-activated protein kinase (MAPK) pathway. Endoglin attenuated stimulation by TGF-β_1 of both MAPK signalling activity and H-Ras gene expression. Furthermore, endoglin inhibited basal MAPK activity in transformed epidermal cells containing an H-Ras oncogene, as found by analyzing the levels of phospho-ERK1/2. Endoglin inhibited ERK phosphorylation without affecting MEK or Ras activity by an unknown mechanism strongly dependent on the endoglin extracellular domain. Finally, endoglin was able to inhibit the transforming capacity of $H\text{-}Ras^{Q61K}$ and $H\text{-}Ras^{G12V}$ oncogenes in a NIH3T3 focus formation assay.
Conclusions: The ability to interfere with the expression and oncogenic potential of H-Ras provides a new face of the suppressor role exhibited by endoglin in H-Ras-driven carcinogenesis.

729 The impact of hypoxia on differential expression of neurotensin receptors (NTR) in colorectal and prostate carcinoma cells

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Background: Recent studies showed increased expression of neurotensin receptors (NTR), particularly, NTR1 and NTR3, in various tumours, thus NTR is assumed a potential target for tumour imaging and therapy. However, the knowledge about the quantitative expression of NTR on mRNA and protein level, e.g., under hypoxic conditions is limited. The aim of this study was to develop a quantitative method for determination of absolute NTR mRNA amount in tumour and non-tumour cells and tissues. For method evaluation the NTR mRNA amounts in human colorectal (HT-29) and prostate (PC3) carcinoma cell lines under normoxic and hypoxic conditions *in vitro* were compared.
Material and Methods: A novel real-time RT-PCR method using an external standard was established. The elongation factor 1 alpha (EF1α) gene served as housekeeping gene and glucose transporter protein type 1 gene (GLUT1) was used as indicator for cellular hypoxic regulation effects. The derived standard curves allow for calculation of the number of specific mRNA molecules normalized to 1000 molecules of EF1α. Acute and chronic experimental hypoxia was induced by cultivation of cells at an oxygen concentration of 0.6% for 4 to 72 hours.
Results: Both HT-29 cells and PC3 cells show high mRNA expression of NTR1 in normoxia. In acute hypoxia (till 24 hours) the expression level of NTR1 did not change. However, under conditions of chronic hypoxia in HT-29 cells, at the latest after 48 hours, the NTR1 mRNA expression was significantly decreased. In contrast, the NTR1 mRNA in PC3 cells remained at a high level also in hypoxia. The mRNA level of NTR3 was about 5 orders of magnitude lower than NTR1 in both cell lines. Expression of NTR3 in both cell lines showed no significant differences during hypoxia, with a tendency to increase.
Conclusion: A novel standardizable and reproducible quantitative method for measurement of NTR mRNA in cancer cells was established. The use of NTR1 as a target for imaging or therapy strongly depends on tumour cell type and tumour hypoxia. Ongoing investigations will compare quantitative mRNA expression with data on functional expression of NTR, e.g., protein synthesis and radioligand interaction, in human samples and rodent tumour (xenograft) models.

730 MicroRNA-based, p53 dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation

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The tumour suppressor p53 is a sequence specific transcription factor that regulates the expression of many target genes linked, among others, to the control of cell cycle, apoptosis, angiogenesis and DNA repair. Recent studies identified direct p53 regulation of miRNAs and related regulatory circuits.
Using bioinformatics approaches, we identified an additional group of candidate miRNAs for direct p53 transcriptional control. Furthermore, some of those miRNAs can be predicted to target mRNAs in genes relevant to p53-mediated responses. Notably, we found examples of miRNA seed binding sequences at target 3′UTRs that contain SNPs predicted to modulate miRNA binding. Our work aims at the validation of p53-mediated control of the newly predicted miRNA genes and related circuitries that would provide additional negative and/or positive feedback loops for p53 regulation. To validate p53-responsiveness of 13 miRNA promoters not previously described to be under control of this family of transcription factors, we initially evaluated the potential for wild type p53, p63 and p73 to transactivate the predicted p53 response elements (REs) in those miRNA promoters. For these experiments we developed in the model system *S. cerevisiae* a panel of isogenic reporter strains harboring the chosen p53 REs upstream of the firefly luciferase reporter gene. 9 REs (including miR10b, 23b, 106a, 151, 191, 198, 202, 221, 320) were responsive to p53 of which 7 were also inducible by p63 or p73, even though to a lower extent. Moreover, we developed RT-qPCR and ChIP assays in human cell lines where p53 proteins could be ectopically expressed or induced by genotoxic stress. In general, results confirmed p53-dependent transcriptional regulation of the studied miRNAs, although cell line differences were observed. To establish miRNA targeting of selected mRNAs and the functional impact of SNPs at the miRNA binding sites we developed 3′UTRs reporter constructs differing for the SNP status or with mutagenized miR binding sites. We also measured allele imbalance at the endogenous gene level by quantitative RT-PCR analysis in cell lines heterozygous for the SNPs and relative protein levels by western blot to evaluate the impact of the SNP allele as well as of p53-dependent or -independent miR modulation. Specific examples of p53-directed post-transcriptional circuits will be presented.

731 Chemical induction of mitotic slippage by proteolytic degradation of spindle assembly checkpoint proteins

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Background: Chemicals were recently identified that induce cells arrested at mitosis by antimitotic agents to undergo mitotic slippage and enter interphase without chromosome segregation (Riffell *et al.*, Cell Cycle 8(18): 3025−38 (2009)), resulting in DNA endoreduplication and cell death through apoptosis; this project examines the mechanism whereby the mitotic slippage inducers SU6656 and geraldol force cells to escape mitotic arrest.
Materials and Methods: T98G glioblastoma cells were arrested in mitosis by exposure to paclitaxel or vinblastine and induced to undergo mitotic slippage by incubation with SU6656 or geraldol in the absence or presence of protease