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