BRCA1-interacting proteins, and the possible existence of SUMO interacting sites both in BRCA1 and partners point to a intricate network encompassing several pathways related to stress response, disruption of which may contribute to carcinogenesis.

720 Simultaneous HER2/neu and PTEN deregulation correlates with aggressive phenotype in hepatocellular carcinoma: a tissue microarray analysis

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Background: Hepatocellular carcinoma (HCC) is a highly aggressive and chemo resistant type of cancer. Although novel anti-HER2/neu targeted therapeutic strategies have been developed and applied in some types of malignancies, specific mechanisms of deregulation in HER2/neu (17q21) depended – signaling transduction pathway remain under investigation in HCC. Our aim was to investigate the potential role of simultaneous HER2/neu and PTEN (10q21-suppressor gene) dysregulation in HCCs.
Materials and Methods: Using tissue microarray technology, fifty-two (n = 52) formalin fixed and paraffin embedded tissue samples of histologically confirmed primary HCCs were cored and re embedded in the final paraffin block (core diam 1.5 mm). Immunohistochemistry (IHC) was performed by applying anti-HER2/neu and anti-PTEN antibodies. Fluorescence in situ hybridization (FISH) analysis was also performed regarding those genes.
Results: Protein over expression was observed in 12/52 (23%) cases regarding HER2/neu, whereas PTEN decreased or loss of expression in 22/52 (43%) cases. HER2/neu gene amplification was confirmed in 7/52 (13%) cases, whereas no one of the examined cases demonstrated PTEN gene numerical imbalances. Combined HER2/neu and PTEN aberrant expression was observed in 9/52 cases associated to the grade of the examined tumours ($p = 0.01$).
Conclusions: HER2/neu up-regulation combined to PTEN down-regulation is a relatively frequent and critical genetic event in HCC correlated also with an aggressive phenotype. PTEN decreased expression maybe is a negative prognostic factor for applying anti-HER2/neu targeted monoclonal antibody therapy (high chemo-resistance levels) in patients with HCC, based on breast adenocarcinoma management experience.

721 Aromatase inhibitor resistance; a role for estrogen receptor and AIB1 in differential gene regulation

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Background: Aromatase inhibitors (AIs) are fast becoming the first line treatment for post menopausal breast cancer patients. However, it is evident that AIs do not remove all estrogen and molecular studies suggest that this can result in adaptive estrogen hypersensitivity of the estrogen receptor-alpha (ERα) with consequent resistance to therapy. We hypothesised that, in the AI-resistant setting, ERα may have the capacity to recruit its coactivator protein AIB1 to drive transcription of ER sensitive genes and induce tumour proliferation.
Materials and Methods: MCF7 breast cancer cells were stably transfected with the aromatase enzyme to generate an AI-sensitive cell line (MCF7aro). To acquire an AI-resistant cell model, MCF7aro was treated long-term with the AI, letrozole, until it lost sensitivity to letrozole (MCF7aroR-Let).
Cellular proliferation was measured by crystal violet staining; ER target gene expression levels were analysed by PCR, real-time PCR and Western blotting; chromatin immunoprecipitation was used to determine recruitment of ER and AIB1 to the promoters of target genes; the expression of, and interactions between, ER and AIB1, were analyzed by co-immunoprecipitation and quantitative coassociation immunofluorescent microscopy, using cell lines.
Results: In the AI-sensitive cell line, increased proliferation and expression of ER target genes pS2, c-myc and cyclinD1 was observed in response to aromatase substrate androstenedione; this effect was inhibited by letrozole. In the AI-resistant cell line, letrozole failed to inhibit proliferation induced by androstenedione, nor expression of pS2 and c-myc. However, cyclinD1 expression remained sensitive to letrozole treatment.
Chromatin immunoprecipitation studies in these cells demonstrated that treatment with letrozole induced recruitment of both ERα and AIB1 to the promoter of ER target genes pS2 and c-myc in the AI-resistant cell line but not to the promoter of cyclinD1.
Co-immunoprecipitation and co-localisation of ER-α and AIB1 were increased in the AI-resistant cell line following treatment with letrozole in comparison with AI-sensitive MCF7aro.
Conclusions: These data suggest that in the AI-resistant setting ERα can utilise AIB1 to drive tumour progression in the presence of an AI, and that this occurs in a target gene-specific context. An alternative signalling network may be involved in regulating cyclinD1 gene expression and allowing AI-resistant cells to retain some sensitivity to AI treatment.

722 Characterisation of gene expression profiles in HeLa cells expressing BRCA1 missense variants

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Background: Most BRCA1 mutations originate non functional truncated proteins that predispose women to early-onset breast and ovarian cancer. A number of missense mutations whose role in the disease is often difficult to ascertain, however, have also been detected in hereditary breast cancer patients. To investigate the molecular mechanisms that may underlie a pathogenetic role for two missense variants located within the BRCT domain of BRCA1, the M1775R and the A1789T, we compared the expression profiles of HeLa cells transfected with these two BRCA1 variants and HeLa cells transfected with BRCA1 wild type. The M1775R variant has widely been described as deleterious by functional assays, but to date a characterization of its effects on gene expression in human cells has never been reported. The A1789T variant has never been studied before by other groups.
Materials and Methods: The gene expression profiles of five clones of HeLa cells transfected with plasmids expressing each of the two BRCA1 missense variants were compared by microarrays to those of five clones transfected with plasmids expressing BRCA1 wild-type. A reference design was adopted and the reference sample was obtained by pooling the mRNAs from the wild-type clones. Gene expression was investigated by two-colour microarray analysis, using the Whole Human Genome 4x44k Microarray G4112F (Agilent Technologies, Palo Alto, CA, USA).
Results: Compared to BRCA1wild-type, the M1775R variant showed 159 differentially expressed genes, 108 down-regulated and 51 up-regulated, while the A1789T variant showed 188 differentially expressed genes, 77 down-regulated and 111 up-regulated. Out of these genes 15 were differentially expressed with the same fold-change direction by both the mutations.
Pathway analyses mapped 33 out of the 159 and 33 out of the 188 differentially expressed genes in 54 and 55 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways respectively. For both variants many of the pathways with the highest values of impact factor were involved in cancer, including pathways implicated in cancer general network and those describing the co-regulation mechanisms which underlie different type of cancers.
Conclusion: Our findings indicate that the M1775R and the A1789T variants of BRCA1 gene affect the expression of many genes associated with known mechanisms of cancerogenesis and thus contribute to sustain the hypothesis that these two mutations have a role in the pathogenesis of familiar breast cancer.

723 Expression of HDAC1, 2, 3 and 7 as a prognostic markers in hepatocellular carcinoma

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Background: Histone deacetylases (HDAC) are enzymes that are responsible for the transcriptional control of genes through modifications of histone proteins. Among others, they play a factor in the control of tumour suppressor genes. Hypoacetylated histone proteins have been associated with precancerous and malignant lesions and for some tumour entities, such as prostate cancer and colon cancer HDAC expression has been identified as an independent prognostic factor. Since inhibitors of histone deacetylases (HDACi) emerge as promising therapeutics in the management of solid tumours, including hepatocellular carcinoma, we analyze the importance of the expression of 4 HDAC isoenzymes as a prognostic marker in hepatocellular carcinoma.
Method: Tissue micro arrays of primary HCCs and adjacent normal tissue of 170 patients (male n = 145, 85.3%; female n = 25, 14.7%; mean age 61.9 ± 11.0 years) were evaluated immunohistochemically for the expression of HDAC isoenzymes 1, 2, 3, 7 and ki-67 antigen. Intensity and extensity of expression were evaluated by two independent blinded observers, a product score calculated (IRS, immunoreactivity score) and the data was correlated with histopathological and clinical criteria. Based on mean HDAC expression for each isoenzyme, patients were stratified into high and low expression groups and the groups compared in terms of clinical data.

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