its aberrant spliced variants in the hepatocellular carcinoma cells were evaluated through MTT assay, wound healing assay and Boyden Chamber assay.
Results: Twenty aberrant splicing forms of Hugl-1 mRNA were identified in 20% (1/5) hepatocellular carcinoma cell lines and 30.77% (24/78) hepatocellular carcinoma specimens, but not their adjacent noncancerous tissues. Sequence analysis of all aberrant spliced forms of Hugl-1 revealed that a striking feature common to 95% of the aberrant forms is that small direct-repeat sequences (3−10 bp) flank the deleted regions of Hugl-1. In addition, somatic mutation in Hugl-1 was also found in 7.5% (6/78) hepatocellular carcinoma specimens. Statistic analysis shown that the abnormal splicing of Hugl-1 was significantly correlated with poor differentiation of hepatocellular carcinoma ($P = 0.011 < 0.05$) and large tumor size ($P = 0.019 < 0.05$). Interestingly, we also found that 75.9% (41/54) of hepatocellular carcinoma tissues displayed high, even over-expression of Hugl-1 compared to their adjacent noncancerous tissues. Overexpression of Hugl-1/wt inhibited migration and invasion of Sk-Hep1 cells. In contrast, expression of two abnormal spliced forms of Hugl-1 significantly promoted the both actions of the tumor cells.
Conclusions: Our data imply that the aberrant splicing and mutation of Hugl-1 may play an important role in development of hepatocellular carcinoma.

3509 POSTER

Growth inhibitory effects and mechanisms of lapatinib, a dual inhibitor of ErbB1 and ErbB2 tyrosine kinase, in gastric cell lines

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Background: Lapatinib (GW572016) is a dual inhibitor of both ErbB1 (epidermal growth factor receptor: EGFR) and ErbB2 tyrosine kinases. HER-2 gene amplification and protein overexpression occur in 10−20% of gastric cancer. We explore the therapeutic potential of lapatinib by testing its effect on gastric cancer cell lines.
Materials and Methods: We tested the in vitro growth inhibitory effect of lapatinib and molecular mechanism in gastric cancer cell lines (SNU-1, 5, 16, 216, 484, 601, 620, 638, 668, 719, NCI-N87) and ErbB2 amplified breast cancer cell SKBR3 as positive control. ErbB1 and ErbB2 amplification were identified through fluorescence in-situ hybridization (FISH). Growth inhibitory effect was assessed by tetrazolium bromide (MTT) assay. In relatively sensitive cell lines, cell cycle analysis at various conditions of lapatinib was done using flow cytometry and down-stream molecules were analyzed using immunoprecipitation and Western blot analysis. Interaction of lapatinib with cytotoxic agents (5-FU, cisplatin, oxaliplatin, paclitaxel) was evaluated by combination index.
Results: ErbB2 amplification were detected in SNU-216 and NCI-N87 gastric cancer cell lines. These two gastric cancer cell lines were sensitive to lapatinib as much as SKBR3 (IC50 = 0.02, 0.01, 0.018 respectively). None of gastric cancer cell lines showed ErbB1-amplification. Lapatinib induced G1 arrest as dose- and time-dependent manners in SNU-216 and NCI-N87. In NCI-N87, apoptosis was induced dominantly. In lapatinib-treated SNU-216 and NCI-N87, phosphorylation of ErbB1 and ErbB2 was inhibited. And then, phosphorylation of Akt and Erk was down-regulated. In NCI-N87, apoptotic molecules of PARP and casepase-3 were induced. Lapatinib treatment with 5-FU, cisplatin, oxaliplatin or paclitaxel resulted in additive or synergistic inhibitory effect. Lapatinib induced downregulation of thymidylate synthase, which is a target enzyme of 5-FU.
Conclusions: Lapatinib showed the growth inhibitory effect in the ErbB2-amplified gastric cancer cell lines as single agent and with combination of clinically relevant cytotoxic agents. This opens up the possibility of considering lapatinib as a therapeutic agent in gastric cancer.

3510 POSTER

Serum vascular endothelial growth factor (VEGF), VEGF receptor-1 and -2 among gastric cancer patients and healthy subjects

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Background: Vascular endothelial growth factor (VEGF) is a factor promoting vascularization including that of malignant tumors and serum VEGF concentration is high in patients with several cancers. Its receptor proteins-1 (VEGFR-1) and -2 (VEGFR-2) are also detected in serum. To date, VEGF is known to be higher among gastric cancer patients than among healthy subjects, but there is not sufficient data on VEGFR-1 or VEGFR-2.
Subjects and Methods: Subjects are 164 primary gastric cancer patients aged 23 to 69 years and 164 apparently healthy subjects (controls) paired one to one with the patients (matched for age [within 2 years] and gender). Using sera from the subjects, VEGF, VEGFR-1 and VEGFR-2 were measured and compared between the patients and controls. Comparison by paired t test was performed using all pairs and restriction pairs to ones with early (depth was within submucosa), advanced (deeper), intestinal or diffuse type cancer.
Results: Among the controls and patients, VEGF (pg/ml) was 479 ± 351 (mean $\pm$ standard deviation) and 641 ± 517 (164 pairs, $p = 0.001$), VEGFR-1 (pg/ml) was 56.0 ± 34.3 and 48.5 ± 32.5 (147 pairs, $p = 0.066$), and VEGFR-2 (pg/ml) was 8850 ± 1890 and 8400 ± 2010 (164 pairs, $p = 0.022$), respectively. Significant or nearly significant differences between patients and controls were observed among early cancer pairs of VEGF (78 pairs, $p = 0.057$), among advanced cancer pairs of VEGF (86, $p = 0.009$) and VEGFR-2 (86, $p = 0.003$), among intestinal type cancer pairs of VEGF (63, $p = 0.026$), and among diffuse type pairs of VEGF (101, $p = 0.018$), VEGFR-1 (90, $p = 0.064$), VEGFR-2 (101, $p = 0.002$).
Conclusion: VEGF was higher and VEGFR-1 and VEGFR-2 were lower among gastric cancer patients than among controls. Compared with early cancer, advanced cancer showed clearer difference with controls. Diffuse type cancer patients gave clearer difference of VEGFR-1 and VEGFR-2 with controls than intestinal type did, while such effect of pathological was not observed on VEGF.

3511 POSTER

Improved in vitro and in vivo efficacy in pancreatic cancer therapy in SCID mice by a new endostatin–albumin fusion protein

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Background: Endostatin is a potent endogenous inhibitor of angiogenesis. Additionally it could be shown that continuous application via intraperitoneally implanted pumps in mice is superior to bolus injections of endostatin. Aim of the study was to investigate the antiangiogenic and antitumor effects in vitro and in vivo of a new albumin endostatin fusion protein (AFP-endostatin) with increased half-life in a pancreatic cancer model.
Materials and Methods:
1. In a first step recombinant human AFP-endostatin was generated and expressed in yeast.
2. In the second step pharmacokinetic studies of AFP-endostatin versus rh-endostatin (Calbiochem) applicated intravenously (i.v.) and subcutaneously (s.c.) were performed to survey Cmax, half-life and optimal dosage. The measurement of endostatin serum levels were performed by ELISA (Cytimmune). Additionally HUVEC migration assays were conducted with AFP-endostatin and rh-endostatin (Calbiochem) 0.03−40 μg/ml.
3. Finally the in vivo efficacy was investigated. In male immuno-deficient mice (SCID, 6−8 weeks old) BxPC-3 pancreatic cancer cells (2.5×10^6 in 0.2 ml RPMI 1640 medium) were implanted s.c. in the midline dorsa of the mice (n = 7/group). Tumour volume was measured every 3−5 days with the digital calliper. Mice were randomised in therapy and control groups when tumour size reached 100 ± 20 mm3. Animals in the 4 therapy groups were treated by s.c. AFP-endostatin application: 0.5 mg/kg/24h; 0.4 mg/kg/72h; 1.2 mg/kg/72h; 3.6 mg/kg/72h versus daily PBS (placebo) application (n = 7/Gruppe) for 23 days. The applications were performed s.c. in an adequate distance from the tumor. Tumor volume was measured every 3−5 days with the digital calliper.

Results:
1. AFP-endostatin could be successfully generated and expressed in yeast.
2. The half-life for AFP-endostatin (56h) versus rh-endostatin (4.5h) was significantly increased. Migration assay showed 66% inhibition (0.2 μg/ml) for AFP-endostatin versus 87% inhibition (rh-endostatin).
3. Similar tumour inhibition rate could be shown for 0.5 mg/kg/24h (84% Inhibition) and 1.2 mg/kg/72h (78% Inhibition). A clear dose-response for the 72 h application schedule could be demonstrated. For 0.4 mg/kg/72h; 1.2 mg/kg/72h and 3.6 mg/kg/72h AFP-endostatin an inhibition rate of 61%; 78% and 92% respectively could be observed. No side effects or weight loss was observed during the whole experiment.