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.

Conclusion: AFP–endostatin shows comparable in vitro effectivity to rh-endostatin. Efficacy in tumour therapy is significantly higher compared to rh-endostatin (100 mg/kg/24h rh-endostatin versus 1.2 mg/kg/72h AFP-endostatin for an inhibition rate of 80%) published in the literature. The application frequency could be reduced with AFP-endostatin, which leads to better patients compliance and reduced therapy costs.

3512 POSTER

Non-resectable esophageal cancer treated with chemotherapy and radiotherapy of multiple 3D beams

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Purpose: The objective of this study is to evaluate the feasibility of treatment in non-resectable esophageal cancer with high dose radiotherapy by multiple 3D beams.
Methods: From July 2005 to February 2007, 18 patients with non-resectable esophageal cancer were treated in our institution with radical intention. Stage distribution was 3p. (17%) T4 N1 M0, 2p. (11%) T4 N0 M0, 4p (22%) T3 N1 M0, 3p (17%) T3N1M1a, 3p (17%) T3 N0 M0 and 3p (17%) T1-T2 N0 (non operable by comorbility). Esophagous sublocation were: 3p (17%) Cervical, 8 p (44%) Upper-esophageal, and 7 p. (39%) were distal. We administered four cycles of chemotherapy (CDDP 80 mg/m^2/day iv $\times$ 1 day + 5 FU 800 mg/m^2/day iv $\times$ 5 days the first and the fourth week radiotherapy) in 11p. (61%). Gross tumour volume (GTV) was defined according to CT Scan-PET images. Clinical tumour volume (CTV) was defined by GTV with a 3 cm cranial-caudal and 1 cm radial expansion. PTV was defined by CTV with a 1 cm around. Boost dose tumour was defined by GTV with a 1 cm around. A "combined" plan was created to treat the PTV receiving 44 Gy in 22 fractions using 3D planning, followed by a boost until final dose at 66 Gy in 11 fractions. Treatment planning goals were spinal cord max dose <46 Gy, lung dose restricted to a combined V20 < 35% and heart dose restricted to V60 < 30%.
Results: Our doses in critical organs were: the mean of maximum dose received in spinal cord was 40.9 with standard deviation (sd) of 5.9 and 17 p. (94.4%) received doses lower than 46.2 Gy. In heart V60 mean was 4.02% (sd, 7.43, range 0.02−24) and irradiated heart volume was less than 24% in all cases. Finally V20 mean for lung was 23.96 (sd 9.09, range 11−37) and 17 p. (94.4%), V20 was less than 36%. No high grade acute complications were observed during the treatment course.
Conclusions: The irradiation with multiple 3D beams in non-resectable esophageal cancer is a good option for radical treatment, achieving high doses in PTV. However, though we didn't have acute complications, the maxim dose in critical organs is not negligible. Late complications should be analyzed during the follow up of this cohort.

3513 POSTER

Interleukin-13 exerts autocrine growth promoting effects in human pancreatic cancer and its expression correlates with a propensity for lymph node metastases

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Background: Pancreatic cancer cells express receptors for interleukin-13 (IL-13). It is not known, however, whether IL-13 modulates pancreatic cancer cell growth and spread.
Materials and Methods: Cell growth and signaling were analyzed by cell counting, MTT assays, FACS, and kinase activity assays. IL-13 expression and secretion were determined by Northern blot analysis and ELISA, respectively. Localization of IL-13 and IL-13 receptors (IL-4R and IL-13R) in primary pancreatic ductal adenocarcinoma (PDAC) was characterized by immunohistochemistry.
Results: IL-13 significantly enhanced the growth of ASPC-1, CAPAN-1, and COLO-357 pancreatic cancer cells. This was associated with enhanced p44/42 MAPK phoshorylation. In contrast to p44/42 MAPK, IL-13 also induced PI-3 kinase activity in IL-13 unresponsive MIA PaCa-2, PANC-1, and T3M4 cells. All tested cell lines expressed and secreted IL-13. Neutralizing IL-13 antibodies inhibited the growth of ASPC-1 and CAPAN-1 cells. High IL-13 (30/70) and IL-4R (28/70) immunoreactivity was present in PDAC tumor samples. Fifteen of 16 specimen (94%) exhibiting high levels of both IL-13 and IL-4R displayed lymph node metastases, while only 30 of the remaining 54 samples (56%) had positive lymph nodes ($p = 0.0134$).
Conclusions: IL-13 may is an autocrine growth factor in PDAC. Moreover, endogenous expression of IL-13 in conjunction with IL-4R in the cancer cells may facilitate lymph node metastasis by modulating the interactions between tumor cells and immune cells.

3514 POSTER

Altered expression of hepatocyte nuclear factors in pancreatic ductal adenocarcinoma cell lines, varying on the level of differentiation

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The work is devoted to the analysis of the impact of hepatocyte nuclear factors (HNFs) in the control of pancreatic cell lines proliferation and differentiation.
HNFs were identified as the main regulators of liver development and differentiation. We have previously identified the essential role of the dysfunction of HNFs network in the progression of hepatocellular carcinoma. The central player of HNFs network in the liver is HNF4a, which is downregulated in highly malignant tumors. Restoration of HNF4a expression in dedifferentiated hepatocellular carcinoma can promote the reversion of tumors toward a less invasive highly differentiated slow-growing phenotype.
While HNFs were first discovered in the liver, they are expressed in different combinations in the epithelium of other organs including pancreas and clearly participate in gene regulation and tissue differentiation together with other tissue-specific factors. Being derived from the common ontogenetic precursor, the liver and pancreas demonstrate very similar patterns of HNFs expression. The most significant difference in HNF set between the liver and pancreas is substitution of "adult" HNF4a1 isoform of by "embryonic" HNF4a7 one driven by the alternative promoter.
On the basis of these facts we decided to analyze the role of HNFs in differentiation and malignant transformations in the pancreas. For this study we used four cultures derived from pancreatic ductal adenocarcinomas: Capan-2, Panc1, AsPC-1 and MIA PaCa-2. These cultures were arranged by their level of differentiation: first consists of typical epithelial cells, able to form well-defined monolayer and three-dimensional structures resembling ducts and acini, and last – of poor differentiated fibroblastoid single cells, therefore presenting an interesting model for investigation of EMT in the process of malignisation.
Using semi-quantitative RT-PCR we have found that HNF4a7 and vHNF1 expression correlates with the level of differentiation. On the contrary the expression of OC2 and CEBPa in differentiated cell cultures is minimal and increases towards the less differentiated ones. Forced expression of HNF4a7 in dedifferentiated cell culture MIA PaCa-2 reduced the cell proliferation and altered the expression of differentiation markers in this pancreatic cell line.
The work was supported by grants from Russian Foundation for Basic Research and President of Russian Federation award for young Doctors of Science (MD-8329.2006.4).

3515 POSTER

Cisplatin is more cytotoxic than oxaliplatin in oesophageal adenocarcinoma cells demonstrated by Expression of ERCC1 and XPA

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Background: Xeroderma Pigmentosum protein A (XPA), recognises DNA damage, its low levels with decreased expression of Excision Repair Cross Complementing 1(ERCC1), an endonuclease of Nucletide excision repair pathway, were found responsible for pronounced cisplatin sensitivity. While increased levels of XPA and ERCC1has demonstrated cisplatin resistance. We want to compare the levels of XPA and ERCC1 gene expression in oesophageal adenocarcinoma cells (OE33) after treating them with cisplatin and oxaliplatin.
Methods: Lethal doses 50 (LD50) of oxaliplatin and cisplatin in OE 33 cells for 24 hours were determined by the alamarBlue assay. Cells were treated with that LD50 at 2, 4, 6 and 24 hours. Using RT-PCR and specific primers, expression of ERCC1 and XPA gene levels with cisplatin and oxaliplatin treatments were compared at 2, 4, 6 and 24 hours interval, by Syngene Gene Tools programme.
Results: XPA was undetectable with cisplatin treatment, while it was overtly expressed with oxaliplatin treatment at all time intervals. Small amounts of ERCC1 were seen in 2, 6 and 24 hours with below detectable levels at