DHMEQ reduced viable cancer cells in the peritoneal cavity (CPM/mm^2 of DHMEQ treated group was reduced by 34.8±13.8%, while none of DMSO treated group reduced).
Conclusion: DHMEQ, by suppressing cancer cell proliferation and adhesion to peritoneum, may effectively prevent gastric cancer progression in abdominal cavity.

6636 POSTER

Genetic profiling of circulating tumor cells in the blood of patients with local advanced or metastatic upper gastrointestinal carcinomas

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Background: We have developed a new preanalytical enrichment method for circulating carcinoma cells (CTC) based on EpCAM and MUC1 specific antibodies coupled to immunomagnetic beads. Molecular detection and tumor cell characterization was performed with a multimarker panel by real-time RT-PCR. Here we present first results of a universal marker panel for upper gastrointestinal carcinomas (including carcinomas of stomach, duodenum, pancreas and biliary tract).
Methods: Samples from patients were divided in native probes and matched calibrator probes containing 2 and 10 carcinoma tumor cells (ETC). The high affinity antibodies BM7 (MUC-1) and VU1D9 (EpCAM) were used for immunomagnetic tumor cell enrichment from 10 ml peripheral EDTA-blood of patients with documented metastatic disease. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis. Real-time quantitative RT-PCR approaches with SYBR assays (Eurogentec) and FAM-labeled TaqMan probes selected with the UniversalProbeLibrary system (Roche AG, Basel, CH) were developed for the epithelial markers cytokeratin19 and 20 (CK19/20), EpCAM, CEA, Survivin, CD276, metastasis associated in colon cancer (MACC) transketolase TKTL1 and HIF-1alpha.
Results: Sensitivity of the the multimarker panel was validated in calibration tests with 2 cells and 10 cells (embedded tumor cell calibrators, ETC) and the specificity of the panel was confirmed by examination of blood from healthy donors. Positivity rate of ETC controlled real-time RT-PCR on the basis of the multimarker panel was 71% (12 of 17 patients with local advanced and/or metastatic disease). 11 patients (65%) showed two or more positive markers. The marker with the highest prevalence was EpCAM (64%) followed by CK19 (43%), CD276 (43%), CEA (39%), Survivin (29%), CK20 (25%), MACC (14%).
Conclusion: We have used embedded tumor cells (ETC) as internal calibrators for accurate process control and normalization of the immunobead quantitative RT-PCR technique. The newly introduced surrogate marker panel from the networks of apoptosis, invasion, angiogenesis and stem cell phenotype should improve early detection of metastasis, monitoring of therapy response and efficacy and selection of tailored therapy regimes.

6637 POSTER

Expression of Bax predicts outcome in advanced gastric cancer patients treated with 5-fluorouracil, leucovorin, and oxaliplatin palliative chemotherapy

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Background: Platinum and 5-fluorouracil (5-FU)-based regimens have been used the most frequently in palliative chemotherapy for gastric cancer. The present study evaluated the prognostic significance of Bax, excision repair cross-complementation group 1 (ERCC1), and thymidylate synthase (TS) in advanced gastric cancer patients treated with 5-FU, leucovorin, and oxaliplatin (FOLFOX) palliative chemotherapy.
Materials and Methods: Seventy-two patients with metastatic or recurrent gastric cancer were treated with FOLFOX regimen. Pretreatment tumor biopsy specimens were analyzed for Bax, ERCC1, and TS expression by immunohistochemistry.
Results: High expression of Bax, ERCC1 and TS was observed in 31 (43%), 33 (46%), and 35 (49%) patients, respectively. The median overall survival (OS) of patients was 12 months. Low expression of Bax was associated with poor OS (median, 9 months vs. 18 months; 2-year, 10% vs. 48%; P = 0.0005) in univariate analysis, while expression of ERCC1 and TS was not correlated with patient outcome. In multivariate analysis, low expression of Bax was a significant independent predictor of poor OS (p = 0.029).
Conclusions: Low expression of Bax was significantly associated with the poor survival of patients with metastatic or recurrent gastric cancer treated with FOLFOX chemotherapy. Immunohistochemical staining for Bax with pretreatment biopsy specimen may be a useful in selecting FOLFOX regimen as a treatment option for advanced gastric cancer patients.

6638 POSTER

Significance of gene expression of vascular endothelial growth factor and its receptors in therapeutic effect of the hepatic arterial infusion chemotherapy against advanced hepatocellular carcinoma

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Background: Transcatheter arterial infusion chemotherapy using platinum complex was generally performed in patients with advanced hepatocellular carcinoma (HCC) but its prognosis was poor. Recently, it was reported that anti-angiogenic drug, sorafenib, was effective against advanced HCC. This drug is going to use together with anti-cancer reagents. In this study, gene expression of angiogenic factor (vascular endothelial growth factor (VEGF)) and its receptors (KDR and flt-1) was investigated and the relation between gene expression of them and therapeutic effect was investigated to assess any possibility of predicting the therapeutic effect.
Material and Methods: The subjects of this study were 37 HCC patients who received the chemotherapy with platinum complex by hepatic arterial infusion. After informed consent was obtained and prior to the start of treatment, liver biopsy was performed to collect tissue from the tumor site and non-tumor site. The expression amount of each gene was determined by quantitative PCR method using LightCycler. The amount of expression was expressed as a relative ratio to GAPDH.
Results: 1) The median follow-up duration was 9.7 months. The median survival time (MST) and 1-year survival rate were 9.6 months and 53%, respectively. Of 37 enrolled patients (male/female 34/3, median age 69 (range 46−75), Child-Pugh A/B 21/16 and portal vein invasion yes/no 9/28), one patient achieved complete response (CR) and thirteen patients achieved partial responses (PR) and eleven patients achieved stable diseases (SD) and twelve achieved progressive diseases (PD). The therapeutic effect was judged according to the RECIST criteria. 2) CR and PR cases were assessed as responders while SD and PD cases were assessed as non-responders. KDR expression in the former was significantly higher than the latter and VEGF expression in the former tended to be higher than the latter. 3) When the cut-off values were set at the median respectively and the patients were classified into the high expression group and low expression group, MST in the former was significantly longer in the latter in case of KDR (30.5 month/10.5 month, p < 0.05) but MST was no significant difference in case of VEGF and flt-1 respectively.
Conclusions: The substantial involvement of KDR is strongly suggested in predicting the effect of platinum-based chemotherapy and sequentially maintenance of VEGF signaling pathway may be prolonged survival.

6639 POSTER

Skeletal metastases in gastric cancer: analysis of skeletal-related events and plasma endothelin-1

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Background: Skeletal metastases occur approximately in 20% of patients with metastatic disease in gastric cancer. There have been limited reports that described skeletal-related events and their patho-physiologic mechanisms. Endothelin-1 (ET-1) and its receptors play an important role in the development of osteoblastic skeletal metastases, which have been investigated for prostate cancer. It has been reported that plasma ET-1 level is high in prostate cancer patients with skeletal metastases, suggesting its receptor antagonist would be a new therapeutic target. With regard to gastric cancer, ET-1 is not yet assessed for the clinical significance in the development of skeletal metastases.
Material and Methods: Between 2002 and 2008, we retrospectively reviewed the medical records of 85 patients with metastatic gastric cancer in our institute. Out of 108 patients, 19 patients (17%) were found to have skeletal metastases during their clinical course. They were analyzed

for clinical characteristics, radiologic appearance, skeletal-related events (SREs) and plasma ET-1.
Results: The median age was 60 years (range 29 to 71). There were 11 males and 8 females. Major pathologic types were poorly-differentiated adenocarcinoma and signet-ring cell carcinoma. Radiologic appearance included 12 osteoblastic, 5 mixed, and 2 osteolytic pattern. There was no case which developed major SREs, being at least complicated with radiation to bone, pathological fractures, or hypercalcemia. Out of 19 patients with skeletal metastases, 11 patients developed hematological complications, including microangiopathic hemolytic anemia (MAHA), disseminated intravascular coagulation (DIC). In contrast, reviewing non-skeletal metastases (n = 89), there was only two cases with hematological complication. Plasma ET-1 level was measured in 6 out of 19 patients with skeletal metastases. The levels in the skeletal metastases were 2.416±0.6 pg/ml, mean±SD (n = 6), which were higher than those in non-skeletal metastases, 1.817±0.4 pg/ml, mean±SD (n = 7). In addition, serum ALP levels were also high in 6 patients that ET-1 were measured (1245±424 U/L, mean±SD, n = 6).
Conclusions: Our study shows that the major SREs are uncommon in gastric cancer and that their skeletal metastases are characterized as associations with hematologic complications. It suggests that higher plasma level of ET-1 is correlated with skeletal metastases in gastric cancer, as it previously studied in prostate cancer by others.

6640 POSTER

Enhancer of zeste homolog 2 expression is associated with tumour cell proliferation and metastasis in gastric cancer

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Background: Polycomb group proteins are transcriptional repressors that silence specific sets of genes through chromatin modification. The enhancer of zeste homolog 2 (EZH2), considered a member of the polycomb group proteins, plays an important role in cell proliferation and cell cycle regulation. EZH2 is overexpressed in aggressive forms of prostate, breast, bladder, and endometrial cancer. However, the role of EZH2 expression in gastric cancer has not yet been fully determined. This study was conducted to investigate the mechanisms of carcinogenesis and the clinical value of EZH2 expression in gastric cancer.
Materials and Methods: We analyzed EZH2 expression using western blot in AGS, MKN-28, SNU-16, SNU-484, SNU-601, and SNU-638 gastric cancer cell lines. After transfection of for EZH2 siRNA in MKN-28, the change of cell cycle related molecules was assessed by western blot. Expression of EZH2, Ki-67, and p53 was determined by immunohistochemical staining of tissue microarrays from specimens of 137 cases of resected gastric cancer.
Results: Among 6 cell lines we found high expression of EZH2 in all gastric cancer cell lines. RNA interference of EZH2 induced up regulation of p53 and down regulation of cyclin D1 and cyclin E. High EZH2 expression was observed in 60.6% of gastric cancers and in 6.7% of non-neoplastic gastric tissues ($P < 0.01$). 40.1% were positive for p53. High EZH2 expression correlated with Ki-67and p53 expression and was significantly associated with distant metastasis and non-signet ring cells.
Conclusions: These results suggest that high EZH2 expression is associated with tumor cell proliferation and metastasis.

6641 POSTER

Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with gastric carcinoma

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Background: Cyclooxygenases regulate the production of prostaglandins and play a role in tumor development and progression. The authors investigated the prognostic impact of expression of the cyclooxygenase (COX) isoform, COX-2, on disease-free survival and progression-free survival in patients with primary gastric adenocarcinoma (any pN any pT) without distant metastasis as well as the association between COX expression and other clinicopathologic parameters.
Methods: A cohort of 194 patients with gastric cancer (123 males 87 women) without distant metastasis who underwent R0 gastric resection were enrolled in this study. Immunoistochemical immunoreactivity was assessed by the intensity of staining and percentage of positivity areas. Association between factors including clinico-pathological variables and COX-2 scores, were assessed by χ^2 and Student t test. Survival rates were calculated using Kaplan-Majer method and the difference between the groups were analyzed by log-rank test.
Results: A correlation between COX-2 expression, grading and advanced penetration dept (mean COX-2 expression 74% in early gastric cancer (EGC) versus 52% in non-EGC, $p = 0.0017$). There was an association between COX-2 expression and the presence of lymph-node metastasis ($p < 0.0001$, χ^2). We also observed a significant association between COX-2 expression and relapse of disease ($p = 0.05$ KM) but not with poor survival.
Conclusions: High COX-2 protein expression, serosal invasion (pT3-pT4), and presence of lymph-node metastasis are poor prognostic factors in patients with gastric carcinoma without distant metastasis. COX-2 expression in any percentage strongly correlates with lymph-node invasion and penetration dept, so it may indicate tumor aggressiveness. The current data suggest that increased expression of COX-2 may play a role in the progression of primary gastric carcinoma. It remains to be investigated whether treatment with selective inhibitors of COX-2 may be an additional therapeutic option for patients with breast carcinoma.

6642 POSTER

Gap junctional intercellular communication influences the cytotoxic effect of docetaxel in esophageal cancer cells

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Background: Gap junctional intercellular communication (GJIC) mediated by connexin (Cx) plays the important role to maintain homeostasis in multicellular organisms. GJIC has also been reported to be associated with positive therapeutic aspect, such as the bystander effect in HSV/TK gene therapy. The aim of this study was to investigate the influences of GJIC in the cytotoxic effect of anticancer drug in esophageal cancer cells.
Materials and Methods: Human esophageal squamous cell carcinoma cell line (KE-10) without GJIC capacity was transfected with connexin 32 gene (Cx32), and cytotoxic effect of docetaxel (DOC) was investigated in KE-10 and Cx32-transfected KE-10 (KE-10/Cx32). Moreover, the cytotoxic effect of DOC was further examined when GJIC was blocked in KE-10/Cx32 cells.
Results: Restoration of GJIC capacity was confirmed by dye-transfer assay in KE-10/Cx32. Cytotoxic effect of DOC in KE-10/Cx32 increased by 40% compared to that in parental KE-10. Enhancement of cytotoxity of DOC in KE-10/Cx32 was abandoned when exposed with the GJIC blocking agent. MDR gene and its protein, which plays the key role in the drug resistance of DOC, was not observed in both KE-10 and KE-10/Cx32.
Conclusions: These data suggest that gap junctional intercellular communication in esophageal cancer cells has the positive influence on the cytotoxic effect of DOC.

6643 POSTER

Anti-proliferative effect of SOCS-1,-3 through the suppression of JAK/STAT and P38 MAPK signaling pathways in gastric cancer cells

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Background: Cytokines and growth factors are important regulators of cell differentiation and proliferation and their signal transduction is negatively regulated by the suppressors of cytokine signaling (SOCS) family proteins. Elevated serum levels of interleukin-6 (IL-6) cytokine correlates with enhanced disease progression and recurrence in patients with gastric cancer. In this study we investigated an anti-proliferative effect of SOCS-1,-3 gene delivery in gastric cancer cells *via* the inhibition of IL-6 signaling.
Material and Methods: Six gastric cancer cell lines (MKN7, MKN45, MKN74, NUGC-3, NUGC-4, AGS) were used in this study. IL-6 levels in culture supernatants were measured by ELISA. Levels of the IL6-activated proteins STAT3, P38 MAPK and PI3-Kinase protein in cell lines was determined by Western blot analysis. The *in vitro* anti-proliferative effect of SOCS-1/-3 adenovirus-mediated gene delivery in cultured gastric cancer cell lines was measured by MTT assay.
Results: Elevated levels of IL-6 in NUGC-3 (1271 pg/ml) and AGS (159 pg/ml) cell culture supernatants compared to MKN7, MKN45, MKN74 and NUGC-4 cell lines (barely detectable levels) correlated with enhanced phosphorylation of STAT3, P38 MAPK and AKT proteins in NUGC-3 and AGS cells. Ectopic expression of SOCS-1/-3 significantly reduced cell proliferation to 12 % in NUGC-3 cells ($p < 0.0001$) and to 10 % in AGS cells ($p < 0.0001$) compared to control cells at day 5. SOCS-1 gene delivery also reduced cell proliferation in MKN45 (a low IL6-producing cell line). The inhibitory effect of SOCS-1/3 delivery on cell proliferation in NUGC-3, AGS and MKN45 cells correlated with decreased levels of phosphorylation of