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

STAT3 and P38 MAPK. Cell proliferation was also significantly reduced in NUGC-3, AGS and MKN45 cells following treatment with either a JAK inhibitor (JAK-Inhibitor 1) or a P38 MAPK inhibitor (SB203580), however, treatment with anti-IL-6R antibody and wortmannin (PI3-K inhibitor) had no inhibitory effect on cell proliferation.
Conclusions: Our data indicates an important role for both the JAK/STAT and P38 MAPK signaling pathways (independent of IL-6) in the proliferation of gastric cancer cells and highlights a potent anti-proliferative effect of SOCS-1/-3 gene delivery *via* the suppression of these signaling pathways. SOCS-1/-3 gene delivery may thus represent a future novel therapeutic strategy for the treatment of gastric cancer.

6644 POSTER

A novel NF-kB inhibitor DHMEQ suppressed peritoneal dissemination of pancreatic cancer in mouse xenograft model

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Background: Pancreatic cancer in advanced stage is lethal in most patients. NF-kB is frequently and constitutively activated in pancreatic cancer and well known to be involved in the biological aggressiveness of this cancer, and hence can be an attractive therapeutic target. In the present study, we studied the therapeutic potentials of a newly developed NF-kB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), especially in pancreatic cancer metastasis.
Materials and Methods: We evaluated growth inhibitory effect of DHMEQ on AsPC-1 human pancreatic cancer cells using MTT assay. For quantitative analysis of tumor size in vivo, we applied GLuc, a secretory type of luciferase (marine copepod, *G. princeps*) to AsPC-1 cells (AsPC-1-Gluc), which enables us to monitor in living mice 1) tumor growth in mice by bio-luminescence imaging and 2) whole tumor progression by measuring bio-luminescent intensity of extracellularly secreted GLuc from cancer cells into circulating blood. 5×10^6 AsPC-1-GLuc cells were implanted subcutaneously into 6-week-old male BALB/c nu/nu mice. For evaluation of the effect of DHMEQ on pancreatic cancer metastasis, 1×10^7 AsPC-1-Gluc cells were injected intraperitoneally into mice as a peritoneal dissemination model (day 0). We administered vehicle or DHMEQ (15 mg/kg or 30 mg/kg) by i.p. injection into the mice twice a day. We monitored tumor growth by GLuc activity in plasma, and tumor metastasis by bio-imaging every 3 or 4 days. The mice were finally sacrificed on day 30 for evaluation of tumor volume and histological examination.
Results: DHMEQ inhibited proliferation of AsPC-1 cancer cells in a dose-dependent manner in vitro. 5 μg / ml of DHMEQ was enough to inhibit cell growth by 50%. In xenograft model, we firstly confirmed that GLuc activity in the blood reflects tumor mass in mice. A good correlation was observed between subcutaneous AsPC-1-GLuc tumor mass and the Gluc activity in mice plasma, indicating this method is appropriate to evaluate tumor mass. In the mouse peritoneal dissemination experiment, DHMEQ significantly reduced GLuc activity in plasma as well as tumor size and dissemination. Histological examination showed the reduced mitosis and the increased apoptosis in the DHMEQ-treated tumor cells. NF-kB activity in the tumor was also suppressed.
Conclusions: Our results indicate that DHMEQ possesses a good potential for treatment of pancreatic cancer.

6645 POSTER

Increased expression of delta Np73 correlates with short time survival in cholangiocarcinoma patients

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Background: Among p53 family members, p63 and p73 share similar structure and function in cell cycle control and apoptosis. All members can encode truncated N-terminal domain or delta-N isoforms; DNp53, DNp63 and DNp73, which can inhibit trans-activation of full length isoforms (TAp53, TAp63, TAp73). Therefore, over-expression of DN isoforms can exert as epigenetic inhibitor of full length proteins. The aim of this study was to examine expression pattern of p73 isoforms in cholangiocarcinoma(CCA) and investigate whether p73 isoforms can be used to predict cancer prognosis.
Materials and Methods: Six CCA cell lines and 34 CCA patients were used to investigate both p73 isoforms at transcriptional and protein levels using quantitative real time PCR and immunohistochemical staining. CCA patients were categorized into short and long survival group according to their median survival time. The association of expression pattern of p73 isoforms was statistical analyzed with patient survival.
Results: Among CCA cell lines, the increase of DNp73/TA p73 was observed in CCA cell lines with poor histopathological grading. In clinical samples, a significant increase of DNp73 transcripts was observed in the patients with short time survival (Mann Whitney test, $p = 0.007$) whereas, over-expression of DNp73 protein was also associated with poor survival patients (Chi'square test, $p = 0.04$).
Conclusion: The up-regulation of DNp73 might be used to predict the cancer outcome in CCA patients. More data in expression of DNp53 and DNp63 as well as more clinical samples should be included in further study.

6646 POSTER

Correlation between FOXO1A transcription factor and cell cycle regulators in gastric cancer

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Background: The present study investigated the relationship between the expression of phosphorylated FOXO1A (pFOXO1A) and the expressions of cell-cycle regulatory proteins in human gastric carcinoma.
Material and Methods: We used the immunohistochemical staining performed on tissue array slides containing 272 human gastric carcinoma specimens. Antibodies against cyclins D1, E and B3 as well as $p21^{Waf1/Cip1}$ (p21), $p27^{Kip1}$ (p27), p53, and retinoblastoma protein (pRB) were used. Data were analyzed with SPSS program.
Results: The expression of pFOXO1A positively correlated with that of cyclin D1 ($P = 0.014$), p21(Waf1/Cip1) ($P = 0.006$) or p27(Kip1) ($P = 0.001$), suggesting co-regulation of these proteins in gastric carcinoma. Moreover, cyclin D1 seems to determine the association between pFOXO1A and p21 or p27. The survival rate was higher in patients with both pFOXO1A-positive and cyclin D1/p21/p27-positive tumors ($P < 0.05$) than in the remainder of the population. On the other hand, the expression of cyclin E, p53 and pRb showed no association with that of pFOXO1A.
Conclusions: Our results suggest that the combined examination of FOXO1A and cyclin D1 along with p21 or p27 expression may allow a precise estimation of prognosis in patients with gastric carcinoma.

6647 POSTER

MET amplification by qPCR predicts poor outcome in gastric cancer: a novel prognostic marker and a potential therapeutic target

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Purpose: We undertook this study to assess the impact of c-Met overexpression, c-Met activation, and *MET* amplification on survival of gastric cancer patients.
Experimental Design: *MET* amplification and activation of c-Met were tested in various gastric cancer cell lines *in vitro*. Of the 482 tissue samples from gastric cancer patients who underwent curative surgery, *MET* amplification was tested in 472 gastric cancer patients. c-Met protein expression and c-Met activation were evaluated by immunohistochemical staining against c-Met protein and phospho-Met (pY1349), respectively.
Results: Analysis of the gastric cancer cell lines using quantitative real-time PCR (qPCR) identified the increased *MET* copy number which predicted sensitivity to PHA-665,752, a selective c-Met kinase inhibitor. Of the 472 patients who had DNA sample available for qPCR analysis, 100 (21.2%) of the patients had *MET* copy number greater than 4.0 copies. Of the 103 tumor samples with c-Met protein activation identified by staining against phospho-Met (pY1349), 30 (29.1%) had *MET* amplification ($\geqslant$4 copies) ($P = 0.026$). Gastric cancer patients with *MET* amplification demonstrated poorer survival following curative surgery with statistical significance (5-year OS; 50.0% vs 59.1%; *MET* amplification (+) vs *MET* amplification (−); $P = 0.0134$).
Conclusions: *MET* amplification measured by qPCR was associated with shorter DFS and poorer OS but not c-Met protein overexpression or c-Met protein activation.