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Characterisation of the response of pancreatic cancer cells to treatment with chemotherapeutic agents alone and in combination with tyrosine kinase inhibitors

R. Sheikh[1], R. O'connor[2], N. Walsh[2], M. Clynes[2], R. Mcdermott[1]. [1]*Adelaide and Meath Hospital Tallaught Dublin Ireland, Medical Oncology, Dublin, Ireland;* [2]*National Institute of Cellular Biotechnology, Dublin City University, Dublin, Ireland*

Background: Pancreatic cancer is inherently resistant to most chemotherapy treatments. The role of chemotherapy in the treatment of locally advanced and metastatic pancreatic cancer, so far, is quite modest and median survival times of 6 months are typically reported, hence new and improved treatment regimens need to be developed.
Methods: We undertook a toxicological characterisation of the response of three pancreatic cancer cell line models, BxPc-3, SW1990 and MiaPaCa-2, to specific chemotherapeutics (cytotoxics and tyrosine kinase inhibitors (TKIs)). The cytotoxic agents employed were gemcitabine, 5-FU, docetaxel, 5-DFUR (the active intermediate of capecitabine), cisplatin and epirubicin and the TKIs used were erlotinib, gefitinib and lapatinib. 1×10^4 cells/ml were seeded in 96 well plates on day 1 and test drug or combination test agents were added on day 2. Plates were kept in the incubator for 6 days for BxPc-3 and SW1990 and 5 days for MiaPaCa-2 and cell survival was assessed using the acid phosphatase assay. Combination cytotoxic agent and TKI assays were undertaken in BxPc-3 cells (the most sensitive to TKIs). To assess the interactions of TKIs with cytotoxic cancer drugs, we classified the findings into three categories; sub-additive, additive and super-additive, based on the combination index value generated by analysing the data using the CalcuSyn programme. All assays were repeated at least three times.
Results: We found the BxPc-3 cells to be the most sensitive not only to all the cytotoxic agents but also to TKIs employed. MiaPaCa-2 was also sensitive to most cytotoxic agents but was insensitive to TKIs. SW1990 was less sensitive to gemcitabine, epirubicin and the TKIs.
Combinations of epirubicin with lapatinib showed super-additive toxicity, same is the case with docetaxel with lapatinib. 5DFUR combined with lapatinib demonstrated additive activity while the combination of cisplatin with erlotinib and lapatinib produced a clear sub-additive interaction.
Conclusion: This data indicates that pancreatic cell models can differ in their response to cancer drug treatment. Specific combinations including epirubicin with lapatinib and docetaxel with lapatinib demonstrated a super-additive response which may warrant further clinical evaluation. Cisplatin combined with lapatinib or erlotinib produced a poorer response than either agent alone, suggesting that such combinations may have less activity and be poor candidates for further clinical trials.

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Prognostic impact of the NFKB1 insertion/deletion promoter polymorphism on survival in patients with surgically resected gastric cancer

J.G. Kim[1], Y.S. Chae[1], S.N. Kim[1], B.W. Kang[1], S.K. Sohn[1], H.Y. Chung[2], W.S. Yu[2], G.S. Choi[2], S.H. Jun[2], K.H. Lim[2]. [1]*Kyungpook National University Hospital, Oncology/Hematology, Daegu, South Korea;* [2]*Kyungpook National University Hospital, Surgery, Daegu, South Korea*

Background: The present study analyzed the functional insertion/deletion polymorphism in the promoter region of *NKFB1* gene and their impact on the prognosis for patients with gastric adenocarcinoma.
Materials and Methods: Five hundred and three consecutive patients with surgically resected gastric adenocarcinoma were enrolled in the present study. The genomic DNA was extracted from paraffin-embedded tissue and the −94 insertion/deletion ATTG polymorphism of *NFKB1* determined using a PCR-RFLP assay.
Results: The *NFKB1* promoter gene polymorphism was successfully amplified in 97.8% of the cases. There were no sexual differences in relation to the genotype and allele. No correlation was observed between the frequency of the genotype or allele and the T, N, or M stage. The multivariate survival analysis showed no association between the *NFKB1* −94 insertion/deletion promoter polymorphism and the disease-free survival or overall survival of the patients with gastric cancer.
Conclusions: The functional *NFKB1* promoter polymorphism was not found to be a prognostic marker for Korean patients with surgically resected gastric adenocarcinoma.

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Evidence for angiogenesis-independent contribution of VEGFR1 (FLT1) in gastric cancer recurrence

A. Sokolenko[1], A. Kashyap[1], E. Suspitsin[1], K. Shelechova[2], A. Kornilov[3], A. Ivantsov[2], T. Gorodnova[1], G. Yanus[1], A. Togo[1], E. Imyanitov[1]. [1]*N.N. Petrov Scientific Research Institute of Oncology, Group of Molecular Diagnostics, St-Petersburg, Russian Federation;* [2]*N.N. Petrov Scientific Research Institute of Oncology, Department of Pathomorphology, St-Petersburg, Russian Federation;* [3]*N.N. Petrov Scientific Research Institute of Oncology, Department of Surgery, St-Petersburg, Russian Federation*

Background: Angiogenesis plays an important role in cancer progression and involves activation of multiple signaling cascades. This study was aimed to investigate relationships between microvessel density, expression of VEGF, VEGFR1 (FLT1), COX2 and PD-ECGF (TP) angiogenic factors, and gastric cancer (GC) recurrence.
Materials and Methods: Over 600 medical charts of consecutive surgically treated gastric cancer patients have been analyzed. 30 stage II GC with nearly identical initial clinical presentation (histology, grade, treatment scheme) have been selected; 12 of these cases recurred within 3 years, while the remaining 18 did not. Microvessel density was evaluated using CD31 and CD34 immunohistochemical analysis, and RNA expression of angiogenic factors was measured by real-time reverse-transcription PCR.
Results: Microvessel density correlated with VEGF mRNA content, but neither of these parameters was associated with the disease outcome. When tumors were ranked according to the level of expression of angiogenic molecules, 9 out of 10 cases with the highest VEGFR1 expression belonged to the recurrence group, while none of the 10 GC with the lowest content of VEGFR1 mRNA had the disease relapse ($p = 0.000$ by Mann–Whitney U-test). VEGFR1 expression did not show even a trend to correlation with the level of cancer tissue vascularization.
Conclusion: Our data provide indirect support to the evidence for non-angiogenic contribution of VEGFR1 in cancer pathogenesis. Study aimed to identify cell origin of VEGFR1 expression in the described tissue samples is currently underway.

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A novel NF-kB inhibitor DHMEQ could suppress peritoneal dissemination of gastric cancer by anti-tumor/-adhesive effects in mice

K. Mino[1], K. Nakanishi[1], S. Haga[2], M. Sato[1], M. Kina[1], H. Yokoo[1], T. Kamiyama[1], K. Umezawa[3], M. Ozaki[2], S. Todo[1]. [1]*Hokkaido University Graduate School of Medicine, General Surgery, Sapporo, Japan;* [2]*Hokkaido University School of Medicine, Molecular Surgery, Sapporo, Japan;* [3]*Keio University, Faculty of Science and Technology, Yokohama, Japan*

Background: Peritoneal dissemination is a critical prognostic factor in gastric cancer. As an integrin-mediated adhesion of cancer cells to extracellular matrix (ECM) is an essential process in peritoneal dissemination, inhibition of this process might be a pivotal therapeutic target. Recently, dehydroxymethylepoxyquinomycin (DHMEQ), a low molecular weight NF-kB inhibitor, has been newly developed, which possesses some anti-tumor effects by specifically inhibiting p65 DNA binding. The effectiveness of DHMEQ regarding peritoneal adhesion has not been studied in spite of its good therapeutic potency. In the present study, we studied the mechanisms of the inhibitory effects of DHMEQ on gastric cancer progression in mice.
Material and Methods: Two human gastric cancer cell lines, NUGC-4 and 44As3Luc (with luciferase activity) were used for the following experiments. 5×10^6 NUGC-4 or 2×10^6 44As3Luc cells were injected intraperitoneally into 6-week-old male BALB/c-nu/nu mice. DHMEQ was daily administered intraperitoneally at the dose of 10−40 mg/kg twice a day after cancer cell implantation. We evaluated anti-tumor effect of DHMEQ by tumor volume at day 30 macroscopically, histologically (NUGC-4 tumor), and in vivo imaging (44As3Luc tumor). We also evaluated the effects of DHMEQ by the following biological markers: 1) Nuclear NF-kB activity (ELISA), 2) Proliferation (CalceinAM), 3) Adhesion to ECMs (CalceinAM), 4) Cell cycle (FACS), 5) Apoptosis (FACS), and 6) Cell surface integrins (FACS). To evaluate the anti-adhesive effect of DHMEQ, we bio-imaged the remnant cancer cells in the abdominal cavity after the lavage of non-attached cancer cells.
Results: NF-kB was constitutively activated in these cancer cells, which was effectively inhibited by DHMEQ both in vitro and in vivo. In the in vitro study, FACS analysis showed that DHMEQ suppressed DNA synthesis for 12 hours and increased early apoptotic cells (annexinV-positive/PI-negative cells) by up to 10% for 24 hours. Interestingly, expressions of cell surface integrin $\alpha2$, $\alpha3$, $\beta1$ and CD44H in these cells were suppressed by up to 70% by DHMEQ for 24 hours, and eventually the adhesion to ECMs was suppressed. In vivo imaging clearly demonstrated that pretreatment of

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.

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

A. Teubner[1], D. Ernst[2], A. de Albuquerque[3], S. Kaul[4], N. Fersis[5], U. Stölzel[1]. *[1]Klinikum Chemnitz gGmbH, Department of Internal Medicine II, Chemnitz, Germany; [2]Klinikum Chemnitz gGmbH, Department of Surgery, Chemnitz, Germany; [3]Faculty of Medicine Carl Gustav Carus at University of Dresden, Department of Diagnostic at Klinikum Chemnitz gGmbH, Chemnitz, Germany; [4]Klinikum Chemnitz gGmbH, Department of Diagnostic, Chemnitz, Germany; [5]Klinikum Chemnitz gGmbH, Department of Gynecology and Obstetrics, Chemnitz, Germany*

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.

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

H. Lee[1], S. Jeong[1], J. Han[2], J. Kim[2], Y. Hwang[1], S. Kang[1], J. Park[1], J. Choi[1]. *[1]Ajou University Hospital, Hematology-Oncology, Suwon, South Korea; [2]Ajou University Hospital, Pathology, Suwon, South Korea*

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.

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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

S. Okabe[1], M. Yoshikawa[1], T. Toriyabe[1], Y. Ohoka[1], Y. Sinozaki[1], F. Kanai[1], M. Sunaga[2], S. Ueda[3], O. Yokosuka[1]. *[1]Graduate School of Medicine Chiba University, Department of Medicine and Clinical Oncology, Chiba, Japan; [2]Chiba Central Medical Center, Department of Gastroenterology, Chiba, Japan; [3]Graduate School of Pharmaceutical Sciences Chiba University, Department of Drug Information and Communication, Chiba, Japan*

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.

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

T. Mizuno[1], H. Nomura[2], K. Saito[1], Y. Yamashita[1], S. Kitano[1], E. Miyata[1], S. Tamaru[1], A. Hiasa[2], S. Kageyama[1], N. Katayama[1]. *[1]Mie University Hospital, Department of Hematology and Oncology, Tsu, Japan; [2]Toyama Hospital, Internal Medicine, Tsu, Japan*

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