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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

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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

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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

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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

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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