We therefore investigated if a sub-toxic concentration of α-toxin could enhance cisplatin-induced apoptosis, and also performed studies to elucidate how α-toxin affected signal transduction to cisplatin-induced apoptosis.
Cultured human pulmonary mesothelioma cells (P31wt) and a sub-line with acquired cisplatin resistance (P31res) was treated with cisplatin alone or cisplatin supplemented with a sub-toxic concentration (0.1 mg/L) of α-toxin for 48 h. Cell viability was measured with a fluorescein diacetate-based method and the amount of apoptotic cells were determined with TUNEL-staining. Apoptotic signalling was investigated with caspase activity assays, FACS-analysis and Western blot for detection of pro- and anti-apoptotic protein expression.
α-toxin enhanced the cytotoxic effect of cisplatin in P31wt and P31res cells. α-toxin did not affect the number of apoptotic cells in P31wt cells and had only a minor effect on apoptosis of P31res cells. When combined with 5 mg/L cisplatin, α-toxin increased the amount of apoptotic cells from 60 to 75% in P31wt cells and from 25 to 60% in P31res cells. Caspase-3 and -9 were expressed in α-toxin-enhanced cisplatin-induced apoptosis. The pro-apoptotic protein SAPK/JNK was activated by cisplatin in P31wt cells and in P31res cells when combining cisplatin and α-toxin, but not with cisplatin alone.
We conclude that α-toxin significantly increases cisplatin-induced apoptosis in cisplatin-resistant pulmonary mesothelioma cells in vitro. These findings may lead to novel therapeutic strategies to circumvent cisplatin resistance in the treatment of lung cancer.

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Combination therapy with sorafenib and radiation demonstrated improved survival in normal murine gut

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Introduction: Sorafenib and Gleevec are small molecules that inhibit signaling through multiple receptor tyrosine kinases (RTK). Sorafenib inhibits signaling through RAF, VEGFR2 and PDGFRb, whereas Gleevec selectively targets Abl, c-Kit and PDGFRa/b. Both drugs have been shown to enhance response to radiation (RT) in experimental models, and sorafenib has been shown to elicit an anti-proliferative and anti-angiogenic effect. We are conducting a series of phase I-II studies with biological targeted therapies in combination with RT in cervix cancer. Intestinal toxicity occurs in a substantial portion of patients who receive standard treatment for cervix cancer. This pre-clinical study was undertaken to evaluate the potential for these novel targeted therapies to increase acute intestinal toxicity further, which would detract from any therapeutic benefit.
Materials and Methods: The intestinal crypt survival assay was used to examine the toxicity of combination therapy with either drug and RT in C3H and Nude mice. Mice were treated with sorafenib (30 or 50 mg/kg/day) or Gleevec (50 mg/kg) for 5 days prior to a single whole body RT dose of 12, 14, 16 or 18 Gy. Control mice received vehicle. Mice were sacrificed 3 days later and the intestines removed and fixed for H&E staining. The numbers of surviving crypts, determined through evidence of mitosis, were counted (blinded) by two independent investigators.
Results: Combination treatment with sorafenib and RT exerted a significant ($p \leqslant 0.05$) radioprotective effect compared with RT alone in both mouse strains. In C3H mice the dose to achieve 10% crypt survival was increased from 15 Gy in control animals to 18 Gy in sorafenib treated mice (ER 1.2). Similarly in nude mice 10% crypt survival was seen at 18 Gy in sorafenib treated mice compared to 13 Gy in controls (ER 1.4). Gleevec did not increased RT toxicity, with both treatment groups showing 10% crypt survival at 16 Gy in C3H mice and 14 Gy in nude mice.
Conclusions: Combined treatment with RT and sorafenib appears to exert a radioprotective effect on normal intestinal crypts relative to RT alone. Interestingly, Gleevec did not have any effect on toxicity. The molecular mechanisms underlying this observation are currently being investigated. Multiple RTK targets can have different effects on the normal gut, and the crypt survival assay may allow rational selection of combination of RTK inhibitors and RT. We will shortly embark on a phase I/II clinical trial of sorafenib in combination with RT and chemotherapy for locally-advanced cervix cancer. The results of this study suggest that RT and sorafenib can safely be combined to treat cervix cancer, with no expectation of increased acute intestinal toxicity.

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Quantitative prediction of therapeutic potential of cancer drugs including pharmacokinetic interactions for apoptosis in 5 min

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Recently, National Cancer Institutes (USA) provides the public service of in silico screening to help the efficacy prediction of newly developed cancer drugs. However, in vitro rapid cell-based assay is demanded to verify the prediction quantitatively since a cancer patient may have unconventional aspects of tumor development. Conventional cell survival measurement is time-consuming and most of the cell-based assays are based tedious labeling. Here, we show the rapid and non-label quantitative verifying method and instrumentation of apoptosis via mitochondrial pathway for cancer drugs by the cell reaction analysis of living pancreatic cancer cell cultured on a sensor chip using a high sensitive surface plasmon resonance (SPR) sensor. The time-course cell reaction as the SPR angle change rate for 5 min from 35 min cell culture with a drug was significantly related to conventional apoptosis ratio after 48 h. The results obtained are universally valid with $P < 0.001$ ($n = 63$) for various cancer drugs using various pancreatic cancer cell lines, which mean to contain different level of receptor expression and protein mutation or existence similar to individual patients. Furthermore, they included the pharmacokinetic interactions of drugs which often enhances therapeutic potentials of individually used drugs. The detected SPR signal was derived from the decrease in mitochondrial membrane potential relating to apoptosis via mitochondrial pathway by using carbonyl cyanide 3-chlorophenylhydrazone of specific protonophore for mitochondrial membrane and an apoptotic specific inhibitor of mitochondrial membrane potential through the inhibition of voltage-dependent anion channel, BH4 domain of Bcl-2 family fused to the protein transduction domain of HIV TAT protein. In conclusion, we established a rapid and non-label cell-based quantitative screening method and instrumentation of apoptosis via mitochondrial pathway involving potential targets for cancer drug candidates even designed to target specific cell-signaling pathways. Our system towards the application to evaluate custom therapeutic potentials of drugs including pharmacokinetic interactions for apoptosis using live cells sampled from patients in clinical use.

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The combined effect of non-selective cyclooxygenase-2 inhibitor indomethacin and 5-fluorouracil treatment on colorectal cancer cell lines and xenografts

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Background: The high COX-2 enzyme expression in tumours is an unfavourable prognostic factor, however, the influence of COX-2 protein expression levels on tumour response to chemotherapy has been relatively little studied.
The aim of the study was to investigate the effect of 5-fluorouracil (5-FU) combined with the non-selective cyclooxygenase-2 (COX-2) inhibitor, indomethacin (INDO), on HT-29 and HCA-7 human colorectal cancer cell lines and on HCA-7 and HT-29 xenografts bearing SCID mice.
Materials and Methods: Sulphorhodamine B proliferation assay was used to measure the effect of 48 h 5-FU±INDO treatment on HT-29 (low COX-2 protein level) and HCA-7 (high COX-2 protein level) cells. Both cell lines were analysed by Western blot for COX-2 protein levels and by ELISA method for PGE2 production. COX-2 positivity of HCA-7 xenografts was confirmed with IHC. Tumour volume and weight of HCA-7 and HT-29 xenograft bearing SCID mice treated with 6 mg/kg 5-FU s.c. for 5 days ± 2.5 mg/kg INDO p.o. for 20 days were measured. Control mice received vehicle s.c. or 2.5 mg INDO p.o. for 20 days.
Results: 5-FU+INDO treatment compared to 5-FU alone resulted in an enhanced proliferation inhibition on HCA-7 cells ($p = 0.0082$). In contrary, on HT-29 cells no similar effect was observed. After 48 h treatment of HCA-7 cells with 5-FU, INDO or 5-FU+INDO the PGE2 levels were decreased below the detection limit.
Compared to 5-FU treatment the 5-FU+INDO combination caused a significant decrease of relative tumour volume ($p = 0.0236$) and weight ($p = 0.0081$) on HCA-7 xenografts.
COX-2 protein expression of HCA-7 xenografts was markedly reduced after treatment with 5FU+ INDO compared to 5-FU alone.
In contrast, on HT-29 xenografts the 5-FU+INDO combination did not decrease the relative tumour volume and weight. The COX-2 protein levels in HT-29 xenografts were undetectable.

Conclusion: 5-FU+INDO combination significantly increased the proliferation inhibition effect of 5-FU monotherapy on high COX-2 protein expressing HCA-7 colorectal cancer cell lines and xenografts.
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Regulatory pathways of plasma membrane integrity in necrotic leukemia cells

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Tumor cells died by apoptosis may evoke immune tolerance in the host, while necrosis leads to a proinflammatory response against the tumor. Thus instead of enforcing induction of apoptosis, provoked necrosis may help to establish a more effective cancer therapy. While apoptotic pathway has become increasingly well defined, little is known about the types and regulation of necrotic cell death pathways. Despite the idea that necrosis is an uncontrolled form of cell death, accumulating studies have suggested that necrotic cell death can be a regulated event. Recent studies describe several modes of necrotic cell death like secondary necrosis, PARP mediated necrosis or autophagic necrosis. Most recently a potent new pharmacon, necrostatin-1 was discovered that was suggested to halt specifically the death receptor mediated necrosis-like cell death form, termed necroptosis, in caspase compromised cells; although the target of necrostatin was not determined. Earlier we have established a model system to investigate the caspase independent cell death mechanisms in U937 leukemia cells applying non selective caspase inhibitor (z-VD.fmk, 5 μM) and flow cytometry to detect plasma membrane damage (R. Mihalik et al, CDD, 2004, 11:1357). In this model system at 20 hrs treatment condition we found that: (1) h.r.TRAIL (48 ng/ml), staurosporine (STS 1 μM) and H_2O_2 (250 μM) induced secondary necrosis (after caspase activation) was inhibited by PARP inhibitors (PJ-34, 1 μM; DPQ, 10 μM). (2) In the presence of caspase inhibitor, TRAIL-induced necrosis was completely abrogated by necrostatin-1 while STS- and H_2O_2-induced necrosis only partially. (3) Necrostatin-1 and 3-methyladenin (10 mM; an inhibitor of autophagy) additively protected cells from necrosis induced by STS or H_2O_2. (4) Geldanamycin (1 μM), by down regulating the expression of RIP1, rendered caspase-compromised cells resistant to TRAIL- and STS-induced necrosis completely but only partially of H_2O_2-induced necrosis. (5) Geldanamycin and PJ-34 together conferred complete resistance to H_2O_2-induced necrosis in the presence of caspase inhibitor. (6) Geldanamycin has no significant effect on secondary necrosis induced by either drugs.
In conclusion, our results indicate that necrosis can be induced in U937 leukemia cells at least three distinct molecular signal pathways. These forms may have different relevance to rising the immune response against leukemia cells.
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Expression profile of BRAF, RKIP, P53 and the AKT family genes in endometrial cancer and atypical endometrial hyperplasia

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Background: Aberrations in mediators or downstream effectors of the RAS/RAF/MAPK and PI3K/AKT signaling cascades have been suggested to increase the risk of developing endometrial cancer. However there is limited information regarding these genes expression profile and their association with the malignant transformation of the endometrium.
Material and Methods: In the present study we evaluated the mRNA expression pattern of BRAF, RKIP, P53 and the AKT family genes (AKT1, AKT2, AKT3) by Real-Time PCR in tissue samples of 4 patients with complex atypical endometrial hyperplasia (AEH), 26 patients with endometrial cancer and adjacent normal tissues of all patients.
Results: Transcript levels of all genes were found to be similar in endometrial cancer and adjacent normal tissue samples. Cancer specimens exhibited similar mRNA levels with AEH cases. Interestingly, BRAF mRNA was not expressed in 39% of the endometrial cancer tissues and in 25% of the AEH cases ($P = 0.033$, x^2 test), while its inhibitor mRNA (RKIP) was present in all cases. P53 transcript levels were detectable only in 19% of endometrial cancer tissues, and not in AEH cases ($P < 10^{-5}$). AKT1 was the predominant family member whose mRNA was expressed in all cases, whereas AKT3 exhibited mRNA expression only in 11% of cancer cases and not in endometrial hyperplasia. No association was observed between all genes mRNA levels and tumor histological type, FIGO staging or grade. A disruption of co-expression patterns was displayed in cancer compared to adjacent normal specimens. BRAF mRNA was positively correlated with AKT1 and marginally negatively correlated with P53 in the normal but not malignant endometrium ($P = 0.017$, $P = 0.056$ respectively, Spearman Correlation). Only in the cancer specimen group however, AKT3 transcript levels correlated negatively with BRAF and P53 mRNA ($P = 0.018$ and $P = 0.005$ respectively). AKT1 mRNA was co-expressed with RKIP in both cancer and normal specimens.
Conclusions: Deregulation of the mRNA co-expression profile of mediators or downstream effectors of the RAS/RAF/MAPK and PI3K/AKT signaling cascades may be associated with the development of endometrial carcinoma.

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Can antisense oligonucleotides specific to mutated K-ras gene inhibit the tumor growth, invasiveness, and MMP-2 and MMP-9 expression in hamster pancreatic cancer model in vitro and in vivo?

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Background: Matrix metalloproteinases (MMP), especially MMP-2 and MMP-9, are thought to play major roles in pancreatic cancer growth and metastasis. Ras activates a multitude of downstream activities with roles in cellular processing, including invasion and metastasis. Therefore, antisense oligonucleotides (ASO) targeting this K-ras gene may be a therapeutic approach.
Aim: To elucidate the effectiveness of this gene therapy in hamster experimental cancer model.
Materials and Methods: HaP-T1, a cell line derived from BHP-induced pancreatic cancer was used. Transfection with ASO were performed. MTT and MTT agarose assays were done. Chemoinvasion assay was performed. MMP-2 and MMP-9 production by the cell lines was determined by gelatin zymography. For in vivo experiments, subcutaneously resected tumors were implanted orthotopically in Syrian golden hamsters, which were divided in 3 groups: (A) Positive control (PC), (B) Sense treated hamsters (STH), and (C) Antisense treated hamsters (ATH). Oligonucleotides were administered for 2 weeks. Follow up was done. Five animals of each group were sacrificed at Days 10, 17, 24, 31, and 38, to study the local response and metastatic sites. Five animals of each group were left to study the survival time. Specimens were studied histopathologically. Orthotopic pancreatic tumor MMP production was measured by gelatin zymography.
Results: ASO inhibited the tumoral growth and invasiveness. They downregulated active forms of MMP-2 and MMP-9 in a dose dependent manner in vitro. Positive controls, STH, and ATH survived in average 72.7, 74.3, and 82.7 days, respectively. Spontaneous lymph node metastases were found from 31 days in ATH group, while PC and STH groups showed metastases and direct invasion to adjacent organs from 17 days. After death, metastatic sites were similar in the 3 groups. ASO downregulated the activation of MMP-9, more than MMP-2 in vivo.
Conclusions: These experiments suggest that ASO targeted K-ras gene may be a good choice in the management of pancreatic cancer because of the suppression of tumor growth and invasiveness in vitro and in vivo. ASO also downregulated the activation of MMP-9 and MMP-2 in vivo.

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N-glycolyl sialic acids as a cancer vaccine target: developing of a mouse B16 melanoma model by transient or stable expression

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Background: Sialic acids are normal components of the glycocalyx in most normal cells that participate in biological processes such as migration, adhesion and specific receptor recognition. N-glycolyl sialic acids (NeuGc) are a subset of these molecules synthesized by the enzyme CMP-NeuAc hydroxilase in murine cells. Although normal human cells do not express NeuGc, it has been described that the antigen can be detected in the cell membrane in melanoma and breast cancer. These facts support the idea to use NeuGc as a target for cancer vaccines in human beings. On the contrary, mouse B16 melanoma cells, as well as most murine tumors,