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,