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The cytotoxic effects of the anticancer drugs rviscumin and paclitaxel are dependent on cellular HER-2 (c-erbB2/neu) levels

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Recombinant Viscumin (rViscumin) is a new anticancer drug currently in clinical phase 1. It represents the pure, biochemically defined active component of plant derived mistletoe lectin and is a potent apoptosis-inducing compound with a unique mode of action leading to cell death through inactivation of translation of the target cell. Additionally, in low concentration ranges of rViscumin an immunomodulatory activity of the innate immunosystem is observed. Upregulation of HER-2 (c-erbB2/neu), a member of the HER receptor family of receptor tyrosine kinases, has been reported in 20-30 % of human adenocarcinomas of the ovary and has been linked to an unfavorable prognosis in these patients. The relationship between HER-2 receptor levels and drug sensitivity is of considerable interest since this molecular marker may allow to better predict response to chemotherapy. In the present study, we abrogated HER-2 expression in human SK-OV-3 ovarian cancer cells by ribozyme targeting and established stable cell lines with different residual HER-2 levels. In proliferation assays and in anchorage-independent soft agar assays, SK-OV-3 cells responded well to rViscumin. Interestingly, ribozyme-mediated down-regulation of HER-2 protein resulted in markedly decreased cellular sensitivities. This effect was comparable to Paclitaxel (Taxol) which is one of the most important cytotoxic drugs and widely used in cancer therapy. Although the mechanism of cytotoxicity of Paclitaxel is completely different, cellular sensitivity was similarly decreased upon ribozyme-mediated HER-2 depletion. Detailed analysis revealed that the HER-2 dependence of the cellular response to rViscumin or Paclitaxel is at least partially due to differential induction of apoptosis which is decreased in HER-2 depleted SK-OV-3 cells. Furthermore, the Paclitaxel-mediated inhibition of cell cycle was lost upon HER-2 reduction. Finally, downstream signal transduction pathways were analyzed. Western blotting revealed rViscumin-mediated, dose-dependent induction of p42/p44, p38 and SAPK/JNK which, again, was dependent on HER-2 expression levels. Our data introduce rViscumin as potential drug in ovarian cancer treatment. We also show that HER-2 expression levels display multiple effects on rViscumin and Paclitaxel cytotoxicity on molecular levels which may effect the clinical response to cancer chemotherapy.

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Activation of Fas-mediated apoptosis in 5-fluorouracil treated breast and colorectal cancer cell lines

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Fas (CD95/Apo-1) is a member of the TNF cell surface receptor family. Binding of Fas Ligand (FasL) causes trimerization of Fas and leads to the recruitment of the adaptor protein FADD (Fas-associated death domain), which in turn recruits caspase 8 (FADD-like IL-1-converting enzyme, FLICE) to form the death-inducing signalling complex (DISC). Caspase 8 molecules become activated at the DISC and in turn activate pro-apoptotic downstream molecules. c-FLIP (FLICE inhibitory protein) inhibits caspase 8 recruitment and processing at the DISC. We have found that the expression of Fas was up-regulated >10-fold in the p53 wild type MCF-7 breast cancer and HCT116 colorectal cancer cell lines in response to treatment with 5-Fluorouracil (5-FU), however, this did not result in activation of caspase 8. Although FasL expression was unaffected by 5-FU treatment, immunoprecipitation reactions demonstrated that the interaction between receptor and ligand was up-regulated. Analysis of c-FLIP expression in 5-FU treated MCF-7 cells demonstrated that its expression was up-regulated and revealed the presence of a truncated form of the protein that is generated during inhibition of caspase 8 activation at the DISC. MTT cell viability and clonogenic survival assays demonstrated a very strong synergistic interaction between 5-FU and the agonistic Fas antibody CH-11 in both MCF-7 and HCT116 cell lines (combination index <0.1). Cell cycle and PARP cleavage assays revealed that this synergy was due to activation of apoptosis. Furthermore, caspase 8 was activated following co-treatment with 5-FU and CH-11 in MCF-7 cells, but not by single treatment with either drug. In addition, c-FLIP expression was down-regulated prior to caspase 8 activation in 5-FU and CH-11 co-treated MCF-7 cells. We have also observed synergy between CH-11 and both the antifolate raltitrexed and the DNA damaging agent oxaliplatin in MCF-7 and HCT116 cell lines. However, synergy between CH-11 and 5-FU was not observed in p53 null MCF-7 and HCT116 daughter cell lines that fail to up-regulate Fas in response to 5-FU. Our results suggest involvement of c-FLIP in blocking Fas-mediated apoptosis following 5-FU treatment and raise the possibility of using Fas-targeted approaches to stimulate apoptosis in chemosensitised cancer cells.

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Cell cycle arresting and apoptosis-inducing activity of mycobacterial cell wall-DNA complex (MCC) towards human bladder cancer cells

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Intravesicle administration of MCC emulsion in patients with carcinoma in situ of the bladder who have failed BCG therapy and/or chemotherapy results in a significant number of complete clinical responses. The potential for MCC as a treatment for other types of bladder cancer has been examined by determining its activity towards a panel of human bladder cancer cell lines with known defects in cell cycle/apoptosis regulators. The human bladder cancer cell lines RT4 (benign papilloma), Hs 172.T (carcinoma, fibroblast), SCaBER (squamous cell carcinoma), UMUC-3 (TCC), SW 780 (grade 1 TCC), TCC SUP (grade 3 TCC), HT 1197 (grade 3 TCC), HT 1376 (grade 3 TCC), and T24 (grade 3 TCC) were treated with MCC (0.1-100 μg/ml) *in vitro*. Determination of cell cycle arrest (flow cytometry), cell division (MTT reduction), intracellular signaling changes (protein phosphorylation), intracellular free radical levels (dichlorofluorescein acetate probe), caspase-3 levels (flow cytometry), PARP degradation (flow cytometry) and apoptotic nuclei (Hoechst 33258 staining) was carried out at 24-72 hours post treatment. MCC caused cell cycle arrest and inhibited the division of all the bladder cancer cell lines tested. Cell lines derived from high grade TCC appeared to be the most susceptible. There was no correlation between cell cycle arrest/inhibition of division and the presence of mutated or absent cell cycle/apoptosis regulators (p16, p21, p53, pRb). Cell cycle arrest occurred at 24-48 hours post-treatment, predominantly at the G0/G1/S phase of the cell cycle. Significant changes in intracellular protein hypophosphorylation and elevated free radical levels occurred at 48-72 hours post-treatment. Apoptosis was present at 72 hours post-treatment, as demonstrated by activated caspase-3, PARP degradation product and apoptotic nuclei. Treatment of MCC with DNase-I significantly reduced its ability to induce apoptosis in the bladder cancer cell lines, demonstrating that the complexed DNA in MCC is responsible for its anticancer activity. The ability of MCC to cause cell cycle arrest/inhibition of division and induce apoptosis in the bladder cancer cell lines tested appears to be independent of defects in cell cycle/apoptosis regulators. MCC is most effective against bladder cancer cell lines derived from high grade TCC, and may therefore have application in the treatment of high grade bladder cancers that are known to be associated with mutations in cell cycle/apoptosis regulators.

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Anticancer activity of a 6-base length phosphodiester oligonucleotide, Oligomodulator™BT 99-25, against lymphoma

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Oligomodulator™BT 99-25, a synthetic 6-base length phosphodiester oligonucleotide with the sequence 5'-G3TG2-3', has apoptosis-inducing activity towards a number of leukemia cell lines. In this study, the activity of BT 99-25 towards lymphoma cells has been determined. BT 99-25 inhibited the division of murine EL-4 T lymphoma cells, human HH cutaneous T cell lymphoma cells, human HB B cell lymphoma cells and human U 937 histiocytic lymphoma cells in a concentration-dependent manner (0.5 to 50 μM). Inhibition of cellular division was associated with cell cycle arrest in the G0/G1/S phase of the cell cycle. BT 99-25 treatment induced apoptosis of EL-4 and U937 cells as measured by phosphatidylserine plasma membrane translocation. In addition, BT 99-25 triggered the release of soluble nuclear mitotic apparatus protein (NuMA), a marker of apoptosis, in a concentration-dependent manner. The murine syngenic EL-4 lymphoma model was used to assess the *in vivo* anticancer activity of BT 99 25. A single intratumoral injection of BT 99-25 (0.4 mg/kg body weight) inhibited subcutaneous EL-4 tumor growth by 53% and cured 37.5% of treated mice (N=8). Anticancer activity was also observed at a lower dose of BT 99-25 (0.04 mg/kg body weight, 34% inhibition of tumor growth, 12.5% mice