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Development of a MDR-1 gene specific ribozyme: A molecular approach to the reversal of drug resistance of cancer cells

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How cancer cells can become resistant to chemotherapy is not completely understood, but it is believed that resistance is usually associated with overexpression of drug resistance genes. Drug resistance mediated by the MDR-1 gene is the first well characterized form of drug resistance in human cancer. MDR-1 encodes a phosphoglycoprotein, P-GP, that serves as an energy-dependent drug efflux pump, reducing intracellular drug accumulation and thereby cytotoxicity. We have used ribozymes to reverse the multiple drug resistance phenotype. A hammerhead ribozyme recognizing the GUC sequence at position -6 to -4 close to the translation start site of the 4.5 kb MDR-1 mRNA was prepared by in vitro transcription (MDR-1-RZiv) or chemical synthesis (MDR-1-RZs specifically cleaved the MDR-1 mRNA into two parts of the expected size under physiological conditions in an extracellular system with MDR-1-RZiv being more effective. Site-specific cleavage was dependent on time, temperature and [$MgCl_2$]. To examine the in vivo potential of MDR-1-RZ, MDR-1-RZiv and MDR-1-RZS were transfected into a human pleural mesothelioma cell line and into one adriamycin-resistant and one vindesine-resistant subline thereof by liposome-mediated transfer. Incorporation of ribozymes resulted in significantly reduced expression of the MDR-1 gene, with MDR-1-RZs being more potent then MDR-1-RZiv in vitro. MDR-1-RZ reduces P-GP overexpression at the protein level. Liposome-mediated transfer of MDR-1-RZiv or MDR-1-RZs reversed the multiple drug resistance phenotype and restored sensitivity towards chemotherapeutic drugs.

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Active specific immunotherapy with Newcastle Disease Virus modified autologous tumor cells.

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An active specific immunization (ASI) procedure with two types of autologous tumor cell vaccines (ATV) was tested for adjuvant immunotherapy of resected colorectal carcinoma to provide preliminary information on local immunological skin responses, side effects and on survival. For vaccine preparation, the tumor derived freshly isolated and cryopreserved cells were thawed, purified by Percoll density centrifugation and depleted of tumor infiltrating lymphocytes by immunomagnetic beads. After inactivation by 200 Gy, the cells of this ATV were either infected by Newcastle Disease Virus (NDV) or they were admixed with Bacillus Calmette Guérin (BCG) organisms. Vaccination was performed in the arm beginning 6-8 weeks after operation, 3 times at two week intervals. The mean value of delayed hypersensitivity skin reactions (DTH) from ATV-NDV treated patients was 18 mm for the first vaccination and 26 and 29 mm for the following ones. While application of ATV-NDV was associated with only mild side effects, the ATV/BCG vaccine lead to long lasting ulcers and to more serious side-effects. Of 57 patients that received ASI, the two year survival rates obtained with ATV-NDV was 98 % while the survival rate with ATV BCG was 67 % ($p < 0,01$). It is concluded that the type and quality of the tumor vaccine for ASI-treatment is important since the use of ATV BCG did not appear to provide improval of survival rates in comparison to controls. The positive findings with ATV-NDV necessitate corroboration in a prospective randomized control study.

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Targeted Eradication of ErbB-2 Over-expressing Tumor Cells Employing Expression of an Intracellular Antibody Directed Against the ErbB-2 Oncoprotein.

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ErbB-2 is a 185kD oncogenic protein known to be involved in several human malignancies,including breast, ovary, lung and stomach. Its over-expression in these cancers and its central rolein neoplastic progression make it an important target for anti-cancer gene therapy strategies. As a novel strategy to achieve specific targeting of erbB-2 over-expressing tumor cells, gene constructs were designed to encode single chain immunoglobulins (sFvs) with anti-erbB-2 specificity. Transient transduction of the anti-erbB-2 sFvs was accomplished in a variety of human tumor cell lines. Immunohistochemistry employing an anti-erbB-2 polyclonal antibody (Ab) demonstrated that expression of sFv resulted in profound down regulation of cell surface erbB-2. Determination of the proliferative rate of the transduced cells indicated that anti-erbB-2 sFv accomplished greater than 95% inhibition of cell growth. Studies of cell viability indicated that erbB-2 over-expressing tumor cells were eradicated within 96 hours of transduction. Futher evaluation indicated that the mechanism of cell death was through induction of apoptosis. Importantly, this effect was noted only in erbB-2 over-expressing targets; no change in cellular proliferation was noted in non-erbB-2 expressing tumor targets. Furthermore, the specific induction of apoptosis in these cells mediated by the intracellular anti-erbB-2 sFv demonstrates the potential efficacy of this novel therapeutic modality for erbB-2 positive tumors of various tissue types.

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Development and initial clinical studies of TA-HPV in cervical cancer.

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Cervical cancer is associated with human papillomavirus (HPV) infection in >94% of cases, usually HPV strain 16 or 18. In the transformed cells continued expression of two intra-cellular, non-structural viral proteins, termed E6 and E7, is required to maintain the malignant phenotype. These proteins are potential targets for immunotherapy, particularly by cytotoxic T cells.

A recombinant (TA-HPV) was made, in which E6 and E7 genes of both strains were fused and inserted into Wyeth strain vaccinia. Furthermore E7 was modified to inactivate the *Rb* binding site, largely removing its oncogenic potential

This construct has been used to vaccinate 8 patients with established cervical cancer, under contained environmental release conditions. Clinical follow up is still in progress but no side effects of vaccination have been observed, after up to a year, despite subsequent chemo- and radio-therapy. No environmental contamination has been detected and the recombinant is stable after human inoculation.

Serological response to the inserted sequences has been observed in 3 of 3 patients. Assays for human HPV specific CTL are difficult but we have developed an assay, using adenovirus recombinants expressing HPV E6 & E7 for in vitro expansion of CTL from PBMC. Studies are in progress to determine if TA-HPV induces this specific response in vaccine recipients.

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