using the crystal violet method. Tumorigenicity studies were performed in athymia mice. Intratumor coinjection and injection of tumor cells (HaCa4, MSC11A5) and MD-E1a retrovirus-producing cells were also performed.

Results: The E1a gene induced marked sensitivity to cisplatin and to radiation in both cell models (80% greater than in control cells lacking E1a expression). *In vivo* assays showed that constitutive E1a expression increased latency and decreased tumorigenicity. The results with coinjection of MD-E1a virus-producing cells were identical to those obtained *in vitro* using transfected lines. Moreover, intratumor injection of producer cells partially blocked the growth of tumors generated by MSC11A5 cells.

Conclusions: The E1a gene induces chemosensitivity and radiosensitivity in epidermoid carcinoma cell lines regardless of the p53 status. Injection of E1a-producer cells may block tumorigenicity and results in a new approach for gene therapy in cancer.

376 POSTER

Employment of the mdr1 promoter for a conditionally active retroviral vector system in cancer gene therapy

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Purpose: Inducible vectors are attractive tools for the conditional expression of therapeutic genes in cancer gene therapy. Earlier studies demonstrated that the promoter of the multidrug resistance gene (mdr1) harbors responsive elements that are inducible by MDR-associated drugs. This points to the applicability of the mdr1 promoter for the construction of drug-inducible vectors. We linked an mdr1 promoter element to the human TNFα gene in a retroviral vector to evaluate expression efficacy and drug-inducibility of this system.

Methods: The retroviral constructs were transduced into MCF-7 human mammary carcinoma and HCT116 human colon carcinoma cells. For the induction experiments transduced cells were treated with doxorubicin, vincristine, VP-16 and taxol. The expression and induction studies were performed by using RT-PCR and TNFα-specific ELISA.

Results: Transduced MCF-7 and HCT116 cells showed measurable basal expression of TNFα. Treatment of the cells with the MDR-associated drugs led to a 2–3 fold increase in TNFα mRNA followed by an 3–13 fold increase in TNFα secretion. This induction was drug-concentration dependent.

Conclusion: The studies have shown that the mdr1 promoter carrying retroviral vector is suitable for the inducible expression of therapeutic genes and could be employed for gene therapy in the context of cancer chemotherapy.

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Cytosine Deaminase – A suicide system for tumor therapy

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Cytosine Deaminase (CD) is a bacterial and fungal enzyme which is not expressed in mammalian cells. This protein dominates the non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which is used for chemotherapy of colorectal cancer. Therefore, CD can be used as a suicide system for transfecting tumor cells and subsequent selective killing of CD-transfectants by 5-FC. In order to allow the detection of CD expression at the protein level we generated antibodies against this enzyme. Furthermore, we used a syngeneic rat pancreatic tumor model to examine the CD/5-FC system in *vivo* in immunocompetent animals.

The generated antibodies specifically recognize the CD protein from *E. coli* in various test systems including western blots and immunohistochemistry on frozen tissue sections of rat AS/CD-tumors. For *in vivo* experiments we induced tumors in rats by injection of CD-expressing AS-tumor cells (AS/CD). In comparison to control tumors (AS/neo), which grew rapidly, CD-expressing tumors regressed after initial tumor growth, when exposed to 5-FC or PBS. After a second injection of AS/CD cells and the AS parental cells into these animals AS/CD cells were rejected immediately. Complete regression of the parental tumor was observed in a significant number of animals. A third injection of AS parental cells into the surviving rats showed an immediate rejection of the syngeneic AS tumor cells.

The Abs generated against CD from *E. coli* facilitate the fast and direct detection of the CD protein in transfected cells and tumors. Our preliminary data obtained in the rat tumor model suggest the induction of an immune response by CD – a bacterial antigen – which might modulate the tumor microenvironment such that the originally non-immunogenic parental tumor cells become immunogenic. The molecular basis of this phenomenon is currently under investigation.

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Generation and characterization of cytotoxic T lymphocytes (CTL) against mutated *ras* peptides

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Mutated *ras* genes have been implicated in the initiation and progression of cancers. Therefore, peptides encompassing *ras* mutations appear to represent an appealing target for active immunotherapy procedures. In this work peptides encompassing GLY→VAL, 61 GLN→LEU and 61 GLN→LYS *ras* mutations and displaying HLA-A2.1 binding motifs, selected by a computer program, were used to attempt the generation of specific CTL *in vitro*. Initially, peripheral blood mononuclear cells (PBMC) from five HLA-A2.1+ healthy donors were stimulated *in vitro* with a mixture of peptides. Weekly thereafter, PBMC were restimulated with irradiated peptide pulsed, autologous Epstein Barr virus (EBV) transformed B cells. After eight rounds of restimulation reproducible cytotoxic activity against peptide pulsed target cells was detectable in one donor. CTL recognized two nonamers encompassing *ras* 61 Gln→Leu mutation. Killing was mediated by $CD8^+$ T cells displaying $\alpha\beta$ T cell receptor (TCR) and was inhibited by anti-HLA-A2.1 monoclonal antibodies. None was able to exert effective cytotoxic activity aginst tumor cells expressing the specific mutation.

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Improving tumour targeting and decreasing normal tissue uptake by optimizing stoichiometry of a two-step biotinylated monoclonal antibody (Mab)/streptavidin (Strv) based targeting strategy: Studies in a nude mouse xenograft model

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Purpose: To assess the impact of relative protein stoichiometry of first and second step and biotinylation density of first step on the pharmacokinetics, biodistribution and tumour targeting of a two-step biot-MAb/Strv approach.

Methods: The HT-29 xenograft nude-mouse model was used. AUA1 Mab was biotinylated to various degrees (r. 0.8–25 biotins per IgG). Protein stoichiometry of the two steps was studied through a range of 2 logs. Both steps i.e. the biot-Mab (1st step) and Strv (2nd step) were radiolabelled (^{131}I & ^{125}I). A 24 h interval between 1st/2nd step was studied, animals were killed 24 h after the 2nd step.

Results: Strv excess led to a decrease in circulating levels of biot-Mab (7.6 ± 1.0 vs 11.2 ± 1.3% i.d./g) and decreased amounts of biot-Mab in the tumour (3.7 ± 0.7 vs 5.7 ± 0.6% i.d./g). Biot-Mab excess led to increase in circulating levels of Strv (3.6 ± 0.5 vs 7.5 ± 1.7% i.d./g), a decrease in renal uptake of Strv (66 ± 12 vs 24 ± 5.9% i.d./g) and increased targeting of Strv to tumour (6.9 ± 1.4 vs 5.3 ± 0.9% i.d./g). At a constant protein molar ratio of 1st to 2nd step (10:1 Mab excess), varying IgG biotin density resulted in: (i) The circulating levels of Strv increasing from 4.8 ± 0.6 to 21 ± 2.6% i.d./g (ii) Increase in tumour uptake of biot-MAb (6.2 ± 0.8 vs 24.1 ± 7.7% i.d./g) and (iii) Renal uptake falling from 69 ± 5 to 8.8 ± 3.8% i.d./g while liver uptake increased from 8 ± 1 to 40 ± 14% i.d/g.

Conclusion: Factors pertaining to protein stoichiometry and to biotinylation density of the MAb profoundly affect biodistribution, pharmacokinetics and tumour targetting in two-step based strategies.

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How to perform effective IL-2 therapy

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Locoregional cancer treatment with IL-2 induces good therapeutic effects