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Highly efficient nonviral gene transfer into human B-CLL cells

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Efficient and safe gene transfer into tumor cells is an essential prerequisite for initiating clinical vaccination trials using genetically modified lymphoma cells. Compared to viral gene transfer nonviral gene transfer techniques such as lipofection, electroporation and ballistic gene transfer commonly fail to transfect human B-lymphoma cells efficiently. Since, however, nonviral techniques for introduction of therapeutic genes into tumor cells may circumvent some disadvantages of viral gene transfer like insertional mutagenesis, triggering of the immune system and complex safety procedures, improved nonviral techniques are desirable. Here we show that the novel nucleofection method, a improvement of conventional electroporation, allows transfection of primary human B-CLL cells with efficiencies of more than 40% using pMOK or MIDGE (minimalistic immunogenically defined gene expression) vectors. Gene expression in human B-CLL cells after nucleofection of these vectors lasts for 24h to 72h. Our results demonstrate that B-CLL cells can be transfected with high efficiency using a nonviral gene transfer technique.

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ADENOVECTOR GENE TRANSFER IN BLADDER CANCER: EXPRESSION OF RECEPTORS FOR VIRAL ATTACHMENT AND INTERNALIZATION.

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We have earlier shown that adenoviral vector mediated gene transfer of immunostimulatory genes (e.g. CD40L) into tumor cells generates protective immunity in the murine bladder cancer model MB49. In the present study, we wanted to investigate the feasibility of adenovectors for gene delivery in human bladder cancer. The expression of receptors important for adenoviral attachment and internalization, i.e. the Coxsackievirus/Adenovirus receptor (CVADR) and the alfa-v beta-3/5 integrins were analyzed.
The human bladder cancer cell lines J82 and T24 as well as 27 surgical biopsies were analyzed. CVADR expression was investigated by immunohistochemistry and PAGE/Western blot. Gene transfer was studied with an adenovector serotype 5, and blocking of transfer was done by anti-CVADR ab's. Transgene expression was detected with flow cytometry. Integrin expression was analyzed by immunohistochemistry. J82 and T24 were efficiently transduced with adenovectors. Transduction with the AdCD40L vector increased the apoptotic rate of the transduced cells, and the expression of CD40L as well as other immunomodulating molecules like ICAM-I, B7.1, MHC-I, FAS and TNFR were upregulated. The transduction could be blocked/reduced with anti-CVADR ab's. Cell lines and biopsies were broadly positive for CVADR and frequently also positive for integrin alfa-v beta-5.

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Constitutive Expression Of The Homeobox Transcription Factor HOXB4 Amplifies Human Primitive Hematopoietic Progenitor Cells In Vitro And In Vivo.

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A major obstacle of cytokine induced amplification of human primitive progenitor cells *ex vivo* is net loss of stem cell numbers by induction of differentiation. We now directly tested the hypothesis that constitutive expression of the homeobox transcription factor HOXB4 can amplify most primitive human hematopoietic progenitor cells *in vitro* and *in vivo*. Using a MSCV-based retroviral construct with GFP as selection marker we expressed the gene in human highly purified $CD34^+/GFP^+$ cord blood (CB) cells. When the impact on long-term culture initiating cell (LTC-IC) numbers was determined 2-9 days after transduction HOXB4 induced a 3-30fold increase (n=6) of the progenitor frequency compared to the GFP control as assessed by limiting dilution assays (p=0.01). This effect was confirmed *in vivo* using the limiting dilution CRU assay in NOD/SCID mice with a 4fold increase in the CRU frequency of HOXB4 transduced CB cells (p=0.02). HOXB4 also had a profound effect on committed progenitors with a 100-fold increase in the frequency of colony forming cells (CFC) in suspension cultures after 6 weeks (p<0.0005). In addition, HOXB4 transduced progenitors had a greatly increased proliferative capacity as indicated by an 8-fold increase in 2° CFC formation (n=6, p=0.04) in serial replating experiments. Importantly, HOXB4 did not perturb hematopoietic differentiation. These data characterize HOXB4 as an attractive candidate gene for the amplification of human repopulating stem cells *ex vivo*.

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First clinical evidence of a combined AP-2/Sp1 promoter motif as a tumor-specific activator of urokinase-receptor (u-PAR) expression in resected gastrointestinal cancers.

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Evidence for transactivation of genes via specific promoter elements has been given entirely by studies on cell lines in the past and never has been pursued in resected tumors. In this repost, we give first clinical evidence that, in a series of 145 resected gastrointestinal cancers (118 colorectal, 27 gastric), the expression of the invasion-related gene u-PAR is regulated via an AP-2/Sp1 promoter element. In electrophoretic mobility shift assays (EMSA), a strong binding of Sp1 to u-PAR-promoter region −152/-135 in tumor tissue in contrast to an almost absent binding in corresponding normal mucosae was observed in 68 of 118 colorectal and in 15 of 27 gastric cancer patients. Tumor-specific binding of an AP-2α-related factor was seen in 69 of 118 colorectal and in 18 of 27 gastric cancer patients, and of both in 61 of 118 colorectal and in 11 of 27 gastric cancer patients. In linear regression analysis, a significant correlation between AP-2- (p<0.0001) and Sp1 (p=0.0003) binding with high u-PAR-protein (ELISA) was observed in tumor tissue, but not in normal tissue. These data suggest that the invasion-related gene u-PAR is induced via this AP-2/Sp1 promoter element in resected tumors, but not in corresponding normal tissue. We hypothesize that, in the subpopulation in which a transactivation of u-PAR-gene expression in tumors in contrast to normal tissue by region −152/-135 is implicated (here up to 60% of patients), molecular targeting of this region or its activating pathways may be discussed as a new therapy approach.