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Biological Gene Delivery and the Local Activation

H. Corban-Wilhelm, K. Braun, R. Pipkorn, W. Waldeck, H. Spring, J. Jenne, R. Raster, J. Simiantonakis and J. Debus

Background: At the moment the main problem for a successful gene therapy is a safe and efficient gene delivery. We developed a "bioshuttle" which can transport inactivated genes/plasmids into cells with subsequently local activation.
Material & Methods: The bioshuttle consists of the following modules: PTD (protein transport domain) -Cys, a Cysx-NLS (nuclear localization site) -Peptide-unit and a covalent linked clamp-PNA (peptide nucleic acid). The pDNA-EGFP (Clontech) was hybridized with the bioshuttle. HeLa and AT-1 cells were incubated with the conjugate in a final concentration of 100 pM for 60 min. Short increasing of the temperature performed the activation of the transported gene. The transport and the expression of the EGFP in living cells were determined by confocal laser scanning microscopy (CLSM).
Results: One day after the incubation with the DNA-bioshuttle, ~90% of the HeLa and AT-1 cells showed a green fluorescent signal. No green signal was detectable when the temperature for the activation was lower than 42°C. A short pulse of 30 sec by a temperature of 44°C was enough to transfect ~90% of the HeLa and AT-1 cells. A longer temperature pulse showed no higher transfection rates. Also higher temperatures above 47°C demonstrated only cell dying. No cell line specific transfection rate could be observed.
Conclusion: The bioshuttle hybridised with DNA is an efficient and safe tool for gene therapy, because of the selective and local activation of a therapeutic gene.

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„Distant" Bystander-Effect: the immunological component of suicide gene therapy

H. Corban-Wilhelm, G. Becker, D. Greulich and J. Debus

Background: The co-expression of foreign bacterial or viral proteins may lead to enhancement of the immune response to normally weak tumors. The present study examined if it is possible to induce an immunological memory or a distant bystander effect with suicide gene/prodrug systems.
Material & Methods: Cytosine Deaminase (CD) and Thymidinekinase (TK) transfected tumor cells (AT-1/CDglyTK) were injected subcutaneously (s.c.) into the flank of syngeneic Copenhagen rats. A part of the rats (group A) received additionally at the opposite flank the non-transfected AT-1 cells. The prodrug therapy with 5-fluorocytosine and ganciclovir starts at a tumorvolume of 300mm³ over a period of 14 days (group A & B). 60 days later the tumor free rats (group B), which had no AT-1 tumor before, obtained s.c. different concentrations of AT-1 cells. The infiltration of macrophages, NK-cells, $CD4^+$ and $CD8^+$ cells were examined immunohistochemically in tumor sections.
Results: The simultaneous prodrug treatment of AT-1 and AT-1/CDglyTK tumors showed a tumor regression of the AT-1/CDglyTK tumors and no growth delay of the AT-1 tumors (group A). The tumor free rats (group B) showed a growth delay after s.c. injection of $1*10^6$ AT-1 cells, whereas after injection of $1*10^5$ and $1*10^4$ AT-1 cells no tumors were detectable. An infiltration of macrophages, $CD4^+$ and $CD8^+$ cells were verified; but no NK-cells.
Conclusion: These data demonstrate that the immune response after a suicide gene therapy plays an important role of disseminated tumors and the tumor recurrence.

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ANALYSIS OF RETROVIRAL MEDIATED TRANSGENE EXPRESSION AFTER *IN VITRO* DIFFERENTIATION OF MOBILIZED HUMAN PERIPHERAL BLOOD PROGENITOR CELLS

H.G. Eckert, C. Lindemann, A.J. Schilz, A.A. Fauser, C. Baum[1] and K. Kühlcke.
EUFETS AG, Idar-Oberstein, Germany; [1]Medizinische Hochschule, Hannover, Germany.

Retroviral gene transfer is at present most frequently used to transduce hematopoietic cells for *ex vivo* gene therapy.
In *in vitro* studies we have evaluated EGFP expression mediated by a set of different retroviral vectors during lineage committed differentiation. Separated human $CD34^+$ peripheral blood stem cells were transduced, sorted for transgene expression and subsequently differentiated to dendritic cells, granulocytes, monocytes and erythrocytes. We found a tremendous decrease of EGFP positive cells during dendritic cell differentiation. Significant amounts of $CD83^+/EGFP^+$ cells could only be detected after transduction with vectors previously optimized for high expression in early hematopoietic stem/progenitor cells. In contrast, loss of transgene expression in other differentiated cell populations was only low. EGFP expression was detected in up to 97% of $CD15^+$ granulocytes, in 94% of $CD14^+$ monocytes as well as in 71% of the erythroblasts.
In summary we show evidence for differently regulated transgene expression from identical vector backbones in different myeloid lineages. Results from these *in vitro* differentiation studies might help to determine the suitability of vectors for expression in desired target cells.

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HIGHLY EFFICIENT RETROVIRAL GENE TRANSFER BASED ON CENTRIFUGATION-MEDIATED VECTOR PRE-LOADING OF TISSUE CULTURE VESSELS

H.G. Eckert, B. Fehse[1], C. Lindemann, F. Ayuk[1], A.A. Fauser, A.R. Zander[1], K. Kühlcke
EUFETS AG, Idar-Oberstein, Germany; [1]Bone Marrow Transplantation, University Hospital Eppendorf, Hamburg, Germany

Efficient retroviral gene transfer into primary human hematopoietic cells is a prerequisite for various gene therapeutic strategies. Recently, new protocols have been described which exploit centrifugation and/or fibronectin-coating to ensure high transduction of $CD34^+$ and T cells. Several factors, including safety concerns and high costs, may hamper the use of those protocols in the clinics.
We developed a transduction protocol which is based on the pre-loading of tissue culture vessels with retroviral particles by low-speed (1000 x g) centrifugation. We show that those vector-pre-loaded tissue culture vessels allow even higher gene transfer than the most efficient protocols reported so far. Transduction of primary T lymphocytes under clinically relevant conditions using a clinical grade vector developed and produced for suicide gene therapy results in mean in ~25% gene transfer efficiency. $CD34^+$ stem/progenitor cells were reproducibly transduced to 40-58 % after a single infection.
This method is part of our application file for a manufacturing authorization for the genetic modification of T-cell transplants and was approved by German authorities in January 2001.