116

ANTIBODY MEDIATED CELL SPECIFIC RETARGETING OF ADENO-ASSOCIATED VIRUS VECTORS DISPLAYING IMMUNOGLOBULIN BINDING-DOMAINS

M. U. Ried, A. Girod, H. Büning, M. Hallek (Gene Center Munich)

Recombinant adeno-associated virus (rAAV) is a promising vector for gene therapy. The transduction of hematopoietic cells by rAAV is less efficient, probably resulting from the low expression of specific AAV receptors. Therefore, we seek to develop rAAV vectors specifically targeting hematopoietic cells. We developed an AAV vector with ligand insertion at site 587 that could specifically infect AAV resistant B16F10 cells (Girod *et al.*, *Nat Med* 19995(9):1052). Here we report for the first time a new universally targetable-AAV2 capsid mutant that can be loaded with different ligands against specific cell surface receptors. We inserted the Z34C IgG-binding domain from Protein A into the 587 insertion domain of AAV2. Z34C binds to the F_C-part of antibodies which bind with their variable domains to specific cellular receptors. Less infectable Jurkat (T-cell leukaemia), M-07e (acute megakaryoblastic leukemia) and Mec1 (chronic lymphatic leukaemia) cells could be targeted and transduced with antibodies against CD29 (β_1-integrin), CD117 (c-kit-receptor) and CXCR4. The infection was specifically antibody mediated and could be blocked by soluble IgG- and Protein A molecules. Future improved IgG-targeting vectors should then be used as a fast screening system for the suitability of variable antibody domains for receptor binding, uptake and intracellular processing of the virus. These new universally targetable-AAV2 vectors open new avenues for the design of targeted AAV2 vectors, in particular for hematopoietic cells and the selective tranduction of specific target cells for gene therapy applications.

117

Influence of different anticoagulants on rAAV2 infectivity

Franz M. Gerner [1,2], Ulrich T. Hacker [1,3], Hildegard Büning [1], Martin Hutter [1], Hermann C. Reichenspurner [2], Manfred Stangl [4] and Michael Hallek [1,3,5]

[1] Genzentrum of the Ludwig-Maximilians-University of Munich (LMU), Feodor-Lynen-Str. 25, 81377 Munich, Germany, [2] Department of Cardiac Surgery, [3] Medical Department III, [4] Department of Surgery, University Hospital Munich/Grosshadern, LMU,Marchioninistr. 15, 81377 Munich, Germany, [5] GSF, National Research Center for Environment & Health, Marchioninistr. 25, 81377 Munich, Germany

In recent publications it has been shown that recombinant adeno-associated virus type 2 (rAAV2) is a promising vector for gene therapy. Transduction with rAAV2 requires the binding of this virus to heparan sulfate proteoglycan on the target cell surface. This interaction can be blocked by the addition of heparin. Because anticoagulation with heparin is required for intravascular gene transfer, we aimed to identify anticoagulants which have reduced inhibitory effects on rAAV2 transduction. Heparin itself showed significant inhibition of rAAV2 transduction at therapeutic concentrations, whereas inhibition of rAAV2 transduction by the low molecular weight (LMW) heparin tinzaparin and by the LMW heparinoid danaparoid was significantly lower. Recombinant hirudin did not interfere with rAAV2 transduction. In summary, these results demonstrate that inhibition of rAAV2 transduction is a clinically relevant problem and that recombinant hirudin might be an alternative for heparin when vascular gene transfer with rAAV2 requires transient anticoagulation. Of the heparinoids, danaparoid has the least inhibitiory effect.

118

Genomic Targeting of Recombinant Adeno-Associated Virus

N.A. Huttner[1], M. Stark[3], Ch. Plank[4], A. Girod[1], M. Hutter[1], S. Schnittger[2], C. Schoch[2], R. Guckenberger[3], M. Hallek[2], H. Buening[1]

[1]Genzentrum und [2]Medizinische Klinik III, LMU München, München, Germany
[3]Max-Planck-Institut für Biochemie, Martinsried, Germany
[4]Klinikum Rechts der Isar, TU München, München, Germany

The human parvovirus adeno-associated virus type 2 (AAV2) has many features that makes it attractive as a vector for somatic gene therapy. One of the most promising features of AAV is its ability to integrate site-specifically into chromosome 19 (AAVS1). However, commonly used recombinant AAV vectors (rAAV) do not target AAVS1 due to the loss of the viral *rep* gene. In first round of experiments we could show that it is possible to regain targeted integration of rAAV by providing Rep *in trans*. The high integration frequency of 70% obtained for rAAV in this experiment was in the range of wild type virus. This encouraged us to develop more convenient methods to couple Rep to rAAV.
For this purpose we are going to link *rep* as a polylysine/DNA-complex via a biotin/streptavidin bridge to the capsid of the virus. Because the AAV capsid has a diameter of only 25 nm we had to construct smaller PLL/DNA-complexes than those described in the literature. We could show that PLL of a mean chain length of 47 lysine residues is able to condense the plasmid DNA to compact spherical particles of approximately 30 nm in diameter. In addition we could show that all three capsid proteins are accessible for biotinylation and that the biotinylated virus is still able to infect. These results will now be used to generate rAAV-PLL/rep-complexes which are able to target the integration of the transgene to the AAV integration site.

119

REAL-TIME SINGLE MOLECULE IMAGING OF ADENO-ASSOCIATED VIRUS UPTAKE IN LIVING CELLS

Ried M. U., Büning H., Seisenberger G., Endreß T., Braeuchle Ch., Hallek M.
Genzentrum, Medizinische Klinik III, Grosshadern, LMU University, Feodor-Lynen-Str. 25, 81377 Muenchen, Germany
CeNS Center for NanoScience, Institut für Physikalische Chemie, LMU University, 81377 Muenchen, Germany

Recombinant adeno-associated virus (rAAV) is a promising vector for gene therapy. However, its biology of infection is still not really understood. This led us to develop methods based on Single Molecule Detection technologies to study the receptor binding, uptake, endosomal processing and nuclear transport of single Cy5-labeled AAV particles in real time in living cells. More than 1000 AAV trajectories were analyzed. Only in 5 %, entry of AAV into the cell was seen. In 32 %, AAV made a membrane contact and disappeared in solution thereafter. 60 % of AAV particles did not show any interaction with the cell. After binding to the cell membrane, AAV entered the cell within 80 ms. On average, AAV needed 1.2 s from the first contact to the cell membrane to the final entry. After 2 min., AAV was observed in the cytoplasm. 15 min later, AAV was detected in the nucleus of half of the infected cells. 102 trajectories of viral particles in the nucleus were analyzed. When arrived in the nucleus, AAV underwent a directed motion along apparently pre-defined ways, suggesting that AAV moved along tubular structures within the cell nucleus. Taken together, this new technology allows the exact and real-time determination of virus movement from the first membrane contact to the nucleus and shed some light on the poorly understood mechanisms and pathways of AAV infection and rAAV transduction.