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Nonclassical Nuclear Localization Signal Provides for High Efficiency, Nonviral Gene Transfer to Nondividing Endothelium

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Lipofection of nondividing cells is not efficient due to poor endosome escape and poor nuclear import. In vivo, the low division rate of endothelium makes them a difficult target for nonviral gene transfer. Using a cytoplasmic transcription assay by lipofecting pT7bgal with T7 RNA polymerase, we observed that nearly 80 % of confluent bovine aortic endothelium expressed lipofected plasmid, indicating that endosome escape was widespread in a nondividing cell population, albeit inefficient since most of the plasmid was observed in an endosome compartment. We then tested a classical nuclear localization sequences (NLS) to understand its role on plasmid expression during lipofection. We utilized the NLS of the SV40 T antigen (CGYGPKKKRKVGG). Increasing amounts (2 to 80 µg) of SV40 T-antigen were complexed with pCMVb (2 µg) and delivered to dividing subconfluent BAEC with lipofectamine. We found that the SV40 T-antigen sequence caused a dose-dependent enhancement of transfection efficiency in BAEC from about 2 to 10 % (n=3). However, the transfection efficiency was below 10 % suggesting that only the dividing cells in the monolayer were susceptible to lipofection. Similar effects were seen with a scrambled sequence of SV40 T-antigen NLS or lipofection with replication-deficient adenovirus (which enhanced endosome escape by 117-fold). Toward overcoming the final rate limit of nuclear import during nonviral gene transfer, we conjugated a nonclassical NLS, the M9 sequence of hnRNP A1, to a cationic peptide. Lipofection of highly confluent BAEC with plasmid conjugated with the conjugate peptide resulted in 83 % transfection and a 63-fold increase in marker gene expression. The M9-cationic peptide localized fluorescent plasmid into the nucleus of permeabilized cells, and this activity was inhibited by wheat germ agglutinin. Plasmid alone was not imported into the nucleus in this assay. Combining the M9 sequence with DNA binding activity to enhance gene transfer may eventually lead to viral and lipid free proteofection of nondividing cells.

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Assessment of Gene Transfer Using a Non-Viral Liposome Vector in a Monkey Model

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The monkey is an invaluable model to assess gene transfer in human because of high similarity of its biomedical characteristics and gene structure to those of human. In this study, we examined GFP gene transfer into monkeys using naked DNA, liposome vector, or VSVG-liposome vector and assessed these three conditions. Normal adult crab-eating monkeys (Macaca fascicularis) are used. pCMV was employed as expression vector and green fluorescent protein (GFP) plasmid was inserted into the pCMV to prepare CMV-GFP DNA. DOTAP was mixed with CMV-GFP DNA and used as a non-viral liposome vector(CMV-GFP/liposome). DOTAP containing vesicular stomatitis virus G glycoprotein (VSVG) was also used as another liposome vector(CMV-GFP/VSVG-liposome). Naked DNA(CMV-GFP), CMV-GFP/ liposome, and CMV-GFP/VSVG-liposome were intracutaneously injected to monkeys, respectively. Hematological responses and proinflammatory cytokines (TNF, CRP) were assayed. In vivo turnover of GFP DNA was monitored by PCR using a primer set yielding 75-bp product. Expression of GFP protein was observed immnohistochemically

Little change in cell numbers of erythrocytes, leukocytes, and platelets was observed for 9 days after GFP gene transfer (day-9) by the three conditions of naked DNA, CMV-GFP/liposome, or CMV-GFP/VSVG-liposome. No increase in plasma level of TNF or CRP was detected by a sandwich ELISA during the 9 days. Seventy five-bp PCR product was detected frommonkey plasma but not from urine until the 3days after gene transfer (day-3) and its peak was at the next day of gene transfer (day-1), in common, among three conditions. GFP-positve cells were observed at day-1 and reached to peak at day-5. These GFP-positve cells were dendritic cells, resident and exudated macrophages, fibroblasts, or endothelial cells of capillary vessel. Any antibody to GFP did not raise during the 9 days. Thus among three conditions CMV-GFP/VSVG-liposome yielded the most potent expression of GFP protein in monkey.

Among three conditions transferring GFP gene VSVG-liposome vector was the most effective to express GFP protein in monkey model. This VSVG-liposome conditions yielded little side effect to host inflammatory or blood responses.