Comparative evaluation of gene delivery devices in primary cultures of rat hepatic stellate cells and rat myofibroblasts

ABSTRACT

Our results indicate that FuGENE™6-based methods may be optimized sufficiently to offer a feasible approach for gene transfer into rat hepatic stellate cells. The data further demonstrate that adenoviral mediated transfer is a promising approach for gene delivery to these hepatic cells.

INTRODUCTION

Background Transfection is the insertion of foreign molecules such as cDNAs or promoter constructs into eukaryotic cells. This method has become a powerful experimental tool for studying gene functions and to analyze the control of gene expression. Genes of interest can either be transfected transiently or stable into cultured mammalian cells. Detailed protocols for efficient gene transfer to various primary cells and continuous cell lines, irrespective whether these cells are grown as monolayers or in suspension have been established during the last decades. Many methods have been developed to overcome the low transfection efficiency in differentiated cells if classical approaches, such as calcium-phosphate-DNA coprecipitates, diethylaminoethyl (DEAE)-dextran or polylysine mediated gene transfer are applied. Transfection by electroporation, microinjection, biolistic particle delivery, activated dendrimers or cationic liposomes are useful for established cell lines. However, in cells of primary culture most of these transfection systems proved to be inefficient. Direct introduction of genes into rat hepatic stellate cells (rHSCs) and their transdifferentiated phenotype, i.e. the rat myofibroblasts (rMFBs) is difficult to achieve, in part due to the quiescent and fragile phenotype of rHSCs or the extracellular matrix in which the rMFBs are embedded. In normal liver, quiescent HSCs (also called Ito cells, lipocytes, fat-storing cells) are the precursor cells for MFBs, which are responsible for the dramatic increase in the synthesis of extracellular matrix proteins in cirrhotic livers. Upon fibrogenic stimuli, HSCs become activated, a process in which they loose vitamin A granules, proliferate, change morphologically into MFBs, and increase their synthesis of extracellular matrix proteins. Culture of quiescent HSCs on a plastic surface also results in spontaneous activation of these cells similar to that seen in liver fibrosis in vivo. Efficient gene delivery to cultured rHSCs and rMFBs would therefore be of great interest for studying the processes involved in hepatic fibrogenesis and for gene-therapeutic devices. To compare various transfection mediators for their potential to increase the efficiency of gene delivery to rHSC and rMFB we used the reporter plasmid pEGFP-C1 expressing the enhanced green fluorescent protein (EGFP) from the jellyfish Aequorea victoria as a reporter expressed under transcriptional control of the ubiquitously active human cytomegalovirus (CMV) immediate early gene 1 promoter. Transfections were performed with the commercially available cationic liposome reagents Effectene, LipofectAmine Plus, Superfect, a classical calcium phosphate based method with and without glycerol shock, and the lipid-based reagent FuGENE™6, respectively. Furthermore, we cloned an adenovirus type 5 reporter construct (Ad5-CMV-EGFP) harboring the CMV/EGFP transgene and showed that high levels of gene transfer can be achieved in rHSC/rMFB with recombinant replication-deficient viral particles generated thereof. Taken together, we conclude that (i) gene delivery to rHSC can be performed by transfection with FuGENE™6 as mediator and that (ii) Ad5-mediated gene delivery can serve as a useful tool for introduction

foreign DNA into cultured rHSC/rMFB, particularly if high gene delivery rates are required.

CONCLUSION

Conclusions In agreement with other reports on gene delivery to cultured rHSCs by adenoviral based techniques our data show that this method is straightforward particularly when high efficiency of gene transfer is required. Furthermore, our report indicates that introduction of foreign DNA even into rMFBs is possible by use of adenoviral based vector systems. Because of the considerable interest on the rHSC/rMFB transition as a cell culture model for liver fibrogenesis the improvement of efficiency of gene delivery to these cells should facilitate applications such as reproducible reporter vector assays, or bulk expression of signalling proteins for biochemical or cell biological assays. Additional studies will be required to determine the optimal in vivo conditions for adenoviral gene transfer to rHSCs/rMFBs.