USE OF ADENOVIRAL VECTORS TO DELIVER SYNTAXIN DNA INTO 3T3-L1 ADIPOCYTES

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Certain cellular events such as the insulin sensitive glucose transporter, GLUT4 trafficking could be studied only in terminally differentiated adipocytes/ myocytes. Gene transfer is an extremely valuable tool to understand the function of a particular protein in a cell. However, the efficiency of standard transfection methods such as use of DNA precipitates or liposome complexes and electroporation, in transferring DNA into highly differentiated cells such as adipocytes is extremely poor. The ability of adenoviral (AdV) vectors to deliver and express genes at high yields in numerous mammalian cells has been demonstrated over the last 20 yrs. Hence, adenoviruses were used in transferring the genes into 3T3-L1 adipocytes to examine the role of syntaxin 6, 8 and 12 in GLUT4 trafficking of the 3T3-L1 cells.

The objective of this study was to produce high titre recombinant adenoviral stocks and use them efficiently in 3T3-L1 adipocytes to over express the cytosolic domains of syntaxin 6, 8 and 12. Three recombinant AdV (syntaxin 6, 8 and 12) were produced according to a previously described method and optimised the infection of 3T3-L1 adipocytes to over express the particular protein of interest.

AdV were produced successfully at titres between 10^9 and 10^{10} plaque forming units/ml. All three adenoviral constructs resulted in expression of respective syntaxin in 60-80% of 3T3-L1 adipocytes in the culture at 1:100 MOI (multiplicity of infection). In contrast to 3T3-L1 adipocytes, HeLa cells demonstrated 95-100% infectivity at MOI 1:10 showing the relative easiness in transferring genes into these cells.

Recombinant adenoviruses could be used as an efficient method of gene transfer into adipocytes in studying the function of the respective gene products. This approach is currently being used to study the function of syntaxin 16 in GLUT4 trafficking.

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