

Abstract

Cystic fibrosis is a genetic disease caused by a mutation in the *CFTR* gene. This leads to an absence or a malfunction of CFTR chloride channel, which is also a crucial regulator of other ion channels. This thesis was aimed at gene therapy of cystic fibrosis using CFTR-mRNA gene transfer. To determine the expression of CFTR protein, methods of indirect immunofluorescence and Western blot immunodetection were utilized. Also relative gene expression levels of *CFTR* gene were assessed by quantitative PCR. Experiments were carried out on a healthy lung epithelial cell line (NuLi-1), a lung epithelial cell line with F508del mutation (CuFi-1) and an embryonic kidney epithelial cell line (HEK293S).

CFTR protein was visualized by previously mentioned methods using six primary antibodies (432, 450, 570, 596, 769, CF3). Primary antibodies 570 and CF3 were found as optimal for the detection of CFTR protein by the method of indirect immunofluorescence, whereas for the detection of this protein by the method of Western blot only the CF3 antibody was suitable. It was also determined that *CFTR* gene is expressed in overall small levels. Its relative gene expression in the CuFi-1 cell line was approximately six times higher than in the NuLi-1 cell line.

The efficiency of transfection of CuFi-1 cell line by CFTR-mRNA was also studied. The cell line was transfected by two types of *in vitro* synthesised CFTR-mRNA containing: a) unmodified nucleotides, and b) 25 % of pseudouridine and 25 % of 7-methylcytidine. Transfection of the cell line by both types of CFTR-mRNA resulted in increased levels of CFTR protein. CFTR-mRNA with unmodified nucleotides was found to be toxic even at small concentration (0,5 µg/ml). CFTR-mRNA with modified nucleotides was, however, found to be toxic only at concentration 2,0 µg/ml. In conclusion, transfection by CFTR-mRNA is a theoretically possible way of treatment of the cystic fibrosis.

Key words:

Cystic fibrosis, gene therapy, CFTR protein, indirect immunofluorescence, Western blot, quantitative PCR, mRNA transfer.