Abstract

Cystic fibrosis is a genetic disease caused by mutation of the *CFTR* gene coding homonymous protein, whose main function is transport of chloride ions. In this thesis, gene therapy was used for correction of this defect. Two types of stable mRNA was synthetised – both contained at least 200 adenines on the 3‘ end, 25 % of pseudouridine, 25 % of 5-methylcytidine and classical cap (enzyme mRNA) or cap analogue 3‘-O-Me-m7G(5‘)ppp(5‘)G (ARCA cap) on the 5‘ end. Cell lines isolated from healthy volunteer (NuLi-1) and those from patient suffering from cystic fibrosis with F508del mutation (CuFi-1) were used.

The mRNA transfection efficiency was determined using different methods. Increased expression of the CFTR protein was confirmed by visualization of this protein by optimized immunofluorescence method in both cell lines while using both ARCA mRNA and enzyme mRNA.

CFTR protein function was studied using fluorescent probe *N*-(ethoxycarbonylmethyl)-6-methoxyquinoline (MQAE), which is quenched by halogen ions. CFTR channel ion transport was verified using CFTR(inh)-172. This inhibitor specifically inhibits this channel through binding on the R domain of the CFTR protein. CFTR protein function was restored after 24h transfection of the CuFi-1 cell line by ARCA mRNA.

The bacterial adhesion of *Pseudomonas aeruginosa* was the last studied method. The adhesion of *P. aeruginosa* on the CuFi-1 cell line should be higher due to their changed cell surface. After 24h transfection of CuFi-1 cells with ARCA mRNA, the adherence of *P. aeruginosa* strain tested was markedly reduced up to the level of NuLi-1 cells.

In conclusion, the transfection with synthesized CFTR-mRNAs is a promising approach for the gene therapy of CF disorder.

Key words

Cystic fibrosis, epithelial cells, CFTR protein, mRNA, loading of fluorescent probe, indirect immunofluorescence, MQAE, CFTR(inh)-172, bacterial adhesion, *Pseudomonas aeruginosa*