

Abstract

Cystic fibrosis is an autosomal recessive disorder caused by a mutation in the CFTR gene, which leads to inefficiency or absence of CFTR chlorid channel. One way to induce production of CFTR protein in target cells, is to use gene therapy. The principle od gene therapy is to transfer DNA or mRNA molecules inside malfunctioning cells. The aim of this study was to optimise the detection of CFTR protein using the Western blot analysis. Then, using this method the effectiveness of CFTR-mRNA transfection was studied.

To study the CFTR protein, a number of cell lines was used: a healthy human ephitelial cell line (NuLi-1), an ephitelial cell line with $\Delta F508$ mutation (CuFi-1), and a human lung carcinoma cell line (A549). This study compared four different ways of cell lysis – lysis by sonication and lysis by three distinct lysis buffers. Lysis by RIPA buffer with protease inhibitors was determined for the detection of CFTR protein. Moreover, three different primary monoclonal antibodies were also tested. The CF3 antibody, which is specific to an extracellular epitope of CFTR protein, was found able to detect CFTR protein specifically. A couple of different glycosylated forms of CFTR protein was detected. The highest amount of CFTR protein was determined in the NuLi-1 cell line. CFTR protein was also detected in the A549 cell line, which was supposed not to produce it. The amount of CFTR protein in A549 cell line was comparable to the amount in with CuFi-1 cell line.

Using the optimised detection method, the effectiveness of CFTR-mRNA transfection by a cationic liposome lipofectamine was studied. An overall increase in the amount of CFTR protein was detected. Practically the same level of CFTR protein was found in CuFi-1 cell line after transfection in comparison with NuLi-1 cell line without transfection. In conclusion, CFTR-mRNA transfection by lipofektamin is a promising method of restoring the CFTR protein levels in cells affected by cystic fibrosis. However, more experiments are required to determine it's actual usefulness.

Key words

Cystic fibrosis, Western blotting, CFTR protein, gene therapy, mRNA transfer, cationic liposome