Abstract

F_1F_O-ATPsynthase is a key enzyme in energy metabolism of the cell. Its deficit is caused usually by mutations in two structural genes *MT-ATP6* and *MT-ATP8* encoded by the mitochondrial DNA or in nuclear genes *ATPAF2* and *TMEM70* encoding the biogenesis factors and structural gene *ATP5E*. Deficiency of the F_1F_O-ATPsynthase leads to progressive and serious phenotype affecting organs with high energy demands. The first symptoms usually occurs in neonatal age and prognosis of the disease is fatal. Mutations in these genes result in both qualitative and quantitative defects of the F_1F_O-ATPsynthase. The study of molecular bases of mitochondrial disorders including F_1F_O-ATPsynthase deficiency uses large number of biochemical and molecular-genetic methods to determine a proper diagnosis which is essential for the symptomatic therapy and genetic counselling in affected families.

The aim of the diploma thesis was to characterise the F_1F_O-ATPsynthase deficiency in isolated mitochondria from the lines of cultured cells by the determination oligomycin-sensitive ATP-hydrolytic activity of the F_1F_O-ATPsynthase, enzymatic activities of the respiratory chain complexes and to analyse changes in the steady-state levels of the representative subunits and whole complex of the F_1F_O-ATPsynthase in comparison with controls. 3 cell lines HEK293 with stably down-regulated expression of the *TMEM70* gene and 16 cell lines of the skin fibroblasts from patients with the suspicion of the F_1F_O-ATPsynthase deficiency were characterised by the optimalized method for the measurement of the oligomycin-sensitive ATP-hydrolytic activity. In isolated mitochondria from HEK293 cells with stably down-regulated *TMEM70* gene, the decrease in the oligomycin-sensitive ATP-hydrolytic activity among 25-86% of control values was found. Decreased ATP-hydrolytic activity was found in isolated mitochondria from 10 cell lines of the skin fibroblasts as well. Markedly decreased activity of the F_1F_O-ATPsynthase (43% of the control) in 4 fibroblasts lines with mutation 317-2A>G in *TMEM70* gene correlated with decreased abundance of the subunits (α, β, *d*, OSCP) and holoenzyme F_1F_O-ATPsynthase. Furthermore enzymatic activities of the respiratory chain complexes reached up >100% of control values and the increase was in corcondance with increased steady-state levels of corresponding subunits. In 6 fibroblasts lines from patients with suspicion of the F_1F_O-ATPsynthase deficiency, decreased oligomycin-sensitive ATP-hydrolytic activity (43-76% of the controls) was observed but no changes in the abundance of the representative subunits and holoenzyme F_1F_O-ATPsynthase were found out. In *MT-ATP6* and *MT-ATP8* genes, no pathogenic mutations were detected.

In conclusion, the optimalized method to determinate oligomycin-sensitive ATP-hydrolytic activity of the F_1F_O-ATPsynthase in isolated mitochondria from cultivated cells was applied with success in the cell models as in lines of the skin fibroblasts from patient with suspicion of the F_1F_O-ATPsynthase deficiency. It could spread the spectrum of proper methods for diagnostics of the mitochondrial disorders.