Abstract

The F_1F_o-ATP synthase (EC 3.6.3.14) is a key enzyme of the mitochondrial oxidative phosphorylation system (OXPHOS) – using the proton gradient generated by the respiratory chain it synthetizes approximately 90 % of cellular ATP. The subunit arrangement of its F_o domain has not been yet described in detail. At present, the research on ATP synthase research is focused mostly on revealing the structure of the proton channel a so that it is possible to precisely define the molecular mechanism of the ATP synthase rotation generation. The role of the supernumery subunits of F_o domain represents another unresolved issue. These proteins specific for eukaryotic ATP synthases are not essential for synthetic activity, instead they are putatively involved in assembly or stabilization of the enzyme complex. One of such subunits is the nuclear encoded MLQ protein (or also 6.8 kDa proteolipid or MP68), which is conserved only in vertebrates. The aim of this diploma thesis was to reveal the role of this subunit in the structure, assembly and function of the F_1F_o-ATP synthase. For these purposes, cellular model of the HEK293 line with the deficiency of the MLQ protein was established employing the CRISPR/Cas9 method with paired nickases (the knock-out MLQ, MLQ KO) as part of the thesis. Three chosen MLQ KO lines were subjected to electrophoretic analyses of ATP synthase structure (SDS-PAGE, BN-PAGE and CN-PAGE), as well as to functional measurements of mitochondrial respiration and glycolytic capacity. The effect of MLQ absence on the level of transcripts of various OXPHOS genes was also studied. The MLQ KO cells showed decrease in the content of subunits *a*, DAPIT a c in the F_o domain, while subunits of the F_1 were not influenced by the MLQ defect. Native electrophoreses revealed that ATP synthase is not completely assembled in MLQ KO and its F_o domain is destabilized, similarly as in ρ_0 cells lacking the subunits *a* and A6L encoded by mitochondrial DNA (mtDNA). The ATP synthase oligomerization is not influenced by MLQ knock-out. Functional measurements revealed a significant defect in the capacity of mitochondrial ATP synthesis and preference of glycolytic pathway for energy provision. The absence of the protein also resulted in a moderate decrease in the functional capacity of cytochrome c oxidase (complex IV) and decrease in the amount of the mtDNA encoded proteins of this enzyme – COX1, COX2 and COX3. The qPCR analysis uncovered decrease of the level of mRNAs of mtDNA-encoded proteins *a*, A6L and COX3 while the amount of nuclear encoded OXPHOS mRNAs was not affected. The results of the diploma thesis established the role of MLQ in stabilization of the F_o domain of mitochondrial ATP synthase as well as its putative involvement in regulation of mtDNA genes expression.

Key words: MLQ, 6.8 kDa proteolipid, MP68, F_1F_o-ATP synthase, mitochondria, OXPHOS, respiratory chain