ABSTRACT

Cytochrome *c* oxidase (COX) represents the terminal enzyme complex of respiratory chain metabolic pathway and it occurs as monomer, dimer or as a part of respiratory supercomplexes in the inner mitochondrial membrane. COX assembly process is complicated, highly regulated and depends on many ancillary proteins. Mutations in COX subunits, which are encoded by mitochondrial and nuclear DNA, or in genes encoding its assembly proteins are frequent cause of very severe mitochondrial disorders. SURF1 assembly protein participates in the first steps of COX assembly, but its exact function is not yet clarified. In humans, mutations of *SURF1* gene lead to severe COX defect and fatal neurodegenerative disorder, Leigh syndrome. Knockout of *SURF1* gene in mouse causes isolated COX defect as well, but less pronounced and without involvement of CNS. The aim of the thesis was detailed analysis of disturbed COX biogenesis in a condition of *SURF1* gene mutations or *SURF1* gene knockout, from assembly of COX monomer to interaction of COX into supercomplexes, and to the impact of isolated COX defect on other OXPHOS complexes. Mutations of *SURF1* gene in patient's fibroblasts led to marked accumulation of COX assembly intermediates and to a defect in formation of functional COX monomer, which was preferentially built into an I-III_2-IV_1 supercomplex. Consequently, COX deficiency led to increased amount of OXPHOS complexes I, III and V. In *SURF1*$^{-/-}$ mouse, COX defect was markedly tissue specific. The most pronounced decrease of COX was in mouse fibroblasts, but less marked than in *SURF1* patients fibroblasts. In *SURF1*$^{-/-}$ mouse, the COX monomer was also more stable, interacted much less into supercomplexes and COX assembly intermediates were faster depleted than in *SURF1* patients.

The study of another defect of COX biogenesis due to the unique 9205delTA mtDNA microdeletion of *ATP6*/*COX3* genes was focused on different manifestation of the defect in patients and showed, that the reason is heteroplasmy of mtDNA mutation and steep threshold effect. The pathological phenotype thus manifests when more than 90% of mtDNA becomes mutated.

The last part of the thesis was focused on possible interactions of COX in respiratory supercomplexes with FAD-dependent dehydrogenases. We have found that succinate dehydrogenase as well as glycerol-3-phosphate dehydrogenase form higher molecular weight complexes, which were rather oligomeric and without any involvement of COX.

Key words: Cytochrome *c* oxidase, Leigh syndrome, *SURF1* gene, SURF1 protein, *SURF1*$^{-/-}$ knockout mouse, respiratory supercomplexes, 9205delTA microdeletion