Abstract

Oxidative phosphorylation apparatus (OXPHOS) is responsible for production of majority of ATP in mammalian organisms. This process, occurring in the inner mitochondrial membrane, is partly regulated by nuclear-encoded subunits of cytochrome *c* oxidase (COX), the terminal enzyme of electron transport chain. Cox4 subunit, participating in OXPHOS regulation, is an early-assembly state subunit, which is necessary for incorporation of Cox2 catalytic subunit, thus for assembly of catalytically functional COX enzyme. Moreover, regulated expression of two isoforms (Cox4i1, Cox4i2) of Cox4 subunit is hypothesized to optimize respiratory chain function according to tissue oxygen supply. However, the functional impact of the isoform switch for mammalian tissues and cells is still only partly understood.

In the present thesis, unique HEK293 cell line-based model with complete absence of subunit Cox4 (knock-out, KO) was prepared employing novel CRISPR CAS9-10A paired nickase technology and further characterized. Knock-out of both isoforms Cox4i1 and Cox4i2 (COX4i1/4i2 KO clones) showed general decrease of majority of Cox subunits resulting in total absence of fully assembled COX. Moreover, detected Complex I subunits as well as the content of assembled Complex I were decreased in COX4i1/4i2 KO clones. On the contrary, levels of Complex II, Complex III, and Complex V were not significantly affected. Pulse-chase metabolic labelling of 13 mtDNA-encoded proteins synthesized in mitochondria uncovered impairment of COX and Complex I subunits proteosynthesis, while Complex III and Complex V subunits were not significantly affected. Correspondingly, partial impairment of mitochondrial proteosynthesis correlated with decreased level of mitochondrial ribosomal proteins. As expected, mitochondrial respiration was undetected in COX4i1/4i2 KO cells, and was compensated by increased glycolytic capacity.

In summary, the HEK293 cell line-based cellular model of COX4i1/4i2 KO displayed phenotype of total COX absence, making cells fully reliant on OXPHOS-independent ATP production. In addition, we hypothesise that the impairment of mitochondrial proteosynthesis represents a secondary effect of electron transport chain dysfunction. COX4i1/4i2 double KO prepared in this project will serve as a tool for knock-in of either Cox4i1 or Cox4i2 isoform to clarify biological role of these isoforms.

Key words: cytochrome *c* oxidase, COX, Cox4 isoforms, Cox4i1, Cox4i2, CRISPR