Abstract

Mitochondrial ATP synthase represents the final complex of oxidative phosphorylation (OXPHOS) system located in the inner mitochondrial membrane. Its primary role is to utilize mitochondrial membrane potential ($\Delta\psi_m$) generated by respiratory chain complexes to produce energy in the form of ATP. Mammalian ATP synthase comprises of 17 different subunits organized into membranous F_o and matrix-oriented F_1 domains. Defects of complex V and their manifestation have been studied on mitochondrial, cellular, tissue and organism levels using different models, including human cell lines and cell lines derived from patient tissues. In many cases mitochondrial diseases display threshold behaviour, when genetic defect is phenotypically manifested only bellow certain threshold in particular enzyme complex activity and/or content.

This work was aimed at elucidation of functional consequences of ATP synthase deficiency in HEK293 cell lines with suppressed gene expression of γ, δ or ε subunits of ATP synthase central stalk. We have analysed range of clones with respective subunits knockdown and found varying decrease in assembled ATP synthase content, which was mirrored by the decrease in individual ATP synthase subunits. The only exception was subunit F_o-c, whose levels remained unchanged or even increased. ATP synthase deficiency translated into limitation of cell viability, which was manifested under nutrient limiting conditions as a threshold in knockdown clones (KD) harbouring less than 50 % of residual ATP synthase content. Decreased functional capacity of ATP synthase was further characterized in the most severely affected clone, where we observed decrease in respiratory control indexes. While ATP synthase content showed decrease in the range of 90-15 % compared to controls, other OXPHOS complexes displayed variable compensatory upregulation.

Next, we adapted the technique of ATP synthase affinity purification through the IF_1 inhibitory protein. We prepared sufficient quantities of recombinant IF_1 protein construct, verified its ability to bind ATP synthase and performed proof-of-concept isolation of ATP synthase from rat liver mitochondria. Isolated ATP synthase was devoud of contamination by other OXPHOS complexes. This approach will be available for future characterisation of ATP synthase dysfunction models, such as knockdowns of the central stalk.

Key words: mitochondria, ATP synthase, deficiency, threshold effect, biogenesis, affinity purification