number of important differences from the wild type were revealed, indicating a different articulation of the stages of coupled proton pumping in the catalytic cycle, despite the preservation of functional activity. The study allows us to draw conclusions about significant changes in the electrogenic mechanism of proton pumping in the mutant enzyme, indicates a significant stability of the organization of elements in the mechanism and provides new information about the organization of proton transfer in the D-channel in the mutant oxidase and in the wild type.

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S3.225. The effect of mitochondrial DNA mutation complex on the content and production of ATP in cells

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The number of diseases associated with mitochondrial DNA (mtDNA) mutations is approaching 400 [1]. The possibility of pathology manifestation depends on the localization, the level of heteroplasmy, and the combination of mutations. Mutations in mtDNA can negatively affect ATP synthesis due to disturbances in the electron transport chain (ETC), initiating the appearance and development of a number of diseases [2]. Therefore, the aim of this work is to investigate the effect of mtDNA mutation combinations on ATP content and synthesis.

Materials and methods

The lines of cytoplasmic hybrids (cybrids) (TCP, TCN, TCI, HSM1, HSM2, LSM1, LSM2, MAM1, MAM2, MAM3, 520, 521, 522) based on THP cells, each having from 5 to 10 mtDNA mutations with different heteroplasmy levels, affecting genes one (m.3336 T>C) (depending on the lineage, the level of heteroplasmy varies from 0% to 37%), the second (m.5178C>A) (0% to 22%), the fifth (m.13513G>A) (10% to 68%), the sixth (m.14459 G>A) (0% to 61%) subunits of complex I, cytochrome b (m.15059G>A) (0% to 38%), (m.14846 G>A) (0% to 55%), 12S rRNA (del652G) (0% to 44%), (m.1555A>G) (0% to 28%), and tRNA(Leu) (m.c3256C>T) (0% to 50%), (m.12315G>A) (0% to 44%). To analyze the physiological level of ATP, we used a luciferase method using an ATP determination kit (LifeTechnologies, USA). Luminescence levels were monitored using a FLUOstar Omega fluorimeter. Depletion time of cellular ATP was measured by fluorescence microscopy using magFura-2 probe at excitation wavelengths of 340 nm (Mg-bound form) and 380 nm (free form). Before the study, cells were incubated in 3 μM probe solution. ATP synthesis was blocked by adding oligomycin A (2 μg/ml) and iodoacetic acid (100 μM). The moment of a sharp increase in the fluorescence ratio of 340 nm/ 380 nm was a signal of ATP depletion. To assess the conjugation of oxidative phosphorylation, we performed a polarographic respiration study using an Oxytherm+R respirometer. HBSS with 10 mM glucose content was used as measuring medium. During the study, baseline oxygen consumption rate was analyzed and after adding the ATP synthase inhibitor oligomycin A (2 μg/ml).

Results and discussion.

A statistically significant decrease in ATP content relative to the THP line (from 1.9-fold for TCN to 19-fold for LSM1) was observed in almost all cybrid lines. This change may be a consequence of both impaired ATP synthesis and increased macroerg consumption.

To confirm the theory of increased ATP consumption, studies were performed using the ratiometric fluorescence probe magFura-2. The results showed that most of the lines studied had no less ATP depletion time than THP (from 3.8 h. in 522 to 7.3 h. in LSM2 and 4.9 h. in THP). This parameter does not correlate with the data on ATP content. One reason may be the high level of cell dissociation, which was assessed by a cell respiration rate study. All cybrid lines had reduced respiration rates compared with THP (from 33 ng(O2)/(min*10^6 cells) in LSM1 to 54 ng(O2)/(min*10^6 cells) in MAM2 and 64 ng(O2)/(min*10^6 cells) in THP). A statistically significant response to oligomycin A was observed for THP, MAM1, and MAM2 lines (23%, 15%, and 18%, respectively). Thus, the rate of oxygen consumption associated with ATP synthesis is relatively low in the case of all cell lines (mean change of about 14%) because of possible uncoupling, which may be a tool to reduce the negative effects of mitochondrial dysfunction associated with mutations of the rRNA (del652G), tRNA (m.3256 C>T, m.12315G>A) and ETC complexes (m.15059G>A, m.14846G>A, m.5178C>A) in mtDNA [3]. At the same time, data from the MAM1 and MAM2 lines show a higher level of mitochondrial function despite a significant mutational load. In our opinion, this may be due to a high level of heteroplasmy mutations of complex 5 subunit I (m.13513G>A) and 12S rRNA (m.1555A>G), for which a number of studies have shown a negative correlation with the development of atherosclerosis [4, 5]. This is probably due to the fact that the m.1555A>G mutation prevents the synthesis of defective ETC proteins due to ribosome disruption, while the m.13513G>A mutation leads to an increase in Complex I functionality.

Thus, disruption of ATP metabolism and further development of the pathology is the result not only of the heteroplasmy of individual mutations, but also of their mutual influence, which can have both negative and compensatory character.

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S3.226. The effect of short-term protein-carbohydrate deficiency in nutrition on memory indicators

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It is known that the energy necessary for the vital activity of the body is the substrate of the processes of decomposition of metabolites entering the blood during digestion, which provides metabolism, structural and functional activity of the cell. Metabolites entering the bloodstream during digestion, acting as stimuli, accelerate energy production by intensifying the functions of intracellular metabolism, which in turn activates the genetic apparatus and ensures its functional activity. Along with proteins, carbohydrates are also important as the most