

# Mitochondrial Respiration and Molecular Markers for its Study

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## Abstract

Mitochondria play a key role in providing energy for cellular functions, and on the other hand, they are a target, decoder and switch of intracellular signals, generator of secondary messengers and proapoptotic factors. Modern ideas about the mechanisms of energy transduction in the mitochondrial membrane were formed after Peter Mitchell created the chemiosmotic theory of ATP synthesis. The data presented in the article can serve as a fundamental basis for further study of the brain in normal and pathological conditions with further extrapolation of the obtained data to humans.

**Keywords:** Mitochondria; Neurons; Central nervous system.

## Introduction

Mitochondria play a key role in providing energy for cellular functions, and on the other hand, they are a target, decoder and switch of intracellular signals, generator of secondary messengers and proapoptotic factors [1].

Modern ideas about the mechanisms of energy transduction in the mitochondrial membrane were formed after Peter Mitchell created the chemiosmotic theory of ATP synthesis. The energy resources used by the inner mitochondrial membrane are initially directed at the transport of the conjugating proton through the membrane to an area with a higher concentration (energization of the membrane), then the proton gradient is used by the mitochondria as a driving force capable of leading to the synthesis of ATP. Isolation of mitochondrial proteins by D.E. Green and coworkers led to the formation of the idea of "complexes" of the respiratory chain [2].

### Structural and functional organization of mitochondria

The mitochondria has two membranes: an inner (IMM) and an outer (OMM) and, accordingly, two compartments separated from each other by an inner membrane. The outer mitochondrial membrane is permeable to small molecules and ions that move through transmembrane channels formed by a family of integral membrane proteins – porins. There are potentialsensitive anion channels (porins) OMM, which allow the

exchange of metabolites between mitochondria and cytoplasm. The inner membrane is impervious to H<sup>+</sup>, and this extremely important property of the membrane is key for mitochondrial energy transduction. Chemicals such as ions and small molecules that cross the inner membrane have specific transporters. IMM contains integral proteins key catalysts of oxidative phosphorylation (respiratory complexes of the electron transport chain of electron transport) and ATP synthase complex [3].

### Components of the mitochondrial respiratory chain and respiratory complexes

The mitochondrial respiratory chain consists of a number of electron carriers, representing prosthetic groups of integral proteins that function as redox pairs. There are four electron transporters, or respiratory complexes, which catalyze the process of electron transfer along the ETC. In addition to respiratory complexes, lowmolecular carriers (shuttles), such as ubiquinone and cytochrome c, also participate in electron transfer [4].

Complex I (NADH ubiquinone oxidoreductase; NADH dehydrogenase) consists of 4243 different polypeptides, including FMN (flavin mononucleotide) containing a flavoprotein and 6 ironsulfur centers. Complex I in its structure has an Lshape with two "arms": a long "arm" consisting of a hydrophobic membrane protein located in the lipid layer of the inner mitochondri-

al membrane, and a short “arm” having a hydrophilic part protruding into the matrix and containing FMN and an active NADH binding center. Complex I preparations isolated according to the Hatefi procedure contain 4 moles of ubiquinone per 1 mole of FMN. Ubiquinone is a lipid-soluble benzoquinone with a long side isoprenoid chain, which is able to move by diffusion in each of the two layers of phospholipids of the inner membrane and provide shuttle electron transfer between membrane proteins.

Complex II (succinate dehydrogenase; succinate ubiquinone oxidoreductase) is a membrane-bound component of the citric acid cycle (Krebs cycle), which functions as a fragment of the mitochondrial respiratory chain. The II-integral protein complex contains covalently bound FAD (flavin adenine dinucleotide) and iron-sulfur centers localized in the membrane outer domain, which catalyze electron transfer from succinate to ubiquinone and cytochrome b heme located in the hydrophobic membrane domain.

Complex III (cytochrome bc<sub>1</sub> complex; ubiquinone-cytochrome c oxidoreductase) consists of 910 polypeptides, 3 of which are involved in redox reactions. In particular, these are hemes of cytochromes b<sub>562</sub>, b<sub>566</sub>, c<sub>1</sub> and (2Fe<sub>2</sub>S). In addition, two ubiquinone molecules are associated with two separate domains of complex III. Cytochrome c is a peripheral protein located on the inner membrane from the intermembrane space. Cytochrome c transfers electrons from complex I to the Cu<sub>A</sub> center of complex IV.

Complex IV (cytochrome c oxidase; cytochrome c oxidase; cytochrome cO<sub>2</sub> oxidoreductase) is the final catalyst of the mitochondrial respiratory chain. Function of the complex IV consists in reducing O<sub>2</sub> to H<sub>2</sub>O by transferring four electrons from the reduced cytochrome c using 2 protons from the matrix [5].

Electronic carriers and complexes form functional ensembles (“assemblies”) with characteristic protein-protein interactions that ensure efficient electron transfer. Complexes I, III and IV interact with each other to form supercomplexes characterized by a certain stoichiometric composition [6].

Electrons, successively oxidizing the components of the electron transport chain, come from electron donors – NADH or succinate to the acceptor – O<sub>2</sub>. Complexes I, III and IV function as proton pumps that are “powered” by the free energy of conjugated redox reactions occurring in the mitochondria, while the catalyzed oxidation and H<sup>+</sup> release are vector (strictly directed) and conjugate reactions. The movement of H<sup>+</sup> is carried out in the direction from the matrix to the intermembrane space, due to which the matrix is charged “negatively”, and the intermembrane space is “positively”. The chemical free energy of the fall of the redox potential of electrons passing through respiratory complexes is used to create a proton electrochemical gradient (potential Δμ<sub>H</sub>), expressed in units of electric potential and called proton-driving force Δp:

$$\Delta p \text{ (mV)} = \Delta \Psi_m \text{ (2.3 RT/F)} \Delta \text{pH, where}$$

ΔΨ<sub>m</sub> – transmembrane electrical potential of the inner mitochondrial membrane,

ΔpH – pH gradient on the inner membrane;

R, T, and F – gas constant, absolute temperature and Faraday constant, respectively.

At a temperature of 37°C Δp = ΔΨ<sub>m</sub> 60ΔpH.

The main contribution to the creation of Δp in most cases is

made by ΔΨ<sub>m</sub>, which quantitatively is approximately 150–180 mV, Δp is a 200–220 mV.

Δp the driving force of the process of phosphorylation of ADP and inhibition of electron flow in a controlled metabolic state (in the absence of ADP).

The proton potential Δμ<sub>H</sub> consists of two components: electrical (ΔΨ<sub>m</sub>) and chemical or osmotic (ΔpH).

The biophysical basis of respiratory control is that Δp is able to stop the exergonic oxidation of substrates due to the establishment of an equilibrium between the free energy of the electron flow between the redox pairs of ETC and the H<sup>+</sup> flow between the mitochondrial chambers. In addition, the membrane potential provides the driving force for the transfer of cations into the mitochondria, such as K<sup>+</sup> and Ca<sup>2+</sup> [7].

### The role of the electrochemical potential and F<sub>1</sub> ATPases of the molecular rotor in the phosphorylation of ADP

In general, the process of oxidative phosphorylation critically depends on the integrity and impermeability of the inner mitochondrial membrane. The first part of the process consists in converting the chemical potential of NADH and the oxidation energy of succinic acid (succinate) into H<sup>+</sup> electrochemical gradient, the second part is in the synthesis of ATP catalyzed by ATP synthase through the use of proton gradient energy. This process is thermodynamically possible, since the released energy during electron transfer along the ETC and the driving proton force retain enough free energy, approximately 34 kJ per mole of the electron pair to lead to the resynthesis of one mole of ATP, requiring ~32 kJ [8].

Mitochondrial ATP synthase (complex V) is an F-type atpase having two components: F<sub>1</sub> is a peripheral membrane protein, and F<sub>0</sub> is an integral protein that is an integral part of IMM. The catalytic domain F<sub>1</sub> is a globular community of 5 proteins – α, β, γ, δ, and ε with stoichiometry 3:3:1:1:1, where γ, δ, and ε subunits form the basis of the protein ensemble.

To perform the function of complex V, the presence of ADP is necessary. The gradients of ADP and ATP on the inner membrane are balanced by the activity of adenine nucleotide translocase, which ensures the entry of ADP into the matrix and ATP into the cytosol. It has been experimentally established that the synthesis of one ATP molecule requires the transfer of ~3–4 H<sup>+</sup> to the matrix. The efficiency of phosphorylation in isolated mitochondria is defined as the ratio of ADP/O with experimental values of 2.6–2.7 for NAD-dependent substrates and 1.6–1.7 for succinate oxidation. These values are close to the theoretical ones (2.5 and 1.5, respectively), which are calculated based on the fact that the amount of pumped H<sup>+</sup> during electron pair transfer is 10 in the case of NADH oxidation and 6 in the case of succinate oxidation [9].

### Mitochondrial sequestration of Ca<sup>2+</sup>

The addition of Ca<sup>2+</sup> ions to isolated mitochondria, even in the presence of ATP, leads to their energy-dependent capture (sequestration) by mitochondria upon termination of ATP synthesis. The mitochondria absorb Ca<sup>2+</sup> in exchange for H<sup>+</sup> in a process driven by an ATP or membrane potential that drives a Ca<sup>2+</sup>/H<sup>+</sup> pump with a stoichiometry of 1:1. Mitochondria are capable of accumulating a large amount of Ca<sup>2+</sup> with simultaneous absorption of Pi. Ca<sup>2+</sup> can be deposited in the matrix in the form of hydroxyapatite, which does not lead to a significant change in the concentration of its dissolved fraction. Another route of Ca<sup>2+</sup> entry into the mitochondria is functionally related to the

opening of  $\text{Ca}^{2+}$ -mediated pores of high permeability. Intramitochondrial  $\text{Ca}^{2+}$  is an allosteric activator of some matrix dehydrogenases, such as NADH –isocitrate dehydrogenase, pyruvate dehydrogenase,  $\alpha$ ketoglutarate dehydrogenase and a cofactor of mitochondrial NO synthase (mtNOS) [10].

The direct source of energy that the cell uses to produce work is the energy contained in the structure of adenosine triphosphate (ATP). This is a very valuable evolutionary acquisition: energy extracted from an external source is stored in the form of “highenergy bonds” between phosphate groups. ATP easily gives its phosphate groups to other molecules, being an indispensable intermediary for the transfer of chemical energy.

Due to the peculiarities of its structure, this compound is rich in energy and the breaking of bonds between its phosphate groups can occur in such a way that the released energy is used to produce work. Energy storage in the form of ATP occurs as a result of oxidative phosphorylation, which is a metabolic pathway for the oxidation of nutrients [11].

The dichotomous pathway of aerobic glucose breakdown provides the cell with 38 ATP molecules as a result of three stages. The first –glycolysis–takes place in the cytosol, the rest in the mitochondria. The formation of ATP is associated with the dehydrogenation of the substrate and the oxidation of hydrogen (formation of  $\text{H}_2\text{O}$ ) with the participation of NAD and FADdependent dehydrogenases, ubiquinone dehydrogenase, cytochromes and cytochrome oxidase [12].

The energy for ATP synthesis comes from substrates containing highenergy electrons during their dehydrogenation in the Krebs cycle implemented in the cytosol. During oxidative phosphorylation, electrons are transferred from donor compounds to acceptor compounds during redox reactions [13].

NADH and FADN2 molecules transfer electrons to the respiratory chain localized in the inner mitochondrial membrane. The complex of respiratory chain enzymes is located between the outer and inner membranes.

NAD dependent dehydrogenases contain NAD and NADP as a coenzyme. The pyridine ring of nicotinamide is capable of attaching hydrogen electrons and protons from various substrates, serving as the main collector of the energy of oxidized substances and the main source of electrons with high energy potential for the tissue respiration chain.

Flavin dehydrogenases contain FAD or FMN as coenzymes. These coenzymes are formed in the human body from vitamin  $\text{B}_2$ . Flavin coenzymes are strongly associated with apoenzymes. The isoalloxazine conjugated cyclic system serves as the working part of FAD and FMN. Most FAD dependent dehydrogenases are soluble proteins localized in the mitochondrial matrix that serve as electron acceptors from many substrates [14].

The cytochrome system contains cytochromes and cytochrome oxidase, which contain an ironporphyrin prosthetic group. Among the oxidases, ironcontaining enzymes and carriers belonging to the cytochrome system play an important role. Being included in a certain sequence in the process of electron transfer, transferring them from flavoproteins to molecular oxygen, when electrons are transferred by cytochromes, reversible oxidation and reduction of iron occurs, as a result of which, when an electron is given or acquired, their valence changes. In the respiratory chain, the direction of electron transport is determined by the value of the redox potential of cytochromes.

Only cytochrome oxidase, which has the greatest affinity for oxygen, is capable of transferring electrons directly to oxygen. Cytochrome oxidase inhibitors are CO, cyanide, and azide [15].

### Oxidative phosphorylation of ADP

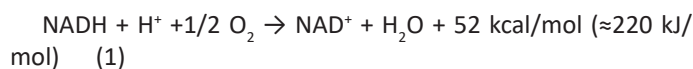
Oxidative phosphorylation requires the energy of electron movement in the respiratory chain. Since electrons tend to move from electronegative systems to electropositive ones, their transport along the electron transport chain (ETC) to oxygen is accompanied by a decrease in free energy.

When comparing the values of the electrochemical potentials of electron carriers, it can be seen that a decrease in free energy occurs at each stage of the ETC, and the energy of the electrons is released in portions.

There are 3 sections in the respiratory chain in which electron transfer is accompanied by a relatively large decrease in free energy. These steps are able to provide energy for the synthesis of ATP, since the amount of free energy released is approximately equal to the energy required for the synthesis of ATP from ADP and phosphate. It has been experimentally confirmed that the process of electron transfer by ETC and the synthesis of ATP are energetically coupled [16].

The first process is the transfer of electrons from the reduced coenzymes NADH and FADH2 through the ETC to oxygen–exergonic.

For example:



The second process – phosphorylation of ADP, or synthesis of ATP, is endergonic:



Synthesis of ATP from ADP and  $\text{H}_3\text{PO}_4$  due to the energy of electron transfer by ETC, it is called oxidative phosphorylation.

### Proton gradient and electrochemical potential

The transfer of electrons along the respiratory chain from NADH to oxygen is accompanied by the pumping of protons from the mitochondrial matrix through the inner membrane into the intermembrane space. This work consumes part of the energy of the electrons transported by the ETC.

Protons transferred from the matrix to the intermembrane space cannot return back to the matrix due to the impenetrability of the inner membrane for protons. Thus, a proton gradient is created in which the concentration of protons in the intermembrane space is greater and the pH is lower than in the matrix. Due to the fact that each proton carries a positive charge, as a result of which there is a potential difference on both sides of the membrane: a negative charge on the inside and a positive charge on the outside. Together, the electrical and concentration gradients make up the electrochemical potential  $\Delta\mu\text{H}^+$  –energy source for ATP synthesis. Since the most active transport of protons into the intermembrane space is necessary for the formation of  $\Delta\mu\text{H}^+$ , occurs at the sites of the ETC corresponding to the location of complexes I, III and IV, these sites are called the points of conjugation of respiration and phosphorylation.

The mechanism of proton transport through the mitochondrial membrane at the interface points is not clear enough. It is known that KOQ plays an important role in this process. The

mechanism of proton transfer with the participation of KOQ has been studied in the most detail at the level of complex III [17].

KOQ transfers electrons from complex I to complex III and protons from the matrix to the intermembrane space, performing a kind of cyclic transformations called Qcycles. The electron donor for complex III is reduced ubiquinone (QH<sub>2</sub>), and the acceptor is cytochrome c. Cytochrome c is located on the outside of the inner membrane of the mitochondria; there is also the active center of cytochrome c, from which electrons are transferred to cytochrome c.

Energy of the proton potential (electrochemical potential  $\Delta\mu\text{H}^+$ ) it is used for the synthesis of ATP if protons return to the matrix through the ion channels of ATP synthase.

The reduced ubiquinone (QH<sub>2</sub>) interacts with Fe<sup>3+</sup> of heme b<sub>1</sub> and, restoring it, releases a proton into the aqueous phase, turning into semiquinone (HQ<sup>•</sup>). The electron from heme b<sub>1</sub> is transferred to Fe<sup>3+</sup> of heme b<sub>2</sub>. HQ<sup>•</sup> gives the second electron to the FeS center located closer to the outer surface of the membrane; in this case, the second proton is in the intermembrane space; the electron is transferred to cytochrome b<sub>1</sub>, and then to cytochrome c. The oxidized Q diffuses to the inner side of the membrane, where it receives an electron from heme b<sub>2</sub> and a proton from the matrix, turning into HQ<sup>•</sup>. receives an electron from complex I and a proton from the matrix; QH<sub>2</sub> is formed in the membrane, and the whole process is repeated from the beginning.

There is a stationary Q/QH<sub>2</sub> pool in the membrane, from which each QH<sub>2</sub> molecule in one cycle ensures the transfer of protons from the matrix to the intermembrane space and electrons, which eventually enter oxygen. The work performed when pumping protons consumes part of the free energy, which is released during the transfer of electrons along the gradient of the redox potential. The energy of the electrochemical potential ( $\Delta\mu\text{H}^+$ ) is used for the synthesis of ATP if protons return to the matrix through the ion channels of ATP synthase [18].

### Structure of ATP synthase and synthesis of ATP

ATP synthase (H<sup>+</sup>ATPase) is an integral protein of the inner membrane of mitochondria. It is located in close proximity to the electron transport chain. ATP synthase consists of 2 protein complexes, designated as F<sub>0</sub> and F<sub>1</sub>.

The hydrophobic complex F<sub>0</sub> is immersed in the membrane. It serves as a base that fixes ATP synthase in the membrane. The F<sub>0</sub> complex consists of several subunits forming a channel through which protons are transferred to the matrix.

The composition of F<sub>0</sub> includes polypeptide chains that form a channel that penetrates the membrane through. Through this channel, protons return to the matrix from the intermembrane space; protein F<sub>1</sub> protrudes into the matrix from the inside of the membrane and contains 9 subunits, 6 of which form 3 pairs of  $\alpha$  and  $\beta$  ("head") covering the core part, which consists of 3 subunits  $\gamma$ ,  $\delta$  and  $\epsilon$ .  $\gamma$  and  $\epsilon$  are mobile and form a rod rotating inside a fixed head and connected to the F<sub>0</sub> complex.

In the active centers formed by pairs of  $\alpha$  and  $\beta$  subunits, ADP, inorganic phosphate (P<sub>i</sub>) and ATP bind [19].

The catalytic cycle of ATP synthesis includes 3 phases, each of which takes place alternately in 3 active centers:

1. Binding of ADP and H<sub>3</sub>PO<sub>4</sub>.

2. Formation of the phosphoanhydride bond of ATP.

3. Formation of the final product.

Each time protons are transferred through the F<sub>0</sub> channel to the matrix, all 3 active centers catalyze the next phase of the cycle. The energy of the electrochemical potential is spent on turning the rod, as a result of which the conformation of  $\alpha$  and  $\beta$  subunits changes cyclically and ATP synthesis occurs.

The F<sub>1</sub> complex protrudes into the mitochondrial matrix. It consists of 9 subunits (3 $\alpha$ , 3 $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\delta$ ). The  $\alpha$  and  $\beta$  subunits are stacked in pairs, forming a "head"; between the  $\alpha$  and  $\beta$  subunits there are 3 active centers in which ATP synthesis occurs; the  $\gamma$ ,  $\epsilon$ ,  $\delta$  subunits bind the F<sub>1</sub> complex to the F<sub>0</sub>.

An increase in the concentration of protons in the intermembrane space activates ATP synthase. The electrochemical potential  $\Delta\mu\text{H}^+$  causes protons to move through the ATP synthase channel into the matrix. In parallel, under the action of  $\Delta\mu\text{H}^+$ , conformational changes occur in the pairs of  $\alpha$ ,  $\beta$  subunits of the F<sub>1</sub> protein, as a result of which ATP is formed from ADP and inorganic phosphate. The electrochemical potential generated at each of the 3 interface points in the ETC is used to synthesize one ATP molecule [19].

### Coefficient of oxidative phosphorylation

The oxidation of the NADH molecule in the ETC is accompanied by the formation of 3 ATP molecules; electrons from FAD-dependent dehydrogenases enter the ETC at KOQ, bypassing the first interface point, so only 2 ATP molecules are formed. The ratio of the amount of phosphoric acid (P) used for phosphorylation of ADP to the oxygen atom (O) absorbed during respiration is called the coefficient of oxidative phosphorylation and is designated P/O. Therefore, for NADH P/O=3, for succinate P/O<sub>2</sub>. These values reflect the theoretical maximum of ATP synthesis, in fact, this value is less.

The P/O coefficient shows the number of Inorganic Phosphate (Pi), molecules that have passed into the organic state (ATP) per each oxygen atom absorbed. In fact, it is equal to the number of ATP molecules that are formed during the transportation of 2 electrons along the respiratory chain per oxygen atom [20].

Respiratory control ratio is a direct effect of the electrochemical gradient on the speed of movement of electrons along the respiratory chain (i.e., on the amount of respiration). In turn, the magnitude of the gradient directly depends on the ratio of ATP / ADP, the quantitative sum of which in the cell is almost constant ( $[\text{ATP}] + [\text{ADP}] = \text{const}$ ).

Oxidation of substrates and phosphorylation of ADP in mitochondria are closely related. The rate of use of ATP regulates the rate of electron flow in the ETC. If ATP is not used and its concentration in cells increases, then the flow of electrons to oxygen stops. On the other hand, the consumption of ATP and its conversion into ADP increases the oxidation of substrates and the absorption of oxygen. The dependence of the mitochondrial respiration intensity on the concentration of ADP is called respiratory control ratio. The mechanism of respiratory control ratio is characterized by high accuracy and is important, since as a result of its action, the rate of ATP synthesis corresponds to the energy needs of the cell. There are no reserves of ATP in the cell. The relative concentrations of ATP / ADP in tissues vary within narrow limits, while the energy consumption of the cell, i.e. the frequency of revolutions of the ATP and ADP

cycle, can vary tenfold.

### Transport of ATP and ADP through mitochondrial membranes

In most eukaryotic cells, the synthesis of the main amount of ATP occurs inside the mitochondria, and the main consumers of ATP are located outside it. On the other hand, a sufficient concentration of ADP should be maintained in the mitochondrial matrix. These charged molecules cannot independently pass through the lipid layer of the membranes. The inner membrane is impervious to charged and hydrophilic substances, but it contains a certain number of transporters that selectively transfer such molecules from the cytosol to the matrix and from the matrix to the cytosol.

There is an ATP/ADP antiporter protein in the membrane that transports these metabolites across the membrane. The ADP molecule enters the mitochondrial matrix only if the ATP molecule exits the matrix [21].

The driving force of such an exchange is the membrane potential of electron transfer along the ETC. Calculations show that about a quarter of the free energy of the proton potential is spent on the transport of ATP and ADP. Other transporters can also use the energy of the electrochemical gradient. This is how inorganic phosphate, necessary for the synthesis of ATP, is transferred into the mitochondria. The proton potential, not the ATP energy, also serves as a direct source of free energy for the transport of  $\text{Ca}^{2+}$  into the matrix.

The flows of various substances (ATP, ADP,  $\text{H}_3\text{PO}_4$ ,  $\text{Ca}^{2+}$ ) pass through specific transporters, while the energy of the electrochemical potential of the membrane is consumed.

### Conjugation of respiration and phosphorylation

According to Mitchell's chemiosmotic theory, there is no direct connection of respiratory chain enzymes with phosphorylation enzymes in mating membranes. The conjugation of respiration and phosphorylation is carried out through the membrane. When protons are transferred through the membrane, it passes into an energized state with the appearance of proton concentration gradients and an electric potential difference across the membrane. At the same time, forces arise that seek to return protons to the matrix. Under the influence of these forces, protons pass through the channel of enzyme  $F_0$  from the outer region to enzyme  $F_1$  located on the inner side of the membrane. The proton transfer energy is used by this enzyme to synthesize the ATP molecule in the matrix. The electrons go along the respiratory chain, part of the energy from these movements is spent for pumping protons into the intermembrane space, as a result of which the enzymes  $F_0$  and  $F_1$  convert the kinetic energy of the moving proton into macroergic energy with phosphorylation of ADP [22].

### Separation of respiration and phosphorylation

Some chemicals (protonophores) can transfer protons or other ions (ionophores) from the intermembrane space through the membrane into the matrix, bypassing the proton channels of ATP synthase. As a result, the electrochemical potential disappears and ATP synthesis stops. This phenomenon is called the disconnection of respiration and phosphorylation. As a result of separation, the amount of ATP decreases, and ADP increases. In this case, the rate of oxidation of NADH and  $\text{FADH}_2$  increases, the amount of oxygen absorbed also increases, but energy is released in the form of heat, and the P/O coefficient decreases

sharply. As a rule, the disconnectors are lipophilic substances that easily pass through the lipid layer of the membrane. One of such substances is 2,4 dinitrophenol, which easily passes from an ionized form to a nonionized one by attaching a proton in the intermembrane space and transferring it to the matrix.

The protonated form of 2,4 dinitrophenol transports protons through the inner membrane of mitochondria and prevents the formation of a proton gradient [23].

Some medications can also be examples of disconnectors, for example, the anticoagulant dicoumarol or metabolites that are formed in the body, bilirubin (a product of heme catabolism), thyroxine (a thyroid hormone). All these substances exhibit a disconnecting effect only at their high concentration.

There are a number of molecular markers, the use of which makes it possible to study in detail the activity of mitochondria under various experimental influences. About a thousand mitochondrial markers are known, but this review presents the main ones.

### Markers of energy function of mitochondria.

**ATP synthase** belongs to the alpha/beta ATP synthase family. The enzyme consists of two structural domains (F1 is an extramembrane catalyst and F0 is a proton channel of the membrane) connected by a central rod. The central core of mitochondrial ATP synthase consists of the  $\gamma$ ,  $\delta$  and  $\epsilon$  subunits and, together with the oligomer of the membrane subunit, represents the rotary domain of the enzyme. It is assumed that the function of the  $\epsilon$  subunit is to assemble the remaining domains of the enzyme. Mutation or deficiency of this subunit reduces the activity of ATP synthase. The study of the genes necessary for the differentiation of stem cells revealed the important role of mitochondrial ATP synthase in this process. ATP synthase promotes maturation of mitochondrial crystals during differentiation by dimerization and specific regulation of the ATP synthase complex. There are two types of ATP synthase  $\alpha$  and  $\beta$ .

The high activity of ATP synthase in cancer is due to the activation of glycolysis, resistance to apoptosis, increased migration and intensive proliferation of tumor cells.

**SDHA** (Succinate dehydrogenase complex, subunit A). It belongs to the family of oxidoreductases. It is a subunit of the enzyme succinate dehydrogenase, which participates in the complex of the mitochondrial electron transport chain and is responsible for the transfer of electrons from succinate to ubiquinone (coenzyme Q).

It has been shown that mutations of subunits (SDH) contribute to the development of pheochromocytoma and renal carcinoma [1,23].

**Recombinant protein COX7A2L** (Cytochrome c oxidase subunit 7A related protein). It belongs to the cytochrome C oxidase VIIa family. The expected function is the regulation of cytochrome C oxidase activity.

Evaluation of COX7A2 expression is a valuable predictive marker of glioma outcome. The analysis of glioma survival showed that patients with higher expression of glioma had a longer life expectancy.

In mice with insufficient expression of COX7A2, lower blood glucose levels were found after injection of insulin and pyruvate, compared with intact animals. It is noteworthy that the

rate of ATP synthesis in the liver of such mice was reduced.

The data obtained indicate that COX7A2 mediated mitochondrial respiration plays an important role in maintaining glucose homeostasis, and therefore its violation will contribute to the pathology of metabolic processes.

**COX<sub>IV</sub>** (Cytochrome c oxidase subunit 4) is a protein that is one of the polypeptide chains of cytochrome oxidase involved in electron transfer in mitochondria. It belongs to the cytochrome C oxidase IV family. It characterizes the efficiency of the tissue respiration chain.

COX<sub>IV</sub> can be used as a marker of colorectal cancer. Most of the energy for tumor transformation is of mitochondrial origin. Differences in mitochondrial efficiency may affect the degeneration of colorectal adenomatous polyps into adenocarcinoma. High levels of COX<sub>IV</sub> expression were noted in tubular, villous adenomas and adenocarcinoma, compared with tubular polyps, which confirms the important role of COX<sub>IV</sub> in the energy metabolism of colorectal cells as they transition from polyps to carcinoma.

**MTc1** (Mitochondrial encoded cytochrome with oxidase I). It belongs to the family of heme-copper respiratory oxidases.

Cytochrome oxidase is a component of the respiratory chain that catalyzes the reduction of oxygen. Subunits 13 form the functional core of the enzyme complex. Cytochrome c oxidase I is a catalytic subunit of the oxidative phosphorylation enzyme.

**HADHA** (HydroxyacylCoA dehydrogenase trifunctional multi enzyme complex subunit alpha). It belongs to the family of 3-hydroxyacyl CoA dehydrogenases. It is a subunit of an enzyme involved in the beta oxidation of fatty acids. Mutations in HADHA genes cause a rare autosomal recessive metabolic disorder characterized by a decrease in the activity of mitochondrial  $\beta$ oxidation of fatty acids.

#### Markers of the mitochondrial pathway of apoptosis.

**VDAC<sub>1</sub> (Voltage dependent anion selective channel 1)/Porin.** Protein belongs to the porin family. Provides diffusion of hydrophilic molecules, participates in the regulation of cell volume and apoptosis. Promotes the formation of pores in the external mitochondrial membrane during apoptosis [4,13].

In the study of cervical cancer tumor cells, it was found that the high expression of VDAC<sub>1</sub> significantly correlates with the high frequency of metastases to the lymph nodes and the depth of invasion. Further clinical study confirmed that high expression of VDAC<sub>1</sub> is associated with lower survival and can be used as a potential prognostic marker for patients with cervical cancer. A functional study showed that suppression of VDAC<sub>1</sub> expression reduces the proliferation, migration and invasion of tumor cells.

**AIF** (Apoptosis inducing factor) belongs to the FAD dependent family of oxidoreductases. It is an oxidoreductase that plays a dual role in the control of the cell cycle. During apoptosis, it is translocated from mitochondria to the nucleus, where it functions as a proapoptotic factor in the caspase independent pathway, whereas in normal conditions it functions as an antiapoptotic factor through oxidoreductase activity. The soluble form (AIFsol) found in the nucleus induces caspase-independent fragmentation of chromosomal DNA, and also activates

caspase7 to activate apoptosis. It plays an important role in caspase-independent pyknotic cell death induced by hydrogen peroxide [4,15].

#### Markers of mitochondrial plastic processes

**Hexokinase 1** is a protein belonging to the hexokinase family. Participates in the regulation of carbohydrate metabolism and hexose metabolism. It contains 2 domains N and C. Catalytic activity is associated with the C domain, and regulatory activity is associated with the N domain. Thus, during experimental modeling of Parkinson's disease, the accumulation of hexokinase 1 in the cytosol was found to be far from its usual location in the mitochondria [16].

**HSP<sub>60</sub>** (Heat Shock Protein), or heat shock protein belongs to the chaperone family (HSP<sub>60</sub>), which play an important role in the implementation of adaptive mechanisms of the cell. Participates in the transport of mitochondrial protein, the assembly of macromolecules and the maturation of polypeptides formed in the mitochondrial matrix. A possible source of autoantigens expressed in tissues during inflammation are heat shock proteins. Studies have shown that HSP<sub>60</sub> can function as vaccines to suppress autoimmune inflammatory reactions, including rheumatoid arthritis [2,8].

**TOMM20** (Translocase of outer mitochondrial membrane 20) functions as a transit peptide receptor on the surface of the external mitochondrial membrane, facilitating the translocation of synthesized mitochondrial preproteins in the cytosol. TOMM20 has different expression in tumor cells. Reed Sternberg lymphoma cells have a high expression of TOMM20, unlike malignant macrophages and malignant degenerated lymph nodes. High expression in cancer cells is a sign of high metabolic heterogeneity between cancer cells and the tumor microenvironment and serves as a marker of an unfavorable outcome of the disease [5,8].

**Prohibitin** inhibits DNA synthesis, it is possibly participates in the regulation of mitochondrial respiration activity. There is an assumption that this protein plays a role in the activation of mRNA. Membrane prohibitin regulates cellular transmission of membrane transport signals, nuclear prohibitin controls transcription activation and the cell cycle, mitochondrial prohibitin complex stabilizes the mitochondrial genome and modulates the mitochondrial internal apoptotic pathway. In addition, prohibitin can be translocated into the nucleus or mitochondria during apoptosis [11,15].

**Frataxin.** It belongs to the family of frataxins- proteins that play an important role in the removal of ferrum from the near-mitochondrial space.

Promotes heme biosynthesis and the assembly of ferrous and sulfur clusters by delivering ferrous ions to the protein. It plays a role in protecting against oxidative stress due to its ability to catalyze the oxidation of Fe<sub>2</sub><sup>+</sup> to Fe<sub>3</sub><sup>+</sup>. It has the ability to store a large amount of iron in the form of a ferrohdyrite mineral. Violation of mitochondrial functions is accompanied by any disease, therefore, further study of the functional features of mitochondria in various pathologies in the clinic and experiment, as well as the search for new diagnostic markers is promising and relevant [16,20]. The data presented in the article can serve as a fundamental basis for further study of the brain in normal and pathological conditions with further extrapolation of the obtained data to humans.

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