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„Udział ludzkich paralogów białka VDAC w przeciwdziałaniu skutkom stresu oksydacyjnego wywołanego brakiem dysmutaz wewnątrzkomórkowych”

## Abstract

The process of cellular respiration takes place in the mitochondria, whose substrates and metabolites are transported through the VDAC channel located in the outer mitochondrial membrane. This channel protein also transports small molecules and inorganic ions and interacts with many mitochondrial and cellular proteins, thus mediating the regulation of mitochondrial and whole cell function. VDAC can exist in the form of paralogs, e.g. in yeast there are 2 paralogs (yVDAC1 and yVDAC2), and in humans there are 3 (hVDAC1, hVDAC2 and hVDAC3), as in other mammals and vertebrates.

During the process of cellular respiration, in addition to molecules "carrying" energy, reactive oxygen species (ROS) are also produced, the excess of which in the cell increases the risk of damage and mutations. Naturally, the excess of ROS is removed by antioxidant enzymes, among which the key role is played by superoxide dismutase (SOD), which occurs in cells in the form of two forms. SOD1 (CuZnSOD) is found in various cellular structures, including the mitochondrial intermembrane space, while SOD2 (MnSOD) is localized in the mitochondrial matrix. Increased ROS levels are an indicator of oxidative stress, which in turn can be counteracted by the cell by activating appropriate defense mechanisms. Available data indicate that hVDAC3 may play an important role in the response of human cells to oxidative stress, which is attributed to the function of a sensor of the reduction-oxidation state (redox state; redox sensitive VDAC; rsVDAC), which in turn may be due to the number and specific location of cysteine residues in the sequence of this protein.

Therefore, the aim of this study was to determine the frequency of rsVDAC occurrence outside the vertebrate group and to construct a yeast model to analyze the functionality of individual human VDAC paralogs under oxidative stress conditions. In pursuit of this goal, tools were used to predict the secondary structures of the studied proteins, followed by the distribution of cysteine residues, in order to determine the frequency of rsVDACs depending on the number of VDAC paralogs and the level of complexity of the organisms, their living environment and their lifestyle, and the CRISPR/Cas9 technique to obtain a model based on *Saccharomyces cerevisiae* yeast cells, in which the genes encoding hVDAC1, hVDAC2 and hVDAC3 (hVDAC3 also in a variant devoid of cysteine residues - hVDAC3ΔCys) were heterologously expressed in the absence of the genes encoding yVDAC1 and yVDAC2 and the genes encoding SOD1 and SOD2 were deleted.

The obtained results indicate that: (1) rsVDAC may be the only VDAC variant in mitochondria and its presence may correlate with habitat conditions, as rsVDAC seems to be common in parasites, which in turn suggests that this channel may mediate sensing and adaptation to environmental conditions; (2) the genotype of *S. cerevisiae* yeast cells may be important for their use in hVDAC studies, including hVDAC3 in particular, due to its effect on the intracellular redox state and (3) hVDAC3 may protect the cell under oxidative stress conditions in a way that does not require the presence of superoxide dismutases and comes down to the activation of a specific bioenergetic state of mitochondria associated with intensive ATP synthesis, which in turn requires the presence of cysteine residues in this protein. These results expand the knowledge on the prevalence of rsVDAC in animal mitochondria and the specific role of hVDAC3 under oxidative stress conditions.