nuclear pools of FH, whose tumor suppressor functions rely on DNA damage repair and stabilization of HIF-1α-signaling that induces pseudohypoxia.

References


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11P8

Mitochondrial potassium channels in Dictyostelium discoideum

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Mitochondria are crucial not only in energy metabolism but also in regulation of cell senescence and apoptosis. The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondria functioning. Potassium ion homeostasis is an important process for mitochondrial optimal functioning. Potassium channels such as ATP-regulated, large conductance calcium activated and voltage dependent channels were observed in inner mitochondrial membrane in various mammalian tissues. Recently, we have identified potassium channels in inner mitochondrial membrane of potato Solanum tuberosum and Acanthamoeba castellanii. Currently we characterize mitochondrial potassium channels from one of Dictyostelium species. It is commonly used as a model organism to study cell differentiation, metabolism and programmed cell death. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayers. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from −50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca2+ and Mg2+ was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of Dictyostelium discoideum functioning.

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11P9

Reactive oxygen species in proinflammatory response of endothelial cells

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Endothelium is a thin layer of cells lining all cardiovascular system. It plays crucial role in such diseases as atherosclerosis, hypertension and diabetes. It is well documented that the inflammation is responsible for these diseases, however very little is known about the role of mitochondria in development of proinflammatory state. It is known that reactive oxygen species (ROS) produced by mitochondria can be involved in the proinflammatory process.

As a model of our study we used endothelial immortalized cell line EA.hy 926 and as a marker molecule the ICAM-1 (intracellular adhesion molecule 1) expression was measured using flow cytometry method. The inflammation was induced by cytokine TNF-α (tumor necrosis factor α). We examined the role of reactive oxygen species (ROS) in the proinflammatory process using fluorescent probe DCF-DA. The main source of ROS production in cells is mitochondria, therefore we checked the effect of rotenone, the complex I of respiratory chain inhibitor, on ROS level in EA.hy 926 cells.

In our study TNF-α caused time and dose dependent increase of ICAM-1 from hardly detected residual level. Additionally our results show that TNF-α increases ROS production in EA.hy 926 cells in dose dependent manner. Rotenone was ineffective in changing the ROS production level in EA.hy 926 cells. Our results related to rotenone are slightly different to literature data that suggests that different cellular models can response to rotenone in different ways.

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11P10

NCLX, but not Letm1, mediates matrix Ca2+ extrusion and modulates the mitochondrial redox state during HeLa cell stimulation

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Mitochondria sense and shape intracellular Ca2+ signals, acting as a cell signaling hub. The uptake of Ca2+ into the mitochondrial matrix activates intermediary and energy metabolism, and Ca2+ extrusion mechanisms ensure that this Ca2+ signal is transient. After a long quest, the proteins promoting Ca2+ uptake and release have been discovered: a mitochondrial Ca2+ uniporter was shown to mediate Ca2+ uptake, and two ion exchangers, NCLX and Letm1, were proposed to exchange Ca2+ against Na+ or H+ respectively.

To relate mitochondrial Ca2+ extrusion to mitochondrial function, we have manipulated the expression levels of NCLX and Letm1 and measured by single cell imaging their impact on matrix Ca2+, matrix redox state, and NAD(P)H concentration evoked by Ca2+ mobilizing agonists.

We find that the histamine stimulated mitochondrial Ca2+ rise is highly variable in individual HeLa cells. The rate of Ca2+ extrusion is a function of this amplitude, being highest for large matrix Ca2+...