S2.P18

Effect of dichloroacetate (DCA) treatment in auto/Mitophagy in SH-SY5Y cells
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The dichloroacetate (DCA) has beneficial effect in cancer, inducing apoptosis and decreasing cancer growth in vitro and in vivo, without affecting noncancerous cells. DCA stimulates the activity of the enzyme pyruvate dehydrogenase kinase by inhibiting the enzyme pyruvate dehydrogenase. It exerts multiple effects on pathways of intermediary metabolism. One of these effects is, DCA favors the oxidation of glucose rather than perform glycolysis, and therefore suffering remodeling the energy metabolism and it could affect to mitochondrial network. Thus, we propose to study the effect that DCA produces in the dynamics of the mitochondrial network using autophagy markers by western blot. With this purpose, we cultivated SH-SY5Y cells and distributed in 3 groups (control, NH\textsubscript{4}Cl & chloroquine) and each group was treated with DCA at 0 mM, 5 mM, 30 mM & 60 mM overnight. Samples were analyzed by western blot using TFAM, FIS1, OPA1, PARKIN & PINK1 antibodies. The results reveal that DCA causes a decrease in a dose-dependent manner with significant protein expression of FIS1 and PARKIN in all groups while only minor decrease was observed in PINK1. TFAM levels were stable in all samples. OPA1 was stable at low- and mid-doses of DCA, but has decreased in the highest dose of DCA, especially when autophagy blockers were used. OPA1 was dramatically reduced in the chloroquine group for all doses of DCA, especially the highest dose of DCA. We can conclude that DCA produces a change in the protein expression of key proteins in the mitochondrial dynamics but apparently does not significantly affect the total number of mitochondria, suggesting that DCA favors a remodeling of the structure of the mitochondrial network. “This work was supported within the project The Centre of Biomedical Research (CZ.1.07/2.3.00/30.0025). This project is co-funded by the European Social Fund and the state budget of the Czech Republic.”

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S2.P19

Lateral pH gradient between OXPHOS complex IV and F\textsubscript{0}F\textsubscript{1} ATP-synthase in folded mitochondrial membranes
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Ion-driven ATP synthesis by rotary F\textsubscript{0}F\textsubscript{1} ATP synthase powers aerobic life. Since Mitchell’s seminal hypothesis, this synthesis has been discussed in terms of the proton-motive force between two bulk phases, each in equilibrium. In eukaryotic cells, ATP is produced at the cristal membrane of mitochondria. Cristae are flat invaginations of the mitochondrial inner membrane extruding into the matrix space. The therein located proton pumps and the proton-driven ATP synthase can be segregated. Complexes I–IV are mainly found in the flat sheet membrane, while Immuno-EM and EM-tomography have revealed ribbons of F\textsubscript{0}F\textsubscript{1} dimers lining the highly curved rim. These ribbons seem to be involved in folding the crista membrane. In active mitochondria, a steady proton flow cycles between pumps and the distant ATP synthase. By attaching a ratiometric fluorescent pH-sensitive GFP variant to OXPHOS complex IV and the dimeric F\textsubscript{0}F\textsubscript{1}, ATP synthase, we determined the lateral pH profile along the p-side of cristae in situ. To achieve an activated oxidative phosphorylation in HeLa cells, glucose was replaced by galactose in the glutamine-containing growth medium. We observed that the local pH at F\textsubscript{0}F\textsubscript{1} dimers (proton sink) is by 0.3 units less acidic than at CIV (proton source) in these cells. This finding is consistent with the calculated pH profile for steady proton diffusion from a proton pump in the crista sheet to F\textsubscript{0}F\textsubscript{1} as proton consumer at the rim. The observed lateral variation in the proton-motive force necessitates a modification to Peter Mitchell’s chemiosmotic proposal. The experimental technique can be extended to other pH-dependent reactions in membrane microcompartments.

S2.P20

Spin control of oxidative phosphorylation
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According to our previous investigations [1], under conditions of low-amplitude mitochondrial swelling (in hypotonic medium, 120 mOsm) electron transport chain and ATPase system operate as a tightly coupled supercomplex. The temperature-dependent efficiency of the mitochondrial oxidative phosphorylation was measured within 15–36 °C. Abnormally narrow peak of the parameter ADP/O was detected at 19 ± 1 °C. This finding suggests the existence of highly organized process that controls ATP synthesis. The effect could not be explained in terms of classical biochemical kinetics. The temperature dependence of pyrene-induced quenching of tryptophan fluorescence in the mitochondrial membrane-bound proteins revealed their conformational transition observed under the conditions facilitating supercomplex formation at the same narrow temperature range of 19 ± 2 °C [2]. This abnormal range of both (functional and structural) temperature dependencies coincides exactly with the point where the conversion of two distinct quantum states of water (ortho–para water differs in their mutual hydrogen spin orientation) is facilitated [3]. These spin-isomers of water were recently discovered experimentally [4], including a liquid water phase [5]. We explain narrow temperature-dependent effects as a phenomenon based on strongly different affinities [6] of ortho–para isomers of water to the proteins involved in oxidative phosphorylation.

References
S2.P21

The role of the mitochondria-shaping protein Opa1 in the BCR vs. OxPhos subset of diffuse large B cell lymphoma
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Diffuse large B cell lymphomas (DLBCLs) are a genetically heterogeneous group of tumors that can be further divided in several subsets, identified by their distinct molecular signatures. Genome wide arrays and multiple clustering algorithms defined a B cell receptor/proliferation cluster (BCR–DLBCL), which displays the upregulation of genes encoding BCR signaling components, and an OxPhos cluster (OxPhos–DLBCL) which is enriched in genes involved in mitochondrial oxidative phosphorylation. The OxPhos subset lacks an intact BCR signaling network, suggesting dependence on alternative survival mechanisms, which are not yet defined. A proteomic analysis identified increased levels of the mitochondria shaping protein Optic atrophy 1 (Opa1), that regulates mitochondrial fusion and cristae biogenesis, in the OxPhos subset. Here we present evidence that mitochondrial morphology, metabolism, and ultrastructure are different between the BCR and the OxPhos subsets that display different levels of Opa1. Our data indicate a role for Opa1 in DLBCL features.

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S2.P22

Submitochondrial protein distributions at the nanoscale
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Mitochondria exhibit a complex architecture which serves to separate different biochemical pathways. Two membranes divide the interior of the organelle into several reaction rooms. The smooth mitochondrial outer membrane surrounds the entire organelle. At contact sites, this membrane is connected to the highly folded inner membrane. Relatively little is known on the distribution of proteins within these membranes. Arguably, the most suitable approach to quantitatively study the distribution of protein complexes in intact cells is far-field fluorescence microscopy. Because mitochondria exhibit a diameter of around 300 nm, which is in the range of the diffraction limited resolution of conventional light microscopes (~200 nm in the focal plane), a detailed analysis of the distribution of proteins within mitochondria is challenging or even impossible with conventional light microscopy. In this study, we employed diffraction-unlimited stimulated emission depletion (STED) microscopy together with automated algorithms for image analysis to investigate sub-mitochondrial distributions of various proteins. Amongst others, we found components of the translocase of the mitochondrial outer membrane (TOM complex) to be mainly concentrated in individual clusters whose nanoscale distribution is finely adjusted to the cellular growth conditions. The distributions of these clusters follow an inner-cellular gradient from the perinuclear to the peripheral mitochondria. The nanoscale distribution of many mitochondrial proteins is finely adjusted to the cellular conditions, resulting in distribution gradients both within single cells and between adjacent cells.

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