S2.P10

Uncouplers preserve the respiratory function in the heteroplasmic *Saccharomyces cerevisiae* cells carrying wild type and non-functional mitochondrial DNAs

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A number of pathologies are associated with the presence of non-identical mitochondrial DNA (mtDNA) molecules within a single cell (heteroplasmy). The latter is usually due to the large deletions in a subset of mtDNAs. The short copies of mtDNA have an advantage in replication and eventually displace the functional mtDNA. We proposed that mitochondrial uncouplers can change the competition conditions in favor of the functional mtDNAs. Here we tested this by mating of the two *Saccharomyces cerevisiae* strains: *mat a* with the wild type mtDNA (*rho*$^{+}$) and *mat alpha* with hypersuppressive mutant mtDNA (*HS rho*$^{-}$). Then we measured the percentages of the obtained diploid cells which were not able to grow on non-fermentable carbon source. These percentages reflect the efficiency of *HS rho*$^{-}$ mtDNA in the displacement of wild type *rho*$^{+}$ mtDNA. It was found that uncouplers FCCP and pentachlorophenol decrease the frequency of such displacement. Unlike the uncouplers, the inhibitors of respiratory chain, an inhibitor of ATP synthase oligomycin or antioxidants (alpha-tocopherol, N-acetylcysteine) did not show such an effect. Furthermore, it was found that suppression of *DNM1* (a key mediator of mitochondrial fission) significantly diminishes the effect of FCCP. We argue that uncouplers decrease the frequency of mitochondria fission–fusion cycles thus preventing the mixing of functional and non-functional mtDNAs. As a result, the mitochondrial quality control machinery has more time for the removal of mitochondria with non-functional variants of mtDNA. We speculate that similar strategy could be applied to multicellular eukaryotes for preventing clonal expansion of mtDNA with common deletion.

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S2.P11

Phenotypic cell analysis using automated mitochondrial morpho-functional fingerprinting and supervised machine learning

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Human mitochondrial complex I (CI) dysfunction is often paralleled by increased ROS levels and aberrant mitochondrial membrane potential and morphology. We generated novel therapeutic molecules ("KH-compounds") and studied their multifactorial effects in primary human skin fibroblasts of Leigh syndrome (LS) patients with isolated CI deficiency. KH-compounds specifically altered the amount/activity of fully assembled CI, as well as cellular ROS level. Cluster analysis highlighted various compound classes with distinct physicochemical properties and cellular effects. The best performing KH compounds were analyzed at the single cell level using high-content microscopy, mitochondrial morpho-functional fingerprinting and machine learning techniques. Compound KH003 which best counterbalanced mitochondrial morpho-functional aberrations in LS cells, was not cytotoxic and displayed ROS scavenging properties. KH003 is currently tested in animal models of mitochondrial disease to determine its therapeutic potential.

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S2.P12

Reduction of the mitochondrial content in cardiolipins decreases O2 consumption and increases ATP synthesis efficiency in human hepatocyte-like HepaRG cells

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Cardiolipins (CL) are major and specific phospholipids of the mitochondrial inner membrane known to be essential for mitochondrial functions such as the oxidative phosphorylation. However the implication of CL in mitochondrial bioenergetics is not fully understood, particularly in mammalians. In fact most of the data previously published have used models of defective yeast mutants lacking CL or cells derived from patients affected by the Barth syndrome (tafazzin mutation). We have previously demonstrated an implication of cardiolipins in cancer cachexia, a paraneoplasic syndrome, characterized by a negative energy balance resulting from variable combination of reduced food intake and hypermetabolism with a systemic inflammation. We have demonstrated CL involvement in the alteration of rat liver mitochondria bioenergetics in cancer-induced cachexia. The increase in CL content ($+55\%$ $p < 0.05$) was positively correlated to the increase in energy wasting observed in liver mitochondria of cancer cachectic rats ($R^2 = 0.64$, $p < 0.05$). Energy wasting was associated to a significant decreased in ATP synthesis efficiency. We confirmed in vitro that liver mitochondria enrichment with CL induced the increase in energy wasting and the reduction in ATP synthesis efficiency. The aim of the present study was to investigate the impact of the reduction in CL content on hepatic cell bioenergetics, with a focus on efficiency of energy production. For this purpose, we established recombinant HepaRG cells stably expressing shRNA triggering the cardiolipin synthase (shCLS) that efficiently reduced CL content (-55% $p < 0.05$). A significant decrease in O_2 consumption (-30%, $p < 0.05$) was observed at phosphorylating, non-phosphorylating and uncoupling states using different substrates such as succinate, glutamate + malate, palmitoyl-l-carnitine, palmitoyl CoA and ascorbate + TMPD. These data demonstrated the impact of CL reduction on the overall OXPHOS system, independently of nature of the substrate and respiratory complexes involved. Interestingly, the

ATP/O ratio, reflecting efficiency of ATP synthesis, was higher in shCLS HepaRG cells (+30% $p < 0.05$) without modification of ATP synthesis rate (using succinate as substrate). Mechanisms involved in these modifications are under investigation. In conclusion, our work clearly demonstrated, for the first time, that the reduction of cardiolipin content regulates directly ATP synthesis in human hepatic cells.

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S2.P13

Effects of reaction probability and occupation area in the electron transfer kinetics of the mitochondrial chain segment involving complexes I, II and ubiquinone: A Monte-Carlo simulation study

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The components of mitochondrial chain were conceived to move freely and interact by random collisions, e.g. ubiquinone moving more rapidly and shuttling electrons from complexes I and II to complex III [1]. The behavior of ubiquinone and cytochrome c pools failed to support the diffusion model of independent complexes randomly distributed [2]. Given the molecular crowding (and likely heterogeneous distribution of reactants), lateral diffusion is expected to be restricted in this membrane. So, the kinetic analysis should take on theories considering irregular lateral diffusion, reaction-controlled and domain-limited reactivity [1]. In this work, the effects of reaction probability varying upon collision and two-dimensional concentration, involving complexes I, II and ubiquinone, are studied using Monte-Carlo techniques. The simulations are based on continuous Pearson-type random walk [3], considering the reactants as non-overlapping disks, whose diameters mimic the molecular areas of the lipid and proteins. The probability varied between 1 (the diffusion-control limit considers the entire perimeter as active), 0.5 (half the perimeter is inactive), 0.25, 0.125 and 0.0625. The regular lateral diffusion of ubiquinone is equivalent to phospholipids and proteins are much less mobile (10^{-10} cm^2/s). The stoichiometry is taken as 1:2:30, for complex I, complex II and ubiquinone, respectively. The analysis is based on the half-time for the decay of initial concentration of reactants (within μs time range). For a disk occupation area of 17.28%, bellow what is expected in vivo, the outcomes indicate obstructed diffusion effects, comparing with simulations with overlapping particles. The results emphasize the importance of reaction kinetics and molecular crowding in models of mitochondrial electron transport. This work was supported by national Portuguese funding through FCT — Fundação para a Ciência e a Tecnologia, project ref. PEst-OE/EQB/LA0023/2013.

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S2.P14

Targeting mitochondria through modulation of eIF5A hypusination protects from anoxia-induced cell death

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eIF5A, for eukaryotic initiation factor 5 A, is a highly conserved protein throughout evolution. Firstly described as a translation initiation factor, it became more noteworthy for its unique post-translational activation through hypusination. This modification, a 4-aminobutyl moiety from spermidine transferred onto a lysyl residue, is sequentially catalyzed by two enzymatic steps involving respectively the deoxyhypusine synthase (DHPS) and the deoxyhypusine hydroxylase (DOHH). We took advantage of this unique characteristic and use the highly specific DHPS competitive inhibitor GC7 (N-guanyl-1,7-diaminoheptane) to demonstrate an unsuspected link between eIF5A hypusination, mitochondria activity and the cellular resistance to anoxia. To investigate the involvement of this pathway in the resistance to low oxygen level, renal proximal tubular cells (PCT) were exposed to anoxia (<0.1% O_2, 24 h). GC7 pre-treatment (30 μM) or RNA silencing-mediated inhibition of DHPS or DOHH was largely protected from the anoxia-induced cell death. This tolerance to anoxia is paralleled by a marked increase in glucose consumption and lactate production reflecting a reversible metabolic shift from aerobic OXPHOS to anaerobic glycolysis, preserving the cellular energetic status. We also studied the effect of GC7 on mitochondrial status and showed that GC7 induced a reversible "mitochondrial silencing" characterized by a decrease in mitochondrial potential ($\Delta\Psi m$) and a drastic mitochondrial structure remodeling associated to a down-regulation of respiratory chain complex expression. The resulting effect is a decrease in O_2 requirements associated with a reduction of the deleterious reactive oxygen species produced during anoxia. Thus, targeting mitochondria through modulation of eIF5A hypusination pathway may offer an innovative therapeutic strategy for ischaemic human diseases—e.g., stroke or myocardial infarction—or organ transplantation.

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S2.P15

Mitochondrial nucleoid visualization in isolated pancreatic β-cells

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Quality of mitochondrial (mt) network and mtDNA is widely discussed in relationship to reactive oxygen species (ROS) production and development of the type 2 diabetes mellitus. Photoactivatable fluorescence proteins like Green fluorescence protein (GFP) and Eos tagged to different lentiviral protein vectors are utilized to study respective targets in vivo. As an approach to study mt morphology and mt nucleoid distribution in isolated pancreatic β-cells, we implemented lentiviral transfection of matrix-addressed GFP (Mito-GFP) to visualize mt network and Eos-tagged Single-strand DNA binding protein-1 (SSBP1-Eos) to image nucleoids. Diabetic Goto-Kakizaki (GK) rats,