elevations but approaching 0 as the amplitudes decrease. We therefore propose a single-cell bi-parametric analysis that correlates the rate of matrix Ca\(^{2+}\) extrusion with the amount of Ca\(^{2+}\) uptake, to determine the impact of NCLX and Letm1. Over-expression of NCLX revealed enhanced mitochondrial Ca\(^{2+}\) extrusion in cells with initial high accumulation of Ca\(^{2+}\) into the matrix. On the other hand, expression (or depletion) of Letm1 had no impact on Ca\(^{2+}\) extrusion, at any level of matrix ion. Ca\(^{2+}\) has a dual effect on mitochondrial redox status, stimulating Ca\(^{2+}\)-activated dehydrogenases (reducing) and accelerating respiration with associated enhanced formation of reactive oxygen (oxidizing). The histamine-induced Ca\(^{2+}\) rise increased the signal of the redox sensitive probe roGFP showing that this physiological Ca\(^{2+}\) rise causes a net reduction of the matrix. Enhancing Ca\(^{2+}\) extrusion following NCLX over-expression abolished the Ca\(^{2+}\) effect. Consistent with these findings, the matrix Ca\(^{2+}\) rise increased cellular NAD(P)H; an effect strongly reduced following NCLX over-expression. Pharmacological inhibition of mitochondrial Ca\(^{2+}\) extrusion, completely rescued both the reduced matrix redox status and NAD(P)H production in NCLX-expressing cells.

We conclude that the extrusion of mitochondrial matrix Ca\(^{2+}\) is mediated by NCLX but not by Letm1. By controlling the duration of matrix Ca\(^{2+}\) rises, NCLX contributes to the regulation of NAD(P)H production and modulates the mitochondrial redox state.

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11P11

Bioenergetic characterization of a neurofibromatosis type-1 cell model

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Neurofibromatosis type-1 is an autosomal dominant genetic disorder affecting one in 2500/3500 persons worldwide. It is caused by loss-of-function type mutations in the NF1 gene which encodes for the protein neurofibromin (NF1) and predisposes patients to tumor development following additional mutations on the remaining normal allele.

Neurofibromin is a large polypeptide, ubiquitously expressed, with a functional GTPase-activating protein (GAP) domain by which it negatively regulates the activity of the proto-oncoprotein p21-Ras. Therefore, NF1 acts as a typical tumor suppressor gene. However, the phenotype of NF1 patients is complex and variable, and efforts to clearly define the molecular pathology of the disease need a complete characterization of the biochemical functions of the NF1 protein. Indeed, apart from the GAP domain, the remaining 90% of neurofibromin is poorly characterized, and it is likely to harbour additional domains and to be involved in other signalling pathways with an unclear relevance for the onset and development of the NF1-associated phenotype. Our group has previously shown that the Ras/ERK signalling axis has a mitochondrial branch (Rasola A. et al., PNAS 2010), whose biological function is only partially understood.

Here we have investigated whether the hyperactivation of the Ras signalling pathway induced by neurofibromin inactivation can affect mitochondria bioenergetics. We report that NF1/−/− mouse embryonic fibroblasts (MEFs) have a decreased Oxygen Consumption Rate (OCR) and a lower Complex I (NADH dehydrogenase) activity compared to wild type MEFs, suggesting that KO cells have a more glycolytic metabolism. This metabolic switch (Warburg effect) is a typical marker of cancer cells that favours tumor progression. Accordingly, we observe that the absence of neurofibromin confers to MEFs the capability to form colonies in an in vitro tumorigenesis assay, and that the use of an ERK inhibitor completely abrogates colony formation. We hypothesize that Ras/ERK signalling is upstream to the regulation of mitochondrial bioenergetics, and that the metabolic rewiring prompted by Ras/ERK activation can contribute to the transformed phenotype that we observe in NF1/−/− MEFs.

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11P12

Abnormal HIF1 regulation of mitochondrial metabolism upon hypoxic adaptation

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Cell adaptation to hypoxic conditions is initiated by stabilization of HIF1α, in coordination with reactive oxygen species (ROS) burst originating in Complex III of respiratory chain. In principle, ROS oxidize Fe\(^{2+}\) of proline hydroxylase domain enzymes and/or of asparaginase hydroxylase FIH [1], which leads to HIF1α stabilization, its further dimerization with HIF1β in nucleus and subsequent regulation of >100 proper gene expression. Canonical response leads to suppression of mitochondrial ATP production/ oxidative phosphorylation in favor to glycolytic metabolism [2,3]. Such response involves elevation of glycolytic enzymes, lactate dehydrogenases included; and drop of mitochondrial oxygen consumption caused by inactivation of pyruvate dehydrogenase by its kinase. In contrast, we have found that hepatocellular carcinoma HepG2 cells metabolizing glutamine and galactose at aglycemia, thus preferentially providing ATP by oxidative phosphorylation, do maintain respiration in hypoxia (5% O\(_2\)). Moreover, their respiratory chain performance runs in optimal way, thus superoxide production extensively falls down to ~10%. This revealed phenotype is regulated in HIF-dependent manner as HIF1α stabilization at 5 h in 5% O\(_2\) was observed with parallel ROS burst initiating HIF pathways. Interestingly, observed morphology changes of mitochondrial network after hypoxic adaptation were not dependent on energy metabolism of the cell. They were pronounced as a thinner, cobweb-like mitochondrial network, compared to atmospheric conditions.

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References

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11P13

Mitochondrial Nm23-H4 can switch between phosphotransfer and lipid transfer activities

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[1] Bioenergetic characterization of a neurofibromatosis type-1 cell model

[2] Abnormal HIF1 regulation of mitochondrial metabolism upon hypoxic adaptation

[3] Mitochondrial Nm23-H4 can switch between phosphotransfer and lipid transfer activities
Nm23-H4 forms symmetrical hexameric complexes in the mitochondrial intermembrane space and is proposed here to have two different functions in mitochondrial metabolism. Nm23-H4 is known for its phosphotransfer activity as an isoform of nucleoside diphosphate kinase [1]. By using mitochondrial ATP, it regenerates GTP and other nucleotide triphosphates e.g. for mitochondrial GTases like OPA1, with which Nm23-H4 also interacts as shown here by immunoprecipitation. This enzymatic reaction also regenerates ADP, which stimulates respiration as we have shown earlier [2]. However, Nm23-H4 also interacts with anionic phospholipids, mainly cardiolipin in the inner mitochondrial membrane [2,3] and can induce membrane domains enriched in cardiolipin [4]. Such binding to anionic lipids is essential for a putative second function of Nm23-H4, which stimulates respiration as we have shown earlier [2]. However, these changes remained unchanged in both HeLa cell lines. We propose that Nm23-H4 is a part of a machinery that is involved in cardiolipin trafficking and thus has a dual function within mitochondria.

References

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11P15

CGS7184 a potassium channel opener modulates activity of mitochondria and Ca^{2+} homeostasis
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CGS7184 is a synthetic large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel opener. The existing literature suggests that potassium channels are involved in cardioprotection, particularly during ischemia–reperfusion events. CGS7184 acts on endothelium in the aorta and coronary circulation, NO production, calcium homeostasis, and mitochondrial function, especially on mitochondrial membrane potential, and mitochondrial respiration in cultured endothelial cells.

11P14

Cisd2 deficiency leads to impaired white adipogenesis and insulin insensitivity in mice
Chih-Hao Wang, Yi-Fan Chen, Chia-Yu Wu, Ting-Fen Tsai, Yau-Huei Wei

In a previous study, we demonstrated that Cisd2 deficiency leads to abnormalities of the structural integrity and function of mitochondria. However, the physiological functions of Cisd2 in a specific cell type remain unclear. It is unknown as to how the loss of Cisd2 causes metabolic defects in patients with the Wolfram syndrome type 2 (WFS2). White adipose tissue (WAT) is an integrator in the maintenance of energy metabolism and glucose homeostasis in the human. In this study we showed significant reduction of adiposity, decrease in the size and weight of epidymidal WAT and expression of adipogenic genes in conventional Cisd2−/− mice compared with the age-matched wild-type mice. Based on these results, we hypothesize that Cisd2 is essential for the development and function of the white adipocytes in mammals. In order to test this hypothesis, we established Cisd2-deficient cells, including Cisd2−/− MEFs and Cisd2-knockdown 3T3-L1 preadipocytes and investigated the essential role and cell autonomous effect of Cisd2 in the differentiation of white adipocytes in vitro. The results revealed decreased intracellular lipids, down-regulation of the expression of adipogenic genes, declined mitochondrial biogenesis and respiratory function in adipocytes differentiated from Cisd2-deficient precursor cells. In addition, we found impaired physiological function of these adipocytes indicated by insulin insensitivity, defective lipolysis, and decreased secretion of adiponectin. Most importantly, we demonstrated that the cytosolic Ca^{2+} level was increased in Cisd2−/− MEFs. Besides, the activity of calcineurin, a calcium/calmodulin-dependent phosphatase, was elevated significantly in Cisd2−/− MEFs. By using cyclosporine A and FK-506 to inhibit the activity of calcineurin, the data revealed a recovery in impaired ability of adipogenic differentiation in Cisd2−/− MEFs. The above findings suggest that Cisd2 deficiency may induce Ca^{2+}+-calcineurin-dependent retrograde signaling to interrupt the process of adipogenesis. In addition, we clarified the novel importance of Cisd2 in differentiation and function of adipocytes and provide a possible role of Cisd2 in the pathogenesis of systemic dysregulation of energy metabolism in WFS2.

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11P14

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