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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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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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 asparagine 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

[1] E.L. Bell, T.A. Klimova, J. Eisenbart, C.T. Moraes, M.P. Murphy, G.R. Budinger, N.S. Chandel, J. Cell Biol. 177 (2007) 1029–1036.
[2] G.L. Semenza, Cell 148 (2012) 399–408.
[3] P. Ježek, L. Plecitá-Hlavatá, K. Smolková, R. Rossignol, Int. J. Biochem. Cell Biol. 42 (2010) 604–622.

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11P13

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

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