Microscopy, showed two populations of mutant mitochondria: high frequency of small round-shaped organelles, consistent with inhibition of mitochondrial fusion, and a group of enlarged mitochondria displaying partially empty cristae. Finally, mitochondrial motility was significantly elevated in Mfn2-M378V fibroblasts, suggesting that Mfn2 GTPase to HR1 connector may have a role in the interaction with the mitochondrial motility machinery. Thus, CMT2A-causing Mfn2 specific-domain mutations display differential defects in human mitochondrial ultrastructure and fusion dynamics. Funding FONDECYT 1150677 to VE.

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Yeast VDAC2 has Pore Forming Activity: Functional Consequences

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The pore-forming proteins Voltage Dependent Anion Channel (VDAC) on the mitochondrial outer membrane (MOM) mediate the metabolic exchanges between cytosol and mitochondria. The pore has been characterized in eukaryotes spanning from insects [1,2] to mammals [3]. In *S. cerevisiae*, the *por1* gene encodes for the main porin yVDAC1, with electrophysiological features similar to the human homologous [4]. yVDAC1 regulates yeast viability in non-fermentable conditions since the genetic inactivation of *por1* (Δ*por1* strain) reduces dramatically the yeast growth on glycerol [4]. In yeast genome a *por1* paralog, *por2*, was found, but the putative porin yVDAC2 was definitely less characterized than VDAC1, despite 50% of sequence identity. Many evidences have questioned the ability of yVDAC2 to act as a pore on the MOM. E.g., the *por2* inactivation does not affect yeast growth on glycerol; furthermore, no pore-forming activity of yVDAC2 was noticed so far in electrophysiological systems. Nevertheless, if overexpressed, yVDAC2 can functionally complement the absence of yVDAC1 in Δ*por1* [4]. This was recently confirmed by our work, showing that *por2* overexpression, a consequence of human SOD1 expression, completely restores Δ*por1* yeast growth on glycerol, as well as the mitochondrial functionality [5]. In this work, we have characterized the electrophysiology of recombinant yVDAC2 and its features in model cell. The influence of the cysteine oxidation was investigated following recent achievements in other eukaryotic VDAC isoforms [6-7].

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Oxidative Stress Induced by Vdac Opening in Cancer Cells Depends on Cytosolic Free Tubulin and is Blocked by ROS Scavenging and Suppression of Superoxide Formation by Complex III

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Background: Voltage dependent anion channels (VDAC) control flux of metabolites into mitochondria. Free α,β-tubulin decreases VDAC conductance and high free tubulin in cancer cells decreases mitochondrial membrane potential (ΔΨ). Erastin antagonizes the inhibitory effect of tubulin on VDAC. Here, we hypothesized that erastin and erastin-like compounds (X1 and X2) discovered during high-throughput screening of a small molecule library, increase mitochondrial formation of reactive oxygen species (ROS) and activate c-jun N-terminal kinase (JNK), culminating in mitochondrial dysfunction and death of cancer cells. Our **AIM** was to evaluate the effects of N-acetyl-L-cysteine (NAC), S3QEL-3 and paclitaxel (PTX) on mitochondrial dysfunction induced by erastin/erastin-like compounds.
Methods: Confocal fluorescence microscopy assessed ΔΨ (TMRM) and ROS (cmDCF; MitoSOX Red). JNK was assessed by Western blotting and cell killing by propidium iodide fluorometry.
Results: In HepG2 and Huh 7 hepatocellular carcinoma cells, erastin and X1 increased ΔΨ and NAD(P)H and activated JNK with maximal phosphorylation within 1-2 h. Initial increases of ΔΨ were followed by mitochondrial depolarization occurring 1-2 h after X1 and X2 and 3-4 h after erastin. X1 did not alter free/polymerized tubulin. The microtubule stabilizer PTX (10 μM) depleted cytosolic free tubulin and prevented depolarization induced by X1. The antioxidant NAC (100 μM) blocked mitochondrial dysfunction and cell death induced by X1. S3QEL-3 (100 μM), a suppressor of superoxide production at site III_{Qo} and JNK inhibitor SP600125 prevented mitochondrial superoxide formation and mitochondrial dysfunction.
Conclusion: Antagonists of VDAC-tubulin interaction promote mitochondrial metabolism and increase mitochondrial ROS formation, which in turn leads to JNK activation, mitochondrial dysfunction and selective death of cancer cells that is prevented by antioxidants and JNK inhibition.

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Manipulation of Amyloid Precursor Protein Processing Impacts Brain Bioenergetics and Glucose Metabolism

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The amyloid precursor protein (APP) and one of its terminal cleavage products, amyloid beta (Aβ) are proposed to play a central role in the pathogenesis of Alzheimer's disease (AD). These proteins have also been shown to co-localise within mitochondria with Aβ directly inhibiting the electron transport chain. Cleavage of APP occurs down distinct pathways. Initial cleavage of the full-length protein is mediated by either α- or β-secretase (BACE1). Subsequent cleavage by γ-secretase releases p3 or Aβ protein fragments into the extracellular milieu. Whilst these interactions with mitochondria have been noted, the impact of manipulating APP processing by these distinct pathways on cellular metabolism and bioenergetics has yet to be elucidated. We utilised the human SH-SY5Y neuronal cell line stably overexpressing BACE1 and cortical slices from mice bearing inducible overexpression of APP harbouring the Swedish and Indiana mutations, favouring Aβ production. Protein expression and enzyme activity were determined via western blotting and enzyme-linked immunosorbance assays (ELISA). A Seahorse extracellular flux analyser and radio-labelled substrate assays were used to determine cellular bioenergetics in real-time. All data are expressed as mean ± standard error of the mean and statistical significance determined by Student's t-test. Manipulation of APP cleavage resulted in alterations of 2-deoxyglucose uptake into cells. Chronic elevation in BACE1 resulted in impaired functioning of a key fuel-partitioning enzyme, pyruvate dehydrogenase (PDH, activity reduced to 69 ± 8%, $p < 0.05$, n=6). This reduced substrate delivery to the mitochondria and increased reliance upon aerobic glycolysis for ATP generation (oxygen consumption rate (OCR) reduced to 65 ± 9%, $p < 0.01$, n=7 and extracellular acidification rate (ECAR) increased to 165 ± 16%, $p < 0.01$, n=7). Thus BACE1 overexpression impairs neuronal glucose oxidation resulting in an increased reliance upon aerobic glycolysis for ATP generation. Acute cortical overexpression of APP favouring amyloidogenic processing resulted in a significant reduction in PDH protein expression (reduced to 62 ± 6%, $p < 0.01$, n = 9-10) and activity of key tricarboxylic acid cycle enzymes, isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase (reduced to 62 ± 7%, $p < 0.01$, n=6 and 88 ± 2%, $p < 0.001$, n = 6). Therefore, manipulation of APP processing towards Aβ production in cells and tissue significantly impacts cellular metabolic pathways at the level of glucose uptake, substrate entry to the mitochondria and the TCA cycle.

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Mitochondrial NM23-H4/NDPK-D and OPA1: Partners in Shaping Mitochondria and Initiating Mitophagy?

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The well-established function of the mitochondrial intermembrane space protein NM23-H4/NDPK-D is phosphotransfer activity as a nucleoside diphosphate kinase (NDPK). However, recent data have revealed a second function in lipid signaling that triggers mitophagy, a critical process for cell homeostasis (1,2). This latter function involves NM23-H4-mediated intermembrane transfer of cardiolipin (CL) from the mitochondrial inner membrane to the mitochondrial surface. Interestingly, both functions seem to involve an interaction of NM23-H4 with OPA1, a dynamin-like GTPase of the mitochondrial inner membrane. First, NM23-H4 directly fuels OPA1 with GTP via its NDPK bioenergetic function (3). In addition, also the CL transfer activity of NM23-H4 seems to depend on OPA1, since knock-down of OPA1 in HeLa cells reduces CL transfer in NM23-H4 WT expressing cells as compared to those expressing CL-transfer incompetent NM23-H4 mutants. Thus, OPA1 seems to be a negative regulator of the