1849-Plat
Mitochondrial NM23-H4/NDPk-D is Multifunctional: Fueling Mitochondrial GTPase OPA1 and Triggering Mitophagy
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NM23-H4/NDPK-D forms symmetrical homohexameric complexes in the mitochondrial inter-membrane space. The well-established function of NM23-H4 is phosphotransfer activity as a nucleoside diphosphate kinase, using mitochondrial ATP to regenerate NTPs, especially GTP. NM23-H4 also strongly binds in vitro to anionic phospholipids, mainly cardiolipin (CL), and in vivo to the mitochondrial inner membrane (MIM). Membrane-binding seems to be important for close co-localization of NM23-H4 with mitochondrial OPA1, a dynamin-like GTPase, involved in fusion of MIM. NM23-H4/OPA1 association increases GTP-loading on OPA1. Like OPA1 loss-of-function, silencing of NM23-H4, but not cytosolic NM23-H1/H2, results in mitochondrial fragmentation, reflecting fusion defects. Thus, NM23-H4 interacts with and provides GTP to OPA1, similar to what is observed for cytosolic NM23 isoforms which interact with endocytic dynamin-1 and −2 and provide GTP for efficient dynamin-mediated endocytosis (Boissan et al.2014, Science 344:1510). Such close association allows these motor proteins to work with high thermodynamic efficiency. Earlier, we have shown that NM23-H4, when fully bound simultaneously to MIM and outer membrane (MOM), loses its kinase activity, but becomes competent to support intermembrane lipid transfer. This depends on the presence of the mitochondria-specific CL, and allows CL to move from its site of synthesis, MIM, to the opposed MOM (Schlattner et al.2013, JBC 288:111). Once CL is externalized at the mitochondrial surface, it can serve as a recognition signal for the autophageal machinery, leading to the elimination of damaged mitochondria. In cells treated with a protonophoric uncoupler, CCCP, CL externalization and mitophagy are stimulated only by transfection with NM23-H4 wild-type, but not R90D-mutant, incapable of CL binding. Similarly, in mouse lung epithelial cells, knocking-down NM23-H4 suppresses CL externalization and mitophagy. These findings suggest that NM23-H4 has dual functions in bioenergetics and lipid signaling leading to autophagy. Support: FRM,ARC,GEFLUC, NIH(U19AIO6802/HL114453), HFSP(RGP0013/2014).

1850-Plat
VDAC Opening Drugs to Induce Mitochondrial Dysfunction and Cell Death
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Background: Mitochondrial membrane potential ($\Delta\Psi$) and reactive oxygen species (ROS) formation depend on metabolite flux into mitochondria through voltage dependent anion channels (VDAC). Free tubulin closes VDAC, and high free tubulin levels decrease $\Delta\Psi$ in cancer cells. Erastin opens VDAC by antagonizing the inhibitory effect of tubulin. Here, we hypothesized that erastin and erastin-like compounds open VDAC, increase mitochondrial metabolism and ROS formation, and activate c-jun N-terminal kinase (JNK), leading to mitochondrial dysfunction and cell killing. Our AIM was to evaluate the effects of erastin/erastin-like compounds on $\Delta\Psi$, ROS, NADH, JNK activation, cell killing and protection by antioxidants in HepG2 cells.
Methods: Confocal/multiphoton fluorescence microscopy assessed $\Delta\Psi$ (tetramethylrhodamine methylester), ROS (chloromethyldichlorofluorescein [cmDCF], MitoSOX Red) and NADH (autofluorescence). JNK was assessed by Western blotting and cell killing by propidium iodide assay.
Results: Erastin increased $\Delta\Psi$ by 46% and NADH by 30% and blocked the depolarizing effect of microtubule destabilizers in HepG2 human hepatoma cells. Increased $\Delta\Psi$ after erastin/erastin-like compounds induced mitochondrial hyperpolarization that was followed by depolarization. Small molecules X1 and X2, identified in a high-throughput screening, caused a more rapid drop of $\Delta\Psi$ (<1 h) compared to erastin (3-4 h). Erastin, X1 and X2 also maximally increased cmDCF and MitoSOX Red fluorescence after 1-2 h. Additionally, JNK activation peaked at 60 min. JNK activation and ROS formation preceded mitochondrial depolarization. Cell killing promoted by X1 (93%) and X2 (76%) after 12 h was blocked by the antioxidant N-acetyl cysteine (100 μM).
Conclusion: Mitochondrial hyperpolarization caused by VDAC opening drugs causes oxidative stress, which in turn leads to JNK activation, mitochondrial dysfunction and cell death that is prevented by antioxidants. Grants DK073336, DK037034 and 14.Z50.31.0028 to JJL and ACS 13-043-01 to ENM.

1851-Plat
The 18kDa Translocator Protein Interacts with VDAC1 and Triggers a Ros-Mediated Inhibition of Mitochondrial Autophagy
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The 18kDa Translocator Protein (herein TSPO) co-localises on the Outer Mitochondrial Membrane (OMM) with the Voltage Dependent Anion Channel 1 (VDAC1) and partakes in the transport of cholesterol. Overexpressed in several types of mammalian cancers it positively correlates with the aggressiveness of lesions as well as inflammation of the brain therefore exploited as biomarker and target for suitable therapies.
In Mouse Embryonic Fibroblasts (MEFs), the recombinant TSPO overexpression prevents Parkin mediated ubiquitination of mitochondrial proteins and recruitment of p62/SQTSM1 and LC3, thus leading to accumulation of dysfunctional mitochondria. Live cell imaging approaches demonstrate that TSPO enriched, mitophagy-evading, mitochondria present: i) defective Ca^{2+} signalling, ii) reduced coupling, iii) low ATP synthesis and iv) aberrant network morphology.
The inhibition of mitochondrial ubiquitination by TSPO is independent from cholesterol trafficking and consequent of the overproduction of Reactive Oxygen Species (ROS) caused by defective mitochondrial metabolism. The prevention of ubiquitination likewise the suppression of cellular mitophagy are lost in MEFs knocked out for VDAC1 ($VDAC1^{-/-}$) with which TSPO interacts to impair mitochondrial signalling and trigger oxidative stress.
These data proposes TSPO as a novel regulatory element of the mitochondrial quality control and a further molecular determinant for the Parkin-mediated ubiquitination and removal of disposable mitochondria.

Workshop: Managing Data and Statistics in the Informatics Era

1852-Wkshp
Informatics Approaches to Data Preservation and Analysis in Protein Electrostatics
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Scientific data curation has presented a challenge to multiple disciplines in terms of preserving data, supporting its reproducibility, and enabling ready access of the data for future statistical analysis. We have addressed this challenge in the context of experimental and computational protein titration data. In particular, we have leveraged the ISA-TAB community-supported data-sharing standard (http://www.isa-tools.org/) to collect and preserve protein pKa data. This data was collected from a variety of published and unpublished sources associated with pKa Cooperative (http://pkacoop.org), a group of researchers dedicated to advancing the understanding of protein electrostatics. Additionally, we have demonstrated the utility of collecting data in a standard format such as ISA-TAB by developing a new statistical pKa prediction approach which combines computational results from the pKa Cooperative effort into an aggregate classifier with significantly improved predictive power.