Molecular bas
is of LONP1 binding to DNA

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Abstract

LonP1, the Human Homolog of Lon Protease Isoform 1, is a universally conserved AAA proteinessential for life and mitochondrial homeostasis. The mitochondria is the center of oxidative phosphorylation, and Lon plays a crucial role in regulating the replication of mtDNA by degrading the replication initiation transcription factor TFAM in the mitochondrial matrix. Additionally, HsLon has retained the ability to bind to DNA inherited from ancestral Lon but lacks the ability to bind dsDNA as bacterial Lon does, instead binding ssDNA which has been shown that HsLon's proteolytic activity can be regulated by binding to DNA. HsLon was found to preferentially bind G-rich sequences that have a tendency to form DNA secondary structures known as G-Quadruplexes. The abundance of these structures in the mtDNA of different types, and particularly directly in the promoter regions of the mitochondria and other parts of the control regions, has raised questions about Lon's DNA binding ability in its homeostatic role. Here, we show that G-quadruplex secondary structures interact with HsLon and modulate its ability to proteolyze substrate in a dose-dependent manner, while simultaneously stimulating it's ATPase domain turnover greater than the increase in ATPase activity associated with ATP-dependent proteolysis, suggesting a heretofore unknown mechanism of DNA interaction in HsLon and thus, mtDNA maintenance.

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LPCAT3 supports endothelial cell homeostasis

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Abstract

Introduction: LPCAT3 modulates cellular membrane lipid composition by catalyzing the incorporation of polyunsaturated fatty acids. Literatures have shown that the absence of LPCAT3 leads to phospholipid remodeling, decreased membrane fluidity, defects in triacylglycerol transport, endoplasmic reticulum stress and inflammation in the liver and intestine. However, the role of LPCAT3 has not studied in the vascular endothelium. The purpose of this study was to investigate the role of LPCAT3 on endothelial cell (EC) homeostasis.

Methods/Results: To investigate the role of LPCAT3 in the vascular endothelium, human aortic endothelial cells (HAECs) were transfected with siRNA targeting LPCAT3 and exposed to laminar flow for 48hrs. LPCAT3 deficient HAECs had reduced eNOS mRNA and protein expression, less nitric oxide (NO) production and increased levels of accessible cholesterol with a negative correlation between eNOS expression and plasma membrane accessible cholesterol level. In addition, RNAseq analysis using EC-enriched RNA from control and endothelial-specific Lpcat3 knockout mice ($Lpcat3^{ECKO}$) revealed that loss of Lpcat3 elevated proinflammatory genes via NF-κB pathway activation with RelA (p65) identified as an upstream regulator. This finding was consistent with an elevation of Nfkbia mRNA expression levels for Lpcat3ECKO mice. Confocal imaging of the descending aorta confirmed that loss of Lpcat3 increased NF-κB activation, as quantified by p65 nuclear localization and VCAM-1 expression. Further analysis of the RNAseq data revealed that loss of Lpcat3 elicited a reduction of PGC-1α mRNA and genes involved in electron transport chain complex activity. *In vitro* studies using MitoTracker, mitoSOX, and Seahorse assays revealed that mitochondrial density and mitochondrial respiration with complex I, II, IV activities were all significantly reduced and associated with an increase in mtROS production for HAECs lacking LPCAT3.

Conclusion: Our data indicates that LPCAT3 is critical for EC homeostasis as its presence and activity support flow-induced NO production, prevents mitochondrial dysfunction, and suppresses pro-inflammatory activation.

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Actin-dependent glycolytic activation downstream of mitochondrial damage: identification of the actin-activated glycolytic step

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Abstract

Mitochondrial damage represents a dramatic change in cellular homeostasis. One rapid response is peri-mitochondrial actin polymerization, termed ADA (acute damage-induced actin). We have shown that ADA is linked to rapid glycolytic activation upon mitochondrial damage in mouse embryonic fibroblasts and effector $CD8^+$ T lymphocytes (PMID: 36102863). ADA-inducing treatments include mitochondrial depolarization (CCCP), electron transport chain inhibition (antimycin, rotenone, oligomycin), and hypoxia. ADA is activated by two signals: release of mitochondrial calcium and decreased ATP, activating Arp2/3 complex-dependent and FMNL formin-dependent actin polymerization pathways, respectively (PMID: 35290799). The Arp2/3 complex inhibitor CK666 and the mitochondrial sodium- calcium exchange (NCLX) inhibitor CGP37157 inhibit both ADA and the glycolytic increase, supporting ADA's role in glycolytic stimulation. Two situations causing chronic reductions in mitochondrial ATP production, mitochondrial DNA depletion and mutation to the NDUFS4 subunit of complex 1 of the electron transport chain, cause persistent peri-mitochondrial actin filaments similar to ADA. CK666 treatment causes rapid mitochondrial actin loss and a drop in ATP in these chronic conditions.

My focus is on the mechanism by which ADA activates glycolysis. My ^{13}C-glucose fluxomic analysis suggests a surprising glycolytic step that is by ADA. We are currently testing this possibility biochemically and in cells. I also find that the ADA response varies between cancer cell types, with cells falling into three categories: 1) ADA-responsive (increased glycolysis with CCCP), 2) ADA-unresponsive (no CCCP- induced glycolytic stimulation), or constitutive ADA (high basal glycolysis that is not further stimulated by CCCP, but is inhibited by CK666). I propose that ADA is necessary for rapid glycolytic activation upon mitochondrial impairment, to re-establish ATP production. The cell type variability of the ADA response might allow targeted ADA modulation in cancer therapy

Oxygen toxicity causes cyclic damage by destabilizing specific Fe-S cluster-containing protein complexes

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Abstract

Excess oxygen (hyperoxia) is toxic to all cellular life. However, the molecular mechanisms underlying oxygen toxicity are poorly understood. Starting with a genome-wide CRISPR-Cas9 KO screen, we systematically investigate the major cellular pathways perturbed by excess oxygen in human cells. We find that hyperoxia leads to depletion of a specific subset of Fe-S cluster (ISC)-containing proteins. Loss of these proteins results in impaired electron transport chain (ETC) function, diphthamide synthesis, purine synthesis, and nucleotide excision repair. Of these pathways, the ETC is the most sensitive to oxygen toxicity. We translate the findings to human lung cells and a mouse model of lung oxygen toxicity. We show that hyperoxia initially depletes ETC proteins, resulting in decreased mitochondrial oxygen consumption and higher levels of unused oxygen. This results in a cycle of damage, whereby higher oxygen levels result in perturbation of the additional ISC- containing pathways. In support of this model, we show that in a mouse model of primary ETC dysfunction (NDUFS4 KO), the lungs are dramatically sensitive to oxygen toxicity and hyperoxia-mediated ISC damage. In addition to mitochondrial disorders, this work has important implications for various diseases caused by excess oxygen

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A novel protein interaction between INF2 and VAP implicated in ER-mitochondria functions

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Abstract

INF2 belongs to the formin family of actin filament nucleators and exists in two isoforms: non-CAAX being mainly cytosolic and the CAAX isoform being ER-bound. VAP proteins are integral ER membrane proteins, that interact with a myriad of partners mediating contact between the ER and essentially all other cellular membranes. These interactions are mediated through the MSP domain of VAP and a loose consensus sequence called the FFAT motif (2 phenylalanines in acidic tract) in the interacting protein. We discovered a novel protein interaction between both VAPA and VAPB and INF2 through BioID screens, which we further confirmed by immunoprecipitations and direct binding of purified proteins. Interestingly, VAPA/B interact with both ER-resident INF2-CAAX and cytosolic INF2-non-CAAX, albeit to a lesser extent for non-CAAX. We mapped the VAP-interaction site to an FFAT-like sequence within INF2's C- terminus and determined that the binding is independent of INF2's actin polymerization activity. Mutation of the two aromatic residues in this motif reduces INF2 – VAP interaction. Using VAPA/B CRISPR KO cells, we determined that VAP proteins are not essential for INF2-CAAX's recruitment to the ER or for the calcium-induced actin polymerization by INF2. However, depleting INF2 or VAPA/B, either by siRNA or CRISPR-mediated KO, caused a dramatic rearrangement of the ER from a tubular-reticular architecture towards an extended, sheet-like ER structure. Furthermore, genetic removal of VAPA/B caused profound mitochondria morphology changes, with mitochondria being fragmented, spherical, and bulky. Future studies will address the potential roles of this novel protein interaction in shaping ER tubules as well as ER-mitochondria contact formation and calcium signaling.

Delivery of exogenous Coenzyme Q to mitochondria in Saccharomyces cerevisiae relies on the dynamin homolog Vps1 and autophagic machinery

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Abstract

Coenzyme Q (CoQ) is a redox-active lipid that plays important roles in the electron transport chain for cellular respiration, antioxidant protection, redox homeostasis, and ferroptosis suppression. It is synthesized within the mitochondria and trafficked to other cellular membranes. Deficiencies in CoQ can be ameliorated by high dose supplementation. However, mechanisms of uptake and intracellular transport remain poorly understood. Previous work identified six genes in *Saccharomyces cerevisiae* whose deletion in CoQ-less mutants (*ORFΔcoq2Δ* mutants) significantly decreased growth in non-fermentable medium with exogenous CoQ, indicating that these genes are required for the efficient uptake and transport of CoQ to mitochondrial respiratory complexes. Deletion of *VPS1*, a dynamin homolog, had the strongest growth defect. Vps1 facilitates membrane remodeling through fusion and fission events in many cellular processes, but the essential roles of Vps1 for CoQ uptake and transport are unknown. The *vps1Δcoq2Δ* mutant was transformed with a set of plasmids expressing *vps1* point mutants. Transformation with three of the plasmids failed to restore growth in non-fermentable medium with exogenous CoQ, indicating that these *vps1* point mutants are unable to restore CoQ uptake and transport. Other genes involved in membrane trafficking events were also investigated. Several genes required for autophagy were identified as essential for CoQ uptake and transport, including *ATG6*, *ATG8*, *ATG9*, *ATG12*, and *PEP4*. Interestingly, other autophagic genes, *ATG1* and *ATG7,* are not essential, indicating a non-autophagic lipid trafficking role in the identified essential genes. Analysis of whole-cell lipid extractions revealed that *ORFΔcoq2Δ* mutants have similar or higher CoQ content compared to a *coq2Δ* mutant when supplemented with exogenous CoQ, indicating that CoQ accumulates outside respiratory complexes. These results suggest that uptake and trafficking of exogenous CoQ to the mitochondria relies on some autophagic machinery, likely through vesicular transport that is facilitated in part through membrane fusion and fission via Vps1.

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Mitochondrial elongation protects against fibrosis of proximal tubule cells

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Abstract

Mitochondria have evolved to efficiently produce energy from available nutrients, but this proficiency is impeded when substrate availability persistently exceeds energetic demand. In cardiometabolic diseases, such as diabetes, diabetic nephropathy, and NASH, disease progression is coupled to reduced mitochondrial oxidative capacity. Utilizing a diverse range of cellular assays to assess mitochondrial dynamics, bioenergetic capacity, and fuel metabolism, we seek to pursue novel drug targets that aim to restore aberrant mitochondrial function. Previous studies revealed ALCAT1, a lysocardiolipin acyltransferase is upregulated in diet-induced obesity (DIO) models and lead to mitochondrial dysfunction, oxidative stress, and insulin resistance. Deficiency of ALCAT1 restored lipid oxidation, oxidative phosphorylation, mitochondrial architecture, and insulin signaling, and prevented the onset of DIO. Here we investigated the role of ALCAT1 in mitochondrial dysfunction and its potential as a drug target in both hepatic and proximal tubule cell lines. Our results demonstrate that incubation of clonal cells with the saturated fatty acid palmitate yielded upregulation of ALCAT1, disruption of cardiolipin synthesis, and induced mitochondrial fragmentation. Depletion of ALCAT1 under excess nutrient conditions prevented fragmentation events suggesting a role in regulating cardiolipin remodeling and mitochondrial dysfunction. Acute overexpression of ALCAT1 recapitulated fragmentation events but failed to change bioenergetic capacity or fuel metabolism, suggesting an alternative mechanism is involved in the observed pathological remodeling and oxidative stress. Taken together, cardiolipin remodeling without the excess nutrient environment is not sufficient to induce pathological fuel switching. We thereby hypothesize that changes to fuel storage and mitochondrial architecture contribute to pathological fuel metabolism. Further, we are currently investigating the relationship between mitochondrial architecture and fuel metabolism under excess nutrient conditions for potential drug targets. Ultimately, by understanding the impact of our targets on fundamental regulatory processes, we can determine their potential to prevent, intercept, and treat cardiometabolic disease.

Mitochondrial morphology controls fatty acid utilization by changing CPT1 sensitivity to malonyl-CoA

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Abstract

Changes in mitochondrial morphology are associated with nutrient utilization, but the precise causalities and the underlying mechanisms remain unknown. Using cellular models representing a wide spectrum of mitochondrial shapes, we show a strong linear correlation between mitochondrial fragmentation and increased fatty acid oxidation (FAO) rates. Forcedmitochondrial elongation following MFN2 over-expression or DRP1 depletion diminishes FAO, while forced elongation upon knockdown/knockout of MFN2 augments FAO as evident from respirometry and metabolic tracing studies. Importantly, genetic induction of fragmentation phenocopies the distinct cell type-specific biologic functions of FAO. These includes stimulation of gluconeogenesis in hepatocytes, induction of insulin secretion in islet β-cells exposed to fatty acids, and survival of FAO-dependent lymphoma subtypes. We find that fragmentation stimulates long-chain but not short-chain FAO, identifying CPT1 as the downstream effector of mitochondrial morphology in regulation of FAO. Mechanistically, we determined that fragmentation reduces malonyl-CoA inhibition of CPT1, while elongation increases CPT1 sensitivity to malonyl-CoA inhibition. Overall, these findings underscore a physiologic role for fragmentation as a mechanism whereby cellular fuel preference and FAO capacity are determined.

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SEL1L-HRD1 ERAD and autophagy synergistically maintain mitochondrial homeostasis in BAT

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Abstract

ER-associated degradation (ERAD) is a principal protein quality control mechanism which targets misfolded proteins in the ER for cytosolic proteasomal degradation. We previously showed that the SEL1L-HRD1 protein complex, the most conserved ERAD branch, regulates ER-mitochondria contacts and mitochondrial dynamics in brown adipose tissue (BAT) upon cold exposure. However, the underlying mechanism remains largely unclear. Here we show that ERAD and autophagy synergistically regulates mitochondrial dynamics in BAT under room temperature. ERAD deficiency triggers the activation of autophagy and increases the formation of autophagosomes in BAT. Brown adipocytes lacking both SEL1L and ATG7 exhibit megamitochondria and increased mitochondrial fusion even in the absence of cold exposure, unlike those lacking SEL1L alone. ER-mitochondria contacts are elevated in brown adipocytes lacking both SEL1L and ATG7, leading to the formation of intra-mitochondrial ER tubules. Mitochondrial function and thermogenic response in BAT lacking both SEL1L and ATG7 are impaired, as demonstrated by decreased oxygen consumption rate and cold sensitive of the mice. This study provides an integrative view of the crosstalk between ERAD and autophagy in brown adipocytes and shed new important light on the importance of mitochondrial quality control in health and disease.

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Mitochondria isolated from lipid droplets in WAT reveal functional differences based on lipid droplet size

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Abstract

Recent studies in brown adipose tissue (BAT) described a unique subpopulation of mitochondria bound to lipid droplets (LDs), peridroplet mitochondria (PDM). PDMs can be isolated from BAT by differential centrifugation and salt washes (Benador et al, 2018). Contrary to BAT, this approach has so far not led to successful isolation of PDMs from white adipose tissue (WAT). Here, we developed a method to isolate PDM from WAT with high yield and purity by an optimized proteolytic treatment that preserves the respiratory function of mitochondria. Using this approach, we show that, contrary to BAT, WAT PDM have lower respiratory and ATP synthesis capacity compared to WAT CM. Furthermore, by isolating PDM from LDs of different sizes, we find a negative correlation between LD size and the respiratory capacity of their PDM in WAT. Thus, our new isolation method reveals tissue-specific characteristics of PDM and establishes the existence of heterogeneity in PDM function determined by LD size.

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Hepatic Lipid droplet associated mitochondria is bioenergetically active but compromised for fatty acid oxidation in nonalcoholic steatohepatitis

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Abstract

Currently, there are limited evidence on the role of lipid droplet associated mitochondria (LDM) in healthy liver metabolism both during fed and overnight fasted conditions. Nevertheless, the role of LDM function in diseased liver such as during non-alcoholic steatohepatitis (NASH) progression remains unknown. Here we isolated both LDM and cytoplasmic mitochondria (CM) from a mouse model of diet-induced NAFLD/NASH to characterize their relative function during simple steatosis to advanced NASH progression. As a healthy control, we isolated both LDM and CM from chow-fed mice. In all our conditions, we fasted the mice for four hours before euthanasia. Our studies show that while the CM content remains almost the same, the LDM content decreases from simple steatosis to advanced NASH. We next found that, compared to CM, LDM are bioenergetically active with higher pyruvate oxidation capacity in both healthy and diseased liver. Additionally, we found that higher respiration capacity of LDM was associated with higher levels of OXPHOS protein complexes as well as higher TCA cycle flux as measured by citrate synthase activity. On the contrary, LDM had higher fatty acid oxidation capacity in both healthy and early steatotic liver, which declined with NASH progression. Current and future experiments include transmission electron microscopy (TEM) of the liver and proteomics of the two mitochondrial populations isolated from different stages of the disease. Preliminary TEM images revealed enhanced LD-mitochondria contacts during early steatosis, while those contacts were reduced in advanced NASH. Altogether, the high degree of differences between LDM and CM population during NASH progression highlights their distinct role in disease progression towards NASH.

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Reaching beyond the mitochondrial fold: Interactome mining of the MICOS from different tissues

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Abstract

Mitochondria are highly specialised organelles characterised by its double membrane structure. The inner membrane folds into cristae thereby increasing the surface area-to-volume ratio and providing an increased area for oxidative phosphorylation to occur. Strikingly, cristae organisation can vary across tissues suggesting that they are responsive to different metabolic needs of the tissue. The Mitochondrial Contact Site and Cristae Organising System, MICOS, is a multi-subunit heterooligomeric complex of the inner membrane involved in cristae shaping. Disruption of the MICOS leads to aberrant cristae and mitochondrial disease. The MICOS complex also makes contact with components of the mitochondrial outer membrane, pointing to an additional role in signalling events. To study the MICOS interactome across tissues, a transgenic mouse was generated that harbours a C-terminal Flag-epitope tag on the core MICOS subunit, Mic60. Affinity enrichment mass spectrometry from several tissues revealed a suite of interactions including previously undefined protein interactors. Functionally, these novel interactors are not confined to only cristae formation and involved in signalling pathways such as protein transport, metabolism, calcium homeostasis and some with yet undefined roles. The analysis of select components will be presented. These results further support the role of this complex as a communication hub between mitochondrial sub-compartments and other cellular organelles.

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Multifaceted role of LACTB in mitochondria and lipid droplet dynamics

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Abstract

LACTB, a filament-forming protease localized in mitochondria, is a potent tumor suppressor in several cancermodels. Initial studies proposed that LACTB exerts its function by degrading the mitochondrial enzyme PISD. However, recent research has revealed a more intricate tumor suppression mechanism involving direct interactions with cytoplasmic factors, such as p53 and PP1A. The precise subcellular location where LACTB exerts its tumor suppression activity remains elusive. Furthermore, emerging evidence suggests an additional metabolic role for LACTB, with associations identified between LACTB and obesity, as well as type-2 diabetes.

We investigated the effects of LACTB knockdown on the morphology and function of mitochondria and other organelles. Our findings demonstrate that LACTB depletion leads to an increase in mitochondrial length and a decrease in mitochondrial membrane potential. Intriguingly, LACTB suppression also results in an increase in the size and number of lipid droplets. Using thin-layer chromatography, we find that LACTB depletion results in two-fold increase in cholesterol esters and slight decrease in triacylglycerol. Interestingly, we also observed that upon induction of apoptosis, LACTB translocates to the cytosol and directly binds lipid droplets.

Together these results suggest that, under stress conditions LACTB might regulate lipid droplet metabolism, which might contribute to its tumor suppressor activity. Alternately, LACTB might exert its effects from its previously documented location in the mitochondrial inter-membrane space. Supporting this second mechanism, we have discovered that LACTB preferentially binds cardiolipin-containing membrane nanotubes, promoting their fission in a nucleotide-independent manner. Additionally, we identified interactions between LACTB and two key mitochondrial proteins, ATAD3 and ATP synthase. The ATAD3 interaction raises the possibility that LACTB is involved in lipid droplet mitochondria contact sites, thereby impacting lipid droplet metabolism. Our study sheds light on the multifaceted cellular functions of LACTB, which might exert its tumor suppressor through impacting lipid droplet metabolism.

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Mitochondrial uptake by macrophages alters cell function

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Abstract

Mitochondrial quality control (MQC) mechanisms remove dysfunctional mitochondria (mito). These include mito-targeted autophagy (mitophagy) and a lysosome-independent process that involves the release of vesicle-encapsulated mito fragments (mitoEVs) to the extracellular space followed by their clearance by tissue macrophages (Mac). Studies discovered that mito migrate between cells within an organ or through the circulation, under both homeostasis and stress conditions such as in heart. Failure to effectively clear MitoEVs leads to their accumulation in the extracellular space, eliciting cardiac inflammation. However, intracellular fate of engulfed mito and how it affects Mac function which in turn alters the tissue environment have not been studied. Using an in-vitro model, we isolated mitochondria and large extra-cellular vesicles (LEV) from mouse hearts expressing mito-targeted Keima (mtKeima) and presented them to bone marrow-derived macrophages (BMDM). The BMDMs were treated with mito/mitoEV for 3 or 24 hours and assessed immediately after the removal of mito/mitoEV and every 24 hours thereafter up to 72 hours. My preliminary confocal studies demonstrated that Mac could ingest isolated mito as well as mitoEVs harvested from cardiac tissue as early as at 3 hours. Flow-cytometry further confirmed the uptake of isolated mito by 98.05±0.1% Macs at 24 hours. Furthermore, Keima signal gradually reclined over time at 72h post-treatment. Inhibition of lysosome by bafilomycin A in BMDM resulted in accumulation of engulfed mito protein, with Keima signal at neutral pH (488 nm, green) being dominant. These results suggest that exogenous mito engulfed by Macs are largely degraded in lysosome. We also found that OCR/ECAR was unaltered at 0 hour after Mac was treated with mito but OCR declined 24 hours later coupled with increased ECAR as compared to control. Moreover, mito uptake stimulated BMDM proliferation. These findings suggested that processing of exogenous mito triggered glycolysis and cell proliferation.

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Cytoprotection by the novel compound SBT-588 across models of Leigh syndrome

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Abstract

Leigh syndrome (LS) is a fatal neurometabolic disease characterized by progressive loss of central nervous system function. There are over 100 genes that can have mutations that cause LS, most of which involve proteins associated with the mitochondrial electron transport chain (ETC). There are currently no approved therapies for LS. As LS involves dysfunctional ETC activity and potentially iron-mediated cell death (ferroptosis), we sought to determine the effect of the novel compound SBT-588 in cell models of LS. We have previously shown that SBT-588 restored bioenergetics by bypassing Complex I (CI) of the ETC and ameliorated iron-mediated cell death. We have confirmed that SBT-588 crosses the blood-brain-barrier and has homogenous biodistribution throughout regions of the rodent brain. In this study, we examined the efficacy of SBT-588 in two LS patient-derived fibroblast lines. When ferroptosis was induced by RAS-selective lethal 3 (RSL3) stress, SBT-588 dose-dependently attenuated cell death and preserved intracellular ATP levels (two-way ANOVA, $p<0.001$). Fibroblasts stressed with the glutathione synthesis inhibitor erastin were also protected with SBT-588 treatment. Interestingly, SBT-588 showed higher potency on cell viability than vatiquinone, which has been used in clinical trials for LS. To assess the impact of SBT-588 on 15-lipoxygenase (15-LO) inhibition, the metabolic by-product 15-HETE was measured in the supernatant of cultured LS fibroblasts undergoing ferroptosis. Co-treatment of erastin or RSL3-injured fibroblasts with 1 μM SBT-588 led to a significant reduction in 15-HETE compared to untreated controls (unpaired t-test, $p=0.0053$ and $p=0.0014$, respectively). The impact of SBT-588 on bioenergetics in the presence of a CI inhibitor was evaluated using high-resolution respirometry. After chemical inhibition of CI, treatment with SBT-588 restored oxygen consumption rates to near basal levels ($EC50 =3.4$ μM). These data highlight the therapeutic potential of SBT-588 as a disease-modifying treatment for individuals living with LS.

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Lipid associated mitochondria promotes fatty acid oxidation through a distinct bioenergetic pattern to ameliorate NAFLD

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Abstract

Mitochondria empower the liver to regulate lipid homeostasis by enabling fatty acid oxidation during starvation and lipogenesis during nutrient-rich conditions. It is unknown if mitochondria can seamlessly control these two distinct processes or if two discrete populations of mitochondria achieve these two functions in the liver. For the first time in the liver, we report the isolation of two distinct populations of mitochondria from rats on an ad-libitum diet: cytoplasmic mitochondria and lipid droplet-associated mitochondria. Our studies show that while lipid droplet mitochondria exhibit higher fatty acid oxidation and are marked by enhanced levels of pACC2, Mfn2, and CPT1 activity, cytoplasmic mitochondria are associated with higher respiration capacity. Notably, lipid droplet-associated mitochondria isolated from a non-alcoholic fatty liver disease (NAFLD) rat model are compromised for fatty acid oxidation. We demonstrate the importance of functional segregation of mitochondria as any aberration in lipid droplet- associated mitochondria may lead to NAFLD.

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Sexual dimorphism of calcium homeostasis in isolated cardiac mitochondria

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Abstract

Heart disease is the leading cause of death in the United States. Notably, the male population has significantly higher rates of heart failure than premenopausal women, yet the influence of sex hormones on these differences is still ambiguous. As key elements of cardiac dysfunction are calcium overload and oxidative stress, uncovering the sexual dimorphism associated with these stressors could elucidate the mechanisms responsible for the cardio-protection afforded to women. Thus, we conducted a sex as a biological variable study using male and female Sprague Dawley (SD) rats and quantified their calcium tolerance (5 - 20 μM $CaCl_2$) and free radical emission profiles. We found that isolated cardiac mitochondria from female and male SD rats respond to and handle calcium differently. Specifically, females have higher Na^+/Ca^{2+} cycling and ATP production rates for a given calcium load, while free radial emission rates between the sexes decrease in response to calcium challenges. Rates of calcium uptake and efflux were also different in both sexes. Absorbance measurements rule out large amplitude swelling and support a genuine calcium effect that impairs oxidative phosphorylation independent of the mitochondrial permeability transition phenomenon. These data call into question the abundance of the mitochondrial calcium uniporter (MCU) and the $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) in males and females, as well as their energization state and structural differences. From this, we suspect that female cardiac mitochondria respire more efficiently during situations of calcium overload as they have lower concentrations of matrix calcium and more stabilized cristae.

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Inducible SOD2 knockdown impairs mitochondrial pyruvate oxidation and OxPhos capacity by reversible oxidative post translational modification in mouse skeletal muscle

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Abstract

Mitochondria demonstrate remarkable plasticity in skeletal muscle adapting morphologically and biochemically to various stressors like exercise and dietary stimuli. Redox signaling mechanisms via mitochondrial ROS production likely play a role in this adaptive process. However, there remain many unanswered questions and paradoxical findings in mitochondrial redox biology that warrant the development of new models to better understand these processes. Here we validate a new model of inducible SOD2 knockdown (iSOD2 KD) in skeletal muscle to produce a mild mitochondrial redox stress and characterize the physiological effects. The model induces SOD2 KD via a TET-ON RNA interference system, where the presence of doxycycline induces expression of a short hairpin RNA that degrades SOD2 mRNA. We demonstrate the model induces skeletal muscle specific SOD2 KD with three weeks of doxycycline (DOX) treatment. There were no unwanted off-target effects of shRNA in other tissues or on SOD1 protein expression in young adult mice. Eight weeks of SOD2 KD showed a mild decrease in in vivo muscle function in female KD mice compared to controls ($p=0.0249$). Ten weeks of SOD2 KD in young adult mice show significant SOD2 KD compared to controls ($p<0.0001$). iSOD2 KD resulted in significant deficits of pyruvate driven respiration ($p<0.0001$), and max Oxphos capacity ($p=0.0001$) in permeabilized muscle fibers. There were no differences in pyruvate dehydrogenase (PDH) protein content or phosphorylation status between groups. Pre-treating fibers with the thiol reductant dithioerythritol (DTE) partially restored pyruvate oxidation indicating metabolic control by a reversible protein thiol PTM. Taken together, these results show that inducing a short mitochondrial redox stress (3-10 weeks) produces changes in physiological function in skeletal muscle, which are regulated in part by reversible thiol PTMs. Future work will utilize this model to explore adaptive and maladaptive mitochondrial driven redox signaling effects through reversible oxidative PTMs in skeletal muscle.

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Sex differences in adipose mitochondrial activity in sedentary and exercised mice

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Abstract

Background. Exercise reduces fat while increasing lean mass. In addition, exercise induces remodeling of white adipose tissue, with formation of multilocular adipocytes (known as beige adipocytes) that have increased thermogenic properties. This metabolic adaptation has not been thoroughly compared between males and females.

Objectives. We sought to characterize the sex differences on exercise-induced changes in adipose tissue using the Four Core Genotypes (FCG) mouse model. This model generates XX mice with testes or ovaries and XY mice with testes or ovaries, allowing detection of effects of sex chromosomes, gonadal hormones, or interactions between the two.

Methods. FCG mice were maintained under sedentary conditions or had access to voluntary wheel running for one month. We assessed body composition, adipose weight and morphology, mitochondrial respiration, mitochondrial density, and gene expression.

Results. In all genotypes, exercise reduced gonadal white fat (gWAT) and inguinal WAT (iWAT) mass, while increasing brown adipose tissue (BAT) mass. Histologically, iWAT showed more multilocular adipocytes after exercise compared to gWAT. We observed sex differences in mitochondrial DNA (mtDNA) content in both WAT and BAT. Exercise increased iWAT respiration in XY mice with testes, and decreased respiration in gWAT from XX and XY mice with ovaries. We further explored the increase in iWAT respiration. The combination of XY chromosomes with testes increased mtDNA content in response to exercise, which was associated with increased mRNA and protein levels for electron transport chain components. XY mice with testes also showed greater exercise-induced expression of genes for mitochondrial biogenesis,
beiging, and fatty acid oxidation when compared to the other three genotypes.

Conclusions. Chronic exercise alters adipose tissue morphology, mitochondrial DNA content, and respiratory activity in an adipose tissue depot- and sex-dependent manner. Our findings reveal roles for both gonadal and chromosomal sex as determinants of adipose tissue metabolic adaptation to exercise.

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Location matters: glycolytic enzymes go to mitochondria upon oxidative stress

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Abstract

Mitochondria are multifaceted organelles that play crucial roles in cellular physiology, beyond being the “powerhouses” of the cell. Mitochondria are the most prominent contributors to the production of reactive oxygen species (ROS) in most cell types, which regulate the activity of different signaling pathways that control cellular survival, proliferation, and fate. However, when produced in excess, such as in conditions of mitochondrial dysfunction, mitochondrial ROS cause oxidative stress and underlie the pathogenesis of age-associated conditions, such as Alzheimer’s disease. Different antioxidant systems are activated in response to an increase in the levels of mitochondrial ROS to keep the balance between their production and detoxification. To investigate the mitochondrial responses to oxidative stress, we have performed a screen for proteins going to mitochondria in cells treated with menadione, a redox cycling compound, using proximity labeling. Notably, we have detected most of the enzymes of glycolysis on the surface of mitochondria upon oxidative stress and confirmed that enolase and pyruvate kinase M go to mitochondria in these conditions. Interestingly, both enzymes are also enriched in mitochondrial fractions from primary fibroblasts derived from patients of Alzheimer’s disease, when compared to age- and sex-matched controls. We show that primary fibroblasts recapitulate some metabolic alterations described for Alzheimer’s disease, namely an increase in mitochondrial superoxide levels, and treatment with the antioxidant N-acetylcysteine reduces the mitochondrial localization of enolase and pyruvate kinase. Therefore, we propose that the translocation of glycolytic enzymes into stressed mitochondria may be a “mito-protective” mechanism to reestablish cellular homeostasis. The characterization of a potential moonlighting function of glycolytic enzymes in mitochondria is an exciting new area of research that could offer new biomarkers and/or targets for the therapy of neurodegenerative diseases.

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A role for SLC25A46 in mediating mitochondrial dynamics and protein quality control

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Abstract

Maintenance of a healthy mitochondrial network is essential to the health of an organism and its cells. It relies on a delicate balance between mitochondrial fission and fusion, which must be coordinated across the inner and outer mitochondrial membranes. If disrupted, several diseases can result, including Pontocerebellar Hypoplasia (PCH), Charcot-Marie-Tooth Disease Type 2 (CMT2), and Optic Atrophy. SLC25A46, a member of the mitochondrial Solute Carrier Family, which localizes to the outer mitochondrial membrane instead of the inner membrane, may mediate these processes. SLC25A46 interacts with several proteins including fusion regulators Mitofusin 1+2 (MFN1+2) on the outer membrane and OPA1 on the inner membrane. When SLC25A46 expression is downregulated, or when the gene encoding the protein undergoes a point mutation at Position 341 from leucine to proline (L341P), MFN1+2 levels increase and the mitochondrial network becomes hyperfused. SLC25A46 L341P is properly imported into the mitochondria, but is promptly identified, extracted, and degraded by the ubiquitin-proteasome system (UPS) independently of mitophagy, making SLC25A46 L341P a model substrate to study the process of Outer Mitochondrial Membrane Associated Degradation (OMMAD.) SLC25A46 may also play a role in metabolism, as it appears to have a competing role with Mitochondrial Carrier Homolog 2 (MTCH2 aka SLC25A50). When SLC25A46 undergoes the L341P mutation, MTCH2 expression levels increase. Both proteins belong to the solute carrier family and are localized to the mitochondrial outer membrane yet have no known transporter function. MTCH2 downregulation results in lower levels of lipid droplet formation and fragmented mitochondria. Although SLC25A46 does not appear to affect lipid droplet formation, it may act as a sensor for metabolic shifts between glycolysis-based and oxidative phosphorylation-based metabolism. This project seeks to determine the role of SLC25A46 in mediating mitochondrial processes and how deficiencies in its function can result in disease states.

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Control of calcium efflux from mitochondria by Mfn2 and its possible relevance for a peripheral neuropathy

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Abstract

Mitochondrial fusion and fission play a critical role in maintaining functional mitochondria especially under stress conditions. Mitochondrial fission is driven by dynamin-related protein Drp1. Fusion is induced by two mitochondrial outer membrane proteins Mitofusin Mfn1 and Mfn2 and by the mitochondrial intermembrane space protein Opa1. Mutations in Mfn2 cause a peripheral neuropathy (Charcot Marie Tooth disease or CMT type 2A) while mutations in Opa1 cause blindness though loss of retinal ganglion cells (autosomal dominant optic atrophy or ADOA). Previous studies from our lab show that, in the presence of the stressor the fungal toxin Phomoxanthone A (PXA) induces calcium release from mitochondria, which in turn causes the mitochondrial matrix to become highly condensed while triggering mitochondrial fission and mitophagy or apoptosis. Now we present evidence that stress-induced calcium release is mediated by NCLX, inner mitochondrial membrane Na^{+}/Ca^{2+} exchange protein, and this release requires Mfn2. Surprisingly, we found that Mfn2 is the direct target of the drug PXA by using biochemical assay Cellular shift thermal temperature (CETSA). We further tested the interactions between Mfn2, NCLX and other proteins that are implicated with this process using Co-IP and proximity ligation assay (PLA). We found that Mfn2 and NCLX increase interactions in the presence of PXA under glycolytic conditions and in the presence of oligomycin under respiratory conditions. In addition, we found morphological effects of PXA can be suppressed by a deletion in the mitochondrial outer membrane fusion protein Mfn2. This unexpected role of Mfn2 under stress induced conditions, implicate a new mechanism of Mfn2-dependent calcium release targets direct to NCLX, which is critical for the switch between healthy mitochondrial and stress-induced fission. We propose a novel mechanistic approach between Mfn2 and NCLX, which is triggered by stress induced conditions. Our overarching goal is a better understanding of fundamental questions about the mechanistic relationships between Mfn2, NCLX and calcium release that triggers fission as a novel regulatory mechanism, and how this relates to neuropathies. These new insights will help understand the underlying causes of CMT disease and maybe suggest novel treatment strategies..

Regulatory mechanism of formin INF2 by Transgelin

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Abstract

Inverted formin 2 (INF2) is a metazoan formin protein that activates actin polymerization for a number of purposes in mammalian cells including regulation of mitochondrial division, which is crucial for mitochondrial homeostasis. In unstimulated cells, INF2 is 'off' (does not polymerize actin) but is activated by an increase in cytoplasmic calcium.

My primary focus has been on INF2 regulation, and how calcium activates its actin polymerization activity. For this purpose, I have developed a simple cell-free assay system to rapidly isolate ER, involving the following steps: 1) homogenizing mammalian culture cells, 2) isolating the low-speed (300xg) pellet (LSP), and 3) mixing the LSP with actin monomers, profilin, and capping protein. I monitor actin polymerization spectrofluorimetrically using pyrene-labeled actin or through fluorescence microscopy using rhodamine-labeled actin. Actin polymerization from LSP is both INF2-dependent and calcium/calmodulin-dependent.

I am utilizing a combination of this cell-free system and proximity labeling by BioID, to identify the factors that hold INF2 in an inactive state in the absence of calcium. Unlike other formin proteins, which are auto-inhibited, purified INF2 is constitutively active. The fact that INF2 in cells is tightly regulated suggests that other factors are needed to keep its actin polymerization activity in check. One protein that appears to act in this capacity is transgelin 2 (TG2), which I identified as an INF2-interacting protein and whose knock-down/knock-out causes constitutive INF2-mediated actin polymerization in multiple cell types.

Interestingly, we have observed that transgelin2 is recruited to INF2-mediated actin filaments during INF2 activation, and the absence of transgelin2 leads to delayed actin disassembly. Consequently, we have observed increased mitochondrial division during INF2 activation, impacting mitochondrial morphology and function. Taken together, these results suggest that 1) transgelin2 might act as an INF2 regulator in cells, or 2) transgelin2 is crucial for disassembling INF2-mediated actin filaments to regulate mitochondrial division.

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Investigation of the endoplasmic reticulum-mitochondria encounter structure (ERMES) as a regulator of CoQ biosynthesis

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Abstract

Coenzyme Q (CoQ) is an essential redox-active lipid that plays a major role in the electron transport chain, driving mitochondrial ATP synthesis. Reduced $CoQH_2$ functions as an antioxidant, slowing lipid peroxidation and inhibiting ferroptosis. Deficiency of CoQ causes a wide range of clinical deficiencies, highlighting the need to study the biosynthesis of this lipid. In *Saccharomyces cerevisiae*, CoQ biosynthesis takes place exclusively in the mitochondrial matrix using a mega complex, the CoQ synthome, comprised of Coq3-Coq9 and Coq11. A recently identified regulator of CoQ Synthome assembly and CoQ production is the ER-mitochondria encounter structure (ERMES). ERMES is a tethering complex that bridges the two organelles, and the CoQ Synthome resides in specific membrane niches directly adjacent to this complex. Loss of ERMES results in a destabilized CoQ synthome, resulting in impaired CoQ biosynthesis. In this work, *ERMESΔcoq11Δ* mutants have been generated in an effort to correct this defect, as deletion of *COQ11* enhanced CoQ synthome stability and rescued the respiratory deficient *coq10Δ* mutant. Here, we show that deletion of *COQ11* can rescue the respiratory defect of the *mmm1Δ* mutant. Immunoblotting and fluorescence microscopy display a stabilized CoQ synthome in the *mmm1Δcoq11Δ* mutant, suggesting deletion of *COQ11* may recruit an auxiliary ER-mitochondrial contact to take the place of ERMES. Using RNA-sequencing, we aim to identify candidates that may detail the mechanism of rescue induced by the deletion of *COQ11*. Additionally, we have expressed artificial tethers in the absence of ERMES and ascertained the effects on CoQ biosynthesis using stable isotope labeling and LC-MS/MS. Collectively, this work seeks to investigate the relationship between the ERMES complex and the CoQ biosynthetic complex to better understand the regulation of CoQ biosynthesis and aid in the development of more effective therapeutics for diseases linked to CoQ deficiencies.

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Visualizing mitochondria under various biochemical conditions with cryogenic electron tomography

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Abstract

Optic nerve drusen are build ups of calcified deposits within the anterior optic nerve that occur in 2% of the population including children and adults, often resulting in vision loss. The process through which acellular calcified deposits occur is unknown but is thought to be related to mitochondrial calcification. Cryogenic electron tomography (cryo-ET) can be used to help identify structural abnormalities in cells under pathological conditions. We utilized cryo-ET to visualize mitochondria in cultured human skin fibroblasts and human retinal ganglion cells induced to calcify, highlighting the feasibility of using this exciting high resolution imaging tool to detect ultrastructure signatures inherent in cellular models of human disease and possibly assay benefits of neuroprotective therapies. We observed a larger granule size, abnormal circular cristae, and an uneven distribution of granules along the edges of the mitochondria compared to the control. In our ongoing studies, we are identifying the composition of the granules and quantifying their size with AI.

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Spatial and temporal mapping of mitochondrial networks and bioenergetics in lung cancer

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Abstract

Tumor metabolism presents a potential target for therapeutic interventions. The configuration and temporal dynamics of cellular organelles play a pivotal role in regulating metabolism within cancer cells, subsequently influencing therapeutic outcomes. We explored a multi-modality imaging platform, integrating longitudinal PET/CT imaging, microCT imaging and 3-dimensional serial block-face electron microscopy (SBEM), aiming to capture the intricate spatial and temporal architecture of mitochondrial networks in non-small cell lung cancer (NSCLC). Combining two radiotracers--^{18}F-fluorodeoxyglucose (^{18}F-FDG) and ^{18}F-4-fluorobenzyl triphenylphosphonium [^{18}F]FBnTP)[1]—which indicate variations in glucose flux and mitochondrial membrane potential ($\Delta\Psi$), we were able to track the dynamic shifts in mitochondrial metabolism during NSCLC progression. Notably, our observations pinpointed that tumors with an LKB1 deficiency undergo a metabolic transition from oxidative to glycolytic processes as the tumor develops. To elucidate the underlying mechanism of this metabolic shift, we employed 3D SBEM imaging to deliver a roadmap of cellular organelles with nanometer resolution at a spatial scale. Capitalizing on machine learning, specifically convolutional neural networks, we automated the segmentation and deep analysis on mitochondrial morphology, spatial organization, and interactions with other cellular organelles. This in-depth structural-functional examination unveiled a distinct mitochondrial subset, termed peri-droplet mitochondria (PDM), in which mitochondria are bound to lipid droplets. Importantly, PDM abundant tumors exhibited heightened oxidative phosphorylation (OXPHOS^{HI}) tendencies were demonstrated to be resistant to treatments. Through this research, we probed into the potential of targeting PDMs within OXPHOS^{HI} tumors to improve treatment outcomes.

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Unraveling the role of Angiotensin Converting Enzyme (ACE) in macrophage metabolism

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Abstract

Angiotensin-converting enzyme (ACE) is best known for its role in regulating blood pressure through its activity in the renin-angiotensin pathway. However, a role for ACE in innate immunity has only recently been discovered. We have previously shown that upon activation, myeloid cells display increased expression of ACE. ACE over-expression in murine macrophages also caused increased pro-inflammatory activation and enhanced mitochondrial metabolism.While these results have been observed in murine cells, the effects of ACE over-expression in human myeloid cells haven't been evaluated. To assess the role of ACE in human macrophage function and metabolism, we generated THP-1 human monocytic cells which over-express ACE. Utilizing gene expression analysis and respirometry we demonstrate that THP-1 cells overexpressing ACE display increased inflammatory activation and enhanced mitochondrial respiration. These results suggest a previously underappreciated role of ACE in regulating human macrophage inflammation and metabolism.

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Structural snapshots of AAA+ protease YME1 reveal substrate-free ADP-bound states that are proteolytically inactive

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Abstract

The AAA+ protease YME1 is an essential mitochondrial protein quality control complex that functions as a protein degradation machine. A structure of YME1 trapped in the act of processing substrate revealed a hand-over-hand mechanism for substrate translocation into the proteolytic chamber. However, the mechanisms underlying other stages of activity remain unknown. Here, we use cryo-EM to explore the conformational landscape of active YME1 in the presence of an engineered Tim10-derived substrate. We identify 4 distinct, coexisting states: 2 substrate-bound configurations that confirm hand-over-hand translocation, and 2 substrate-free, ADP-bound configurations. These novel conformers reveal dramatic rearrangements of the ATPases that are allosterically transmitted to the protease domains. A FRET-based peptide cleavage assay shows that the proteases are inhibited in the ADP-bound states, indicating that the substrate-free organizations represent “off” states of the enzyme. Linear interpolations between the 4 states provide insights into the complete mechanochemical cycle of YME1, including substrate engagement, release, and resetting of the complex for reloading. These findings allow us to propose a model for how AAA+ proteases like YME1 decide which substrates to target and how to process them for the first time.

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NMNAT2 links bioenergetics and proteostasis in cortical glutamatergic neurons

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Abstract

Bioenergetic maladaptations are often found in the early stage of neurodegenerative diseases associated with proteostasis failure and protein misfolding, e.g., the glucose hypometabolism and the aberrant Amyloid Precursor Protein (APP) proteolytic processing in Alzheimer's disease. How does bioenergetics and proteostasis intertwine and interfere with each other in neuronal cells? We focused on a putative AD target gene, NMNAT2 (Nicotinamide mononucleotide adenylyl transferase 2), whose expression level positively correlates with cognitive resilience and negatively correlates with pathological severity in human subjects. Our studies found that NMNAT2 is critical in maintaining cytosolic NAD+/NADH ratio in distal axons to drive glycolysis as well as for fueling fast axonal transport of vesicular cargos. Such glycolysis deficits in NMNAT2 KO neurons results in enhanced mitochondria function. To delineate the bioenergetic and proteomic consequence of NMNAT2 loss in cortical neurons, we conducted Seahorse assays and proteomic analyses. I will summarize our data revealing both functional and proteomic changes in mitochondria upon NMNAT2 loss and how such changes may link to alterations in proteostasis, such as lysosomal and ubiquitin-proteasome system function. Taken together, we hypothesize that NMNAT2 loss impairs mitochondrial machinery, which in turn disrupts neuronal proteostasis. Intriguingly, we found supplying NMN (Nicotinamide Mononucleotide, NAD precursor) alleviated both bioenergetic and proteostatic deficits in NMNAT2 KO neurons, however the mechanisms requires further investigation.

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Regulation of nuclear transcription by mitochondrial RNA

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Abstract

Mitochondrial-nuclear communication is vital for cellular homeostasis and response to environmental stress. The known messengers for mitochondria to communicate their functional status to the nucleus include ATP, ROS, and Ca^{2+}. It remains unclear whether the immediate output of mitochondrial transcription, i.e., the mitochondrial RNAs (mtRNAs), are a type of messenger to communicate to the nucleus. Herein, we show that mtRNAs are attached to the nuclear genome and thus constitute a subset of the chromatin-associated RNA (mt-caRNA). Mt-caRNAs preferentially attach to promoter regions and the attachment levels change in response to cellular stress, i.e. high glucose and TNFa (HT). We identify the mitochondrial non-coding RNA SncmtRNA as a mt-caRNA. In human endothelial cells (ECs), suppression of SncmtRNA attenuates HT stress induction of nascent RNA transcribed from the nuclear genome, including the cell adhesion molecules ICAM1 and VCAM1, and abolishes stress-induced monocyte-EC adhesion. In addition to SncmtRNA, we show nuclear localization of other mtRNAs such as mt-CYB and mt-ND5, which is increased in the diabetic human condition. Collectively, our findings suggest the potential involvement of many mtRNAs in mitochondrial-nuclear communications and that mt-caRNAs may regulate nuclear transcription and thus play a role in mediating cellular response in health and disease.

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Mitochondrial ATP hydrolysis is linked to impaired mitophagy in aging

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Abstract

Mitophagy, the process by which damaged or dysfunctional mitochondria are selectively removed and recycled, plays a crucial role in maintaining cellular health. Mitophagy impairment in aging can lead to the accumulation of defective mitochondria, resulting in increased oxidative stress, decreased energy production, and a higher susceptibility to age-related diseases, such as neurodegenerative and cardiovascular disorders. The mitochondrial F1Fo-ATP synthase uses a rotary mechanism to synthesize ATP. Under conditions of impaired respiration, however, this mechanism can operate in reverse, pumping protons at the expense of ATP hydrolysis. Here we hypothesize that mitochondrial ATP hydrolysis is increased with aging, particularly in metabolically active tissues. Furthermore, we propose that by triggering ATP hydrolysis, dysfunctional mitochondria can maintain their membrane potential and thereby evade mitophagy, creating a viscous cycle that can contribute to aging pathophysiology.

Results presented here indicate that mitochondrial ATP hydrolytic capacity, measured by hydrolysis in frozen samples (HyFS), is increased in the brains of aging rats and mice compared to their younger counterparts. This observation strongly correlates with a decrease in expression of ATPIF1, the endogenous inhibitor of ATP hydrolysis, that is observed in aging human brain. In agreement with our in vivo data, we also observe increased ATP hydrolysis in primary skin fibroblasts from a patient with atypical Werner syndrome, a cellular model of accelerated aging. Remarkably, this increase in ATP hydrolysis coincides with reduced mitophagy, as evidenced by mitoQC imaging. Importantly, blocking of ATP hydrolysis with a selective inhibitor, (+)-epicatechin, enhances mitophagy in these cells, possibly via a PINK1-Parkin independent pathway.

Taken together, our results suggest that the increased consumption of ATP by mitochondria is a maladaptive response that drives energy depletion during aging. Furthermore, we demonstrate that inhibiting ATP hydrolysis can break the viscous cycle between increased ATP hydrolysis and impaired mitophagy. Therefore, these findings not only shed light on the intricate interplay between mitochondrial ATP hydrolysis and aging, but also offer a promising avenue for potential therapeutic interventions to alleviate age-related mitochondrial dysfunction.

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Developing mammalian cell model system for identifying small molecule modulators of mitochondrial protein import in the context of PH1

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Abstract

Primary Hyperoxaluria type one (PH1) is an autosomal recessive metabolic disorder that is caused by an accumulation of the metabolic intermediate glyoxylate which is converted to oxalate. This build of oxalate is caused by the deficiency of the protein alanine-glyoxylate aminotransferase (AGT). In humans, AGT is present in the peroxisome however, a large subset of cases are caused by two respective mutations in the gene which produces a small mitochondrial targeting sequence and impairs proper folding in the cytosol, resulting in localization to the mitochondrion. This is an example of a dual-localized protein. If this protein can be restored to its natural location, the diseased phenotype present in PH1 may be attenuated. A previous study done in collaboration with this lab showed data from a small molecule screen done in yeast. The data presented a number of small molecules shown to block mitochondrial import and restore metabolically active AGT to the peroxisome. Here, we engineered a HepG2 cell line to stably express the mutant AGT P11L G170R with a unique bi-molecular split GFP reporting system. We authenticated the effectiveness of these small molecules with fluorescence microscopy and protein stabilization studies to elucidate the small molecule targets. Additionally, we ran a small molecule, high content screen using our mammalian cell model and were able to identify more compounds that restore AGT to better effect in a mammalian context. This study will identify new compounds as possible therapeutics and explore how dual-localized proteins can be potential drug targets.

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Characterizing the NAD metabolism of senescent macrophages

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Abstract

Dysregulation of Nicotinamide Adenine Dinucleotide (NAD) metabolism is an underlying feature of aging and its related diseases. NAD is an essential bioenergetic molecule and age-related declines contribute to the impairment of cellular homeostasis through: mitochondrial dysfunction, genomic instability, and altered intracellular signaling. NAD de-novo synthesis and salvage pathways and consuming enzymes like sirtuins, CD38, and poly (ADP ribose) polymerases, finely regulate NAD metabolism and thus cellular function. However, the cellular mechanisms driving NAD decline with age remain largely unknown. Our lab has shown that 1) senescent cells drive tissue NAD decline with age via the activation of CD38 expressing macrophages and 2) macrophages make up a major senescent cell type with age. These studies uncover a link between senescence, NAD metabolism, and macrophage activation. We hypothesize that senescent macrophages contribute to NAD depletion through the dysregulation of NAD regulating pathways. Using senescent macrophages derived from mice, we will test this hypothesis by RT-qPCR and Western, and quantify NAD hydrolysis rates. Preliminary NADase data suggest that senescent macrophages display low grade consumption of NAD compared to passage control macrophages. This research will provide insight into the regulation of NAD metabolism in senescent macrophages to assess novel therapeutic targets to regulate tissue NAD levels with age.

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Evaluating the role of nitric oxide in macrophage metabolism and the pro-inflammatory response

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Abstract

Within the innate immune system, pro-inflammatory macrophages function in host defense against pathogens partly by producing nitric oxide (NO), a signaling and effector molecule. Previous studies have shown that nitric oxide interacts with the electron transport chain to collapse mitochondrial respiration and inhibits enzymes such as mitochondrial aconitase (ACO2) in the tricarboxylic acid (TCA) cycle. While this metabolic reprogramming is widely thought to repurpose mitochondria away from oxidative phosphorylation to assume the role of accumulating signaling metabolites, the influence of NO on the pro-inflammatory response is not well-defined. Thus, we set out to characterize how a loss of NO impacts pro-inflammatory gene expression as well as metabolite accumulation and enrichment from uniformly labeled ^{13}C-glucose. The production of NO is catalyzed by inducible nitric oxide synthase (iNOS), which is encoded by the Nos2 gene in mice. Using a loss-of- function experiment with Nos2$^{-/-}$ murine macrophages, our data reaffirm that in the absence of NO production, mitochondrial respiration fails to collapse upon pro-inflammatory activation by various stimuli. Nos2$^{-/-}$ murine macrophages also show increased flux from citrate to α-ketoglutarate, possibly due to the loss of ACO2 inhibition. Interestingly, we show that the enhancement of pro-inflammatory gene expression and metabolite accumulation upon activation are mostly unchanged. Therefore, our studies provide evidence that while NO is important in modulating mitochondrial respiration in pro- inflammatory macrophages, it may not play an essential role in enhancing the pro-inflammatory response.

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The “Seeder Model” of MICOS assembly: MIC13 and SLP2 seeds the assembly of MIC60-subcomplex to promote crista junction formation

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Abstract

Mitochondrial functions depend on their internal structural integrity, intricately governed by crista architecture. The Mitochondrial Contact Site and Crista Organizing System (MICOS) complex plays a pivotal role in shaping the inner mitochondrial membrane (IM) into crista which are critical for cellular respiration. Mutations in the MICOS subunit MIC13 result in severe mitochondrial hepatic encephalopathy (MHE) spurring an in-depth interactome analysis that spotlights indispensable role of Stomatin-like Protein 2 (SLP2) in MICOS complex assembly. SLP2 emerged as a central interaction hub within the MICOS complex, forming specific associations with multiple MICOS subunits independently. Notably, SLP2 stabilizes MIC26, a key MICOS component, through its interaction with YME1L, preventing YME1L-mediated proteolysis. The importance of SLP2 extends to crista morphology and junction maintenance, as its loss results in swollen crista and reduced number of crista junctions. Furthermore, our findings reveal a functional synergistic relationship between SLP2 and MIC13, with their double knockout severely impairing MIC60 assembly and crista structure, where SLP2 specifically influences the assembly kinetics of the MIC60-subcomplex. We unravel a novel MIC13-YME1L axis independent of SLP2, stabilizing MIC10 and MIC27, pivotal for crista junction formation, illustrating early molecular pathogenesis towards mitochondrial hepatic encephalopathy. We further introduce a ‘Seeder Model’ of MICOS assembly, wherein SLP2, along with MIC10-subcomplex forming the ‘Seeder Complex’, dictates formation of MIC60 puncta thereby facilitates the efficient assembly of MIC60-subcomplex-MIB leading to formation of crista junctions.

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Pro-inflammatory macrophage memory as a source of immune metabolic dysfunction

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Abstract

Aging is a primary risk factor for severe infection and the incidence of metabolic disease. With age, the innate immune system loses the ability to mount an effective response against pathogens like bacteria, viruses, and cancer cells, and this age-related program is known as immunosenescence. Current efforts have revealed novel regulatory mechanisms of immunosenescence, but its underlying sources remain understudied. Tissue-resident macrophages are self-renewing phagocytes that lose the ability to generate mitochondrial-mediated anti-microbial metabolites in response to pathogens with age. Bacterial activation causes endotoxin tolerance to resolve inflammation in macrophages, and the long-term fate and function of tolerant macrophages can provide novel insights into immunosenescence. *Due to its long-lived nature, we hypothesized that macrophages can inherent tolerance-induced epigenetic marks that attenuate the long-term immune response to a broad range of pathogens*. To test this, we employed an *in vitro* model of pro-inflammatory memory using gram-negative bacterial carbohydrate structures known as lipopolysaccharides (LPS). Our results show repeated LPS insults cause immunosenescence in macrophages and impair a range of effector functions. We also observed that macrophages fail to generate mitochondrial-derived reactive oxygen species that serve as antimicrobial species and signaling metabolites to activate inflammatory gene expression. These results suggest that frequent exposure to pathogens imprints and attenuates long-term macrophage function and serves as a novel therapeutic target to reverse the effects of an aged immune system.

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Physiological adaptation to hypoxia involves organ-specific rewiring of glucose and fatty acid metabolism

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Abstract

Acute oxygen deprivation can be toxic, but mammals can adapt to hypoxia over time. Moreover, populations at high altitude exhibit lower rates of cardiometabolic disease, and hypoxia rescues models of mitochondrial disease. These findings suggest that hypoxia induces significant metabolic rewiring. The existing model of hypoxia metabolism stems largely from the study of immortalized cells, which exhibit distinct metabolic profiles from organs in a physiological context. Here, we show that mice exhibit a remarkable ability to adapt to even severe hypoxia over time. Over the course of their physiological adaptation to hypoxia, mice exhibit systemic metabolic reprogramming, including diminished blood glucose levels and adiposity. Using in vivo metabolic imaging and stable isotope-labeled fuel tracers, we find that organs partition fuels differently during acute and chronic hypoxia. Acutely, most organs ramp up glucose uptake while diminishing the mitochondrial oxidation of glucose-derived carbons, which is consistent with the highly anaerobic metabolism of hypoxic cells in vitro. However, brown adipose tissue and skeletal muscle become “glucose savers,” significantly diminishing their glucose uptake. In chronic hypoxia, each organ adopts a distinct metabolic profile; while the heart continues prioritizing glucose metabolism, the kidney, liver, and brain all surprisingly increase their uptake and oxidation of fatty acids. Altogether, these findings suggest that different organs have distinct mitochondrial responses.

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MiD49/51 function as long-chain fatty acyl-coenzyme A sensors on mitochondria to activate Drp1

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Abstract

Mitochondrial fission occurs in many cellular processes, but the factors inducing fission are poorly understood. Here, we show that long-chain acyl coenzyme A (LCACA) is an activator of two related mitochondrial fission proteins, MiD49 and MiD51, which are receptors for the dynamin GTPase Drp1. LCACAs such as palmitoyl-, oleoyl-, and stearoyl-CoA induce MiD49 and MiD51 oligomerization, activating their ability to stimulate Drp1 GTPase activity. The activation ability decreases with acyl chain length, becoming negligible for octanoyl-CoA. Palmitic acid or coenzyme A alone, acetyl-CoA, malonyl-CoA or lyso-phosphatidic acid do not induce MiD oligomerization, suggesting specificity for LCACAs. The stoichiometry of LCACA:MiD in the oligomer is 1:1, suggesting binding to the previously identified nucleotide-binding pocket. A point mutation in the nucleotide binding pocket reduces LCACA binding and LCACA-induced oligomerization for MiD51. In cells, this LCACA binding mutant does not assemble into puncta on mitochondria or rescue MiD49/51 knock-down effects on mitochondrial length and Drp1 recruitment. These results suggest that LCACA is an endogenous ligand for both MiDs, inducing Drp1 recruitment and mitochondrial fission, and might provide a mechanism for fatty acid-induced mitochondrial fragmentation.

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Targeting resistance to cancer therapy through translational control of metabolism

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Abstract

Tumor resistance to anticancer drugs is a well-known clinical phenomenon that impinges on a patient's quality of life and survival. While most cancers initially respond to targeted therapies (such as oncogenic kinase inhibitors), they often develop mechanisms of resistance to treatment. Although this phenomenon is well established, the underlying mechanisms are not fully understood. We recently discovered a new mechanism of adaptation that involves translational regulation of metabolism in response to therapies that target oncogenic kinases (e.g. EGFR/HER2, BCR/ABL, BRAF). Translation of mRNAs encoding metabolic regulators, including those involved in serine (PHGDH, PSAT1), aspartate (PC), and asparagine (ASNS) synthesis, was dependent on the mTORC1/eIF4F/4E-BP axis. Pathway dysregulation caused by 4E-BP1/2 depletion induces metabolic plasticity and partial resistance to combinations of kinase inhibitors and biguanides, suggesting that translational regulation of metabolic genes via the mTORC1/4E-BP pathway plays a major role in energy stress response in cancer and that the efficiency of cancer strategies targeting metabolic vulnerabilities is dependent on the translation machinery.

Intriguingly, we found that drugs that interfere with the translation machinery (such as molecules targeting the eIF4A subunit of the translation initiation complex eIF4F; eIF4Ainh) are very effective in the low nM range against BRAF inhibitor-resistant melanoma cells, inducing apoptosis in a dose-dependent manner. Classic translational targets of mTORC1/eIF4F, such *CyclinD3*, *CDK4*, *BCL-2, MCL-1*, encoding for cell cycle and pro-survival proteins, are downregulated by eIF4Ainh. Metabolic characterization of BRAFinh-resistant melanoma cells treated with eIF4Ainh shows a strong metabolic rewiring, particularly for TCA cycle intermediates and derivate metabolites. Bioenergetic assessment using Seahorse technology indicates a strong reduction in respiration, drastically amplified by the combination with BRAFinh vemurafenib. Ongoing translatome analyses aim to uncover eIF4A-dependent translationally-regulated genes that mediate the efficacy of eIF4Ai in BRAFi-resistant melanoma cells and the associated metabolic reprogramming. Our studies highlight (i) the importance of the crosstalk between mRNA translation and metabolic regulation and (ii) that direct inhibition of translation represents an appealing therapeutic strategy for clinical cases of targeted therapy resistance.

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Characterization of the Q367H MFN2 variant from a patient with distal myopathy reveals a novel disease mechanism via mtDNA/TLR9-mediated inflammation

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Abstract

We describe a novel heterozygous MFN2 variant, Q367H, identified in a patient diagnosed with late-onset distal myopathy, but lacking the typical MFN2-associated pathology of peripheral neuropathy. Indicative of MFN2 dysfunction, characterization of patient fibroblasts revealed mitochondrial fragmentation, reduced mitochondrial ER contacts, and mtDNA alterations, but only when cultured in galactose media, where mitochondrial function is required for energy production. Meanwhile, under 'normal' glucose media cell culture conditions, we observed an increased abundance of cellular lipid droplets and alterations to mitochondrial respiration, suggesting these MFN2-related phenotypes were more adversely impacted by the Q367H variant. Given the myopathy presentation of the patient, we next explored the expression of inflammatory genes, as impaired mitochondrial dynamics can cause sterile muscle inflammation leading to myopathy. Excitingly, we discovered an increased expression of several inflammation-related genes connected to TLR9-NFκB signaling (e.g., PYCARD(ASC), IL6, NLRP3, S100A9, TNF). Notably, we also observed the presence of mtDNA co-localizing with endosomes. Taken together, these observations are consistent with mtDNA activation of the TLR9-NFκB pathway. To gain tissue-specific insight, we transdifferentiated Q367H fibroblasts into myoblasts. We also observed elevated expression of TLR9 inflammation genes in Q367H myoblasts, which notably was 10-fold higher than in fibroblasts. The endosomal presence of mtDNA and TLR9 inflammatory signature, which was aggravated in myoblasts, provides a possible mechanistic explanation for the patient myopathy. Overall, this work highlights myopathy in the absence of peripheral neuropathy as a novel disease phenotype associated with MFN2 dysfunction, and identifies mtDNA activation of TLR9 inflammation as novel pathomechanism of MFN2 dysfunction.

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New insights into idebenone therapy in relation to NQO1

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Abstract

Idebenone, the only approved treatment for Leber hereditary optic neuropathy (LHON), promotes recovery of visual function in up to 50% of patients, but we can neither predict nor understand the non-responders. Idebenone is reduced by the cytosolic NAD(P)H oxidoreductase I (NQO1) and directly shuttles electrons to respiratory complex III, bypassing complex I affected in LHON. We show here that two polymorphic variants drastically reduce NQO1 protein levels, when homozygous or compound heterozygous. This hampers idebenone reduction. In its oxidized form idebenone inhibits complex I, decreasing respiratory function in cells. By retrospectively analysing a large cohort of idebenone treated LHON patients, classified by their response to therapy, we show that patients with homozygous or compound heterozygous NQO1 variants have the poorest therapy response, particularly if carrying the m.3460G>A/MT-ND1 LHON mutation. These results suggest to consider patient NQO1 genotype and mitochondrial DNA mutation in the context of idebenone therapy.

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Development of a cell free model of mitochondria-lipid droplet attachment and detachment identifies signals that detach mitochondria from lipid-droplets

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Abstract

Proper regulation of cellular lipid storage and oxidation is important for energy homeostasis and health. In brown adipose tissue (BAT), mitochondria play a key role in lipid storage and thermogenesis. Upon thermogenesis, BAT mitochondria that are bound to lipid droplets detach and undergo -oxidation. Recently, it has been shown that there are two populations of mitochondria: cytoplasmic mitochondria (CM) and peridroplet mitochondria (PDM). PDM are mitochondria attached to lipid droplets. Compared to CM, PDM have a higher capacity to oxidize pyruvate and malate but lower capacity for fat oxidation. However, what determines the formation of PDM and the detachment of PDM from lipid droplets remains unclear. Here, we describe an approach to detach PDM from lipid droplets in a cell free system where we reproduced mitochondria-lipid droplet binding in an in-vitro system. The advantage of the cell free model as a reductionist system is that it excludes out contributing factors from signaling pathways. By establishing a method to assess how mitochondria bind and detach from lipid droplets in a cell free system, we can identify the mechanism responsible for PDM formation. Our preliminary data shows that cytosolic calcium and palmitoyl-carnitine are strong detachment signals. Here, we describe a novel approach to assess the mechanistic detachment of PDM from lipid droplets, leading to their transformation into CM. Overall, this method can be utilized to study the interactions between other organelles.

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Glucose stimulation activates mitochondrial dynamics in pancreatic β-cells

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Abstract

Insulin secretion from pancreatic β-cells in response to nutrients depends on their metabolic flexibility to switch between glucose, amino acids, and fatty acids for ATP generation. Mitochondria undergo structural changes, collectively referred to as mitochondrial dynamics, to regulate their own function depending on nutrient availability. While it is evident that loss of mitochondrial function underlies insulin secretory defects in all forms of diabetes, the impact of mitochondrial dynamics on metabolic flexibility in β-cells has not yet been examined. Herein, we investigate mitochondrial dynamics in β-cells following acute exposure to glucose, amino acids (glutamine and leucine) and fatty acids (palmitate). By utilizing genetically encoded, mitochondrial photoactivatable fluorophores, we observed an increase in mitochondrial dynamics with stimulatory concentrations of glucose and leucine, but not palmitate, in mouse and human β-cells. Pharmacological inhibition of mitofusins 1 and 2 (Mfn1/2) with a novel pharmacological agent, MFI8, blocked mitochondrial dynamics and insulin secretion with glucose stimulation. Our results further indicated reduced levels of Mfn2 in type 2 diabetic human pancreatic islets indicating defects in mitochondrial dynamics. Further studies are underway to quantify mitochondrial dynamics and ultrastructure in mouse and human β-cell models of diabetes. Together, our studies will demonstrate the involvement of mitochondrial dynamics in regulating nutrient preference in β-cells and investigate if imbalances in fission/fusion machinery could lead to metabolic inflexibility and β-cell dysfunction.

F_oF_1-ATP synthase: when it pays to go reverse

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Abstract

F_oF_1-ATP synthase is a reversible nanomachine that synthesizes or hydrolyzes ATP depending on the values of the inner mitochondrial membrane potential ($\Delta\Psi m$). ATP-consuming activity of this complex is undesirable for an aerobic eukaryotic cell in the long run and is inhibited by inhibitory peptide 1, IF1. We have shown that the lack of IF1, and therefore the reversal of F_oF_1-ATP synthase, is beneficial for cellular differentiation of the unicellular parasite *Trypanosoma brucei*, a causative agent of human sleeping sickness. This organism undergoes complex programmed development in its insect vector, the tsetse fly, as it migrates from the midgut to the salivary glands. In the midgut, the parasite consumes almost exclusively proline, which is oxidized in the parasite's single mitochondrion. The F_oF_1-ATP synthase harvests the $\Delta\Psi m$ generated by the canonical electron transport chain and produces ATP by oxidative phosphorylation. Using the in vitro differentiation system that mimics the complex development in the fly, we showed that during the transition from midgut to salivary gland-like forms, there is an increase in alternative oxidase abundance and activity along with a decrease in IF1 expression. This allows a portion of the F_oF_1-ATP synthase to reverse its activity leading to an increased cellular ADP/ATP ratio, followed by activation of AMP-activated kinase - a key sensor of energy stress. This is accompanied by extensive remodeling of mitochondrial metabolism as shown by proteomics and metabolomics data. In addition, we detected highly elevated levels of cellular respiration, which is followed by an increase in mitochondrial and cellular ROS, known signaling molecules. By overexpressing the ROS scavengers, superoxide dismutase and catalase, in the parasite mitochondrion, we demonstrate that both superoxide and H_2O_2 are essential molecules driving a stress response pathway involved in parasite cellular differentiation.

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Mitoribosomes and genes encoding mitochondrial respiration represent major phenotypic interface of b-cell fitness in diabetes

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Abstract

Despite the clear link between the accumulation of dysfunctional β-cells and the progressive loss of β-cell function, it is difficult to target these β-cells in a therapeutic attempt to modify or reverse type-2 diabetes (T2D). We probed the existence of an intrinsic tissue property called cell fitness competition (CFC) to which we can attribute the specific recognition and clearance of the dysfunctional β-cells using CFC model of T2D (b-cell specific proteotoxicity and high fat diet, termed PFKFB3 WT DS). 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) knockout in PFKFB3 bKO DS mice led to the elimination of β-cells that adopted glycolytic metabolism, hypomaturity and/or bihormonal identity. Dysfunctional β-cell clearance resembled CFC, which enables functional cell regeneration by replacement of aged, injured, or dysfunctional (“loser”) cells with healthy, functional (“winner”) cells. We sought to investigate the signature of human dysfunctional “loser” β-cells across health, prediabetes (AAB), type-1 diabetes (T1D), and T2D utilizing Human Pancreas Analysis Program (HPAP) scRNA-Seq database and the spatial transcriptomics on the Network for Pancreatic Organ Donors with Diabetes (nPOD) T2D pancreata. We applied dual- pronged criteria to identify “loser” β-cells based on 1) the bona fide “loser algorithm” based on the literature (PMCID:PMC7611553) and 2) the PFKFB3 expression from the geotranscriptional profiling of the nPOD T2D pancreata. Comprehensive bioinformatic tools were employed to characterize “loser” b-cells while our findings were functionally validated with inhibition of PFKFB3 in PFKFB3 WT DS mice. The overlapping “loser” β-cell signatures across all disease states were marked by the distorted stoichiometry of mito- and ribosomal proteins and genes encoding mitochondrial respiration that eclipsed the impact of all other insufficiencies. Thus, the mitoribosomes and mitochondrial respiration represent a major phenotypic interface of b-cell fitness that in the presence of PFKFB3 can create an epiphenomenon of positive epistasis between individually negative effects, enabling survival and progressive accumulation of dysfunctional b-cells in diabetes.

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Mitochondrial calcium concentration is an important mediator of mitochondria-lipid droplet detachment

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Abstract

Peridroplet mitochondria (PDM) are a distinct population of mitochondria bound to lipid droplets. Compared to cytoplasmic mitochondria, PDM exhibit a unique metabolic phenotype of decreased β-oxidation and increased ATP generation, which drives triacylglycerols (TAG) synthesis and the accumulation and expansion of lipid droplets. Elucidating the mechanisms of PDM attachment and detachment from lipid droplets is vital to controlling mitochondria-lipid droplet association, thereby deciphering the role of PDM in disease. We previously identified SEN-1 as an inducer of PDM detachment from lipid droplets in a cellular imaging-based screen for mitochondria-lipid droplet attachers and detachers. Here we confirm the detachment effect of SEN-1 and show that an increase in mitochondrial calcium is an essential component of the mechanism by which SEN-1 induces detachment. Furthermore, inhibition of mitochondrial calcium extrusion, via knockout of the mitochondrial sodium calcium exchanger NCLX, results in reduced mitochondria-lipid droplet association. Inhibition of mitochondrial calcium influx prevents the SEN-1-mediated detachment of mitochondria from lipid droplets. We propose that elevation of mitochondrial matrix calcium concentration is a key event in the detachment of PDM from lipid droplets.

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Saliva as a non-invasive sampling biomarker to measure mitochondrial respirometry in humans

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Abstract

Mitochondria have been well studied as the primary producers of ATP in the cell, but more recent research has shown that mitochondria have a myriad of roles in the diagnosis and progression of multiple different diseases. At the forefront of monitoring mitochondrial status is respirometry, an assay which uses oxygen consumption as a surrogate readout for mitochondrial activity. While the current standards for mitochondrial respirometry assess the primary tissue for mitochondrial monitoring, these techniques require invasive sampling methods and intensive processing requirements, making them largely prohibitive to utilize. Recent research has identified a possible solution to this challenge though; the respirometry profiles of systemic markers like PBMCs may be used indirectly measure the mitochondrial status of the tissue of interest. While this breakthrough has made repeated patient sampling for mitochondrial monitoring more feasible, the intensive processing requirements still prevent large-scale adoption of respirometry methods for clinical and research use. Our lab has identified that PBMCs from salivary mouthwash may be used as a systemic marker of mitochondrial health. These cells may be repeatedly sampled in a non-invasive manner and require very little processing and storage requirements, overcoming the hurdles preventing large-scale assessments of mitochondrial respirometry. Our poster will detail the isolation of this novel sample type and the optimizations required to run respirometry on these cells using a Seahorse respirometer.

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Calcium overload, cristae remodeling, and oxidative phosphorylation impairment

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Abstract

Mitochondrial calcium homeostasis has been of great interest to many scientific communities. This is because mitochondria respond to calcium in a manner that can either promote health or cause disease. The orthodox view is that calcium stimulates energy metabolism through a variety of mechanisms if kept below toxic levels. When mitochondrial calcium levels get too high, a phenomenon known as mitochondrial permeability transition occurs which turns mitochondria from ATP producers into ATP consumers. However, before this transition event occurs, mitochondria store excess calcium as calcium phosphate precipitates of heterogenous composition. In this calcium overloaded state, mitochondria remain intact and functional, albeit with reduced oxidative capacity, and reveal dramatically altered cristae morphologies when imaged with cryo-electron tomography. We show that calcium phosphate precipitates appear to modulate isolated mitochondrial membrane morphology and depress ADP-stimulated respiration which implies a link between structure and function. From these results, a new model of mitochondrial operation is coming into focus, and a testable hypothesis emerges. In this model, cristae junctions play a key role in energy homeostasis, and as a corollary, maintaining cristae junction integrity preserves mitochondrial oxidative capacity. Overall, these findings establish a mechanism of calcium- induced mitochondrial dysfunction which may be causal in many diseases. Thus, new emerging therapeutics that maintain cristae structure and integrity may translate into preserved or enhanced mitochondrial function during stress.

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Novel variants in ATP6V0a1 are associated with dysfunction of autophagy, nutrient sensing and mitochondria in skeletal muscle

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Abstract

The Vacuolar H+-ATPase is a multi-subunit proton pump involved in the acidification of lysosomes during autophagy. ATP6V0a1, a gene implicated in neurodevelopmental and neurodegenerative disease, encodes the a1 subunit of this proton pump. Whilst these disorders predominantly affect the nervous system, milder phenotypes may be associated with hypotonia and mobility impairments. The importance of variants of this gene outside of the nervous system is largely unknown, as well as processes affected by the variants associated with milder neurodevelopmental phenotypes.

We investigated the role of ATP6V0a1 in muscle homeostasis by siRNA-mediated knockdown and overexpression of wild type or mutant ATP6V0a1 discovered in patients, in human myoblasts. In addition to lysosomal accumulation and changes in nutrient sensing pathways, changes in autophagy proteins, e.g. p62 and mitochondria-associated proteins, such as mitofusins, Pgc1a, or TOM20, were observed as a result of knockdown or variant overexpression. Differences in morphology of mitochondria observed by Mitotracker staining were also observed. RNA-Seq revealed changes in proteins associated with redox homeostasis. NFkB activation was also significantly reduced following ATP6V0a1 knockdown which may be associated with lysosome sensing of pro-inflammatory cytokines as a result of ineffective lysosomal acidification by Vacuolar H+-ATPases. Myogenic potential was negatively influenced by ATP6V0a1 knockdown or variant expression. Together, these data support the critical nature of ATP6V0a1 function in muscle homeostasis and suggest another layer of pathogenesis in patients with V-ATPase conditions.

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Examining the role of cytosolic mtDNA during Drosophila aging

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Abstract

Mitochondrial dysfunction and pro-inflammatory signaling are each thought to be key drivers of aging. However, a clear understanding of the connections between mitochondrial homeostasis, immune signaling and aging remains elusive. Mitochondrial DNA (mtDNA) is normally kept within the mitochondria. However, under conditions of mitochondrial stress or damage, mtDNA can be released into the cytosol thus encountering cytosolic DNA sensors and activating pro-inflammatory responses. Cytosolic mtDNA has been reported in the context of age-related disease. However, fundamental questions remain regarding the occurrence and role of cytosolic mtDNA in aging and age-related health decline. In preliminary work, we find that aging leads to a striking decline in mitochondrial autophagy and a concurrent accumulation of cytosolic mtDNA, which is linked to pro-inflammatory signaling in different organ systems of Drosophila including the brain. Critically, we have discovered that adult-onset, neuron-specific silencing of EYA, a molecule involved in sensing cytosolic DNA, dampens inflammatory signaling in the aged brain and extends lifespan. In addition, we have developed genetic approaches to reduce cytosolic mtDNA, via increased lysosomal DNase activity, in aged flies. Overexpression of DNase II reduces the accumulation of cytosolic mtDNA during aging and prolongs Drosophila lifespan. Our findings indicate that targeting cytosolic mtDNA accumulation could help to alleviate the symptoms cause by “inflammaging”, and provide an important first step towards understanding the mechanistic interplay between cytosolic mtDNA, immune signaling and healthspan.

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Sexual dimorphism in renal metabolism, hemodynamics and diseases

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Abstract

Mammalian organs exhibit distinct physiology, disease susceptibility and injury responses between the sexes. Especially, age-related decline of renal function is faster in males than in females, and males experience increased susceptibility to both chronic and acute kidney diseases. In the mouse kidney, sexually dimorphic gene activity maps predominantly to proximal tubule (PT) segments, where most reabsorption of water and salt happens, with a high energetic demand for mitochondria. Recent work suggests that male PTs undergo excessive oxidative stress to meet the high energetic demand, while female PTs exhibit an anti-oxidation state. However, it remains elusive how the observed molecular differences relate to sex disparities in renal physiology and pathophysiology. Widely known as an indicator of renal function, glomerular filtration rate (GFR) is tightly regulated by tubuloglomerular feedback (TGF) within the nephron to optimize ultrafiltration of plasma and reabsorption of essential molecules. Importantly, GFR shows a sustained oscillatory pattern over time with a period of 30-45 seconds in rodents, and loss of GFR oscillations is associated with cessation of reabsorption activities and ischemia-reperfusion injuries in the kidney, as well as systemic hypertension. To study the relationship between intracellular events and organ physiology, we developed a mathematical model of TGF linking metabolic regulation within PT cells to fluid handling and salt reabsorption in the nephron, using intra-vital imaging data from both sexes. Bifurcation analysis revealed that due to heightened oxidative profile to supply energy for reabsorption, male PTs operate near the critical boundary between sustaining and losing oscillatory behavior in the system, hence they are prone to damage upon metabolic stress. In contrast, female PTs situate further away from the boundary in the parameter space and are more resilient to failure. Combining physiological measurement and mathematical modeling of renal hemodynamics, this study provides a mechanistic explanation for sexual dimorphism in kidney diseases.

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Mitochondrial dysfunction in retinal degeneration-a pilot study based on the model of inherited optic atrophy

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Abstract

Autosomal Dominant Optic Atrophy (ADOA) is a primary inherited optic neuropathy caused by mutations in the *OPA1* gene. Despite its prevalence, there have been no reports of *Opa1* mouse models with missense mutations, which are the most common protein-coding mutations found in patients with ADOA. To gain insights into ADOA pathophysiology and study the impact of mitochondrial function on retinal ganglion cell (RGC) survival under degeneration conditions, we generated an *Opa1* knock-in mouse model based on a patient mutation. Homozygous mutations of *Opa1* are embryonically lethal. However, heterozygous *Opa1* mutant mice exhibit normal longevity and fertility. Over time, they gradually develop RGC dysfunction, which anatomically and functionally resembles human ADOA. This dysfunction includes statistically significant RGC loss observed in immunohistochemical staining, decreased pattern electroretinogram amplitudes, and reduced retinal nerve fiber layer thickness, as measured by spectral domain optical coherence tomography. Transmission electron microscopy revealed various abnormalities in the mitochondria of *Opa1* mutant mice, including vacuolation of the myelin sheath, separation of the inner and outer mitochondrial membranes, loss of cristae, and vacuolation within the mitochondria. Additionally, these mutant mice have fewer myelinated axons compared to their wild-type littermates. Immunoblotting analyses indicated reduced levels of PGC1α, antioxidant proteins, and PARKIN, along with elevated pLKB1 expression in *Opa1* mutant mouse retinas. These findings suggest reduced mitobiogenesis and mitophagy, increased oxidative stress, and higher ATP demands. Seahorse metabolic analyzer data demonstrated decreased Complex I activity in the retinas of one-month-old Opa1 mutant mice. Comprehensive RNA data provided further insights into the molecular shifts occurring in this model, shedding light on mechanisms related to RGC degeneration associated with *OPA1* mutations. In conclusion, our novel *Opa1* mutant mice accurately mimic ADOA seen in patients, making them a valuable model for investigating underlying mechanisms and testing potential gene therapies for this condition.

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Loss of the mitochondrial inorganic phosphate transporter impairs β cell glucose-stimulated insulin secretion despite a maintenance of ATP levels

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Abstract

The classical model for β cell glucose-stimulated insulin secretion (GSIS) depends on mitochondrial metabolism to generate ATP, subsequent increases in the ATP/ADP ratio, thus leading to closure of the ATP sensitive potassium channel (also known as the triggering pathway). However, recent studies have challenged the importance of mitochondrial ATP in the regulation of GSIS. *Slc25a3* encodes the mitochondria-specific inorganic phosphate transporter, PiC, which is vital for phosphate transport, and ultimately ATP generation. Mutations in *SLC25A3* lead to myocardial and skeletal myopathies and premature mortality in humans; however, the role of PiC in pancreatic β cells and diabetes is unclear. Thus, we aimed to determine the importance of mitochondrial inorganic phosphate transport on ATP generation and GSIS in β cells.

To characterize the role of *Slc25a3* in β cell function *in vivo*, we generated β cell specific knockout animals utilizing the Ins1-Cre knockin model. PiC knockout (β-*Slc25a3*^{KO}) mice developed elevated random blood glucose levels at 2 weeks of age with glucose intolerance beginning at 3 weeks that dramatically worsened with age. Despite increases in mitochondrial mass, β-*Slc25a3*^{KO} islets had significantly impaired glucose-stimulated oxygen consumption. Further, we detected a 2-fold decrease in β cell mass at 6 weeks of age that was associated impaired β cell replication, without an induction of apoptosis or immaturity. Contrary to our expectations, ATP concentrations were not reduced in β-*Slc25a3*^{KO} islets. In addition, ATP/ADP ratio and Ca^{2+} oscillations were *elevated* at 2 mM glucose and were not significantly induced following glucose stimulation when compared to controls.

Together, our data will determine how β cells survive without a functional phosphate carrier for the purposes of mitochondrial ATP generation, including previously unknown pathways to compensate for the severe bioenergetic changes following *Slc25a3* loss. Additionally, insights garnered from this model may further clarify mechanisms underlying β cell GSIS, particularly in the contribution of mitochondrial ATP production to the triggering pathway.

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Novel small-molecule improves mitochondrial function and mitophagy in a complex III deficiency model

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Abstract

Mitochondrial diseases are clinically, and genetically heterogeneous conditions characterized by defects in oxidative phosphorylation. In this way, accumulation of damaged mitochondria in these cells can be a common pathophysiological mechanism and a target for treatment. Mitophagy, the selective degradation of mitochondria by autophagy, is one robust mechanism to induce the elimination of damaged mitochondria and can be used as a broad-spectrum treatment for mitochondrial dysfunctions. Screening of mitochondrial disease patients' derived fibroblasts with different mutations showed impaired mitophagy in most of the cells analyzed. Here, we report on a novel mitophagy inducer CAP-1902 and the assessment of its effect on mitochondria disease patient fibroblasts. CAP-1902 is an agonist of the MAS G-Protein Coupled Receptor. Analysis of mitophagy was performed in mutant fibroblasts carrying the BCS1L mutation, which impairs CIII assembly, leading to reduced activity of CIII and impaired respiration, as well as the accumulation of depolarized and fragmented mitochondria and defects in lysosomal distribution. RNA-seq analysis revealed that treatment with CAP-1902 effected mitochondrial biogenesis and mitophagy related targets. The analysis using the mito-QC reporter showed an accumulation of mitolysosomes in CIII deficient cells when compared to control, and that accumulation was cleared after treatment with CAP-1902. Clearance of damaged mitochondria was accompanied by an improvement of bioenergetic function, decreased mitochondrial stress, and corrected lysosomal distribution. In summary, we describe the first potential GPCR-mediated treatment of a mitochondrial disease model.

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Ca^{2+} signaling in cancer: learning from Uveal Melanoma

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Abstract

Most cases of uveal melanoma (UM) have a constitutively activating mutation in the α subunit of the heterotrimeric G protein $G\alpha_q$ or $G\alpha_{11}$. These mutations are predominantly α_qQ209L, α_qQ209P and α_qR183C. Activated $G_{q/11}$ binds and stimulates phospholipase C-β leading to production of the second messengers diacylglycerol and inositol trisphosphate (IP3). The role for α_qQ209L/P/R183C-induced IP3 upregulation in activation of Ca^{2+} signaling remains unclear. We hypothesized that the constitutively activating mutations induce IP3-linked cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) oscillations in unstimulated cells leading to derangement of Ca^{2+} signaling.

Simultaneous $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ single cell imaging in HEK293 $\alpha_{q/11}$ CRISPR KO cells expressing wild type (WT) and α_q mutants was performed for 10min. α_q mutants-rescued cells showed asynchronous spontaneous $[Ca^{2+}]_c$ oscillations in 40% of the cells, whereas the WT α_q rescued only in 10%. The spontaneous $[Ca^{2+}]_c$ oscillations also propagated to the mitochondria inducing a resting $[Ca^{2+}]_m$ rise measured by the fluorescence of a mitochondrial matrix-targeted Ca^{2+} sensing fluorescent protein that was twice brighter in the α_qQ209L/P/R183C as compared to WT α_q-rescued cells. Upon stimulation of the cells with Carbachol, $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ were unaltered in α_qQ209L/P/R183C-rescued cells, whereas the WT α_q-rescued cells showed a robust rise.

Next, we studied patient-derived UM and melanoma cell lines. Five UM cell lines with α_qQ209L/P or α_{11}Q209L/P mutations (UM004, OMM1.3, 92.1, MEL202 and UM002B) showed spontaneous $[Ca^{2+}]_c$ oscillations in >40% of the cells, whereas a melanoma cell line without mutant $\alpha_{q/11}$ (OCM-3) in 10%. Upon stimulation with ATP, the $[Ca^{2+}]_c$ rise in UM cell lines was suppressed as compared to that in OCM-3.

In conclusion, $\alpha_{q/11}$ mutations result in sustained $[Ca^{2+}]_c$ oscillations in both UM and HEK cells, which also become desensitized to agonist-induced $[Ca^{2+}]_c$ signaling. In turn, $[Ca^{2+}]_m$ is dysregulated. The impact of altered calcium homeostasis on cell survival and tumorigenesis will be addressed by ongoing studies.

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Investigating the role of IP3receptors in Non-alcoholic fatty liver disease induced changes in hepatic ER-mitochondria contacts

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a widespread chronic liver disease affecting a significant global population, with limited therapeutic cure demanding better comprehension to identify potential biomarkers. Closely apposed membrane regions of ER and mitochondria, termed as ER-mitochondria contacts (ERMCs) participate in locally conveying Ca^{2+} signals, lipid exchange, ROS regulation among other essential functions governing cell metabolism. The key player mediating release of Ca^{2+} from ER is the inositol 1,4,5 triphosphate receptor (IP3R). Dysregulated ER Ca^{2+} handling, IP3R linked Ca^{2+} transfer and mitochondrial dysfunction have been implicated in NAFLD but the mechanistic details are obscure. We hypothesize that ERMCs are altered structurally under NAFLD leading to functional changes. In this work we studied the structure of ERMCs in NAFLD in genetic models of control and IP3R knockdown (KD) mice. NAFLD was induced with 60% High-fat choline-deficient diet (HFD) for 3 and 7 weeks mimicking fatty liver and more sever non-alcoholic steatohepatitis (NASH) respectively. Efficacy of the diet was validated with histological and biochemical marker measurements showing lipid droplet accumulation and higher triglyceride (TG) levels. The IP3R triple knockdown (TKD) and IP3R type2 (IP3R2) knockdown (IP3R2KD) were validated at the level of protein abundance and Ca^{2+} specific phenotype with fluorometric measurements and imaging studies in hepatocytes derived from control and KD mice which together showed time-dependent abolition of IP3R expression and function. IP3R2 isoform has been shown to be the most effective in delivering Ca^{2+} to mitochondria at the ERMCs in cell lines. We intended to look at the role of IP3R in modulating hepatic ERMCs in NAFLD and if there is any isoform specific relevance. Electron microscopic (EM) analysis of ERMCs along with histological and biochemical studies in different genetic mice models of IP3R KD have enabled us to examine the structure-function of hepatic ERMCs under NAFLD vs regular diet along with a look at the isoform specific contribution of IP3R2.

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Novel functions of mitochondrial transcription factor A in damaged mitochondrial DNA turnover

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Abstract

In higher eukaryotic cells, mitochondria are essential subcellular organelles for energy production, cell signaling, and the biosynthesis of biomolecules. The mitochondrial DNA (mtDNA) genome is indispensable for mitochondrial function because it encodes protein subunits of the oxidative phosphorylation system and a full set of transfer and ribosomal RNAs. Although the fast turnover of mtDNA has been known for decades, it is only in the recent decade or so that the emerging role of mtDNA turnover as an active damage response mechanism has been recognized. Nonetheless, the molecular mechanisms of mtDNA turnover remain poorly understood. Mitochondrial transcription factor A (TFAM) is a major mtDNA packaging protein and a transcription factor. TFAM plays a critical role in mtDNA replication and transcription. Our recent research demonstrates a novel role of TFAM in regulating mtDNA turnover at abasic (AP) sites. AP sites are among the most abundant endogenous DNA modifications and DNA repair intermediates. We demonstrate that the stability of AP sites is reduced dramatically upon binding to TFAM. The half-life of AP lesions within TFAM-DNA complexes is 2- to 3 orders of magnitude shorter than that in free DNA, depending on their position. The TFAM-catalyzed AP-DNA turnover produces TFAM-DNA cross-links and single-strand breaks conjugated with glutathione in human cells. We developed assays to quantify TFAM-DNA cross-links and glutathione-DNA conjugates in cultured human cells. Using next-generation sequencing-based mapping for AP sites, we found that the accumulated AP sites correlate with low TFAM density sites from published TFAM ChIP-seq data. Collectively, our research points to a novel role of TFAM in facilitating the damaged mtDNA turnover. Our ongoing research is investigating the signaling role of TFAM-DNA cross-links and glutathione-DNA conjugates in triggering mtDNA turnover, with a goal of fully elucidating the molecular mechanism of mtDNA turnover in the future.

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Glycolytic metabolon assembly on mitochondria via O-GlcNAcylation

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Abstract

Glucose is the major fuel of cells, and its metabolism starts with the activity of the first rate-limiting enzyme Hexokinase (HK). HK1 is the dominant isoform in the brain, and it is mostly associated with the mitochondrial outer membrane. The positioning of HK1 on mitochondria is critical because it couples two energy generation pathways: Glycolysis and mitochondrial oxidative phosphorylation. Here, we report a new molecular mechanism which regulates HK1 activity and its localization on mitochondria via metabolic sensing enzyme O-GlcNAc transferase (OGT). OGT catalyzes a reversible post-translational modification by adding a GlcNAc sugar moiety to serine and threonine residues, this step is called O-GlcNAcylation. The catalytic activity of OGT is regulated by intracellular UDP-GlcNAc concentrations, which in response to glucose flux through the hexosamine biosynthetic pathway. In this study, we show that HK1 is dynamically modified with O-GlcNAcylation at its regulatory domain. O-GlcNAcylation of HK1 is elevated when OGT activity is genetically or pharmacologically upregulated. We further characterized that O-GlcNAc modification increases mitochondrial HK1, Aldolase A and Pyruvate kinase isozyme M. Increasing O-GlcNAcylation also enhances both glycolytic and mitochondrial ATP production rates. By mutating O-GlcNAcylation site of HK1, we see a decrease in both glycolytic and mitochondrial ATP production rates and dysfunction of presynaptic vesicle releasing in neuron. Our findings may reveal key molecular pathways that couple neuronal metabolism to mitochondrial function via OGT, and how their dysregulation leads to neurological disorders.

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Is STX4 the next contender in lipotoxic-stressed skeletal muscle mitophagy?

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Abstract

Excessive intramyocellular lipid accumulation in skeletal muscle via chronic high fat diet (HFD) is a major contributor for lipotoxic-induced promotion of inefficient and damaged fragmented mitochondrial networks that exacerbates reactive oxygen species (ROS) production. An important quality control mechanism to mitigate these effects is mitophagy, a selective form of macro-autophagy that removes and disposes of damaged mitochondria. Although studies have shown that mitophagy serves as an early adaptive response to mitigate ROS production under short-term HFD exposure, chronic HFD culminates in reduced mitophagy, leading to uncontrolled mitochondrial damage, suggesting that mitophagy may serve as a useful strategy to improve mitochondrial health in lipotoxic-stressed skeletal muscle. With mitophagy a complex process that utilizes ubiquitin-dependent disposal via the PINK1-PARKIN pathway or receptor-mediated disposal by outer- and inner-mitochondrial membrane (OMM and IMM) proteins with LC3 interacting motifs, there are a number of known and novel therapeutic targets. Recently, we discovered that Syntaxin 4 (STX4), traditionally a cell surface transmembrane SNARE protein required for glucose uptake, was also associated with the OMM in mouse skeletal muscle. Furthermore, we demonstrated that STX4 enrichment in skeletal muscle-specific STX4 transgenic (skmSTX4-dTg) male mice reversed HFD-induced fragmented mitochondrial phenotype and reduced activation of mitochondrial fission protein Drp1, suggesting a novel role in mitochondrial quality control. However, whether this phenotype is due to STX4 expediting disposal and/or repair of damaged mitochondria remains to be determined. Using the immortalized human LHCN-M2 skeletal muscle cell line our preliminary transmission electron microscopy images for the first time have shown the presence of OMM-associated STX4 in human skeletal muscle. Furthermore, enrichment of STX4 in lipotoxic-stressed LHCN-M2 myotubes elevated LC3 presence in mitochondrial fractions, suggesting a possible role in mitophagy. Thus, we propose a strategy of determining whether STX4 alleviates lipotoxic mitochondrial damage through mitophagy.

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Imaging mitochondrial cristae in live cellular models of mitochondrial diseases

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Abstract

The inner mitochondrial membrane (IMM) is comprised of inner boundary membrane (IBM) from which cristae involute into the mitochondrial inner space. OXPHOS complexes reside primarily within the cristae membrane. Different cristae within the same mitochondrion show significant membrane potential (ΔΨm) differences, suggesting that each mitochondrion can have heterogeneous cristae structures and function. Whether the intra-mitochondrial diversity of cristae structure and function play a role in disease has not been investigated. Our objective is to utilize skin-derived fibroblasts from primary mitochondrial disease patients to investigate the extent of this heterogeneity and the impact of various mutations on live IMM organization. We hypothesize that specific OXPHOS deficiencies and genetic backgrounds may manifest as a change in membrane potential distribution across the cristae population within a mitochondrion, which may have direct implications on the organelle energetics and fate. To image mitochondria at individual cristae resolution, cells were stained with mitoOrange dye (Abberior) and imaged using Stimulated Emission Depletion (STED) microscopy (STEDycon). To assess mitoOrange dye accumulation in the IMM, we first measured membrane potential to understand if the dye accumulation is dependent on it. We used TMRE plus MitoTracker green staining in control cells and compared the effects of Oligomycin-induced hyperpolarization and FCCP-induced depolarization with mitoOrange plus MitoTracker green stained cells. While TMRE yielded expected results, no differences were observed in mitoOrange under any tested conditions, suggesting that the dye is not dependent on membrane potential. Subsequently, we evaluated morphological parameters, such as the number of cristae per mitochondria and the distance between cristae. Our findings demonstrate that mitoOrange dye can detect differences in cristae number in OXPHOS deficiency models.

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Peripheral blood mononuclear cells from older adults exhibit sex-associated differences in mitochondrial function

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Abstract

Blood based mitochondrial bioenergetic profiling is a feasible, economical, and minimally invasive approach that can be used to examine mitochondrial function and energy metabolism in human subjects. In this study, we use two complementary respirometric techniques to evaluate mitochondrial bioenergetics in both intact and permeabilized peripheral blood mononuclear cells (PBMCs) to examine sex dimorphism in mitochondrial function among older adults. Employing equal numbers of PBMCs to assess mitochondrial bioenergetics, we observe significantly higher respiration rates in female compared to male participants. Bioenergetic differences remain significant after controlling for independent factors including demographic parameters (age, years of education), and cognitive parameters (mPACC5, COGDX). Our study illustrates that circulating blood cells, immune cells in particular, have distinctly different bioenergetic profiles between females and males. These differences should be taken into account as blood based bioenergetic profiling is now commonly used to understand the role of mitochondrial bioenergetics in human health and aging.

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Mitochondrial calcium signaling regulates branched chain amino acid catabolism in fibrolamellar carcinoma

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Abstract

Fibrolamellar liver cancer (FLC) is a rare cancer that affects adolescents and young adults and has a 5-year survival rate of less than 50%. One of the distinguishing histological features of FLC is abundance of mitochondria. FLC mitochondria appear swollen and harbor electron dense particles, indicative of calcium overload. Such overload is typically associated with mitochondrial damage and cell death, which is evidently absent in FLC. The function of excess mitochondrial calcium in the absence of apoptosis remains unknown.

Calcium ions enter the mitochondria through the mitochondrial calcium uniporter, a multi-protein complex composed of MCU, MCUb, EMRE, MICU and MICU2. MCU forms the pore, other components regulate channel activity. The main function attributed to physiological increases in mitochondrial calcium levels is stimulation of the TCA cycle. Other roles for calcium in the mitochondria are longstanding open questions in the field. To better understand calcium regulation of mitochondria, we characterized cells that lack MCU or overexpress it. RNA sequencing and proteomics revealed that branched chain amino (BCAA) catabolism is an MCU-regulated pathway: MCU loss activates BCAA catabolism, whereas MCU overexpression inhibits this pathway. Tumors from FLC patients also show strong inhibition of BCAA catabolism. This downregulation is transcriptionally regulated through the transcriptional activator KLF15 in a calcium-dependent manner. In addition, MCU is required for faster growth of FLC cells compared to controls. We conclude that mitochondrial calcium overload in FLC leads to reduced BCAA degradation downstream of a transcriptional program that involves KFL15. We posit that this leads to BCAA accumulation, and activation of growth pathways, such as mTORC1.

This work identifies a new calcium-regulated mitochondrial metabolic pathway. Our results also point the presence of an MCU-responsive transcriptional network that supports cell growth through regulation of BCAA catabolism in FLC, and possibly other disease contexts with increased mitochondrial calcium levels.

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Mitochondrial Pearling: an emerging class of mitochondrial dynamics

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Abstract

Mitochondrial networks exhibit remarkable dynamics, traditionally attributed to fission and fusion events. These processes facilitate the transition of mitochondria between isolated segments and interconnected networks. However, certain network reorganizations occur independently of fission and fusion. A notable example is the elusive ‘beads-on-a-string’ morphology. Here, mitochondrial branches, which are normally thought of as uniform cylinders, transiently undergo shape changes forming "pearls" connected by thin tubes. This phenomenon has been understood through the biophysical properties of tubular fluid membranes. Under tension, membranes reach an instability threshold, triggering a transformation into compartmentalized units. In this study, we delve into this unique class of dynamics, termed mitochondrial pearling. We begin by observing that mitochondrial pearling occurs spontaneously in a variety of cell cultures including primary human fibroblasts, immortalized epithelial cells, and budding yeast. Our observations reveal that these pearling events coincide with “flashes”; in membrane potential, pH, reactive oxygen species (ROS), and calcium levels. This event is further premted by alterations in mitochondrial membrane tension, a precursor to the pearling instability. Furthermore, we introduce several novel methods to induce mitochondrial pearling, including the ability to trigger pearling within a single contiguous mitochondrion. We also explore the compartmentalization of mitochondria, presenting a novel mechanism for the organization of mitochondrial DNA, independent of fission and fusion. Finally, we deploy live-cell STED microscopy to establish the structural reorganization of cristae membranes during mitochondrial pearling. Together, this work establishes a new frontier for mitochondrial dynamics governed by the biophysics of a pearling instability.

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Characterizing the role of polynucleotide phosphorylase in mitochondrial double-stranded RNA (mtdsRNA) escape and elicitation of the innate immune response

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Abstract

The inner and outer membranes of mitochondria contain specific transporters to regulate the movement of macromolecules. In addition to proteins and mitochondrial DNA, a diverse cohort of RNA species including mitochondrial double stranded RNAs (mtdsRNAs), cytosolic tRNAs, small and long non-coding RNAs, and viral RNAs, are imported as well as exported from mitochondria. However, the specific channels for RNA transport have not been demonstrated and the translocation route may vary by species. Here we begin to characterize candidates that participate in an export pathway for mtdsRNAs from the mitochondrial matrix to the cytosol in cultured cells. Downregulation of SUV3 resulted in accumulation of mtdsRNAs in the matrix, whereas downregulation of PNPase resulted in export of mtdsRNAs to the cytosol. Inhibiting or downregulating outer membrane proteins VDAC and BAK/BAX or inner membrane proteins PHB1/2 strongly attenuated the export of mtdsRNAs to the cytosol. The cytosolic mtdsRNAs subsequently localized to large granules that also contained the stress protein TIA-1 and activated the Type-1 Interferon pathway. Abundant mtdsRNAs were detected in a subset of Non-Small Cell Lung Cancer cell lines that were glycolytic, indicating relevance in cancer biology. In sum, we propose that mtdsRNA is a new damage-associated molecular pattern that is exported in a regulated manner under conditions of mitochondrial stress.

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Myc inhibition impairs pancreatic β-cell function, identity, and mitochondrial bioenergetics while enhances mitophagy markers

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Abstract

Glucose enhances mitochondrial function, insulin secretion and Myc expression in β-cells. β-cell-specific Myc knockout mice show glucose intolerance, hypoinsulinemia and lack of adaptive β-cell mass expansion following high-fat diet feeding. However, whether Myc regulates insulin secretion and mitochondrial function in β-cells is unknown. Here, we tested the effects of the Myc inhibitor 10058-F4 (1RH) in glucose-stimulated insulin secretion (GSIS), expression of β-cell identity markers, mitochondrial bioenergetics, and markers of mitochondrial biogenesis, dynamics and mitophagy using islet perifusion, Seahorse, transcriptomics, qPCR and western blot approaches. Mouse and human islets incubated 6h with 40μM 1RH displayed impaired insulin secretion induced by 11mM glucose. Since adequate mitochondrial function is essential for GSIS, we measured oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) to analyze mitochondrial respiration and glycolytic flux in these islets. 1RH significantly reduced OCR, ECAR and glucose-induced ATP production. RNAseq of mouse islets treated with 1RH in 11mM glucose revealed reduced expression of both oxidative phosphorylation and β-cell identity genes but enhanced expression of autophagy/mitophagy genes. These changes were validated by qPCR and western blot. No changes were observed in markers of mitochondrial biogenesis and dynamics. In conclusion, Myc is required for GSIS and mitochondrial function in β-cells. Impaired Myc action may lead to unbalanced metabolism and enhanced mitophagy resulting in β-cell dysfunction.

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Molecular mechanism of oxidative mitohormesis in heart

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Abstract

Cardiovascular diseases (CVD) are the leading cause of global mortality and studies strongly implicate mitochondrial dysfunction in CVD and cardiac aging. While this encompasses declines in mitochondrial energetics, multiple defects in mitochondrial function also drive CVD including decreased mitochondrial biogenesis and enhanced mitochondrial reactive oxygen species production. Despite this, few mitochondria-targeted therapies have been successful in clinical trials, in part because therapeutics target singular mitochondrial functions or cause unanticipated alterations that exacerbate pathology. Thus, there is a need to identify therapies that optimally modulate multiple mitochondrial parameters in parallel to restore cardiac homeostasis. Oxidative mitohormesis describes a form of adaptive signaling where transient mitochondrial oxidative stress activates sustained, beneficial cytoprotective programs. This increases lifespan in invertebrates, confers protection from oxidative stress in mouse embryonic fibroblasts (MEFs), and increases mitochondrial biogenesis and basal antioxidant capacity in mouse liver. To test the effect of oxidative mitohormesis in murine cardiac tissue, we developed a mouse model of oxidative mitohormesis termed iSOD2 mice. These mice allow for transient induction of mitochondrial oxidative stress during development via reversible knockdown of the mitochondrial antioxidant protein superoxide dismutase 2 (SOD2). Following a 6-week recovery period, adapted iSOD2 cardiac tissue exhibited increased mitochondrial content and expression of the mitochondrial biogenesis transcriptional coactivator PPRC1. Additionally, iSOD2 hearts displayed increased basal expression of antioxidant enzymes, implying heightened antioxidant buffering capacity. To understand how transient mitochondrial stress confers sustained, adaptive responses, we asked how oxidative mitohormesis affects cellular epigenetics by probing global histone post-translational modifications in iSOD2 MEFs, liver, and cardiac tissue. MEFs and liver tissue had increased H3K27ac while cardiac tissue specifically upregulated H3K9ac, indicating there are cell-type specific responses to oxidative mitohormesis. Overall, these results suggest that cardiac oxidative mitohormesis may reprogram the epigenetic landscape to increase mitochondrial biogenesis and basal antioxidant protein expression, which could potentially treat CVD.

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Regulation of mitochondrial respiration by interferon stimulated gene-15 (ISG15) in melanoma

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Abstract

Interferons (IFN) modulate both innate and adaptive immune responses not only against viruses as originally described, but also in pathologies like autoimmune diseases and cancer. Exposure to a single high dose of IFN is often cytotoxic and pro-apoptotic; however, sustained stimulation by low doses of IFN, as often seen in cancers, can provide a pro-survival advantage. IFNs exert their effect through expression of IFN-stimulated genes (ISGs), of which one of the most abundant is ISG15. ISG15 functions in either its free form or through post-translation conjugation to cellular proteins, a process termed ISGylation. Interestingly, about 5-7% of proteins ISGylated upon an IFN response have been predicted to be localized to the mitochondria, however, precise biochemical function of these protein modifications on or in mitochondria remains unclear. Since mitochondrial metabolism underlies tumor proliferation and cell death phenotypes, we decided to determine the effect of ISG15 in cancer cells treated acutely or chronically with IFN. We generated *Isg15* knockout melanoma model YUMM 1.7 (Yale University Mouse Melanoma; $Braf^{V600E}$/ $Pten^{-/-}$/ $Cdkn2a^{-/-}$) cells and treated them with type I and II IFNs either for 24 hours or for 28 days. Chronic stimulation of sg*Isg15* YUMM cells with either type I or II IFNs reduced mitochondrial oxygen consumption compared to control ($ISG15^{+}$) cells, with maximal respiratory capacity most affected. Interestingly, mitochondrial mass was lower and mitochondrial membrane potential was higher in sg*Isg15* YUMM cells. Knockout of *Uba7*, encoding a key enzyme required for ISGylation that abrogates ISG15-conjugation with an accumulation of the free form, also decreased respiration upon chronic exposure to IFNs. These results suggest a possible role for ISGylation in regulating mitochondrial biogenesis and function during IFN-signaling. Mitochondrial proteomic studies are underway to address this further and how *Isg15* knockout affects tumor growth and immune responses in mice will be assessed in future experiments.

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Mitochondrial stress in the gut epithelium of mice overexpressing α-Synuclein

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Abstract

Parkinson's Disease (PD) may initiate in the gut, spreading to the brain via α-Synuclein through the vagus nerve. Propagation alone isn't sufficient for lasting CNS PD pathology; additional risk factors are required. Mitochondrial stress results from oxidative damage, inflammation, and mitochondrial dysfunction. Mitophagy reduces inflammation in PD and colitis models due to mitochondrial stress. Aging, a key PD risk factor, causes mitochondrial problems and reduced mitophagy.

Aim: To compare intestinal epithelial mitochondria in PD vs WT mice.

Methods: We confirmed hαSyn expression in Thy1-hαSyn mice's colon using a custom nCounter assay. Intestinal crypts from these mice were analyzed for Oxygen consumption rate (OCR) in a Seahorse XF96 analyzer at UCLA's Mitochondrial and Metabolism Core, normalized using manual crypt counts. Transmission electron microscopy was conducted at UCLA's Brain Research Institute Electron Microscopy Core Facility. ImageJ with 'analyze particles' and the JACoP plugin were used for analysis. Intestinal epithelial organoid diameters and Tom20 levels were assessed and analyzed with ImageJ. Cytokines were quantified via ELISA.

Results: OCR declined with age (10-12m vs. 1-4m) in both Thy1-hαSyn and WT mice ($p < 0.005$). Thy1- hαSyn mice showed higher OCR than WT ($p < 0.005$), especially in adults, with no significant age-genotype interaction due to limited samples. In adults, Thy-hαSyn mice displayed increased OCR across all bioenergetic components, notably in non-mitochondrial respiration ($p = 0.03$). Tom20 levels decreased in Thy1-hα-Syn mice, indicating enhanced mitophagy and increased mitochondrial fragmentation. Tissue and isolated crypts from 12-month-old Thy1-hα-Syn mice exhibited elevated IL-1β and KC (IL-8 equivalent) levels. Colonic epithelial organoids from Thy1-hα-Syn mice had smaller diameters, suggesting potential gut epithelial health decline compared to WT.

Conclusion: These findings support heightened gut epithelial mitochondrial stress in PD, possibly contributing to inflammation and gut barrier issues in patients. Understanding this stress is crucial for early PD comprehension and potential therapeutic targets.

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PCR-based enrichment methods bias detection and characterization of mitochondrial DNA deletions using long-read sequencing

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Abstract

The mitochondrial genome (mtDNA) contains essential genetic information and plays a critical role in cellular function. Given its impact on various cellular processes, accurately detecting and characterizing mitochondrial DNA mutations is of significant importance. Mitochondrial DNA deletions, particularly large deletion mutations, are known to be associated with various diseases and aging, however their specific locations and frequencies are challenging to identify in sequencing data. Due to the relatively low representation of mtDNA abundance in whole genomic DNA preparations, various methods have been used to enrich mtDNA in sequencing. Many of these methods use PCR amplification, either linear, long range, amplification or circular, isothermal, amplification. While these methods successfully enrich mtDNA sequence, the potential bias introduced by these is unknown. In this study, we investigated the bias introduced by different methods of PCR-based mtDNA enrichment on structural variant calling in long-read sequencing data. We specifically explored the impact of circular amplification and long-range PCR amplification on the identification and quantification of mtDNA deletion mutations using nanopore sequencing. Through analysis of two samples for which mtDNA deletion frequency and location have been established using gold standards, we found that the choice of PCR amplification method significantly influenced the representation of deletion events in the sequencing data. Circular amplification resulted in increased deletion frequencies and artificial deletion events, while long-range PCR amplification increased deletion frequency and showed a distinct bias towards specific large deletion events. These results emphasize the importance of considering the biases associated with PCR-based mtDNA enrichment methods when identifying and characterizing mitochondrial DNA deletions. This work provides insights into the challenges and considerations for accurately mapping and quantifying mtDNA deletions, which are essential for understanding their implications in various cellular processes and diseases. As sequencing technology continues to evolve, understanding these biases is crucial for accurate interpretation of mtDNA structural variations.

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Disentangling the MDM12-COQ10 relationship: a reassessment of the roles of Coq10 and the ER-Mitochondrial Encounter Structure (ERMES) in Coenzyme Q (CoQ) biosynthesis

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Abstract

Coenzyme Q (CoQ) is a redox-active lipid molecule that acts as an electron carrier in the mitochondrial electron transport chain, aiding in the mitochondrial production of ATP. In *Saccharomyces cerevisiae* (yeast), CoQ is synthesized in the mitochondrial matrix by a multi-subunit, high-molecular-mass protein-lipid complex termed the CoQ Synthome. Recently, the Endoplasmic Reticulum-Mitochondria Encounter Structure (ERMES) was identified as a regulator of CoQ Synthome formation and CoQ production. ERMES is a four-subunit tethering complex that forms a bridge between the ER and mitochondrial outer membrane while also colocalizing with the CoQ Synthome. The gene encoding the cytosolic subunit of ERMES, *MDM12*, is coexpressed with *COQ10*, which encodes the putative CoQ chaperone Coq10, via a shared promoter. Deletion of *COQ10* results in respiratory deficiency, impaired CoQ biosynthesis, and reduced spatial coordination between ERMES and the CoQ Synthome. While deleting *MDM12* maintains Coq10 expression, we show that deletion of *COQ10* results in a significant decrease in Mdm12 protein content. Since deletion of individual ERMES subunits leads to a loss of ERMES formation, we asked whether the phenotypes observed upon deletion of *COQ10* are solely due to the loss of Coq10 or if some of the *coq10Δ* phenotypes may result from disrupted ERMES formation. To separate the *coq10Δ* mutant phenotypes from those of the *mdm12Δ* mutant, we constructed strains expressing either a functionally impaired (Coq10-L96S) or truncated (Coq10-R147*) Coq10 isoform using CRISPR-Cas9 genome editing. Using Western blot analysis, we show that Mdm12 protein content is preserved in strains expressing these Coq10 point mutants. Importantly, we show that respiratory capacity is impaired in strains expressing either of the *coq10* mutants, a phenotype that recapitulates the *coq10Δ* mutant. Overall, this study seeks to further our understanding of the functions of Coq10 and ERMES in CoQ biosynthesis through phenotypic characterization of select *coq10* mutants.

Exploring the effect of dimerization on the activity of COQ5, a C-methyltransferase in coenzyme Q biosynthesis

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Abstract

Coenzyme Q (CoQ) is an essential redox-active lipid that acts as an electron carrier in the mitochondrial electron transport chain and a chain-terminating antioxidant. The biosynthesis of CoQ relies on a multi-subunit complex within the mitochondrial matrix deemed the CoQ synthome. Pathogenic mutations in the components of the synthome lead to primary CoQ deficiency, a condition with highly variable phenotypes including cardiovascular, kidney, and neurodegenerative disorders. One particular component is COQ5,an S-adenosylmethionine (AdoMet)-dependent C-methyltransferase. Crystal structures and previous studies have shown that *Saccharomyces cerevisiae* Coq5 is present as a dimer both in crystals and in solution. Using yeast as a model organism, we examine human missense single nucleotide variants (SNVs) with unknown clinical significance that occur on the dimer interface. Spot dilution assays surprisingly reveal that our two mutants, Coq5-A255T and Coq5-Y261H, are respiratory-competent when overexpressed. However, LC-MS/MS experiments show an apparent reduction of *in vivo* steady state and de novo CoQ levels compared to over-expression of wild-type Coq5. Future work will explore the mechanism of this biosynthetic defect by verifying wild-type Coq5, Coq5-A255T, and Coq5-Y261H protein content and disruption of dimerization through immunoblotting, protein purification, and gel filtration. To elucidate if dimerization is required for activity, we aim to perform molecular dynamic simulations to computationally assess the stability of the substrate-bound protein in monomeric or dimeric form. Given the intricate relationship between the components of the CoQ synthome, our work presents an example where a residue distal to the active site may have an effect on activity. These results will reveal the molecular mechanism underlying two SNVs that may give rise to primary CoQ deficiency in humans.

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Mutating substrate-binding residues in a promiscuous enzyme – the case of COQ5, a C-methyltransferase in coenzyme Q biosynthesis

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Abstract

Coenzyme Q (CoQ) is a vital lipid that functions as an electron carrier in the mitochondrial electron transport chain. In its reduced form ($CoQH_2$), it can also act as a chain-terminating antioxidant providing protection against lipid peroxidation. Defects in the CoQ biosynthetic pathway in humans cause a wide array of illnesses, including cardiovascular, kidney, and neurodegenerative disorders, through a condition known as primary CoQ deficiency. COQ5, an *S*-adenosylmethionine (AdoMet)-dependent *C*-methyltransferase, is one such enzyme in the pathway and is required for the benzoquinol ring modification. Using the yeast *Saccharomyces cerevisiae*, we examine mutations and human missense single nucleotide variants (SNVs) with unknown clinical significance that occur on highly conserved putative substrate binding residues. Surprisingly, mutations affecting several putative catalytic and hydrogen bonding residues in yeast Coq5 result in respiratory competent cells. Their *in vivo* steady state and *de novo* CoQ levels, as examined by LC-MS/MS, are also unaffected or only moderately reduced, suggesting modest effects on catalytic activity. Our work and recent findings also suggest that the enzyme and its eukaryotic homologs are unexpectedly active towards demethylmenaquinone-8 ($DMeMK_8$), a bulkier naphthoquinone involved in prokaryotic anaerobic respiration that is non-native to eukaryotes. We will examine the effect of mutations affecting the binding of the benzoquinol substrate on activity toward $DMeMK_8$ in *Escherichia coli*. Furthermore, we aim to computationally elucidate the molecular basis of catalysis and substrate binding in this promiscuous enzyme in the context of mutations and previously identified human SNVs. Our results will shed light on the structure-and-function relationship of COQ5 and facilitate the screening and diagnosis of pathogenic SNVs likely to cause primary CoQ deficiency.

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FASN-deficiency induces a cytosol-to-mitochondria citrate flux to mitigate detachment-induced oxidative stress

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Abstract

Fatty acid synthase (FASN) maintains de novo lipogenesis (DNL) to support rapid growth in most proliferating cancer cells. Lipogenic acetyl CoA is primarily produced from carbohydrates but can arise from glutamine-dependent reductive carboxylation. Here we show that reductive carboxylation also occurs in the absence of DNL. In FASN-deficient cells, reductive carboxylation is mainly catalyzed by isocitrate dehydrogenase-1 (IDH1), but IDH1-generated cytosolic citrate is not utilized for supplying DNL. Metabolic flux analysis (MFA) reveals that FASN deficiency induces a net cytosol-to- mitochondria citrate flux through mitochondrial citrate transport protein (CTP). Previously, a similar pathway has been shown to mitigate detachment-induced oxidative stress in anchorage-independent tumor spheroids. We further demonstrate that tumor spheroids show reduced FASN activity, and FASN-deficient cells acquire resistance to oxidative stress in a CTP- and IDH1-dependent manner. Collectively, these data indicate that by inducing a cytosol-to-mitochondria citrate flux, anchorage-independent malignant cells can gain redox capacity by trading off FASN-supported rapid growth.

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Structural remodeling of microglial mitochondria across brain regions and developmental stages

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Abstract

Microglia play essential roles in CNS development by engulfing newborn cells, pruning excess synapses, and secreting trophic and inflammatory factors that shape neuronal maturation. Microglia also undergo prominent morphological changes as their functional roles change throughout development. In peripheral macrophages, mitochondria act as central signaling hubs to regulate cell morphology and phagocytic and secretory function in response to changes in the microenvironment. Recent studies in microglia indicate that cell metabolism may similarly shape microglial functional state in different contexts. Yet the specific roles for mitochondria in regulating these key cellular functions have not been explored. In this study, we use a combination of transgenic mice and imaging approaches to generate data about microglial mitochondria across development and adulthood. Additionally, we investigate mitochondrial roles in key functions such as microglial surveillance and response to an inflammatory insult using lipopolysaccharide (LPS). To investigate these organelles, we generated mice with GFP-tagged mitochondria in microglia. We analyzed microglial mitochondria in nucleus accumbens (NAc) and ventral tegmental area (VTA), where they display differences in various cell attributes during development and adulthood. At P8, when microglia show very simplified morphology, mitochondrial mass was elevated. In young adult mice, mitochondrial mass was significantly higher in VTA microglia, which display less complex branching of cell processes. We also found a change in microglial mitochondrial mass after LPS administration during adulthood. To understand microglial dynamics, we used multiphoton imaging in acute brain slices to understand how mitochondria reorganize during microglial motility and laser injury. Altogether, our data reveal the in vivo structure of microglial mitochondria from postnatal development to adulthood. The data suggests mitochondrial status and microglial morphological complexity are linked in different contexts including maturation and inflammatory insult. This study will provide key insight into how mitochondria may regulate key microglial properties during maturation.

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Mapping the mitochondrial landscape in microglia during aging and models of PD

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Abstract

Microglia are dynamic cells that play essential roles in CNS homeostasis and CNS aging, and likely shape vulnerability to neurodegenerative disease. Recent findings indicate that cellular metabolism plays a crucial role in regulating microglial attributes, particularly during shifts between resting and activated states. This raises the exciting possibility that mitochondria act as regulatory signaling hubs within microglia and that they can be targeted to manipulate microglial function in numerous contexts, including Parkinson's Disease (PD). However, little is known about microglial mitochondrial status during aging and PD. Furthermore, it is unknown which microglial attributes (inflammatory profile, phagocytosis, etc.) are regulated by these organelles. Here we utilize novel transgenic crosses and molecular biology and imaging-based approaches to profile microglial mitochondria during aging. Using transgenic mice with EGFP-labeled mitochondria in microglia, we analyzed microglial mitochondria in the nucleus accumbens (NAc) and ventral tegmental area (VTA), where microglia show distinct cellular phenotypes and aging profiles. Confocal microscopy revealed both hyper-elongated and fragmented microglial mitochondria in 12-13 month (mo) and 16-18mo mice compared to 2-3mo mice, suggesting altered mitochondrial fission and fusion. Using FACS-based analyses, we observed decreases in microglial mitochondrial membrane potential beginning at 12-13mo, which were further reduced by 16-18mo. These reductions were larger in the midbrain, where we have previously shown that microglia exhibit early aging and inflammatory profiles. Our results support key links between mitochondrial status and regional differences in microglial responses to aging. Moving forward, we are using RNAseq and transgenic manipulation of microglial mitochondria to define how specific changes in microglial mitochondria during aging regulate discrete microglial attributes. Similar characterization is also being performed in an alpha-synuclein mouse model of PD. Together, these data begin to reveal the *in vivo* microglial mitochondrial landscape and provide a foundation for exploring an exciting potential therapeutic pathway to manipulate microglial function.

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Selective removal of deleterious mtDNA mutations from mammalian cells

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Abstract

As we grow older, mutations in the mitochondrial genome (mtDNA) accumulate in various tissues, including brain, heart, skin, intestine and skeletal muscle. When these mutations expand, they compromise mitochondrial energy production and enhance the generation of reactive oxygen species. Together, these molecular changes accelerate the natural aging process and contribute to various age-related diseases, including cancer, sarcopenia, Alzheimer's disease and Parkinson's disease. mtDNA mutations are also responsible for a wide array of pediatric diseases that are characterized by epilepsy, muscle weakness and neuronal dysfunction. To this day though, no child has been cured of an mtDNA disease, nor is there a treatment for the mtDNA component of age-related diseases. *We hypothesize that by manipulating mitochondrial fusion and mitophagy, it will be possible to clear living cells of mitochondria that carry mutant genomes*. To test this hypothesis, we used a genetic switching mechanism to create a cell line that carries a large number of mitochondrial DNA mutations. We are now using CRISPR-Cas9 technology, small chemicals and dietary restriction to manipulate mitochondrial fusion and mitophagy in an attempt to deplete these cells from deleterious mutations. These mutations are monitored with a novel DNA sequencing tool called MADDD-Seq, which allows mitochondrial DNA mutations to be detected, alongside DNA damage, in vast quantities with unprecedented precision. Together, these experiments have the potential to transform our understanding of mitochondrial genetics in aging organisms and set the stage for the development of treatments aimed at preventing or ameliorating age-related and pediatric diseases associated with mtDNA mutations.

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Unveiling the Complex problem with Mdivi-1

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Abstract

Several human diseases, from cancer to neurodegeneration, are associated with excessive mitochondrial fission. The mitochondrial division inhibitor (Mdivi-1) has been tested as a therapeutic target for inhibiting fission-related protein Dynamin-like protein 1 (Drp1) [1] [2]. A study in 2017 raised a debate about significant off-target effects such as inhibiting complex I [3].

Here, we show that the effects of Mdivi-1 on mitochondrial morphology and function, ultimately leading to cellular dysfunction of neurons, are mechanistically based on direct complex I inhibition at the IQ site. This leads to destabilization of the complex I and supercomplexes, increased ROS production and ultimately reduced mitochondrial ATP. Furthermore, the calcium homeostasis of cells is dampened, which in the long run attenuates the activity of neurons. Given the results presented here, a putative therapeutic application of Mdivi-1 will need to be reconsidered in terms of dose- and time-dependent effects on mitochondrial energy metabolism, particularly OXPHOS activity.

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Alcohol intake disproportionately effects specific hepatic mitochondrial subpopulations

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Abstract

Purpose: It is now well established that mitochondria exist as heterogenous subpopulations with different characteristics while associated with other organelles. Although it is known that hepatic mitochondria in general undergo changes in substrate oxidation rates in response to chronic alcohol intake and during the progression of alcoholic liver disease, it is not yet understood how these changes present across mitochondrial subpopulations. This study thus sought to investigate changes in hepatic subpopulations following alcohol feeding to mice.

Methods: Mitochondrial subpopulations (cytosolic-mitochondria (CM), endoplasmic reticulum-attached-mitochondria (ERM), peridroplet-mitochondria (PDM)) were isolated from previously frozen APP/PS1 double transgenic Alzheimer's mouse livers, collected at 8 weeks of age. These mice were intragastrically fed a control diet with or without alcohol for 4 weeks. CM were harvested by conventional differential centrifugation, ERM were pulled through a sucrose gradient to maintain attachment, and PDM were stripped from the fat layer taken from homogenized liver after a slow spin to achieve separation. Bioenergetic testing of mitochondrial complex I, II, and IV was conducted via Agilent Seahorse Analyzer, utilizing NADH, Succinate+Rotenone, and TMPD+Ascorbate respectively. Analyses to help determine whether these changes were based in changes in protein expression or function were conducted via western blot and RT-qPCR.

Results: Mitochondrial Complex I activity was found to be significantly different between peridroplet mitochondria in ethanol versus control mice. Alcohol feeding and its subsequent enhanced steatosis trended towards increased levels of hepatic peridroplet mitochondria.

Conclusion: It is well known that alcohol intake causes stress and mitochondrial remodeling within the liver. By determining if there is a bioenergetic shift specific to specific subpopulations of mitochondria, we may better understand not only the progression of alcoholic liver disease, but the etiology of various other diseases with a bioenergetic basis as well.

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Dexamethasone impairs mitochondrial function in trabecular meshwork cells

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Abstract

Primary open-angle glaucoma (POAG) is the second leading cause of irreversible blindness worldwide. POAG is driven by failure of the trabecular meshwork (TM), which precedes vision loss and correlates with disease severity. In POAG, TM cells show several forms of mitochondrial damage, including damage to mitochondrial DNA (mtDNA). While the molecular drivers of TM damage remain poorly understood, application of dexamethasone (Dex) and transforming growth factor β2 (TGF-β2) are commonly used to model glaucoma-like changes in TM cells. We sought to quantify the impact of Dex and TGF-β2 on ATP production and mitochondrial performance in TM cells using 3-day treatments.

We used the Agilent Seahorse XFp system to measure ATP production, sources, as well as mitochondrial performance. Treatment with TGF-β2 did not show any changes; however, Dex treatment resulted in a significant decrease in both total ATP production and ATP produced by respiration, with relative ATP levels from respiration 51% ± 5.4% ($p = 0.012$) of control. Dex treatment also resulted in significant decreases in non-mitochondrial O2 consumption, maximal respiration, and O2 consumption for ATP production, with values 74.2%±5.0% ($p = 0.012$), 62.1%±5.6% ($p = 0.007$), and 62%±4.8% ($p = 0.032$) of the control respectively. There were also non-significant decreases in spare respiratory capacity, basal O2 consumption, and a non-significant increase in proton leak.

Overall, we showed Dex can decrease mitochondrial ATP production and performance, in alignment with some reports showing that Dex can lead to increased mitochondrial damage and reduced performance. However, other reports show Dex can lead to mitochondrial biogenesis and increased performance, especially at longer time courses. For this reason, we are currently studying the mtDNA depletion in TM cells via two models; early data shows increases in levels of glaucoma-related mRNA transcripts.

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Storable mitochondria organelle complex – structural integrity, incorporation into cell and energy production

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Abstract

Introduction: Mitochondrial transplantation therapy is one of emerging approaches to treat or prevent a number of diseases which have impaired or damaged mitochondrial function in cells. We have developed the proprietary technology (*WO2021132735A2)* to isolate mitochondria that keeps mitochondrial structural and functional integrity even after storage in liquid nitrogen, which enables us to supply consistent quality of mitochondria and facilitates understanding of interaction between exogenous mitochondria and cells.

Methods and Results: Spherical mitochondria were isolated from various cultured cells. Freeze-throw mitochondria from different cultured cells maintain their inner and outer membrane integrity based on measurement of citrate formation and oxidation of cytochrome c, respectively. They can generate ATP and have anti-ROS activity against hydrogen peroxide. ATP production capability lasted more than 12 weeks after storage. mtDNA from exogenous mitochondria were detected in recipient cells as early as 2 hours after incubation and blockade of mitophagy with chloroquine further increased the level of mtDNA. When RFP-tagged mitochondria were incubated with recipient cells, dose-dependent and time-dependent incorporation of red fluorescent particles was observed. However, no fusion was observed between RFP-tagged mitochondria and endogenous mitochondria stained with mitoBright green. The FBS-starved recipient cells increased OCR as well as level of intracellular ATP 24 hours after incubation with exogenous mitochondria. Cell incorporated exogenous mitochondria reduced susceptibility against oxidative stress with hydrogen peroxide.

Conclusion: Storable mitochondria were obtained and they can incorporate into various cells without merging into endogenous mitochondria. Incorporated mitochondria were able to increase OCR as well as ATP in the stressed cells and protected cells from oxidative stress. These indicate that storable mitochondria have potential to treat diseases with impaired/damaged mitochondrial functions.

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Can calcium stand in as an alternative mechanism for thermogenesis?

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Abstract

Mammals have thermogenic brown adipose tissue (BAT) with specialized mitochondria that prioritize heat generation instead of ATP. BAT maintains body temperature and regulates metabolism by burning calories. It increases energy expenditure and thus plays a key role in combating metabolic syndrome. Rodent BAT mitochondria utilize the uncoupling protein 1 (UCP1) to dissipate the H^+ buildup across the inner membrane to produce heat. Mice without UCP1 ($UCP1^{-/-}$) do not survive in acute cold but withstand it in progressive cold, implicating an alternative mechanism of mitochondrial thermogenesis independent of UCP1. Since human brown fat has very little UCP1, the mechanism underlying heat production without this uncoupler is poorly understood. Hence, $UCP1^{-/-}$ mouse models under progressive cold exposure can reveal an alternative pathway of thermogenesis more closely reflective of that in humans. Using patch-clamp analysis to study the thermogenic ability of mitochondria after progressive cold adaptation, we found a drastic increase in Ca^{2+} current via the mitochondrial Calcium Uniporter (MCU). Western blots showed increased expression of MCU, increase in endoplasmic reticulum (ER) biomass, and changes in the adrenergic pathway. These changes suggest a remodeling of Ca^{2+} signaling within BAT adipocytes which are likely to involve mitochondria-ER membranes (MAMs) that regulate calcium homeostasis. We first studied key MAM proteins that could play a role in mitochondrial calcium-dependent thermogenesis such as GRP75, VDAC and IP3R. Using the proximity ligation assay (PLA), immunohistochemistry and hematoxylin-eosin staining, we can characterize how changes in the composition of MAMs can accommodate an alternative calcium pathway in $UCP1^{-/-}$ BAT. Taken together, these studies will shed light on how heat production can be increased to burn fat in humans and reveal a therapeutic means to combat obesity and diabetes type 2.

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Alternative of UCP1-dependent thermogenesis in brown and beige adipocytes under cold adaptation

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Abstract

Brown adipocytes are highly thermogenic due to optimized mitochondria for heat production that burns fat and thus, increases energy expenditure, which is one of the best ways to fight metabolic syndrome. Uncoupling protein 1(UCP1), is a mitochondrial transporter specific to brown and beige fat mitochondria, it is responsible for mitochondrial thermogenesis. The thermogenic program of brown adipocytes is potentiated through a cold challenge. White fat (storage fat) also transforms under cold exposure into a brown-like fat depot called beige fat, becoming a fat burner tissue. Humans do have brown and beige fat that increase energy expenditure when activated but they don't have much UCP1, suggesting other mechanisms of mitochondrial thermogenesis. WT mice with intact brown fat resist acute cold challenge, in contrast to UCP1$^{-/-}$ mice that eventually die. Interestingly, when UCP1$^{-/-}$ mice are put under a progressive cold challenge, they withstand the cold suggesting alternative mechanism of mitochondrial thermogenesis. UCP1$^{-/-}$ mice thus provide a great model to study UCP1-independent pathways of mitochondrial heat production. To determine the mitochondrial thermogenic capacity of brown fat after a progressive cold exposure, we first performed patch-clamp applied to brown and beige mitochondria. We found a calcium current amplitude ~5x greater in UCP1$^{-/-}$ mice when exposed to cold. We propose this calcium current through mitochondrial calcium uniporter (MCU) to be responsible for mitochondrial calcium futile cycle, to compensate for the UCP1 loss in mitochondria. Thermogenic profile of UCP1$^{-/-}$ brown and beige fat studied via mRNA expression is induced under cold. Protein levels also reveal a significant increase in MCU and a difference in the OXPHOS profile of WT compared to UCP1$^{-/-}$ brown and beige fat. Thus, we propose calcium cycling to be one of the main fundamental heat producing pathways in thermogenic mitochondria which can be present in humans.

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Brown adipose tissue CoQ deficiency reshapes mitochondrial morphology and activates the ISR

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Abstract

Mitochondrial dynamics in brown adipocytes are intricately regulated to support their specialized role in thermogenesis. Brown adipose tissue (BAT) orchestrates non-shivering thermogenesis, primarily mediated by the uncoupling protein 1 (UCP1) located in the mitochondrial inner membrane. Tremendous efforts are carried out to unravel the mitochondrial changes associated with brown adipose tissue (BAT) energy expenditure. Mitochondrial dynamics, especially fission, synergistically acts with fatty acid-induced uncoupling to activate BAT metabolism. [1]

Studies suggest that manipulating fission or fusion could offer protection against damage, enhancing mitochondrial resistance through essential component exchange in the Stress-Induced Mitochondrial Hyperfusion (SIMH) pathway [2]. In parallel reduced fragmentation may be associated with lower UCP1 levels and uncoupling activity in CoQ-deficient cells [3]. Coenzyme Q (CoQ) is indispensable for mitochondrial respiration and plays a pivotal role in BAT thermogenic activity. While CoQ deficiency leads to various pathological manifestations, the specific mechanistic consequences in mitochondria-rich tissue, such as BAT, remain poorly understood. Moreover different consequences of CoQ deficiency such as perturbation in ETC, ROS overproduction, reported in several studies [4] , could be considered possible triggers of the integrated stress response. This study sheds light on the impact of pharmacological and genetic CoQ deficiency in BAT, unveiling stress signals that result in the accumulation of cytosolic mitochondrial RNAs, decreased oxygen consumption rates (OCR), a notable alteration in mitochondrial morphology, towards elongation, and cristae reorganization. RNA-seq data reveals upregulation of mitochondrial-localized chaperones and proteases, suggesting a potential stress adaptation during the unfolded protein response in mitochondria (UPRmt). These findings have several implications for both primary and secondary CoQ deficiencies, underscoring the complex interplay of mitochondrial dynamics in such diseases. The study not only advances our understanding of BAT function but also opens avenues for therapeutic interventions targeting mitochondrial health in CoQ-related disorders.

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Common mitochondrial deletions in RNA-Seq: methodological considerations and analyses of aging, tissues, brain regions and cortical layers

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Abstract

Common mitochondrial DNA (mtDNA) deletions are large structural variants in the mitochondrial genome that accumulate in metabolically active tissues with age and have been investigated in various diseases. We applied the Splice-Break2 pipeline (designed for high-throughput quantification of mtDNA deletions) to human RNA-Seq datasets and describe the methodological considerations for evaluating common deletions in bulk, single-cell, and spatial transcriptomics datasets. A robust evaluation of 1,570 samples from 14 RNA-Seq studies (including 1,107 samples across 11 tissues from the Genotype-Tissue Expression (GTEx) Project) showed: (i) the abundance of common deletions detected in PCR-amplified mtDNA correlates with levels observed in RNA-Seq data; (ii) RNA-Seq library preparation method has a strong effect on deletion detection, with the most valuable RNA-Seq wet lab protocols for mtDNA deletion detection being bulk sequencing without ribosomal depletion (e.g., polyA), LCM RNA-Seq, and spatial transcriptomics; (iii) deletions had a significant, positive correlation with age in brain and muscle; (iv) deletions were enriched in cortical grey matter, specifically in layers 3 and 5; and (v) brain regions with dopaminergic neurons (i.e., substantia nigra, ventral tegmental area, and caudate nucleus) had remarkable enrichment of common mtDNA deletions.

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Mitochondrial DNA deletions and copy number in whole genome sequencing (WGS) data: analyses of cortical/cerebellar aging and Parkinson's disease effects

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Abstract

Mitochondrial DNA (mtDNA) exists as a polyploid feature of eukaryotic cells, and the absolute ratio of mitochondria to nuclei (mitochondrial copy number) can change in response to a cell's energy demands. MtDNA can also be affected by deletions. Several bioinformatics methods exist to quantify mitochondrial copy number and mtDNA deletions.

In this study, we evaluated mitochondrial copy number and mtDNA deletions from human brain-derived whole genome sequencing (WGS) data using fastMitoCalc and Splice-Break. Analysis of 292 samples from the cortex and cerebellum from the North American Brain Expression Consortium (NABEC) demonstrated the following: (1) there is a significant decrease in mitochondrial copy number in the cortex with aging; and (2) there is a significant increase in mtDNA deletions in both brain regions with aging, more dramatically observed in the cortex.

Additional analysis of 341 Parkinson's Disease (PD) cerebellum samples was performed and compared to 74 age-matched controls from the NABEC. These studies demonstrated that in the cerebellum specifically, there was no difference in mtDNA deletion levels between PD and controls. However, a robust and significant increase in mitochondrial copy number was observed in PD cerebellum, and this effect was reproducible across brain banks. Furthermore, PD subjects with very high mitochondrial copy number in the cerebellum had lower Unified Parkinson's Disease Rating Scale (UPDRS) scores than expected, relative to the total Lewy Body burden in their brain. This supports the hypothesis that enhanced cerebellar activation may protect against some progression of PD motor symptoms, and that this hyperactivation may be captured with the quantification of mitochondrial copy number in this brain region.

Together, these studies demonstrate that brain-derived WGS data can be evaluated for mtDNA abundance and structural variation, and can reveal interesting mitochondrial effects as they relate to aging and neurodegenerative disease diagnosis.

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MitochondriaWorld: a new web platform to organize and promote the global mitochondrial research community

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Abstract

MitochondriaWorld.org is an evolving web portal bringing together researchers, advocacy networks, and clinical resources to accelerate advances in mitochondrial biology and patient care while communicating the central role of mitochondria in disease and health to the broader public. The overarching goal of MitochondriaWorld is to enable the global mitochondrial research and clinical communities to make breakthroughs in basic science and advance metabolic medicine. To facilitate this, the platform aims to become a nexus for mitochondrial researchers to build community, organize research foci, and coordinate large-scale collaborative endeavors. Despite a recent explosion of mitochondrial research activity and discovery identifying their involvement across many seemingly unrelated diseases and dysfunctions, mitochondria are often still thought of as only ATP-generating "powerhouses" by many investigators and the general public. These major advances in mitochondrial basic and translational biology need to be effectively and consistently organized so researchers and physicians can leverage them to produce meaningful changes in healthcare. Central to the impact and longevity of this mission is clearly communicating progress to the public to develop greater support and funding for investigating the functions of mitochondria in disease and health.
MitoWorld™ is dedicated to building community and catalyzing collaboration to speed the time to discovery, understanding, and therapy.
Please join us at www.mitoworld.org or email us at info@mitoworld.org.

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Isolation and processing of salivary PBMCs as a biomarker for mitochondrial respiration in humans

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Abstract

Novel research has characterized mitochondria as highly dynamic organelles that play roles in signaling, cell cycling, metabolism, and bioenergetics, underscoring a need for sensitive and reliable measurements of mitochondrial function. Due to the growing importance of mitochondrial function as a biological parameter in humans, there has been an increased focus on bringing these measurements into the clinical space. High-resolution respirometry, which measures O_2 consumption, represents the best technology for measuring mitochondrial function. However, current sampling and processing protocols are extensive and time-constrained, restricting respirometry usage as an indicator of mitochondrial dysfunction in humans. We have identified that saliva, more specifically the cells of the oral cavity, represent a biomarker that would be non-invasive and could be frozen for mitochondrial analysis, alleviating issues with respirometry measurements in humans. Oral cell isolation can be done with higher frequency and less expertise than the current sampling methods that require muscle tissue or whole blood PBMC fractions. We have outlined a protocol to isolate salivary PBMCs and run their respirometry analysis and are currently focused on the characterization of the protocol and cells and further analysis of their viability. I will explore the accuracy of the respirometry measurements produced by these cells. Since the sample cells are exposed to the oral microbiome, bacteria in the sample that are also actively respiring could interfere with the respirometry readings. We have observed that bacteria in the sample elevates basal respirometry readings and disrupts oxygen consumption readings. The absence of bacteria in the sample could indicate respirometry performance in the future, which justifies the inclusion of certain processing steps in the protocol. Our poster will outline the steps taken to isolate the salivary PBMCs and describe how these steps reduce bacterial content in the sample to provide an accurate measurement of mitochondrial respirometry in humans.

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LRRK2 promotes β cell apoptosis following inflammatory damage

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing pancreatic islet β cells. T1D is caused by a combination of β-cell dysfunction and immune system dysregulation, leading to autoimmune attack and β-cell demise. As β-cells are uniquely sensitive to inflammatory damage, therapies targeting β-cells present opportunities for treatment of T1D. Recently, leucine-rich repeat kinase 2 (LRRK2) inhibitors were identified as potential candidates to reverse inflammatory signatures found within T1D β-cells. In other cell types, LRRK2 is induced in response to pro-inflammatory cytokine exposure and limits mitochondrial autophagy (mitophagy). However, LRRK2 has not been studied in β-cells. We exposed mouse islets to pro-inflammatory cytokines *ex vivo* and observed upregulation of LRRK2 in response to IFN-γ stimulation. Additionally, we found that genetic deletion or pharmacologic inhibition of LRRK2 attenuates cytokine-induced β-cell apoptosis. Previous work from our lab showed that pro-inflammatory cytokines induce mitophagy in β-cells to promote cell survival. We observed that LRRK2 pharmacologic inhibitors enhance cytokine-induced mitophagy in β-cells. Furthermore, LRRK2 deletion or inhibition blocks phosphorylation of Rab10, a small GTPase that recruits autophagy adaptors to damaged mitochondria for clearance. Intriguingly, we found that deletion of the mitophagy regulator Parkin is protective in response to pro-inflammatory cytokine exposure. LRRK2 inhibitors provide no additive benefit to β-cell survival in Parkin-knockout islets, suggesting that LRRK2 and Parkin function in a common pathway to regulate β-cell survival following cytokine exposure. Together, our results suggest that LRRK2 may promote β-cell apoptosis through dysregulation of mitophagy via Rab10 and Parkin.

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Unraveling the link between ATP hydrolysis and mitochondrial dynamics in progeria through the influence of (+)-epicatechin

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Abstract

Progeria, a genetic disorder characterized by accelerated aging, is associated with changes in mitochondrial structure and function. We have previously observed alterations in mitochondrial morphology in fibroblasts from a patient with progeroid syndrome. Interestingly, we have also detected increased mitochondrial ATP hydrolysis in these cells. However, the relationship between ATP hydrolysis and mitochondrial dynamics remains unclear. The dynamic process of mitochondrial fission is regulated by DRP1, as it becomes recruited to the outer mitochondrial membrane via interaction with mitochondrial fission factor (MFF). In contrast, mitochondrial fusion is regulated by the outer mitochondrial membrane proteins Mitofusin 1 and 2 (MFN 1 and MFN2), while Optic Atrophy 1 (OPA1) promotes inner mitochondrial membrane fusion. AMP-activated protein kinase (AMPK) plays a crucial role in the regulation of fission activity and mitophagy. This research addresses this gap by investigating the impact of the newly reported ATP hydrolysis inhibitor (+)-epicatechin (EPI) on mitochondrial dynamics and morphology. By performing Western blot analysis, we found that EPI treatment reduced MFN2 expression, while increasing MFF expression in progeria fibroblasts, indicating decreased fusion and enhanced fission activity upon ATP hydrolysis inhibition. Additionally, epicatechin treatment reduced expression of pS637 Drp1- the inactive form of Drp1 and increased expression of pS616 Drp1- the active form of Drp1, further supporting our finding that blocking ATP hydrolysis can promote mitochondrial fission. In contrast, OPA1 levels remained largely unaffected by EPI treatment. We further propose that enhanced fission upon EPI treatment is mediated by increase in AMPK signaling. Taken together, these results suggest a modulation of mitochondrial dynamics by ATP hydrolysis inhibition in the context of progeria, providing insights into the complex relationship between ATP hydrolysis and mitochondrial morphology.

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Individualized multi-tissue mitochondrial distribution patterns in mice and humans

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Abstract

Energy transformation capacity is generally assumed to be an individual trait driven by genetic and environmental factors. This predicts that measures of mitochondrial content and respiratory capacity will display strong correlations between tissues, where some individuals have high and others have low mitochondrial content across all tissues. Here, we first test this assumption using multi-tissue enzymatic activities (citrate synthase and complexes I, II, IV) and mtDNA copy number across two mouse cohorts. In cohort 1 (n=27 male mice, 22 organs/tissues), tissues were minimally correlated (median spearman r=0.16; r=0.03 excluding the brain). This finding was replicated in cohort 2 (n=16 male mice, 5 tissues), marked by lack of correlation between tissues (median r=0.01; r=0.00 excluding the brain). Extending these analyses using RNAseq from 45 tissues in n=948 women and men (GTEx), we observed similarly weak between-tissue correlations in the expression of nuclear-encoded mitochondrial genes (r=0.12), mtDNA genes (r=0.14), and functional pathways (e.g. OxPhos r=0.11), meaning that individuals high in some tissues can be low in other tissues. We confirmed this finding using a data-driven approach in a subset of GTEx (n=101, 6 tissues), identifying individualized patterns with high mitochondrial gene expression in some tissues (e.g., brain) but low expression in others (e.g., skeletal muscle). These tissue-specific differences in mitochondrial gene expression are attributable in part to the activation of canonical energy stress sensing pathways. The transcriptional coactivator PGC1α and the integrated stress response (ISR) correlated positively with mitochondrial gene expression in 32 and 44 tissues ($p<0.05$), respectively. People with high mitochondrial gene expression in the brain had high expression of PGC1α ($r=0.74$, $p<0.0001$) and ISR ($r=0.80$, $p<0.0001$), suggesting that tissue-specific metabolic stress sensing contributes to distinct mitochondrial distribution strategies in each person. We speculate that these individualized mitochondrial distribution patterns could explain why some people are predisposed to certain diseases.

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Mapping human mitochondrial diversity and dynamics in human tissues and aging cells

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Abstract

Cellular and organismal metabolic needs are met by multifunctional mitochondria that specialize in their molecular composition, morphology, behavior and functions across cell types and tissues. However, we lack a specific quantitative atlas of molecular features that define mitochondria in specific cell types and with aging. To address this gap, we use a novel computational mitochondrial phenotyping approach – termed mitotyping – to quantitatively map transcriptome-based mitochondrial specialization in two contexts. First, using the Human Protein Atlas multi-tissue dataset together with pathway annotations from the human MitoCarta3.0 dataset, we systematically define how the mitochondrial transcriptome differs between anabolic tissues, contractile muscles, and the brain. The human brain (n=14 brain areas) mitotype differs from other vital organs by their overexpression of OxPhos complex I relative to other supercomplex components (+59%, $p<0.001$), and the upregulation of the Tetrahydrobiopterin (BH4) pathway (+9.5-fold, $p<0.001$), among other features. Second, we examine whether the mitotype of a differentiated cell, once established, remains stable as it ages over time. Multi-omics profiles of primary human fibroblasts cultured over up to 9 months revealed a profound mitotype shift in the second half of the lifespan. In particular, aging cells exhibited a downregulation of the cholesterol-associated mitochondrial pathway (-49%, $p<0.001$), which is correlated with telomere length (Spearman's Rho 0.74, $p<0.001$). This pathway also is downregulated in cells with pharmacologically-induced or genetic OxPhos defects (-41%, $p<0.001$) that accelerate telomere shortening and DNA methylation-based aging rate. In contrast, iron-homeostasis-related genes increased by 33% ($p<0.05$) throughout the lifespan. To conclude, our large-scale mitotyping approach highlights the multifaceted and malleable nature of mitochondria, and provides quantitative evidence of their domain-specific plasticity in the context of aging and OxPhos defects.

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HSP75 inhibition as a therapeutic target for lung squamous cell carcinoma

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Abstract

In 2023, approximately 130,000 people will die of non-small cell lung cancer (NSCLC) in the United States. Lung squamous carcinoma (LUSC) is an aggressive subtype of NSCLC, which accounts for approximately 40,000 patients per year in the U.S. The 5-year survival rate for advanced NSCLC is 6%, with squamous patients having a worse prognosis than those with adenocarcinomas, underscoring the need to identify targeted therapies for the treatment of squamous tumors. Recent breakthroughs in immunotherapy have improved the treatment of this deadly disease, but unfortunately the majority of patients still do not benefit from this therapy and frequently develop resistance. LUSCs frequently carry high mutation burdens and often lack defined genetic mutations that can be successfully paired with targeted therapies such as a tyrosine kinase inhibitors (TKIs). The genetic heterogeneity intrinsic to LUSC makes it difficult to target these tumors with targeted therapies paired to defined genetic mutations. However, at a metabolic level, LUSC retain a homogeneous metabolic signature defined by high glycolytic flux. Our lab has performed in vivo analysis of metabolic signatures in combination with spatial mapping of mitochondrial networks in LUSC (Momcilovic et al., 2018; Han et al., 2023). In addition to homogeneous metabolic signatures, we identified that LUSC have homogeneous structural and functional mitochondrial phenotypes – characterized by high levels of mitochondrial stress. In this study I aim to dissect the metabolic and molecular vulnerabilities regulating mitochondrial stress responses in aggressive glycolytic tumors. I will test the hypothesis that high glucose flux in lung squamous tumors drives mitochondrial stress phenotypes and sensitivity to inhibition of heat shock proteins. The overarching goal of this proposal is to identify new therapeutic strategies to target therapy resistant LUSC while deepening our understanding of the molecular mechanisms that regulate mitochondrial stress responses in these tumors.

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The role of Ca^{2+} in UCP1-independent thermogenesis in beige and brown fat

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Abstract

Mitochondrial thermogenesis is one of the best ways to increase energy expenditure and combat metabolic syndrome. Brown adipose tissue (BAT) have mitochondria specialized in thermogenesis using the Uncoupling Protein 1 (UCP1) to produce heat and burn fat. White fat, an energy-storing fat, can be transformed into thermogenic beige fat. Humans have beige and brown fat but lack UCP1, which suggests that there are UCP1-independent mechanisms of mitochondrial thermogenesis. Using $UCP1^{-/-}$ mice, we hope to reveal UCP1-alternative pathways of heat production in beige and brown fat by challenging them with cold adaptation. Under acute cold exposure, $UCP1^{-/-}$ mice die. However, progressive cold exposure allows them to survive, suggesting a compensatory mechanism of mitochondrial thermogenesis. Using patch-clamp technique applied to mitochondria to study their thermogenic capacity, we found increased Ca^{2+} current across the inner mitochondrial membrane (IMM) through the mitochondrial calcium uniporter (MCU) as a potential alternative to UCP1-dependent thermogenesis. Tissue respiration revealed that $UCP1^{-/-}$ BAT following cold adaptation is not stimulated by β3-adrenergic receptor agonists, which target receptors specific to beige and brown fat, but instead responds to α1-adrenergic receptor agonist. Western blots also show a decrease in β3-adrenergic receptor expression and an increase in expression of the α1-adrenergic receptor. This suggests remodeling of the adrenergic receptor signaling balance to support Ca^{2+}-dependent thermogenesis. Primary brown adipocytes will help us study the role of MCU, β3- and α1-adrenergic receptors in WT and $UCP1^{-/-}$ brown fat by knocking them down and observing the impact on mitochondrial thermogenesis. These experiments will further characterize and assess the thermogenic ability of $UCP1^{-/-}$ BAT, and our findings can be transposed to human beige and brown adipocytes.

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Redox regulation of proton transport through the ADP/ATP carrier

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Abstract

All mitochondria convert nutrients into two forms of energy, ATP and heat, due to transport of protons across the inner mitochondrial membrane (IMM). How mitochondria regulate this fine bioenergetic balance to meet the complex metabolic needs of the cell remains largely unknown. Our previous patch-clamp experiments revealed that proton transport across the IMM is a primary function of the ADP/ATP carrier protein (AAC), a uniporter of ADP into the mitochondrial matrix and ATP into the intermembrane space. As such, AAC is positioned as the master regulator of bioenergetic distribution between ATP and heat within non-adipose tissues. Controlled activation of AAC-dependent H^+ current would enable the targeted potentiation of mitochondrial thermogenesis to increase energy expenditure which poses significant therapeutic potential in the treatment of metabolic diseases. One such physiological activation occurs during periods of high oxidative stress. H^+ transport through AAC is increased to lower the membrane potential across the IMM, thereby increasing the efficiency of the electron transport chain and limiting production of reactive oxygen species (ROS). Additional patch-clamp experiments revealed heightening of the H^+ current through AAC in the presence of oxidizers Tributyltin and 4-Hydroxynonenal. Potentiation of AAC-dependent H^+ current is thus redox sensitive likely due to a single, or combination of, its four cysteine residues given the sensitivity of thiol groups to oxidative conditions. We generated an AAC1/AAC2 double knockout C2C12 cell line in which AAC1 containing point mutations of cysteine to alanine was reintroduced. Using this cell line, we will characterize oxygen consumption during cellular respiration assays, H^+ current potentiation by mitochondrial patch-clamp experiments, and ROS production through cellular toxicity assays. Delineating the role of redox regulation in heightening AAC-dependent thermogenesis upon oxidative stress serves to advance the development of treatments against pressing metabolic diseases by leveraging mitochondrial thermogenesis to increase energy expenditure.

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Mitochondrial control of intercellular communication: how mitochondria alter gap junctional communication between cells

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Abstract

To survive, cells must appropriately coordinate their actions to respond to stressors by transitioning from one metabolic state to another, which involves the use of energy and (inter)cellular communication. Mitochondria energetically support all cellular behaviors, and defects in oxidative phosphorylation (OxPhos) are known to cause intracellular changes such as a decrease in growth capacity, trigger the integrated stress response (ISR), and decrease lifespan. If, and how, OxPhos defects affect intercellular communication is not well understood, nor is how energetic and cell-cell communication processes influence the ability of cells to respond and adapt to stress. Both work within our lab and in the literature has shown that OxPhos defects cause alterations to cell-cell communication by differential release of cytokines from fibroblasts, and in RNAseq data of different mitochondrial heteroplasmy levels contributing to altered transmembrane and G-protein-coupled receptor (GPCR) expression. We hypothesize that OxPhos defects alter intercellular communication by decreasing gap junctional communication (GJC), such that cells exhibit a more isolated, stressed state, which contributes to the hypermetabolic state of OxPhos perturbed cells. Our results show that in fibroblasts, OxPhos defects via SURF1 mutation and oligomycin treatment, and chronic stressor dexemethosone exposure decreases the 3 mostly highly expressed connexin genes by 39%, 17% and 19% respectively, suggestive of a decrease in GJC between cells. These fibroblasts also express adrenergic receptors in 3 different wildtype lines, and are metabolically responsive to norepinephrine (NE) in dose dependent manner as shown via seahorse assay, which demonstrates that NE can be used as a stressor in testing the metabolic flexibility of the cells. Initial data suggests that a short duration (3 hours) of oligomycin to perturb OxPhos increases GJC via the scrape loading-dye transfer assay, and further work is being done to investigate whether longer durations or SURF1 mutations result in a decrease of GJC.

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High-throughput identification of calcium regulated proteins across diverse proteomes

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Abstract

Calcium-regulated proteins play important roles in nearly every biological process. The characterization of calcium-regulated proteins is integral to understanding cellular homeostasis and signaling. Yet methods for rapid and unbiased quantification of calcium engagement by proteins remain challenging. Currently, there are 18 known mitochondrial Ca^{2+}-binding proteins, which were identified using targeted biochemical assays or computational detection of EF-hand domains [1,2]. The existence of other calcium-regulated mitochondrial proteins with non-canonical Ca^{2+}-binding domains, which are resistant to computational detection, is unknown. To identify calcium-regulated proteins, we adapted protein thermostability assays. We focus on calcium-binding proteins in three species - yeast, human, and mouse - to highlight the generalizability of these methods and to enable comparison of protein-calcium binding conservation. In total, we quantified 2824 putative calcium-regulated proteins in yeast, cultured human cells, and in situ mouse mitochondria. These data revealed divalent cation engagement with key cellular signaling hubs and diverse cellular processes including the engagement of spliceosomal proteins, metabolic enzymes, and signal transduction hubs. Multi-species, quantitative comparison of calcium-protein engagement identified residue-specific effectors of divergent cation specificity even within well-known EF-hand domains. Validation of proteomic data established that the mitochondrial dienoyl-CoA reductase DECR1 binds calcium at physiologically relevant calcium concentrations with substrate-specific affinity suggesting a direct role of calcium regulation in mitochondrial fatty acid oxidation and identifying a novel mitochondrial Ca^{2+}-binding protein. Together, the three proteome-wide thermal stability datasets offer a key starting point for dissecting divalent cation engagement and mechanistic effects across multiple species and diverse biological processes in and outside of the mitochondria.

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PAK1-enriched skeletal muscle promotes islet β-cell insulin secretion

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Abstract

The p21-activated kinase 1 (PAK1) is required for non-canonical insulin-stimulated glucose uptake and mitochondrial function in skeletal muscle cells. The detailed mechanism(s) by which PAK1 improves whole-body glucose homeostasis remains to be elucidated. We hypothesized that PAK1-enriched skeletal muscle secretes a circulating factor that can interact with pancreatic islet β-cells. This is based on our preliminary observation that rat L6 muscle cell-containing media could enhance INS-1 832/13 clonal β-cell function *in vitro*. Furthermore, our mass spectrometry analysis of the PAK-1-enriched L6 rat muscle-containing conditioned media (CM) revealed elevated levels of the secreted myokine, hepatocyte growth factor activator (HGFAC). Secreted HGFAC in circulation was previously shown to proteolytically cleave and activate HGF via paracrine- or endocrine-effects. Further, HGF is vital for islet β-cell mass maintenance and function. Thus, here we investigated if the HGFAC-enriched CM from PAK1-enriched skeletal muscle had the capacity to enhance islet β-cell insulin secretion *in vitro*. Using sandwich ELISA we confirmed elevated HGFAC protein levels in PAK1-enriched skeletal myotubes and in the associated CM compared to GFP-transduced control myotubes. The serum-free CM collected from PAK1-enriched L6.GLUT4myc myotubes containing elevated HGFAC levels significantly enhanced glucose-stimulated insulin secretion in rat clonal β-cells vs. the control CM. Taken together, we revealed a new mechanism by which PAK1-enrichment in skeletal muscle may impart improved overall whole-body glucose homeostasis. Future studies will investigate if Pak1-induced muscle-pancreas crosstalk alters β-cell mitochondrial health and function.

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In-vitro investigations into post-translational control of mitochondrial gene expression

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Abstract

The human mitochondrial DNA (mtDNA) is a ~16 kb circular DNA encoding 13 essential OXPHOS proteins, 22 mitochondrial tRNAs, and 2 mitochondrial ribosomal RNAs. mtDNA is compacted by a dual transcription factor/compaction protein, mitochondrial transcription factor A (TFAM), whose binding and compaction is proposed to regulate processes involving mtDNA like transcription and replication. Possible modes for regulating TFAM include changing its expression, degrading it, or adding post-translational modifications (PTMs) such as phosphorylation by protein kinase A (PKA) and nonenzymatic acetylation by acetyl-CoA that may tune TFAM affinity for DNA. PTMs on TFAM would represent an efficient way to modulate mtDNA compaction and gene expression in response to the changing cellular energy needs. While these modifications have been detected in cells, there is yet to be a targeted quantification of TFAM PTMs specifically. We investigated these modifications to TFAM *in vitro* to test the impact of PTMs on TFAM's compaction and transcription initiation functions. We demonstrated that DNA-bound TFAM is less susceptible to both enzymatic phosphorylation and non-enzymatic acetylation. Surprisingly, we showed using electrophoretic mobility shift assays that pre-phosphorylated or pre-acetylated TFAM compacted circular double-stranded DNA comparably to unmodified TFAM. Additionally, we provided an in-depth analysis of TFAM's lysine reactivity towards acetyl-CoA using isotopic labelling and liquid chromatography-tandem mass spectrometry. Finally, we showed that phosphorylation and acetylation of TFAM increased the processivity of transcription through TFAM-imposed barriers on DNA, but that TFAM bearing either modification maintained full activity in transcription initiation. We conclude that phosphorylation by PKA and nonenzymatic acetylation by acetyl-CoA are unlikely to occur on TFAM that are bound to the mtDNA (Reardon and Mishanina, *J Biol Chem* 2022). Driven by the *in vitro* discoveries, we are currently investigating the possibility of PTM regulation of TFAM in cells, in particular how changes in cellular metabolism impact mitochondrial transcription.

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Machine-Learning-derived pixel-wise cell health score for high-throughput Single Molecule Tracking imaging

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Abstract

Single Molecule Tracking (SMT) has provided the ability to capture changes in diffusion coefficients for a protein-target of interest under a variety of genetic or chemical perturbations with the aim of understanding its spatiotemporal regulation and mechanism of action. However, most SMT implementations are not amenable to high-throughput and high-volume imaging. Our platform has adapted SMT to 384-well plates and automated the process of data acquisition and processing, enabling the testing of thousands of perturbations per day in high-throughput SMT (htSMT). While some compounds elicit on-target or on-pathway effects, it is expected that several will yield cytotoxic effects that may impact protein dynamics non-specifically.

For this purpose, we present here an ML-derived model to identify cell health as a function of compound treatment in Hoechst-labeled fluorescence microscopy images. Briefly, we generated a training-set by exposing U2OS cells with varying concentrations of staurosporine and sorbitol over a range of compound incubation times. A weakly supervised training method was used to train a U-NET model to provide pixel-level binary classifications describing whether the pixel belongs to an unhealthy cell or not. The model takes a single 2D Hoechst image as input, which we demonstrate is sufficient to successfully capture several different subtypes in which unhealthiness can visually manifest. During high-throughput screening (HTS), the model functions in conjunction with a separate segmentation model to label full cells by using the mean pixel score per mask instance.

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LACTB deletion alters mitochondrial metabolism and impacts intermembrane contacts

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Abstract

Mitochondria are crucial regulators of cellular physiology, but many aspects of their functions, including roles of many conserved mitochondrial proteins remain unclear. The mitochondrial protease LACTB is a homolog of the penicillin-binding protein found in Gram-negative bacteria. This serine protease is localized in the mitochondrial intermembrane space where it forms long filamentous structures, the significance of which is unknown. The protease is postulated to support membrane organization and metabolic regulation and influence mitochondrial lipid metabolism, thereby acting as a tumor suppressor in breast cancer, but its exact physiological role is unclear. Here, we generated a LACTB-knockout cell lines to systematically investigate its role in mitochondrial functions. Our data indicate that whereas the proliferation and doubling rate of cells lacking the enzyme remain unaltered, the migratory properties of LACTB-deleted cells are dramatically changed. LACTB-devoid cells exhibit reduced mitochondrial respiration as well as ultrastructural alterations in response to homeostatic challenges. Consistently, LACTB depletion upregulates the constituents of the mitochondrial contact site and cristae organizing system (MICOS) complex, suggesting the enzyme's role in regulation of contacts between the inner and outer mitochondrial membranes. Overall, our data provide new insights into LACTB's role in regulating physiological outputs related to mitochondrial membrane remodelling.

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A conserved SKY insert regulates human Fis1's mitochondrial fission functions

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Abstract

FIS1 is conserved in all eukaryotes, yet its function in metazoans is thought divergent. Structure-based sequence alignments of FIS1 revealed a conserved but noncanonical, three-residue insert in its first tetratricopeptide repeat (TPR), suggesting a conserved function. In vertebrates, this insert is serine (S45), lysine (K46), and tyrosine (Y47). To determine the biological role of the "SKY insert," three variants (ΔSKY, ΔSKYD49G, and AAA) were tested in HCT116 cells for altered mitochondrial morphology and recruitment of fission mechanoenzyme DRP1 and mitophagic adaptor TBC1D15. Surprisingly, we observed bi-directional FIS1 regulation as supported by stark gains and losses of function phenotypes for the AAA and deletion variants, respectively. These drastic phenotypes were largely attributed to major changes in Fis1's regulatory N-terminal arm region. Collectively, these results support a unifying model whereby FIS1 activity is effectively governed by intramolecular interactions between its regulatory arm and a noncanonical TPR insert that is conserved across eukaryotes.

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A hunt for mitochondrial restrictors of Toxoplasma growth

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Abstract

As immune signaling platforms, mitochondria are critical in the host defense against intracellular pathogens. Consequently, mitochondria are a target of microbial effectors, several of which alter mitochondrial morphology and function independently of their role in innate immune signaling. This raises the question of whether mitochondria mediate noncanonical cell autonomous defenses during infection. In previous work, we found that host mitochondria compete with the human parasite *Toxoplasma gondii* for fatty acids, thereby restricting parasite growth. Here, we set out to more broadly define how a host cell weaponizes mitochondrial function. To identify mitochondrial modulators of *T. gondii* growth, we performed a mitochondria-specific loss-of-function CRISPR/Cas9 screen. To define the impact of mitochondrial respiration on cellular defenses, we included in our screen conditions of high or low mitochondrial oxidative function. Our results shed light on how mitochondria regulate microbial growth, and conversely, how *Toxoplasma* exploits mitochondrial function to its benefit.

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Interplay between mitochondrial ATP synthase reverse activity and cristae architecture

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Abstract

Mitochondrial ATP Synthase or Complex V (CV) is the main producer of Adenosine Triphosphate (ATP) for the cell. However, when respiratory function is impaired, CV can also function in reverse to hydrolyze ATP. Dimerization of CV facilitates the formation of invaginations in the mitochondrial inner membrane (IMM) called cristae, which respond to and maintain the proton motive force. The dynamics of the interaction between the hydrolysis of ATP by CV and cristae architecture maintenance are not fully understood. Here we show that impairment of cristae architecture in the mitochondrial contact site and cristae organizing system (MICOS) knockout cell models is linked to changes in mitochondrial CV ATP hydrolytic capacity. Moreover, we have found that the selective inhibitor of CV reverse activity (+)-Epicatechin can reduce hydrolytic capacity in these models, potentially rescuing the defects in mitochondrial architecture. Previous data has also shown that mitochondrial membrane potential is heterogenous, with cristae maintaining distinct electrochemical domains. We hereby propose that cristae architecture and CV ATP hydrolysis are linked by the dimerization of CV and maintenance of the proton motive force.

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Neuronal activity-driven O-GlcNAcylation promotes mitochondrial plasticity

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Abstract

Neuronal activity is an energy-intensive process that is largely sustained by instantaneous fuel utilization and ATP synthesis. However, how neurons couple ATP synthesis rate to fuel availability is largely unknown. Here, we demonstrate that the metabolic sensor enzyme O-GlcNAc transferase regulates neuronal activity-driven mitochondrial bioenergetics. We show that neuronal activity upregulates O-GlcNAcylation mainly in mitochondria. Mitochondrial O-GlcNAcylation is promoted by activity-driven fuel consumption, which allows neurons to compensate for high energy expenditure based on fuel availability. To determine the proteins that are responsible for these adjustments, we mapped the mitochondrial O-GlcNAcome of neurons. Finally, we determine that neurons fail to meet activity-driven metabolic demand when O-GlcNAcylation dynamics are prevented. Our findings suggest that O-GlcNAcylation provides a fuel-dependent feedforward control mechanism in neurons to optimize mitochondrial performance based on neuronal activity. This mechanism thereby couples neuronal metabolism to mitochondrial bioenergetics and plays a key role in sustaining energy homeostasis.

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TMEM65 regulates NCLX-dependent mitochondrial calcium efflux

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Abstract

The balance between mitochondrial calcium ($_mCa^{2+}$) uptake and efflux regulates ATP production, but if perturbed causes energy starvation or $_mCa^{2+}$ overload and cell death. The mitochondrial sodium-calcium exchanger, NCLX, is a critical route of $_mCa^{2+}$ efflux in excitable tissues, such as the heart and brain, and animal models support NCLX as a promising therapeutic target to limit pathogenic $_mCa^{2+}$ overload. However, the mechanisms that regulate NCLX activity remain largely unknown. We used proximity biotinylation proteomic screening to identify the NCLX interactome and define novel regulators of NCLX function. Here, we discover the mitochondrial inner membrane protein, TMEM65, as an NCLX-proximal protein that potently enhances sodium (Na^+)-dependent $_mCa^{2+}$ efflux. Mechanistically, acute pharmacologic NCLX inhibition or genetic deletion of NCLX ablates the TMEM65-dependent increase in $_mCa^{2+}$ efflux. Further, loss-of-function studies show that TMEM65 is required for Na^+-dependent $_mCa^{2+}$ efflux. Co-fractionation and *in silico* structural modeling of TMEM65 and NCLX suggest these two proteins exist in a common macromolecular complex in which TMEM65 directly stimulates NCLX function. In line with these findings, knockdown of *Tmem65* in mice promotes $_mCa^{2+}$ overload in the heart and skeletal muscle and impairs both cardiac and neuromuscular function. We further demonstrate that *TMEM65* deletion causes excessive mitochondrial permeability transition, whereas TMEM65 overexpression protects against necrotic cell death during cellular Ca^{2+} stress. Collectively, our results show that loss of TMEM65 function in excitable tissue disrupts NCLX-dependent $_mCa^{2+}$ efflux, causing pathogenic $_mCa^{2+}$ overload, cell death and organ-level dysfunction, and that gain of TMEM65 function mitigates these effects. These findings demonstrate the essential role of TMEM65 in regulating NCLX-dependent $_mCa^{2+}$ efflux and suggest modulation of TMEM65 as a novel strategy for the therapeutic control of $_mCa^{2+}$ homeostasis.

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Inhibition of ATP synthase reverse activity restores energy homeostasis in mitochondrial pathologies

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Abstract

The maintenance of cellular function relies on the close regulation of adenosine triphosphate (ATP) synthesis and hydrolysis. ATP hydrolysis by mitochondrial ATP Synthase (CV) is induced by loss of proton motive force and inhibited by the mitochondrial protein ATPase inhibitor (ATPIF1). The extent of CV hydrolytic activity and its impact on cellular energetics remains unknown due to the lack of selective hydrolysis inhibitors of CV. We find that CV hydrolytic activity takes place in coupled intact mitochondria and is increased by respiratory chain defects. We identified (+)-Epicatechin as a selective inhibitor of ATP hydrolysis that binds CV while preventing the binding of ATPIF1. In cells with Complex-III deficiency, we show that inhibition of CV hydrolytic activity by (+)-Epichatechin is sufficient to restore ATP content without restoring respiratory function. Inhibition of CV–ATP hydrolysis in a mouse model of Duchenne Muscular Dystrophy is sufficient to improve muscle force without any increase in mitochondrial content. We conclude that the impact of compromised mitochondrial respiration can be lessened using hydrolysis-selective inhibitors of CV.

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Lower mitochondrial genome turnover and greater mutation frequency in skeletal muscle of aged compared to adult OKC-HET rats

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Abstract

Mitochondrial genomic integrity, which is supported by processes such as replication, repair, and turnover, is critical in supporting metabolism and organismal health. Accumulation of mitochondrial DNA (mtDNA) mutations contributes to mitochondrial and whole-tissue dysfunction, which lead to chronic diseases including sarcopenia. The mechanisms that promote generation and age-related accumulation of mtDNA mutations are unclear. mtDNA mutants may have a replicative advantage that propagates the mutation burden. However, there is limited in vivo data regarding effects of age on mtDNA replication. We hypothesized mtDNA synthesis rates would increase with age as mutation burden increased in skeletal muscle in both male and female rats.

We measured mtDNA synthesis over 14 days using the stable isotope tracer deuterium oxide and assessed mtDNA copy number and mutation deletion frequency using digital PCR in quadriceps muscle of 9- and 26 month-old (mo) male and female OKC-HET rats, which have heterogenous mtDNA backgrounds.

There were no differences in mtDNA copy number between sexes or ages. However, 26 month-od rats had lower rates of mtDNA synthesis compared to 9 month-old rats (9 mo: 0.509 ± 0.009 %/day, 26 month: 0.371 ± 0.048 %/day; p=0.0024) and greater mtDNA half-lives (9 mo: 132 ± 1.79 days, 216 ± 25.63 days; p=0.0009). Concomitantly, 26 month-old rats had greater (p=0.003) mtDNA deletion mutation frequency (2.023e-004 ± 7.18e-005) than 9 month-old rats (9.310e-005 ± 2.404e-005). 26 month-old female rats (1.309e-004 ± 2.386e-005) had a lower (p=0.008) mutation burden than male rats (2.74e-004 ± 4.484e-005).

Contrary to our hypothesis, mtDNA synthesis declined with age as mutation burden increased in the quadriceps. Because mtDNA copy number was not different between ages as synthesis decreased, mtDNA turnover declined with age. Altogether, these results suggest lower mtDNA turnover rates contribute to age-related mtDNA mutation burden. Future studies should test how modulating mtDNA turnover affects mutation frequency.