From cristae membrane structure and dynamics to metabolic implications

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Abstract

Altered inner mitochondrial membrane (IMM) ultrastructure is well known to be implicated in ageing, diabetes and numerous neurological and muscular disorders. Still, the IMM was for a long time seen as quite static, despite the fact that it can adapt under different physiological conditions or during apoptosis. We showed using state-of-the-art live-cell stimulated emission depletion (STED) super-resolution nanoscopy that cristae as well as crista junctions (CJs) are dynamic and apparently undergo fission and fusion events in a reversible and balanced manner at a timescale of seconds. Moreover, several lines of evidence strongly suggest the formation of transient cristae vesicles with dynamic changes of the membrane potential. These dynamics processes depend on the MICOS complex, known to be required for formation of crista junctions and contact sites. We showed that loss of Mic13, a subunit of the MICOS complex which is causally linked to mitochondrial encephalopathy with liver dysfunction in humans, impairs cristae dynamics. The bioenergetic requirements of cristae dynamics were analyzed in detail revealing that inhibition of OXPHOS complexes does hardly impair cristae dynamics in living cells, yet inhibiting ADP/ATP exchange via adenine nucleotide translocators (ANTs) using bongkrekic acid does. I will discuss why cristae have nowadays to be seen as dynamic entities and why this has fundamental implications for the generation of ATP via oxidative phosphorylation and other mitochondrial functions. Finally, I will propose and discuss the 'Dynamic-Trap-and-Flux' (DynaTrux) model.

Mitochondrial double-stranded RNA is a new DAMP for mitochondrial stress

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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.

Chaperones, contact sites, and membrane lipid transport – a nexus for biosynthesis and trafficking of CoQ

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Abstract

Coenzyme Q (CoQ) is an essential lipid molecule utilized in energy metabolism. It also serves as a vital antioxidant that protects cellular membranes from lipid peroxidation. CoQ is synthesized within mitochondria and must be trafficked from mitochondria to non-mitochondrial membranes. CoQ-deficient cells supplemented with exogenously supplied CoQ are able to take up this insoluble lipid and transport it from the plasma membrane to the mitochondrial inner membrane, where it restores the function of respiratory electron transport complexes. However, the uptake and membrane trafficking pathways that mediate the intracellular distribution of either endogenously synthesized CoQ or exogenously supplied CoQ remain poorly understood. To advance our understanding of the mechanisms that traffic CoQ to membrane destinations within the cell, we are characterizing three proteins deemed essential for the transport and function of CoQ in the yeast *Saccharomyces cerevisiae*. The Coq10 polypeptide functions as a CoQ chaperone that is peripherally associated with the matrix-side of the mitochondrial inner membrane, and is necessary for respiration, antioxidant function of CoQ, and the organization of the CoQ synthome adjacent to ER-mitochondrial contact sites. Coq11 is also peripherally associated with the mitochondrial inner membrane and is a partner protein of the CoQ synthome, a multi-subunit complex that is required for CoQ biosynthesis. The Coq11 polypeptide mediates the intracellular distribution of endogenously synthesized CoQ, but its biochemical function is still mysterious. The third protein, Vps1, is a cytosolic protein required for several aspects of membrane trafficking. We have shown that yeast harboring mutations in Vps1 are unable to utilize exogenously supplied CoQ. A better understanding of how these proteins function to modulate CoQ biosynthesis and its subcellular locations will shed light on how a highly hydrophobic molecule like CoQ is trafficked to and from mitochondria, and its use as a therapeutic in CoQ deficiencies.

CryoET reveals mitochondrial phenotypes in Huntington's disease patient iPSC-derived and mouse primary neurons

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Abstract

Huntington's disease (HD) is a neurodegenerative disease with a genetic cause; an expanded CAG repeat in the Huntingtin gene. The resulting protein contains an expanded polyglutamine tract and the mutant protein is known to alter mitochondrial dynamics in HD. The genetics of the disease allow us to use differentiated induced pluripotent stem cells from patients to study the disease, however defining pathologies remains challenging. Here, we used cryogenic electron tomography to visualize organelles within neurites of HD iPSC-neurons and primary neurons from HD mice. Although not visible using standard microscopy, cryo-ET showed that mitochondrial structure was aberrant, with distorted cristae, and the presence of enlarged granules that we show are mitochondrial RNA granules. We used artificial intelligence to quantify the granules, and proteomics to reveal the differential protein content in the isolated HD mitochondria. Finally, reduction of the E3 SUMO ligase Protein Inhibitor of Activated STAT1 (PIAS1) ameliorated the HD-associated mitochondrial phenotypes observed, demonstrating that an ultrastructural approach may inform future mechanistic studies and therapeutic interventions.

Mitochondria and lipid droplet intimacy

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Abstract

As mitochondria are the primary site for lipid oxidation, dynamic relationships between lipid droplets (LDs) and mitochondria have been extensively reported but inadequately characterized. While original studies conjectured that interactions between these organelles existed merely to facilitate lipid transfer for oxidation, a growing body of evidence suggests these organelle dynamics are more nuanced. We and others have identified a key role for mitochondria-associated membranes (MAMs) – ER subdomains near mitochondria - in facilitating lipid anabolic pathways that link mitochondria and LDs. Although MAM formation and LD and mitochondrial interactions are known to form in response to fasting or nutrient deprivation, the mechanisms that underlie these effects are not well understood. Herein, we show data suggesting that acyl-CoAs are a key metabolite that facilitates LD and mitochondria interactions. The addition of exogenous fatty acids under full media (i.e., fed) conditions is sufficient to drive LD and mitochondria interactions, a process that is blocked by the inhibition of acyl-CoA synthetases and acyl-CoA formation. Inhibition of lipolysis during fasting negates the increase in LD and mitochondria interactions known to occur with nutrient deprivation. Manipulation of other pathways involved in acyl-CoA metabolism further supports a key role for acyl-CoAs in organelle tethering under different metabolic conditions. Moreover, we show that ABHD5, a lipolytic co-activator and acyl-CoA binding protein, plays a key role in LD and mitochondria interactions. ABHD5 appears to work, at least in part, through its interactions with PLIN5, a well-established LD and mitochondria tethering protein. Overall, these data provide new insights into the regulation of LD and mitochondria tethering, a process that is being further interrogated in ongoing studies.

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.

Extracellular Mitochondria in HFpEF

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Abstract

Heart failure with preserved ejection fraction (HFpEF) represents 50% of all heart failure patients. Mitochondrial dysfunction is a shared pathogenic mechanism for HFpEF comorbidities and several studies demonstrated mitochondrial malfunction in HFpEF hearts. These observations have led to an emerging interest in the mechanistic role of mitochondrial dysfunction in HFpEF. Mitochondria play a fundamental role in energy metabolism. However, aberrant mitochondria can also instigate innate immunity or trigger cell death. Hence, preserving a healthy mitochondrial population is crucial for cell survival. To sustain normal mitochondrial function cells have developed multiple mitochondrial quality control (MQC) mechanisms, which converge in clearance of damaged mitochondria within the lysosomes. Mitochondria and lysosomes present contact sites due to their close proximity (~10nm) and it has been shown that the malfunction of either organelle resonates in the function of the other in a reciprocal manner. Despite the central role of lysosomes in MQC, it was recently demonstrated that in the heart and brown adipose tissue mitochondrial homeostasis (mitostasis) is also achieved via a lysosomal independent mechanism. Such mechanism involves the release of mitochondrial fragments packaged within vesicles to the extracellular space (mitochondria containing extracellular vesicles; mitoEVs) followed by their uptake and clearance by tissue macrophages. We have found that lysosome-dependent MQC mechanisms were impaired in the heart of a HFpEF mouse model. Furthermore, a greater number of mitochondria were released into the extracellular space of HFpEF hearts, which also presented increased number of CD68+ macrophages. In addition, we have shown that macrophages can uptake mitoEVs in vitro, which are degraded over time. These results suggest an increased release of extracellular mitochondria in HFpEF, as an alternative MQC mechanism. The role of extracellular mitochondria in HFpEF has not been investigated.

Novel roles for the mitochondrial protein cyclophilin D in skin wound healing and collagen secretion

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Abstract

Central for wound healing is granulation tissue formation, largely consisting of collagen which role stretches past wound healing as it is implicated in fibrosis and skin aging. Cyclophilin D (CyD) is a mitochondrial protein that regulates the permeability transition pore, known for its role in apoptosis and ischemia-reperfusion. To date the role of CyD in human wound healing and collagen generation is largely unexplored. Here, we show that CyD is upregulated in normal wounds and venous ulcers, likely adaptive as CyD inhibition impairs re-epithelization, granulation tissue formation and wound closure in human and pig models. Overexpressing CyD increased keratinocyte migration and fibroblast proliferation, whereas its inhibition reduced migration. Independent of wound healing, CyD inhibition in fibroblasts reduced collagen secretion and caused endoplasmic reticulum collagen accumulation, while overexpression increased collagen secretion. This was confirmed in CyD knockout mice that showed reduction in skin collagen. This study reveals novel roles of CyD in the skin with implications for wound healing and beyond.

Mitochondrial bioenergetics as an emerging target for pan small cell neuroendocrine cancer

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Abstract

Epithelial cancers from multiple tissues can converge to a small cell neuroendocrine (SCN) state, linked with therapy resistance and poor prognosis. SCN oncogenes are intricately linked with cellular metabolism, yet how they influence metabolic reprogramming during SCN differentiation remains unknown. Using our human tissue-derived SCN transformation system (termed PARCB), we found that SCN prostate cancer (NEPC) progression is driven by PGC1a, a master regulator of mitochondrial biogenesis and function. PGC1a inhibition during PARCB transformation blocked OXPHOS and tumor formation, whereas its overexpression upregulated OXPHOS to drive an ASCL1-expressing neuroendocrine lineage. Consistent with its role in neuroendocrine differentiation, PGC1a correlates tightly with the neuroendocrine marker ASCL1 in PARCB prostate tumors and in multiple clinical SCN cancers. Furthermore, PGC1a and OXPHOS are elevated in prostate cancer patients following androgen deprivation therapy (ADT) and in NEPC compared with castration-resistant prostate cancer patients. PGC1a is also elevated in clinical small cell lung cancer (SCLC) tumors and OXPHOS gene deletion is a potent inducer of SCLC cell death. These findings designate PGC1a-induced OXPHOS as a novel therapeutic target for NEPC and SCLC, suggesting promising avenues for targeted therapies against these aggressive cancers.

Manipulating mitochondrial electron flow enhances tumor immunogenicity

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Abstract

Although tumor growth requires the mitochondrial electron transport chain (ETC), the relative contribution of complex I (CI) and complex II (CII), the gatekeepers for initiating electron flow, remains unclear. In this work, we report that the loss of CII, but not that of CI, reduces melanoma tumor growth by increasing antigen presentation and T cell–mediated killing. This is driven by succinate-mediated transcriptional and epigenetic activation of major histocompatibility complex–antigen processing and presentation (MHC-APP) genes independent of interferon signaling. Furthermore, knockout of methylation-controlled J protein (MCJ), to promote electron entry preferentially through CI, provides proof of concept of ETC rewiring to achieve antitumor responses without side effects associated with an overall reduction in mitochondrial respiration in noncancer cells. Our results may hold therapeutic potential for tumors that have reduced MHC-APP expression, a common mechanism of cancer immunoevasion." Mangalhara et al. Science (2023), 381:1316-1323.

A battle between immune cell mitochondria and weight loss

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Abstract

Objective. It is yet to be determined whether mitochondria (MITO) play a role as a counter regulatory mechanism that contributes to metabolic adaptation and propensity to regain weight following weight loss. This study aimed to compare differences in respiratory capacity of peripheral mononuclear cells (PBMCs) in subjects with obesity (OB) who were in phases of active weight loss (OB-WL), plateau (OB-PL), or regain (OB-RG).

Methods. Fresh blood samples were collected from fasted participants with BMI of 30-56 kg/m^2 at either their baseline, or their 6-month, or 12-month visit following 24-weeks of behavioral weight loss intervention. Blood was also collected from healthy weight-control participants (HWC, n=11) (BMI of 18.5-24.9). PBMCs were isolated through density gradient centrifugation, and CD3+ (T-cell) and CD14+ (monocytes) were subsequently separated though magnetic activated cell sorting. MITO function was assessed by Agilent Extracellular Flux Analysis (XFe). To determine the phase of WL at each visit, body weight trajectories were tracked for each participant throughout 18 months using a remote electronic scale. Threshold regression modeling was used to determine if the participant was in OB-WL (BW loss ≥0.5lbs/wk, n=7), OB-PL (±0.25lbs/wk, n=10), or OB-RG (BW gain ≥0.5lbs/wk, n=6).

Results. There was a consistent reduction in MITO capacity of T-cells in subjects with obesity (2.0±0.6 pmol O_2/10^6 cells) compared to HWC (2.8±0.8). No significant differences were found in monocyte mitochondrial function between the two groups. We found lower MITO respiratory capacity in T-cells from the OB-PL (1.4±0.4) and OB-RG (1.6±0.8) groups relative to OB-WL (2.5±0.4).

Conclusions. T-cells exhibit reduced MITO respiratory capacity in participants with obesity compared to those in the normal weight range. MITO respiratory capacity was further reduced in participants following a plateau and during weight regain. These findings suggest that reductions in oxygen consumption are exacerbated rather than improved in response to diet-induced WL at the level of immune cells. More work is needed to determine how these bioenergetic changes influence metabolic and immune health following weight loss.

Histopathological and molecular characterization in ocular post-mortem analyses following AAV2 gene therapy for Leber hereditary optic neuropathy

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Abstract

Leber hereditary optic neuropathy (LHON) is a rare disease that causes severe vision loss. Lenadogene nolparvovec is a novel AAV2 gene therapy for LHON patients carrying the m.11778G>A MT-ND4 mutation.

During the RESCUE trial, a 29-year-old man with MT-ND4-LHON received an intravitreal injection of lenadogene nolparvovec (AAV2-ND4) in his right eye (OD) and a sham injection in his left eye (OS). One month after treatment, he developed moderate intraocular inflammation that resolved with topical corticosteroids. At 1 year, BCVA was +1.5 (OD) and +1.1 (OS) LogMAR and 4 months later the patient was found dead.

Post-mortem histopathologic eye analysis revealed an angiocentric lymphocytic infiltration in the optic nerve head OD. T-cells, but also B-cells and macrophages were highlighted by specific immunohistochemical markers. A sparse infiltrate was also found in the innermost peripheral retina on the temporal side OD as was a retinal “pearl”.

Molecular investigation after retinal laser microdissection revealed an asymmetric distribution of AAV2-ND4 as detected by ddPCR in OD, most prevalent in retinal ganglion cells of the temporal side, and only 5-10% in photoreceptors, and absent in optic nerve, vessels, retinal pigmented epithelium and choroid. About hundred times less amount of AAV2-ND4 was also detected in the OS retina.

This is the first postmortem study of human eyes unilaterally treated with AAV2-based gene therapy. In the injected eye, more than a year later, persisted evidence of angiocentric lymphocytic infiltration. There was also strong evidence of AAV2-ND4 retinal transfection in the injected eye, prevalent on the temporal hemiretina, and it was also detected in the contralateral fellow eye at much lower amount, confirming previous primate studies showing that unilateral injection provides some AAV2 dissemination from one eye to the other. Whether this AAV2 leakage was enough for clinical significance remains to be understood.

Mitochondria transfer-based therapy reduces the morbidity and mortality of Leigh Syndrome

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Abstract

Intercellular mitochondria transfer is a recently described phenomenon in which some cell types export their mitochondria for delivery to developmentally unrelated cells. This process has been linked to regulation of cellular metabolism, cancer, the immune system, mitochondria quality control, wound healing, and adipose tissue homeostasis. Recently, we demonstrated that administering purified wildtype (WT) mitochondria to *Ndufs4*$^{-/-}$ mice, which lack mitochondrial Complex I activity and develop Leigh Syndrome (LS)-like disease, is sufficient to rescue cell-intrinsic defects in mitochondrial metabolism in peritoneal macrophages (Borcherding et al., *Cell Metabolism*, 2022). However, the therapeutic potential of using mitochondria transfer to treat inherited mitochondrial diseases, such as LS, is unclear. Here we show that repeated administration of purified WT mitochondria to *Ndufs4*$^{-/-}$ mice extends their lifespan, improves their neurologic function, and increases whole-body energy expenditure. Based on these findings, we transplanted *Ndufs4*$^{-/-}$ mice with bone marrow from WT or mitochondria reporter mice to engraft every organ with a continuous, self-renewing source of healthy mitochondria. Engrafted hematopoietic cells released their mitochondria into circulation and transferred their mitochondria to host cells in multiple organs, processes that were associated with improved morbidity and mortality of *Ndufs4*$^{-/-}$ mice. These data suggest that mitochondria transfer-related pathways can be harnessed to treat inherited mitochondrial diseases such as LS.

Nucleotide imbalance and mitochondrial innate immune signaling

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Abstract

Mitochondria are master regulators of metabolism and have emerged as key signaling organelles of the innate immune system. Each mitochondrion harbors potent inflammatory agonists, such as mtDNA, which elicit inflammatory cascades when exposed to the cytosol. Mitochondrial damage and dysfunction can result in the translocation of mtDNA to the cytosol, but recent evidence has shown that metabolic cues can also stimulate mtDNA release and innate immune signaling. The i-AAA protease YME1L rewires the mitochondrial proteome in response to mTORC1 inhibition to ensure the synthesis of pyrimidines, thereby maintaining the neurogenic potential of adult neural stem cells and promoting the growth of pancreatic ductal adenocarcinoma cells. Perturbations in pyrimidine synthesis in YME1L deficient cells or after pharmacological inhibition of cytosolic enzymes synthesizing pyrimidines lead to cellular nucleotide imbalance, inducing mtDNA release and innate immune signaling along the cGAS-STING-TBK1 axis. These findings identify metabolic cues as triggers of a mitochondria-dependent inflammatory response. However, it remains enigmatic how pyrimidine deficiency affects mtDNA and cause its release into the cytosol under physiological conditions. Experiments addressing these questions are discussed.

Energizing neurons: Unfolding metabolic plasticity mechanisms

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Abstract

We investigate the mechanisms of metabolic plasticity in neurons, essential for the proper functioning of neural networks and cognitive integrity. Our research focuses on how neurons adapt their metabolic processes to meet changing energy demands and nutrient conditions. Integrating findings from neurobiology, biochemistry, and molecular genetics, we aim to elucidate the regulatory systems controlling neuronal energy homeostasis. Our study reveals key molecular pathways that allow neurons to modulate their metabolic activities efficiently. These insights enhance our understanding of neuronal physiology and could lead to new therapeutic approaches for neurological disorders linked to metabolic imbalances.

Mitochondrial control of cellular immunity

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Abstract

Mitochondria are the evolutionary product of the endosymbiosis between the ancestral eukaryotic cell and an obligate aerobe bacterium, which brought new functionalities to eukaryotic cells. During their evolution, mitochondria transferred more than 99% of their known genetic material to the nucleus – with the exception of a small, multi-copy genome referred to as mtDNA. In Homo sapiens, the 16.6 Kbp of mtDNA is circular and encodes for 13 members of the oxidative phosphorylation chain (OXPHOS) and other structural RNAs. Beyond their canonical role in the generation of ATP through OXPHOS, the mitochondria are also critical stakeholders in several cellular processes from metabolite fluxes and calcium signaling to cell death and aging. Recently, mitochondrial stress has been implicated in the aberrant activation of innate immunity, mediated by the release of organellar components, such as mitochondrial nucleic acids, recognized by cytosolic sensors as foreign and potentially dangerous. I recently uncovered a previously unknown source of stress conducive to aberrant immunity: stress to the mtDNA in the form of double-stranded breaks (mtDSBs). The presence of this stressor is relayed to the cytosolic compartment via mitochondrial herniation, a recently described form of Bax/Bak mediated organelle permeabilization, that exposes mitochondrial contents to the cytosol. After mtDSBs, mitochondrial RNA – rather than the recipient of the stress, mtDNA – initiated the innate immunity cascade by activating the sensor RIG-I. My team's research plan builds on this past work to ask essential questions like: (1) which sources of mtDNA stress are conducive to aberrant immunity and what is the impact of dysfunctional mitochondrial transcription or translation; (2) what are the distinctive features of mitochondrial RNA activation of RIG-I and where do they originate?

Our ultimate goal is to understand how mitochondria integrate and translate stress signals, particularly in the context of innate immunity. My lab will probe different sources of mtDNA stress and interrogate the mechanisms, effectors, and mitochondrial moieties engaging the cytosolic stress pathways, with the hope of developing a long-lasting impact on the foundational and translational knowledge of cellular stress responses going beyond mitochondrial biology into a pathway with further implications in human health and disease: innate immunity.

Developing new therapies for mitochondrial diseases

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Abstract

Mitochondrial diseases are a family of genetically inherited conditions impairing the oxidative phosphorylation system. No cure is currently available for most of the mitochondrial diseases. Several different pharmacological and genetic approaches have been proposed to mitigate or cure mitochondrial disorders. Interesting results have been obtained for improvement of isolated or predominant mitochondrial myopathies, for instance by using the pro-autophagic anti-TORC1 rapamycin, activators of the mitochondriogenic PGC1-alpha, moderate overexpression of the cristae organizer OPA1 and, AAV-based gene replacement. I will summarize our recent findings on the use of AAV vectors for the therapy of Leigh disease and on the hunt for pharmacological compounds activating mitophagy.

Leigh disease is a genetically heterogeneous condition characterized by defective mitochondrial bioenergetics and is the most common oxidative phosphorylation disease in infancy. A knockout mouse for complex I subunit Ndufs4 recapitulates the main features of Leigh syndrome. We recently exploited double-stranded self-complementary AAV9 (scAAV9) vectors, to deliver the wild-type form of human NDUFS4 to Ndufs4-/- mice by either single intra-vascular or double intra-vascular and intra-cerebro-ventricular injections at post-natal Day 1. The first strategy ubiquitously conveyed the human NDUFS4 gene product in Ndufs4-/- mice, doubling the lifespan from 45 to ≈100 days after birth, when the mice developed rapidly progressive neurological failure. However, the double, contemporary intra-vascular and intra-cerebroventricular administration of self-complementary-adeno-associated viral NDUFS4 prolonged healthy lifespan up to 9 months of age. Robust expression of hNDUFS4 was detected in different cerebral areas preserving normal morphology and restoring Complex I activity and assembly. Future work is warranted to explore translatability of scAAV9-NDUFS4 in the prodromal phase of the disease in mice and eventually humans.

Activation of mitophagy is a very attractive way to eliminate dysfunctional mitochondria. We previously showed that rapamycin, a well-known mTORC1 inhibitor, improved mitochondrial myopathy due to cytochrome c oxidase deficiency in skeletal muscle specific Cox15 knockout mice via promoting the translocation of TFEB. We carried out a two-steps high-content screening of FDA-approved compounds to identify drugs able to activate TFEB and mitophagy and identified 8 compounds that effectively activate these pathways, that will be tested on pathological models.

A Na^+ gradient controls Δψ through the Na^+/H^+ antiporter activity of respiratory complex I and is impaired in LHON disease

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Abstract

The mitochondrial electron transport chain (mETC) converts the energy of substrate oxidation into a H^+ electrochemical gradient (Δp), which is composed by an inner mitochondrial membrane (IMM) potential (ΔΨmt) and a pH gradient (ΔpH). So far, ΔΨmt has been assumed to be composed exclusively by H^+. Mitochondrial Ca^{2+} and Na^+ homeostasis, which are essential for cellular function, are controlled by exchangers and antiporters in the inner mitochondrial membrane (IMM). In the last few years, some of them have been identified, except for the mitochondrial Na^+/H^+ exchanger (mNHE). Here, using a rainbow of mitochondrial and nuclear genetic models, as well as reconstitution into proteoliposomes, we have identified it as, specifically, the P-module of complex I (CI). In turn, its activity creates a Na^+ gradient across the IMM, parallel to ΔpH, which accounts for half of the ΔΨmt in coupled respiring mitochondria. We have also found that a deregulation of this mNHE function in CI, without affecting its enzymatic activity, occurs in Leber hereditary optic neuropathy (LHON), which has profound consequences in ΔΨmt and mitochondrial Ca^{2+} homeostasis, explains the previously unknown molecular pathogenesis of this neurodegenerative disease and provides an explanation for the differences in the molecular pathogenesis with other CI-based neurological disorders.

Mitochondrial dynamics actively drive cell fate decisions

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Abstract

Transitions into distinct cellular states, for example from proliferation into quiescence or senescence, require fundamental metabolic reprogramming and mitochondrial remodeling. Mitochondrial dynamics, through fission and fusion, facilitate structural and functional adjustments of cellular metabolism and are associated with cell state changes. Accumulating evidence implies that mitochondrial remodeling is not only a consequence of altered cellular demand, but that mitochondrial dynamics actively determine cellular fate.

The aim of this project is to reveal how mitochondria are remodeled to satisfy the demand of different cell states and to what degree they actively drive cell fate transitions. For this we use diploid, non-transformed hTERT-immortalized retinal pigment epithelial cells (RPE1) as a model system in which we induce a permanent or non-permanent cell cycle arrest and put them into a quiescent or senescent state. To investigate mitochondrial remodeling during cell state transitions, we perform live-cell super-resolution microscopy (SIM, STED) and automated AI-based image analysis of mitochondrial membrane markers and fluorescent biosensors to access mitochondrial ultrastructure, dynamics and metabolic function simultaneously with single-organelle resolution. We combine this with mass spectrometry analysis of phosphorylation sites on mitochondrial fission and fusion proteins to identify upstream signaling pathways inducing mitochondrial shape changes during cell fate transitions.

Our data show that mitochondrial dynamics, including organelle biogenesis and quality control, strongly decline when cells enter quiescence resulting in an extremely static mitochondrial network. We suggest that this is due to altered biophysical properties of the mitochondrial matrix. In senescent cells, mitochondria remain dynamic but display reduced quality control and elevated ROS production. In addition, we frequently observe release of mtDNA into the cytoplasm and activation of the cGAS-STING pathway. Our preliminary data indicate that these processes actively drive a permanent cell cycle arrest and that promoting or inhibiting fission is a sufficient strategy to accelerate or inhibit senescence progression.

Role of the MICOS subunit MIC26 in the early development of insulin resistance

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Abstract

Altered levels of the mitochondrial contact site and cristae organizing system (MICOS) complex are correlated to many human diseases such as Parkinson's disease, cardiomyopathy and diabetes. The MICOS complex is comprised of seven different subunits including MIC60, MIC19, MIC25, MIC13, MIC10 and the apolipoproteins MIC27 and MIC26/ApoO and was shown to be essential for cristae architecture, crista junction (CJ) formation, as well as cristae membrane dynamics. Previous studies showed a correlation between altered MIC26 levels and dysregulation of fatty acid metabolism causing lipotoxicity as well as an upregulation of MIC26 in the diabetic heart. Diet-induced obesity resulted in reduced expression of MIC26, while adipose tissue specific MIC26 depletion caused increased adiposity. Hence, we approached to reveal the underlying cellular mechanism of MIC26 in the early development of obesity and insulin resistance. We performed a chronic normo- and hyperglycemic treatment and investigated cellular changes using a multi-omics approach including transcriptomics, proteomics and targeted metabolomics. As anticipated, we observed that MIC26 depletion led to changes in mitochondrial function and structure. Moreover, we found that depletion of MIC26 led to a cellular metabolic rewiring and a switch in fuel preferences. Based on our findings we hypothesize MIC26 to be a mitochondrial fuel sensor with a possible role in the early development of insulin resistance and obesity upon nutrition overload.

Mitochondrial subpopulations in cardiomyocytes are shaped by their intracellular environment

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Abstract

The heart is one of the tissues with the highest energy demand, making mitochondrial function indispensable for cardiomyocyte health and thereby organismal survival. Interestingly, it has been shown that cardiomyocyte mitochondria are segregated into functionally distinct mitochondrial types (or mitotypes), the interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM). These mitotypes differ in their morphology, protein composition, and bioenergetics capacity. Importantly, certain pathologies like aging, diabetes, and acute myocardial infarction have been linked to a marked decline in IFM abundance and functionality, however, the exact mechanisms driving this decline remain elusive.

While the existence and functional distinctions of cardiomyocyte mitotypes are well-accepted and extensively researched, it is still unclear by which mechanism these two distinctive mitochondrial population can be generated and maintained within a single cell. Using heteroplasmic mice that harbor two distinct mtDNA types, giving rise to functionally different mitochondria, we provide new evidence that SSM and IFM are not intrinsically distinct, but are shaped by the subcellular environment in which they are. Moreover, we performed proteomics analyses of SSM and IFM, and found unique cytoplasmic ribosomal proteins associated with each intracellular mitotype within the heart. These results suggest that the proteomic differences between the mitotypes could be a consequence of a locally specialized translation machinery. We have identified Rpl3l, whose role in shaping cardiomyocyte function was recently described, as one of the ribosomal proteins significantly enriched in the IFM fraction. We show that loss of Rpl3l significantly alters IFM respiratory capacity, in particular complex I driven respiration. These findings suggest that intracellular distribution of ribosomal proteins could be a key driver of functional specialization of intracellular mitotypes, which could provide new insights in cardiac disease therapies.

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.

Mouse and human brain mitochondrial diversity

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Abstract

The brain and animal behavior are under energetic constraints, limited by mitochondrial energy transformation capacity. However, the mitochondria-behavior relationship has not been systematically studied on a brain-wide scale. Here we examined the association between multiple features of mitochondrial respiratory chain capacity and stress-related behaviors in mice with diverse behavioral phenotypes. Miniaturized assays of mitochondrial respiratory chain enzyme activities and mitochondrial DNA (mtDNA) content were deployed on 571 samples across 17 mouse brain areas, defining specific patterns of mito-behavior associations. By applying multi-slice network analysis to our brain-wide mitochondrial dataset, we identified three large-scale networks of brain areas with shared mitochondrial signatures. A major network composed of cortico-striatal areas exhibited the strongest mitochondria-behavior correlations, accounting for a up to 50% of animal-to-animal behavioral differences, suggesting that this mito-based network is functionally significant. The mito-based brain networks also overlapped with regional gene expression and structural connectivity, and quantitatively diverged in their molecular mitochondrial phenotype (i.e., mitotype) signatures. Therefore, this work provides convergent multimodal evidence anchored in enzyme activities, gene expression, and animal behavior that distinct, behaviorally-relevant mitochondrial phenotypes exist across the mouse brain. Extending these findings to the human brain, we apply the same molecular and enzymatic mitotyping approach to >700 systematically dissected physical voxels from cortical and subcortical structures of a single post-mortem human brain. The resulting atlas of mitochondrial variation across the human brain provides a basis to understand the energetic basis of brain connectivity and computations, and how the brain generates and sustains complex energy patterns related to the mind.