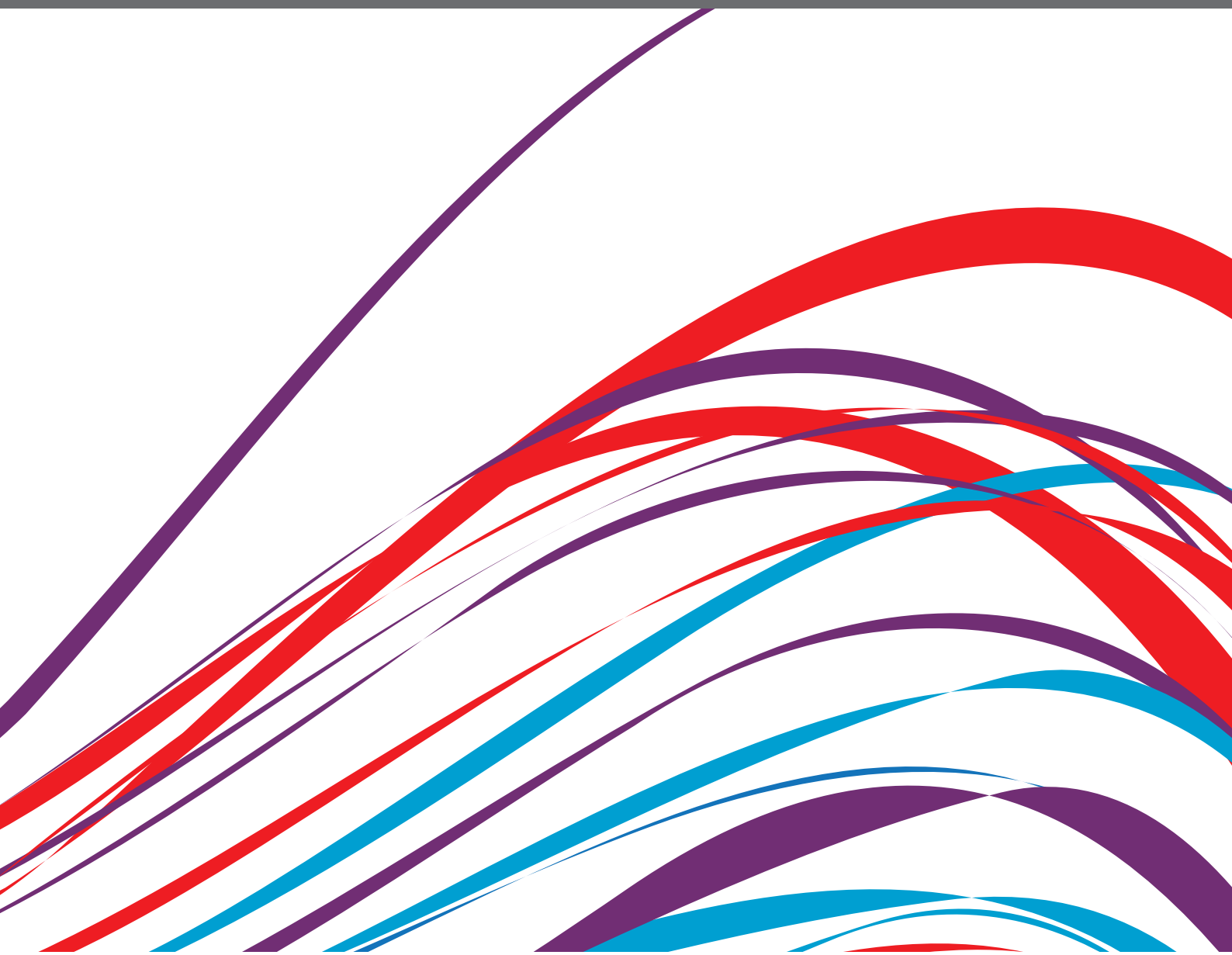
MITOCHONDRIAL DYSFUNCTION AND CARDIOVASCULAR DISEASES

EDITED BY: Sebastiano Sciarretta, Richard N. Kitsis and Junichi Sadoshima
PUBLISHED IN: Frontiers in Cardiovascular Medicine



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-556-3
DOI 10.3389/978-2-88966-556-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MITOCHONDRIAL DYSFUNCTION AND CARDIOVASCULAR DISEASES

Topic Editors:
Sebastiano Sciarretta, Sapienza University of Rome, Italy
Richard N. Kitsis, Albert Einstein College of Medicine, United States
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Citation: Sciarretta, S., Kitsis, R. N., Sadoshima, J., eds. (2021). Mitochondrial Dysfunction and Cardiovascular Diseases. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88966-556-3

Table of Contents

frontiers in Cardiovascular Medicine

EDITORIAL
published: 22 January 2021
doi: 10.3389/fcvm.2021.645986

Editorial: Mitochondrial Dysfunction and Cardiovascular Diseases

Junichi Sadoshima[1], *Richard N. Kitsis*[2] *and Sebastiano Sciarretta*[3,4]*

[1] Department of Cell Biology and Molecular Medicine, Rutgers New Jersey Medical School, Newark, NJ, United States, [2] Albert Einstein College of Medicine, Bronx, NY, United States, [3] Department of Medical and Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy, [4] IRCCS Neuromed, Pozzilli, Italy

Keywords: mitochondrial dysfunction, mitophagy, cardiovascular diseases, mitochondrial dynamics, mitochondrial ROS

Editorial on the Research Topic

Mitochondrial Dysfunction and Cardiovascular Diseases

A deeper understanding of the molecular mechanisms underlying the development and progression of cardiovascular diseases represents a major goal in cardiovascular medicine. Mitochondrial dysfunction has emerged as major player in the development of cardiovascular diseases, with potential therapeutic implications. Mitochondrial dysfunction encompasses mitochondrial complex disruption, mitochondrial uncoupling, and cristae remodeling and swelling, which in turn cause ROS accumulation, energy stress, and cell death.

This Research Topic is a collection of original and state-of-the art review articles discussing and extending our current knowledge about molecular mechanisms responsible for mitochondrial dysfunction in cardiovascular diseases. Many aspects of mitochondrial biology and therapies targeting damaged mitochondria have been highlighted.

One of the main feature of mitochondrial dysfunction observed in several cardiovascular diseases is the exaggerated generation of mitochondrial ROS (1), which represents the common pathological substrate underlying diabetes-induced complications, such as cardiomyopathy, as comprehensively described by Kaludercic and Di Lisa in their review article. Mitochondrial ROS are generated from multiple sources in cardiomyocytes during diabetes by a feed-forward/amplification mechanism, which further exacerbates oxidative stress and causes contractile dysfunction. The authors reviewed current therapies aimed at reducing ROS and improving cardiac function in diabetic patients. While some systemic antioxidants failed to exert cardiac protection in clinical trials, mitochondrial-targeted antioxidants such as MitoTEMPO were shown to be cardioprotective in preclinical models of diabetic cardiomyopathy.

Sodium glucose cotransporter 2 (SGLT2) inhibitors also appear to be promising drugs to reduce cardiovascular events in diabetic patients. In this regard, Maejima provided a detailed overview about the mitochondrial-mediated mechanisms underlying the beneficial effects of SGLT2 inhibitors in heart failure. SGLT2 inhibitors increase ketone bodies, which represent a suitable source of energy in failing hearts, and also improve sodium metabolism and mitochondrial dynamics. However, further studies are needed to identify other targets modulated by SGLT2 inhibitors, since SGLT2 does not appear to be expressed in human and rodent cardiomyocytes, at least in unstressed conditions. A modulation of mitochondrial dynamics may contribute to the beneficial effects of this class of drugs on mitochondrial function in response to metabolic derangements (2).

Targeting mitochondria, and in particular mitochondrial ROS, has also emerged as a potential therapy for patients with dilated cardiomyopathy with ataxia syndrome (DCMA), a rare genetic disorder caused by a mutation of DNAJ Heat Shock Protein Family (Hsp40) Member

OPEN ACCESS

Edited and reviewed by:
Ichiro Manabe,
Chiba University, Japan

***Correspondence:**
Sebastiano Sciarretta
sebastiano.sciarretta@uniroma1.it

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *24 December 2020*
Accepted: *05 January 2021*
Published: *22 January 2021*

Citation:
Sadoshima J, Kitsis RN and Sciarretta S (2021) Editorial: Mitochondrial Dysfunction and Cardiovascular Diseases. Front. Cardiovasc. Med. 8:645986. doi: 10.3389/fcvm.2021.645986

C19 (DNAJC19), a protein localized in the inner mitochondrial membrane. Machiraju et al. demonstrated that SS-31, a mitochondrial targeted antioxidant, also known as elamipretide or Bendavia, rescues mitochondrial fragmentation, oxidative stress, and improves mitochondrial fusion in skin fibroblasts extracted from DCMA patients. However, the therapeutic potential of SS-31 in improving cardiac function in patients with DCMA should be assessed in further studies.

Mitochondrial health is facilitated by specific quality control mechanisms, such as mitophagy, a cargo-specific form of autophagy selective for elimination of damaged mitochondria (3). Damaged mitochondria are degraded by mitophagy and defects in mitophagy were reported to lead to harmful cardiovascular effects, because of accumulation of defective mitochondria. In their original article, Thomas et al. found decreased levels of Parkin protein in the heart of obese mice. Parkin is a ubiquitin E3 ligase, which represents a canonical regulator of mitophagy and proteasome degradation. The authors also observed a modest increase of infarct size in obese mice undergoing ischemia/reperfusion (IR) *ex-vivo* and a cardiac accumulation of ubiquitinated mitochondrial proteins at baseline and in response to IR in obese animals. This study suggested that mitophagy may be impaired in the context of obesity because of Parkin downregulation, thereby predisposing the heart to develop increased injury in response to stress. However, a direct assessment of mitophagy was not performed in this study and further work is necessary to clarify the impact of metabolic alterations on Parkin-dependent and independent mitophagy in the heart.

The importance of autophagy and mitophagy abnormalities in aging-induced cardiovascular abnormalities was the main focus of the review article by Liang and Gustafsson. The authors reviewed relevant literature supporting the concept that autophagy declines with aging, leading to age-related cardiovascular diseases, due to alterations in cellular energy metabolism and adaption to stress. Either genetic or pharmacological activation of mitophagy appears to attenuate aging-related abnormalities, whereas its inhibition seems to accelerate them (4). It will be important to understand in the future how aging affects Parkin-dependent and independent mitophagy in the heart, and the exact molecular mechanisms through which autophagosome formation and fusion are impaired by the aging process. Increased oxidative stress and inflammation appear to play a critical role.

Aside from mitophagy and mitochondrial dynamics, mitochondrial proteostasis is also emerging as an important mechanism regulating mitochondrial quality control in the heart, as described in the paper by Arrieta et al. Mitochondrial proteostasis regulates biogenesis, folding, and degradation of mitochondrial proteins and this process appears to be altered during cardiac stress. In the presence of misfolded protein accumulation in mitochondria, mitochondrial unfolded protein response (mtUPR) is activated by means of accumulation of ATF5, which translocates to the nucleus and stimulates the upregulation of an adaptive gene response aimed at the restoration of mitochondrial protein folding and proteostasis. Previous work showed that stimulation of mtUPR improves mitochondrial function and reduces cardiac damage in response to I/R injury and pressure overload. The elucidation of the integration points between mitochondrial and endoplasmic reticulum proteostasis represents an important aspect to be clarified in future studies.

Mitochondria are also massively damaged by anthracycline-based chemotherapy, and mitochondrial dysfunction contributes to the development anthracycline-induced cardiotoxicity, as reviewed by Murabito et al. Doxorubicin, a well-known drug belonging to the anthracycline class, directly binds cardiolipin and accumulates into mitochondria, causing disruption of electron transport chain complexes, thereby contributing to ROS accumulation. The latter triggers several adverse events, such as mitochondrial uncoupling, oxidative stress, apoptosis, ferroptosis, and impairment of calcium metabolism, which then lead to cardiomyopathy development. In addition, mitochondrial dynamics and autophagy are impaired by doxorubicin treatment, further aggravating mitochondrial damage. Different therapeutic strategies have been suggested to reduce anthracycline-induced mitochondrial dysfunction and cardiotoxicity. These include mitochondria-targeted antioxidants, autophagy activators, or inhibitors of mitochondrial fatty acid beta-oxidation. However, the signaling pathways involved in the perpetuation of mitochondrial damage in response to doxorubicin treatment still need to be clarified. The elucidation of this aspect will be very important for the discovery of new therapeutic targets for the prevention of doxorubicin-induced cardiotoxicity and for the identification of subjects with potentially higher susceptibility to develop cardiac injury after chemotherapy.

Mitochondrial biogenesis is also critical for the regulation of mitochondrial turnover and function in cardiovascular pathophysiology (5). The transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1 alpha (PGC-1α) represents a major regulator of mitochondrial biogenesis and metabolism, as discussed in detail by Oka et al. A dysregulation of PGC-1α signaling during heart failure occurs at transcriptional and post-transcriptional level, contributing to the development of cardiac dysfunction, due to alterations of multiple mechanisms, particularly those involved in mitochondrial metabolism.

Perturbations of epigenetic mechanisms regulating mitochondrial function also contribute to cardiovascular diseases, as reviewed by Mohammed et al. Epigenetic changes impair mitochondrial function, resulting in a decrease in mitochondrial metabolites (i.e., NAD, FAD) used as cofactors by components involved in chromatin modifications. The latter further exacerbates epigenetic remodeling. Among epigenetic modulators, HDAC inhibitors or SIRT1-3 activators were shown to preserve mitochondrial function in different cardiovascular diseases by reducing epigenetic remodeling.

In conclusion, this Research Topic highlights that alterations in different mechanisms regulating mitochondrial quality control and function directly contribute to the development of cardiovascular diseases. Mitochondrial dysfunction determines an impairment of energy production, which is detrimental for heart function. In addition, mitochondrial damage triggers cell death pathways. Although the reduction of mitochondrial

ROS appears to be a valid approach to reduce mitochondrial dysfunction, an improvement of mitochondrial quality control and epigenetic mechanisms may also represent an efficacious strategy in future clinical applications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Kornfeld OS, Hwang S, Disatnik MH, Chen CH, Qvit N, Mochly-Rosen D. Mitochondrial reactive oxygen species at the heart of the matter: new therapeutic approaches for cardiovascular diseases. *Circ Res*. (2015) 116:1783–99. doi: 10.1161/CIRCRESAHA.116.305432
2. Forte M, Schirone L, Ameri P, Basso C, Catalucci D, Modica J, et al. The role of mitochondrial dynamics in cardiovascular diseases. *Br J Pharmacol*. (2020) doi: 10.1111/bph.15068. [Epub ahead of print].
3. Saito T, Hamano K, Sadoshima J. Molecular mechanisms and clinical implications of multiple forms of mitophagy in the heart. *Cardiovasc Res*. (2020) cvaa340. doi: 10.1093/cvr/cvaa340. [Epub ahead of print].
4. Sciarretta S, Maejima Y, Zablocki D, Sadoshima J. The role of autophagy in the heart. *Annu Rev Physiol*. (2018) 80:1–26. doi: 10.1146/annurev-physiol-021317-121427
5. Vega RB, Horton JL, Kelly DP. Maintaining ancient organelles: mitochondrial biogenesis and maturation. *Circ Res*. (2015) 116:1820–34. doi: 10.1161/CIRCRESAHA.116.305420

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 15 November 2019
doi: 10.3389/fcvm.2019.00167

SS-31 Peptide Reverses the Mitochondrial Fragmentation Present in Fibroblasts From Patients With DCMA, a Mitochondrial Cardiomyopathy

*Pranav Machiraju[1†], Xuemei Wang[1†], Rasha Sabouny[2], Joshua Huang[1], Tian Zhao[2], Fatima Iqbal[1], Melissa King[1], Dimple Prasher[2], Arijit Lodha[1], Nerea Jimenez-Tellez[1], Amir Ravandi[3], Bob Argiropoulos[4,5], David Sinasac[4,5], Aneal Khan[1,4,5], Timothy E. Shutt[2,4,5] and Steven C. Greenway[1,2,5,6,7]**

[1] Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [2] Department of Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [3] Department of Physiology and Pathophysiology, St. Boniface Hospital Research Centre, Institute of Cardiovascular Sciences, University of Manitoba, Winnipeg, MB, Canada, [4] Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [5] Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [6] Department of Cardiac Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [7] Libin Cardiovascular Institute of Alberta, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

We used patient dermal fibroblasts to characterize the mitochondrial abnormalities associated with the dilated cardiomyopathy with ataxia syndrome (DCMA) and to study the effect of the mitochondrially-targeted peptide SS-31 as a potential novel therapeutic. DCMA is a rare and understudied autosomal recessive disorder thought to be related to Barth syndrome but caused by mutations in *DNAJC19*, a protein of unknown function localized to the mitochondria. The clinical disease is characterized by 3-methylglutaconic aciduria, dilated cardiomyopathy, abnormal neurological development, and other heterogeneous features. Until recently no effective therapies had been identified and affected patients frequently died in early childhood from intractable heart failure. Skin fibroblasts from four pediatric patients with DCMA were used to establish parameters of mitochondrial dysfunction. Mitochondrial structure, reactive oxygen species (ROS) production, cardiolipin composition, and gene expression were evaluated. Immunocytochemistry with semi-automated quantification of mitochondrial structural metrics and transmission electron microscopy demonstrated mitochondria to be highly fragmented in DCMA fibroblasts compared to healthy control cells. Live-cell imaging demonstrated significantly increased ROS production in patient cells. These abnormalities were reversed by treating DCMA fibroblasts with SS-31, a synthetic peptide that localizes to the inner mitochondrial membrane. Levels of cardiolipin were not significantly different between control and DCMA cells and were unaffected by SS-31 treatment. Our results demonstrate the abnormal mitochondria in fibroblasts from patients with DCMA and suggest that SS-31 may represent a potential therapy for this devastating disease.

Keywords: mitochondria, cardiomyopathy, fibroblasts, SS-31, DCMA, cardiolipin

OPEN ACCESS

Edited by:
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Reviewed by:
Wang Wang, University of Washington, United States
Shinichi Oka, Rutgers, The State University of New Jersey, United States

****Correspondence:***
Steven C. Greenway
scgreenw@ucalgary.ca

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *16 June 2019*
Accepted: *31 October 2019*
Published: *15 November 2019*

Citation:
Machiraju P, Wang X, Sabouny R, Huang J, Zhao T, Iqbal F, King M, Prasher D, Lodha A, Jimenez-Tellez N, Ravandi A, Argiropoulos B, Sinasac D, Khan A, Shutt TE and Greenway SC (2019) SS-31 Peptide Reverses the Mitochondrial Fragmentation Present in Fibroblasts From Patients With DCMA, a Mitochondrial Cardiomyopathy. Front. Cardiovasc. Med. 6:167. doi: 10.3389/fcvm.2019.00167

INTRODUCTION

The dilated cardiomyopathy with ataxia syndrome (DCMA), also known as 3-methylglutaconic aciduria type V, is a rare and understudied autosomal recessive disorder caused by mutations in the poorly characterized gene DNAJ Heat Shock Protein Family (Hsp40) Member C19 (*DNAJC19*) (1–4). The DNAJ family of proteins act as molecular chaperones and are defined by their J-domains which regulate the function of HSP70 chaperones (5). DNAJC19 is localized to the inner mitochondrial membrane and although some of its interacting partners have been identified (4), its precise role is unknown. DCMA was first described in the Dariusleut Hutterite population of southern Alberta who represent the largest population of patients in the world with only sporadic cases reported elsewhere (3, 6, 7). In the Hutterites, a genetically-isolated population that share a common European ancestry and a communal lifestyle, DCMA is caused by a single homozygous *DNAJC19* intronic pathogenic variant NG_022933.1:c.130-1G>C (rs137854888) that leads to abnormal splicing and a truncated, non-functional protein (2). DCMA is a heterogeneous disorder characterized by 3-methylglutaconic aciduria, dilated cardiomyopathy, developmental delay, neuromotor abnormalities, growth failure, prolongation of the QT interval, and various other systemic features (8). End-stage heart failure leading to death in early childhood is common and, until recently, no effective therapeutic had been identified (9). However, the mechanism of disease remains unknown.

DCMA is phenotypically related to Barth syndrome (3-methylglutaconic aciduria type II) which is caused by mutations in the X-linked *TAZ* gene and whose clinical features partially overlap those seen in DCMA (10, 11). *TAZ* encodes the tafazzin protein which is involved in the remodeling of cardiolipin (CL), a phospholipid predominantly localized to the inner mitochondrial membrane (11). CL has important roles in stabilizing mitochondrial membrane protein complexes and maintaining mitochondrial structure and membrane curvature (12). CL acyl chain remodeling is disrupted in cardiomyopathy, including Barth syndrome, and heart failure (13–16). In cultured cells, knock-down of *DNAJC19* expression was reported to affect CL remodeling, which may explain the related clinical features of DCMA and Barth syndrome (4). Although this *in vitro* data demonstrated that *DNAJC19* deficiency resulted in changes in CL composition and abnormal mitochondrial structure and dysfunction, results from DCMA patients have been conflicting. Both decreased and normal electron transport chain complex activities in tissues and cells have been reported (3, 6, 7), with Al Teneiji et al. reporting normal mitochondrial morphology in skeletal muscle (7). Despite the conflicting findings, the potential for abnormal mitochondrial structure and function in DCMA may represent a possible target for therapeutic intervention.

The Szeto-Schiller peptide SS-31 (also known as elamipretide or Bendavia) interacts specifically with CL to affect membrane curvature and prevent peroxidative damage (17–19) and has shown pre-clinical promise as a treatment for mitochondrial disorders and heart failure (20–22). Our study aimed to characterize the structure of mitochondria found in primary dermal fibroblasts isolated from pediatric DCMA patients and to evaluate the effect of treatment with SS-31.

MATERIALS AND METHODS

Fibroblasts

After obtaining informed consent, clinically-indicated skin biopsies were obtained from pediatric patients undergoing investigation for metabolic disease. Fibroblasts were expanded in the Molecular Genetics Laboratory at the Alberta Children's Hospital and subsequently frozen at −80°C until use. Four fibroblast strains from patients with biochemically and/or genetically-confirmed DCMA were selected for this study. Commercially-available control fibroblast strains derived from healthy adults or children were obtained from ThermoFisher Scientific or the Coriell Institute. All fibroblasts were grown in T25 or T75 cell culture flasks (ThermoFisher Scientific) with Minimum Essential Medium Eagle supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM glutamine, 200 μM uridine, and 100 U/ml penicillin-streptomycin (Sigma-Aldrich). Cells were maintained under mycoplasma-free and sterile conditions in a tissue culture incubator equilibrated with 5% CO_2 at 37°C and medium was changed every 5 days. SS-31 (D-Arg-2′6′-dimethylTyr-Lys-Phe-NH_2) was synthesized by China Peptides (23). Experiments using SS-31 were performed by incubating fibroblasts for 24 h with 100 nM SS-31. A peptide lacking the methylated tyrosine (D-Arg-Tyr-Lys-Phe-NH_2) which we have named 366401 was synthesized for us by China Peptides and incubated with fibroblasts for 24 h using two different concentrations (100 and 300 nM) to assess the effect of the methylated tyrosine group.

Imaging

To prepare cells for immunocytochemistry, confluent cells were dissociated using trypsin-EDTA then collected by centrifugation at 2,000 rpm for 10 min. Cell pellets were resuspended in fresh medium post-passage and seeded onto individual sterilized microscope coverslips placed on the bottom of a 24-well tissue culture plate. Cells were then allowed to grow for 48-h prior to staining. Cells on glass coverslips were washed twice with Dulbecco's phosphate-buffered saline (DPBS) then fixed with pre-warmed 4% paraformaldehyde (J. T. Baker) in DPBS and incubated at 37°C for 15 min. Cells were then washed three times with DPBS, quenched with 50 mM NH_4Cl for 15 min at room temperature (RT) then washed again with DPBS and stored at 4°C. When ready to stain, cells were permeabilized with 0.2% Triton X-100 in PBS for 15 min then washed three times with DPBS, blocked with 10% FBS for 25 min at RT then incubated with 1:1000 TOMM20 primary antibody (Sigma-Aldrich, cat. HPA011562) diluted in 5% FBS for 1-h at 37°C. Cells were then washed three times (5 min per wash) with 5% FBS diluted in DPBS. Cells were then incubated with the AlexaFluor 488 secondary antibody (1:1000, ThermoFisher Scientific, cat. A11034) in 5% FBS for 1-h at RT. Cells were washed then stored at 4°C in the dark until imaged on a Zeiss LSM880 confocal microscope using a 63X oil objective.

To quantify mitochondrial fragmentation in fibroblasts, thirty TOMM20-stained cells per cell line and treatment were manually graded based on a set fragmentation scale. Hyperfused cells were assigned a grade of (1), cells with intermediate fragmentation were assigned (2), and a grade of (3) was assigned to cells exhibiting substantial mitochondrial fragmentation (24). Significance was determined using a two-way ANOVA with a Holm-Sidak correction for multiple comparisons. To quantitatively assess cellular mitochondrial networks in an objective manner, a semi-automated ImageJ plug-in Mitochondrial Network Analysis (MiNA) toolset was used (25). Briefly, TOMM20-stained fibroblasts were imaged using a Zeiss LSM880 high-resolution confocal microscope. Images were then randomly cropped to select 30 individual cells. These 30 cells were identical to the those used for manual quantification. Cells were then pre-processed by using ImageJ functions unsharp mask, CLAHE, and median filtering then batch processed through MiNa. Raw data from MiNa was put through R Studio (ggbiplot, vegan, readxl, plyr, scales, and grid packages) to generate the PCA plots and calculate significant differences in clustering through Adonis tests. MiNa output displays mean network size, mean fragment length, and mitochondrial footprint. Mean network size is calculated through counting the number of mitochondrial branches per network. Mean fragment length refers to the average mitochondrial rod/branch length. Mitochondrial footprint is described as the total area in the cell expressing mitochondrial marker TOMM20. Significance was determined using a two-way ANOVA with a Holm-Sidak correction for multiple comparisons.

To assess mitochondrial ultrastructure using transmission electron microscopy, DCMA, and control fibroblasts were cultured in 24-well plates to over 80% confluence. Once grown, cells were fixed and sent to University of Calgary's Microscopy and Imaging Facility. Processed cells were imaged using a Hitachi H7650 transmission electron microscope.

Reactive Oxygen Species (ROS) Production

Fibroblasts cultured on 35 mm glass plates (World Precision Instruments) to 50% confluence were co-stained with MitoSOX Red (ThermoFisher Scientific, cat. M36008) and MitoTracker Green (ThermoFisher Scientific, cat. M7514). Fresh fibroblast medium (2 mL) containing 5 μM MitoSOX Red and 70 nM MitoTracker Green was added to the cells and incubated for 20 min at 37°C. Cells were then washed with DPBS and new medium was added. Cells were incubated at 37°C for 20 min for de-staining and then imaged using an Olympus spinning disc confocal system (Olympus SD OSR) operated using Metamorph software. Cells were then analyzed using ImageJ. Briefly, each cell was isolated through a selection tool on both treatment images. Once identified, remaining fluorescence in the image was cleared. The cells were then subjected to a defined threshold to keep brightness consistent. Both channels were then combined using the image calculator resulting in a cell expressing co-localized fluorescence. Mean gray intensity of the cells was then calculated and plotted using GraphPad Prism 7. Seventy individual cells were quantified per cell line and treatment. Significance was determined through a one-way ANOVA with a Holm-Sidak correction for multiple comparisons.

Western Blotting

Control and patient fibroblasts were seeded onto T25 flasks and allowed to grow overnight at 37°C and 5% CO_2. Cells were then treated with 100 nM SS-31 or vehicle control for 24-h. Subsequently, cells were harvested, cell pellets washed and lysed with RIPA buffer containing protease inhibitors (Amersco, cat. M250). Total cell lysates (20 μg) were resolved on SDS-PAGE gels and transferred onto PVDF membranes. Blots were probed with antibodies against OPA1 (BD Bioscience, cat. 612606) at 1:1000 final dilution followed by horseradish peroxidase-conjugated secondary antibodies. Blots were finally incubated with Clarity ECL substrate (Biorad) according to manufacturer's instructions and imaged on an Amersham Imager AI600. Densitometric analysis of band intensities were performed using ImageJ and normalized to a loading control (HSP60). Data was plotted using Prism 7 (GraphPad Software) and significance was determined using an one-way ANOVA followed by a Tukey correction for multiple comparisons.

RNA Preparation and RNA-Seq Analysis

Total RNA was extracted from DCMA fibroblasts ($n = 4$) and control fibroblasts ($n = 4$) using the RNA extraction Mini kit (Invitrogen) according to the manufacturer's protocol. RNA purity was assessed and quantified using Nanodrop and a Qubit 2.0 fluorometer (ThermoFisher Scientific). The sequencing library was prepared using 2 μg of RNA and the TruSeq Stranded mRNA library preparation kit (Illumina). RNA sequencing generating single-end 100 base pair reads was performed on the Illumina NextSeq500 platform. Raw FASTQ files were generated using Illumina NextSeq Control software (version 2.02). For RNA-Seq analysis, initial sequencing quality was inspected using FASTQC. Next, transcript counts were estimated using kallisto (26) with reference genome GRCh37 and the default settings. Kallisto-estimated counts were then summarized to the gene level using the tximport package in RStudio. Differential gene expression from the counts data was performed using the Bioconductor package DESeq2. Read counts for control and patient fibroblasts were compared to determine the log2-fold change in abundance for each transcript. Raw p-values were adjusted for multiple comparisons with the Benjamini-Hochberg method.

Cardiolipin Analysis

CL mass and species composition were determined as previously described (27).

Statistical Analysis

The data are presented as mean ± standard deviation (SD) and analyzed as described above. A p value < 0.05 was considered significant.

RESULTS

Patient Characteristics

Dermal fibroblasts have often been used to study mitochondrial dysfunction in human diseases (28–30). The fibroblasts used in this study came from four individual Hutterite children from three different families with distinct clinical phenotypes

(**Table 1**) despite harboring the same homozygous pathogenic variant. All patients had evidence of dilated cardiomyopathy by echocardiography with a globular and/or dilated left ventricle. Two patients (D1 and D2) had mild left ventricular dysfunction with a left ventricular ejection fraction (LVEF) of 40–50% (normal > 50%) and two patients (D3 and D4) had severe dysfunction with a LVEF < 35%. Each patient also had other comorbidities, most commonly developmental delay, a prolonged QT interval on the electrocardiogram and failure to thrive. The patients with severe cardiac dysfunction were both deceased at the time of this study. All studies were approved by the Conjoint Health Research Ethics Board at the University of Calgary.

TABLE 1 | Patient information.

ID	Family	Sex	Age (months)	Cardiac phenotype	Other features	Status
D1	1	F	24	mild LV dysfunction	dystonia, DD, FTT, LQT	Alive
D2	2	M	33	mild LV dysfunction	seizures, DD, FTT, LQT	Alive
D3	2	F	13	severe LV dysfunction	DD, FTT, LQT	Deceased
D4	3	F	19	severe LV dysfunction	DD, FTT, LQT	Deceased

Clinical characteristics for the four DCMA fibroblast strains used in this study. F, female; M, male, age in months at the time fibroblasts were collected; LV, left ventricular; DD, developmental delay; FTT, failure to thrive; LQT, prolonged QT interval.

Mitochondrial Fragmentation in DCMA Fibroblasts Is Reversible by Incubation With SS-31

TOMM20, an outer mitochondrial membrane protein, was stained to elucidate mitochondrial structure in DCMA and control fibroblasts. Qualitatively, mitochondrial networks in all DCMA fibroblasts appeared fragmented and disorganized in contrast to control cells which displayed intact and reticular mitochondrial networks (**Figure 1A**). After 24 h of incubation with 100 nM SS-31, the mitochondrial networks in the DCMA fibroblasts qualitatively appeared to be less fragmented and more net-like with increased branching of mitochondrial networks and longer fragments (**Figure 1B**). Semi-automated analysis of mitochondrial structure was used to quantify network

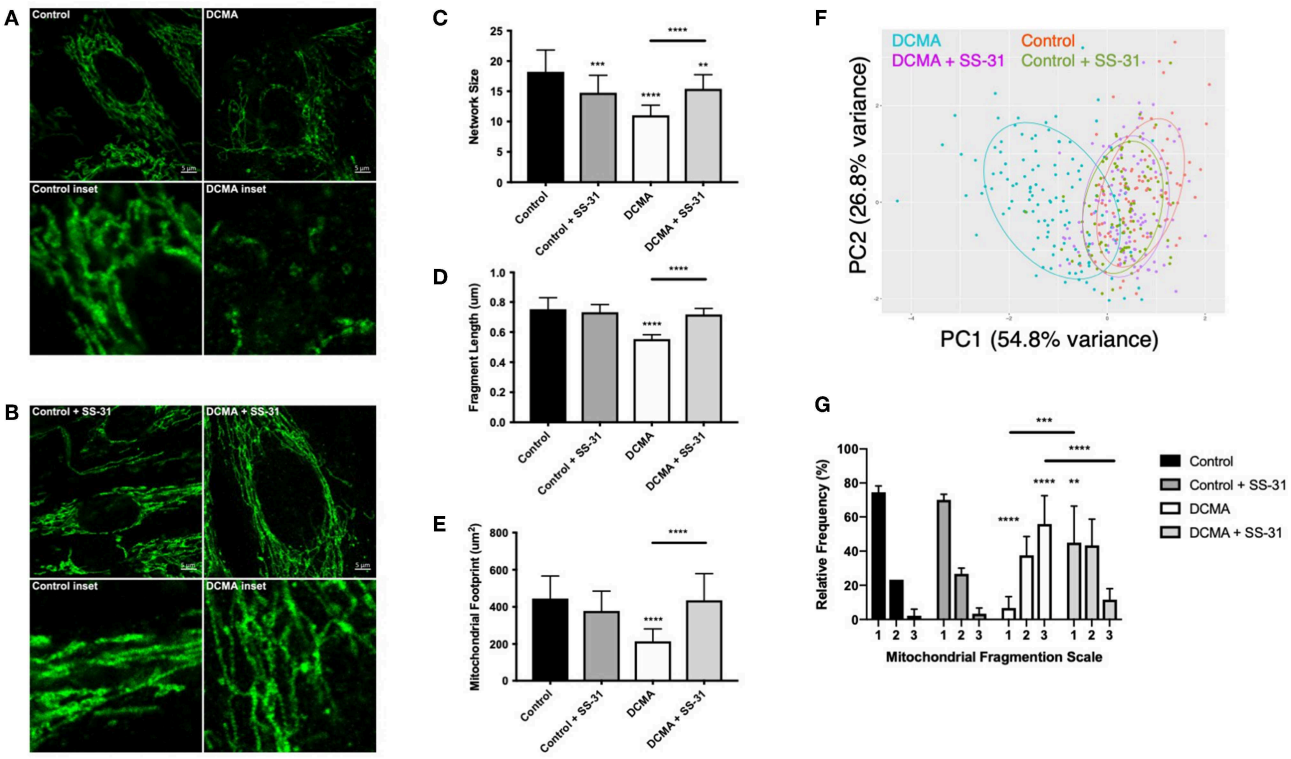


FIGURE 1 | Visualization and quantification of TOMM20 staining of mitochondria. **(A)** Representative example of TOMM20 staining of control and DCMA fibroblasts. **(B)** TOMM20 staining of control and DCMA fibroblasts treated with SS-31 (100 nM for 24 h). Scale bar measures 5 μm. Inset boxes represent the corresponding region at higher magnification. **(C)** Mean network size for control and DCMA fibroblasts representing the number of mitochondrial branches per network. DCMA mitochondria have significantly smaller mitochondrial networks that were restored by SS-31. **(D)** Mean fragment length is the average mitochondrial rod/branch length with DCMA cells having significantly smaller fragments compared to controls that increased with SS-31. **(E)** Mitochondrial footprint is the total area in the cell expressing mitochondrial marker TOMM20 and was significantly smaller in DCMA fibroblasts when compared to controls but increased significantly with SS-31. Data are the mean ± SD of measurements from 30 individual cells for each cell strain ($n = 3$–4). Groups were compared using a two-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. **(F)** PCA plot incorporating data for all mitochondrial morphological metrics (network size, fragment length, and mitochondrial footprint) from control fibroblasts and DCMA fibroblasts before and after exposure to SS-31. **(G)** Manual quantification of mitochondrial morphology from 30 individual cells for control ($n = 3$) and DCMA ($n = 4$) fibroblasts before and after treatment with SS-31 (100 nM for 24 h). Quantification according to a three-point fragmentation scale: (1) hyperfused, (2) intermediate, and (3) fragmented. Data represent mean ± SD. Significance was determined using a two-way ANOVA with a Holm-Sidak correction for multiple comparisons. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

size, fragment length and mitochondrial footprint (25). All parameters were found to be significantly lower in the DCMA cells in comparison to controls and the addition of SS-31 resulted in significant improvement in all three mitochondrial metrics (**Figures 1C–E**). Principal components analysis (PCA) encompassing all three mitochondrial morphological metrics was performed for all cells in the presence and absence of SS-31 (**Figure 1F**). The DCMA patient cells clustered together and were significantly different ($p < 0.0001$) from the control cells. In the presence of SS-31, the DCMA cells exhibited significant improvement ($p < 0.0001$) in the combined mitochondrial metrics, migrating away from the untreated cells and toward the control cluster. Manual grading of mitochondrial fragmentation was performed to confirm the accuracy of our semi-automated quantification. Fibroblasts from DCMA patients displayed a higher percentage of intermediate and fragmented cells compared to control and, following treatment with SS-31, DCMA patient cell lines exhibited more hyperfused and intermediate mitochondria with a lower relative percentage of fully fragmented cells (**Figure 1G**). To further evaluate mitochondrial structure and the effect of SS-31, transmission electron microscopy (TEM) of a single DCMA strain (D1) and control fibroblast strain was performed before and after treatment with 100 nM SS-31 for 24 h. The resulting high-magnification images showed that, qualitatively, mitochondria in the DCMA cells appeared less dark, indicating a lower electron density, and had thinner individual cristae, abnormalities that disappeared after SS-31 exposure. Incubating control and DCMA fibroblasts (D1 and D3) with peptide 366401 (SS-31 lacking the methylated tyrosine) had no significant effect on the mitochondrial fragmentation seen in the DCMA cells.

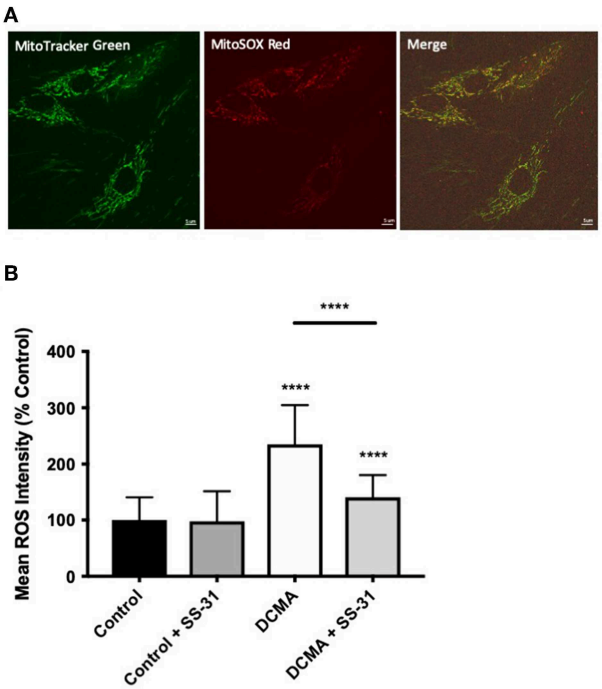


FIGURE 2 | Live-cell imaging of mitochondrial ROS production. **(A)** Example of fibroblasts stained with MitoTracker Green, MitoSOX Red, and merged images. Scale bar measures 5 μm. **(B)** Intensity of mitochondrial ROS staining in control and DCMA fibroblasts with and without treatment with SS-31 (100 nM for 24 h). ROS intensity was significantly increased in DCMA fibroblasts strains but significantly decreased by SS-31. Data are mean ± SD of measurements from 70 individual cells from each control ($n = 1$) and DCMA ($n = 4$) fibroblast strain. Significance was determined with a one-way ANOVA and a Holm-Sidak correction for multiple comparisons. ****$p < 0.0001$.

Increased ROS Production in DCMA Fibroblasts Is Reversible by Incubation With SS-31

Mitochondrial ROS production was measured using live-cell imaging and specific dyes (MitoTracker Green and MitoSOX Red) to co-localize the mitochondrial network with the relative fluorescence of mitochondrial superoxide. Semi-automated mean intensity analysis of the co-localized signals showed significantly higher ($p < 0.0001$) mitochondrial ROS formation in the DCMA fibroblasts compared to controls. Treatment with SS-31 significantly ($p < 0.0001$) reduced mitochondrial ROS production in the DCMA cells and had no effect on the control cells (**Figure 2**).

Changes in the Length of OPA1 Are Reversed by SS-31 in DCMA Fibroblasts

Western blotting was performed on DCMA patient fibroblasts and a control to ascertain the relative ratio of the long (L-OPA1) and short (S-OPA1) isoforms of OPA1. All four DCMA patient lines showed a significant reduction in the ratio of the long and short forms that was reversed by treatment with SS-31 (**Figure 3**).

Total Cardiolipin Is Not Reduced in DCMA

Analysis of 22 individual molecule species of CL did not identify any significant differences between DCMA and control fibroblasts. Similarly, total CL was not significantly different between patient and control cells with or without exposure to SS-31 (**Figure 4**).

RNA-Seq Identifies Changes in Gene Expression Related to DCMA

Comparison between DCMA and control fibroblasts identified 262 transcripts that were significantly differentially-expressed ($p < 4.9 \times 10^{-5}$). However, there were five transcripts that were highly significantly different ($p < 10^{-18}$) (**Table 2**). Implicated genes of particular note included *DNAJC19* and those involved in oxidative stress (*GSTM1*) and mitochondrial biogenesis (*GATD3A*) (31, 32).

DISCUSSION

Using dermal fibroblasts collected from four individual children with DCMA, we have identified defects in mitochondria, specifically abnormal morphology and

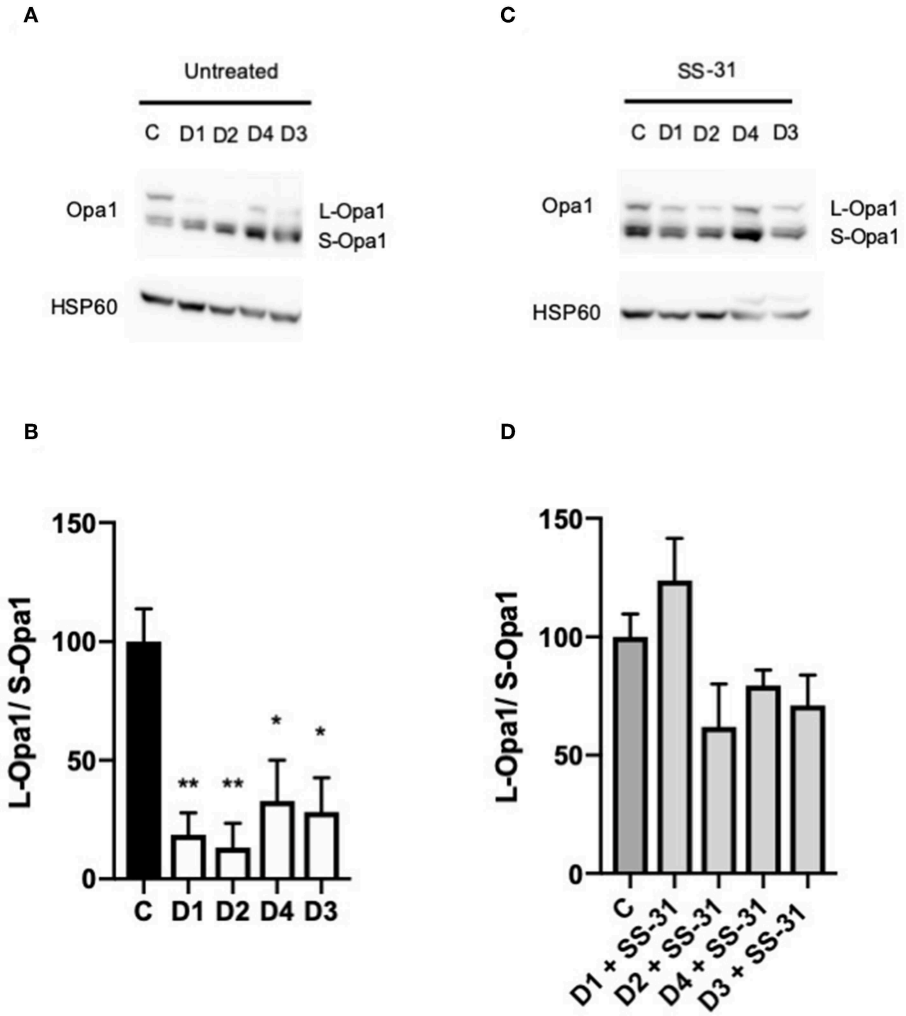


FIGURE 3 | Western blotting of changes in the ratio of OPA1 isoforms. **(A)** Western blot of untreated control (C1) and DCMA (D1-D4) fibroblasts showing the long and short isoforms of OPA1. **(B)** Densitometric analysis of untreated fibroblasts with D1-D4 plotted relative to C1. The quantity of L-OPA1 is significantly reduced in DCMA cells. Data represent mean ± SD from two separate replicates. Significance was determined using a Tukey *post-hoc* test. $^{*}p < 0.05$; $^{**}p \leq 0.01$. **(C)** Western blot of C1 and D1-D4 fibroblasts treated with 100 nM SS-31 for 24 h showing the long and short isoforms of OPA1 and the HSP60 loading control. **(D)** Densitometric analysis of SS-31 treated fibroblasts. There were no significant differences between any of the groups.

increased ROS production. Our results support previous *in vivo* and *in vitro* observations characterizing DCMA as a mitochondrial disease, provide a previously-lacking characterization of mitochondria in DCMA patient fibroblasts and demonstrate a striking response to the novel peptide therapeutic SS-31.

Immunocytochemistry for the outer mitochondrial membrane protein TOMM20 demonstrated that mitochondria in DCMA fibroblasts were severely fragmented with significantly reduced mitochondrial fragment length, network size, and total mitochondrial footprint. TEM provided additional insight into the mitochondrial abnormalities induced by mutated DNAJC19, demonstrating that the electron density and cristae thickness were severely reduced. Reduced electron density, reflected by a decrease in the relative darkness of the mitochondrial matrix, suggests that DCMA mitochondria are likely to be in a lower energetic state in comparison to control mitochondria that are in a more condensed state and therefore actively phosphorylating ADP to produce cellular energy (33). However, in this study, only a single DCMA cell line was studied and these preliminary observations still require validation.

The abnormal mitochondrial structure in DCMA fibroblasts was associated with significantly higher ROS production. ROS are implicated in numerous roles, including cellular signaling, and in the correct balance are critically important for maintaining homeostasis and proper cellular function (34, 35). An increase in ROS production can cause oxidative stress and subsequent peroxidative damage, particularly of cardiolipin which is very susceptible to this type of injury due to its composition and

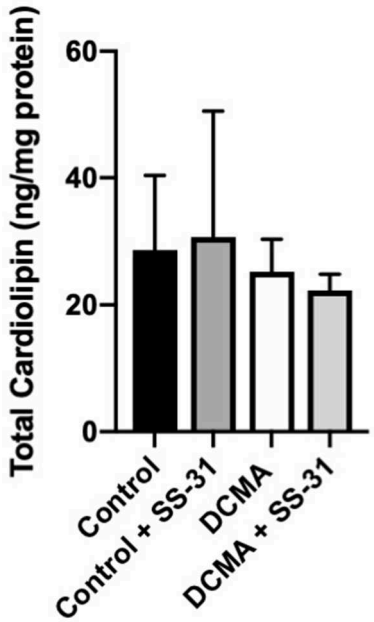


FIGURE 4 | Total cardiolipin content. For control ($n = 3$) and DCMA ($n = 4$) fibroblasts, the total cardiolipin content was measured before and after incubation with SS-31 (100 nM for 24 h). No significant differences were found between any of the groups.

TABLE 2 | RNA-Seq results.

ID	Log2-fold change	Adjusted *P*-value	Gene symbol
XM_005270782.5	4.95	5.46×10^{-24}	*GSTM1*
NM_145261.3	−10.87	1.79×10^{-23}	*DNAJC19*
XM_017028479.1	−11.59	4.33×10^{-23}	*GATD3A*
NM_001282418.1	10.21	1.70×10^{-18}	*STAG2*
NM_005049.2	−10.64	4.22×10^{-18}	*PWP2*

Most significantly differentially-expressed genes in DCMA fibroblasts.

location (11). From our observations, it is not clear if the increased ROS production is the primary insult or secondary to the abnormal mitochondrial structure. The mitochondrial structural abnormalities that we visualized are consistent with an imbalance between mitochondrial fission and fusion. This conclusion is supported by our finding that DCMA cells exhibited significantly lower proportions of the L-OPA1 isoform which is required for mitochondrial fusion and cristae formation (36). A similar loss of L-OPA1 was observed in genetically-modified HEK293T cells and associated with abnormalities in CL composition (4). However, despite the abnormalities in mitochondrial structure and OPA1 isoform proportions, we did not see a significant difference in either total CL or individual CL species between DCMA and control fibroblasts. Given the purported link between DCMA and Barth syndrome (based on the presence of excess 3-methylglutaconic acid), this finding was unexpected. Given the documented abnormalities in CL in Barth syndrome (37), this finding suggests that the underlying cause of disease in DCMA and Barth syndrome will be different. Our RNA-Seq results support our observations of abnormal mitochondrial structure and function but do not immediately suggest mechanism.

Despite our lack in insight into disease mechanism, the mitochondrially-targeted peptide SS-31 shows promise as a therapeutic for DCMA, paralleling results seen for other mitochondrial disorders and heart failure (21, 22). Incubating cells with SS-31 for just 24 h, and using a concentration similar to that previously documented to be safe and effective *in vitro* (38), the overall mitochondrial structure in patient fibroblasts improved significantly both qualitatively and quantitatively. In addition to improved mitochondrial structure, the amount of mitochondrially-produced ROS also significantly decreased with exposure to SS-31. Although SS-31 improved mitochondrial structure and reduced oxidative stress in DCMA cells, similar to the effects observed in Friedreich ataxia (a neurodegenerative disease also associated with cardiomyopathy related to mitochondrial dysfunction), the precise mechanism of action is not known (21). However, recent work suggests that SS-31 improves coupling of electron transport chain complexes CI and CIV which may be responsible for reducing ROS production (22). We have observed reduced CI and CIV complex activity in skeletal muscle and liver from DCMA patients (Khan, unpublished data). Alternatively, due to its antioxidant activity, SS-31 may be reducing ROS abundance or it may be specifically protecting cardiolipin from peroxidative damage. Our results showing no significant changes in the levels of CL are consistent with those recently published showing that SS-31 appears to exert its effect by influencing the function of the electron transport chain rather than affecting CL directly (22). Interestingly, SS-31 significantly improved the expression of L-OPA1 and resulted in a healthier balance of L-OPA1/S-OPA1 in our DCMA cells. CL has been associated with L-OPA1 and it is hypothesized that their interaction results in adequate mitochondrial fusion (39). Through protection of CL, SS-31 could be improving the interaction of L-OPA1 with CL and may provide an explanation for the improved mitochondrial structure seen in our cells post-treatment. Although metabolically quiescent, our research has shown dermal fibroblasts to be an adequate *in vitro* model for mitochondrial structural abnormalities. However, it remains unknown if SS-31 localizes to the mitochondria in DCMA and further work is required to assess potential mitochondrial energetic dysfunction. As such, the effect of other potential therapeutics could be evaluated using our fibroblasts. For example, the cardiac glycoside digoxin has recently been shown to improve myocardial function and structure in children with DCMA but the impact of digoxin on mitochondrial structure and function remains to be evaluated (9).

CONCLUSION

We have completed a novel *in vitro* study of the rare mitochondrial disease DCMA using patient-derived dermal

fibroblasts. Analysis of mitochondrial morphology identified multiple abnormalities of mitochondrial structure that may be contributing to elevated ROS production and decreased organelle fusion. The observation that SS-31 is able to ameliorate all of these abnormalities is also a novel and exciting finding. Since dysfunctional mitochondria most likely underlie the lethal cardiomyopathy frequently found in this disorder, identification of SS-31 as a potential therapeutic may have important future clinical implications.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in GEO under the accession number GSE133754.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Conjoint Health Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PM, XW, AK, TS, and SG conceived and designed the experiments. PM, XW, TZ, NJ-T, RS, MK, and AR performed experiments and acquired data. PM, JH, and FI performed data analysis. BA and DS provided reagents. PM, XW, and SG wrote the manuscript. All authors read and approved the manuscript.

FUNDING

This work was supported by a research grant from the Children's Cardiomyopathy Foundation to SG with additional financial support from the Department of Pediatrics at the University of Calgary and the Alberta Children's Hospital Foundation to SG.

ACKNOWLEDGMENTS

We would like to thank Vincent Ebacher in the Hotchkiss Brain Institute for his image analysis support. We also acknowledge the imaging resources of the Charbonneau Microscopy Facility and the Microscopy and Imaging Facility at the University of Calgary.

REFERENCES

1. Blomen VA, Májek P, Jae LT, Bigenzahn JW, Nieuwenhuis J, Staring J, et al. Gene essentiality and synthetic lethality in haploid human cells. *Science*. (2015) 350:1092–96. doi: 10.1126/science.aac7557
2. Davey KM, Parboosingh JS, McLeod DR, Chan A, Casey R, Ferreira P, et al. Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. *J Med Genet*. (2006) 43:385–93. doi: 10.1136/jmg.2005.036657
3. Ojala T, Polinati P, Manninen T, Hiippala A, Rajantie J, Karikoski R, et al. New mutation of mitochondrial DNAJC19 causing dilated and noncompaction cardiomyopathy, anemia, ataxia, and male genital anomalies. *Pediatr Res*. (2012) 72 : 432–7. doi: 10.1038/pr.2012.92
4. Richter-Dennerlein R, Korwitz A, Haag M, Tatsuta T, Dargazanli S, Baker M, et al. DNAJC19, a mitochondrial cochaperone associated with cardiomyopathy, forms a complex with prohibitins to regulate cardiolipin remodeling. *Cell Metab*. (2014) 20 : 158–71. doi: 10.1016/j.cmet.2014.04.016
5. Kampinga HH, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol*. (2010) 11 : 579–92. doi: 10.1038/nrm2941
6. Ucar SK, Mayr JA, Feichtinger RG, Canda E, Çoker M, Wortmann SB. Previously unreported biallelic mutation in DNAJC19: are sensorineural hearing loss and basal ganglia lesions additional features of dilated cardiomyopathy and ataxia (DCMA) syndrome? *JIMD Rep*. (2016) 35:39–45. doi: 10.1007/8904_2016_23
7. Al Teneiji A, Siriwardena K, George K, Mital S, Mercimek-Mahmutoglu S. Progressive cerebellar atrophy and a novel homozygous pathogenic DNAJC19 variant as a cause of dilated cardiomyopathy ataxia syndrome. *Pediatr Neurol*. (2016) 62:58–61. doi: 10.1016/j.pediatrneurol.2016.03.020
8. Sparkes R, Patton D, Bernier F. Cardiac features of a novel autosomal recessive dilated cardiomyopathic syndrome due to defective importation of mitochondrial protein. *Cardiol Young*. (2007) 17:215–7. doi: 10.1017/S1047951107000042
9. Greenway SC, Dallaire F, Hazari H, Patel D, Khan A. Addition of digoxin improves cardiac function in children with the dilated cardiomyopathy with ataxia syndrome: a mitochondrial cardiomyopathy. *Can J Cardiol*. (2018) 34:972–7. doi: 10.1016/j.cjca.2018.02.019
10. Clarke SL, Bowron A, Gonzalez IL, Groves SJ, Newbury-Ecob R, Clayton N, et al. Barth syndrome. *Orphanet J Rare Dis*. (2013) 8:23. doi: 10.1186/1750-1172-8-23
11. Dudek J, Maack C. Barth syndrome cardiomyopathy. *Cardiovasc Res*. (2017) 113:399–410. doi: 10.1093/cvr/cvx014
12. Osman C, Voelker DR, Langer T. Making heads or tails of phospholipids in mitochondria. *J Cell Biol*. (2011) 192:7–16. doi: 10.1083/jcb.201006159
13. Chicco AJ, Sparagna GC. Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am J Physiol Cell Physiol*. (2007) 292:C33–44. doi: 10.1152/ajpcell.00243.2006
14. Saini-Chohan HK, Holmes MG, Chicco AJ, Taylor WA, Moore RL, McCune SA, et al. Cardiolipin biosynthesis and remodeling enzymes are altered during development of heart failure. *J Lipid Res*. (2009) 50:1600–8. doi: 10.1194/jlr.M800561-JLR200
15. Sparagna GC, Chicco AJ, Murphy RC, Bristow MR, Johnson CA, Rees ML, et al. Loss of cardiac tetralinoleoyl cardiolipin in human and experimental heart failure. *J Lipid Res*. (2007) 48:1559–70. doi: 10.1194/jlr.M600551-JLR200
16. Acehan D, Xu Y, Stokes DL, Schlame M. Comparison of lymphoblast mitochondria from normal subjects and patients with Barth syndrome using electron microscopic tomography. *Lab Invest*. (2007) 87:40–8. doi: 10.1038/labinvest.3700480
17. Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br J Pharmacol*. (2014) 171:2029–50. doi: 10.1111/bph.12461
18. Szeto HH, Birk AV. Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. *Clin Pharmacol Ther*. (2014) 96:672–83. doi: 10.1038/clpt.2014.174
19. Szeto HH, Schiller PW. Novel therapies targeting inner mitochondrial membrane–from discovery to clinical development. *Pharm Res*. (2011) 28:2669–79. doi: 10.1007/s11095-011-0476-8
20. Birk AV, Liu S, Soong Y, Mills W, Singh P, Warren JD, et al. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. *J Am Soc Nephrol*. (2013) 24:1250–61. doi: 10.1681/ASN.2012121216
21. Zhao H, Li H, Hao S, Chen J, Wu J, Song C, et al. Peptide SS-31 upregulates frataxin expression and improves the quality of mitochondria: implications in the treatment of Friedreich ataxia. *Sci Rep*. (2017) 7:9840. doi: 10.1038/s41598-017-10320-2

22. Chatfield KC, Sparagna GC, Chau S, Phillips EK, Ambardekar AV, Aftab M, et al. Elamipretide improves mitochondrial function in the failing human heart. *JACC*. (2019) 4:147–57. doi: 10.1016/j.jacbts.2018.12.005
23. Wu J, Hao S, Sun XR, Zhang H, Li H, Zhao H., et al. Elamipretide (SS-31) ameliorates isoflurane-induced long-term impairments of mitochondrial morphogenesis and cognition in developing rats. *Front Cell Neurosci*. (2017) 11:119. doi: 10.3389/fncel.2017.00119
24. Sabouny R, Fraunberger E, Geoffrion M, Ng AC, Baird SD, Screaton RA., et al. The Keap1-Nrf2 stress response pathway promotes mitochondrial hyperfusion through degradation of the mitochondrial fission protein Drp1. *Antioxid Redox Signal*. (2017) 27:1447–59. doi: 10.1089/ars.2016.6855
25. Valente AJ, Maddalena LA, Robb EL, Moradi F, Stuart JA. A simple Imagej macro tool for analyzing mitochondrial network morphology in mammalian cell culture. *Acta Histochem*. (2017) 119:315–26. doi: 10.1016/j.acthis.2017.03.001
26. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol*. (2016) 34:525–7. doi: 10.1038/nbt.3519
27. Ravandi A, Leibundgut G, Hung MY, Patel M, Hutchins PM, Murphy RC, et al. Release and capture of bioactive oxidized phospholipids and oxidized cholesteryl esters during percutaneous coronary and peripheral arterial interventions in humans. *J Am Coll Cardiol*. (2014) 63:1961–71. doi: 10.1016/j.jacc.2014.01.055
28. James AM, Wei YH, Pang CY, Murphy MP. Altered mitochondrial function in fibroblasts containing MELAS or MERRF mitochondrial DNA mutations. *Biochem J*. (1996) 318 (Pt 2):401–7. doi: 10.1042/bj3180401
29. Onesto E, Colombrita C, Gumina V, Borghi MO, Dusi S, Doretti A., et al. Gene-specific mitochondria dysfunctions in human TARDBP and C9ORF72 fibroblasts. *Acta Neuropathol Commun*. (2016) 4:47. doi: 10.1186/s40478-016-0316-5
30. Kogot-Levin A, Saada A, Leibowitz G, Soiferman D, Douiev L, Raz I, et al. Upregulation of mitochondrial content in cytochrome c oxidase deficient fibroblasts. *PLoS ONE*. (2016) 11:e0165417. doi: 10.1371/journal.pone.0165417
31. Masuda T, Wada Y, Kawamura S. ES1 is a mitochondrial enlarging factor contributing to form mega-mitochondria in zebrafish cones. *Sci Rep*. (2016) 6:22360. doi: 10.1038/srep22360
32. Ponamarev MV, She YM, Zhang L, Robinson BH. Proteomics of bovine mitochondrial RNA-binding proteins: HES1/KNP-I is anew mitochondrial resident protein. *J Proteome Res*. (2005) 4:43–52. doi: 10.1021/pr049872g
33. Hackenbrock CR. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. II. Electron transport-linked ultrastructural transformations in mitochondria. *J Cell Biol*. (1968) 37:345–69. doi: 10.1083/jcb.37.2.345
34. Tsutsui H, Kinugawa S, Matsushima S. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. *Cardiovasc Res*. (2009) 81:449–56. doi: 10.1093/cvr/cvn280
35. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal*. (2012) 24:981–90. doi: 10.1016/j.cellsig.2012.01.008
36. Olichon A, Guillou E, Delettre C, Landes T, Arnauné-Pelloquin L, Emorine LJ, et al. Mitochondrial dynamics and disease, OPA1. *Biochim Biophys Acta*. (2006) 1763:500–9. doi: 10.1016/j.bbamcr.2006.04.003
37. Mejia EM, Zinko JC, Hauff KD, Xu FY, Ravandi A, Hatch GM. Glucose uptake and triacylglycerol synthesis are increased in barth syndrome lymphoblasts. *Lipids*. (2017) 52:161–5. doi: 10.1007/s11745-017-4232-7
38. Hao S, Ji J, Zhao H, Shang L, Wu J, Li H., et al. Mitochondrion-targeted peptide SS-31 inhibited oxidized low-density lipoproteins-induced foam cell formation through both ROS scavenging and inhibition of cholesterol influx in RAW264.7 cells. *Molecules*. (2015) 20:21287–97. doi: 10.3390/molecules201219764
39. Liu R, Chan DC. OPA1 and cardiolipin team up for mitochondrial fusion. *Nat Cell Biol*. (2017) 19:760–2. doi: 10.1038/ncb3565

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 08 January 2020
doi: 10.3389/fcvm.2019.00186

SGLT2 Inhibitors Play a Salutary Role in Heart Failure via Modulation of the Mitochondrial Function

*Yasuhiro Maejima**

Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Three cardiovascular outcome trials of sodium glucose cotransporter 2 (SGLT2) inhibitors, including the EMPA-REG OUTCOME trial, CANVAS Program, and DECLARE TIMI 58 trial, revealed that SGLT2 inhibitors were superior to a matching placebo in reducing cardiovascular events, including mortality and hospitalization for heart failure, in patients with type 2 diabetes. However, the detailed mechanism underlying the beneficial effects that SGLT2 inhibitors exert on cardiovascular diseases remains to be elucidated. We herein review the latest findings of the salutary mechanisms of SGLT2 inhibitors in cardiomyocytes, especially focusing on their mitochondrial function-mediated beneficial effects. The administration of SGLT2 inhibitors leads to the elevation of plasma levels of ketone bodies, which are an efficient energy source in the failing heart, by promoting oxidation of the mitochondrial coenzyme Q couple and enhancing the free energy of cytosolic ATP hydrolysis. SGLT2 inhibitors also promote sodium metabolism-mediated cardioprotective effects. These compounds could reduce the intracellular sodium overload to improve mitochondrial energetics and oxidative defense in the heart through binding with NHE and/or SMIT1. Furthermore, SGLT2 inhibitors could modulate mitochondrial dynamics by regulating the fusion and fission of mitochondria. Together with ongoing large-scale clinical trials to evaluate the efficacy of SGLT2 inhibitors in patients with heart failure, intensive investigations regarding the mechanism through which SGLT2 inhibitors promote the restoration in cases of heart failure would lead to the establishment of these drugs as potent anti-heart failure drugs.

Keywords: SGLT2, mitochondria, ketone body, NHE, fusion, fission

OPEN ACCESS

Edited by:
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Reviewed by:
Junco Shibayama Warren, The University of Utah, United States
Yoshiyuki Ikeda, Kagoshima University, Japan

***Correspondence:**
Yasuhiro Maejima
ymaeji.cvm@tmd.ac.jp

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *03 November 2019*
Accepted: *10 December 2019*
Published: *08 January 2020*

Citation:
Maejima Y (2020) SGLT2 Inhibitors Play a Salutary Role in Heart Failure via Modulation of the Mitochondrial Function. Front. Cardiovasc. Med. 6:186. doi: 10.3389/fcvm.2019.00186

INTRODUCTION

Sodium glucose cotransporter (SGLT) is a channel protein that imports glucose into the intracellular space together with sodium ions (Na^+) using the gradient of the Na^+ concentration between inside and outside of the cells (**Figure 1A**) (1). SGLTs are expressed in limited organs, including the brain, small intestine, and renal tubule of mammals. Phlorizin, a phloretin that connects with glucose via glucoside bonding, is a natural compound derived from the bark of the apple tree root (**Figure 1B**). The administration of phlorizin leads to renal glycosuria, as this compound can inhibit SGLT1/2 located on the renal tubule, which results in the alleviation of hyperglycemia by discharging glucose to urine (**Figure 1A**) (2, 3). However, the intake of phlorizin causes severe diarrhea because this compound also inhibits small intestinal SGLT1, thereby suppressing the reabsorption of glucose together with water in the intestinal tract. To overcome this

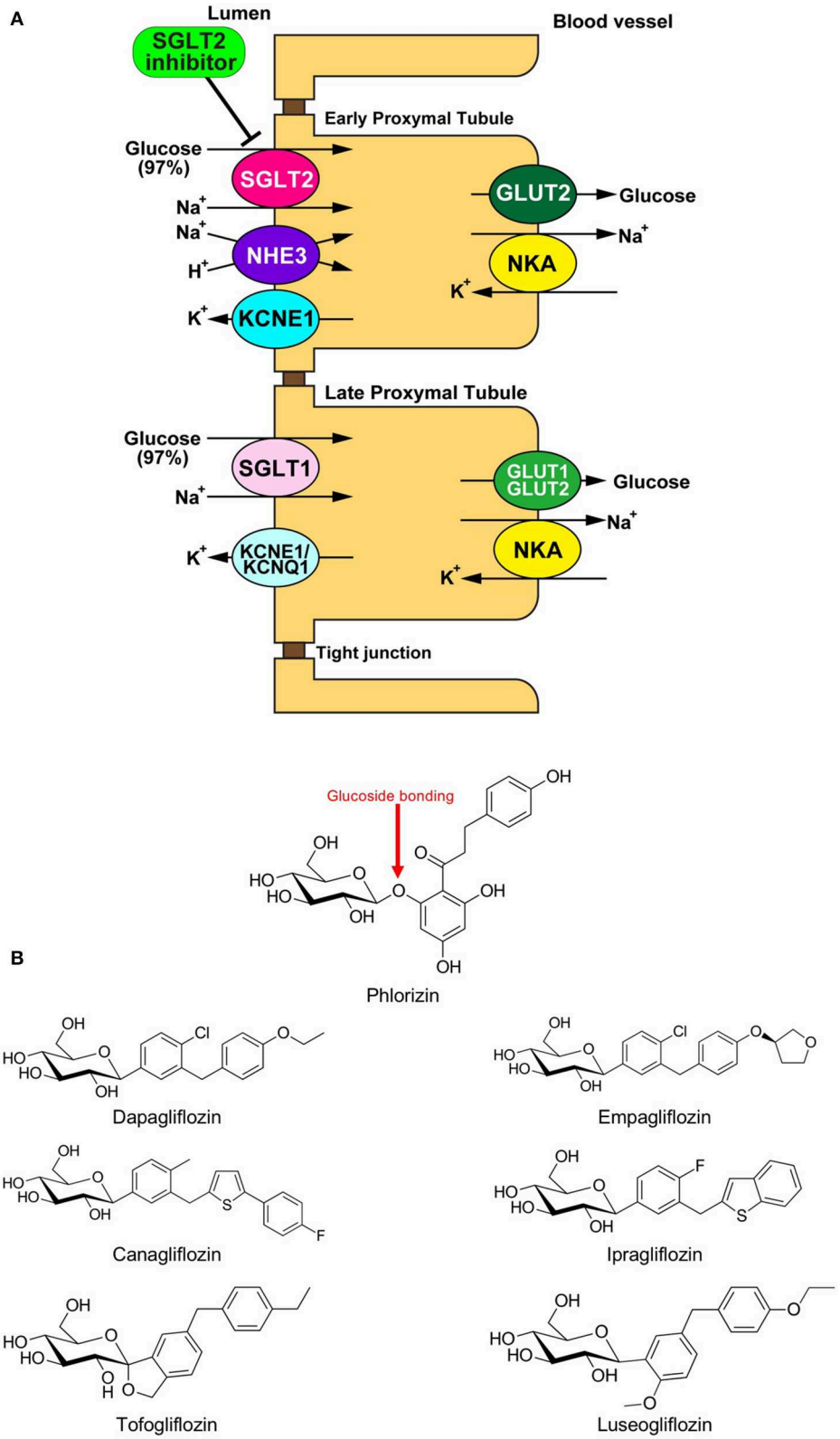


FIGURE 1 | (A) Physiology of glucose reabsorption in the renal proximal tubules and the target of SGLT2 inhibitors. GLUT, glucose transporter; KCNE1, potassium voltage-gated channel Isk-related family member 1; KCNQ1, potassium voltage-gated channel KQT-like subfamily member 1; NHE, Na^+/H^+ exchanger; NKA, Na^+/K^+ ATPase; SGLT, sodium-dependent glucose transporter. **(B)** Chemical structural formulas of Phlorizin and SGLT2 inhibitors (Dapagliflozin, Empagliflozin, Canagliflozin, Ipragliflozin, Tofogliflozin, and Luseogliflozin).

weakness of phlorizin, intensive analyses were conducted to investigate the molecular structures of both phlorizin and the SGLT receptor. Based on these analyses, highly selective SGLT2 inhibitors were developed as a novel type of anti-diabetes drug (**Figure 1B**) (4). In recent years, several cardiovascular outcome studies to test the safety of glucose-lowering drugs have demonstrated that SGLT2 inhibitors have a potential protective effect against cardiovascular events that is comparable to existing anti-heart failure drugs. However, it remains unknown how SGLT2 inhibitors exert such beneficial effects in patients with cardiovascular diseases. One of the major reasons why this has not been elucidated is that SGLT2 is not expressed in cardiomyocytes (5). Thus, it is largely believed that SGLT2 inhibitors play a protective role via the modulation of the internal environment outside of the myocardium (6). On the other hand, several investigators have shown that SGLT2 inhibitors directly manifest protective effects in the heart (6). In both cases, it is assumed that SGLT2 inhibitors exert their protective effects by restoring the mitochondrial function in cardiomyocytes. We herein review the current understanding on how SGLT2 inhibitors mitigate cardiac dysfunction through mitochondrial protection-mediated mechanisms.

CLINICAL EVIDENCE OF THE CARDIOPROTECTIVE EFFECTS OF SGLT2 INHIBITORS

The EMPA-REG OUTCOME trial, a cardiovascular outcome trial (CVOT) of the SGLT2 inhibitor empagliflozin, demonstrated that empagliflozin was superior to a matching placebo in reducing cardiovascular events, including mortality and hospitalization for heart failure in patients with type 2 diabetes and established cardiovascular diseases (7, 8) (**Table 1**). The CANVAS Program, which consists of the CANVAS study and CANVAS-R, CVOTs assessed the cardiovascular safety and efficacy of the SGLT2 inhibitor canagliflozin in patients with type 2 diabetes and established cardiovascular disease, and also revealed that canagliflozin reduced the risk of a composite outcome of major adverse cardiovascular events in comparison to a matching placebo (9) (**Table 1**). Furthermore, the DECLARE TIMI 58 trial demonstrated that the SGLT2 inhibitor dapagliflozin reduced the risk of cardiovascular death or hospitalization for heart failure in comparison to a matching placebo in patients with type 2 diabetes and either a high cardiovascular risk or established atherosclerotic cardiovascular disease (10) (**Table 1**). As most patients in these trials did not have a diagnosis of heart failure at the time of study entry, the merit of treatment with an SGLT2 inhibitor largely reflected the prevention of heart failure development (11). Furthermore, the fact that reduction in the risk of hospitalization for heart failure emerged early after randomization raised the possibility that the mechanisms of the SGLT2 inhibitor-mediated cardiovascular benefits differ from those of existing glucose-lowering therapies that exert their effects independently of glycemic control. Indeed, a series of preclinical investigations demonstrated the effectiveness of SGLT2 inhibitors in animal models of non-diabetic heart failure. Byrne et al. revealed that the administration of empagliflozin alleviated left ventricular systolic dysfunction in non-diabetic mice subjected to pressure overload both *in vivo* and *ex vivo* (12). Andreadou et al. and Yurista et al. demonstrated that the administration of empagliflozin reduced the infarcted area of the myocardium, thereby improving the cardiac function in experimental non-diabetic myocardial infarction models (13, 14). In this background, randomized clinical trials were designed to explore the effects of SGLT2 inhibitors in patients with established heart failure with or without diabetes. Recently, the DAPA-HF trial demonstrated the significant advantage of dapagliflozin in reducing major adverse outcomes, such as unexpected hospitalization due to the exacerbation of heart failure, in patients with established heart failure with a reduced ejection fraction (HFrEF) (15). However, for SGLT2 inhibitors to be safely used for the treatment of non-diabetic heart failure, it is essential to elucidate their mechanism of action in detail. Thus far, a number of hypothesized mechanisms have proposed to explain the benefits of SGLT2 inhibitors in heart failure (6). Some investigators suggested that SGLT2 inhibitor-mediated natriuresis reduces the plasma volume or interstitial fluid, thereby favorably influencing ventricular remodeling by reducing the cardiac volume (16). Other investigators suggested that SGLT2 inhibitors alleviate heart failure through the suppression of sympathetic nervous activity, as evidenced by the reduction in arterial blood pressure without an increase in heart rate (7, 17). Still others hypothesized that SGLT2 inhibitors enhance the synthesis of erythropoietin by restoring the activity of "neural crest-derived" fibroblasts surrounding the renal proximal tubules, which, in turn, increases the delivery of oxygen to the failing myocardium (18). Thus, the targets through which SGLT2 inhibitors exert their protective effects against heart failure are mainly located outside of the heart. However, some investigations regarding this issue demonstrated that SGLT2 inhibitors have the potential to directly protect cardiomyocytes. Most such investigations have argued that SGLT2 inhibitors directly alleviate cardiac dysfunction through the modulation of mitochondria-associated mechanisms, including ketone body metabolism, sodium metabolism, and mitochondrial dynamics.

SGLT2 INHIBITORS INCREASE THE AMOUNT OF KETONE BODIES, THEREBY PROMOTING CARDIOPROTECTIVE EFFECTS

The inhibition of SGLT2 induces glucosuria, which thereby lowers plasma glucose levels, resulting in a reduction in the insulin level and an increase in the glucagon level during the fasting state. Such hormonal changes facilitate lipolysis in adipose tissue, and—at the same time—promote the conversion of carbohydrate to fat in whole-body substrate utilization. Thus, the administration of SGLT2 inhibitors could elevate ketone body levels in humans (**Figure 2**) (19). Ketone bodies, which are composed of acetoacetate (AcAc), β-hydroxybutyrate (βOHB), and acetone, are exclusively generated in the liver when the supply of glucose is impaired

TABLE 1 | Summary of cardiovascular outcome trials with SGLT2 inhibitors.

	EMPA-REG Outcome	CANVAS Program	Declare-TIMI 58
Study drug	Empagliflozin	Canagliflozin	Dapagliflozin
Drug class	SGLT2 inhibitor	SGLT2 inhibitor	SGLT2 inhibitor
Comparator	Placebo	Placebo	Placebo
Selected inclusion criteria	Adults with T2D at high risk of CV disease; BMI ≤45 kg/m^2; no glucose-lowering therapy in previous 12 weeks and HbA1c 7.0–9.0%, or stable glucose-lowering therapy and HbA1c 7.0–10.0%	T2D; HbA1c 7.0–10.5%; age ≥30 years with a history of CV events, or age ≥50 years with a high risk of CV events; eGFR ≥30 ml/min/1.73 m^2	T2D; HbA1c ≥6.5–
Selected exclusion criteria	ACS, stroke, or TIA in previous 2 months; planned cardiac surgery or angioplasty; liver disease; eGFR 2	T1D; diabetic ketoacidosis; pancreas or beta-cell transplantation; diabetes secondary to pancreatitis or pancreatectomy; severe hypoglycaemic episode in previous 6 months	T1D; CrCl
Number of patients	7,020	10,142	17,160
Study aim	Assess CV safety outcomes with empagliflozin compared with placebo, on top of standard of care, in patients with T2D at high CV risk	To pool results from the CANVAS and CANVAS-R trials to assess CV safety outcomes with canagliflozin compared with placebo, on top of standard of care, in patients with poorly controlled T2D and a history of CV events, or high risk of CV events	Assess CV outcomes with dapagliflozin compared with placebo, on top of standard of care, in patients with T2D who either have or are at risk of atherosclerotic CV disease
Primary outcome	3P-MACE (CV death, non-fatal MI or non-fatal stroke)	3P-MACE (CV death, non-fatal MI or non-fatal stroke)	Primary safety outcome: non-inferiority for 3P-MACE (CV death, non-fatal MI or non-fatal ischemic stroke). Co-primary efficacy outcomes: 3P-MACE; CV death or hospitalization for heart failure
Other key outcomes	4P-MACE (3P-MACE or hospitalization for unstable angina); CV death; hospitalization for heart failure; all-cause mortality; incident or worsening nephropathy	Individual components of composite endpoint; all-cause mortality; hospitalization for heart failure; progression of albuminuria	Composite kidney outcome (sustained ≥40% reduction in eGFR to 2, new ESKD, or kidney or CV death); all-cause mortality; hospitalization for heart failure
Number of events	772	1,011	–
Start date	2010-07-01	2014-01-01	2013-04-01
Median follow-up	3.1 years	CANVAS: ∼5.7 years; CANVAS–R: ∼2.1 years; CANVAS Program: ∼2.4 years	4.2 years
Date of completion	2015-04-01	2017-02-01	2018-09-01
Key results	Primary outcome: HR 0.86 (95% CI 0.74, 0.99; $p = 0.04$ for superiority); 4P-MACE: HR 0.89 (95% CI 0.78, 1.01; $p = 0.08$ for superiority); CV death: HR 0.62 (95% CI 0.49, 0.77; $p < 0.001$) hospitalization for heart failure: HR 0.65 (95% CI 0.50, 0.85; $p = 0.002$); all-cause mortality: HR 0.68 (95% CI 0.57, 0.82; $p < 0.001$) incident or worsening nephropathy: HR 0.61 (95% CI 0.53, 0.70; $p < 0.001$)	CANVAS Program ITT analysis Primary outcome: 3P-MACE: HR 0.86 (95% CI 0.75, 0.97; $p = 0.02$ for superiority); all-cause mortality: HR 0.87 (95% CI 0.74, 1.01); CV death: HR 0.87 (95% CI 0.72, 1.06); hospitalization for HF: HR 0.67 (95% CI 0.52, 0.87); progression of albuminuria: HR 0.73 (95% CI 0.67, 0.79)	Co-primary efficacy outcomes—3P-MACE: HR 0.93 (95% CI 0.84, 1.03; $p = 0.17$ for superiority); CV death or hospitalization for heart failure: HR 0.83 (95% CI 0.73, 0.95; $p = 0.005$ for superiority); exploratory outcomes—kidney composite outcome: HR 0.76 (95% CI 0.67, 0.87); all-cause mortality: HR 0.93 (95% CI 0.82, 1.04); hospitalization for heart failure: HR 0.73 (95% CI 0.61, 0.88); CV death: HR 0.98 (95% CI 0.82, 1.17)
References	Zinman et al. *N Engl J Med* 2015; 373:2117; Wanner et al. *N Engl J Med* 2016; 375:323; NCT01131676	Neal et al. *N Engl J Med* 2017; 377:644; Neal et al. *Diabetes Obes Metab* 2017;19:926	Wiviott et al. *N Engl J Med* 2019; 380:347; NCT01730534
Sponsor	Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance	Janssen Research and Development, The George Institute for Global Health	AstraZeneca

due to either a reduction of exogenous influx or deterioration of insulin signaling, or when the amount of free fatty acids (FFAs) is excessive due to the hyperactivation of lipolysis (20). During such situations, fatty acid β-oxidation is upregulated, thereby increasing the NADH/NAD$^+$ ratio, which in turn promotes the conversion of AcAc to βOHB in the mitochondria of the liver (**Figure 2**). FFAs, a major source of ketone bodies, are taken up into hepatocytes, and β-oxidation transforms FFAs into acetyl-CoA and acetoacetyol-CoA (AcAc-CoA). 3-hydroxy-3-methylglutaryl-coenzyme A

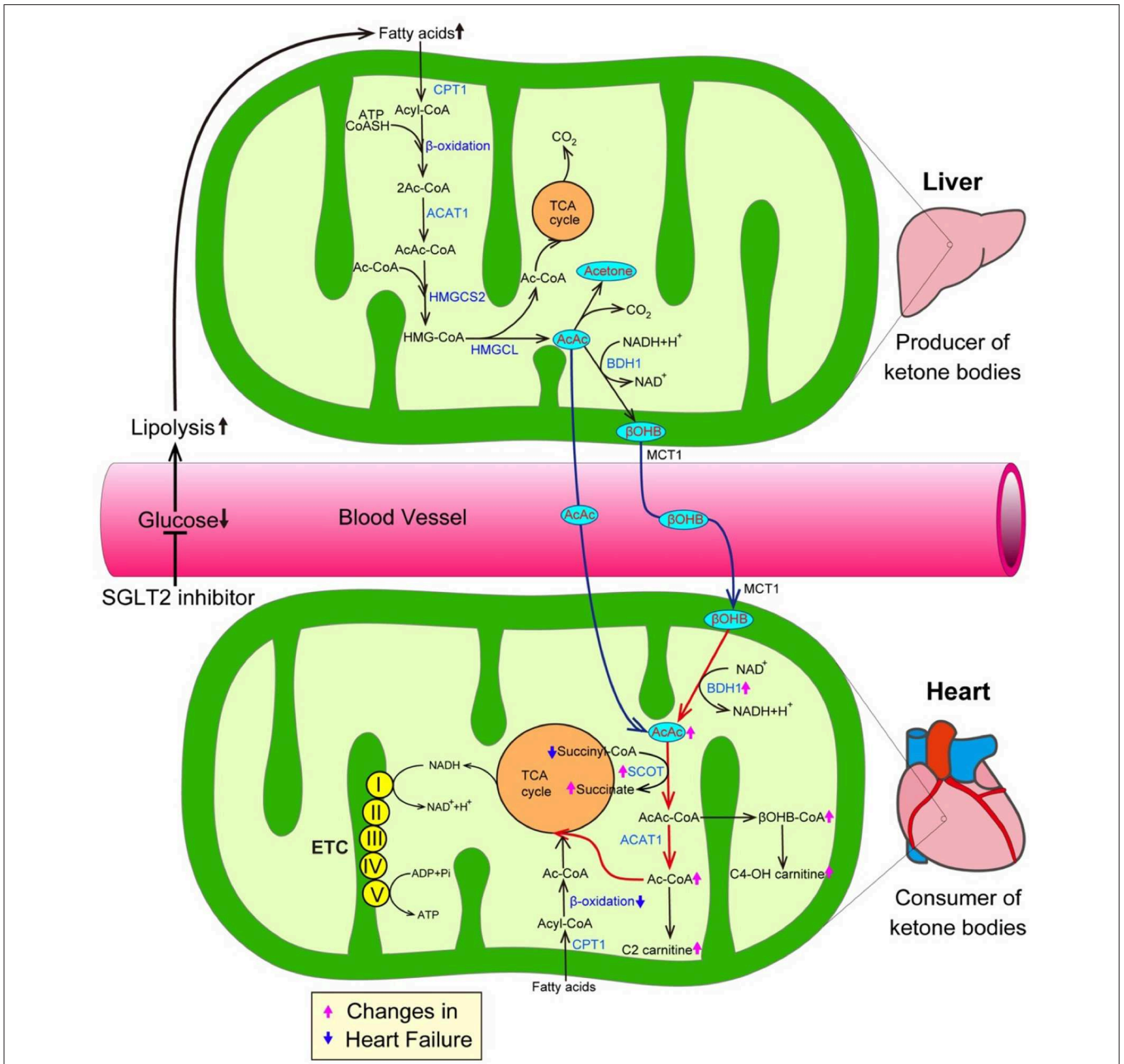


FIGURE 2 | SGLT2 inhibitors increase the amount of ketone bodies, thereby promoting cardioprotective effects. The inhibition of SGLT2 reduces plasma glucose levels, thereby promoting lipolysis in adipose tissue, which in turn enhances the generation of ketone bodies. On the other hand, a growing body of evidence suggests that ketone bodies are favorable substrates in energy production because the conversion of ketone bodies to acetyl-CoA is much easier in comparison to the conversion of FFAs and glucose to acetyl-CoA. Furthermore, transcriptional level changes of ketone oxidation-related genes would be associated with the substrate shift to ketone bodies in the failing heart. Both pink and blue arrows show the changes in heart failure. AcAc CoA, Acetoacetyl CoA; ACAT1, Acetyl-CoA acetyltransferase; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; BDH1, Mitochondrial β-hydroxybutyrate dehydrogenase; βOHB, β-hydroxybutyrate; βOHB CoA, β-hydroxybutyryl CoA; C2-carnitine, Acetylcarnitine; C4-OH carnitine, Hydroxybutyrylcarnitine; CPT1, Carnitine palmitoyltransferase 1; ETC, Electron transport chain; HMGCL, 3-hydroxy-3-methylglutaryl-coenzyme A lyase; HMGCS2, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2; and SCOT, Succinyl-CoA:3-oxoacid-CoA transferase.

synthase 2 (HMGCS2), a rate-limiting mitochondrial enzyme, catalyzes the condensation of acetyl-CoA and AcAc-CoA to generate 3-hydroxy-3-methtylglutaryl-CoA (HMG-CoA) (21). Subsequently, 3-hydroxy-3-methylglutaryl-coenzyme A lyase (HMGCL) sequentially cleaves HMG-CoA into acetyl-CoA and AcAc (22, 23). Then, D-β-hydroxybutyrate dehydrogenase

(BDH1) converts AcAc to βOHB, a more stable form of ketone body. In addition, both the kidneys and intestines play a critical role in maintaining ketone body homeostasis by regulating the ketone-reabsorptive capacity through sodium-dependent monocarboxylate transporter (SMCT) 1 and 2. Ketone bodies diffuse into the circulation and are used as an energy source in various organs (24). In the mitochondria of the heart, ketone bodies are rapidly converted to acetyl CoA through catalyzation with several enzymes, such as βOHB dehydrogenase (BDH1), succinyl-CoA:3-oxoacid-CoA transferase (SCOT), and mitochondrial acetyl-CoA acetyltransferase 1 (25).

The mammalian heart requires a vast amount of energy to maintain a normal contractile function and intracellular energy storage is limited. Thus, cardiomyocytes must generate an enormous amount of adenosine triphosphate (ATP) via the oxidation of carbon fuel. Under normal conditions, the predominant energy source of cardiomyocytes is FFAs, which provide 60% of the myocardial ATP demand by β-oxidation (26). The remaining 40% of the myocardial ATP demand is provided by carbohydrate oxidation, including glycolysis. The proportions of the energy sources of cardiomyocytes dynamically changes according to conditions such as exercise, feeding and starvation. When the mitochondrial oxidative metabolism balance of cardiomyocytes is seriously damaged due to various stresses including hypoxia and pressure overload, the major origin of ATP shifts from β-oxidation-mediated FA degradation to carbohydrate oxidation-mediated glucose catabolism. Such metabolic adaptation during hypoxia is reasonable because the glycolysis pathway can work, even under anaerobic conditions. However, as the efficiency of ATP production in glycolysis is significantly lower than that in mitochondrial oxidative metabolism, more efficient energy sources are required in the failing heart, in which the oxygen supply is impaired for an extended period of time (27). From this perspective, ketone bodies are a favorable substrate for energy production because the conversion of ketone bodies to acetyl-CoA is much easier in comparison to the conversion of FFAs and glucose (**Figure 2**) (28, 29). More importantly, ketone bodies can lead to the more efficient oxidation of the mitochondrial coenzyme Q couple and enhance the free energy of cytosolic ATP hydrolysis. Furthermore, changes at the transcriptional level of ketone oxidation-related genes would be associated with the substrate shift to ketone bodies in the failing heart. Indeed, previous investigations revealed that ketone metabolism is increased accompanied with the decrease of fatty acid oxidation in failing heart, as evidenced by the elevation of the levels of BDH1 and ketone body-derived materials, such as hydroxybutyryl-carnitine (C4OH-carnitine), βOHB-CoA and acetyl-carnitine (C2-carnitine) (28, 29) (**Figure 2**). In addition, a number of studies have demonstrated that the intake of a ketogenic diet extends longevity and the health span (30). Shimazu et al. revealed part of its mechanism. Treatment with βOHB inhibits histone deacetylase, thereby promoting FoxO3A and MT2 activity, which, in turn, markedly reduce oxidative stress and extend the life span in mice (31). Furthermore, ketone bodies possess anti-inflammatory activity (32). Youm et al. demonstrated that ketone bodies play an anti-inflammatory role by inhibiting the activity of the NOD-like receptor pyrin domain containing protein 3 (NLRP3) inflammasomes in animal models (33).

Thus, the elevation of ketone levels by SGLT2 inhibition might have a beneficial effect in patients with heart failure through multiple mechanisms.

SGLT2 INHIBITORS PROMOTE SODIUM METABOLISM-MEDIATED CARDIOPROTECTIVE EFFECTS

As the inhibition of SGLT2 induces natriuresis as well as glycosuria because SGLT2 cotransports glucose with sodium, SGLT2 blockade could alter intracellular sodium homeostasis. Sodium plays an important role in mitochondrial redox regulation and excitation-contraction coupling in cardiomyocytes (**Figure 3**) (34, 35). Indeed, to produce energy in the form of ATP, cardiomyocytes primarily depend on the mitochondrial oxidative phosphorylation system (OXPHOS). Nicotinamide adenine dinucleotide (NADH), a reducing equivalent that is produced from the tricarboxylic acid (TCA) cycle, donates its electron to complexes I, III and IV of the electron transport chain (ETC), thereby promoting the translocation of H^+ to the mitochondrial intermembrane space. The reduced form of flavin adenine dinucleotide ($FADH_2$) also participates in the ETC reaction by donating its electron to complex II. The concentration gradient of H^+ translocation generated by this reaction drives the conversion from ADP to ATP at the F1/F0-ATP synthase. The increase of ADP caused by the increased energy demand enhances the production of ATP at the F1/F0-ATP synthase, thereby promoting the oxidization of NADH to NAD^+. Concurrently, the increase of cytosolic Ca^{2+} transients by β-adrenergic stimulation promotes the uptake of mitochondrial Ca^{2+} through the mitochondrial Ca^{2+} uniporter (MCU) (36). Then, Ca^{2+} activates the dehydrogenases of the TCA cycle to promote the regeneration of NADH (37). Thus, OXPHOS acts in concert with the TCA cycle to preserve constant ratios of ATP/ADP and NADH/NAD^+ (38). In addition, nicotinamide adenine dinucleotide phosphate (NADPH) which is produced from NADH and TCA cycle products such as malate and isocitrate, plays a critical role in maintaining oxidative defense by donating electrons to reduced glutathione, thioredoxin, and glutaredoxin pools. Thus, the mitochondrial Ca^{2+} uptake is crucial for preserving the mitochondrial antioxidative capacity as well as for matching the energy supply to the demand (39). Ca^{2+} handling in cardiomyocytes is closely coordinated with Na^+ handling through the activity of the sarcolemmal Na^+/Ca^{2+} exchanger (NCX) and the mitochondrial Na^+/Ca^{2+} exchanger (NCLX). The cardiac NCX entirely bails out Ca^{2+} to the extracellular space under physiological conditions. However, NCX sets out to import Ca^{2+} to the cytosol in the early phase of the action potential, depending on the membrane potential and the Na^+ and Ca^{2+} transmembrane gradients

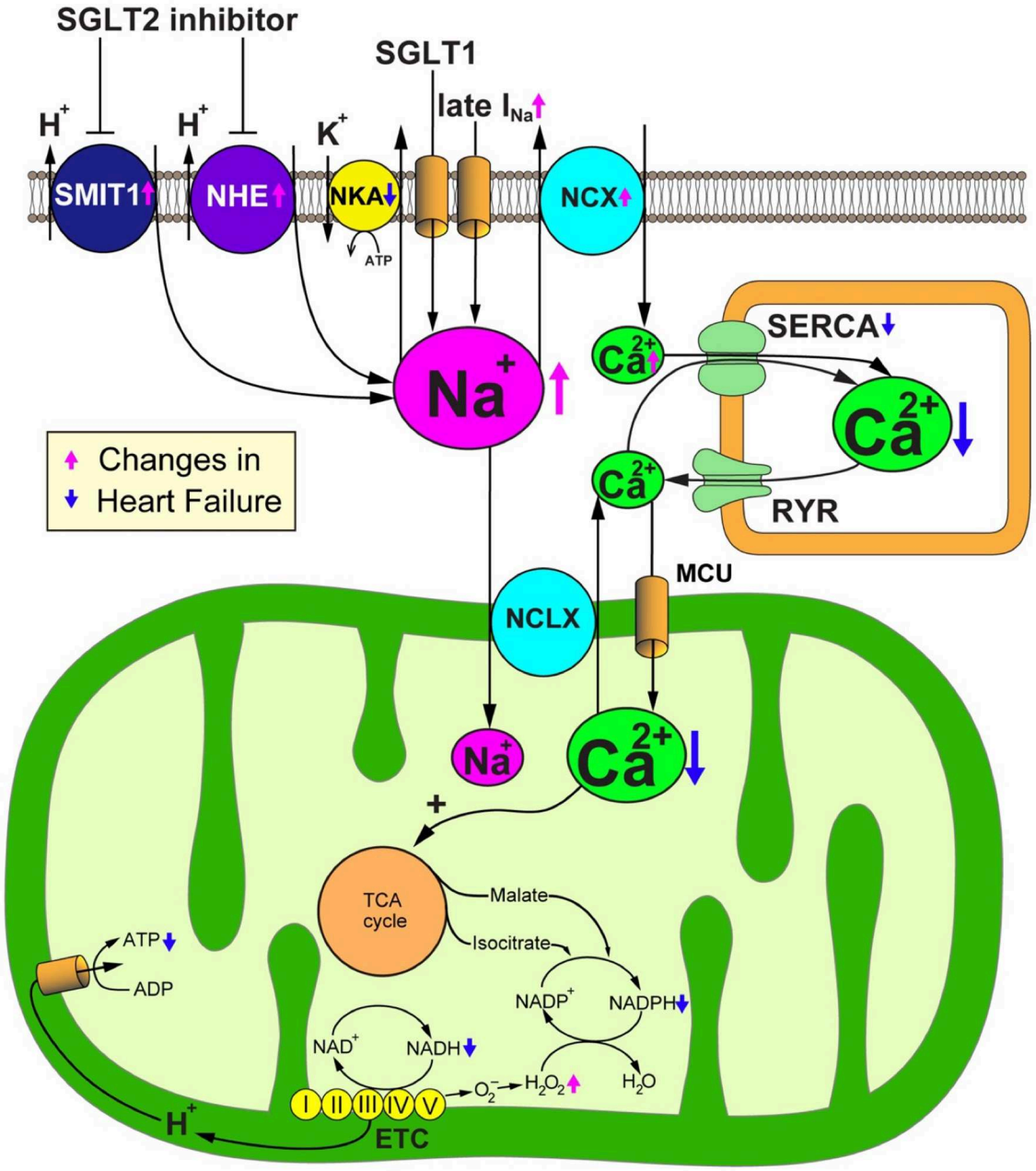


FIGURE 3 | SGLT2 inhibitors promote sodium metabolism-mediated cardioprotective effects. Failing cardiomyocytes show elevated intracellular Na^+ concentrations due to (1) increased Na^+ influx via the late Na^+ current (I_{Na}), (2) enhanced sarcolemmal Na^+/H^+ exchanger (NHE) activity, (3) reduced Na^+/K^+ ATPase (NKA) activity, and in the case of the diabetic heart, (4) the increased expression and activity of Na^+-glucose cotransporter 1 (SGLT1). Intracellular overload of Na^+ promotes Ca^{2+} efflux from mitochondria through the mitochondrial Na^+/Ca^{2+} exchanger (NCLX). The reduction of the Ca^{2+} concentration in the mitochondrial matrix deteriorates the Ca^{2+}-induced upregulation of TCA cycle dehydrogenases in response to workload transition, thereby disturbing the regeneration of reducing equivalents that are essential for preserving the antioxidative capacity and matching the energy supply to the energy demand. SGLT2 inhibitors would have a salutary role in failing cardiomyocytes through their alleviation of Na^+ and Ca^{2+} handling through NHE inhibition. ADP, adenosine diphosphate; ATP, adenosine triphosphate; ETC, electron transport chain; MCU, mitochondrial Ca^{2+} uniporter; NAD^+/NADH, nicotine amide dinucleotide oxidized/reduced; NCX, sarcolemmal Na^+/Ca^{2+} exchanger; NKA, Na^+/K^+ ATPase; RyR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase.

(40). The cardiac NCLX is mainly responsible for the extrusion of Ca^{2+} from mitochondria. However, as the kinetics of NCLX are slower in comparison to the uptake of Ca^{2+} via the MCU, it is susceptible to the accumulation of Ca^{2+} in mitochondria after increasing the rate and amplitude of cytosolic Ca^{2+} transients.

Heart failure is closely associated with the impairment of both Ca^{2+} and Na^+ handling in cardiomyocytes. Indeed, the amplitude and velocity of cytosolic Ca^{2+} transients are decreased in failing cardiomyocytes. Furthermore, the elevation of diastolic cytosolic Ca^{2+} ($[Ca^{2+}]_c$) and Na^+ concentrations ($[Na^+]_c$) is observed in failing cardiomyocytes (41, 42). The impairment of Ca^{2+} handling is due to the decrease of the Ca^{2+} uptake by the sarco/endoplasmic reticulum Ca^{2+}- ATPase (SERCA) and the leak of Ca^{2+} from the sarcoplasmic reticulum (SR) via ryanodine receptors (43, 44). The increase in the expression and activity of the NCX promotes the export of Ca^{2+} into the extracellular space, and thereby also reduces the Ca^{2+} load of the SR (45). Furthermore, the reduction of the release of Ca^{2+} from the SR results in the impairment of the mitochondrial Ca^{2+} uptake and steady-state Ca^{2+} concentration ($[Ca^{2+}]_m$) (46). On the other hand, excessive influx of Ca^{2+} into the mitochondria is detrimental to cardiomyocytes. The elevation of $[Ca^{2+}]_m$ triggers depolarization of mitochondrial inner membrane potential, generation of reactive oxygen species (ROS), and opening the mitochondrial permeability transition pore (47, 48), thereby promoting the release of pro-apoptotic proteins, such as cytochrome c, into the cytosol (49).

Increasing lines of evidence suggest that $[Na^+]_c$ is significantly elevated in failing cardiomyocytes as a result of (1) increased Na^+ influx via the late Na^+ current (I_{Na}) (41), (2) enhancement of sarcolemmal Na^+/H^+ exchanger (NHE) activity (50), (3) reduction of Na^+/K^+ ATPase (NKA) activity (51), and—in the case of diabetic heart—(4) the increased expression and activity of the Na^+-glucose cotransporter 1 (SGLT1) (52) (**Figure 2**). Generally, the increase of $[Na^+]$c should trigger positive effects on cytosolic Ca^{2+} handling because intracellular Na^+ overload prevents the NCX from exporting Ca^{2+} during the diastolic phase and promotes the reverse-mode function of the NCX during the action potential—thereby enhancing additional trans-sarcolemmal Ca^{2+} influx to achieve the elevation of Ca^{2+} in the SR and increase the amplitude of cytosolic Ca^{2+} transients. However, from a metabolic point of view, the elevation of $[Na^+]_c$ results in detrimental effects, especially in mitochondria. As Ca^{2+} is pumped out of mitochondria to the cytosol by an NCLX, the elevation of $[Na^+]$c enhances the driving force for mitochondrial Ca^{2+} efflux. The decrease of $[Ca^{2+}]_m$ suppresses the Ca^{2+}-induced upregulation of dehydrogenases in the TCA cycle, thereby attenuating the production of both NADH and NADPH (46). The decreased production of NADH causes ATP depletion. The reduction of the amount of NADPH causes the impairment of mitochondrial antioxidative defense because the donation of electrons from NADPH is indispensable for antioxidative enzymes, such as peroxiredoxin, glutathione peroxidase, and glutaredoxin. Thus, the elevation of $[Na^+]_c$ enhances oxidative stress, thereby aggravating the vulnerability of the heart to arrhythmias and neurohormonal hyperactivation. Furthermore, the increase of $[Na^+]_c$ eventually causes the emission of mitochondrial ROS, which results in the further deterioration of the intracellular Na^+ overload (35). Based on these facts, reducing the intracellular Na^+ overload to improve mitochondrial energetics and oxidative defense could be a promising therapeutic strategy for heart failure (**Figure 3**).

With regard to the beneficial effects of SGLT2 inhibitors on heart failure, it was initially considered that SGLT2 inhibitors have no direct effect on cardiomyocytes because SGLT2 is not expressed in the heart in either healthy subjects or under pathological conditions (5). However, a recent investigation demonstrated that empagliflozin reduced $[Na^+]_c$ and $[Ca^{2+}]_c$ in isolated cardiomyocytes (53). According to this report, empagliflozin directly reduced myocardial $[Na^+]_c$ and $[Ca^{2+}]_c$ and elevated $[Ca^{2+}]_m$ by suppressing myocardial NHE flux, independently of glucose transport. Habibi et al. demonstrated that the administration of empagliflozin mitigates diastolic dysfunction in db/db mice (54). The author of the present study found that empagliflozin suppresses the expression of serum- and glucocorticoid-inducible kinase 1 (SGK1) in the myocardium. As SGK1 activity may modulate NHE activity through Akt-mediated signaling, these results suggest that empagliflozin could restore myocardial $[Na^+]_c$ in a sustained manner (55). Examinations using $^{23}Na^+$ magnetic resonance imaging revealed that the tissue Na^+ content in diabetic patients was markedly reduced by treatment with dapagliflozin (56). An *in silico* docking study demonstrated that three SGLT2 inhibitors, empagliflozin, dapagliflozin, and canagliflozin, showed high binding affinity with the extracellular Na^+-binding site of NHE (57). In this study, the authors confirmed—by *in vitro* experiments—that empagliflozin, dapagliflozin and canagliflozin directly inhibit the cardiac NHE flux and reduce $[Na^+]_c$.

The expression of NHE is upregulated in the failing heart, possibly through the acidification of the intracellular environment due to increased conversion of pyruvate to lactate (58). Similarly, the NHE activity of cardiomyocytes of the animal models of type 2 diabetes and the suppression of $[Na^+]_c$ by NHE inhibition with cariporide was found to be cardioprotective (59, 60). Specifically, cariporide significantly suppressed the elevation of $[Na^+]_c$ at the end of ischemia and inhibited ventricular arrhythmia during reperfusion in a db/db mouse model of ischemia/reperfusion (59). In the Goto–Kakizaki rat model of type 2 diabetes, which does not develop hypertension, obesity or hyperlipidemia, the NHE activity of cardiomyocytes is markedly upregulated, which results in an increase in $[Na^+]_c$. In this model, the intracellular Na^+ overload was closely associated with the Akt-mediated progression of left ventricular hypertrophy. Consistently, the administration of cariporide significantly suppressed both $[Na^+]_c$ and Akt activation, resulting in the attenuation of cardiac hypertrophy (60).

There are seven SGLT isoforms (SGLT1 to 6 and sodium-myoinositol cotransporter 1, SMIT1). Among these, only SGLT1 and SMIT1 are expressed in the mammalian heart. The overexpression of SMIT1 activates NOX2, increases ROS, and exacerbates glucotoxicity in cardiomyocytes. Consistently, the deletion of SMIT1 prevented hyperglycemia-induced NOX2 activation (61). Thus far, the physiological role of SMIT1 in the heart remains unknown, as the deletion of SMIT1 does not alter the cardiac phenotype. Interestingly, however, SMIT1 is hardly associated with the glucose uptake in the heart, regardless of any glycemic conditions. Thus, SMIT1-mediated NOX2 activation would modulate glucose sensitization, which

could trigger ionic signaling ($[Na^+]_c$ and $[Ca^{2+}]_c$ via the NCX) into cells in association with the changes in the extracellular glucose concentration. Concomitantly, intracellular signaling via protein kinase C (PKC)-β, a calcium-dependent serine/threonine kinase, could be the link to ionic changes downstream of SMIT1. The IC_{50} of empagliflozin and canagliflozin for SMIT1 are estimated to be 8.3 and 5.6 μM, respectively (62, 63). Indeed, empagliflozin is even able to reduce $[Na^+]_c$ in the absence of glucose (53).

SGLT2 INHIBITORS COULD MODULATE MITOCHONDRIAL DYNAMICS RESULTING IN CARDIOPROTECTION

Mitochondria continuously fuse and divide in highly regulated manners to maintain their functions, which include metabolism, energy production, intracellular signaling, and the regulation of apoptosis. The enhancement of mitochondrial fusion would allow for the making up of "healthy" mitochondria, resulting in the normalization of the overall mitochondrial function. In response to various stresses, mitochondria undergo stress-induced mitochondrial hyperfusion (64), which thereby enhances ATP production, which—in turn—plays a pro-survival role. On the other hand, damaged mitochondria must be removed to preserve mitochondrial homeostasis. To this end, mitochondrial fission could be enhanced to more easily remove dysfunctional mitochondria via mitochondria-selective autophagy, termed mitophagy (65). Several key regulators are required for the operation of such mitochondrial dynamics. Mitochondrial fusion is regulated by mitofusin1 (Mfn1), mitofusin2 (Mfn2), and Opa1 (**Figure 4**) (66). On the other hand, mitochondrial fission is regulated by the recruitment of Dynamin-related protein 1 (Drp1) to specific sites on the outer mitochondrial membrane in coordination with mitochondrial fission 1 (Fis1) and mitochondrial fission factor (Mff) (**Figure 4**) (67).

The impairment of mitochondrial fusion via the downregulation of Mfn1 and Mfn2 aggravates cardiac dysfunction both at baseline and in response to stress (68). On the other hand, the inhibition of mitochondrial fission by the pharmacological suppression of Drp1 with Mdivi-1 reduces the size of infarcts that develop in response to ischemia/reperfusion (I/R) (69). In contrast, the inhibition of mitochondrial fission by genetic modulation, such as the knockdown of Fis1 mRNA or the expression of dominant-negative mutation in Drp1, inhibits mitophagy which results in metabolic dysfunction in INS1 cells (70), suggesting that mitochondrial fission has a two-sided nature with respect to cell survival in the myocardium.

Increasing lines of evidence suggest that SGLT2 inhibitors may modulate mitochondrial dynamics. Ipragliflozin alleviates the mitochondrial dysfunction induced by a high-fat diet by restoring the levels of Opa1 and Mfn2 to normal values *in vivo* without reducing body weight or blood glucose levels in rat models (71). Similarly, dapagliflozin normalizes the Mfn1/Mfn2 ratio in the rat model of metabolic syndrome, thereby suppressing prolonged ventricular repolarization (72). Empagliflozin restores the AMP/ATP ratio, thereby activating adenosine monophosphate (AMP)-activated protein kinase (AMPK) (73). The activation of AMPK causes an increase in $Drp1^{S637}$ phosphorylation and a decrease in $Drp1^{S616}$ phosphorylation, which results in the suppression of mitochondrial fission. Another study demonstrated that empagliflozin normalized the size and number of mitochondria in the OLETF diabetic rat heart and that the diabetes-induced excessive reduction in mitochondrial size after MI was inhibited by empagliflozin via the suppression of Fis1 upregulation and following ROS production, which results in the reduction of the MI size (74).

Thus, inhibition of SGLT2 is closely associated with the mitochondrial dynamics through the regulation of fusion and fission of mitochondria. Although several hypotheses have been proposed (71, 74, 75), the detailed molecular mechanism through which mitochondrial fusion and fission are modulated by the administration of SGLT2 inhibitors is largely unknown. Furthermore, it remains to be determined whether the effect of SGLT2 inhibitors on AMPK activity, one of the key molecules in the regulation of mitochondrial fission, is a class effect or a drug-specific effect. Indeed, Mancini et al. reported that canagliflozin, but not dapagliflozin or empagliflozin, could enhance AMPK activity both in human umbilical vein endothelial cells and human arterial endothelial cells (76). In addition, the precise roles of mitochondrial fission and fusion in the development of heart failure remain to be determined.

FUTURE DIRECTIONS

We reviewed the proposed cardioprotective effect of SGLT2 inhibitors, which is mediated through the improvement of the mitochondrial function by (1) increasing ketone body usage, (2) the mitigation of sodium metabolism, and (3) the modulation mitochondrial dynamics. However, many questions remain to be solved to validate these hypotheses. Indeed, it remains controversial whether SGLT2 inhibitors could be directly involved in the protective effects of cardiomyocytes, which do not express SGLT2. In particular, regarding the regulation of mitochondrial dynamics, previous studies merely observed the change in the expression levels of factors that regulate the mitochondrial dynamics (e.g., Mfn1 or Drp1) in response to the administration of SGLT2 inhibitors. Thus, the molecular mechanism through which these compounds modulate mitochondrial fusion and fission remains to be elucidated. Regarding the association with ketone body metabolism, it is necessary to determine whether the favorable effects induced by the increase in ketone bodies would be limited in the alteration of the mitochondrial energy metabolism. Furthermore, the possibility that these drugs could regulate different target molecule(s) other than SGLT2 (i.e., have off-target effects) should be examined. Indeed, the hypothesis that SGLT2 inhibitors regulate sodium metabolism is based on the fact that SGLT2 inhibitors possess the potential to inhibit both NHE and SMIT1.

As stated above, the DAPA-HF trial demonstrated that dapagliflozin plays a protective role in patients with established HFrEF, regardless of the presence of diabetes (15). Currently,

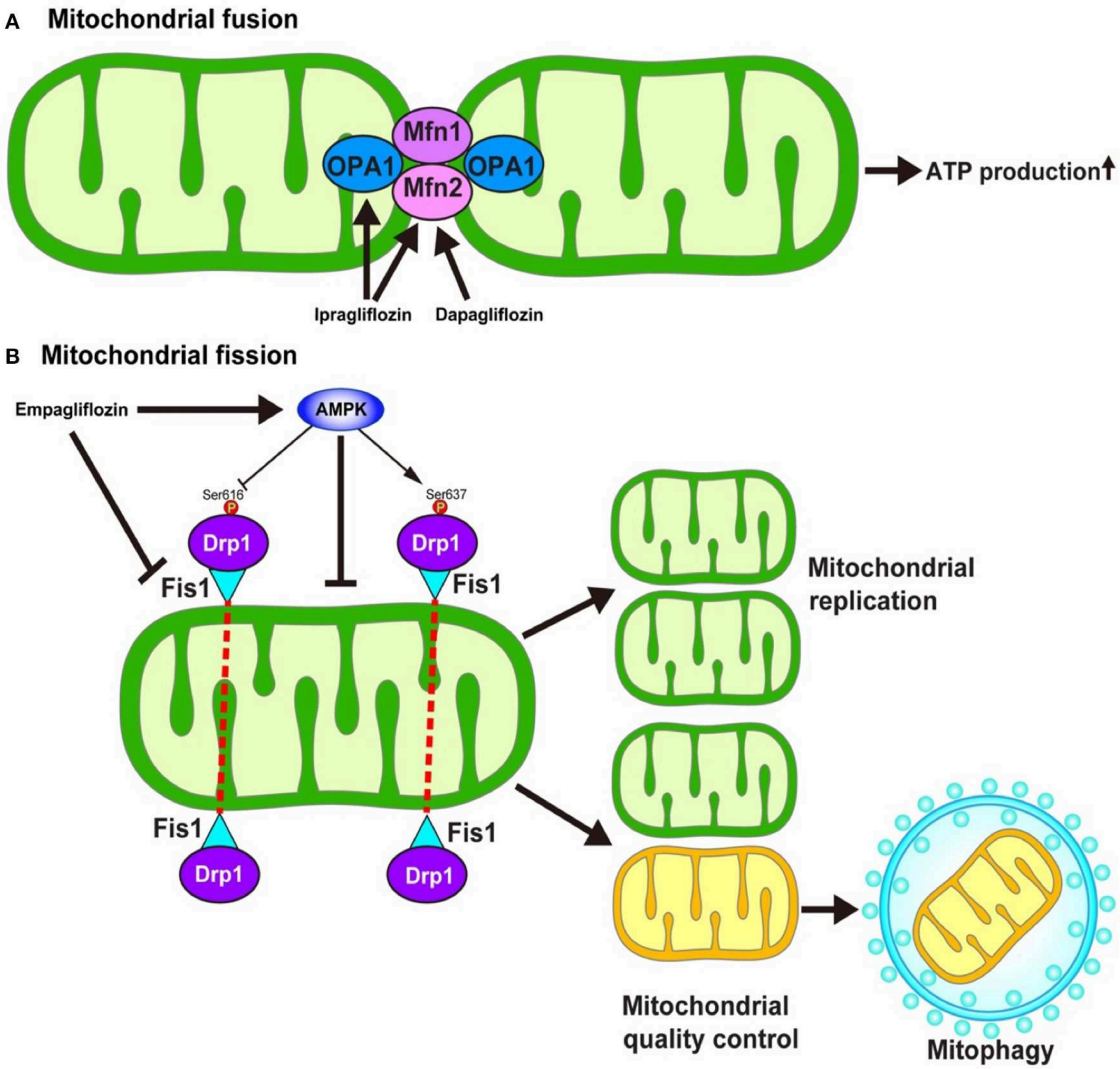


FIGURE 4 | Hypothesized mechanism of the modulation of mitochondrial dynamics by SGLT2 inhibitors. The inhibition of SGLT2 might be associated with the mitochondrial dynamics through the regulation of **(A)** mitochondrial fusion and **(B)** mitochondrial fission. However, the detailed mechanism as to how SGLT2 inhibitors modulate the regulators of mitochondrial dynamics is largely unknown. AMPK, AMP-activated protein kinase; Drp1, Dynamin-related protein 1; Fis1, Mitochondrial fission 1 protein; Mfn, Mitofusin; Ser, Serine.

the EMPEROR-Reduced trial [NCT03057977] to evaluate the efficacy of empagliflozin vs. placebo on top of guideline-directed medical therapy in HFrEF patients with or without diabetes is ongoing (77). If empagliflozin is proven to be beneficial in patients with HFrEF based on the results of this trial, it would provide more robust evidence of the beneficial effect of SGLT2 inhibitors on heart failure. At the same time, two randomized clinical trials are evaluating the effects of SGLT2 inhibitors in patients with established heart failure with a preserved ejection fraction (HFpEF), regardless the presence of diabetes. One is the EMPEROR-Preserved trial [NCT03057951] with empagliflozin (78), and the other one is the DELIVER trial [NCT03619213] with dapagliflozin. Several preclinical studies proposed the mechanism how SGLT2 inhibitor alleviates cardiac diastolic dysfunction, a major cause of HFpEF. For example, Juni et al. demonstrated that Empagliflozin suppresses TNF-α-induced mitochondrial and cytoplasmic ROS accumulation, thereby restoring cardiac microvascular endothelial cell-derived NO delivery, which in turn leads to reinstatement of cardiac relaxation and contraction (79). There are great expectations regarding the result of these clinical trials because, at the time of writing, no drugs have been demonstrated to be effective for the treatment of HFpEF (80).

As is the case with the positive effects, unfavorable aspects of SGLT2 inhibitor administration for the heart failure patients should be considered. Increasing lines of evidence suggest that sarcopenia is one of the major risk factors for morbidity and mortality of heart failure. Past clinical observations demonstrated that the skeletal muscle mass reduction is observed in a given number of patients

with diabetes who were treated with SGLT2 inhibitors. Also, the decreased exercise capacity, one of the major causes of sarcopenia which is the consequence of mitochondrial dysfunction in skeletal muscles, is an independent predictor of the poor prognosis of patients with heart failure (81). Thus, basically, the patients who are susceptible to sarcopenia should not be prescribed SGLT2 inhibitors. On the other hand, a recent investigation demonstrated the intriguing result that Empagliflozin restored decreased exercise endurance capacity by alleviating skeletal muscle fatty acid oxidation in an animal heart failure model (82). In any case, we should carefully determine which kind of patients are optimal for the treatment with SGLT2 inhibitors.

Taken together, unremitting efforts to elucidate the molecular mechanism through which the administration of SGLT2 inhibitors alleviates heart failure, as well as clinical studies of these compounds for non-diabetic heart failure could shift their classification from merely anti-diabetic drugs to potent anti-heart failure drugs.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This work was supported in part by a JSPS KAKENHI Grant-in-Aid for Scientific Research (C) (17K09570), the Smoking Research Foundation, and Bristol-Myers Squibb Research Grant 2018.

ACKNOWLEDGMENTS

The author would like to thank Dr. Shun Nakagama, Dr. Yuka Shiheido-Watanabe, Dr. Noriko Tamura, Dr. Tetsuo Sasano, and Ms. Noriko Tamura, for their excellent contributions. Also, the author thanks Brian Quinn (Japan Medical Communication) for critical reading.

REFERENCES

1. Pajor AM, Wright EM. Cloning and functional expression of a mammalian Na+/nucleoside cotransporter. A member of the SGLT family. *J Biol Chem*. (1992) 267:3557–60.
2. Diedrich DF. Competitive inhibition of intestinal glucose transport by phlorizin analogs. *Arch Biochem Biophys*. (1966) 117:248–56. doi: 10.1016/0003-9861(66)90409-7
3. Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, et al. The concise guide to PHARMACOLOGY 2013/14: transporters. *Br J Pharmacol*. (2013) 170:1706–96. doi: 10.1111/bph.12450
4. Chao EC, Henry RR. SGLT2 inhibition–a novel strategy for diabetes treatment. *Nat Rev Drug Discov*. (2010) 9:551–9. doi: 10.1038/nrd3180
5. Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. *Diabetes Ther*. (2010) 1:57–92. doi: 10.1007/s13300-010-0006-4
6. Chin KL, Ofori-Asenso R, Hopper I, von Lueder TG, Reid CM, Zoungas S, et al. Potential mechanisms underlying the cardiovascular benefits of sodium glucose cotransporter 2 inhibitors: a systematic review of data from preclinical studies. *Cardiovasc Res*. (2019) 115:266–76. doi: 10.1093/cvr/cvy295
7. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
8. Fitchett D, Zinman B, Wanner C, Lachin JM, Hantel S, Salsali A, et al. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. *Eur Heart J*. (2016) 37:1526–34. doi: 10.1093/eurheartj/ehv728
9. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. (2017) 377:644–57. doi: 10.1056/NEJMoa1611925
10. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. (2019) 380:347–57. doi: 10.1056/NEJMoa1812389
11. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet*. (2019) 393:31–9. doi: 10.1016/S0140-6736(18)32590-X
12. Byrne NJ, Parajuli N, Levasseur JL, Boisvenue J, Beker DL, Masson G, et al. Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. *JACC Basic Transl Sci*. (2017) 2:347–54. doi: 10.1016/j.jacbts.2017.07.003
13. Andreadou I, Efentakis P, Balafas E, Togliatto G, Davos CH, Varela A, et al. Empagliflozin limits myocardial infarction in vivo and cell death *in vitro*: role of STAT3, mitochondria, and redox aspects. *Front Physiol*. (2017) 8:1077. doi: 10.3389/fphys.2017.01077
14. Yurista SR, Sillje HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, et al. Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. *Eur J Heart Fail*. (2019) 21:862–73. doi: 10.1002/ejhf.1473
15. McMurray JJV, Solomon SD, Inzucchi SE, Kober L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med*. (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303
16. Dekkers CCJ, Sjostrom CD, Greasley PJ, Cain V, Boulton DW, and Heerspink HJL. Effects of the sodium-glucose co-transporter-2 inhibitor dapagliflozin on estimated plasma volume in patients with type 2 diabetes. *Diabetes Obes Metab*. (2019) 21:2667–73. doi: 10.1111/dom.13855
17. Blood Pressure Lowering Treatment Trialists' Collaboration. Blood pressure-lowering treatment based on cardiovascular risk: a meta-analysis of individual patient data. *Lancet*. (2014) 384:591–8. doi: 10.1016/S0140-6736(14)61212-5
18. Sano M, Takei M, Shiraishi Y, Suzuki Y. Increased Hematocrit During sodium-glucose cotransporter 2 inhibitor therapy indicates recovery of tubulointerstitial function in diabetic kidneys. *J Clin Med Res*. (2016) 8:844–7. doi: 10.14740/jocmr2760w
19. Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME Trial: a "thrifty substrate" hypothesis. *Diabetes Care*. (2016) 39:1108–14. doi: 10.2337/dc16-0330
20. Puchalska P, Crawford PA. Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. *Cell Metab*. (2017) 25:262–84. doi: 10.1016/j.cmet.2016.12.022
21. Qiu H, Novikov A, Vallon V. Ketosis and diabetic ketoacidosis in response to SGLT2 inhibitors: basic mechanisms and therapeutic perspectives. *Diabetes Metab Res Rev*. (2017) 33:e2886. doi: 10.1002/dmrr.2886
22. Williamson DH, Lund P, Krebs HA. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Biochem J*. (1967) 103:514–27. doi: 10.1042/bj1030514
23. Krebs HA, Wallace PG, Hems R, Freedland RA. Rates of ketone-body formation in the perfused rat liver. *Biochem J*. (1969) 112:595–600. doi: 10.1042/bj1120595
24. Ganapathy V, Thangaraju M, Gopal E, Martin PM, Itagaki S, Miyauchi S, et al. Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. *Aaps J*. (2008) 10:193–9. doi: 10.1208/s12248-008-9022-y

25. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME Study? A unifying hypothesis. *Diabetes Care*. (2016) 39:1115–22. doi: 10.2337/dc16-0542
26. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev*. (2010) 90:207–58. doi: 10.1152/physrev.00015.2009
27. Neubauer S, The failing heart–an engine out of fuel. *N Engl J Med*. (2007) 356:1140–51. doi: 10.1056/NEJMra063052
28. Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, et al. The failing heart relies on ketone bodies as a fuel. *Circulation*. (2016) 133:698–705. doi: 10.1161/CIRCULATIONAHA.115.017355
29. Bedi KC, Jr, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation*. (2016) 133:706–16. doi: 10.1161/CIRCULATIONAHA.115.017545
30. Johnson AA, Stolzing A. The role of lipid metabolism in aging, lifespan regulation, and age-related disease. *Aging Cell*. (2019) 18: e13048. doi: 10.1111/acel.13048
31. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science*. (2013) 339:211–4. doi: 10.1126/science.1227166
32. Ruskin DN, Kawamura M, Masino SA. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. *PLoS One*. (2009) 4:e8349. doi: 10.1371/journal.pone.0008349
33. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med*. (2015) 21:263–9. doi: 10.1038/nm.3804
34. Clancy CE, Chen-Izu Y, Bers DM, Belardinelli L, Boyden PA, Csernoch L, et al. Deranged sodium to sudden death. *J Physiol*. (2015) 593:1331–45. doi: 10.1113/jphysiol.2014.281204
35. Bay J, Kohlhaas M, Maack C. Intracellular Na($^{+}$) and cardiac metabolism. *J Mol Cell Cardiol*. (2013) 61:20–7. doi: 10.1016/j.yjmcc.2013.05.010
36. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na+ decreases mitochondrial Ca2+ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ Res*. (2006) 99:172–82. doi: 10.1161/01.RES.0000232546.92777.05
37. Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria. *Circ Res*. (2018) 122:1460–78. doi: 10.1161/CIRCRESAHA.118.310082
38. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol*. (2007) 102:369–92. doi: 10.1007/s00395-007-0666-z
39. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, et al. Elevated cytosolic Na+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation*. (2010) 121:1606–13. doi: 10.1161/CIRCULATIONAHA.109.914911
40. Weber CR, Piacentino V III, Houser SR, Bers DM. Dynamic regulation of sodium/calcium exchange function in human heart failure. *Circulation*. (2003) 108:2224–9. doi: 10.1161/01.CIR.0000095274.72486.94
41. Despa S, Islam MA, Weber CR, Pogwizd SM, Bers DM. Intracellular Na(+) concentration is elevated in heart failure but Na/K pump function is unchanged. *Circulation*. (2002) 105:2543–8. doi: 10.1161/01.CIR.0000016701.85760.97
42. Lambert R, Srodulski S, Peng X, Margulies KB, Despa F, Despa S. Intracellular Na+ concentration ([Na+]i) is elevated in diabetic hearts due to enhanced Na+-glucose cotransport. *J Am Heart Assoc*. (2015) 4:e002183. doi: 10.1161/JAHA.115.002183
43. O'Rourke B, Kass DA, Tomaselli GF, Kaab S, Tunin R, Marban E. Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart failure, I: experimental studies. *Circ Res*. (1999) 84:562–70. doi: 10.1161/01.RES.84.5.562
44. Hobai IA, Maack C, O'Rourke B, Partial inhibition of sodium/calcium exchange restores cellular calcium handling in canine heart failure. *Circ Res*. (2004) 95: 292–9. doi: 10.1161/01.RES.0000136817.28691.2d
45. Studer R, Reinecke H, Bilger J, Eschenhagen T, Bohm M, Hasenfuss G, et al. Gene expression of the cardiac Na(+)-Ca2+ exchanger in end-stage human heart failure. *Circ Res*. (1994) 75:443–53. doi: 10.1161/01.RES.75.3.443
46. Kohlhaas M, and Maack C, Adverse bioenergetic consequences of Na+-Ca2+ exchanger-mediated Ca2+ influx in cardiac myocytes. *Circulation*. (2010) 122:2273–80. doi: 10.1161/CIRCULATIONAHA.110.968057
47. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol*. (2004) 287:C817–33. doi: 10.1152/ajpcell.00139.2004
48. Halestrap AP, McStay GP, Clarke SJ. The permeability transition pore complex: another view. *Biochimie*. (2002) 84:153–66. doi: 10.1016/S0300-9084(02)01375-5
49. Crow MT, Mani K, Nam YJ, Kitsis RN. The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ Res*. (2004) 95:957–70. doi: 10.1161/01.RES.0000148632.35500.d9
50. Baartscheer A, Schumacher CA, Belterman CN, Coronel R, Fiolet JW. [Na+]i and the driving force of the Na^{+}/Ca^{2+}-exchanger in heart failure. *Cardiovasc Res*. (2003) 57:986–95. doi: 10.1016/S0008-6363(02)00848-9
51. Swift F, Birkeland JA, Tovsrud N, Enger UH, Aronsen JM, Louch WE, et al. Altered Na^{+}/Ca^{2+}-exchanger activity due to downregulation of Na+/K+-ATPase alpha2-isoform in heart failure. *Cardiovasc Res*. (2008) 78:71–8. doi: 10.1093/cvr/cvn013
52. Banerjee SK, McGaffin KR, Pastor-Soler NM, Ahmad F. SGLT1 is a novel cardiac glucose transporter that is perturbed in disease states. *Cardiovasc Res*. (2009) 84:111–8. doi: 10.1093/cvr/cvp190
53. Baartscheer A, Schumacher CA, Wust RC, Fiolet JW, Stienen GJ, Coronel R, et al. Empagliflozin decreases myocardial cytoplasmic Na(+) through inhibition of the cardiac Na(+)/H(+) exchanger in rats and rabbits. *Diabetologia*. (2017) 60:568–73. doi: 10.1007/s00125-016-4134-x
54. Habibi J, Aroor AR, Sowers JR, Jia G, Hayden MR, Garro M, et al. Sodium glucose transporter 2 (SGLT2) inhibition with empagliflozin improves cardiac diastolic function in a female rodent model of diabetes. *Cardiovasc Diabetol*. (2017) 16:9. doi: 10.1186/s12933-016-0489-z
55. Voelkl J, Lin Y, Alesutan I, Ahmed MS, Pasham V, Mia S, et al. Sgk1 sensitivity of Na(+)/H(+) exchanger activity and cardiac remodeling following pressure overload. *Basic Res Cardiol*. (2012) 107:236. doi: 10.1007/s00395-011-0236-2
56. Karg MV, Bosch A, Kannenkeril D, Striepe K, Ott C, Schneider MP, et al. SGLT-2-inhibition with dapagliflozin reduces tissue sodium content: a randomised controlled trial. *Cardiovasc Diabetol*. (2018) 17:5. doi: 10.1186/s12933-017-0654-z
57. Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, et al. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na(+)/H(+) exchanger, lowering of cytosolic Na(+) and vasodilation. *Diabetologia*. (2018) 61:722–6. doi: 10.1007/s00125-017-4509-7
58. Takewaki S, Kuro-o M, Hiroi Y, Yamazaki T, Noguchi T, Miyagishi A, et al. Activation of Na(+)-H+ antiporter (NHE-1) gene expression during growth, hypertrophy and proliferation of the rabbit cardiovascular system. *J Mol Cell Cardiol*. (1995) 27:729–42. doi: 10.1016/S0022-2828(08)80063-6
59. Anzawa R, Bernard M, Tamareille S, Baetz D, Confort-Gouny S, Gascard JP, et al. Intracellular sodium increase and susceptibility to ischaemia in hearts from type 2 diabetic db/db mice. *Diabetologia*. (2006) 49:598–606. doi: 10.1007/s00125-005-0091-5
60. Darmellah A, Baetz D, Prunier F, Tamareille S, Rucker-Martin C, Feuvray D. Enhanced activity of the myocardial Na+/H+ exchanger contributes to left ventricular hypertrophy in the Goto-Kakizaki rat model of type 2 diabetes: critical role of Akt. *Diabetologia*. (2007) 50:1335–44. doi: 10.1007/s00125-007-0628-x
61. Van Steenbergen A, Balteau M, Ginion A, Ferte L, Battault S, Ravenstein CM, et al. Sodium-myoinositol cotransporter-1, SMIT1, mediates the production of reactive oxygen species induced by hyperglycemia in the heart. *Sci Rep*. (2017) 7:41166. doi: 10.1038/srep41166
62. Suzuki M, Honda K, Fukazawa M, Ozawa K, Hagita H, Kawai T, et al. Tofogliflozin, a potent and highly specific sodium/glucose cotransporter 2 inhibitor, improves glycemic control in diabetic rats and mice. *J Pharmacol Exp Ther*. (2012) 341:692–701. doi: 10.1124/jpet.112.191593
63. Scheen AJ. Evaluating SGLT2 inhibitors for type 2 diabetes: pharmacokinetic and toxicological considerations. *Expert Opin Drug Metab Toxicol*. (2014) 10:647–63. doi: 10.1517/17425255.2014.873788

64. Tondera D, Grandemange S, Jourdain A, Karbowski M, Mattenberger Y, Herzig S, et al. SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J*. (2009) 28:1589–600. doi: 10.1038/emboj.2009.89
65. Sciarretta S, Maejima Y, Zablocki D, Sadoshima J. The role of autophagy in the heart. *Annu Rev Physiol*. (2018) 80:1–26. doi: 10.1146/annurev-physiol-021317-121427
66. Maejima Y, Chen Y, Isobe M, Gustafsson AB, Kitsis RN, Sadoshima J. Recent progress in research on molecular mechanisms of autophagy in the heart. *Am J Physiol Heart Circ Physiol*. (2015) 308:H259–68. doi: 10.1152/ajpheart.00711.2014
67. Shirakabe A, Zhai P, Ikeda Y, Saito T, Maejima Y, Hsu CP, et al. Drp1-Dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure. *Circulation*. (2016) 133:1249–63. doi: 10.1161/CIRCULATIONAHA.115.020502
68. Chen Y, Liu Y, Dorn GW II. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res*. (2011) 109:1327–31. doi: 10.1161/CIRCRESAHA.111.258723
69. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. *Circulation*. (2010) 121:2012–22. doi: 10.1161/CIRCULATIONAHA.109.906610
70. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. (2008) 27:433–46. doi: 10.1038/sj.emboj.7601963
71. Takagi S, Li J, Takagaki Y, Kitada M, Nitta K, Takasu T, et al. Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet. *J Diabetes Invest*. (2018) 9:1025–32. doi: 10.1111/jdi.12802
72. Durak A, Olgar Y, Degirmenci S, Akkus E, Tuncay E, Turan B. A SGLT2 inhibitor dapagliflozin suppresses prolonged ventricular-repolarization through augmentation of mitochondrial function in insulin-resistant metabolic syndrome rats. *Cardiovasc Diabetol*. (2018) 17:144. doi: 10.1186/s12933-018-0790-0
73. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. *Redox Biol*. (2018) 15:335–46. doi: 10.1016/j.redox.2017.12.019
74. Mizuno M, Kuno A, Yano T, Miki T, Oshima H, Sato T, et al. Empagliflozin normalizes the size and number of mitochondria and prevents reduction in mitochondrial size after myocardial infarction in diabetic hearts. *Physiol Rep*. (2018) 6:e13741. doi: 10.14814/phy2.13741
75. Esterline RL, Vaag A, Oscarsson J, Vora J. MECHANISMS IN ENDOCRINOLOGY: SGLT2 inhibitors: clinical benefits by restoration of normal diurnal metabolism? *Eur J Endocrinol*. (2018) 178:R113–25. doi: 10.1530/EJE-17-0832
76. Mancini SJ, Boyd D, Katwan OJ, Strembitska A, Almabrouk TA, Kennedy S, et al. Canagliflozin inhibits interleukin-1β-stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms. *Sci Rep*. (2018) 8:5276. doi: 10.1038/s41598-018-23420-4
77. Packer M, Butler J, Filippatos GS, Jamal W, Salsali A, Schnee J, et al. Evaluation of the effect of sodium-glucose co-transporter 2 inhibition with empagliflozin on morbidity and mortality of patients with chronic heart failure and a reduced ejection fraction: rationale for and design of the EMPEROR-reduced trial. *Eur J Heart Fail*. (2019) 21:1270–8. doi: 10.1002/ejhf.1536
78. Anker SD, Butler J, Filippatos GS, Jamal W, Salsali A, Schnee J, et al. Evaluation of the effects of sodium-glucose co-transporter 2 inhibition with empagliflozin on morbidity and mortality in patients with chronic heart failure and a preserved ejection fraction: rationale for and design of the EMPEROR-preserved trial. *Eur J Heart Fail*. (2019) 21:1279–87. doi: 10.1002/ejhf.1596
79. Juni RP, Kuster DWD, Goebel M, Helmes M, Musters RJP, van der Velden J, et al. Cardiac microvascular endothelial enhancement of cardiomyocyte function is impaired by inflammation and restored by empagliflozin. *JACC Basic Transl Sci*. (2019) 4:575–91. doi: 10.1016/j.jacbts.2019.04.003
80. Bonsu KO, Arunmanakul P, Chaiyakunapruk N. Pharmacological treatments for heart failure with preserved ejection fraction-a systematic review and indirect comparison. *Heart Fail Rev*. (2018) 23:147–56. doi: 10.1007/s10741-018-9679-y
81. Kinugawa S, Takada S, Matsushima S, Okita K, Tsutsui H. Skeletal muscle abnormalities in heart failure. *Int Heart J*. (2015) 56:475–84. doi: 10.1536/ihj.15-108
82. Nambu H, Takada S, Fukushima A, Matsumoto J, Kakutani N, Maekawa S, et al. Empagliflozin restores lowered exercise endurance capacity via the activation of skeletal muscle fatty acid oxidation in a murine model of heart failure. *Eur J Pharmacol*. (2019) 172810. doi: 10.1016/j.ejphar.2019.172810

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 15 January 2020
doi: 10.3389/fcvm.2019.00193

Integrating ER and Mitochondrial Proteostasis in the Healthy and Diseased Heart

*Adrian Arrieta, Erik A. Blackwood, Winston T. Stauffer and Christopher C. Glembotski**

Department of Biology, San Diego State University Heart Institute, San Diego State University, San Diego, CA, United States

The integrity of the proteome in cardiac myocytes is critical for robust heart function. Proteome integrity in all cells is managed by protein homeostasis or proteostasis, which encompasses processes that maintain the balance of protein synthesis, folding, and degradation in ways that allow cells to adapt to conditions that present a potential challenge to viability (1). While there are processes in various cellular locations in cardiac myocytes that contribute to proteostasis, those in the cytosol, mitochondria and endoplasmic reticulum (ER) have dominant roles in maintaining cardiac contractile function. Cytosolic proteostasis has been reviewed elsewhere (2, 3); accordingly, this review focuses on proteostasis in the ER and mitochondria, and how they might influence each other and, thus, impact heart function in the settings of cardiac physiology and disease.

Keywords: mitochondria, proteostasis, UPR, endoplasmic reticulum, protein folding

OPEN ACCESS

Edited by:
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Reviewed by:
Yibin Wang, University of California, Los Angeles, United States
Asa Gustafsson, University of California, San Diego, United States

****Correspondence:***
Christopher C. Glembotski
cglembotski@sdsu.edu

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *30 September 2019*
Accepted: *18 December 2019*
Published: *15 January 2020*

Citation:
Arrieta A, Blackwood EA, Stauffer WT and Glembotski CC (2020) Integrating ER and Mitochondrial Proteostasis in the Healthy and Diseased Heart. Front. Cardiovasc. Med. 6:193. doi: 10.3389/fcvm.2019.00193

ER PROTEOSTASIS

Most secreted and membrane proteins are made in the ER, making it a major site for proteostasis (4, 5). Moreover, the specialized ER in cardiac myocytes, which includes the sarco/endoplasmic reticulum, is responsible for contractile calcium handling (6–9), and most of the proteins that are required for this important function of the heart are made at the ER (10, 11). Thus, ER proteostasis in the heart, and in particular in cardiac myocytes, is critical for proper cardiac function. ER proteostasis requires an environment that optimizes a balanced synthesis, folding and degradation of proteins made in this location (**Figure 1A**). Conditions, including cardiac pathologies can perturb the ER environment in ways that decrease the efficiency of ER protein folding, leading to the accumulation of potentially toxic misfolded proteins, which imbalance and dysregulate proteostasis, leading to activation of the unfolded protein response (UPR) (**Figure 1B**) (12). Misfolded proteins in the ER are detected by 3 well studied transmembrane proteins, ATF6 (activating transcription factor 6), IRE1 (inositol requiring enzyme 1) and PERK (protein kinase R [PKR]-like ER kinase), each of which exhibits a unique mechanism of activation in response to the accumulation of misfolded proteins in the ER; thus, ATF6, IRE1, and PERK initiate three different but complementary branches of the ER unfolded protein response (UPR^{ER}) (**Figure 1C**) (13). The UPR^{ER} can also be activated by other cellular stresses that could impact proteostasis, or may be independent of it, including changes in ER lipid content (14), hypoxia (15, 16), growth stimuli and reactive oxygen species (17). Thus, while ATF6, IRE1 and PERK were originally found to all be activated by overt ER protein misfolding, it is now clear that they are activated differentially by different pathophysiological stresses and, as a result, the downstream signaling events initiated by each stress are different yet complementary, as far as their ultimate effects on cell function.

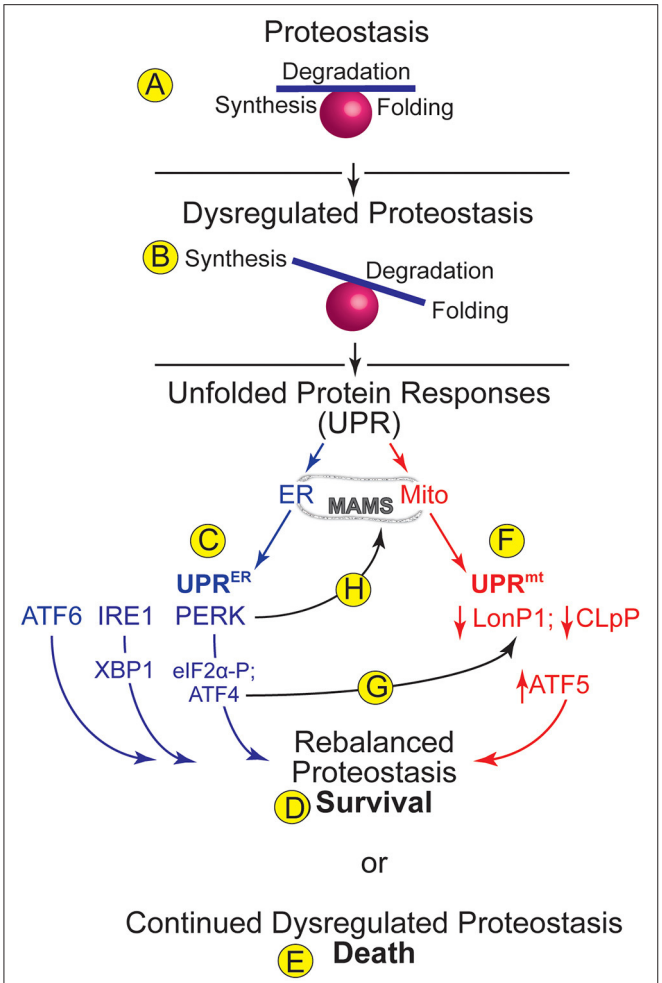


FIGURE 1 | ER and Mitochondrial Proteostasis- **(A)** Proteostasis encompasses processes such as protein synthesis, degradation, and folding. A balance amongst such processes supports optimal proteome integrity. **(B)** Dysregulated proteostasis occurs when environmental conditions, including cardiac pathology, cause an imbalance in these processes, which activates adaptive compensatory responses, such as the unfolded protein responses (UPRs) in various organelles. **(C)** The UPR in the endoplasmic reticulum (ER) is called the UPR^{ER}. Increased levels of misfolded proteins in the ER activate three ER transmembrane proteins, ATF6, IRE1, and PERK, which cause increases in the transcription factors ATF6, XBP1, and ATF4, which together regulate genes designed to rebalance ER proteostasis. PERK also phosphorylates eIF2a, which arrests translation of most mRNAs, thus relieving the protein-folding burden on the ER and allowing for cell survival **(D)**. **(E)** Continued dysregulation of proteostasis leads to chronic activation of the UPR^{ER} proximal sensors and cell death. **(F)** The UPR in mitochondria (mt) is called the UPR^{mt}. The levels of the mitochondrial proteases, LonP1 and CLpP decrease upon dysregulation of mitochondrial proteostasis. Decreased LonP1 and CLpP contribute to increasing the level of the transcription factor, ATF5, which regulates genes designed to rebalance mitochondrial proteostasis. **(G)** A potential integration point between the UPR^{ER} and the UPR^{mt} is the ability of the UPR^{ER}-activated transcription factor, ATF4 to increase expression of the UPR^{mt} protease, LonP1. **(H)** Another potential integration point between the UPR^{ER} and the UPR^{mt} is the ability of PERK to tether the ER to mitochondria at contact sites called mitochondrial associated membranes (MAMS).

In terms of the canonical role for ER stress, initially, UPR^{ER} signaling is designed to restore proper protein folding to the ER, constituting an adaptive return to proteostasis and cell survival (**Figure 1D**). This restoration takes place at many levels, including enhanced expression of chaperones to facilitate protein-folding, increases in the rate at which misfolded proteins in the ER are degraded through a process called ER associated degradation (ERAD) (18), and decreases in translation of mRNAs that encode proteins that are not required for the restoration of ER proteostasis (19, 20). However, if these complex initial events of the UPR^{ER} are not sufficient to restore proteostasis, then continued dysregulation of proteostasis leads to chronic activation of the proximal sensors and cell death (**Figure 1E**), and is thus considered maladaptive (21).

ER PROTEOSTASIS IN CARDIAC PATHOLOGY

A number of studies have demonstrated important roles for the UPR^{ER} in the heart; most of these studies have focused on examining ER proteostasis in cardiac myocytes. For example, the ATF6 branch of the UPR^{ER} is mainly adaptive and can protect the heart during pathophysiological maneuvers involving ischemia/reperfusion (I/R) and pressure overload in mice (13, 17, 22–26), the latter of which mimics hypertension and stimulates pathological growth of the heart. The adaptive effects of ATF6 are considered to be largely due to its abilities to serve as a transcription factor following its activation (17, 25, 27). Consistent with this are findings that the genes induced by ATF6 as part of the UPR^{ER} are known to participate in adaptive restoration of proteostasis in the heart by inducing canonical adaptive UPR genes, such as those proteins that constitute the ER protein-folding machinery (**Figure 2A**), thus serving protective roles (28). Surprisingly, upon activation, ATF6 has been shown to induce a number of genes not previously thought to be involved in restoring ER protein folding capacity. For example, the induction of catalase during cardiac I/R (29), was a surprise, since catalase is not an ER protein, nor is it known to be involved in restoration of ER proteostasis. However, in that study it was shown that ATF6 can transcriptionally induce catalase during I/R and, as a result, catalase neutralizes damaging reactive oxygen species that accumulate during reperfusion, which decreases myocardial damage, thus describing catalase as a non-canonical adaptive UPR gene (**Figure 2A**, ischemia/reperfusion). In another study, it was shown that during acute pressure overload, ATF6 is necessary for the initial growth of the heart, which is an adaptive effect (17). In that study, using mice in which ATF6 was deleted specifically in cardiac myocytes, it was shown that ATF6 transcriptionally induces the small GTP binding protein, Rheb, which is an activator of mTORC1 (**Figure 2A**, Cardiac Hypertrophy), a well-studied pathway responsible for myocardial growth during development and pathology, thus describing Rheb as a non-canonical adaptive UPR gene induced during cardiac hypertrophy. Another study involved global deletion of ATF6 and showed that after acute pressure overload compensatory hypertrophy was impaired by ATF6 deletion, while ATF6 deletion led to increased hypertrophy and impaired function after chronic pressure overload (25). It is interesting to

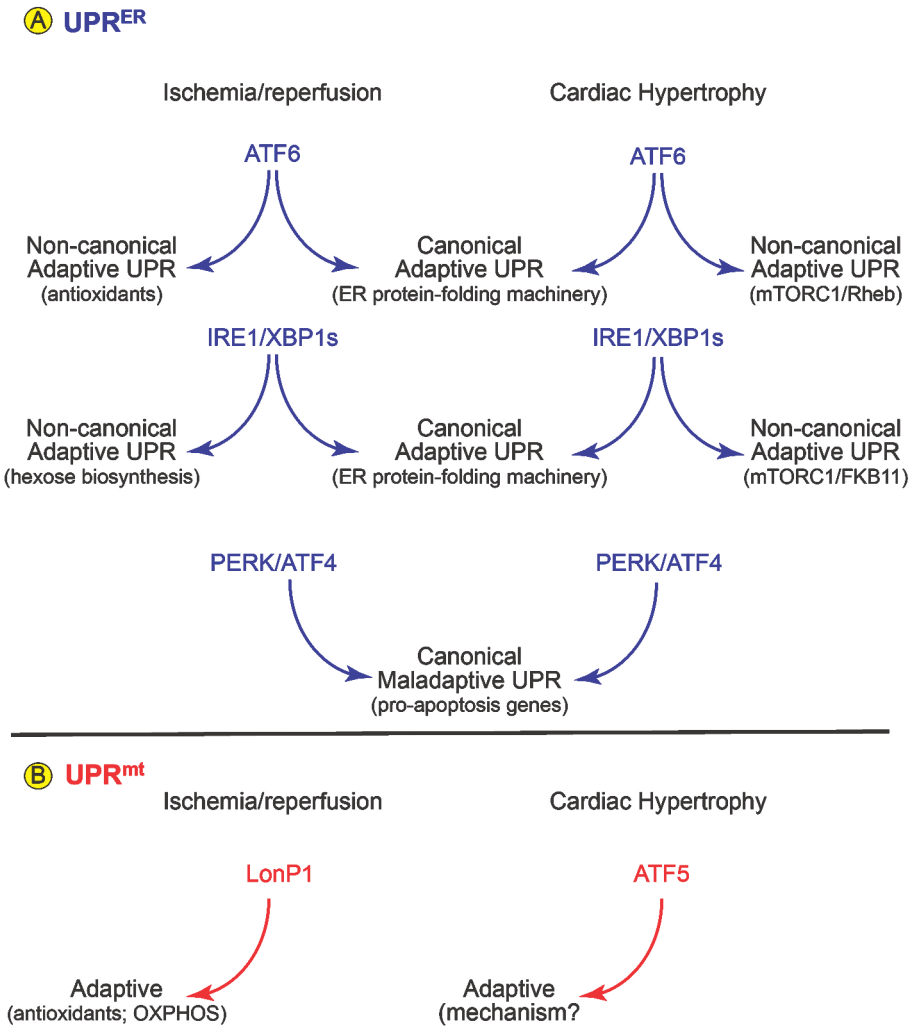


FIGURE 2 | Roles for the UPR^{ER} and UPR^{mt} in Cardiac Pathology- **(A)** In mouse models of cardiac ischemia/reperfusion and pathological cardiac hypertrophy there is evidence for activation of all three arms of the UPR^{ER}. (Center) Upon activation each arm of the UPR induces canonical ER stress response genes which support protection for ATF6 and IRE1/XBP1s and damage for PERK/ATF5. However, the ATF6 and IRE1/XBP1s arms of the UPR^{ER} also induce non-canonical gene programs that foster protection in the heart (left and right). **(B)** In mouse models of cardiac pathology the LonP1 and ATF5 aspects of the UPR^{mt} are activated and both are protective in these disease settings.

note that while it was not studied in the context of activating ATF6, Rheb-mediated mTORC1 activation has been shown to suppress mitophagy, which is generally considered adaptive during cardiac pathology (30–32), suggesting that mTORC1 activation via Rheb is not always adaptive in the heart.

Other branches of the UPR^{ER} have also been implicated in the adaptive responses of the heart of pathological stress. For example, in mouse hearts ischemia/reperfusion activates the IRE1 branch of the UPR^{ER}, leading to the formation of an active transcription factor, XBP1, which protects cardiac myocytes from I/R damage, in part, by inducing canonical adaptive UPR genes (**Figure 2**, IRE1/XBP1s; canonical) (33). In that study it was subsequently shown that XBP1 protects the mouse heart from I/R damage in a non-canonical manner by transcriptionally inducing key genes responsible for the hexosamine biosynthetic pathway, which is required for protein O-GlcNAcylation (**Figure 2**, I/R non-canonical). Cardiac I/R was shown to increase protein O-GlcNAcylation in the hearts of mice (34), suggesting that O-GlcNAcylation is protective. Moreover, inhibition of O-GlyNAcases increased mitochondrial OXPHOS enzyme activities, implying that this is one way that O-GlcNAcylation might be protective (35); however, the mechanism by which XBP1-mediated protein O-GlcNAcylation results in cardioprotection remains unclear. In terms of heart failure, it was shown that XBP1s stimulates adaptive cardiac growth through activation of mTORC1, which is mediated via FKBP11 (FK506-binding protein 11), a novel transcriptional target of XBP1s, thus describing a non-canonical protective for IRE1/XBP1s in pathological hypertrophy (**Figure 2A**, IRE1/XBPs cardiac hypertrophy) (36). It has also been shown that in a

mouse model of heart failure with preserved ejection fraction (HFpEF), activation of IRE1 is deficient and restoration of activated XBP1 ameliorated the HFpEF phenotype (37). While this study indicates that IRE1 and perhaps the transcription factor, XBP1, protect against the development of HFpEF, the genes that are responsible for this protection have not been identified.

The PERK branch of the UPR^{ER} has also been studied in the heart. In many tissues, including the heart, PERK is known to be involved in numerous signaling pathways, one of which leads to activation of the transcription factor, ATF4, which increases expression of the pro-apoptotic transcription factor, C/EBP homologous protein (CHOP) (38). Since apoptosis is a major contributor to the decline in cardiac function observed during heart failure and other cardiac pathologies (39), and since CHOP expression is increased in experimental models of heart disease (40), several studies have focused on the effects of CHOP gene deletion in the mouse heart. For the most part, those studies have shown that PERK-mediated induction of CHOP in the ischemic or hypertrophic heart exacerbates cardiac pathology, in large part by increasing cardiac myocyte dropout by apoptosis (41). However, other studies that examined the effects of PERK deletion suggest that PERK is adaptive in the setting of pressure overload induced heart failure (42) (**Figure 2A**, PERK/ATF4). Studies such as these demonstrate the complex nature of the UPR^{ER}, indicating that depending on the circumstances, the UPR^{ER} can be adaptive or maladaptive.

MITOCHONDRIAL PROTEOSTASIS

Many cardiac physiology and pathology studies have focused on mitochondria, as they play an undeniably central role in energy generation in the metabolically demanding cardiac myocyte. Thus, processes that comprise mitochondrial quality control, which encompass proteostasis, biogenesis, dynamics (fusion and fission) and mitophagy, are critical for maintaining cardiac myocyte viability and heart contractile function (43). Among the features of mitochondrial quality control, relatively little is known about mitochondrial proteostasis in the heart. In non-cardiac cell and tissue types, stresses similar to those occurring during cardiac pathology cause the misfolding of mitochondrial proteins, as well as impaired mitochondrial protein import and decreased translation of mRNAs in mitochondria (44). Mitochondrial ATP production is at risk when mitochondrial proteostasis is dysregulated because it often leads to alterations in the relative quantities of the hundreds of proteins necessary for oxidative phosphorylation (OXPHOS) (45, 46). Moreover, an imbalance between nuclear-encoded and mitochondrial-encoded OXPHOS proteins affects mitochondrial proteostasis in ways that extend lifespan in mice and worms (47). In fact, since the mitochondrial proteome comprises proteins made in the cytosol as well as in mitochondria, the proteostasis balancing act that must be maintained in mitochondria is particularly challenging (46). One important first line of defense against mild mitochondrial damage is carried out by several mitochondrial proteases, which contribute to the mitochondrial unfolded protein response (UPR^{mt}) (**Figure 1F**). In the mitochondrial matrix, protein turnover is controlled by three AAA proteases: the soluble mitochondrial Lon protease homolog (LonP1) and mitochondrial ATP-dependent CLp protease (CLpP), and the mitochondrial inner membrane-bound m-AAA protease. In the intermembrane space, mitochondrial protein quality is ensured by the membrane-bound ATP-dependent zinc metalloproteinase, YME1L1, the soluble mitochondrial serine protease, HTRA2, the mitochondrial metalloendopeptidase, OMA1, and the mitochondrial presenilins-associated rhomboid-like protein (PARL). These proteases play a variety of roles, such as degradation of misfolded proteins and balancing various mitochondrial constituents, such as OXPHOS proteins. However, most evidence suggests that Lon1 and CLpP are central to the UPR^{mt}, while the other proteases may play roles in other aspects of mitochondrial proteostasis and dynamics (48). Moreover, because of the dire functional consequences of reductions in the quality of the mitochondrial proteome, dysregulation of mitochondrial proteostasis is communicated to various parts of the cell through at least five different pathways, including peptide-derived signaling, mitochondrial backup-signaling, mitochondrial translation control (MTC) loss-induced signaling and the mitochondrial unfolded protein response, UPR^{mt}.

Although the UPR^{mt} is beginning to be understood more clearly in mammals (49), much of our knowledge of this process comes from studies of the nematode, *Caenorhabditis elegans*. In fact, UPR^{mt} activation protects *C. elegans* against ischemic injury, further supporting potential roles for the UPR^{mt}, in the ischemic mammalian heart (50). A key regulator of the UPR^{mt} is the transcription factor, ATFS-1 in *C. elegans*, which in mammals is ATF5, a transcription factor that is imported into mitochondria in an ATP-dependent manner when mitochondrial function and protein folding is optimal (46, 51). Under such conditions, LonP1 and CLpP proteases degrade ATF5 (52). However, when dysregulated OXPHOS and other stresses lead to dysregulated mitochondrial proteostasis, LonP1 and CLpP are diverted toward degrading those misfolded proteins to minimize their toxic effects; this diversion leads to the accumulation of intact ATF5 (**Figure 1F**) (46, 49, 52). Upon accumulation ATF5 is then exported from mitochondria to the nucleus where it acts as a transcription factor that induces genes encoding proteins designed to improve mitochondrial protein folding and rebalance mitochondrial proteostasis (**Figure 1F**), such as HSPA9, LonP1, and YME1L. ATF5 also serves as a communicator of metabolic stress by temporarily limiting the transcription of OXPHOS genes encoded in nuclear and mitochondrial genomes, while simultaneously increasing nuclear encoded gene transcription of all glycolysis components, and this is thought to maintain cellular ATP levels until mitochondrial dysfunction is resolved (45, 53).

MITOCHONDRIAL PROTEOSTASIS IN CARDIAC PATHOLOGY

Little is known about the UPR^{mt} in the heart; however, several recent publications have provided initial evidence that the UPR^{mt}

is important for optimal cardiac function and recovery from I/R injury, as well as in the setting of pathological cardiac hypertrophy (**Figure 2B**). For example, using LonP1 transgenic mice, as well as mice that are haploinsufficient for the LonP1 gene, it was shown that this UPR^{mt} protease mitigates cardiac injury during I/R by preventing oxidative damage, in part by rebalancing OXPHOS complex subunit levels in an adaptive manner (54). Moreover, pressure overload in mice was shown to activate the UPR^{mt}. Additionally, pharmacologic boosting of the UPR^{mt} reduced cardiac pathology in this model (55, 56). In the same study it was also shown that hearts from patients with aortic stenosis, which is often associated with left ventricular overload, exhibited increased expression of genes associated with the UPR^{mt}. In another study, mice in which ATF5 was genetically deleted were used to show that the UPR^{mt} protected the heart against I/R in an ATF5-dependent manner (53). Moreover, in the same study RNAseq results demonstrated the induction of numerous genes in an ATF5-dependent manner during pharmacological induction of the UPR^{mt}. While these studies implicate roles for the UPR^{mt} in the setting of cardiac pathology, much remains to be determined about the role of this mitochondrial proteostasis pathway in the heart. Underscoring the need for additional studies is a recent report where it was shown that CLpP, which plays a central role in the UPR^{mt} in *C. elegans*, and thought to be important for the UPR^{mt} in mammals was not required for the mammalian UPR^{mt} (52). In fact, in that study it was found that CLpP contributes to mitochondrial cardiomyopathy, such that deletion of CLpP increased *de novo* synthesis of OXPHOS proteins leading to increased ATP and improved cardiac function in mice. On the other hand, a different study, while not in the heart, but done with C2C12 myoblasts, demonstrated that knockdown of CLpP altered mitochondrial morphology and expression of OXPHOS proteins, reduced oxygen consumption, increased reactive oxygen species and impaired myoblast differentiation (57). Interestingly, in this same study it was shown that knocking down CLpP leads to increases in the phosphorylation of EIF2α, which is a hallmark feature of the UPR^{ER}.

INTEGRATING ER AND MITOCHONDRIAL FUNCTION IN CARDIAC MYOCYTES

There is some evidence suggesting that there is a potential for integration between the UPR^{mt} and the UPR^{ER}. One important piece of this evidence is the physical linkage between mitochondria and the ER at mitochondrial associated membranes, or MAMs (58). Although physical linkages between mitochondria and the ER were reported beginning in the 1960's, the term MAM was christened by Jean Vance, who identified a function for the mitochondrial-ER contact sites in phospholipid transport between these organelles (59). Subsequently, numerous studies of MAMs have identified the proteins that tether the two organelles, including mitofusin2 (60), as well as important physiological roles for their juxta-positioning, which, in the heart, have been centered mostly around the movement of calcium from the ER into mitochondria (61). In this way, MAMs are responsible for coordinating ER calcium flux with a variety of mitochondrial functions, including the ATP generation, as well as apoptosis and mitophagy (62, 63). More recent studies have implicated specific mitochondrial-ER tethering proteins, such as FUNDC1, as having important roles in maintaining normal cardiac contractility in mice (64–66). Studies outside the cardiac context have shown that there are numerous components of the UPR^{ER} that are associated with MAM structure and function, including ER chaperones, the IP_3 receptor and PERK, which, if deleted decreases calcium movement from the ER to mitochondria (67). Relatedly, PERK deletion in the heart disrupts calcium signaling in cardiac myocytes in mice, *in vivo* (68).

INTEGRATING ER AND MITOCHONDRIAL PROTEOSTASIS

While studies on MAMs imply that the proteostasis pathways in these organelles must be integrated, to date there have been no studies in the heart that have addressed the molecular details of such integration beyond those involving MAMs. However, studies of molecular integration points between mitochondrial and ER proteostasis pathways have been done in other cell and tissue types, and the results of such studies could begin to inform us about whether such integration might also occur in the heart and, if so, what the functional consequences of this integration might be in terms of cardiac physiology and pathology. One potential molecular integration point between the UPR^{mt} and UPR^{ER} that has been studied extensively in non-cardiac cells and tissues is the integrated stress response (IRS). The IRS is an elaborate signaling pathway in eukaryotic cells that is activated in response to an array of stresses including hypoxia, amino acid starvation, glucose deprivation, ER stress and viral infection (42, 69, 70). All of these pathways converge on the activation of kinases, such as PERK, which phosphorylate eIF2α on serine 51. In addition to causing global translational repression, a feature that reduces the protein-folding burden on nearly all of cellular proteostasis, eIF2α phosphorylation leads to the preferential translation of some transcription factors that have upstream ORFs in their 5′ UTRs, such as ATF4 (71, 72) and to subsequent changes in gene expression that are adaptive upon acute ATF4 activation, but can culminate in apoptosis and necrosis upon chronic ATF4 activation. Importantly, the PERK/ATF4 signaling axis, which plays a central role in the IRS and UPR^{ER} (38), is also involved in the UPR^{mt} (69). In fact, like ATF4, the ATF5 transcript also has an ORFs in its 5′ UTR, so ATF5 levels also increase upon PERK upon activation of either the UPR^{ER} or the UPR^{mt} (73). PERK-mediated increases in ATF4 enhance the expression of the UPR^{mt} component, LonP1 (**Figure 1G**) (74). Related to this finding, but somewhat perplexing is the observation that chemical inhibition of LonP1 protease activity using CDDO activates the UPR^{mt}, as well as increasing ATF4 mediated gene induction (75), indicating a possible bidirectional regulatory linkage between ATF4 and LonP1. Another finding that could serve as a molecular integration point between the UPR^{ER} and UPR^{mt} was shown in chondrocytes, where

the ATF6 family member, BBF2H7, induces typical UPR^{ER} genes, as well as the regulator of the UPR^{mt}, ATF5 (76). In an examination of the effects of drugs that dysregulate mitochondrial proteostasis in HeLa, 293T, and COS7 cells, as well as maneuvers that cause mitochondrial proteostatic stress, *in vivo*, it was shown that via activation of the ISR, ATF4 but not ATF5 responds to dysregulated mitochondrial proteostasis and activates the expression of cytoprotective genes (77). Moreover, the PERK/ATF4 signaling axis can affect mitochondrial morphology and functional integrity, presumably having these effects at least partly through regulating mitochondrial proteostasis (78). Thus, it seems possible that through PERK-mediated increases in ATF4 and ATF5, and perhaps through PERKs role as a tether which holds MAMs together (**Figure 1H**), a function that does not require PERK enzyme activity (79), the UPR^{ER} and UPR^{mt} could be integrated and, in some cases co-activated, which could improve both ER and mitochondrial function during stresses that dysregulate proteostasis in these two organelles.

CONCLUSION

The processes that govern mitochondrial and ER proteostasis are of critical importance for the adaptation of eukaryotic cells to environmental changes that risk proteome integrity. Even though the processes involved in mitochondrial proteostasis have gone relatively unstudied in the heart, it seems likely that in combination with those that regulate ER proteostasis, they are critical for cardiac function and, in particular, cardiac myocyte viability and contractility. In light of this, it is apparent that mitochondrial and ER proteostasis, which are regulated by many processes in addition to the UPRs in these organelles, provide fertile opportunities for future studies that could lead to the design of novel therapeutics for treating cardiac pathologies, ranging from ischemic to hypertrophic and dilated cardiomyopathies. Our hope is that this review has brought such potential intervention points to light amongst the heart research community and that it will spawn investigation into these aspects of proteostasis, with an objective of developing much needed new therapies for treating cardiac pathologies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by NIH grants 1HL135893, 1HL141463, 1HL149931 to CG and NIH grant 1F31HL140850 and the Inamori Foundation to EB, and the ARCS Foundation, Inc., San Diego Chapter to EB and WS. AA, EB, and WS are Rees-Stealy Research Foundation Phillips Gausewitz, M.D., Scholars of the SDSU Heart Institute.

REFERENCES

1. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science*. (2008) 319:916–9. doi: 10.1126/science.1141448
2. Henning RH and Brundel B. Proteostasis in cardiac health and disease. *Nat Rev Cardiol*. (2017) 14:637–53. doi: 10.1038/nrcardio.2017.89
3. Jayaraj GG, Hipp MS, Hartl FU. Functional modules of the proteostasis network. *Cold Spring Harb Perspect Biol*. (2019) 4:a033951. doi: 10.1101/cshperspect.a033951
4. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*. (2007) 8:519–29. doi: 10.1038/nrm2199
5. Reid DW, Nicchitta CV. Diversity and selectivity in mRNA translation on the endoplasmic reticulum. *Nat Rev Mol Cell Biol*. (2015) 16:221–31. doi: 10.1038/nrm3958
6. Bers DM. Cardiac excitation-contraction coupling. *Nature*. (2002) 415:198–205. doi: 10.1038/415198a
7. Santana LF, Kranias EG, Lederer WJ. Calcium sparks and excitation-contraction coupling in phospholamban-deficient mouse ventricular myocytes. *J Physiol*. (1997) 503(Pt1):21–9. doi: 10.1111/j.1469–7793.1997.021bi.x
8. Bers DM, Shannon TR. Calcium movements inside the sarcoplasmic reticulum of cardiac myocytes. *J Mol Cell Cardiol*. (2013) 58:59–66. doi: 10.1016/j.yjmcc.2013.01.002
9. Eisner DA, Caldwell JL, Kistamas K, Trafford AW. Calcium and excitation-contraction coupling in the heart. *Circ Res*. (2017) 121:181–95. doi: 10.1161/CIRCRESAHA.117.310230
10. Glembotski CC. Roles for the sarco-/endoplasmic reticulum in cardiac myocyte contraction, protein synthesis, and protein quality control. *Physiology*. (2012) 27:343–50. doi: 10.1152/physiol.00034.2012
11. Doroudgar S, Glembotski CC. New concepts of endoplasmic reticulum function in the heart: programmed to conserve. *J Mol Cell Cardiol*. (2013) 55:85–91. doi: 10.1016/j.yjmcc.2012.10.006
12. Glembotski CC. Endoplasmic reticulum stress in the heart. *Circ Res*. (2007) 101:975–84. doi: 10.1161/CIRCRESAHA.107.161273
13. Glembotski CC, Rosarda JD, and Wiseman RL. Proteostasis and beyond: ATF6 in ischemic disease. *Trends Mol Med*. (2019) 25:538–550. doi: 10.1016/j.molmed.2019.03.005
14. Tam AB, Roberts LS, Chandra V, Rivera IG, Nomura DK, Forbes DJ, et al. The UPR activator ATF6 responds to proteotoxic and lipotoxic stress by distinct mechanisms. *Dev Cell*. (2018) 46:327–43.e7. doi: 10.1016/j.devcel.2018.04.023
15. Martindale JJ, Fernandez R, Thuerauf D, Whittaker R, Gude N, Sussman MA, et al. Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res*. (2006) 98:1186–93. doi: 10.1161/01.RES.0000220643.65941.8d
16. Doroudgar S, Thuerauf DJ, Marcinko MC, Belmont PJ, Glembotski CC. Ischemia activates the ATF6 branch of the endoplasmic reticulum stress response. *J Biol Chem*. (2009) 284:29735–45. doi: 10.1074/jbc.M109.018036
17. Blackwood EA, Hofmann C, Santo Domingo M, Bilal AS, Sarakki A, Stauffer W, et al. ATF6 regulates cardiac hypertrophy by transcriptional induction of the mTORC1 activator, Rheb. *Circ Res*. (2019) 124:79–93. doi: 10.1161/CIRCRESAHA.118.313854
18. Sun Z, Brodsky JL. Protein quality control in the secretory pathway. *J Cell Biol*. (2019) 218, 3171–87. doi: 10.1083/jcb.201906047
19. Pavitt GD, Ron D. New insights into translational regulation in the endoplasmic reticulum unfolded protein response. *Cold Spring Harb Perspect Biol*. (2012) 4:a012278. doi: 10.1101/cshperspect.a012278
20. Preissler S, Ron D. Early events in the endoplasmic reticulum unfolded protein response. *Cold Spring Harb Perspect Biol*. (2019) 11:a033894. doi: 10.1101/cshperspect.a033894

21. Sano R, Reed JC. ER stress-induced cell death mechanisms. *Biochim Biophys Acta*. (2013)1833:3460–70. doi: 10.1016/j.bbamcr.2013.06.028
22. Lynch JM, Maillet M, Vanhoutte D, Schloemer A, Sargent MA, Blair NS. A thrombospondin-dependent pathway for a protective ER stress response. *Cell*. (2012) 149:1257–68. doi: 10.1016/j.cell.2012.03.050
23. Wang X, Xu L, Gillette TG, Jiang X, Wang ZV. The unfolded protein response in ischemic heart disease. *J Mol Cell Cardiol*. (2018) 117:19–25. doi: 10.1016/j.yjmcc.2018.02.013
24. Zhang G, Wang X, Gillette TG, Deng Y, Wang ZV. Unfolded protein response as a therapeutic target in cardiovascular disease. *Curr Top Med Chem*. (2019) 19:1902–17. doi: 10.2174/1568026619666190521093049
25. Correll RN, Grimes KM, Prasad V, Lynch JM, Khalil H, Molkentin JD. Overlapping and differential functions of ATF6alpha versus ATF6beta in the mouse heart. *Sci Rep*. (2019) 9:2059. doi: 10.1038/s41598–019-39515–5
26. Blackwood EA, Azizi K, Thuerauf DJ, Paxman RJ, Plate L, Kelly JW, et al. Pharmacologic ATF6 activation confers global protection in widespread disease models by reprograming cellular proteostasis. *Nat Commun*. (2019) 10:187. doi: 10.1038/s41467–018-08129–2
27. Belmont PJ, Tadimalla A, Chen WJ, Martindale JJ, Thuerauf DJ, Marcinko M, et al. Coordination of growth and endoplasmic reticulum stress signaling by regulator of calcineurin 1 (RCAN1), a novel ATF6-inducible gene. *J Biol Chem*. (2008) 283:14012–21. doi: 10.1074/jbc.M709776200
28. Wang X, Bi X, Zhang G, Deng Y, Luo X, Xu L, et al. Glucose-regulated protein 78 is essential for cardiac myocyte survival. *Cell Death Differ*. (2018) 25:2181–94. doi: 10.1038/s41418–018-0109–4
29. Jin JK, Blackwood EA, Azizi K, Thuerauf DJ, Fahem AG, Hofmann C, et al. ATF6 decreases myocardial ischemia/reperfusion damage and links ER stress and oxidative stress signaling pathways in the heart. *Circ Res*. (2017) 120:862–75. doi: 10.1161/CIRCRESAHA.116.310266
30. Ranek MJ, Kokkonen-Simon KM, Chen A, Dunkerly-Eyring BL, Vera MP, Oeing CU, et al. PKG1-modified TSC2 regulates mTORC1 activity to counter adverse cardiac stress. *Nature*. (2019) 566:264–9. doi: 10.1038/s41586–019-0895-y
31. Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci USA*. (2011) 108:9572–7. doi: 10.1073/pnas.1106291108
32. Saito T, Sadoshima J. Molecular mechanisms of mitochondrial autophagy/mitophagy in the heart. *Circ Res*. (2015) 116:1477–90. doi: 10.1161/CIRCRESAHA.116.303790
33. Wang ZV, Deng Y, Gao N, Pedrozo Z, Li DL, Morales CR, et al. Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell*. (2014) 156:1179–92. doi: 10.1016/j.cell.2014.01.014
34. Jensen RV, Andreadou I, Hausenloy DJ, Botker HE. The Role of O-GlcNAcylation for protection against ischemia-reperfusion injury. *Int J Mol Sci*. (2019) 20:E404. doi: 10.3390/ijms20020404
35. Ma J, Liu T, Wei AC, Banerjee P, O'Rourke B, Hart GW. O-GlcNAcomic profiling identifies widespread o-linked beta-N-Acetylglucosamine modification (O-GlcNAcylation) in oxidative phosphorylation system regulating cardiac mitochondrial function. *J Biol Chem*. (2015) 290:29141–53. doi: 10.1074/jbc.M115.691741
36. Wang X, Deng Y, Zhang G, Li C, Ding G, May HI, et al. Spliced X-box binding protein 1 stimulates adaptive growth through activation of mTOR. *Circulation*. (2019) 140:566–579. doi: 10.1161/CIRCULATIONAHA.118.038924
37. Schiattarella GG, Altamirano F, Tong D, French KM, Villalobos E, Kim SY, et al. Nitrosative stress drives heart failure with preserved ejection fraction. *Nature*. (2019) 568:351–6. doi: 10.1038/s41586–019-1100-z
38. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell*. (2003) 11:619–33. doi: 10.1016/S1097–2765(03)00105–9
39. Rutkowski DT, Kaufman RJ. All roads lead to ATF4. *Dev Cell*. (2003) 4:442–4. doi: 10.1016/S1534–5807(03)00100-X
40. Toth A, Nickson P, Mandl A, Bannister ML, Toth K, Erhardt P. Endoplasmic reticulum stress as a novel therapeutic target in heart diseases. *Cardiovasc Hematol Disord Drug Targets*. (2007) 7:205–18. doi: 10.2174/187152907781745260
41. Fu HY, Okada K, Liao Y, Tsukamoto O, Isomura T, Asai M, et al. Ablation of C/EBP homologous protein attenuates endoplasmic reticulum-mediated apoptosis and cardiac dysfunction induced by pressure overload. *Circulation*. (2010) 122:361–9. doi: 10.1161/CIRCULATIONAHA.109.917914
42. Liu X, Kwak D, Lu Z, Xu X, Fassett J, Wang H, et al. Endoplasmic reticulum stress sensor protein kinase R-like endoplasmic reticulum kinase (PERK) protects against pressure overload-induced heart failure and lung remodeling. *Hypertension*. (2014) 64:738–44. doi: 10.1161/HYPERTENSIONAHA.114.03811
43. Picca A, Mankowski RT, Burman JL, Donisi L, Kim JS, Marzetti E, et al. Mitochondrial quality control mechanisms as molecular targets in cardiac ageing. *Nat Rev Cardiol*. (2018) 15:543–54. doi: 10.1038/s41569–018-0059-z
44. Andreasson C, Ott M, Buttner S. Mitochondria orchestrate proteostatic and metabolic stress responses. *EMBO Rep*. (2019) 20:e47865. doi: 10.15252/embr.201947865
45. Nargund AM, Fiorese CJ, Pellegrino MW, Deng P, Haynes CM. Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPR(mt). *Mol Cell*. (2015) 58:123–33. doi: 10.1016/j.molcel.2015.02.008
46. Shpilka T, Haynes CM. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. *Nat Rev Mol Cell Biol*. (2018) 19:109–20. doi: 10.1038/nrm.2017.110
47. Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, et al. Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature*. (2013) 497:451–7. doi: 10.1038/nature12188
48. Wai T, Garcia-Prieto J, Baker MJ, Merkwirth C, Benit P, Rustin P, et al. Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice. *Science*. (2015) 350:aad0116. doi: 10.1126/science.aad0116
49. Fiorese CJ, Schulz AM, Lin YF, Rosin N, Pellegrino MW, Haynes CM. The transcription factor ATF5 mediates a mammalian mitochondrial UPR. *Curr Biol*. (2016) 26:2037–43. doi: 10.1016/j.cub.2016.06.002
50. Kaufman DM, Crowder CM. Mitochondrial proteostatic collapse leads to hypoxic injury. *Curr Biol*. (2015) 25:2171–6. doi: 10.1016/j.cub.2015.06.062
51. Neupert W and Brunner M. The protein import motor of mitochondria. *Nat Rev Mol Cell Biol*. (2002) 3:555–65. doi: 10.1038/nrm878
52. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science*. (2012) 337:587–90. doi: 10.1126/science.1223560
53. Wang YT, Lim Y, McCall MN, Huang KT, Haynes CM, Nehrke K, et al. Cardioprotection by the mitochondrial unfolded protein response requires ATF5. *Am J Physiol Heart Circ Physiol*. (2019) 317:H472–8. doi: 10.1152/ajpheart.00244.2019
54. Venkatesh S, Li M, Saito T, Tong M, Rashed E, Mareedu S, et al. Mitochondrial LonP1 protects cardiomyocytes from ischemia/reperfusion injury *in vivo*. *J Mol Cell Cardiol*. (2019) 128:38–50. doi: 10.1016/j.yjmcc.2018.12.017
55. Smyrnias I, Gray SP, Okonko DO, Sawyer G, Zoccarato A, Catibog N, et al. Cardioprotective effect of the mitochondrial unfolded protein response during chronic pressure overload. *J Am Coll Cardiol*. (2019) 73:1795–806. doi: 10.1016/j.jacc.2018.12.087
56. Glembotski CC, Arrieta A, Blackwood EA. Unfolding the roles of mitochondria as therapeutic targets for heart disease. *J Am Coll Cardiol*. (2019) 73:1807–10. doi: 10.1016/j.jacc.2018.12.089
57. Deepa SS, Bhaskaran S, Ranjit R, Qaisar R, Nair BC, Liu Y, et al. Down-regulation of the mitochondrial matrix peptidase ClpP in muscle cells causes mitochondrial dysfunction and decreases cell proliferation. *Free Radic Biol Med*. (2016) 91:281–92. doi: 10.1016/j.freeradbiomed.2015.12.021
58. de Brito OM, Scorrano L. An intimate liaison: spatial organization of the endoplasmic reticulum-mitochondria relationship. *EMBO J*. (2010) 29:2715–23. doi: 10.1038/emboj.2010.177
59. Vance JE. Phospholipid synthesis in a membrane fraction associated with mitochondria. *J Biol Chem*. (1990) 265:7248–56.
60. de Brito OM, Scorrano L. Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. *Antioxid Redox Signal*. (2008) 10:621–33. doi: 10.1089/ars.2007.1934
61. Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL, Bers DM. Measuring local gradients of intramitochondrial [Ca(2+)] in cardiac myocytes during sarcoplasmic reticulum Ca(2+) release. *Circ Res*. (2013) 112:424–31. doi: 10.1161/CIRCRESAHA.111.300501

62. Dorn GW II, Maack C. SR and mitochondria: calcium cross-talk between kissing cousins. *J Mol Cell Cardiol*. (2013) 55:42–9. doi: 10.1016/j.yjmcc.2012.07.015
63. Seidlmayer LK, Mages C, Berbner A, Eder-Negrin P, Arias-Loza PA, Kaspar M, et al. Mitofusin 2 is essential for IP3-mediated SR/Mitochondria metabolic feedback in ventricular myocytes. *Front Physiol*. (2019) 10:733. doi: 10.3389/fphys.2019.00733
64. Wu S, Lu Q, Wang Q, Ding Y, Ma Z, Mao X, et al. Binding of FUN14 domain containing 1 With inositol 1,4,5-trisphosphate receptor in mitochondria-associated endoplasmic reticulum membranes maintains mitochondrial dynamics and function in hearts *in vivo*. *Circulation*. (2017) 136:2248–66. doi: 10.1161/CIRCULATIONAHA.117.030235
65. Wu S, Lu Q, Ding Y, Wu Y, Qiu Y, Wang P, et al. Hyperglycemia-driven inhibition of AMP-activated protein kinase alpha2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes *In vivo*. *Circulation*. (2019) 139:1913–36. doi: 10.1161/CIRCULATIONAHA.118.033552
66. Wu S, Zou MH. Mitochondria-associated endoplasmic reticulum membranes in the heart. *Arch Biochem Biophys*. (2019) 662:201–12. doi: 10.1016/j.abb.2018.12.018
67. Carreras-Sureda A, Pihan P, Hetz C. The unfolded protein response: at the intersection between endoplasmic reticulum function and mitochondrial bioenergetics. *Front Oncol*. (2017) 7:55. doi: 10.3389/fonc.2017.00055
68. Liu Z, Cai H, Zhu H, Toque H, Zhao N, Qiu C, et al. Protein kinase RNA-like endoplasmic reticulum kinase (PERK)/calcineurin signaling is a novel pathway regulating intracellular calcium accumulation which might be involved in ventricular arrhythmias in diabetic cardiomyopathy. *Cell Signal*. (2014) 26:2591–600. doi: 10.1016/j.cellsig.2014.08.015
69. Rainbolt TK, Saunders JM, Wiseman RL. Stress-responsive regulation of mitochondria through the ER unfolded protein response. *Trends Endocrinol Metab*. (2014) 25:528–37. doi: 10.1016/j.tem.2014.06.007
70. Lu Z, Xu X, Fassett J, Kwak D, Liu X, Hu X, et al. Loss of the eukaryotic initiation factor 2alpha kinase general control nonderepressible 2 protects mice from pressure overload-induced congestive heart failure without affecting ventricular hypertrophy. *Hypertension*. (2014) 63:128–35. doi: 10.1161/HYPERTENSIONAHA.113.02313
71. Ron D. Translational control in the endoplasmic reticulum stress response. *J Clin Invest*. (2002) 110:1383–8. doi: 10.1172/JCI0216784
72. Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat Cell Biol*. (2013) 15:481–90. doi: 10.1038/ncb2738
73. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress response. *EMBO Rep*. (2016) 17:1374–95. doi: 10.15252/embr.201642195
74. Hori O, Ichinoda F, Tamatani T, Yamaguchi A, Sato N, Ozawa K, et al. Transmission of cell stress from endoplasmic reticulum to mitochondria: enhanced expression of Lon protease. *J Cell Biol*. (2002) 157:1151–60. doi: 10.1083/jcb.200108103
75. Munch C, Harper JW. Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation. *Nature*. (2016) 534:710–3. doi: 10.1038/nature18302
76. Izumi S, Saito A, Kanemoto S, Kawasaki N, Asada R, Iwamoto H, et al. The endoplasmic reticulum stress transducer BBF2H7 suppresses apoptosis by activating the ATF5-MCL1 pathway in growth plate cartilage. *J Biol Chem*. (2012) 287:36190–200. doi: 10.1074/jbc.M112.373746
77. Quiros PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, et al. Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. *J Cell Biol*. (2017) 216:2027–45. doi: 10.1083/jcb.201702058
78. Lebeau J, Saunders JM, Moraes VWR, Madhavan A, Madrazo N, Anthony MC, et al. The PERK arm of the unfolded protein response regulates mitochondrial morphology during acute endoplasmic reticulum stress. *Cell Rep*. (2018) 22:2827–36. doi: 10.1016/j.celrep.2018.02.055
79. Verfaillie T, Rubio N, Garg AD, Bultynck G, Rizzuto R, Decuypere JP, et al. PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ*. (2012) 19:1880–91. doi: 10.1038/cdd.2012.74

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 20 January 2020
doi: 10.3389/fcvm.2019.00191

Decrease of Cardiac Parkin Protein in Obese Mice

*Amandine Thomas[1], Stefanie Marek-Iannucci[1], Kyle C. Tucker[2], Allen M. Andres[1] and Roberta A. Gottlieb[1]**

[1] Cedars-Sinai Medical Center, Smidt Heart Institute, Los Angeles, CA, United States, [2] Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, United States

Mitophagy plays a major role in heart physiology. Impairment of Parkin-dependent mitophagy in heart is known to be deleterious. Obesity is a known cardiovascular risk factor. Impaired autophagy has been reported in models of obesity or hyperlipidemia/hypercholesterolemia; however less is known regarding obesity and mitophagy. The aim of this study was to evaluate the regulation of Parkin expression in hearts of mice fed a high fat diet. Interestingly, we found a significant decrease in Parkin protein in hearts of HFD mice compared those fed a low-fat diet. This was associated with mitochondrial dysfunction in the context of ischemia/reperfusion (I/R). This downregulation was not associated with a decrease in Parkin mRNA expression. We did not detect any change in the degradation rate of Parkin and only a slight decrease in its translation. The reduction of Parkin protein abundance in HFD hearts remains a mystery and will need further studies. However, Parkin depletion in the setting of obesity may contribute to cardiovascular risk.

Keywords: mitophagy, Parkin, obesity, ischemia/reperfusion, myocardium, mitochondria

OPEN ACCESS

Edited by:
Sebastiano Sciarretta, Sapienza University of Rome, Italy

Reviewed by:
Petra Kienesberger, Dalhousie University, Canada
Alessandra Ghigo, University of Turin, Italy

***Correspondence:**
Roberta A. Gottlieb
roberta.gottlieb@cshs.org

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *16 July 2019*
Accepted: *18 December 2019*
Published: *20 January 2020*

Citation:
Thomas A, Marek-Iannucci S, Tucker KC, Andres AM and Gottlieb RA (2020) Decrease of Cardiac Parkin Protein in Obese Mice. Front. Cardiovasc. Med. 6:191. doi: 10.3389/fcvm.2019.00191

INTRODUCTION

Mitochondrial clearance through mitophagy is a major element of mitochondrial homeostasis and plays an important role in maintaining cardiac well-being at baseline as well as during stress (1). Mitophagy occurs through different pathways involving Parkin, BNIP3, or FUNDC1. These appear to be complementary and differentially activated according to the stimulus (2, 3). Parkin-mediated mitophagy is generally triggered by mitochondrial inner membrane depolarization, which leads to PINK1 accumulation on the outer membrane and phosphorylation of targets that recruit Parkin. Parkin-dependent mitophagy has been well studied in the context of myocardial injury after ischemia/reperfusion (I/R) (4). Its role in the heart has been reevaluated in the light of the fact that Parkin deficiency at baseline did not induce cardiac dysfunction; however, Parkin is required for cardioprotection by ischemic preconditioning or statin administration (5, 6) and we previously reported that diet-induced obesity increases ischemic injury (7). Moreover, Parkin deficiency increases severity of ischemia/reperfusion (I/R) injury (8). Interestingly, Parkin plays an important role in the heart's transition from fetal to postnatal life involving a metabolic switch from carbohydrates to fatty acids and amino acids for fuel utilization; this highlights its potential significance in metabolic remodeling of mitochondria (4). Related to that, obesity is known to induce metabolic reprogramming of mitochondria as well as mitochondrial dysfunction (3). However, little is known about the regulation of cardiac mitophagy in the context of obesity. The aim of this study was to examine how Parkin-mediated mitophagy was regulated in a model of diet-induced obesity in mice.

METHODS

Animals and Experimental Design

Eight-week-old male C57Bl/6J mice were housed under standard conditions in conventional cages with *ad libitum* food and water. Ambient temperature was maintained at 20–22°C. The mice were fed a low-fat diet (LFD: 10% energy derived from fat; D12450b; Research Diets) or a high-fat diet (HFD: 60% energy derived from fat; D12492; Research Diets) for 12 weeks. For the inhibition of proteasome and autophagy, HFD mice were treated, respectively, with intraperitoneal injection of Bortezomib (1 mg/kg) and Chloroquine (50 mg/kg). Mice were sacrificed 6 h after injections.

Isolated Heart Perfusion

Hearts from anesthetized mice (i.p. pentobarbital 70 mg/kg) were rapidly excised and cannulated onto the Langendorff apparatus and perfused in a retrograde manner with Krebs-Henseleit bicarbonate buffer consisting of: (in g/L) NaCl 6.9, KCl 0.35, $MgSO_4$ 0.14, KH_2PO_4 0.16, $NaHCO_3$ 2.1, $CaCl_2$ 0.37, glucose 2.0, gassed with 95%O_2 /5%CO_2 (pH 7.4). The buffer reservoir height was adjusted to achieve a perfusion pressure of 60–80 mm Hg and perfusate temperature was maintained at 37°C. Hearts were allowed to stabilize for 15 min prior to induction of global no-flow ischemia via cessation of perfusion for 30 min. Temperature was maintained during ischemia by immersing the heart in perfusate maintained at 37°C. Hearts were then reperfused by restoring flow and maintained for 30 min. Pre-ischemic and reperfusion flow rates were measured. At the end of the experiment atria and ventricles were rapidly excised and immediately snap frozen in liquid nitrogen or further processed for mitochondrial isolation. For infarct size measurement, the hearts were cut into five transverse slices. Each slice was incubated for 20 min in 1% triphenyltetrazolium chloride solution at 37°C to differentiate infarcted from viable myocardial areas. Extension of the area of necrosis was quantified by planimetric analysis (ImageJ software).

Western Blot Analysis

Total cell lysates were obtained after lysing frozen heart samples (∼50 mg) in ice-cold RIPA buffer containing: (in mM) Tris-HCl 50, NaCl 150, EDTA 2, NaF 50, and detergents Na-deoxycholate 0.5%, SDS 0.1%, NP40 1%, and protease inhibitors cocktail (Complete, Roche). Mitochondrial fractions were obtained after homogenization of fresh heart samples (30–50 mg) in ice-cold mitochondrial isolation buffer (250 mM sucrose; 1 mM EDTA; 10 mM HEPES, pH 7.4) containing protease and phosphatase inhibitors (Complete, Roche). Nuclei and unbroken cells were eliminated by low-speed spin (1,000 g, 4°C, 10 min). Postnuclear supernatant was centrifuged (7,000 g, 4°C, 15 min) to obtain the final mitochondria-enriched pellet and supernatant (crude cytosol). The mitochondria-enriched fraction was resuspended in isolation buffer and centrifuged (7,000 g, 4°C, 5 min). The final pellet was resuspended in ice cold RIPA buffer with inhibitors. Both total cell lysate and mitochondrial fractions were probed with primary antibodies against Parkin (sc-32282, Santa Cruz Biotechnology), Ubiquitinated protein (ab-7780, Abcam), HSP60 (Cell signaling #12165) and CHOP (Cell signaling #5554). Bands were visualized by enhanced chemiluminescence and quantified using Image lab (Biorad). All protein expression levels have been normalized to ponceau staining.

Polysome Profiling

Polysome profiling has been done as previously described (9). Briefly, heart samples were homogenized in a buffer containing: (in mM) KCl 100, Tris 20, $MgCl_2$ 5, pH 7.5, with 0.4% NP-40, 100 μg/ml cycloheximide and 0.1 U/μl RNase inhibitor (Invitrogen). Homogenates were incubated 15 min on ice and centrifuged at 14,000 rpm for 15 min at 4°C. The supernatants were loaded onto 15–50% (w/v) sucrose gradients and centrifuged at 37,000 rpm in a Beckman SW41 Ti rotor for 2 h at 4°C. Gradient fractions were collected with a BioLogic LP System. Total RNA was isolated from fractions with Trizol following the manufacturer's suggested procedure.

RNA Purification and qRT-PCR

RNA was extracted from snap-frozen heart (∼25 mg) using Trizol RNA isolation reagent. Total RNA (0.5 μg) was reverse-transcribed and quantitative real-time PCR was then performed with SYBR Green Core Kit on a thermal cycler (Bio-Rad). mRNA expression was normalized to 18S or Rplp0 mRNA content and expressed as fold change compared to control mice using the ΔΔ CT method. Primer sequences are shown in **Table 1**.

Statistical Analysis

All data are expressed as mean ± SEM. Statistical analysis was performed using Graphpad Prism 6 software package for Windows with two-tailed unpaired Student's test (LFD vs. HFD) or two-way ANOVA with multiple comparisons followed by *post hoc* Fisher's LSD test (LFD vs. HFD on either basal or I/R conditions). Differences between groups were considered statistically significant when $p < 0.05$.

RESULTS

Mice fed with a high-fat diet (HFD) exhibit a significant decrease in Parkin protein level (**Figures 1A,B**). In order to validate the model of diet-induced obesity, metabolic phenotype parameters were evaluated. The HFD fed mice presented a higher body weight (**Figure 1C**) and increased fat mass (**Figure 1D**). Blood

TABLE 1 | Primer Sequences.

	Forward	Reverse
Parkin	CGTGTGTAGCTGGCTGTCCCAA	ACCTCCCATTTGCAGCACGCA
HSP60	CCCGCAGAAATGCTTCGACT	ACTTTGCAACAGTGACCCCA
mt-HSP70	TGCCTCCAATGGTGATGCTT	CAGCATCCTTAGTGGCCTGT
18S	GACTCAACACGGGAAACCTC	AGACAAATCGCTCCACCAAC
Rplp0	TCTGGAGGGTGTCCGCAACG	GCCAGGACGCGCTTGTACCC

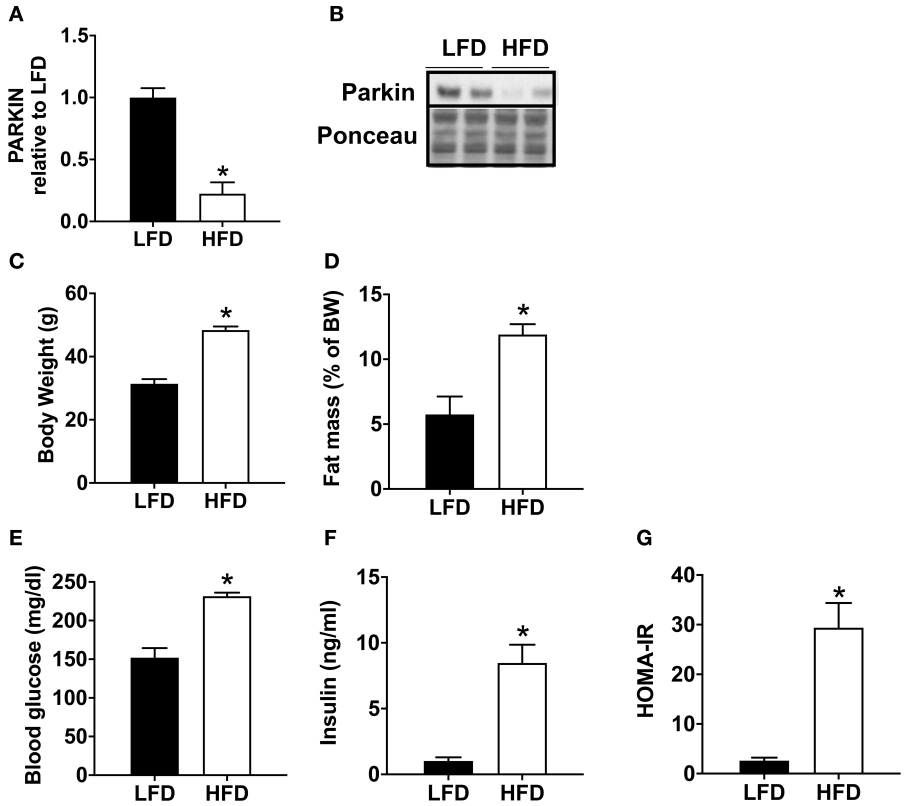


FIGURE 1 | Decrease of cardiac Parkin protein level in mice after 12 weeks of HFD. The protein expression of Parkin was quantified by densitometric analysis **(A)** after Western blot analysis **(B)** in LFD and HFD mice at baseline (no ischemia reperfusion, fed *ad libitum*). Body weight **(C)** was monitored after 12 weeks of LFD or HFD. The fat mass was calculated after measurement of adipose tissue mass after sacrifice **(D)**. After 1 weeks of HFD, plasma glucose **(E)** and insulin **(F)** levels were determined in mice fasted for 6 h and HOMA-IR was calculated **(G)**. Results (n = 5–8/group) are expressed in mean ± SEM; $^*p < 0.05$ vs. LFD.

glucose (**Figure 1E**) and insulin levels were higher (**Figure 1F**), leading to an increase in HOMA-IR (**Figure 1G**).

To determine if Parkin level changed acutely during cardiac ischemia and reperfusion, we isolated hearts from low fat diet (LFD) and HFD mice and subjected them to 30 min global ischemia and 3 h reperfusion via Langendorff perfusion. We found that the level of Parkin protein remained low in the hearts of HFD mice compared to LFD after I/R (**Figure 2A**). In our acute I/R model, we saw a modest trend toward increased infarct size (**Figure 2B**) and a significant decrease of coronary reflow in hearts of HFD mice (**Figure 2C**). Pre-ischemic coronary flows were not different between LFD and HFD mice (data not shown). Under basal conditions, the level of mitochondria-associated Parkin is low in hearts of both LFD and HFD mice; however, after I/R, Parkin translocated to mitochondria only in the LFD mice (**Figure 2D**). Consistent with this, the quantity of ubiquitinated protein in the mitochondrial fraction increased after I/R only in the LFD group (**Figure 2E**). Interestingly, mitochondrial protein ubiquitin was already high in the basal state in HFD mice. This likely reflects reduced clearance of Ub-tagged mitochondrial proteins via mitophagy or proteasomal degradation. As mitochondrial dysfunction can trigger the mitochondrial unfolded protein response (10), we measured mRNA and protein level for HSP60 and CHOP. mRNA levels of both HSP60 (**Figure 2F**) and CHOP (**Figure 2G**) are increased in the HFD group under basal conditions with a more pronounced change for CHOP mRNA. I/R tends to upregulate both targets, but no significant differences between LFD and HFD are observed. The densitometry analysis showed that the increase of HSP60 is maintained at the protein level (**Figure 2H**) with a slight but statistically significant upregulation of the protein upon HFD and I/R. The increase of CHOP mRNA level is not reflected by an increase of its protein level (**Figure 2I**) under basal conditions. Like CHOP mRNA, CHOP protein level is upregulated by I/R but no significant differences appear between LFD and HFD groups.

In order to understand the basis for reduced Parkin protein in HFD mice, we assessed the mRNA expression of Parkin and found no difference between the groups (**Figure 3A**). The observed lack of change in mRNA expression in our model suggested increased Parkin degradation. To determine if the decrease in Parkin was related to increased protein degradation, we treated mice for 6 h with either bortezomib or chloroquine *in vivo* to block, respectively, proteasome activity or autophagic flux. Neither treatment restored Parkin protein levels in HFD mice hearts (**Figure 3B**). We then analyzed if there was a change in translational activity for Parkin, using polysome profiling (9). When we consider the mRNA distribution, we observed that

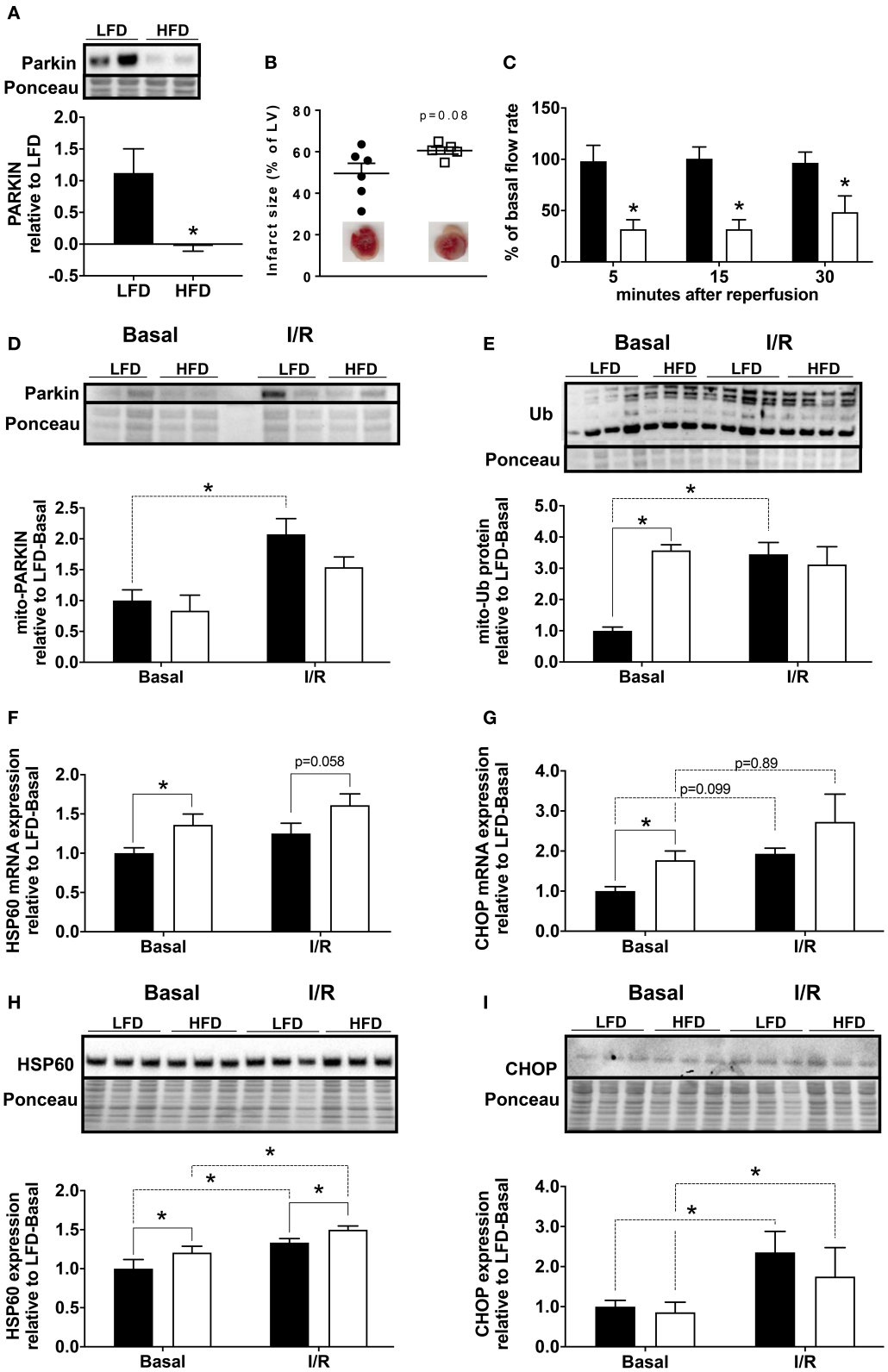


FIGURE 2 | Loss of Parkin, cardiac and mitochondrial homeostasis alteration in HFD mice subjected to ischemia/reperfusion (I/R). Protein expression of Parkin was quantified by densitometric analysis of Western blots of heart lysates **(A)** from LFD and HFD mice after I/R. Infarct size was determined by colorimetry and quantified

(Continued)

FIGURE 2 | by planimetry **(B)**, examples of heart slices are shown on the graph. Flow rate recovery was measured at indicated time point after reperfusion **(C)**. Parkin **(D)** and ubiquitinated proteins **(E)** were detected by Western blot in mitochondrial extracts from hearts of LFD and HFD mice after I/R. Cardiac expression of genes involved in mitochondrial stress: HSP60 **(F)** and CHOP **(G)** were measured by RT-qPCR. The HSP60 **(H)** and CHOP **(I)** protein expression levels were quantified by densitometric analysis of Western blots. Results (n = 4–6/group) are expressed in mean ± SEM; *$p < 0.05$.

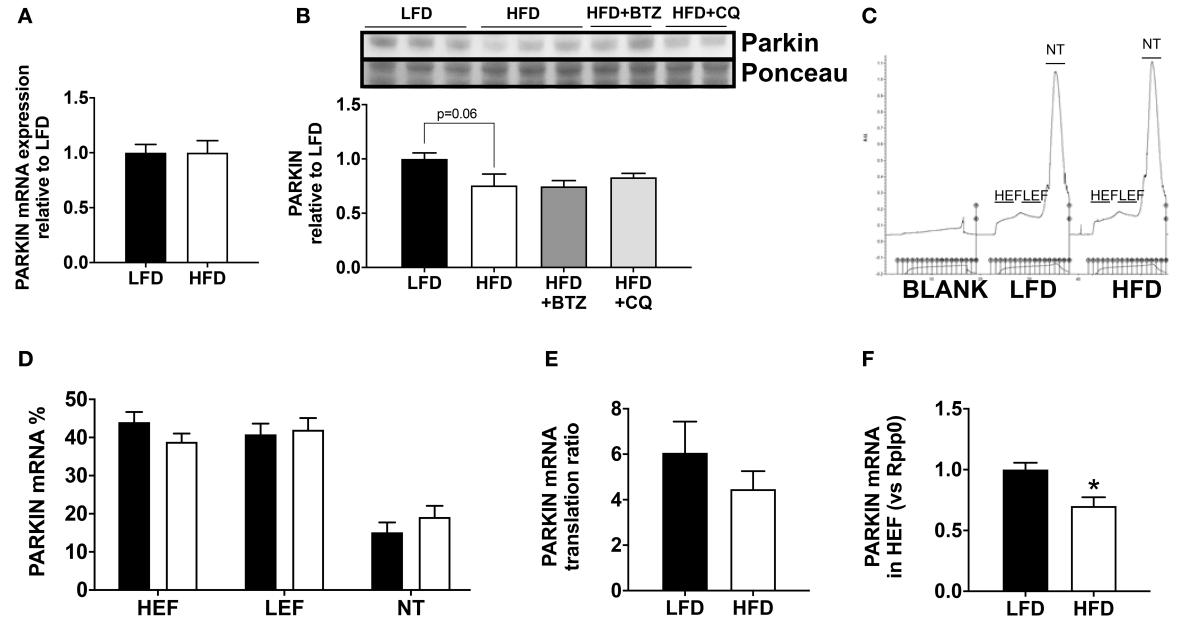


FIGURE 3 | Regulation of Parkin protein abundance. The cardiac mRNA expression of Parkin **(A)**. The protein expression of Parkin was analyzed Western blot analysis **(B)** in LFD and HFD mice and HFD mice treated with Bortezomib (1 mg/kg) or Chloroquine (50 mg/kg). UV densitometry tracing of RNA in the sucrose gradient for polysome profiling **(C)**. Polysome profiling to detect distribution of Parkin mRNA in high-efficiency (HEF) and low-efficiency (LEF) polysomes and the non-translating (NT) fraction **(D)**. Quantitation of Parkin mRNA translation ratio [(HEF + LEF)/NT] **(E)**. Quantitation of Parkin mRNA level in the HEF **(F)**. Results (n = 5–6/group) are expressed in mean ± SEM.

Parkin mRNA is less abundant in the translating fraction and more present in the non-translating fraction (**Figures 3C–E**). This is confirmed by the significant decrease of Parkin mRNA in the high efficiency translating fraction (HEF) (**Figure 3F**).

DISCUSSION

Few studies have examined the regulation of Parkin protein in the setting of obesity. Parkin is upregulated in vascular walls (11) or adipose tissue (12) but decreased in the brain substantia nigra (SN) (13) of obese or diabetic mice. In liver, studies show both an increase (14) or a decrease (15) in Parkin level upon obesity. Contrary to our results, Tong et al. observed an increase in cardiac parkin protein during HFD consumption, although their paper did not indicate how many weeks of HFD were performed prior the analysis of Parkin (16). In their study, Parkin KO mice developed more severe cardiac hypertrophy and cardiac diastolic dysfunction in response to HFD feeding, suggesting that upregulation of Parkin-dependent mitophagy is a homeostatic response to HFD. These data suggest that obesity affects expression of Parkin protein and mitophagy capacity. Interestingly, these changes appear to be tissue specific and affected by the duration of the HFD. Further studies are needed to understand the effect of Parkin expression variations. In our case, we demonstrated a significant decrease of Parkin level in hearts of obese mice fed HFD for 12 weeks. The loss of Parkin is known to be deleterious for heart physiology (8, 17). Under basal conditions, Parkin deficient mice did not present a major phenotype. However, ischemic preconditioning cannot protect Parkin-deficient mice from ischemia/reperfusion injury (6). Also, these mice develop more severe cardiac remodeling after permanent ligation of left ventricular artery (8). Overall, the lack of Parkin protein in hearts of obese mice is associated with myocardial injury after I/R as reflected by the trend toward increased infarct size and the no-reflow phenomenon in the HFD group. The decrease of total Parkin level may be responsible for the impairment of its translocation to the mitochondria in the context of an ischemic stress, as we observed less Parkin translocated to the mitochondria upon I/R. Moreover, the latter seems to be associated with an increase in mitochondrial stress marker in a basal state. This is in agreement with the idea that Parkin plays a major role in mitochondrial stress, with or without apparent cardiac dysfunction (4). We cannot exclude compensation by other mitophagy pathways that may mitigate injury linked to the reduction of Parkin in the hearts of HFD mice. This result is consistent with the results of Khang et al. (13), who described a decrease in Parkin protein level in the substantia nigra of HFD or db/db mice without any change

in mRNA expression of Parkin. Interestingly, they showed that insulin treatment in SH-SY5Y cell line induced a decrease of Parkin, suggesting a role for insulin signaling in the regulation of Parkin protein expression. We hypothesize that this modest decrease in translational efficiency of Parkin mRNA can result in a gradual decrease in Parkin protein, as well as a limited ability to rapidly upregulate Parkin translation in response to stress in HFD mice. However, further studies are needed to understand how Parkin is regulated in the context of obesity.

CONCLUSION

In conclusion, this paper showed a substantial reduction of Parkin protein level in the hearts of HFD mice, although we were unable to discern the mechanism. Moreover, while Parkin is known to initiate mitophagy (and perhaps other unrecognized targets) via ubiquitination, little is known regarding regulation of Parkin abundance itself.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

Animal experiments were performed in accordance with the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center (IACUC5000).

AUTHOR CONTRIBUTIONS

AT performed experiments, analyzed data, and wrote the manuscript. SM-I performed experiments, analyzed data, and critically reviewed the manuscript. KT performed experiments and contributed to discussion. AA performed experiments and contributed to discussion. RG supervised the project and edited the manuscript.

FUNDING

This study was funded by a project program grant (P01-HL112730, 07/01/13-06/30/18) awarded by the National Institutes of Health entitled Mitochondrial Quality in Cardioprotection: Overcoming Co-Morbidities. The principal investigator of this project is RG.

REFERENCES

1. Moyzis AG, Sadoshima J, Gustafsson AB. Mending a broken heart: the role of mitophagy in cardioprotection. *Am J Physiol Heart Circ Physiol.* (2015) 308:H183–92. doi: 10.1152/ajpheart.00708.2014
2. Eiyama A, Okamoto K. PINK1/Parkin-mediated mitophagy in mammalian cells. *Curr Opin Cell Biol.* (2015) 33:95–101. doi: 10.1016/j.ceb.2015.01.002
3. Gottlieb RA, Thomas A. Mitophagy and mitochondrial quality control mechanisms in the heart. *Curr Pathobiol Rep.* (2017) 5:161–9. doi: 10.1007/s40139-017-0133-y
4. Dorn GW II. Central Parkin: the evolving role of Parkin in the heart. *Biochim Biophys Acta.* (2016) 1857:1307–12. doi: 10.1016/j.bbabio.2016.03.014
5. Andres AM, Hernandez G, Lee P, Huang C, Ratliff EP, Sin J, et al. Mitophagy is required for acute cardioprotection by simvastatin. *Antioxid Redox Signal.* (2014) 21:1960–73. doi: 10.1089/ars.2013.5416
6. Huang C, Andres AM, Ratliff EP, Hernandez G, Lee P, Gottlieb RA. Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1. *PLoS ONE.* (2011) 6:e20975. doi: 10.1371/journal.pone.0020975
7. Andres AM, Kooren JA, Parker SJ, Tucker KC, Ravindran N, Ito BR, et al. Discordant signaling and autophagy response to fasting in hearts of obese mice: implications for ischemia tolerance. *Am J Physiol Heart Circ Physiol.* (2016) 311:H219–28. doi: 10.1152/ajpheart.00041.2016
8. Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, et al. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem.* (2013) 288:915–26. doi: 10.1074/jbc.M112.411363
9. Stastna M, Thomas A, Germano J, Pourpirali S, Van Eyk JE, and Gottlieb RA. Dynamic proteomic and miRNA analysis of polysomes from isolated mouse heart after langendorff perfusion. *J. Vis. Exp.* (2018) e58079. doi: 10.3791/58079
10. Pellegrino MW, Nargund AM, Haynes CM. Signaling the mitochondrial unfolded protein response. *Biochim Biophys Acta.* (2013) 1833:410–6. doi: 10.1016/j.bbamcr.2012.02.019
11. Wu W, Xu H, Wang Z, Mao Y, Yuan L, Luo W, et al. PINK1-Parkin-mediated mitophagy protects mitochondrial integrity and prevents metabolic stress-induced endothelial injury. *PLoS ONE.* (2015) 10:e0132499. doi: 10.1371/journal.pone.0132499
12. Cui C, Chen S, Qiao J, Qing L, Wang L, He T, et al. PINK1-Parkin alleviates metabolic stress induced by obesity in adipose tissue and in 3T3-L1 preadipocytes. *Biochem Biophys Res Commun.* (2018) 498:445–52. doi: 10.1016/j.bbrc.2018.02.199
13. Khang R, Park C, Shin JH. Dysregulation of parkin in the substantia nigra of db/db and high-fat diet mice. *Neuroscience.* (2015) 294:182–92. doi: 10.1016/j.neuroscience.2015.03.017
14. Kim KY, Stevens MV, Akter MH, Rusk SE, Huang RJ, Cohen A, et al. Parkin is a lipid-responsive regulator of fat uptake in mice and mutant human cells. *J Clin Invest.* (2011) 121:3701–12. doi: 10.1172/JCI44736
15. Shao N, Yu XY, Ma XF, Lin WJ, Hao M, Kuang HY. Exenatide delays the progression of nonalcoholic fatty liver disease in C57BL/6 mice, which may involve inhibition of the NLRP3 inflammasome through the mitophagy pathway. *Gastroenterol Res Pract.* (2018) 2018:1864307. doi: 10.1155/2018/1864307
16. Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, et al. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res.* (2019) 124:1360–71. doi: 10.1161/CIRCRESAHA.118.314607
17. Li Y, Ma Y, Song L, Yu L, Zhang L, Zhang Y, et al. SIRT3 deficiency exacerbates p53/Parkinmediated mitophagy inhibition and promotes mitochondrial dysfunction: implication for aged hearts. *Int J Mol Med.* (2018) 41:3517–26. doi: 10.3892/ijmm.2018.3555

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 30 January 2020
doi: 10.3389/fcvm.2020.00002

Multiple Levels of PGC-1α Dysregulation in Heart Failure

Shin-ichi Oka[1], *Amira D. Sabry*[2], *Keiko M. Cawley*[2] *and Junco S. Warren*[2,3,4]*

[1] Department of Cell Biology and Molecular Medicine, Rutgers New Jersey Medical School, Newark, NJ, United States, [2] Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, United States, [3] Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT, United States, [4] Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan

Metabolic adaption is crucial for the heart to sustain its contractile activity under various physiological and pathological conditions. At the molecular level, the changes in energy demand impinge on the expression of genes encoding for metabolic enzymes. Among the major components of an intricate transcriptional circuitry, peroxisome proliferator-activated receptor γ coactivator 1 alpha (PGC-1α) plays a critical role as a metabolic sensor, which is responsible for the fine-tuning of transcriptional responses to a plethora of stimuli. Cumulative evidence suggests that energetic impairment in heart failure is largely attributed to the dysregulation of PGC-1α. In this review, we summarize recent studies revealing how PGC-1α is regulated by a multitude of mechanisms, operating at different regulatory levels, which include epigenetic regulation, the expression of variants, post-transcriptional inhibition, and post-translational modifications. We further discuss how the PGC-1α regulatory cascade can be impaired in the failing heart.

Keywords: PGC-1α, heart failure, epigenetics, histone methylation, transcriptional control, cardiac metabolism, mitochondria

OPEN ACCESS

Edited by:
Richard N. Kitsis, Albert Einstein College of Medicine, United States

Reviewed by:
Susumu Minamisawa, Jikei University School of Medicine, Japan
Konstantinos Drosatos, Lewis Katz School of Medicine, Temple University, United States

***Correspondence:**
Junco S. Warren
junco.warren@utah.edu

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *23 October 2019*
Accepted: *08 January 2020*
Published: *30 January 2020*

Citation:
Oka S, Sabry AD, Cawley KM and Warren JS (2020) Multiple Levels of PGC-1α Dysregulation in Heart Failure. Front. Cardiovasc. Med. 7:2. doi: 10.3389/fcvm.2020.00002

INTRODUCTION

Peroxisome proliferator-activated receptor γ coactivator 1 alpha (PGC-1α) belongs to a small family of transcriptional coactivators, including PGC-1β and PGC-1-related coactivator (PRC), which possess a common function in mitochondrial physiology. PGC-1α was first identified as a cofactor for the nuclear hormone receptor peroxisome proliferator-activated receptor gamma (PPARγ) in adipocytes required for the adaptive thermogenic responses to lower temperature (1). PGC-1α is expressed in several tissue types and highly expressed in metabolically active tissues, which includes brown fat and skeletal and cardiac muscle. In the heart, PGC-1α is an essential molecule in mitochondrial biogenesis and muscle maturation and shares its role with PGC-1β (2). Cardiac-specific ablation of both PGC-1α and PGC-1β is embryonically lethal due to cardiomyopathy (2).

In the past two decades, our understanding of the mechanisms by which PGC-1α regulates cardiac energetics has significantly advanced. PGC-1α binds to several transcription factors, including PPARγ, PPARα, estrogen-related receptor alpha (ERRα), and nuclear respiratory factor 1 (NRF1) [reviewed in (3)]. This explains how PGC-1α signaling can be amplified to a number of metabolic pathways. Therefore, PGC-1α target genes are primarily determined by the transcription factors that PGC-1α interacts with. Gene expression analysis of PGC-1α knockout mice and transgenic mice that overexpress PGC-1α has revealed PGC-1α target pathways, which include mitochondrial biogenesis, oxidative phosphorylation (OXPHOS), fatty acid β-oxidation (FAO),

and glycolysis (4–9). Recent studies showed that PGC-1α also enhances autophagy (10–12), apoptosis (13–15), and aging (11), and activates genes that encode enzymes involved in reactive oxygen species (ROS) detoxification in the brain (9, 16).

PGC-1α is a metabolic sensor that enables the body to respond to a plethora of stimuli, including exercise, fasting, and changes in metabolic substrate availability (17). Thus, PGC-1α expression and function are key determinants of energetic states in the heart. Numerous studies have shown that PGC-1α target genes are downregulated in the failing heart (18–20). Some reports have suggested that downregulation of PGC-1α is a major cause of mitochondrial impairment and metabolic defects in the failing heart (7, 8, 21, 22). However, other studies, including ours, suggest that the expression levels of PGC-1α *per se* cannot always explain downregulation of PGC-1α target genes in the failing heart (23–25). In this review, we carefully analyze recent findings in an attempt to construct a holistic picture of the complex mechanisms contributing to impaired PGC-1α regulatory function in the failing heart. We show that these mechanisms operate on multiple levels, including epigenetic and post-transcriptional regulation of PGC-1α expression, as well as altered PGC-1α function occurring under pathophysiological stress (**Figure 1**). We hope that our analysis helps to identify knowledge gaps in the complex pattern of PGC-1α regulatory network in the heart, and to provide guidance for future studies in this exciting field.

PGC-1α EXPRESSION IN HEART FAILURE

Pathological cardiac hypertrophy is a common response to hypertension, aortic stenosis, and myocardial infarction (37). Transverse aortic constriction (TAC) is a primary animal model for cardiac hypertrophy and heart failure (38, 39). A ligature or a clip is placed across the ascending or descending aorta, leading to the increase of intracardiac pressure ("pressure overload"). TAC initially leads to compensated hypertrophy of the heart, manifested by maintained ejection fraction and temporary enhancement of cardiac contractility (40), in association with metabolic substrate switch from fatty acid to glucose metabolism (41) and a slight increase of glucose oxidation capacity (40). However, over prolonged periods of a chronic hemodynamic overload, an apparently inevitable transition to a decompensated phase takes place, manifested by reduced ejection fraction and cardiac dilation (19, 23, 28, 42). Despite variability of cardiac phenotype in the TAC model (43), most studies using this model have reported energetic abnormalities (19, 20, 23, 44), culminating in a ∼30% reduction of myocardial ATP (45), a vitally important physiological constant normally kept within very narrow limits (46). Metabolic remodeling in the setting of pathological cardiac hypertrophy and failure includes decreased myocardial capacity for FAO, reduced ATP production rate, and increased reliance on glucose, concurrent with downregulation of genes that are involved in FAO and mitochondrial oxidative phosphorylation (OXPHOS) (41, 47–50). PGC-1α plays a central role in transcriptional control of those metabolic genes in the heart. PGC-1α knockout mice exhibit deficiencies in cardiac energy reserve and function (7, 21) and the accelerated development of heart failure, in association with downregulation of OXPHOS genes (8). In cultured primary rat neonatal cardiomyocytes, PGC-1α expression was reduced by α1-adrenergic receptor agonist phenylephrine, which recapitulates myocardial remodeling under pressure overload (8). Thus, the decreased expression of PGC-1α has been postulated as an important molecular mechanism for energy starvation and metabolic defects in the failing myocardium. However, the dynamics of PGC-1α expression in the failing heart may be more complex. In animal models of failure, most of the studies showed downregulation of PGC-1α (8, 51–57), but some studies found no change (24, 25, 58). Likewise, analysis of tissue samples obtained from patients at the advanced stage of heart failure showed a variability of outcomes, including decreased gene (59) or protein (60) expression, unchanged gene expression (61, 62), or even a slightly increased gene expression of PGC-1α (63). Of note, in the latter study PGC-1α target genes were coordinately downregulated, underscoring the fact that PGC-1α signaling may be compromised at multiple levels.

The divergent outcomes of these different studies regarding PGC-1α expression in heart failure might be, in part, due to assessment of PGC-1α expression at *different time points* of the disease progression (i.e., early vs. advanced stages, compensated vs. decompensated phases), when PGC-1α expression fluctuates with respect to time, reflecting a combination of adaptive and maladaptive responses to the increased workload. Note that human studies obtain information predominantly from hearts at advanced or terminal stages of heart failure. These stages of the disease are rarely reached in animal studies. Moreover, in human patients with heart failure, PGC-1α expression dynamics may additionally be confounded by different therapeutic interventions (60, 61). Patients with heart failure were treated with various inotropic agents such as β-blockers, diuretics, and angiotensin-converting enzyme (ACE) inhibitors (60). Additionally, human heart failure is pathophysiologically heterogeneous. Depending on the underlying cause, several distinct pathophysiologic conditions, such as ischemia, volume and pressure overload, and metabolic disorders (i.e., diabetes) may contribute to various results of PGC-1α expression. A recent study demonstrates that ischemia triggers distinct epigenetic modifications in heart failure patients (64). Diabetes and obesity are another layer of complexity. In diabetic and prediabetic humans, there is a consistent decrease in the expression of OXPHOS genes that are regulated by PGC-1α and PGC-1β in muscle (65–68). However, cardiac PGC-1α is upregulated in mice that are fed a high-fat diet and in genetically obese (*ob/ob*) mice (69). Thus, it remains unclear how PGC-1α expression is altered in heart failure patients with diabetes and insulin resistance. Differences in age when comparing samples from patients with heart failure and control subjects may also confound results because PGC-1α levels decrease with aging (70). Nevertheless, it is clear that numerous mitochondrial genes and other known targets of PGC-1α, such as glycolytic and FAO genes, are repressed in human heart failure (61, 63), suggesting that dysregulation of PGC-1α may play a role in the pathogenesis of this disease.

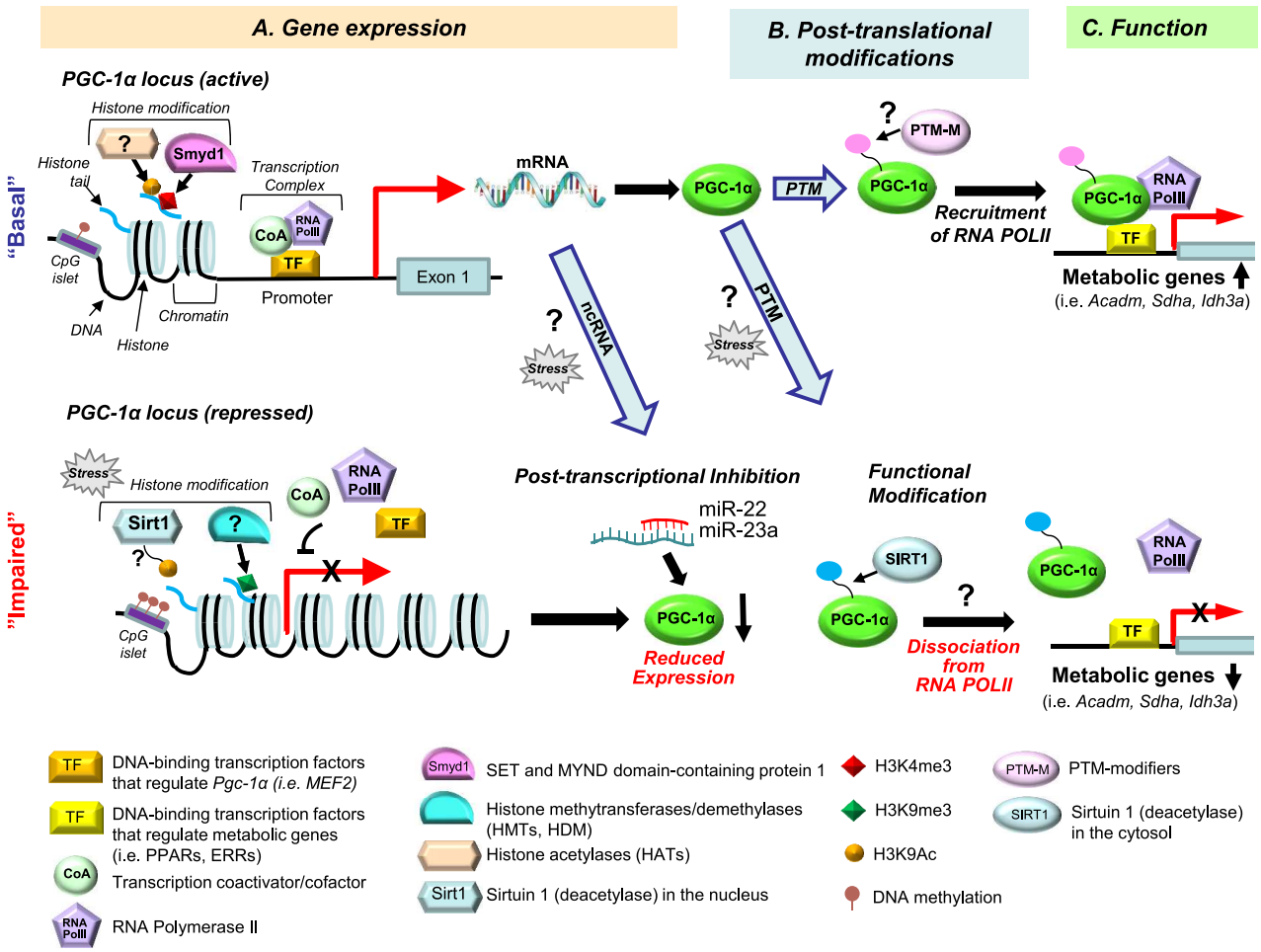


FIGURE 1 | Multiple levels of PGC-1α signaling dysregulation in heart failure. Dysregulation of the PGC-1α regulatory cascade can occur at the level of gene expression **(A)**, post-translational modifications (PTM) **(B)**, and PGC-1α function **(C)**. **(A)** At the gene expression level, the *PGC-1α* cascade can be modulated via histone modifications ((de)methylation and (de)acetylation), DNA (de)methylation, by various transcription factors [TFs, reviewed in (17)], and via post-transcriptional inhibition of the *PGC-1α* gene by non-coding (nc) RNAs. The histone methyltransferase Smyd1 increases promoter activity of *PGC-1α* through regulating the enrichment of the H3K4me3 levels (a gene activation mark) (26). In different animal models of heart failure, reduced expression of PGC-1α was associated with increased H3K9me3 level (a gene repression mark) (27) or a decreased level of H3K9Ac (a gene activation mark) (28). Sirtuin 1 (Sirt1) is a plausible histone deacetylase (HDAC) for gene repression of PGC-1α under pressure overload (28), but this remains to be established. DNA hypermethylation is known to suppress *PGC-1α* in the skeletal muscle (29–31), but little is known about its role in *PGC-1α* regulation in the heart (32). The protein expression level of PGC-1α can be reduced through post-transcriptional inhibition by miRNAs, such as miR-22 (33) and miR-23a (34), but it is unknown whether small ncRNA-mediated PGC-1α repression occurs in heart failure. **(B)** PGC-1α activity is known to be regulated by posttranslational modifications (PTMs), including phosphorylation and acetylation [reviewed in (17)]. However, which PTMs of PGC-1α are specific for the development of heart failure remains unknown. Sirt1 deacetylases the PGC-1α protein (35), but it is not known whether this PTM is a part of PGC-1α dysregulation in the failing heart. **(C)** PGC-1α's transcriptional control of metabolic genes (i.e., *Acadm, Sdha, Idh3a*) largely depends on interaction with DNA-binding transcriptional factors (TF) [i.e., ERRs, PPARs, reviewed in (36)]. In addition, our recent study showed that PGC-1α recruits RNA Polymerase II (RNA PolII) to the promoter regions of PGC-1α target genes (25). Moreover, PGC-1α is dissociated from the promoters of its target genes and RNA PolII in the failing mouse heart (25). We propose that this alteration of PGC-1α behavior in the failing heart is secondary to a PTM of the PGC-1α protein, and intend to test this hypothesis in our future studies.

Gain and loss of function studies in mice have confirmed the pivotal role of PGC-1α in cardiac energetics (**Tables 1**, **2**). Several gain-of-function models showed increased mitochondrial biogenesis, however, the sarcomeric structure of the heart was disrupted due to uncontrolled mitochondrial proliferation (4, 6). More importantly, those transgenic mice developed dilated cardiomyopathy. In contrast, gain-of-function models with a modest PGC-1α overexpression do not induce heart failure (23, 24) (∼3 and ∼2 fold at the mRNA level, respectively), suggesting that the development of heart failure in the transgenic mice was due to excessive PGC-1α expression. More importantly, maintaining PGC-1α expression during pressure overload did not show any protective effects on contractile function in this setting (23, 24).

TABLE 1 | Cardiac and energetic phenotypes of PGC-1α overexpression mouse models.

Systemic/Tissue-specific	Constitutive/ Inducible	Cardiac phenotype	References
Cardiac-specific	Constitutive	Uncontrolled mitochondrial proliferation, loss of sarcomeric structure, dilated CM	(4)
Cardiac-specific	Inducible	↑Mitochondrial number and size and upregulation of genes involved in mitochondrial biogenesis during neonatal stages	(6)
		↑Mitochondrial proliferation, derangements of mitochondrial ultrastructure, reversible CM, and ↑venticular mass and chamber dilation in adult mice	
Systemic	Constitutive	↑FAO and cardiac output at baseline and restored expression levels of FAO genes and OXPHOS genes at baseline, no protective effect on TAC-induced cardiac dysfunction, ↑VEGF, and ERRα during pressure overload	(24)
Cardiac and skeletal muscle-specific	Constitutive	No change in cardiac function and energetics with slight decrease in mitochondrial number at baseline, no protective effect on TAC-induced cardiac dysfunction	(23)

↑: *increase*, ↓: *decrease*.

In loss-of-function models, two independent lines of global PGC-1α knockout mice were generated. Spiegelman and colleagues showed normal cardiac phenotype and mitochondrial contents under basal conditions (8). However, gene expression analyses revealed upregulation of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and β-MHC, indicative of the presence of cardiac dysfunction (7). The PGC-1α$^{-/-}$ mice generated by the Kelly group exhibited cardiac systolic dysfunction under basal conditions, and cardiac inotropic and chronotropic responses to exercise were both blunted (21). Interestingly, no cardiac dysfunction was observed in those mice when characterized by the other investigators (53). Despite the phenotypic variation in these two lines of global PGC-1α knockout mice, hemodynamic challenge in the form of transverse aortic banding consistently led to pronounced cardiac failure in PGC-1α null mice (8, 53). To further investigate the role of cardiac PGC-1α, three independent groups, including us, have generated cardiac-specific PGC-1α knockout line with identical PGC-1α flox and αMHC-Cre lines (25, 71, 72). The Tavi group and we observed the similar degree of cardiac dysfunction in cardiac-specific PGC-1α knockout mice under basal conditions (25, 72). In contrast, Patten et al. reported normal cardiac function in cardiac-specific PGC-1α knockout mice, but the hearts of female mice exhibited dilated cardiomyopathy after their second delivery (71). Of note, the peripartum cardiomyopathy has not been reported in systemic PGC-1α knockout mice. Taken together, cardiac-specific, rather than systemic PGC-1α knockout mice, prone to develop heart failure. The mechanisms by which the cardiac phenotypes are more pronounced in cardiac-specific PGC-1α knockout mice than systemic knockout mice are currently unknown. Since loss of PGC-1α leads to metabolic derangements in various tissues (**Table 2**), the complex compensatory mechanisms might take place and mask the effect of PGC-1α deletion on cardiac function.

Overall, the sum of available knowledge strongly suggests that dysregulation of PGC-1α expression is an important factor in cardiac dysfunction and energetic defects in the heart. We will now review recent advances in our understanding of the epigenetic regulation of the *PGC-1α* gene and PGC-1α function.

TRANSCRIPTIONAL REGULATION OF PGC-1α GENE IN THE HEART

Several transcriptional regulators associated with cardiac pathophysiology are involved in transcriptional control of PGC-1α, which include cAMP response element-binding protein (CREB), nuclear factor of activated T-cells (NFAT), myocyte enhancer factor 2 (MEF2), Yin Yang 1 (YY1), PPARs, and Sirt1. Several lines of evidence suggest that some transcription factors that positively regulates PGC-1α transcription are activated or upregulated in the failing heart, such as CREB, NFAT, MEF2, and YY1 (73–75) (**Figure 2**). The isoforms of PPARs differentially regulate PGC-1α in the healthy and diseased hearts. The mouse proximal PGC-1α promoter contains a typical PPAR response element (PPRE), which is conserved in rat and human (76). PPARδ, but not PPARα, stimulates PGC-1α transcription, although both PPARδ and PPARα bind to the PPRE. PPARγ also stimulates PGC-1α transcription (77). Interestingly, PPARγ-induced PGC-1α transcription is inhibited by PPARα possibly through competition of the binding to PPRE (77). Cardiac-specific PPARα overexpression inhibits PGC-1α transcription (78). Thus, PPARδ and PPARγ positively regulate PGC-1α transcription, whereas PPARα negative regulates the transcription. In the failing heart, PPARα, a negatively regulator for PGC-1α transcription, is inactivated (79). On the other hand, PPARδ and PPARγ, which positively regulate PGC-1α transcription, may also be inactivated, since PPAR target genes involved in fatty acid metabolism are mostly downregulated in the failing heart. Taken together, simultaneous stimulation of the pathways that downregulates and upregulates PGC-1α transcription may be a mechanism responsible for the diverging outcome of PGC-1α expression in the failing heart (**Figure 2**).

Sirt1 deacetylates lysine residues in both histones and non-histone proteins and regulates the function and transcription

TABLE 2 | Phenotypes of the heart and other organs in PGC-1α knockout mouse models.

Systemic/Tissue-specific	Constitutive/Inducible	Cardiac phenotype	Effects on other organs	References
Systemic	Constitutive		Constitutively active gluconeogenesis with reduced mitochondrial function in the liver, lean, and resistant to diet-induced obesity due to hyperactivity, lesions in the striatal region of the brain that controls movement	(5)
Systemic	Constitutive	↓Fractional shortening, ↓cardiac performance response to exercise and dobutamine	↓Mitochondrial number and respirator capacity in slow-twitch skeletal muscle with reduced exercise capacity, loss of thermogenic response, ↓oxidative capacity in hepatocytes with hepatic steatosis after short-term starvation, vacuolar lesions in the central nervous system	(21)
Systemic	Constitutive	Normal mitochondrial volume and cardiac function in adult mice (3 months), downregulation of OXPHOS genes, reduced mitochondrial enzymatic activities with energy deficiency (↓ATP, ↓PCr), cardiac dysfunction in old mice (7–8 months)		(7)
Systemic	Constitutive	Normal cardiac function at baseline, accelerated cardiac dysfunction, and chamber dilation under pressure overload		(8)
Systemic	Constitutive		↑Sensitivity to oxidative stress and neurodegeneration in the brain	(9)
Cardiac-specific	Constitutive	Normal cardiac function at baseline, peripartum cardiomyopathy		(71)
Cardiac-specific	Constitutive	Dilated CM, ↓glucose, and fatty acid oxidation, blunted anaerobic metabolism at baseline		(72)
Cardiac-specific	Constitutive	Cardiac hypertrophy and failure, ↓OXPHOS genes, accelerated cardiac dysfunction, accelerated cardiac dysfunction during TAC		(25)

↑: increase, ↓: decrease.

of PGC-1α (80). In general, deacetylation of the PGC-1α protein leads to the transactivation of PGC-1α and is known to coactivate PPARα to enhance the gene expression of mitochondrial fatty acid oxidation genes (81). However, Sirt1 can either activate or inhibit PGC-1α through deacetylation in a context dependent manner (35, 82). What determines the PGC-1α function via Sirt1-mediated deacetylation remains unclear. In the heart of systemic Sirt1 knockout mice, PGC-1α is downregulated, indicating that Sirt1 positively regulates PGC-1α (83). However, PGC-1α is also downregulated in cardiac-specific Sirt1 overexpression mouse lines, suggesting that gain of Sirt1 function rather inhibits PGC-1α (84, 85). Whether Sirt1 activates or inhibits PGC-1α in the context of heart failure remains unknown. In the nucleus, Sirt1 acts as an epigenetic modifier and deacetylases histone H3K9/H3K14, leading to chromatin silencing, which occurs at the promoters of myogenin and myosin heavy chain (MHC) in development (86). In our previous study, we demonstrated that Sirt1 deacetylates histone H3K9 in the PGC-1α promoter in the failing heart (28) (**Figure 1**), which presumably leads to inactivation of the gene. Thus, upregulation of Sirt1 in the failing heart (28) might contribute to the reduced abundancy of PGC-1α.

The transducer of regulated CREB (cAMP response element-binding protein) binding protein (TORC)1, a coactivator of CREB, is another transcription factor that induces PGC-1α, which was identified through screening of 10,000 putative human full-length cDNA in Hela cells for the induction of PGC-1α promoter (87). The other two members of the TORC family, TORC2 and TORC3, also strongly activate PGC-1α transcription. TORC1, 2, and 3 increase the expression of PGC-1α and PGC-1α target genes (Cyt c; CoxII; IDH3α) in mouse primary myotubes (88). In the heart, CREB is activated in response to both physiological and pathological hypertrophic stimuli, which is correlated with upregulation of PGC-1α and increased mitochondrial respiratory rate (89). However, whether TORCs induce PGC-1α and its target genes in the heart needs to be elucidated.

An autoregulatory loop controls PGC-1α expression. The positive feedback loop exists between PGC-1α and MEF2 family of transcription factors: MEF2s bind to the PGC-1α promoter and activate it, predominantly through coactivation by PGC-1α itself (90, 91). This feedback loop allows a stable induction of PGC-1α. It is worth to note that in cardiac-specific PGC-1α knockout mice, the mRNA regions of PGC-1α corresponding to

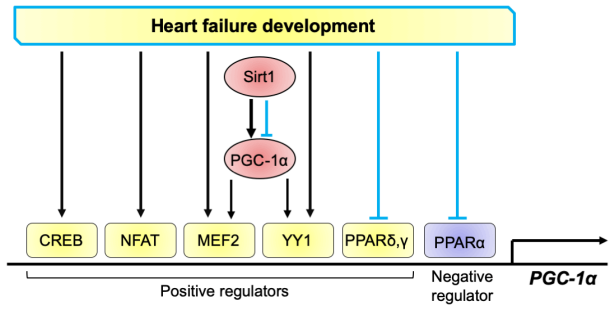


FIGURE 2 | Transcriptional regulation of PGC-1α in the heart. Positive regulators for PGC-1α transcription include CREB, NFAT, MEF2, YY1, PPARδ, and PPARγ, whereas those negatively regulate PGC-1α include PPARα. These factors are activated (black arrows) or inhibited (blue lines) in the progression of heart failure. PGC-1α promotes its transcription through co-activation of MEF2 and YY1. Sirt1 either activates and inhibits PGC-1α, thereby positively and negatively regulates PGC-1α transcription.

targeted (floxed) exons are significantly downregulated while the other intact regions are rather upregulated (25). This observation suggests that although the autoregulatory transcription loop can enhance PGC-1α induction in response to physiological stimuli, PGC-1α itself might not be essential for PGC-1α transcription.

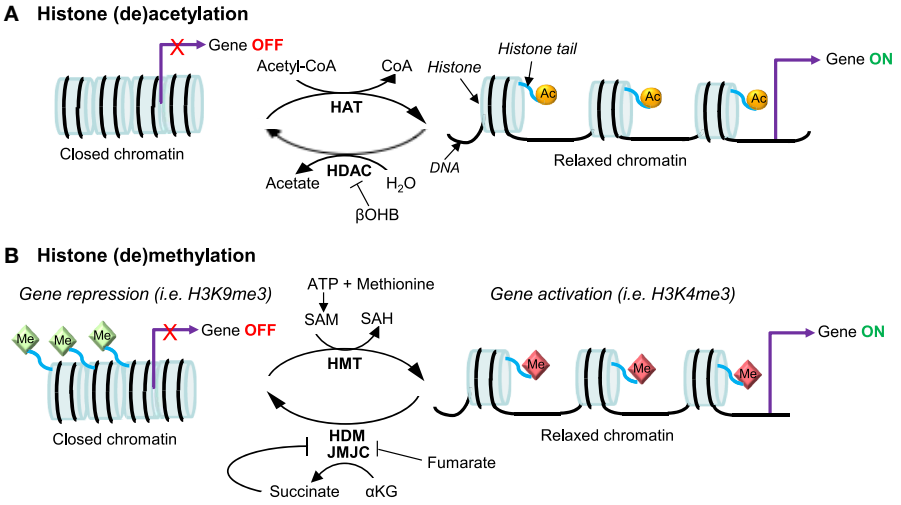
EPIGENETIC REGULATION OF *PGC-1α* IN THE HEART

Epigenetics refers to reversible modifications of the phenotype without a change in the DNA sequence. In other words, epigenetic regulatory mechanisms can switch genes on or off and determine which proteins are transcribed without changing the inherited genetic program. Epigenetic modifications encompass histone modifications, DNA methylation, and RNA-associated silencing (i.e., microRNAs) (92). The histone landscape is an important part of transcriptional activation (93). The best characterized histone post-translational modifications (PTMs) are acetylation and methylation (94) (also summarized in **Figure 3**). Histone acetylation is usually associated with gene activation since this process "relaxes" the chromatin allowing for the recruitment of the transcription factors and RNA polymerase (101). This process is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which add or remove the acetyl groups from histones, respectively. On the other hand, histone methylation is more complex and can occur in various forms: mono- (me), di- (me2), or tri-methylation (me3), with each methylation leading to either gene activation or repression. Histone methylation is catalyzed by histone methyltransferases (HMTs) and histone demethylases (HDMs) (102). Although a large body of work has implicated epigenetic modifications in the development of cardiac disease (102–105), there is a limited number of studies that have examined epigenetic modifications of the *PGC-1α* promoter. Below, we summarize and discuss recent studies reporting histone methylation or acetylation, and DNA methylation in the *PGC-1α* gene. We will also briefly discuss the potential significance of PGC-1α variants, currently well-established in the skeletal muscle but largely unknown in the myocardium.

Histone Methylation and Acetylation Across *PGC-1α* Promoters

Histone methylation can be associated with either transcriptional repression or activation. For example, trimethylation of histone H3 at lysine 4 (H3K4me3) is an active mark for transcription, while methylation of histone H3 at lysine 9 (H3K9me3) is frequently associated with gene silencing or repression. An excellent overview of histone modifications can be found in Bannister and Kouzarides (93). Our recent study identified the striated muscle-specific histone methyltransferase Smyd1 (SET and MYND domain-containing protein 1) as a novel regulator of PGC-1α in the heart (26) (**Figure 1A**, top). Smyd1 is known to tri-methylate histone H3K4 (H3K4me3), which generally leads to gene activation (93). Bioinformatic analysis of the heart from the cardiac-specific Smyd1 knockout mice revealed that OXPHOS and the TCA cycle were the most perturbed biological pathways, concomitant with downregulation of the key metabolic regulators PGC-1α, PPARα and RXRα. Furthermore, knockdown of Smyd1 with siRNAs in neonatal rat ventricular cardiomyocytes led to a significant reduction in PGC-1α expression, without significant changes in gene expression of PPARα and RXRα (26). Overall, these data suggested that PGC-1α is a downstream target of Smyd1. Chromatin immunoprecipitation (ChIP) and luciferase reporter assay confirmed that Smyd1 transcriptionally regulates PGC-1α through modulating the H3K4me3 marks on its promoter region (26). In the hypertrophied mouse heart chromatin-bound Smyd1 is increased, while overexpression of Smyd1 in cardiomyocytes prevents cellular hypertrophy under phenylephrine-induced hypertrophic stress (106). Thus, it is plausible that Smyd1 plays a role in maintaining PGC-1α expression as part of adaptive responses to pathological stress through modulating the H3K4me3 marks on its promoter. To support this notion, the ablation of Smyd1 gene in the adult mouse heart led to fulminant heart failure (26). Of note, Smyd1 also acts as a repressor of genes controlling cell growth (106), suggesting the intriguing possibility that Smyd1 is multifunctional in epigenetic regulation of genes involved in metabolic and structural remodeling of the myocardium in response to chronic hemodynamic stress.

The unique histone methylation marks in the *PGC-1α* locus have also been reported in a rat model of high-salt induced-cardiac hypertrophy and failure. In this model, a downregulation of PGC-1α and the reduced mitochondrial respiration capacity in the failing heart were associated with an elevated level of H3K9me3, a marker of gene repression, on the *PGC-1α* promoter (27) (**Figure 1A**, bottom). Inhibition of histone H3K9 methyltransferases by chaetocin partially normalized PGC-1α expression and H3K9me3 levels in the *PGC-1α* gene (27), confirming a mechanistic link between H3K9me3 marks and *PGC-1α* expression. However, it remains unknown which specific enzymes are responsible for the elevation of H3K9me3 levels in the *PGC-1α* loci in the failing heart.



C Modification sites of histone tails via acetylation and methylation

Modification	*Role in transcription*	*Modification site*
Acetylation	Activation	H3(K9, K14, K18, K56), H4(K5, K8, K12, K16), H2B(K6, K7, K16, K17)
Methylation	Activation	H3(K4me2, K4me3, K36me3, K79me2)
Methylation	Repression	H3(Kme3, K27me3), H4(K20me3)

FIGURE 3 | Regulation in gene expression via histone acetylation and methylation. The protruding amino tails of histone proteins can undergo post-translational modifications that affect the expression of genes in close proximity. **(A)** Histone acetylation and deacetylation. Histone lysines are acetylated by histone acetyltransferases (HATs), which use acetyl-CoA as a cosubstrate. Histone deacetylases (HDACs) are grouped in four classes: Classes I, II, and IV are Zn^{2+}-dependent and release acetate as a coproduct while sirtuins (class II HDACs) consume NAD^{+} and produce nicotinamide and O-acetyl-ADP-ribose. β-hydroxybutyrate (βOHB) is a ketone body and can inhibit class I and IIa HDACs, being structurally related to be well-known HDAC inhibitor butyrate (95). **(B)** Histone methylation and demethylation. Histones are methylated by histone methyltransferases (HMTs), which require S-adenosylmethionine (SAM) as a consubstrate, yielding S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed to homocysteine and adenosine by SAH-hydrolase (96) Two classes of histone demethylases (HDMs) can remove a methyl group: Lysine-specific demethylase 1 (LSD1) requires the reduction of flavin adenine dinucleotide (FAD) (97), while the Jumonji C (JMJC) domain-containing lysine demethylases catalyze a different demethylation reaction that requires α-ketoglutarate (αKG), O^2, and Fe(II) (98). Fumarate and succinate, the intermediates in the TCA cycle, are the competitive inhibitors (99, 100). **(C)** Summary of modification sites of histone tails via acetylation and methylation. Other histone post-translational modifications include phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation citrullination, and biotinylation.

As for transcriptional regulation of *PGC-1α* through histone (de)acetylation, we have previously reported that in the TAC mouse model of heart failure, the reduced mRNA level of PGC-1α was associated with a significant decrease in H3 lysine 9 acetylation (H3K9Ac) (**Figure 1A**) (28). Moreover, the reduced H3K9Ac level on the *PGC-1α* promoter was associated with an increase of the promoter occupancy of the histone deacetylase (HDAC) Sirtuin 1 (Sirt1) (28). This raises a possibility that Sirt1 contributes to gene repression of *PGC-1α* under pressure overload through histone deacetylation of the promoter. However, direct evidence indicating the role of Sirt1, or any other HDACs or HATs, in the histone acetylation marks on the *PGC-1α* gene in the heart is lacking. In rat skeletal muscle, the increased level of histone acetylation at H3 lysine 27 (H3K27Ac) of the *PGC-1α* gene was reported in correlation with transcriptional activation after acute exercise (20 min at a speed of 24 m/min on a rodent treadmill) (107).

In summary, cumulative data suggest that posttranslational modifications of histone proteins across *PGC-1α* promoters occur under physiological stimuli and hemodynamic stress. However, the endeavor to understand regulation of the *PGC-1α* gene through histone modifications has just begun. A comprehensive profiling of histone methylation and acetylation marks on the *PGC-1α* promoter in the healthy and diseased heart would greatly advance our understanding of the mechanisms of transcriptional control on the *PGC-1α* gene.

DNA Methylation of the *PGC-1α* Gene

DNA methylation also controls transcriptional activity of genes. The addition of a methyl group on position 5 of cytosine of the cluster of CpG island (a promoter of the regulatory region of genes) is typically associated with a closed chromatin state and leads to gene silencing, which can be passed to the next generation (108).

Little is known about DNA methylation of the *PGC-1α* gene in the heart. Bisphenol A-induced cardiomyopathy caused hypermethylation on the PGC-1α gene, in association with downregulation of PGC-1α (32). More information can be found in studies concerning other organs and tissues. In the skeletal muscle from patients of type 2 diabetes mellitus (T2DM), hypermethylation of the *PGC-1α* gene was observed at cytosine residues (non-CpG nucleotides), which was associated with a reduction in mRNA levels of PGC-1α and mitochondrial DNA (29). The correlation between DNA hypermethylation of the *PGC-1α* promoter and reduced insulin secretion was also demonstrated in pancreatic islet cells from patients with T2DM (30). Moreover, it has been reported that diet can also alter the DNA methylation profile in *PGC-1α* in skeletal muscle. High-fat diet in mice leads to the increase in DNA methylation in *PGC-1α* at −260 nucleotide site in skeletal muscle, concurrent with the reduced expression of total PGC-1α, which was prevented by supplement of bioflavonoid quercetin and quercetin-rich red onion extract (31). Another group showed that quercetin attenuates high-cholesterol-induced cardiac diastolic dysfunction and cholesterol accumulation in rats, in association with the preserved expression level of PGC-1α and the reduced oxidative stress (109). Exercise-induced activation of PGC-1α in the skeletal muscle was associated with DNA hydroxymethylation (110).

Taken together, these studies suggest that DNA methylation of the *PGC-1α* gene may be a general mechanism regulating PGC-1α expression in response to pathophysiological and dietary stimuli. If this is the case, DNA methylation might also play a role in modulation of *PGC-1α* gene expression in the failing heart, but this needs to be determined in future experiments.

Epigenetics and Mitochondria

Mitochondria are the essential source of epigenetic modifiers. There is a growing awareness that central components of intermediary metabolism in mitochondria are cofactors or cosubstrates of chromatin-modifying enzymes (111) (**Figure 3**). The concentrations of those metabolic intermediates constitute a potential regulatory interface between the metabolic and chromatin states. In histone acetylation, S-adenosylmethionine (SAM) is the methyl group donor for both histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs), which is generated from methionine and ATP in mitochondria (**Figure 3B**). Two classes of histone demethylases (HDMs) can remove a methyl group: lysine-specific demethylase 1 (LSD1) requires the reduction of flavin adenine dinucleotide (FAD) (97), and the Jumonji C (JMJC) domain-containing lysine demethylases catalyze a different demethylation reaction that requires α-ketoglutarate (αKG) (98). Fumarate and succinate, the intermediates in the TCA cycle, are the competitive inhibitors of HDMs (99, 100). In histone methylation, acetyl-CoA is used as an acetyl group donor by histone acetyltransferases (HAT), which is also formed in mitochondria from glycolysis or from fatty acid oxidation. β-hydroxybutyrate (β-OHB), a ketone body, can inhibit class I and IIa HDACs, being structurally related to the well-known HDAC inhibitor butyrate (**Figure 3A**). Both caloric restriction of mice and direct administration of βOHB resulted in enhanced global histone acetylation (95), consistent with decreased HDAC activity. Thus, the activity of central chromatin-modifying enzymes is closely linked to changes in the levels of the metabolites/intermediates in mitochondria.

Recent developments suggest that mitochondrial protein lysine acetylation (LysAc) modulates the sensitivity of the heart to stress and is involved in mitochondrial dysfunction and the development of heart failure [reviewed in (112)]. Myocardial acetylproteomics revealed that extensive mitochondrial protein lysine hyperacetylation occurs in the early stages of heart failure in the mouse TAC heart and in end-stage failing human heart, in association with reduced catalytic function in succinate dehydrogenase A and complex II-derived respiration (113), suggesting the role of LysAc in mitochondrial dysfunction as the primary metabolic remodeling of heart failure. Protein LysAc occurs when an acetyl group is added to a lysine residue by non-enzymatic chemical modification with acetyl-CoA, or by enzymatic acetylation with acetyltransferases, while removal of the acetyl group from lysine requires NAD^+ and is mediated by deacetylases, such as sirtuins. Sirtuin 3 (Sirt3) is mainly localized to the mitochondria (114), and loss of Sirt3 in mice leads to the increased mitochondrial LysAc (115). NAD^+/NADH ratio is the other determinant of energetic states and mitochondrial LysAc, and the elevated NADH/NAD^+ ratio has been reported in the human failing heart, in association with the increased LysAc levels (116). A recent study demonstrated that increasing myocardial NAD^+ level via the supplementation of its precursor prevents mitochondrial hyperacetylation and cardiac hypertrophy during pressure overload, concurrent with improved cardiac function (116).

Myocardial contents of the TCA-cycle intermediates (α-KG; fumarate; malate) are decreased in the failing heart, where the mitochondrial capacity of fatty acid oxidation is reduced (117). Interestingly, the changes in metabolome occur earlier than downregulation of OXPHOS genes, suggesting that the regulatory modifications between the metabolic and chromatin states may occur at the early stage of heart failure. Can PGC-1α be involved in this mechanism? Recent study of metabolomic profiling of cardiac-specific PGC-1α knockout mice revealed that cardiac metabolite contents are significantly altered, which includes the decreased level of acetyl-CoA (72), an essential source of epigenetic modifiers (**Figure 3**). Whether PGC-1α plays a role in the maintenance of the supply for cofactors or cosubstrates of epigenetic modifications needs to be investigated.

Post-transcriptional Inhibition of PGC-1α Expression

Non-coding RNAs (ncRNAs), which are encoded within the genomes, are generally not translated into proteins. However, ncRNAs play an important role in the regulation of gene expression at the post-transcriptional level. ncRNAs that appear to be involved in epigenetic processes are generally classified into two subgroups based on their length; the long ncRNAs (>200 nt) and the small ncRNAs (<30 nt), the latter of which have three major classes: microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) [reviewed in

(118, 119)]. Among those, miRNAs are the important regulators of gene expression. miRNAs generally bind to a specific target mRNA with a complementary sequence to induce cleavage, degradation, or block translation. It has been estimated that miRNAs are able to modulate up to 60% of protein-coding genes in the human genome at the translational level (120). Thus, they are known to have the potential to fine-tune the expression of numerous genes.

Several miRNAs have been reported to inhibit PGC-1α expression in various organs, which include miR-696 and miR-761 in skeletal muscle (121, 122), miR-199a/214 in brown and beige adipocyte (123), miR29b in cochlear hair cells (124), miR19b/221/222 in vessels (125), and miR485-3p and mi485-5p in breast cancer cells (126). However, very little is known about miRNAs that post-transcriptionally inhibit PGC-1α expression in the heart. miR-23a directly downregulates PGC-1α expression in cardiomyocytes via binding to the 3′UTR of PGC-1α mRNA. Overexpression of miR-23a led to downregulation of PGC-1α and mitochondrial damage in culture cardiomyocytes (34). miR-22 is a muscle-enriched miRNA and post-transcriptionally inhibits PGC-1α as well as PPAR-α and Sirt1 expression (33). Cardiomyocyte-specific overexpression of miR-22 in mice promotes hypertrophic growth and cardiomyopathy, concurrent with downregulation of PGC-1α, PPAR-α, and Sirt1 (33). Whereas, genetic manipulations on miR-23a and miR-22 strongly suggest their involvement in regulation of cardiac metabolism and growth, it remains to be determined whether these or other miRNAs contribute to downregulation of PGC-1α in the failing heart.

Expression of PGC-1α Variants in the Heart

Transcription of a single *PGC-1α* gene is controlled by multiple promoters coupled to alternative splicing, which give rise to coactivator variants with distinct transcript and protein structure (127). To date, more than ten isoforms of PGC-1α are known to exist, arising from a combination of various promoters and alternative splicing. Currently, two promoters have been identified in the PGC-1α loci of the mouse skeletal muscle: *canonical (proximal)* and *alternative* (**Figure 4**). The *canonical* promoter originates at exon 1a (E1a), where the canonical PGC-1α-a mRNA isoform and the canonical PGC-1α protein are generated (the 797 amino acid-long murine full-length protein, of which 94.7% of the sequence identifies with the 798 amino acid-long human PGC-1α) (**Figure 4**). The *alternative* promoter is located ∼14 kb upstream of the *proximal promoter,* which is highly conserved between species and has been shown to be active in human skeletal muscle (129, 130). Through alternative splicing, the *alternative* promoter directs the transcription of two different first exons (exon 1b and exon 1c), which generates the PGC-1α-b and PGC-1α-c mRNA isoforms, respectively (130, 131) (**Figure 4**). The PGC-1α-b and PGC-1α-c proteins differ only in the N-termini while the rest of the protein is identical to the canonical PGC-1α-a. The proteins PGC-1α-a, PGC-1α-b, and PGC-1α-c are all capable in activating PPARs (α, δ, and γ) (132). The combination of these two promoters and splicing provide more variants, such as NT-PGC-1α-1, NT-PGC-1α-c, PGC-1α2, PGC-1α3, and PGC-1α4 (NT-PGC-1αb) [reviewed in (127)].

The PGC-1α gene generates a variety of mRNAs under different biological conditions. Emerging evidence suggests that specific isoforms are induced by physiological stimuli and hypertrophic stress in the skeletal muscle. The mRNA transcripts driven from the *alternative* promoter of PGC-1α were increased by exercise in humans (130, 133, 134) and mice (110), while the mRNA levels of PGC-1α-a driven from the *canonical* promoter remained unchanged in the post-exercised mouse skeletal muscle (132). Interestingly, the protein from the spliced PGC-1α-b from the *alternative* promoter [NT-PGC-1α-b, "PGC-1α-4" in (130)] does not regulate most known PGC-1α targets, such as the mitochondrial OXPHOS, rather it regulates insulin growth factor 1 (IGF1) and myostatin pathways and induces myotube hypertrophy (130). The other study also demonstrated that the administration of β-adrenergic agonist clenbuterol to mice increase the PGC-1α mRNA levels (PGC-1α-b and PGC-1α-c) from the *alternative* promoter without exercise (132). These studies suggest that the expression derived from the *alternative* promoter of PGC-1α is regulated via activation of an β-adrenergic receptor.

Amid this wealth of data obtained from the skeletal muscle, little is known about PGC-1α variants in the heart. One study reported that the mRNA level of a PGC-1α variant NT-PGC-1α is decreased in a mouse model of myocardial infarction (32). It remains unknown whether hemodynamic stress alters the expression of PGC-1α variants in the heart. It is worth to point out that the variability of reported mRNA levels of PGC-1α in the failing heart (8, 23, 24, 55–57), might in part be due to detecting transcripts of different PGC-1α isoforms. Indeed, it is not necessary that all those reported PGC-1α transcripts in the failing heart are the canonical PGC-1α (PGC-1α-a) derived from the *canonical* promoter. For instance, detecting total PGC-1α by targeting exon 2 might mask important changes in the levels of specific isoforms because most variants include the sequence from exon 2. On the other hand, the primers that target the exon 1a will fail to measure the induction or repression of the *alternative* promoter.

What determines the activation and repression of the *alternative* promoters? In other words, what epigenetic modifications regulate the *alternative* PGC-1α promoter? It is possible that the *canonical* and *alternative* promoters are individually regulated by different histone methylation marks and modifiers. Our recent study assessed the H3K4me3 levels at the PGC-1α promoter in Smyd1-knockout mice (26), which is ∼1 kb upstream from the *canonical* promoter (**Figure 4**, indicated as a yellow star). The reduced enrichment of the H3K4me3 by loss of Smyd1 (26) suggests that Smyd1 is likely to regulate the *canonical* promoters. This is consistent with global downregulation of OXPHOS genes (26), which are mainly regulated by PGC-1α-a derived from the *canonica*l promoter (128). It remains unknown whether Smyd1 can also methylate the histone proteins within the *alternative* promoter. A recent study of the skeletal muscle showed that exercise leads to the elevation of histone H3K4me3 marks in the *alternative* promoter region of *PGC-1α,* which was correlated with the increases of the

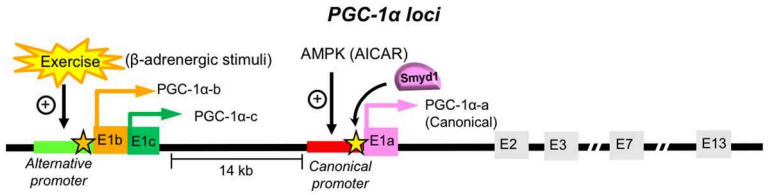


FIGURE 4 | PGC-1α promoters and isoforms. The PGC-1α loci contains two promoters in the skeletal muscle: the *canonical* and *alternative* promoters [Reviewed in (128)]. Transcription initiated from the upstream alternative promoter of the PGC-1α gene results in the inclusion of new exons E1b or E1c, which generate PGC-1α-b and PGC-1α-c, respectively. The PGC-1α-b and PGC-1α-c proteins contain two distinct N-termini, which are different from the canonical PGC-1α-a derived from the exon (E1a) from the canonical promoter. Exercise increases the PGC-1α mRNA levels originated from the *alternative promoter*, which is correlated with the elevated H3K4me3 marks in the *alternative promoter* region of PGC-1α (110) (indicated with an orange star). However, it remains elusive what histone methylation modifiers are responsible for the increase of the H3K4me3 levels on the *alternative* protomer of PGC-1α by exercise. In our previous study, the enrichment of the H3K4me3 marks were assessed in the Smyd1-knockout mouse heart, which was reduced at the canonical promoter (∼−1 kb from E1a, indicated with a yellow star), suggesting that Smyd1 regulates the expression of the PGC-1α-a mRNA isoform in the heart. It remains unknown whether the PGC-1α variants from the alternative promoter are involved in metabolic remodeling in the hypertrophied and failing heart.

PGC-1α mRNA levels originated from the *alternative* promoter (110) (**Figure 4**, indicated as a red star). However, it remains unknown what histone modifiers are responsible for methylation of the *alternative* promoter in response to exercise.

Summarizing, the expression and function of PGC-1α variants in heart muscle have been somewhat overlooked. However, by analogy with data obtained in the skeletal muscle, it is likely that the profile of PGC-1α isoforms is changing in response to pathological stress, and thus may play a role in adaptive or maladaptive metabolic alterations occurring during the development of heart failure.

REGULATION OF PGC-1α ACTIVITY BY POST-TRANSLATIONAL MODIFICATIONS IN THE HEART

PTMs, which are equally important as the transcriptional mechanisms, also extensively regulate PGC-1α. To date, phosphorylation, acetylation, ubiquitination, methylation, acetylation, and GlcNAcylation of the PGC-1α protein have been reported. The PTM sites and modulators of the PGC-1α protein are well-described in (17). In particular, phosphorylation of PGC-1α via p38 mitogen-activated protein kinase (MAPK) is clinically relevant. Several diseases, such as heart failure and cancer, cause the elevation of the circulating levels of TNFα and other inflammatory cytokines (i.e., ILα and IL-β) (135), which leads to the nuclear translocalization of p38 MAP kinase, resulting in phosphorylation of PGC-1α at T272, S265, and T298 (136). The phosphorylated PGC-1α via p38 MAP kinase is more stable to degradation and more transcriptionally active, in association with increased mitochondrial respiration capacity and upregulation of OXPHOS genes (136). The expression and activation of p38 MAPK transiently increase in the mouse heart during pressure overload (2 and 4 weeks of TAC) (137). The inhibition of p38 MAPK is beneficial in a mouse model of right ventricular hypertrophy and failure that was induced by pulmonary artery banding (138). It remains elusive whether phosphorylation of PGC-1α via p38 MAPK plays a role in metabolic remodeling in response to hemodynamic stress. Furthermore, which types of PGC-1α's PTMs occur under pathological stress in the heart remains largely unknown. Our previous study demonstrated that the NAD-dependent deacetylase Sirt1 is upregulated in pressure overload-induced heart failure in mice, concurrent with the increased interaction with PPARα (PGC-1α's binding partner), resulting in downregulation of genes involved in OXPHOS and FAO (28). Given that the PGC-1α protein is deacetylated by Sirt1 (35), it is plausible that the upregulation of Sirt1 in the failing heart leads to the deacetylation of PGC-1α (**Figure 1C**, bottom). The functional consequence of PGC-1α deacetylation in transcriptional control of its target genes and mitochondrial biogenesis is not well-established. In skeletal muscle, most studies have shown that the deacetylation of PGC-1α by Sirt1 increases the co-activation of its target transcription factors (17, 35, 139). However, in one study deacetylation of PGC-1α by Sirt1 did not change exercise-induced mitochondrial biogenesis (140). In the heart, it remains unknown how deacetylation of PGC-1α by Sirt1 modulates its function.

MULTIPLE MECHANISMS BY WHICH PGC-1α REGULATES TRANSCRIPTION OF ITS TARGET GENES

In the past two decades, our understanding of the role of PGC-1α as a co-activator has significantly advanced. PGC-1α regulates the activity of a large number of transcription factors, including PPARγ (1), PPARα (141), ERRα (142), Forkhead Box O1 (FoxO1) (143), and NRF1 (88). PGC-1α also interacts with p300/CBP, which contains a histone acetyltransferase domain (144), the transcription activator TRAP/Mediator (145), and RNA processing factors (146). Thus, PGC-1α can regulate its target genes via a multitude of mechanisms, which include chromatin modification, preinitiation complex assembly, and RNA processing. Our recent study revealed an additional role

of PGC-1α in transcriptional control of its target genes in the heart. We demonstrated that PGC-1α recruits RNA polymerase II (PolII) to the promoters of metabolic genes, which was dissipated in the failing heart (25) (**Figure 1C**). Chromatin immunoprecipitation-sequencing (ChIP-seq) revealed that the occupancy of PolII to the PGC-1α target gene promoters was consistently reduced in the mouse heart after 4 days of TAC surgery, the time point at which neither mRNA nor protein expression of PGC-1α was changed. ChIP-PCR assays of the mouse failing heart also showed a decreased interaction of PGC-1α with the promoters of its target genes (*Mcad; Sdha; Idh3a; Atp5k1*) in the TAC mouse heart, concurrent with the decrease of PolII's promoter occupancy in those genes (25). In cardiomyocytes, overexpression of PGC-1α induced recruitment of PolII to the promoters of PGC-1α target genes, such as *Mcad* and *Idh3a*. Furthermore, *in-vitro* DNA binding assay using biotin-labeled DNA comprising 380 bp of the *Idh3a* promoter showed that PGC-1α enhances recruitment of PolII to the promoter, whereas siRNA-mediated PGC-1α knockdown inhibits it. Therefore, downregulation of OXPHOS genes in the failing heart is, in part, attributed to the dissociation of PGC-1α from the target gene promoters, rather than the decreased expression levels of PGC-1α. In other words, it appears that pathological stress interferes with the ability of PGC-1α to bind to its target promoters. To support this notion, PGC-1α purified from cardiomyocytes treated with α1-adrenergic agonist phenylephrine had reduced ability to bind to the *Idh3a* promoter, mimicking a pathological consequence of heart failure (25).

It remains unknown what regulates the ability of PGC-1α to recruit PolII to the promoters of metabolic genes. It is plausible the PTMs of the PGC-1α protein occur under pressure overload, which leads to the dissociation of PGC-1α from the target gene promoters (**Figure 1C**, bottom). Given that Sirt1 was upregulated in the TAC mouse heart, concurrent with downregulation of PGC-1α target genes (28), it is likely that the deacetylation of PGC-1α by Sirt1 is attributed to the dissociation of PGC-1α from its target gene promoters and PolII. To support this notion, less PGC-1α was dissociated from target gene promoters under pressure overload in Sirt1 knockout mice (81). It is our future study to determine the specific PTMs that are responsible for the recruitment of PolII and that interfere with the ability of PGC-1α to bind to its target gene promoters under pathological conditions.

In cardiac-specific PGC-1α knockout mice, where the protein expression was decreased by ∼50%, the promoter occupancy of PolII in PGC-1α target genes was decreased, similar to the TAC heart (25). However, maintaining PGC-1α expression during pressure overload by PGC-1α overexpression did not prevent mitochondrial impairment in the TAC mouse heart (24). It is possible that maintaining PGC-1α expression during pressure overload is not sufficient to preserve its function in the recruitment of PolII to the promoters of OXPHOS and FAO genes. The appropriate PTMs of PGC-1α might be required to

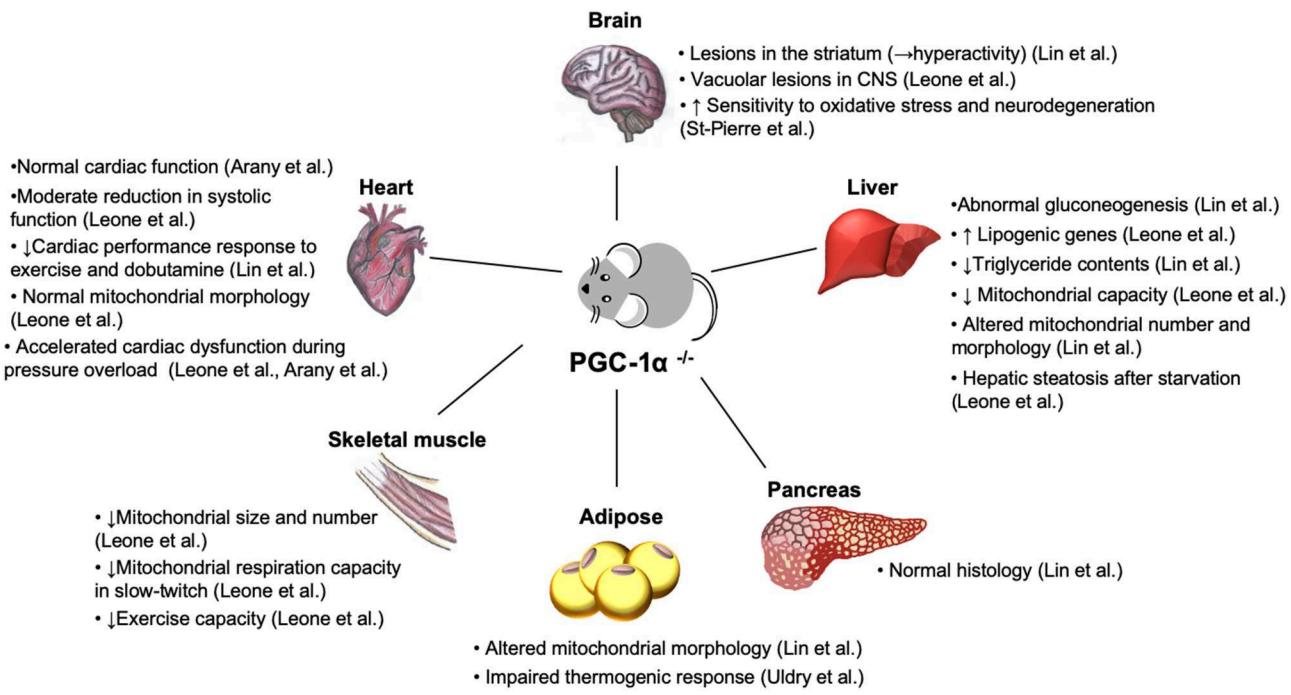


FIGURE 5 | Phenotypes of systemic PGC-1α knockout mice. Loss of PGC-1α leads to impaired energy homeostasis in a variety of organs (5, 21). Despite the reduced density and function of mitochondria in skeletal muscle (21) and abnormality in brown fat tissue with abundant accumulation of large lipid droplets (5), PGC-1α knockout mice are paradoxically lean and resistant to diet-induced obesity due to hyperactivity, resulted from the lesions in the striatum in the brain (5). Normal cardiac function (7, 8) and moderate systolic dysfunction (21) have been reported in two different lines of PGC-1α null mice. Nevertheless, both PGC-1α$^{-/-}$ models show cardiac dysfunction in response to hemodynamic stress and metabolic challenge (8, 21). CNS, central nervous system.

normalize its function under pathological stress. Therefore, it is critical to determine the specific PTMs and PTM modifiers that are responsible for functional modifications of PGC-1α in the failing heart (**Figure 1C**).

ROLE OF PGC-1α IN VARIOUS ORGANS

Heart failure is accompanied by a systemic illness that contributes to its progressive nature. Recent studies suggest that heart failure may itself promote systemic metabolic changes such as insulin resistance, in part through neurohumoral activity (147). Moreover, patients with chronic heart failure are characterized by systemic inflammation, as evidenced by elevated circulating levels of several inflammatory cytokines (148). Thus, interorgan cross-talk might contribute to the detrimental self-perpetuating cycle of heart failure (heart failure→altered metabolism in the other organs→heart failure).

PGC-1α is abundantly expressed in tissues with high energy demand (149). Loss-of-function study in mice suggests that PGC-1α dysfunction leads to multisystem energy metabolic derangements (**Table 2**, **Figure 5**). Systemic PGC-1α knockout mice exhibit neurological disorders, in association with the severe lesions in the striatum of the brain area that controls movement (5, 9), which is affected in certain neurogenerative diseases, such as Huntington's disease (150). Similarly, PGC-1α null mice exhibit the accelerated neurodegeneration in response to oxidative stress (9), indicative of the role of PGC-1α in the defense system to ROS. It remains elusive whether the neurological abnormalities in PGC-1α deficiency contribute to the systemic metabolic abnormalities through the alterations in circulating hormones and/or signals that originated from the central nervous system.

Patients with congestive heart failure decrease exercise capacity. Although cardiac dysfunction is the primary pathological insult, emerging evidence suggests that myocardial remodeling in peripheral skeletal muscle occurs independent of cardiac impairment (151). It has been reported that PGC-1α is downregulated in skeletal muscle in heart failure patients (66, 67). Total skeletal muscle PGC-1α deficiency led to a dramatic reduction in exercise performance, concurrent with rapid depletion of muscle glycogen store and mitochondrial biogenic defects (152). In skeletal muscle-specific PGC-1α-KO mice, reduced mitochondrial function and abnormal glucose homeostasis in skeletal muscle led to pancreatic dysfunction in association with the elevated levels of the circulating IL-6 (153). IL-6 treatment of isolated mouse pancreas islet suppresses glucose-stimulated insulin secretion (153), suggesting the cytokine-mediated crosstalk between skeletal muscle and pancreas.

PGC-1α also plays an essential role in hepatic metabolism. In the liver, loss of PGC-1α led to impaired gluconeogenesis, manifested by lacking hormone-stimulated gluconeogenesis and constitutively activated gluconeogenic gene expression that is completely insensitive to normal feeding controls (5). Interestingly, this phenotype was absent in the different line of PGC-1α knockout mice (21). Consistent with altered mitochondrial number and morphology (5), hepatocytes in PGC-1α knockout mice reduced mitochondrial capacity (21), while the genes involved in lipogenic genes were upregulated with the decreased triglyceride contents (21).

Abnormal morphology was also found in brown fat in PGC-1α knockout mice (5). Induction of thermogenic genes was severely reduced in brown adipose tissue of mice lacking PGC-1α, confirming the essential role of PGC-1α in thermogenesis, while loss of PGC-1α did not affect brown fat differentiation (154). Unexpectedly, PGC-1α knockout mice are lean and resistant to diet-induced insulin resistance (5). This is, in part, due to hyperactivity related to the lesions in striatum in the brain, as described above (5).

It remains unknown whether hemodynamic stress directly leads to the alterations in PGC-1α expression in those organs besides cardiac muscle. The cytokine-mediated metabolic changes might be one of the possible mechanisms leading to multisystem metabolic derangements in heart failure.

CONCLUSIONS

In this review, we summarized multiple mechanisms by which the PGC-1α regulatory cascade can be impaired in the failing heart. Whereas, early studies predominantly considered the regulation of PGC-1α transcription, it is now clear that PGC-1α dysregulation may occur at multiple levels, including epigenetic regulation of the *PGC-1α* gene, post-transcriptional inhibition via miRNAs, the expression of PGC-1α variants, and post-translational modifications of the PGC-1α protein. However, at each of these levels, the current knowledge remains limited and many questions remain to be answered. At the epigenetic level, whereas dynamic changes in histone marks across *PGC-1α* promoters have been documented, the factors inducing these changes are largely unknown. We provided evidence that Smyd1 is one of the factors. Our recent work also suggests that Sirt1 may be involved, but its role needs to be directly demonstrated. What other histone modifiers are involved in epigenetic regulation of the *PGC-1α* gene remains to be established.

A recurrent theme of this review is that the cardiac field is lagging behind other fields of science in the understanding of PGC-1α regulation. Whereas, in several organs and tissues DNA methylation of the *PGC-1α* gene has been implicated in response to pathophysiological and dietary stimuli, the prominence of this mechanism in the heart remains to be established. Likewise, the cardiac field is lagging behind in the understanding the role of PGC-1α splice variants in the regulation of the organ (heart) function and metabolism. Studies performed in skeletal muscle suggest that PGC-1α splice variants may regulate disjoint sets of target genes. We cannot exclude a similar arrangement in the heart muscle. One particular motivation to address this issue is the fact that reported variability in PGC-1α expression in the failing heart might result from an indiscriminate detection of different sets of PGC-1α splice variants in different studies—and a finer analysis might reveal that a certain PGC-1α variant is consistently downregulated.

Whether and which types of PGC-1α's PTMs occur under pathological stress in the heart remains largely unknown. We believe, however, that better understanding of PTMs in this context may be a key to explaining downregulation of PGC-1α target genes in those cases when PGC-1α expression is preserved in the failing heart (19, 23, 25, 61, 62). We have proposed a hypothesis that under pathological stress PGC-1α undergoes PTMs which interferes with its ability to recruit polymerase II to the promoters of OXPHOS and FAO genes. We hope to prove this hypothesis in our future studies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Work in the Warren lab was supported by the Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research and the Nora Eccles Treadwell Foundation while work in the Oka lab was supported by American Heart Association (AHA) Grant in Aid 17GRNT33440031 and Transformational Project Award 19TPA34850170, and New Jersey Health Foundation (NJHF) research grants PC56-16 and PC80-17.

ACKNOWLEDGMENTS

We thank Drs. Alexey V. Zaitsev, Samarjit Das, and Dipayan Chaudhuri for critical feedback on this manuscript.

REFERENCES

1. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell.* (1998) 92:829–39. doi: 10.1016/S0092-8674(00)81410-5
2. Martin OJ, Lai L, Soundarapandian MM, Leone TC, Zorzano A, Keller MP, et al. A role for peroxisome proliferator-activated receptor gamma coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth. *Circ Res.* (2014) 114:626–36. doi: 10.1161/CIRCRESAHA.114.302562
3. Scarpulla RC, Vega RB, Kelly DP. Transcriptional integration of mitochondrial biogenesis. *Trends Endocrinol Metab.* (2012) 23:459–66. doi: 10.1016/j.tem.2012.06.006
4. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest.* (2000) 106:847–56. doi: 10.1172/JCI10268
5. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, et al. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. *Cell.* (2004) 119:121–35. doi: 10.1016/j.cell.2004.09.013
6. Russell LK, Mansfield CM, Lehman JJ, Kovacs A, Courtois M, Saffitz JE, et al. Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1α promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. *Circ Res.* (2004) 94:525–33. doi: 10.1161/01.RES.0000117088.36577.EB
7. Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, et al. Transcriptional coactivator PGC-1α controls the energy state and contractile function of cardiac muscle. *Cell Metab.* (2005) 1:259–71. doi: 10.1016/j.cmet.2005.03.002
8. Arany Z, Novikov M, Chin S, Ma Y, Rosenzweig A, Spiegelman BM. Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1α. *Proc Natl Acad Sci USA.* (2006) 103:10086–91. doi: 10.1073/pnas.0603615103
9. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell.* (2006) 127:397–408. doi: 10.1016/j.cell.2006.09.024
10. Vainshtein A, Tryon LD, Pauly M, Hood DA. Role of PGC-1α during acute exercise-induced autophagy and mitophagy in skeletal muscle. *Am J Physiol Cell Physiol.* (2015) 308:C710–9. doi: 10.1152/ajpcell.00380.2014
11. Garcia S, Nissanka N, Mareco EA, Rossi S, Peralta S, Diaz F, et al. Overexpression of PGC-1α in aging muscle enhances a subset of young-like molecular patterns. *Aging Cell.* (2018) 17:e12707. doi: 10.1111/acel.12707
12. Salazar G, Cullen A, Huang J, Zhao Y, Serino A, Hilenski L, et al. SQSTM1/p62 and PPARGC1A/PGC-1α at the interface of autophagy and vascular senescence. *Autophagy.* (2019). doi: 10.1080/15548627.2019.1659612. [Epub ahead of print].
13. Sano M, Wang SC, Shirai M, Scaglia F, Xie M, Sakai S, et al. Activation of cardiac Cdk9 represses PGC-1 and confers a predisposition to heart failure. *EMBO J.* (2004) 23:3559–69. doi: 10.1038/sj.emboj.7600351
14. Adhihetty PJ, Uguccioni G, Leick L, Hidalgo J, Pilegaard H, Hood DA. The role of PGC-1α on mitochondrial function and apoptotic susceptibility in muscle. *Am J Physiol Cell Physiol.* (2009) 297:C217–25. doi: 10.1152/ajpcell.00070.2009
15. Choi HI, Kim HJ, Park JS, Kim IJ, Bae EH, Ma SK, et al. PGC-1α attenuates hydrogen peroxide-induced apoptotic cell death by upregulating Nrf-2 via GSK3beta inactivation mediated by activated p38 in HK-2 cells. *Sci Rep.* (2017) 7:4319. doi: 10.1038/s41598-017-04593-w
16. Marmolino D, Manto M, Acquaviva F, Vergara P, Ravella A, Monticelli A, et al. PGC-1α down-regulation affects the antioxidant response in Friedreich's ataxia. *PLoS ONE.* (2010) 5:e10025. doi: 10.1371/journal.pone.0010025
17. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr.* (2011) 93:884s—90. doi: 10.3945/ajcn.110.001917
18. Wagner RA, Tabibiazar R, Powers J, Bernstein D, Quertermous T. Genome-wide expression profiling of a cardiac pressure overload model identifies major metabolic and signaling pathway responses. *J Mol Cell Cardiol.* (2004) 37:1159–70. doi: 10.1016/j.yjmcc.2004.09.003
19. Huss JM, Imahashi K, Dufour CR, Weinheimer CJ, Courtois M, Kovacs A, et al. The nuclear receptor ERRalpha is required for the bioenergetic and functional adaptation to cardiac pressure overload. *Cell Metab.* (2007) 6:25–37. doi: 10.1016/j.cmet.2007.06.005
20. Bugger H, Schwarzer M, Chen D, Schrepper A, Amorim PA, Schoepe M, et al. Proteomic remodelling of mitochondrial oxidative pathways in pressure overload-induced heart failure. *Cardiovasc Res.* (2010) 85:376–84. doi: 10.1093/cvr/cvp344
21. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, et al. PGC-1α deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol.* (2005) 3:e101. doi: 10.1371/journal.pbio.0030101
22. Lehman JJ, Boudina S, Banke NH, Sambandam N, Han X, Young DM, et al. The transcriptional coactivator PGC-1α is essential for maximal and efficient cardiac mitochondrial fatty acid oxidation and lipid homeostasis. *Am J Physiol Heart Circ Physiol.* (2008) 295:H185–96. doi: 10.1152/ajpheart.00081.2008
23. Karamanlidis G, Garcia-Menendez L, Kolwicz SC Jr, Lee CF, Tian R. Promoting PGC-1α-driven mitochondrial biogenesis is detrimental in pressure-overloaded mouse hearts. *Am J Physiol Heart Circ Physiol.* (2014) 307:H1307–16. doi: 10.1152/ajpheart.00280.2014
24. Pereira RO, Wende AR, Crum A, Hunter D, Olsen CD, Rawlings T, et al. Maintaining PGC-1α expression following pressure overload-induced cardiac hypertrophy preserves angiogenesis but not contractile or mitochondrial function. *FASEB J.* (2014) 28:3691–702. doi: 10.1096/fj.14-253823

25. Bhat S, Chin A, Shirakabe A, Ikeda Y, Ikeda S, Zhai P, et al. Recruitment of RNA polymerase II to metabolic gene promoters is inhibited in the failing heart possibly through PGC-1α (Peroxisome Proliferator-Activated Receptor-gamma Coactivator-1α) dysregulation. *Circ Heart Fail.* (2019) 12:e005529. doi: 10.1161/CIRCHEARTFAILURE.118.005529
26. Warren JS, Tracy CM, Miller MR, Makaju A, Szulik MW, Oka SI, et al. Histone methyltransferase Smyd1 regulates mitochondrial energetics in the heart. *Proc Natl Acad Sci USA*. (2018) 115:E7871–80. doi: 10.1073/pnas.1800680115
27. Ono T, Kamimura N, Matsuhashi T, Nagai T, Nishiyama T, Endo J, et al. The histone 3 lysine 9 methyltransferase inhibitor chaetocin improves prognosis in a rat model of high salt diet-induced heart failure. *Sci Rep.* (2017) 7:39752. doi: 10.1038/srep39752
28. Oka S, Alcendor R, Zhai P, Park JY, Shao D, Cho J, et al. PPARalpha-Sirt1 complex mediates cardiac hypertrophy and failure through suppression of the ERR transcriptional pathway. *Cell Metab.* (2011) 14:598–611. doi: 10.1016/j.cmet.2011.10.001
29. Barres R, Osler ME, Yan J, Rune A, Fritz T, Caidahl K, et al. Non-CpG methylation of the PGC-1α promoter through DNMT3B controls mitochondrial density. *Cell Metab.* (2009) 10:189–98. doi: 10.1016/j.cmet.2009.07.011
30. Ling C, Del Guerra S, Lupi R, Ronn T, Granhall C, Luthman H, et al. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia.* (2008) 51:615–22. doi: 10.1007/s00125-007-0916-5
31. Devarshi PP, Jones AD, Taylor EM, Stefanska B, Henagan TM. Quercetin and quercetin-rich red onion extract alter Pgc-1α promoter methylation and splice variant expression. *PPAR Res.* (2017) 2017:3235693. doi: 10.1155/2017/3235693
32. Jiang Y, Xia W, Yang J, Zhu Y, Chang H, Liu J, et al. BPA-induced DNA hypermethylation of the master mitochondrial gene PGC-1α contributes to cardiomyopathy in male rats. *Toxicology.* (2015) 329:21–31. doi: 10.1016/j.tox.2015.01.001
33. Gurha P, Wang T, Larimore AH, Sassi Y, Abreu-Goodger C, Ramirez MO, et al. microRNA-22 promotes heart failure through coordinate suppression of PPAR/ERR-nuclear hormone receptor transcription. *PLoS ONE.* (2013) 8:e75882. doi: 10.1371/journal.pone.0075882
34. Sun LY, Wang N, Ban T, Sun YH, Han Y, Sun LL, et al. MicroRNA-23a mediates mitochondrial compromise in estrogen deficiency-induced concentric remodeling via targeting PGC-1α. *J Mol Cell Cardiol.* (2014) 75:1–11. doi: 10.1016/j.yjmcc.2014.06.012
35. Nemoto S, Fergusson MM, Finkel T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{α}. *J Biol Chem.* (2005) 280:16456–60. doi: 10.1074/jbc.M501485200
36. Finck BN, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) regulatory cascade in cardiac physiology and disease. *Circulation.* (2007) 115:2540–8. doi: 10.1161/CIRCULATIONAHA.107.670588
37. Kavazis AN. Pathological vs. physiological cardiac hypertrophy. *J Physiol.* (2015) 593:3767. doi: 10.1113/JP271161
38. Dealmeida AC, Van Oort RJ, Wehrens XH. Transverse aortic constriction in mice. *J Vis Exp.* (2010) 1729. doi: 10.3791/1729
39. Houser SR, Margulies KB, Murphy AM, Spinale FG, Francis GS, Prabhu SD, et al. Animal models of heart failure: a scientific statement from the American Heart Association. *Circ Res.* (2012) 111:131–50. doi: 10.1161/RES.0b013e3182582523
40. Doenst T, Pytel G, Schrepper A, Amorim P, Farber G, Shingu Y, et al. Decreased rates of substrate oxidation *ex vivo* predict the onset of heart failure and contractile dysfunction in rats with pressure overload. *Cardiovasc Res.* (2010) 86:461–70. doi: 10.1093/cvr/cvp414
41. Akki A, Smith K, Seymour AM. Compensated cardiac hypertrophy is characterised by a decline in palmitate oxidation. *Mol Cell Biochem.* (2008) 311:215–24. doi: 10.1007/s11010-008-9711-y
42. Oka S, Zhai P, Yamamoto T, Ikeda Y, Byun J, Hsu CP, et al. Peroxisome proliferator activated receptor-alpha association with silent information regulator 1 suppresses cardiac fatty acid metabolism in the failing heart. *Circ Heart Fail.* (2015) 8:1123–32. doi: 10.1161/CIRCHEARTFAILURE.115.002216
43. Mohammed SF, Storlie JR, Oehler EA, Bowen LA, Korinek J, Lam CS, et al. Variable phenotype in murine transverse aortic constriction. *Cardiovasc Pathol.* (2012) 21:188–98. doi: 10.1016/j.carpath.2011.05.002
44. Kolwicz SC Jr, Olson DP, Marney LC, Garcia-Menendez L, Synovec RE, Tian R. Cardiac-specific deletion of acetyl CoA carboxylase 2 prevents metabolic remodeling during pressure-overload hypertrophy. *Circ Res.* (2012) 111:728–38. doi: 10.1161/CIRCRESAHA.112.268128
45. Ingwall JS. Energy metabolism in heart failure and remodelling. *Cardiovasc Res.* (2009) 81:412–9. doi: 10.1093/cvr/cvn301
46. Balaban RS, Kantor HL, Katz LA, Briggs RW. Relation between work and phosphate metabolite in the *in vivo* paced mammalian heart. *Science.* (1986) 232:1121–3. doi: 10.1126/science.3704638
47. Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *Am J Physiol.* (1994) 267:H742–50. doi: 10.1152/ajpheart.1994.267.2.H742
48. Nascimben L, Ingwall JS, Lorell BH, Pinz I, Schultz V, Tornheim K, et al. Mechanisms for increased glycolysis in the hypertrophied rat heart. *Hypertension.* (2004) 44:662–7. doi: 10.1161/01.HYP.0000144292.69599.0c
49. Sorokina N, O'donnell JM, Mckinney RD, Pound KM, Woldegiorgis G, Lanoue KF, et al. Recruitment of compensatory pathways to sustain oxidative flux with reduced carnitine palmitoyltransferase I activity characterizes inefficiency in energy metabolism in hypertrophied hearts. *Circulation.* (2007) 115:2033–41. doi: 10.1161/CIRCULATIONAHA.106.668665
50. Pound KM, Sorokina N, Ballal K, Berkich DA, Fasano M, Lanoue KF, et al. Substrate-enzyme competition attenuates upregulated anaplerotic flux through malic enzyme in hypertrophied rat heart and restores triacylglyceride content: attenuating upregulated anaplerosis in hypertrophy. *Circ Res.* (2009) 104:805–12. doi: 10.1161/CIRCRESAHA.108.189951
51. Lehman JJ, Kelly DP. Transcriptional activation of energy metabolic switches in the developing and hypertrophied heart. *Clin Exp Pharmacol Physiol.* (2002) 29:339–45. doi: 10.1046/j.1440-1681.2002.03655.x
52. Garnier A, Fortin D, Delomenie C, Momken I, Veksler V, Ventura-Clapier R. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol.* (2003) 551:491–501. doi: 10.1113/jphysiol.2003.045104
53. Lu Z, Xu X, Hu X, Fassett J, Zhu G, Tao Y, et al. PGC-1 α regulates expression of myocardial mitochondrial antioxidants and myocardial oxidative stress after chronic systolic overload. *Antioxid Redox Signal.* (2010) 13:1011–22. doi: 10.1089/ars.2009.2940
54. Barth AS, Kumordzie A, Frangakis C, Margulies KB, Cappola TP, Tomaselli GF. Reciprocal transcriptional regulation of metabolic and signaling pathways correlates with disease severity in heart failure. *Circ Cardiovasc Genet.* (2011) 4:475–83. doi: 10.1161/CIRCGENETICS.110.957571
55. Faerber G, Barreto-Perreia F, Schoepe M, Gilsbach R, Schrepper A, Schwarzer M, et al. Induction of heart failure by minimally invasive aortic constriction in mice: reduced peroxisome proliferator-activated receptor gamma coactivator levels and mitochondrial dysfunction. *J Thorac Cardiovasc Surg.* (2011) 141:492–500.e491. doi: 10.1016/j.jtcvs.2010.03.029
56. Watanabe S, Horie T, Nagao K, Kuwabara Y, Baba O, Nishi H, et al. Cardiac-specific inhibition of kinase activity in calcium/calmodulin-dependent protein kinase kinase-beta leads to accelerated left ventricular remodeling and heart failure after transverse aortic constriction in mice. *PLoS ONE.* (2014) 9:e108201. doi: 10.1371/journal.pone.0108201
57. Piquereau J, Moulin M, Zurlo G, Mateo P, Gressette M, Paul JL, et al. Cobalamin and folate protect mitochondrial and contractile functions in a murine model of cardiac pressure overload. *J Mol Cell Cardiol.* (2016) 102:34–44. doi: 10.1016/j.yjmcc.2016.11.010
58. Hu X, Xu X, Huang Y, Fassett J, Flagg TP, Zhang Y, et al. Disruption of sarcolemmal ATP-sensitive potassium channel activity impairs the cardiac response to systolic overload. *Circ Res.* (2008) 103:1009–17. doi: 10.1161/CIRCRESAHA.107.170795
59. Sebastiani M, Giordano C, Nediani C, Travaglini C, Borchi E, Zani M, et al. Induction of mitochondrial biogenesis is a maladaptive mechanism in mitochondrial cardiomyopathies. *J Am Coll Cardiol.* (2007) 50:1362–9. doi: 10.1016/j.jacc.2007.06.035

60. Garnier A, Zoll J, Fortin D, N'guessan B, Lefebvre F, Geny B, et al. Control by circulating factors of mitochondrial function and transcription cascade in heart failure: a role for endothelin-1 and angiotensin II. *Circ Heart Fail.* (2009) 2:342–50. doi: 10.1161/CIRCHEARTFAILURE.108.812099
61. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, Del Monte F, Tian R. Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. *Circ Res.* (2010) 106:1541–8. doi: 10.1161/CIRCRESAHA.109.212753
62. Hu X, Xu X, Lu Z, Zhang P, Fassett J, Zhang Y, et al. AMP activated protein kinase-α2 regulates expression of estrogen-related receptor-alpha, a metabolic transcription factor related to heart failure development. *Hypertension.* (2011) 58:696–703. doi: 10.1161/HYPERTENSIONAHA.111.174128
63. Sihag S, Cresci S, Li AY, Sucharov CC, Lehman JJ. PGC-1α and ERRalpha target gene downregulation is a signature of the failing human heart. *J Mol Cell Cardiol.* (2009) 46:201–12. doi: 10.1016/j.yjmcc.2008.10.025
64. Pepin ME, Ha CM, Crossman DK, Litovsky SH, Varambally S, Barchue JP, et al. Genome-wide DNA methylation encodes cardiac transcriptional reprogramming in human ischemic heart failure. *Lab Invest.* (2019) 99:371–86. doi: 10.1038/s41374-018-0104-x
65. Sreekumar R, Halvatsiotis P, Schimke JC, Nair KS. Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. *Diabetes.* (2002) 51:1913–20. doi: 10.2337/diabetes.51.6.1913
66. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet.* (2003) 34:267–73. doi: 10.1038/ng1180
67. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA.* (2003) 100:8466–71. doi: 10.1073/pnas.1032913100
68. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science.* (2003) 300:1140–2. doi: 10.1126/science.1082889
69. Wang X, West JA, Murray AJ, Griffin JL. Comprehensive metabolic profiling of age-related mitochondrial dysfunction in the high-fat-fed ob/ob mouse heart. *J Proteome Res.* (2015) 14:2849–62. doi: 10.1021/acs.jproteome.5b00128
70. Ling C, Poulsen P, Carlsson E, Ridderstrale M, Almgren P, Wojtaszewski J, et al. Multiple environmental and genetic factors influence skeletal muscle PGC-1α and PGC-1beta gene expression in twins. *J Clin Invest.* (2004) 114:1518–26. doi: 10.1172/JCI21889
71. Patten IS, Rana S, Shahul S, Rowe GC, Jang C, Liu L, et al. Cardiac angiogenic imbalance leads to peripartum cardiomyopathy. *Nature.* (2012) 485:333–8. doi: 10.1038/nature11040
72. Karkkainen O, Tuomainen T, Mutikainen M, Lehtonen M, Ruas JL, Hanhineva K, et al. Heart specific PGC-1α deletion identifies metabolome of cardiac restricted metabolic heart failure. *Cardiovasc Res.* (2019) 115:107–18. doi: 10.1093/cvr/cvy155
73. Sucharov CC, Mariner P, Long C, Bristow M, Leinwand L. Yin Yang 1 is increased in human heart failure and represses the activity of the human alpha-myosin heavy chain promoter. *J Biol Chem.* (2003) 278:31233–9. doi: 10.1074/jbc.M301917200
74. Mckinsey TA, Olson EN. Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. *J Clin Invest.* (2005) 115:538–46. doi: 10.1172/JCI24144
75. Ichiki T. Role of cAMP response element binding protein in cardiovascular remodeling: good, bad, or both? *Arterioscler Thromb Vasc Biol.* (2006) 26:449–55. doi: 10.1161/01.ATV.0000196747.79349.d1
76. Hondares E, Pineda-Torra I, Iglesias R, Staels B, Villarroya F, Giralt M. PPARdelta, but not PPARalpha, activates PGC-1α gene transcription in muscle. *Biochem Biophys Res Commun.* (2007) 354:1021–7. doi: 10.1016/j.bbrc.2007.01.092
77. Kalliora C, Kyriazis ID, Oka SI, Lieu MJ, Yue Y, Area-Gomez E, et al. Dual peroxisome-proliferator-activated-receptor-α/γ activation inhibits SIRT1-PGC1α axis and causes cardiac dysfunction. *JCI Insight.* (2019) 5:129556. doi: 10.1172/jci.insight.129556
78. Duncan JG, Bharadwaj KG, Fong JL, Mitra R, Sambandam N, Courtois MR, et al. Rescue of cardiomyopathy in peroxisome proliferator-activated receptor-α transgenic mice by deletion of lipoprotein lipase identifies sources of cardiac lipids and peroxisome proliferator-activated receptor-alpha activators. *Circulation.* (2010) 121:426–35. doi: 10.1161/CIRCULATIONAHA.109.888735
79. Barger PM, Brandt JM, Leone TC, Weinheimer CJ, Kelly DP. Deactivation of peroxisome proliferator-activated receptor-alpha during cardiac hypertrophic growth. *J Clin Invest.* (2000) 105:1723–30. doi: 10.1172/JCI9056
80. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *EMBO J.* (2007) 26:1913–23. doi: 10.1038/sj.emboj.7601633
81. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* (2009) 9:327–38. doi: 10.1016/j.cmet.2009.02.006
82. Higashida K, Kim SH, Jung SR, Asaka M, Holloszy JO, Han DH. Effects of resveratrol and SIRT1 on PGC-1α activity and mitochondrial biogenesis: a reevaluation. *PLoS Biol.* (2013) 11:e1001603. doi: 10.1371/journal.pbio.1001603
83. Planavila A, Dominguez E, Navarro M, Vinciguerra M, Iglesias R, Giralt M, et al. Dilated cardiomyopathy and mitochondrial dysfunction in Sirt1-deficient mice: a role for Sirt1-Mef2 in adult heart. *J Mol Cell Cardiol.* (2012) 53:521–31. doi: 10.1016/j.yjmcc.2012.07.019
84. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, et al. Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ Res.* (2007) 100:1512–21. doi: 10.1161/01.RES.0000267723.65696.4a
85. Kawashima T, Inuzuka Y, Okuda J, Kato T, Niizuma S, Tamaki Y, et al. Constitutive SIRT1 overexpression impairs mitochondria and reduces cardiac function in mice. *J Mol Cell Cardiol.* (2011) 51:1026–36. doi: 10.1016/j.yjmcc.2011.09.013
86. Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, et al. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol Cell.* (2003) 12:51–62. doi: 10.1016/S1097-2765(03)00226-0
87. Wu Z, Huang X, Feng Y, Handschin C, Feng Y, Gullicksen PS, et al. Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1α transcription and mitochondrial biogenesis in muscle cells. *Proc Natl Acad Sci USA.* (2006) 103:14379–84. doi: 10.1073/pnas.0606714103
88. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell.* (1999) 98:115–24. doi: 10.1016/S0092-8674(00)80611-X
89. Watson PA, Reusch JE, Mccune SA, Leinwand LA, Luckey SW, Konhilas JP, et al. Restoration of CREB function is linked to completion and stabilization of adaptive cardiac hypertrophy in response to exercise. *Am J Physiol Heart Circ Physiol.* (2007) 293:H246–59. doi: 10.1152/ajpheart.00734.2006
90. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. *Proc Natl Acad Sci USA.* (2003) 100:7111–6. doi: 10.1073/pnas.1232352100
91. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1α transcriptional complex. *Nature.* (2007) 450:736–40. doi: 10.1038/nature06322
92. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet.* (2009) 10:295–304. doi: 10.1038/nrg2540
93. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res.* (2011) 21:381–95. doi: 10.1038/cr.2011.22
94. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature.* (2000) 403:41–5. doi: 10.1038/47412
95. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science.* (2013) 339:211–4. doi: 10.1126/science.1227166

96. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat Rev Cancer.* (2013) 13:572–83. doi: 10.1038/nrc3557
97. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell.* (2004) 119:941–53. doi: 10.1016/j.cell.2004.12.012
98. Walport LJ, Hopkinson RJ, Chowdhury R, Schiller R, Ge W, Kawamura A, et al. Arginine demethylation is catalysed by a subset of JmjC histone lysine demethylases. *Nat Commun.* (2016) 7:11974. doi: 10.1038/ncomms11974
99. Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes Dev.* (2012) 26:1326–38. doi: 10.1101/gad.191056.112
100. Sciacovelli M, Goncalves E, Johnson TI, Zecchini VR, Da Costa AS, Gaude E, et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature.* (2016) 537:544–7. doi: 10.1038/nature19353
101. Bird A. Perceptions of epigenetics. *Nature.* (2007) 447:396–8. doi: 10.1038/nature05913
102. Yerra VG, Advani A. Histones and heart failure in diabetes. *Cell Mol Life Sci.* (2018) 75:3193–213. doi: 10.1007/s00018-018-2857-1
103. Asrih M, Steffens S. Emerging role of epigenetics and miRNA in diabetic cardiomyopathy. *Cardiovasc Pathol.* (2013) 22:117–25. doi: 10.1016/j.carpath.2012.07.004
104. Abi Khalil C. The emerging role of epigenetics in cardiovascular disease. *Ther Adv Chronic Dis.* (2014) 5:178–87. doi: 10.1177/2040622314529325
105. Marin-Garcia J, Akhmedov AT. Epigenetics of the failing heart. *Heart Fail Rev.* (2015) 20:435–59. doi: 10.1007/s10741-015-9483-x
106. Franklin S, Kimball T, Rasmussen TL, Rosa Garrido M, Chen H, Tran T, et al. The chromatin binding protein Smyd1 restricts adult mammalian heart growth. *Am J Physiol Heart Circ Physiol.* (2016) 311:H1234–47. doi: 10.1152/ajpheart.00235.2016
107. Masuzawa R, Konno R, Ohsawa I, Watanabe A, Kawano F. Muscle type-specific RNA polymerase II recruitment during PGC-1α gene transcription after acute exercise in adult rats. *J Appl Physiol.* (2018) 125:238–1245. doi: 10.1152/japplphysiol.00202.2018
108. Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology.* (2013) 38:23–38. doi: 10.1038/npp.2012.112
109. Castillo RL, Herrera EA, Gonzalez-Candia A, Reyes-Farias M, De La Jara N, Pena JP, et al. Quercetin prevents diastolic dysfunction induced by a high-cholesterol diet: role of oxidative stress and bioenergetics in hyperglycemic rats. *Oxid Med Cell Longev.* (2018) 2018:7239123. doi: 10.1155/2018/7239123
110. Lochmann TL, Thomas RR, Bennett JP Jr, Taylor SM. Epigenetic modifications of the PGC-1α promoter during exercise induced expression in mice. *PLoS ONE.* (2015) 10:e0129647. doi: 10.1371/journal.pone.0129647
111. Kalea AZ, Drosatos K, Buxton JL. Nutriepigenetics and cardiovascular disease. *Curr Opin Clin Nutr Metab Care.* (2018) 21:252–9. doi: 10.1097/MCO.0000000000000477
112. Lee CF, Tian R. Mitochondrion as a target for heart failure therapy- role of protein lysine acetylation. *Circ J.* (2015) 79:1863–70. doi: 10.1253/circj.CJ-15-0742
113. Horton JL, Martin OJ, Lai L, Riley NM, Richards AL, Vega RB, et al. Mitochondrial protein hyperacetylation in the failing heart. *JCI Insight.* (2016) 2:e84897. doi: 10.1172/jci.insight.84897
114. Cooper HM, Spelbrink JN. The human SIRT3 protein deacetylase is exclusively mitochondrial. *Biochem J.* (2008) 411:279–85. doi: 10.1042/BJ20071624
115. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, et al. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol Cell Biol.* (2007) 27:8807–14. doi: 10.1128/MCB.01636-07
116. Lee CF, Chavez JD, Garcia-Menendez L, Choi Y, Roe ND, Chiao YA, et al. Normalization of NAD+ redox balance as a therapy for heart failure. *Circulation.* (2016) 134:883–94. doi: 10.1161/CIRCULATIONAHA.116.022495
117. Lai L, Leone TC, Keller MP, Martin OJ, Broman AT, Nigro J, et al. Energy metabolic reprogramming in the hypertrophied and early stage failing heart: a multisystems approach. *Circ Heart Fail.* (2014) 7:1022–31. doi: 10.1161/CIRCHEARTFAILURE.114.001469
118. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep.* (2017) 37:3–9. doi: 10.3892/or.2016.5236
119. Dong Y, Xu S, Liu J, Ponnusamy M, Zhao Y, Zhang Y, et al. Non-coding RNA-linked epigenetic regulation in cardiac hypertrophy. *Int J Biol Sci.* (2018) 14:1133–41. doi: 10.7150/ijbs.26215
120. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* (2009) 19:92–105. doi: 10.1101/gr.082701.108
121. Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, et al. The microRNA miR-696 regulates PGC-1{α} in mouse skeletal muscle in response to physical activity. *Am J Physiol Endocrinol Metab.* (2010) 298:E799–806. doi: 10.1152/ajpendo.00448.2009
122. Xu Y, Zhao C, Sun X, Liu Z, Zhang J. MicroRNA-761 regulates mitochondrial biogenesis in mouse skeletal muscle in response to exercise. *Biochem Biophys Res Commun.* (2015) 467:103–8. doi: 10.1016/j.bbrc.2015.09.113
123. He L, Tang M, Xiao T, Liu H, Liu W, Li G, et al. Obesity-associated miR-199a/214 cluster inhibits adipose browning via PRDM16-PGC-1α transcriptional network. *Diabetes.* (2018) 67:2585–600. doi: 10.2337/db18-0626
124. Xue T, Wei L, Zha DJ, Qiu JH, Chen FQ, Qiao L, et al. miR-29b overexpression induces cochlear hair cell apoptosis through the regulation of SIRT1/PGC-1α signaling: implications for age-related hearing loss. *Int J Mol Med.* (2016) 38:1387–94. doi: 10.3892/ijmm.2016.2735
125. Xue Y, Wei Z, Ding H, Wang Q, Zhou Z, Zheng S, et al. MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1α in the progression of atherosclerosis. *Atherosclerosis.* (2015) 241:671–81. doi: 10.1016/j.atherosclerosis.2015.06.031
126. Lou C, Xiao M, Cheng S, Lu X, Jia S, Ren Y, et al. MiR-485-3p and miR-485-5p suppress breast cancer cell metastasis by inhibiting PGC-1α expression. *Cell Death Dis.* (2016) 7:e2159. doi: 10.1038/cddis.2016.27
127. Martinez-Redondo V, Pettersson AT, Ruas JL. The hitchhiker's guide to PGC-1α isoform structure and biological functions. *Diabetologia.* (2015) 58:1969–77. doi: 10.1007/s00125-015-3671-z
128. Popov DV, Lysenko EA, Kuzmin IV, Vinogradova V, Grigoriev AI. Regulation of PGC-1α isoform expression in skeletal muscles. *Acta Nat.* (2015) 7:48–59. doi: 10.32607/20758251-2015-7-1-48-59
129. Yoshioka T, Inagaki K, Noguchi T, Sakai M, Ogawa W, Hosooka T, et al. Identification and characterization of an alternative promoter of the human PGC-1α gene. *Biochem Biophys Res Commun.* (2009) 381:537–43. doi: 10.1016/j.bbrc.2009.02.077
130. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, et al. A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell.* (2012) 151:1319–31. doi: 10.1016/j.cell.2012.10.050
131. Zhang Y, Huypens P, Adamson AW, Chang JS, Henagan TM, Boudreau A, et al. Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1α. *J Biol Chem.* (2009) 284:32813–26. doi: 10.1074/jbc.M109.037556
132. Miura S, Kai Y, Kamei Y, Ezaki O. Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) mRNA in response to beta2-adrenergic receptor activation and exercise. *Endocrinology.* (2008) 149:4527–33. doi: 10.1210/en.2008-0466
133. Norrbom J, Sallstedt EK, Fischer H, Sundberg CJ, Rundqvist H, Gustafsson T. Alternative splice variant PGC-1α-b is strongly induced by exercise in human skeletal muscle. *Am J Physiol Endocrinol Metab.* (2011) 301:E1092–8. doi: 10.1152/ajpendo.00119.2011
134. Nader GA, Von Walden F, Liu C, Lindvall J, Gutmann L, Pistilli EE, et al. Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. *J Appl Physiol.* (2014) 116:693–702. doi: 10.1152/japplphysiol.01366.2013
135. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol.* (1993) 9:317–43. doi: 10.1146/annurev.cb.09.110193.001533
136. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, et al. Cytokine stimulation of energy expenditure through p38 MAP kinase

activation of PPARgamma coactivator-1. *Mol Cell.* (2001) 8:971–82. doi: 10.1016/S1097-2765(01)00390-2
137. Lei B, Chess DJ, Keung W, O'shea KM, Lopaschuk GD, Stanley WC. Transient activation of p38 MAP kinase and up-regulation of Pim-1 kinase in cardiac hypertrophy despite no activation of AMPK. *J Mol Cell Cardiol.* (2008) 45:404–10. doi: 10.1016/j.yjmcc.2008.06.008
138. Kojonazarov B, Novoyatleva T, Boehm M, Happe C, Sibinska Z, Tian X, et al. p38 MAPK inhibition improves heart function in pressure-loaded right ventricular hypertrophy. *Am J Respir Cell Mol Biol.* (2017) 57:603–14. doi: 10.1165/rcmb.2016-0374OC
139. Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P. Metabolic adaptations through the PGC-1 α and SIRT1 pathways. *FEBS Lett.* (2008) 582:46–53. doi: 10.1016/j.febslet.2007.11.034
140. Philp A, Chen A, Lan D, Meyer GA, Murphy AN, Knapp AE, et al. Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) deacetylation following endurance exercise. *J Biol Chem.* (2011) 286:30561–70. doi: 10.1074/jbc.M111.261685
141. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol.* (2000) 20:1868–76. doi: 10.1128/MCB.20.5.1868-1876.2000
142. Huss JM, Kopp RP, Kelly DP. Peroxisome proliferator-activated receptor coactivator-1α (PGC-1α) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor-α and -gamma. Identification of novel leucine-rich interaction motif within PGC-1α. *J Biol Chem.* (2002) 277:40265–74. doi: 10.1074/jbc.M206324200
143. Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, et al. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1α interaction. *Nature.* (2003) 423:550–5. doi: 10.1038/nature01667
144. Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'malley B, et al. Activation of PPARgamma coactivator-1 through transcription factor docking. *Science.* (1999) 286:1368–71. doi: 10.1126/science.286.5443.1368
145. Wallberg AE, Yamamura S, Malik S, Spiegelman BM, Roeder RG. Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1α. *Mol Cell.* (2003) 12:1137–49. doi: 10.1016/S1097-2765(03)00391-5
146. Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M, Spiegelman BM. Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. *Mol Cell.* (2000) 6:307–16. doi: 10.1016/S1097-2765(00)00031-9
147. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation.* (2007) 116:434–48. doi: 10.1161/CIRCULATIONAHA.107.702795
148. Yndestad A, Damas JK, Oie E, Ueland T, Gullestad L, Aukrust P. Systemic inflammation in heart failure–the whys and wherefores. *Heart Fail Rev.* (2006) 11:83–92. doi: 10.1007/s10741-006-9196-2
149. Liang H, Ward WF. PGC-1α: a key regulator of energy metabolism. *Adv Physiol Educ.* (2006) 30:145–51. doi: 10.1152/advan.00052.2006
150. Chakraborty J, Rajamma U, Mohanakumar KP. A mitochondrial basis for Huntington's disease: therapeutic prospects. *Mol Cell Biochem.* (2014) 389:277–91. doi: 10.1007/s11010-013-1951-9
151. Zizola C, Schulze PC. Metabolic and structural impairment of skeletal muscle in heart failure. *Heart Fail Rev.* (2013) 18:623–30. doi: 10.1007/s10741-012-9353-8
152. Zechner C, Lai L, Zechner JF, Geng T, Yan Z, Rumsey JW, et al. Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. *Cell Metab.* (2010) 12:633–42. doi: 10.1016/j.cmet.2010.11.008
153. Handschin C, Choi CS, Chin S, Kim S, Kawamori D, Kurpad AJ, et al. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1α knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J Clin Invest.* (2007) 117:3463–74. doi: 10.1172/JCI31785
154. Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM. Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab.* (2006) 3:333–41. doi: 10.1016/j.cmet.2006.04.002

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer, KD, declared a past co-authorship with one of the authors, SO, to the handling editor.

frontiers in Cardiovascular Medicine

REVIEW
published: 18 February 2020
doi: 10.3389/fcvm.2020.00012

Mitochondrial ROS Formation in the Pathogenesis of Diabetic Cardiomyopathy

Nina Kaludercic[1]* *and Fabio Di Lisa*[1,2]

[1] Neuroscience Institute, National Research Council of Italy (CNR), Padua, Italy, [2] Department of Biomedical Sciences, University of Padua, Padua, Italy

Diabetic cardiomyopathy is a result of diabetes-induced changes in the structure and function of the heart. Hyperglycemia affects multiple pathways in the diabetic heart, but excessive reactive oxygen species (ROS) generation and oxidative stress represent common denominators associated with adverse tissue remodeling. Indeed, key processes underlying cardiac remodeling in diabetes are redox sensitive, including inflammation, organelle dysfunction, alteration in ion homeostasis, cardiomyocyte hypertrophy, apoptosis, fibrosis, and contractile dysfunction. Extensive experimental evidence supports the involvement of mitochondrial ROS formation in the alterations characterizing the diabetic heart. In this review we will outline the central role of mitochondrial ROS and alterations in the redox status contributing to the development of diabetic cardiomyopathy. We will discuss the role of different sources of ROS involved in this process, with a specific emphasis on mitochondrial ROS producing enzymes within cardiomyocytes. Finally, the therapeutic potential of pharmacological inhibitors of ROS sources within the mitochondria will be discussed.

Keywords: diabetic cardiomyopathy, reactive oxygen species, mitochondria, oxidative stress, diabetic complication

OPEN ACCESS

Edited by:
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Reviewed by:
Susumu Minamisawa, Jikei University School of Medicine, Japan
Yasuhiro Maejima, Tokyo Medical and Dental University, Japan
Leonardo Schirone, Sapienza University of Rome, Italy
Jun Ren, University of Wyoming, United States

***Correspondence:**
Nina Kaludercic
nina.kaludercic@unipd.it

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *31 October 2019*
Accepted: *28 January 2020*
Published: *18 February 2020*

Citation:
Kaludercic N and Di Lisa F (2020) Mitochondrial ROS Formation in the Pathogenesis of Diabetic Cardiomyopathy. Front. Cardiovasc. Med. 7:12. doi: 10.3389/fcvm.2020.00012

INTRODUCTION

Chronic hyperglycemia, the major characteristic of type 1 diabetes (T1D), is a life-threatening risk factor that results in organ and tissue damage in the long term. One of the acute metabolic complications associated with mortality includes diabetic ketoacidosis occurring mainly in T1D (1). Instead, type 2 diabetes (T2D) and obesity are characterized by insulin resistance, hyperlipidemia and hyperinsulinemia that might occur before the onset of hyperglycemia. The heart is an insulin-dependent tissue, since insulin promotes glucose utilization and suppresses fatty acid utilization thereby conferring a certain level of metabolic flexibility, i.e., the ability to adapt substrate oxidation rates to substrate availability, in support of cardiac function (2). This metabolic flexibility is largely impaired in diabetic hearts, resulting in minimal glucose utilization, shift to free fatty acid utilization and energetic inefficiency (3). Vascular complications occurring in diabetes account for increased morbidity and mortality associated with this disease. In the long term, diabetes may cause microvascular disease, resulting from the damage of small blood vessels, and/or macrovascular disease, resulting from the damage of the arteries (4). The latter includes coronary artery disease, peripheral arterial disease, and stroke, while microvascular complications result in retinopathy, nephropathy and neuropathy. Diabetic cardiomyopathy (DCM) is a pathology associated with alterations in the

myocardial structure and function without the coexistence of other cardiac risk factors such as coronary artery disease, hypertension, valvular disease (5). DCM is one of the deadliest complications associated with diabetes (1). The incidence of heart failure is increased in diabetic patients compared with age-matched individuals, independently of obesity, hypertension, dyslipidemia, and coronary artery disease (6). In addition, diabetes has also been associated with increased rates of cancer, physical and cognitive disability, tuberculosis and depression (7–12).

Reactive oxygen species (ROS) and oxidative stress have been linked both to the onset of diabetes and development of complications associated with this disease (13). Here, we will review the pathophysiological features of DCM, the evidence related to the contribution of ROS to DCM and the role of different sources of ROS involved in this process. The present review will focus on mitochondrial sources of ROS in cardiac myocytes (rather than other cell types in the heart) and will briefly discuss the advantages and disadvantages of targeting mitochondrial enzymes to prevent oxidant damage and postpone or prevent the development of cardiac complications in diabetes.

DIABETIC CARDIOMYOPATHY

DCM is a result of diabetes-induced changes in the structure and function of the heart and is diagnosed only if there is cardiac dysfunction not associated with coronary artery disease (14). The clinical outcomes associated with ischemic heart disease, hypertension or heart failure are worse for patients with diabetes and indeed, cardiovascular complications are the leading cause of mortality and morbidity in diabetic patients (5, 15, 16). Thus, a better understanding of DCM-associated pathophysiology and underlying mechanisms is necessary in order to develop tools for early diagnosis and treatment strategies.

As an early complication of diabetes, DCM is characterized by a long latent phase during which the disease progresses, but is completely asymptomatic. This subclinical period includes an increase in the left ventricle (LV) mass, fibrosis, abnormalities in cell signaling and diastolic dysfunction (3, 5). Studies using magnetic resonance imaging demonstrated that hyperglycemia and insulin resistance are associated with an increase in LV mass (3, 17). The increase in cardiac stiffness and fibrosis detected in diabetic patients frequently evolves to heart failure with preserved ejection fraction (HFpEF) (18, 19). In some patients, diastolic dysfunction may progress to pump failure and impairment in systolic function resulting in heart failure with reduced ejection fraction (20, 21). Nevertheless, not all cardiac anomalies observed in T2D are recapitulated in T1D (22, 23). While T2D is characterized by both morphological and functional cardiac abnormalities in patients (i.e., LV hypertrophy, diastolic, and systolic dysfunction), T1D patients show intact systolic function and impairment in diastolic function (23). Moreover, not all studies conducted in T1D patients evidenced an impairment in diastolic function. This may be explained by the fact that T1D patients are normally treated with insulin that normalizes insulin-dependent metabolic processes and therefore likely renders T1D-induced alterations in the heart less evident (23). Regardless of these differences, clinical trials showed that the prevalence of heart failure in diabetic patients ranges from 19 to 26% (24–27), while the mortality rate is 15–20% in diabetic patients with systolic dysfunction (21).

Although the exact mechanism of diabetes-associated LV dysfunction is not known, it appears that hyperglycemia, hyperinsulinemia, and/or lipotoxicity initiate a series of adaptive and maladaptive processes contributing to the development of heart failure. Factors underlying pathological changes in the diabetic heart are multiple. Metabolic alterations such as hyperglycemia, insulin resistance and increased free fatty acid levels, result in the oxidative stress, organelle dysfunction, inflammation, advanced glycation end products (AGEs) formation, activation of protein kinase C (PKC), abnormalities in ion homeostasis, alterations in structural proteins, apoptosis and fibrosis, changes that eventually result in diabetes-induced cardiac dysfunction (**Figure 1**) (28, 29). Despite a myriad of factors has been shown to collectively contribute to the development and progression of DCM, causal relationships and the exact sequence of events among these cellular and molecular mechanisms are still not entirely clear. Moreover, these factors frequently interact with each other, making DCM a very complex disease to treat.

ROS: A COMMON DENOMINATOR IN DIABETES-INDUCED COMPLICATIONS

ROS formation has gained significant experimental and clinical evaluation amongst the various mechanisms proposed (13, 20, 30). Notably, the aforementioned pathogenic factors and changes either induce or result from oxidative stress. ROS can be dangerous for biological systems for their capacity to interact with numerous macromolecules, such as proteins, lipids and DNA. ROS-induced modification of DNA can be mutagenic, especially if DNA damage cannot be repaired (31). ROS may lead to DNA strand breakage and formation of 8-hydroxydeoxyguanosine, a prominent feature in diabetic hearts (32, 33). While protein oxidation can be reversible and serve for signaling purposes, oxidative stress may lead to protein carbonylation that cannot be reversed and results in toxic aggregate accumulation if carbonylated molecules are not promptly degraded (34, 35). Membrane lipids are rich in polyunsaturated fatty acids that can easily be oxidized by ROS, a process that is also involved in the generation of atherosclerotic plaques (36). Lipid oxidation results in excess formation of carbonyl compounds, such as prostanoids and aldehydes, toxic metabolites that can promote numerous pathologies (37). In addition to direct macromolecule targeting, high ROS formation can also decrease the antioxidant capacity of the diabetic myocardium, contributing thereby to oxidative stress and resulting myocardial damage. This concept is further supported by studies demonstrating that overexpression of antioxidant defense proteins, such as metallothionein or catalase, could prevent oxidative stress and maladaptive

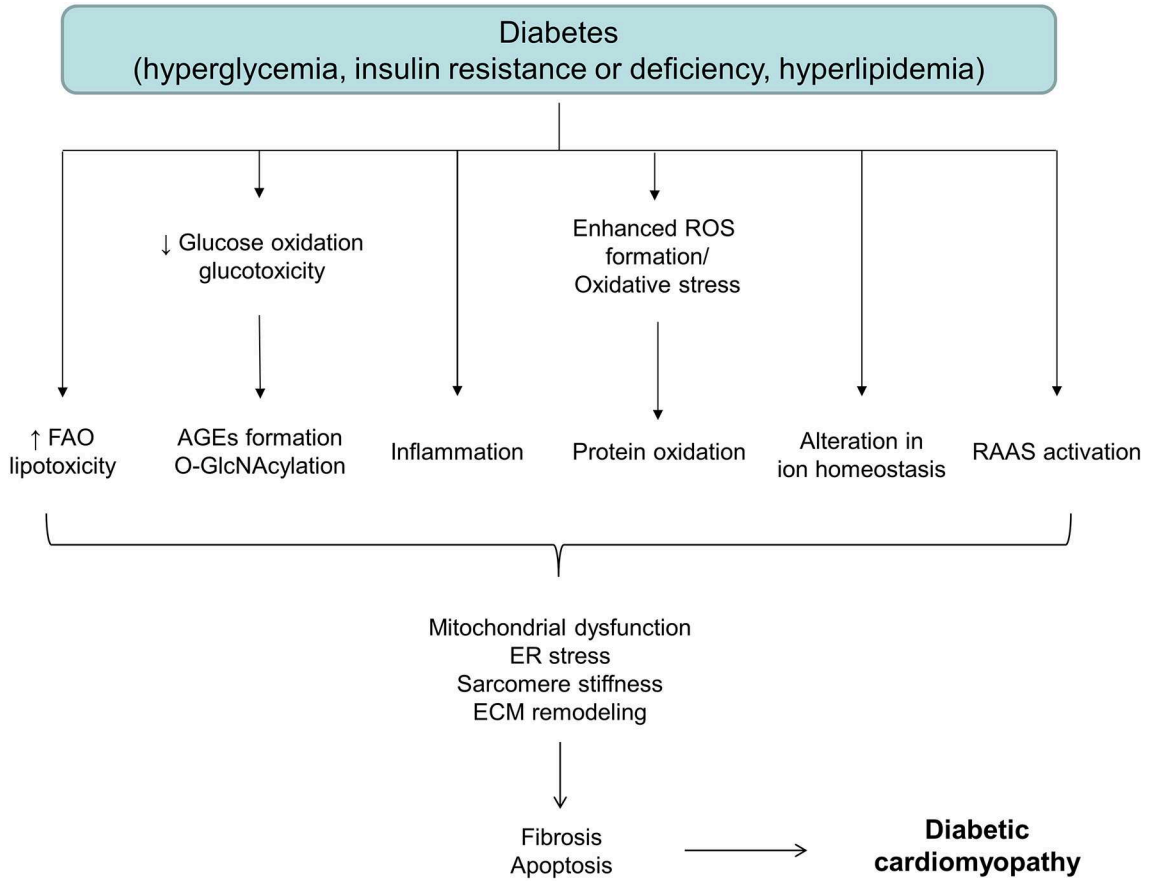


FIGURE 1 | A schematic diagram depicting different factors involved in the onset and development of diabetic cardiomyopathy. AGEs, advanced glycation end products; ECM, extracellular matrix; ER, endoplasmic reticulum; FAO, fatty acid oxidation; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species.

remodeling of the diabetic hearts (22, 38–41). Mitochondrial ROS production underlies several hyperglycemia-induced pathogenic mechanisms, such as GAPDH inhibition, activation of polyol pathway, formation of AGEs, activation of PKC, glucose auto-oxidation, and activation of the 12/15-lipoxygenases pathway (13, 30, 42, 43). Activation of these pathways can, in turn, exacerbate oxidative stress. For instance, the polyol pathway utilizes NAPDH which is required for GSH regeneration, while binding of AGEs to their receptor results in ROS formation (44). Inhibition of AGE formation or AGE receptor gene knock-down attenuates the development of DCM (45). Moreover, activation of p53 signaling in T1D and T2D mouse models by an initial oxidative trigger leads to the upregulation of cytochrome c oxidase assembly protein, mitochondrial respiration, fatty acid oxidation, and mitochondrial ROS generation (46). However, hyperglycemia is not the only factor responsible for cardiac complications in diabetes, as mentioned before. Lipotoxicity and increased oxidation of free fatty acids also lead to oxidative stress, mitochondrial and ER stress, and activation of pro-inflammatory signals (47–51). Damage to mitochondria results in enhanced ROS generation and activation of the NLRP3 inflammasome (52) which, in turn, may promote or exacerbate cardiac fibrosis. Moreover, high glucose and inflammation provide a synergistic effect and further enhance ROS formation and all the downstream events leading to cell dysfunction (53, 54). Inflammation, angiogenesis, cardiomyocyte hypertrophy and apoptosis, fibrosis and contractile dysfunction, are processes susceptible to ROS-dependent modulation in the diabetic heart (55). Diastolic abnormalities observed in HFpEF are largely due to increased collagen and cardiomyocyte stiffness (56). ROS are well-known to target sarcomere proteins thereby inducing oxidative changes that may impact on sarcomere and cardiomyocyte stiffness (57, 58). While oxidation of the proteins forming the thick and thin filaments is mostly associated with impaired contractility, post-translational modifications of the elastic filament protein titin are tightly related to changes in LV stiffness (59). The passive stiffness of cardiac muscle was shown to be redox-dependent through titin oxidation and disulfide bridge formation that lead to increased cardiac stiffness (60). In addition to direct mechanisms, ROS can modulate sarcomere function also by affecting key protein kinases or phosphatases to induce post-translational

modifications (57). In that regard, reduced titin phosphorylation is an important determinant of diastolic stiffness in HFpEF (59, 61, 62). This is particularly relevant in diabetic and obese patients in which oxidative stress impairs NO/cGMP/PKG signaling and leads to titin hypophosphorylation (59, 63) and increased cardiomyocyte stiffness along with collagen and AGEs deposition (59). Thus, enhanced ROS formation and alteration in the redox status are deeply intertwined with numerous alterations observed in diabetic hearts, suggesting that targeting ROS formation/elimination may represent an attractive therapeutic strategy for the treatment of DCM. Several cellular and subcellular sources that may account for enhanced ROS production were described in diabetic cardiovascular system and other tissues. Enzymes involved in deleterious ROS generation associated with diabetic complications include nicotinamide adenine dinucleotide phosphate oxidases (NOXs) (64–66), xanthine oxidase/oxidoreductase (XO) (67, 68), arachidonic acid cascade and microsomal enzymes, uncoupled nitric oxide synthase (NOS) (69), and mitochondria (13, 70–72).

NOX is a family of membrane-bound enzyme complexes composed of plasma membrane spanning and cytosolic components (73, 74). The active NOX complex allows for the transfer of electrons to molecular oxygen to generate superoxide (75). NOXs are considered to be one of the major cellular ROS sources and prominent players in several pathological conditions (74, 76, 77). NOX2, located in the cell membrane, and NOX4, localized in perinuclear ER and/or mitochondria, are expressed in the heart (78, 79). Increased cardiac NOX2 expression/activity has been described both in T1D and T2D, and contributes to hyperglycemia-induced ROS production (64–66, 80). NOX4 expression and NOX4-derived ROS are increased ∼14 days after the induction of T1D in rats and contribute to the development of cardiomyopathy (81). Importantly, reducing either NOX2 or NOX4 activity in streptozotocin-induced diabetic hearts blunts myocardial oxidative stress, remodeling and improves cardiac function (81–83). ROS formation through NOX following high glucose administration has been associated with pathways involving sodium/glucose co-transporter 1 (SGLT1), PKCβ, and calcium/calmodulin dependent kinase II (CaMKII) (84). SGLT1-mediated glucose transport is responsible for NOX2 activation, since its inhibition efficiently abolished ROS production induced by exposure to high glucose (85). Importantly, PKCβ activation by RhoA/Rho kinase pathway activates Rac1 that, in turn, determines $p47^{phox}$ translocation to the membrane, event required for NOX2 activation (86). Indeed, PKCβ inhibition by ruboxistaurin prevented NOX2 activation and subsequent ROS formation in cardiomyocytes treated with high glucose (87). An additional mechanism responsible for NOX activation in hyperglycemic conditions involves CaMKII activation. High glucose causes an increase in intracellular levels of Ca^{2+} that leads to CaMKII hyperphosphorylation and activation (32). Activated CaMKII is likely responsible for activation of PKCβ and downstream cascade of events (86). In that regard, inhibition of CaMKII prevented both the upregulation of $p47^{phox}$ and $p67^{phox}$ as well as oxidative stress in streptozotocin-induced model of T1D (32), suggesting that CaMKII may indeed play a major role in NOX-induced ROS formation.

XO is a cytoplasmic enzyme that catalyzes the oxidation of hypoxanthine to xanthine and further converts xanthine to uric acid (88). It uses oxygen as electron acceptor and produces superoxide and hydrogen peroxide (H_2O_2). In addition to their role in cardiac damage induced by ischemia/reperfusion injury or pacing-induced heart failure in dogs (89), hypoxanthine and XO activity are also increased in diabetic subjects (90). The role of XO in hyperglycemia-induced oxidative stress is documented by increased ROS formation in the muscle and development of fibrosis of hyperglycemic streptozotocin-induced diabetic mice (68, 91, 92). Some investigators reported evidence for beneficial vascular effects of XO inhibitors in hypercholesterolemic and diabetic patients (72, 93). Indeed, in T1D patients XO inhibition reduced the degree of oxidative stress, whereas in T2D patients it led to significant improvements in peripheral endothelium-dependent vasorelaxation (67, 90, 93).

NOS uncoupling results in superoxide formation, oxidative stress and decreased NO bioavailability that may have important vascular effects in diabetic subjects (94). Indeed, a decrease in the dimer to monomer ratio, indicative of the enzyme uncoupling, has been reported within the myocardium of diabetic animals (95). Consequently, inhibition of NOS activity and uncoupling by L-NAME, insulin-like growth factor, sepiapterin, ascorbic acid or N-acetyl-cysteine improved LV function in the diabetic heart (66, 96–100). In addition to uncoupling, NOS expression may also be increased in the diabetic hearts (33, 64, 101) and this is associated with an increase in lipid peroxidation and peroxynitrite generation (72). Peroxynitrite in turn may also lead to NOS uncoupling (102). Taken together, these studies suggest that the increased production of superoxide and peroxynitrite through NOS uncoupling is a major contributor to suppressed contractile performance in diabetes (72, 99, 100).

For detailed discussion related to XO, NOX or uncoupled NOS involvement in DCM, readers are referred to other excellent reviews (67, 72, 74, 84).

MITOCHONDRIAL ROS FORMATION IN DCM

The role of mitochondrial ROS formation and dysfunction in the pathogenesis of diabetes and its complications is well-established (13, 20, 28). Indeed, cardiac mitochondria from diabetic patients are dysfunctional, displaying increased mitochondrial H_2O_2 emission, impaired mitochondrial respiratory capacity and increased levels of oxidized or hydroxynonenal-modified proteins (103–105). Several mechanisms are likely responsible for mitochondrial dysfunction in diabetic hearts, including fatty acid-induced mitochondrial uncoupling, changes in mitochondrial morphology, increased ROS formation, mitochondrial proteome remodeling, impaired mitochondrial calcium handling and altered mitochondrial turnover (20, 28, 106–108). All these events might lead to compromised cardiac ATP generation and ultimately to cardiac dysfunction. Impairment in the activity of ATP synthase also

affects mitochondrial function in the diabetic heart. A recent study very elegantly showed that hyperglycemia-induced calpain-1 upregulation in the mitochondria cleaves the ATP synthase α subunit, resulting in the reduction in the ATP synthase activity and increased mitochondrial ROS formation (109) that eventually contribute to the development of DCM. In addition, excessive mitochondrial ROS formation results in the increased propensity to permeability transition pore (PTP) opening that eventually leads to cell death (110). A tight relationship also exists between alterations in mitochondrial morphology and ROS formation that may reciprocally modulate each other. Cardiomyocytes from animal models of T1D, T2D, and from diabetic patients show increased levels of ROS and altered mitochondrial morphology, including mitochondrial fragmentation, cristae disruption and swelling (107, 108). Of interest, mitochondrial fragmentation induced by chronic hyperglycemia can be reversed with antioxidants, suggesting that ROS are causally related to this pro-fission phenotype and that controlling mitochondrial morphology and dynamics might represent a therapeutic strategy for the treatment of DCM (107, 111). Altered mitochondrial function may inhibit insulin signaling by interfering with oxidation of fatty acyl-CoA, accumulation of intracellular lipid and diacylglycerol, PKC activation and through generation of ROS (112). Both processes lead to insulin receptor substrate 1 phosphorylation and interference with insulin signal transduction. Reduction in mitochondrial ROS formation obtained either through cardiac-specific Mn-SOD overexpression or following stimulation of AMPK activity, prevented mitochondrial damage and many fatty acid- or hyperglycemia-induced events, both *in vitro* and *in vivo* (113–116).

Given the tight relationship between mitochondrial ROS formation, structure/function, and diabetes-induced complications, it is crucial to dissect and identify sites responsible for ROS formation in mitochondria exposed to diabetic milieu. Electron transport chain (ETC), $p66^{Shc}$, and monoamine oxidase (MAO) are the major sources of ROS formation in mitochondria (**Figure 2**).

Electron Transport Chain

ETC is by far the major site of ATP production in mitochondria inside any given cell, and especially in cardiomyocytes (more than 90%). At the inner mitochondrial membrane (IMM), electrons from NADH and $FADH_2$ are transferred across the respiratory chain to oxygen, which is reduced to water at the level of complex IV (117). This process powers the movement of protons into the intermembrane space and generates a proton gradient that drives the synthesis of ATP by the ATP synthase. A small amount of electrons (about 0.1%) can leak from the ETC and cause superoxide formation due to the partial reduction of oxygen (118). Superoxide generation may occur under conditions that decrease the flow of electrons, particularly at the level of the first three complexes where flavins or quinones are able to act as single electron donors (117, 119, 120). Notably, ROS formation can also result from the reverse electron flow through complex I (121). A recent study supported this pathophysiological concept demonstrating that succinate accumulates during cardiac ischemia *in vivo* (121, 122). Upon reperfusion, accumulated succinate is oxidized by complex II leading to dramatic ROS formation that is likely attributable to the reverse electron flow through complex I (122).

Seminal discoveries implicating ETC superoxide production as the central event in hyperglycemia-induced pathogenic mechanisms were provided by Brownlee's group back in 2000 using endothelial cells (123, 124). High intracellular glucose levels and glucose-derived pyruvate promote mitochondrial respiration by increasing the availability of reducing equivalents for the ETC and resulting in mitochondrial membrane hyperpolarization and superoxide production (123, 125). Furthermore, hyperglycemia-induced ROS formation is prevented by several interventions, such as via inhibition of ETC complex II activity, uncoupling of oxidative phosphorylation, by overexpression of uncoupling protein-1 and/or Mn-SOD (123). Normalizing levels of mitochondrial ROS with each of these agents prevents glucose-induced activation of PKC, hexosamine pathway, formation of AGEs, sorbitol accumulation, and NFκB activation. A further confirmation that ETC superoxide production is responsible for these events comes from experiments performed in Rho zero (ρ0) endothelial cells lacking mitochondrial ETC function (30). When exposed to high glucose, ρ0 cells do not display an increase in ROS production. Similar mechanism has also been shown to be at play in cardiomyocytes exposed to high glucose. Indeed, ROS formation is reduced in cardiomyocytes isolated from diabetic animals in which complex I and II activity is inhibited or which overexpress catalase, further denoting the crucial role of ETC in ROS generation in diabetes (41, 86, 126). Interestingly, the protective effect afforded by complex I or II inhibition suggests that ETC superoxide production upon high glucose exposure likely occurs through the reverse electron transport. It remains to be elucidated whether succinate accumulation occurs at some point during the development of cardiovascular complications in diabetes. Cardiac lipotoxicity is also mediated by mitochondrial ROS formation. Indeed, exposure to palmitate enhances mitochondrial ROS generation and leads to increased mitochondrial fission by modulating DRP1 phosphorylation levels and proteolytic processing of OPA1 (47).

An initial ROS trigger produced by ETC can promote activation of processes that eventually amplify the signal and lead to oxidative stress. Such processes involve the occurrence of post-translational modifications, such as (but not limited to) diabetes–induced defects caused by oxidation, increased methylglyoxal adduct formation and increased O-GlcNAcylation, that contribute to the impairment in mitochondrial and systolic function (127–129). Hyperglycemia alters the function of respiratory chain in mitochondria via dysregulation of O-GlcNAcylation (130, 131). O-GlcNAc transferase (OGT) enzyme is located in the IMM and interacts with complex IV of the respiratory chain in normal conditions. In streptozotocin-treated rats this enzyme is improperly localized to the mitochondrial matrix and the impairment in the OGT-complex IV interaction results in the loss of complex IV activity and reduced mitochondrial membrane potential (130). O-GlcNAcylation of proteins involved in

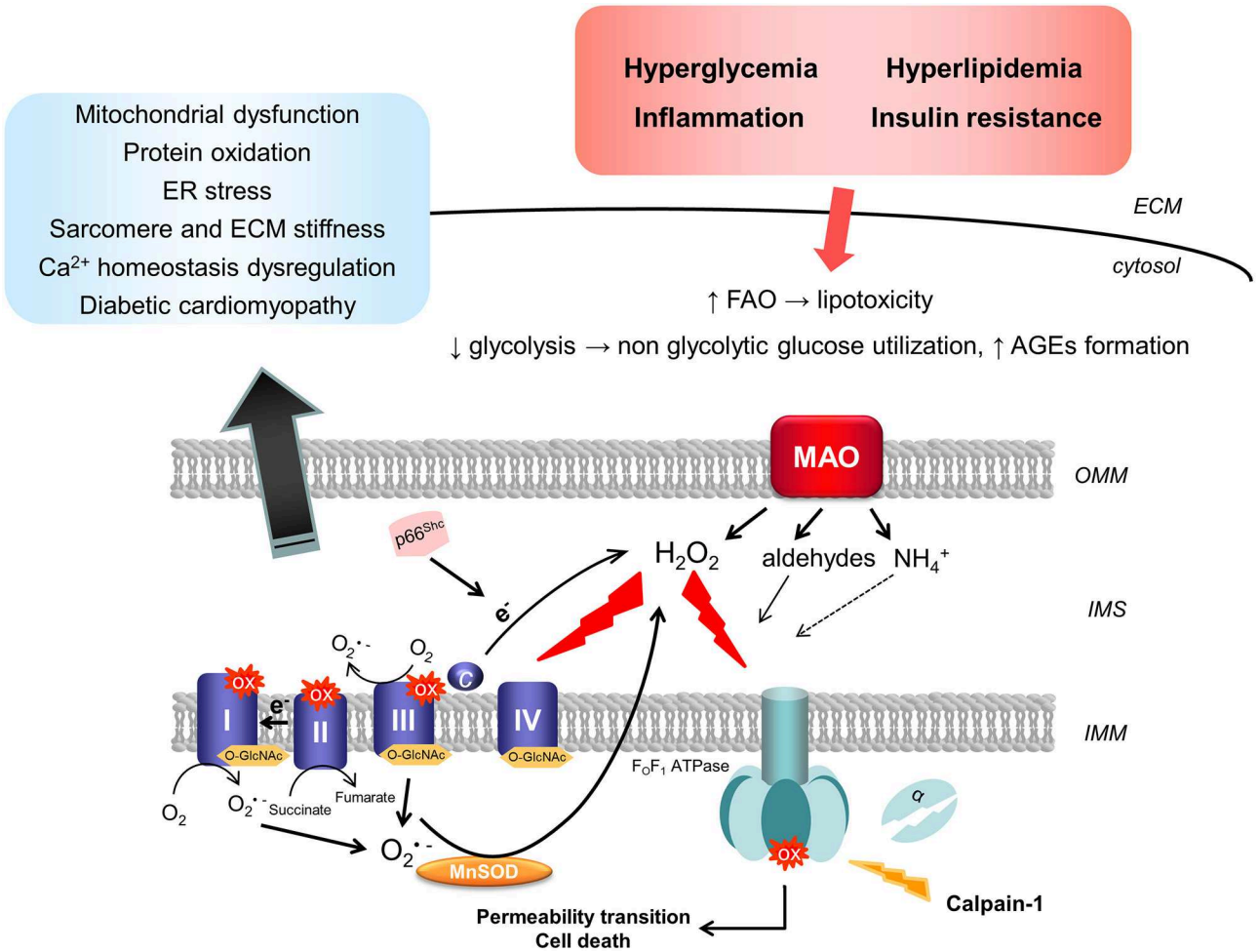


FIGURE 2 | Mitochondrial sources of ROS in diabetic cardiomyopathy. Diabetic milieu, characterized by hyperglycemia, hyperlipidemia, and inflammation, results in the up-regulation in the activity of mitochondrial ROS-producing enzymes. Superoxide can be produced by the respiratory chain through forward or reverse electron transport. In addition, calpain-1 translocates to the mitochondrial matrix in the diabetic heart and cleaves the α subunit of the ATP synthase, leading therefore to the reduction in its activity and mitochondrial dysfunction. On the other hand, in situations of stress, $p66^{Shc}$ is phosphorylated and translocates to the IMS where it catalyzes the electron transfer from cytochrome c to oxygen (O_2) leading to the formation of hydrogen peroxide (H_2O_2). Finally, up-regulation of MAO activity upon exposure to high glucose and pro-inflammatory stimuli results in enhanced formation of H_2O_2 that can directly increase the susceptibility of mitochondria to undergo permeability transition. Post-translational modifications, such as oxidation or O-GlcNAcylation of respiratory chain complexes, can impair mitochondrial bioenergetics and function. All these events are implicated in the pathogenesis of diabetic cardiomyopathy by promoting mitochondrial and ER stress, leading to protein oxidation and Ca^{2+} homeostasis impairment, as well as sarcomere and ECM stiffness. AGEs, advanced glycation end products; ECM, extracellular matrix; ER, endoplasmic reticulum; FAO, fatty acid oxidation; IMM, inner mitochondrial membrane; IMS, intermembrane space; MAO, monoamine oxidase; MnSOD, manganese superoxide dismutase; OMM, outer mitochondrial membrane; O-GlcNAc, β-linked N-acetylglucosamine; ox, oxidation.

mitochondrial dynamics, such as DRP-1 and OPA1, also contributes to mitochondrial fragmentation that further exacerbates organelle dysfunction (132, 133). On the other hand, methylglyoxal-induced modifications affect Ca^{2+} homeostasis and indirectly affect mitochondrial function. Indeed, in the diabetic heart methylglyoxal preferentially forms adducts with proteins involved in the intracellular calcium handling such as ryanodine receptor 2 and SERCA2a (134, 135). Ryanodine receptor glycation is associated with impaired Ca^{2+} cycling, increased mitochondrial Ca^{2+} levels and mitochondrial dysfunction (136). Collectively, these studies underline the importance of ETC-derived superoxide in diabetic conditions and mitochondria as their source and target.

$p66^{Shc}$

$p66^{Shc}$ is another important source of ROS in mitochondria. $p66^{Shc}$ is a cytosolic adaptor protein and, along with $p46^{Shc}$ and $p52^{Shc}$, is encoded by the ShcA gene (137, 138). $p46^{Shc}$ and $p52^{Shc}$ isoforms are formed through alternative translation start sites (137, 139). While $p46^{Shc}$ and $p52^{Shc}$ isoforms are ubiquitously expressed, $p66^{Shc}$ promoter may bear epigenetic modifications resulting in cell type- or specific condition-restricted expression (140). Under stress conditions, PKCβ phosphorylates $p66^{Shc}$ at

Ser-36, event required for its translocation to mitochondria (141). Once in the intermembrane space, $p66^{Shc}$ catalyzes the electron transfer from cytochrome c to oxygen resulting in the formation of H_2O_2 (142). In addition to this mechanism, $p66^{Shc}$ can promote oxidative stress by activating membrane-bound NOX or through down-regulation of antioxidant enzymes synthesis (143). Accordingly, cells and mice lacking $p66^{Shc}$ show reduction in markers of oxidative stress (139, 144).

A number of studies characterized the pathophysiological role of $p66^{Shc}$ in cardiovascular diseases, such as maladaptive hypertrophy, heart failure and ischemia/reperfusion injury (137–139, 145). Importantly, excessive ROS generation is a major contributing factor to those cardiac pathologies (146). Since PKC activation plays a major role in the intracellular signaling leading to oxidative stress, cell dysfunction and tissue damage in hyperglycemia, and is required for $p66^{Shc}$ translocation to mitochondria in response to stress (70), it is tempting to hypothesize that $p66^{Shc}$ may play a role in cardiovascular complications induced by hyperglycemia acting as a downstream target following high glucose-induced PKCβ activation. Indeed, $p66^{Shc-/-}$ mice have an increased resistance to ROS (70), less atherosclerosis and preserved aortic endothelium-dependent vasorelaxation following high-fat diet and in a model of streptozotocin-induced T1D (147, 148). Moreover, lack of $p66^{Shc}$ prevented oxidative damage in cardiac progenitor cells and cardiomyocytes in streptozotocin-induced DCM (149). Unlike diabetic wild type animals characterized by cardiomyocyte loss, diabetic $p66^{Shc-/-}$ hearts displayed preserved cardiac progenitor cell replication and turnover, along with unaltered wall thickness, chamber volume, LV end-diastolic pressure and diastolic wall stress (149).

Monoamine Oxidases

Monoamine oxidases (MAOs) are flavoenzymes localized at the level of the outer mitochondrial membrane. MAOs exist in two isoforms, A and B, differing in structure, substrate preference, inhibitor specificity and tissue distribution (150–153). The physiological role of MAOs consists in the catalysis of the oxidative deamination of its substrates (i.e., endogenous and exogenous amines, neurotransmitters). MAOs generate H_2O_2, ammonia and corresponding aldehydes as products of catalysis (154, 155). Over the last decade, several studies have shown that alterations in redox balance cause by enhanced MAO activity play a prominent role in promoting the development of cardiovascular disorders and causing oxidative damage to cardiomyocytes (37, 146, 156–158). Indeed, MAO contributes to ischemia/reperfusion injury, maladaptive hypertrophy, heart failure and vascular dysfunction (37, 139, 159–162). Of note, evidence for MAO involvement in cardiac disease has also been demonstrated in patients. Up-regulation of MAO activity and consequent ROS formation has been identified as a prominent contributor to the impaired myocardial redox balance in patients and a major risk factor and predictor for the postoperative atrial fibrillation (163). In addition, MAO activity was shown to be increased in left and right ventricles from patients with ischemic heart disease (164). With regard to the possible involvement of MAO in diabetes, one study showed an improvement in blood glucose levels and systolic and diastolic pressures in a patient with T1D administered with the MAO inhibitor tranylcypromine (165). Unexpectedly, it has been demonstrated that pioglitazione, used as an antidiabetic drug in T2D patients, is a specific and reversible MAO-B inhibitor (166). These findings support a possible MAO involvement in diabetes-induced complications.

A clear and undeniable evidence for the role of MAO in the pathogenesis and progression of DCM came from animal models of T1D showing that MAO inhibition prevents cardiac dysfunction, death and fibrosis in diabetic mice and rats (71, 167). Data from our laboratory indicates that MAO activity is responsible for diastolic stiffness and dysfunction, some of the earliest signs of DCM in diabetic mice (71). Indeed, administration of MAO inhibitors is able to prevent oxidative changes, diastolic dysfunction and myocardial fibrosis in streptozotocin-treated hearts. In addition, MAO inhibition prevented mast cell degranulation in diabetic hearts, event that can contribute to fibrotic remodeling of the myocardial tissue. This evidence suggests that MAO-generated ROS are at the basis of diabetes-induced cardiovascular complications and, in addition to cardiomyocytes, affect also other cell types present in the heart. Oxidative stress induced by enhanced MAO activity has also been implicated in cardiomyocyte and mesenchymal stromal cell senescence (168–170). It remains to be elucidated whether ROS produced by MAO may also promote cardiac progenitor cell senescence during remodeling induced by diabetes, as is the case with $p66^{Shc}$.

Up to date, the mechanisms underlying MAO toxicity have mostly been attributed to excessive H_2O_2 and aldehyde formation that leads to impaired mitochondrial function (146). Our recent work showed that incubation of primary cardiomyocytes with high glucose and pro-inflammatory cytokine IL-1β leads to a MAO-dependent increase in ROS that, in addition to causing PTP opening and mitochondrial dysfunction, also results in the endoplasmic reticulum (ER) stress (71). This evidence indicates that, in addition to mitochondrial ROS being a trigger for inflammasome activation, inflammatory processes can also promote mitochondrial ROS formation by up-regulating MAO activity. MAO inhibition prevented mitochondrial dysfunction and ER stress, factors that eventually contribute to the progression of DCM, suggesting that cardiomyocyte targeting of pro-inflammatory stimuli occurs in a MAO-dependent manner (71). Given that MAO is localized at the outer mitochondrial membrane and faces the cytosol, it is conceivable to imagine that H_2O_2 produced by MAO can also affect the function of neighboring organelles. Notably, ER and mitochondria are adjacent organelles, connected both at structural and functional level (171). Although our data suggests that MAO-induced mitochondrial dysfunction occurs upstream of ER stress, it is tempting to hypothesize that MAO may directly modulate ER function also through physical interaction with ER-resident proteins (mitochondria associated membrane proteins, for instance). Finally, it cannot be excluded that other products of MAO activity, such as aldehydes, may also contribute to diabetes-induced alterations. MAO-dependent oxidative stress may lead to the inhibition of aldehyde dehydrogenase 2 (ALDH2) resulting in further accumulation of toxic and reactive aldehydes

(37). In that regard, it has been demonstrated that stimulation of ALDH2 activity protects from streptozotocin-induced cardiac damage (172), suggesting that accumulation of aldehydes may promote cardiac remodeling in diabetes independently or in concert with high ROS levels (173).

FEED-FORWARD/AMPLIFICATION LOOP FOR ROS FORMATION

An intense cross-talk between different cellular ROS sources is likely to exist since many papers report that inhibition of a single ROS source prevents the development of cardiac pathology triggered by oxidative stress (146). For instance, hyperglycemia does not induce ROS formation in the ρ0 cells in which the respiratory chain is disrupted, as well as upon NOX or MAO inhibition (30). In addition, mitochondrial superoxide scavenging using mitochondria-targeted antioxidants is able to reduce NOX2 expression and activity in diabetic myocardium (174), while genetic inhibition of NOX2 and consequent reduction in superoxide formation at the mitochondrial level suggest that mitochondrial ROS formation in hyperglycemic hearts might be NOX2-dependent (82–84). Such evidence strongly supports the existence of an "amplification mechanism," whereby an initial stress (i.e., hyperglycemia and/or inflammation), induces the formation of ROS that, in turn, activates other ROS producing enzymes to start producing free radicals thus amplifying the original oxidative trigger (146). The hypothesis of the feed-forward/amplification mechanism is also supported by the characterization of the so-called ROS-induced ROS release mechanism, whereby an initial ROS trigger induces PTP opening that leads to further ROS formation, instituting thereby a positive feedback loop for the ROS-induced ROS release (175, 176). This is indeed the case in adult cardiomyocytes that, when exposed to high glucose and pro-inflammatory stimuli, display an increase in MAO-dependent ROS formation that causes PTP opening and mitochondrial and ER stress (71). Moreover, other processes may participate in such amplification loop, such as for instance impairment in autophagy. While low/moderate ROS levels are required for autophagy initiation, excessive oxidative damage can impair autophagy resulting in the aberrant clearance of damaged proteins and/or organelles (177–180). For instance, AGE accumulation in an experimental model of diabetes inhibits autophagy, induces ER stress and promotes ROS formation (181). Either autophagy stimulation with rapamycin or inhibition of ER stress due to ER chaperone administration alleviate AGEs-induced deleterious effects on cardiomyocytes, suggesting that these processes are involved in diabetes-induced cardiac remodeling. Impairment in the elimination of damaged and dysfunctional mitochondria in diabetic hearts results in the accumulation of ROS-producing fragmented mitochondria (182, 183). Either inhibition of mitochondrial fragmentation during exposure to high glucose or stimulation of organelle removal through mitophagy in high-fat diet fed animals prevents oxidative stress as well as mitochondrial and cardiac dysfunction (182, 184, 185). However, it appears that autophagy and mitophagy are independently controlled in T2D, since autophagy flux was attenuated following 6 weeks of high-fat diet while mitophagy continued to increase even after 2 months (184). This suggests that mitophagy may occur through a non-canonical, alternative autophagy pathway. In this regard, it has been previously shown that Rab9 is mobilized to the mitochondria in early stages of diabetes where it induces activation of alternative autophagy for mitophagy (186, 187). The mechanisms controlling the activation of canonical vs. non-canonical autophagy remain unknown to date. Mitochondrial ROS are implicated in the activation of the canonical autophagy (178), but on the other hand excessive mitochondrial ROS formation impairs lysosomal biogenesis, function and the autophagy process in the cardiac myocytes (156, 168). Whether alterations in the redox status may represent the switch for autophagy to become maladaptive, and/or for the activation of canonical vs. non-canonical autophagy remains to be defined.

INTERVENTIONS AIMED AT REDUCING ROS BURDEN IN DCM

Given the large body of evidence linking aberrant ROS formation and oxidative stress to the development of cardiac diseases, it is quite straightforward to hypothesize that reducing redox burden would protect the heart against deleterious changes induced by diabetes or other pathologies. Nevertheless, large scale clinical trials using antioxidant therapies have not produced the desired results (188, 189). Whether this is a consequence of particular antioxidant molecules used in clinical trials, their limited absorption and/or reduced cardiac availability, or it can be explained by the fact that a certain level of ROS is beneficial and required for signaling and physiological processes, including the response to insulin mediated by $p66^{Shc}$-dependent ROS (190), remains to be elucidated. Another attractive explanation is that interfering with the complex redox network might result in compensatory changes (191). Currently, there are no efficient therapies to treat HFpEF in patients with diabetes. In that regard, antidiabetic SGLT2 inhibitors (such as empagliflozin) afforded cardioprotective effects in patients with diabetes (192). SGLT2 inhibitors lead to the reduction in plasma volume and reduced preload, events that have a favorable effect on cardiac function and structure (193, 194). Importantly, human and rodent hearts do not express SGLT2 (195–197), suggesting that the direct cardioprotective effects of SGLT2 inhibitors are independent of their action on SGLT2. Indeed, it has been demonstrated that SGLT2 inhibitors can directly affect cardiomyocytes by targeting Na^+/H^+ exchanger 1, reducing intracellular Na^+ and Ca^{2+} levels, improving mitochondrial function and reducing inflammation and AMPK activity (197, 198). In addition, SGLT2 inhibitors are able to reduce oxidative stress through Nrf2/ARE signaling activation and it is likely that these off-target effects contribute to the cardioprotection observed in clinical trials (198–200). Another strategy to modulate an oxidative stress-related pathway is the use of the soluble guanylate cyclase stimulator vericiguat that targets the cGMP pathway in an

ROS/NO-independent manner (201). HFpEF is associated with excessive ROS formation by the coronary microvasculature that limits NO bioavailability, reduces cGMP levels and therefore lowers PKG activity (as discussed in section 3). A recent clinical trial demonstrated an improvement in quality of life in patients with HFpEF receiving vericiguat for 12 weeks, suggesting that it could be a promising therapeutic agent in HFpEF (201). To foster the development of a specific and successful therapy, future studies should aim either at identifying the molecular ROS targets (191), the pathways of redox signaling or the specific sources of ROS that are responsible for deleterious changes in the diseased heart. While the first two options are just beginning to become accessible and are still far from being conclusively elucidated, inhibition of specific ROS sources might prove to be a useful strategy to prevent alterations in the redox status, and myocardial structure and function.

In this regard, inhibitors for some of the ROS sources outlined in this review are being developed and/or tested in the clinic. Data obtained in experimental models of diabetes identified NOX4 as a therapeutic target (81). Indeed, NOX4 inhibitors are currently being tested for various cardiovascular indications (76, 202). For instance, GKT-831 is a NOX1/4 dual inhibitor and the only NOX inhibitor that has reached the clinical trial stage; in fact, it is currently being tested in clinical trial phase II for diabetic nephropathy. It remains to be established whether NOX inhibitors would be effective in limiting cardiovascular complications in diabetic patients.

In line with the concept of mitochondria as major ROS producers, employment of mitochondria-targeted antioxidants such as MitoTEMPO proved to be cardioprotective in experimental models of DCM (203). On the other hand, MitoQ was never tested in such setting and neither of the compounds was ever tested in clinical trials. The paucity of studies concerning the use of mitochondrial antioxidants in DCM urges for studies adopting strategies that target specific mitochondrial ROS sources or their downstream targets (204). In that regard, it is not possible to inhibit the respiratory chain in humans in the long term without jeopardizing a wide array of vital functions. Although genetic inhibition of $p66^{Shc}$ has proven protective in many cardiovascular pathologies, pharmacological inhibitors of $p66^{Shc}$ are not yet available. On the contrary, MAO inhibitors are clinically available and employed for the treatment of depression and neurodegenerative diseases (76, 164, 205–207). As mentioned before, administration of a non-selective MAO inhibitor to a patient with T1D led to several improvements, including those at the cardiovascular level (165). Side-effects associated with the "old" irreversible MAO-A inhibitors have been eliminated since reversible MAO-A inhibitors or selective MAO-B inhibitors have been developed (207). Taking into consideration recent findings obtained in experimental models of DCM, it is worth assessing whether molecules such as moclobemide or safinamide could be repurposed for the treatment of patients with DCM.

CONCLUSIONS

Current consensus is that exacerbated ROS generation due to hyperglycemia and/or fatty acid oxidation causes oxidative stress, that in turn promotes the development and progression of diabetes and its complications. In addition to the cytosolic sources of ROS, it is now well-documented that mitochondrial sources represent the major ROS burden in multiple tissues in both animal and human diabetic subjects. Pharmacological targeting of specific ROS sources may prove as a successful therapeutic strategy for the treatment of DCM. Alternatively, identification of processes and targets downstream of mitochondrial ROS may hold more promise in correcting cellular structural and functional derangements in diabetic individuals.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work has been supported by the Leducq Foundation Transatlantic Network of Excellence (grant no. 16CVD04).

REFERENCES

1. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. (2013) 93:137–88. doi: 10.1152/physrev.00045.2011
2. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*. (1963) 1:785–9. doi: 10.1016/S0140-6736(63)91500-9
3. Maack C, Lehrke M, Backs J, Heinzel FR, Hulot JS, Marx N, et al. Heart failure and diabetes: metabolic alterations and therapeutic interventions: a state-of-the-art review from the translational research committee of the heart failure association-european society of cardiology. *Eur Heart J*. (2018) 39:4243–54. doi: 10.1093/eurheartj/ehy596
4. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clin Diabetes*. (2008) 26:77–82. doi: 10.2337/diaclin.26.2.77
5. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res*. (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
6. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol*. (1974) 34:29–34. doi: 10.1016/0002-9149(74)90089-7
7. Carstensen B, Jorgensen ME, Friis S. The epidemiology of diabetes and cancer. *Curr Diab Rep*. (2014) 14:535. doi: 10.1007/s11892-014-0535-8
8. Lu FP, Lin KP, Kuo HK. Diabetes and the risk of multi-system aging phenotypes: a systematic review and meta-analysis. *PLoS ONE*. (2009) 4:e4144. doi: 10.1371/journal.pone.0004144
9. Wong E, Backholer K, Gearon E, Harding J, Freak-Poli R, Stevenson C, et al. Diabetes and risk of physical disability in adults: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. (2013) 1:106–14. doi: 10.1016/S2213-8587(13)70046-9
10. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS Med*. (2008) 5:e152. doi: 10.1371/journal.pmed.0050152
11. Riza AL, Pearson F, Ugarte-Gil C, Alisjahbana B, van de Vijver S, Panduru NM, et al. Clinical management of concurrent diabetes and tuberculosis

and the implications for patient services. *Lancet Diabetes Endocrinol*. (2014) 2:740–53. doi: 10.1016/S2213-8587(14)70110-X
12. Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. *J Affect Disord*. (2012) 142:S8–21. doi: 10.1016/S0165-0327(12)70004-6
13. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. (2010) 107:1058–70. doi: 10.1161/CIRCRESAHA.110.223545
14. Bugger H, Bode C. The vulnerable myocardium. Diabetic cardiomyopathy. *Hamostaseologie*. (2015) 35:17–24. doi: 10.5482/HAMO-14-09-0038
15. Solang L, Malmberg K, Ryden L. Diabetes mellitus and congestive heart failure. Further knowledge needed. *Eur Heart J*. (1999) 20:789–95. doi: 10.1053/euhj.1998.1472
16. Bauters C, Lamblin N, McFadden EP, Van Belle E, Millaire A, de Groote P. Influence of diabetes mellitus on heart failure risk and outcome. *Cardiovasc Diabetol*. (2003) 2:1. doi: 10.1186/1475-2840-2-1
17. Yoneyama K, Venkatesh BA, Wu CO, Mewton N, Gjesdal O, Kishi S, et al. Diabetes mellitus and insulin resistance associate with left ventricular shape and torsion by cardiovascular magnetic resonance imaging in asymptomatic individuals from the multi-ethnic study of atherosclerosis. *J Cardiovasc Magn Reson*. (2018) 20:53. doi: 10.1186/s12968-018-0472-9
18. Boyer JK, Thanigaraj S, Schechtman KB, Perez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am J Cardiol*. (2004) 93:870–5. doi: 10.1016/j.amjcard.2003.12.026
19. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. *Circulation*. (2008) 117:43–51. doi: 10.1161/CIRCULATIONAHA.107.728550
20. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia*. (2014) 57:660–71. doi: 10.1007/s00125-014-3171-6
21. Teupe C, Rosak C. Diabetic cardiomyopathy and diastolic heart failure – difficulties with relaxation. *Diabetes Res Clin Pract*. (2012) 97:185–94. doi: 10.1016/j.diabres.2012.03.008
22. Wold LE, Ceylan-Isik AF, Ren J. Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. *Acta Pharmacol Sin*. (2005) 26:908–17. doi: 10.1111/j.1745-7254.2005.00146.x
23. Holscher ME, Bode C, Bugger H. Diabetic cardiomyopathy: does the type of diabetes matter? *Int J Mol Sci*. (2016) 17:2136. doi: 10.3390/ijms17122136
24. Ryden L, Armstrong PW, Cleland JG, Horowitz JD, Massie BM, Packer M, et al. Efficacy and safety of high-dose lisinopril in chronic heart failure patients at high cardiovascular risk, including those with diabetes mellitus. Results from the ATLAS trial. *Eur Heart J*. (2000) 21:1967–78. doi: 10.1053/euhj.2000.2311
25. Thrainsdottir IS, Aspelund T, Thorgeirsson G, Gudnason V, Hardarson T, Malmberg K, et al. The association between glucose abnormalities and heart failure in the population-based Reykjavik study. *Diabetes Care*. (2005) 28:612–6. doi: 10.2337/diacare.28.3.612
26. Shindler DM, Kostis JB, Yusuf S, Quinones MA, Pitt B, Stewart D, et al. Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) trials and registry. *Am J Cardiol*. (1996) 77:1017–20. doi: 10.1016/S0002-9149(97)89163-1
27. Aronow WS, Ahn C. Incidence of heart failure in 2,737 older persons with and without diabetes mellitus. *Chest*. (1999) 115:867–8. doi: 10.1378/chest.115.3.867
28. Bugger H, Abel ED. Mitochondria in the diabetic heart. *CardiovascRes*. (2010) 88:229–40. doi: 10.1093/cvr/cvq239
29. Neel S, Singla DK. Induced pluripotent stem (iPS) cells inhibit apoptosis and fibrosis in streptozotocin-induced diabetic rats. *Mol Pharm*. (2011) 8:2350–7. doi: 10.1021/mp2004675
30. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. (2005) 54:1615–25. doi: 10.2337/diabetes.54.6.1615
31. Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis*. (2000) 21:361–70. doi: 10.1093/carcin/21.3.361
32. Nishio S, Teshima Y, Takahashi N, Thuc LC, Saito S, Fukui A, et al. Activation of CaMKII as a key regulator of reactive oxygen species production in diabetic rat heart. *J Mol Cell Cardiol*. (2012) 52:1103–11. doi: 10.1016/j.yjmcc.2012.02.006
33. Farhangkhoee H, Khan ZA, Mukherjee S, Cukiernik M, Barbin YP, Karmazyn M, et al. Heme oxygenase in diabetes-induced oxidative stress in the heart. *J Mol Cell Cardiol*. (2003) 35:1439–48. doi: 10.1016/j.yjmcc.2003.09.007
34. Pacifici RE, Davies KJ. Protein, lipid and DNA repair systems in oxidative stress: the free-radical theory of aging revisited. *Gerontology*. (1991) 37:166–80. doi: 10.1159/000213257
35. Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T, et al. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J*. (2000) 14:1490–8. doi: 10.1096/fj.99-0843com
36. Halliwell B. The role of oxygen radicals in human disease, with particular reference to the vascular system. *Haemostasis*. (1993) 23(Suppl. 1):118–26. doi: 10.1159/000216921
37. Kaludercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, et al. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. *Antioxid Redox Signal*. (2014) 20:267–80. doi: 10.1089/ars.2012.4616
38. Wold LE, Ceylan-Isik AF, Fang CX, Yang X, Li SY, Sreejayan N, et al. Metallothionein alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of Ca2+ cycling proteins, NADPH oxidase, poly(ADP-Ribose) polymerase and myosin heavy chain isozyme. *Free Radic Biol Med*. (2006) 40:1419–29. doi: 10.1016/j.freeradbiomed.2005.12.009
39. Dong F, Li Q, Sreejayan N, Nunn JM, Ren J. Metallothionein prevents high-fat diet induced cardiac contractile dysfunction: role of peroxisome proliferator activated receptor γ coactivator 1α and mitochondrial biogenesis. *Diabetes*. (2007) 56:2201–12. doi: 10.2337/db06-1596
40. Ye G, Metreveli NS, Ren J, Epstein PN. Metallothionein prevents diabetes-induced deficits in cardiomyocytes by inhibiting reactive oxygen species production. *Diabetes*. (2003) 52:777–83. doi: 10.2337/diabetes.52.3.777
41. Ye G, Metreveli NS, Donthi RV, Xia S, Xu M, Carlson EC, et al. Catalase protects cardiomyocyte function in models of type 1 and type 2 diabetes. *Diabetes*. (2004) 53:1336–43. doi: 10.2337/diabetes.53.5.1336
42. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, et al. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest*. (2003) 112:1049–57. doi: 10.1172/JCI18127
43. Nascimento NR, Lessa LM, Kerntopf MR, Sousa CM, Alves RS, Queiroz MG, et al. Inositols prevent and reverse endothelial dysfunction in diabetic rat and rabbit vasculature metabolically and by scavenging superoxide. *Proc Natl Acad Sci USA*. (2006) 103:218–23. doi: 10.1073/pnas.0509779103
44. Zhang M, Kho AL, Anilkumar N, Chibber R, Pagano PJ, Shah AM, et al. Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase. *Circulation*. (2006) 113:1235–43. doi: 10.1161/CIRCULATIONAHA.105.581397
45. Ma H, Li SY, Xu P, Babcock SA, Dolence EK, Brownlee M, et al. Advanced glycation endproduct (AGE) accumulation and AGE receptor (RAGE) up-regulation contribute to the onset of diabetic cardiomyopathy. *J Cell Mol Med*. (2009) 13:1751–64. doi: 10.1111/j.1582-4934.2008.00547.x
46. Nakamura H, Matoba S, Iwai-Kanai E, Kimata M, Hoshino A, Nakaoka M, et al. p53 promotes cardiac dysfunction in diabetic mellitus caused by excessive mitochondrial respiration-mediated reactive oxygen species generation and lipid accumulation. *Circ Heart Fail*. (2012) 5:106–15. doi: 10.1161/CIRCHEARTFAILURE.111.961565
47. Tsushima K, Bugger H, Wende AR, Soto J, Jenson GA, Tor AR, et al. Mitochondrial reactive oxygen species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPA1 that promote mitochondrial fission. *Circ Res*. (2018) 122:58–73. doi: 10.1161/CIRCRESAHA.117.311307
48. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, et al. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes*. (2007) 56:2457–66. doi: 10.2337/db07-0481
49. Li S, Zhang L, Ni R, Cao T, Zheng D, Xiong S, et al. Disruption of calpain reduces lipotoxicity-induced cardiac injury by preventing endoplasmic reticulum stress. *Biochim Biophys Acta*. (2016) 1862:2023–33. doi: 10.1016/j.bbadis.2016.08.005

50. Ertunc ME, Hotamisligil GS. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J Lipid Res*. (2016) 57:2099–114. doi: 10.1194/jlr.R066514
51. Wende AR, Symons JD, Abel ED. Mechanisms of lipotoxicity in the cardiovascular system. *Curr Hypertens Rep*. (2012) 14:517–31. doi: 10.1007/s11906-012-0307-2
52. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. (2011) 469:221–5. doi: 10.1038/nature09663
53. Lafuente N, Matesanz N, Azcutia V, Romacho T, Nevado J, Rodriguez-Manas L, et al. The deleterious effect of high concentrations of D-glucose requires pro-inflammatory preconditioning. *J Hypertens*. (2008) 26:478–85. doi: 10.1097/HJH.0b013e3282f331fb
54. Peiro C, Romacho T, Azcutia V, Villalobos L, Fernandez E, Bolanos JP, et al. Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. *Cardiovasc Diabetol*. (2016) 15:82. doi: 10.1186/s12933-016-0397-2
55. Wilson AJ, Gill EK, Abudalo RA, Edgar KS, Watson CJ, Grieve DJ. Reactive oxygen species signalling in the diabetic heart: emerging prospect for therapeutic targeting. *Heart*. (2018) 104:293–9. doi: 10.1136/heartjnl-2017-311448
56. Kass DA, Bronzwaer JG, Paulus WJ. What mechanisms underlie diastolic dysfunction in heart failure? *Circ Res*. (2004) 94:1533–42. doi: 10.1161/01.RES.0000129254.25507.d6
57. Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. *Free Radic Biol Med*. (2011) 50:777–93. doi: 10.1016/j.freeradbiomed.2011.01.003
58. Steinberg SF. Oxidative stress and sarcomeric proteins. *Circ Res*. (2013) 112:393–405. doi: 10.1161/CIRCRESAHA.111.300496
59. Breitkreuz M, Hamdani N. A change of heart: oxidative stress in governing muscle function? *Biophys Rev*. (2015) 7:321–41. doi: 10.1007/s12551-015-0175-5
60. Grutzner A, Garcia-Manyes S, Kotter S, Badilla CL, Fernandez JM, Linke WA. Modulation of titin-based stiffness by disulfide bonding in the cardiac titin N2-B unique sequence. *Biophys J*. (2009) 97:825–34. doi: 10.1016/j.bpj.2009.05.037
61. Kruger M, Kotter S, Grutzner A, Lang P, Andresen C, Redfield MM, et al. Protein kinase G modulates human myocardial passive stiffness by phosphorylation of the titin springs. *Circ Res*. (2009) 104:87–94. doi: 10.1161/CIRCRESAHA.108.184408
62. Hidalgo C, Hudson B, Bogomolovas J, Zhu Y, Anderson B, Greaser M, et al. PKC phosphorylation of titin's PEVK element: a novel and conserved pathway for modulating myocardial stiffness. *Circ Res*. (2009) 105:631–8. doi: 10.1161/CIRCRESAHA.109.198465
63. Beckendorf L, Linke WA. Emerging importance of oxidative stress in regulating striated muscle elasticity. *J Muscle Res Cell Motil*. (2015) 36:25–36. doi: 10.1007/s10974-014-9392-y
64. Rajesh M, Mukhopadhyay P, Batkai S, Patel V, Saito K, Matsumoto S, et al. Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. *J Am College Cardiol*. (2010) 56:2115–25. doi: 10.1016/j.jacc.2010.07.033
65. Rajesh M, Batkai S, Kechrid M, Mukhopadhyay P, Lee WS, Horvath B, et al. Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes*. (2012) 61:716–27. doi: 10.2337/db11-0477
66. Roe ND, Thomas DP, Ren J. Inhibition of NADPH oxidase alleviates experimental diabetes-induced myocardial contractile dysfunction. *Diabetes Obes Metab*. (2011) 13:465–73. doi: 10.1111/j.1463-1326.2011.01369.x
67. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev*. (2006) 58:87–114. doi: 10.1124/pr.58.1.6
68. Rajesh M, Mukhopadhyay P, Batkai S, Mukhopadhyay B, Patel V, Hasko G, et al. Xanthine oxidase inhibitor allopurinol attenuates the development of diabetic cardiomyopathy. *J Cell Mol Med*. (2009) 13:2330–41. doi: 10.1111/j.1582-4934.2008.00564.x
69. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. (2007) 87:315–424. doi: 10.1152/physrev.00029.2006
70. Francia P, Cosentino F, Schiavoni M, Huang Y, Perna E, Camici GG, et al. p66(Shc) protein, oxidative stress, and cardiovascular complications of diabetes: the missing link. *J Mol Med*. (2009) 87:885–91. doi: 10.1007/s00109-009-0499-3
71. Deshwal S, Forkink M, Hu CH, Buonincontri G, Antonucci S, Di Sante M, et al. Monoamine oxidase-dependent endoplasmic reticulum-mitochondria dysfunction and mast cell degranulation lead to adverse cardiac remodeling in diabetes. *Cell Death Differ*. (2018) 25:1671–85. doi: 10.1038/s41418-018-0071-1
72. Varga ZV, Giricz Z, Liaudet L, Hasko G, Ferdinandy P, Pacher P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. *Biochim Biophys Acta*. (2015) 1852:232–42. doi: 10.1016/j.bbadis.2014.06.030
73. Braunersreuther V, Montecucco F, Asrih M, Pelli G, Galan K, Frias M, et al. Role of NADPH oxidase isoforms NOX1, NOX2 and NOX4 in myocardial ischemia/reperfusion injury. *J Mol Cell Cardiol*. (2013) 64:99–107. doi: 10.1016/j.yjmcc.2013.09.007
74. Casas AI, Dao VT, Daiber A, Maghzal GJ, Di Lisa F, Kaludercic N, et al. Reactive oxygen-related diseases: therapeutic targets and emerging clinical indications. *Antioxid Redox Signal*. (2015) 23:1171–85. doi: 10.1089/ars.2015.6433
75. Sirker A, Zhang M, Shah AM. NADPH oxidases in cardiovascular disease: insights from *in vivo* models and clinical studies. *Basic Res Cardiol*. (2011) 106:735–47. doi: 10.1007/s00395-011-0190-z
76. Dao VT, Casas AI, Maghzal GJ, Seredenina T, Kaludercic N, Robledinos-Anton N, et al. Pharmacology and clinical drug candidates in redox medicine. *Antioxid Redox Signal*. (2015) 23:1113–29. doi: 10.1089/ars.2015.6430
77. Santos CX, Raza S, Shah AM. Redox signaling in the cardiomyocyte: from physiology to failure. *Int J Biochem Cell Biol*. (2016) 74:145–51. doi: 10.1016/j.biocel.2016.03.002
78. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proc Natl Acad Sci USA*. (2010) 107:15565–70. doi: 10.1073/pnas.1002178107
79. Zhang M, Brewer AC, Schroder K, Santos CX, Grieve DJ, Wang M, et al. NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. *Proc Natl Acad Sci USA*. (2010) 107:18121–6. doi: 10.1073/pnas.1009700107
80. Varga ZV, Kupai K, Szucs G, Gaspar R, Paloczi J, Farago N, et al. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. *J Mol Cell Cardiol*. (2013) 62:111–21. doi: 10.1016/j.yjmcc.2013.05.009
81. Maalouf RM, Eid AA, Gorin YC, Block K, Escobar GP, Bailey S, et al. Nox4-derived reactive oxygen species mediate cardiomyocyte injury in early type 1 diabetes. *Am J Physiol Cell Physiol*. (2012) 302:C597–604. doi: 10.1152/ajpcell.00331.2011
82. Shen E, Li Y, Li Y, Shan L, Zhu H, Feng Q, et al. Rac1 is required for cardiomyocyte apoptosis during hyperglycemia. *Diabetes*. (2009) 58:2386–95. doi: 10.2337/db08-0617
83. Li J, Zhu H, Shen E, Wan L, Arnold JM, Peng T. Deficiency of rac1 blocks NADPH oxidase activation, inhibits endoplasmic reticulum stress, and reduces myocardial remodeling in a mouse model of type 1 diabetes. *Diabetes*. (2010) 59:2033–42. doi: 10.2337/db09-1800
84. Hansen SS, Aasum E, Hafstad AD. The role of NADPH oxidases in diabetic cardiomyopathy. *Biochim Biophys Acta Mol Basis Dis*. (2018) 1864:1908–13. doi: 10.1016/j.bbadis.2017.07.025
85. Balteau M, Tajeddine N, de Meester C, Ginion A, Des Rosiers C, Brady NR, et al. NADPH oxidase activation by hyperglycaemia in cardiomyocytes is independent of glucose metabolism but requires SGLT1. *Cardiovasc Res*. (2011) 92:237–46. doi: 10.1093/cvr/cvr230
86. Roul D, Recchia FA. Metabolic alterations induce oxidative stress in diabetic and failing hearts: different pathways, same outcome. *Antioxid Redox Signal*. (2015) 22:1502–14. doi: 10.1089/ars.2015.6311
87. Wang Z, Zhang Y, Guo J, Jin K, Li J, Guo X, et al. Inhibition of protein kinase C βII isoform rescues glucose toxicity-induced cardiomyocyte

contractile dysfunction: role of mitochondria. *Life Sci*. (2013) 93:116–24. doi: 10.1016/j.lfs.2013.06.002
88. Saliaris AP, Amado LC, Minhas KM, Schuleri KH, Lehrke S, St John M, et al. Chronic allopurinol administration ameliorates maladaptive alterations in Ca2+ cycling proteins and β-adrenergic hyporesponsiveness in heart failure. *Am J Physiol Heart Circ Physiol*. (2007) 292:H1328–35. doi: 10.1152/ajpheart.00461.2006
89. Amado LC, Saliaris AP, Raju SV, Lehrke S, St John M, Xie J, et al. Xanthine oxidase inhibition ameliorates cardiovascular dysfunction in dogs with pacing-induced heart failure. *J Mol Cell Cardiol*. (2005) 39:531–6. doi: 10.1016/j.yjmcc.2005.04.008
90. Desco MC, Asensi M, Marquez R, Martinez-Valls J, Vento M, Pallardo FV, et al. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. *Diabetes*. (2002) 51:1118–24. doi: 10.2337/diabetes.51.4.1118
91. Bravard A, Bonnard C, Durand A, Chauvin MA, Favier R, Vidal H, et al. Inhibition of xanthine oxidase reduces hyperglycemia-induced oxidative stress and improves mitochondrial alterations in skeletal muscle of diabetic mice. *Am J Physiol Endocrinol Metab*. (2011) 300:E581–91. doi: 10.1152/ajpendo.00455.2010
92. Gao X, Xu Y, Xu B, Liu Y, Cai J, Liu HM, et al. Allopurinol attenuates left ventricular dysfunction in rats with early stages of streptozotocin-induced diabetes. *Diabetes Metab Res Rev*. (2012) 28:409–17. doi: 10.1002/dmrr.2295
93. Szwejkowski BR, Gandy SJ, Rekhraj S, Houston JG, Lang CC, Morris AD, et al. Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy. *J Am College Cardiol*. (2013) 62:2284–93. doi: 10.1016/j.jacc.2013.07.074
94. Karbach S, Wenzel P, Waisman A, Munzel T, Daiber A. eNOS uncoupling in cardiovascular diseases–the role of oxidative stress and inflammation. *Curr Pharm Des*. (2014) 20:3579–94. doi: 10.2174/13816128113196660748
95. Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest*. (2002) 109:817–26. doi: 10.1172/JCI0214442
96. Jo H, Otani H, Jo F, Shimazu T, Okazaki T, Yoshioka K, et al. Inhibition of nitric oxide synthase uncoupling by sepiapterin improves left ventricular function in streptozotocin-induced diabetic mice. *Clin Exp Pharmacol Physiol*. (2011) 38:485–93. doi: 10.1111/j.1440-1681.2011.05535.x
97. Okazaki T, Otani H, Shimazu T, Yoshioka K, Fujita M, Iwasaka T. Ascorbic acid and N-acetyl cysteine prevent uncoupling of nitric oxide synthase and increase tolerance to ischemia/reperfusion injury in diabetic rat heart. *Free Radic Res*. (2011) 45:1173–83. doi: 10.3109/10715762.2011.605361
98. Ren J, Duan J, Thomas DP, Yang X, Sreejayan N, Sowers JR, et al. IGF-I alleviates diabetes-induced RhoA activation, eNOS uncoupling, and myocardial dysfunction. *Am J Physiol Regul Integr Comp Physiol*. (2008) 294:R793–802. doi: 10.1152/ajpregu.00713.2007
99. Smith JM, Paulson DJ, Romano FD. Inhibition of nitric oxide synthase by L-NAME improves ventricular performance in streptozotocin-diabetic rats. *J Mol Cell Cardiol*. (1997) 29:2393–402. doi: 10.1006/jmcc.1997.0474
100. Esberg LB, Ren J. Role of nitric oxide, tetrahydrobiopterin and peroxynitrite in glucose toxicity-associated contractile dysfunction in ventricular myocytes. *Diabetologia*. (2003) 46:1419–27. doi: 10.1007/s00125-003-1183-8
101. Stockklauser-Farber K, Ballhausen T, Laufer A, Rosen P. Influence of diabetes on cardiac nitric oxide synthase expression and activity. *Biochim Biophys Acta*. (2000) 1535:10–20. doi: 10.1016/S0925-4439(00)00078-8
102. Cassuto J, Dou H, Czikora I, Szabo A, Patel VS, Kamath V, et al. Peroxynitrite disrupts endothelial caveolae leading to eNOS uncoupling and diminished flow-mediated dilation in coronary arterioles of diabetic patients. *Diabetes*. (2014) 63:1381–93. doi: 10.2337/db13-0577
103. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am College Cardiol*. (2009) 54:1891–8. doi: 10.1016/j.jacc.2009.07.031
104. Anderson EJ, Rodriguez E, Anderson CA, Thayne K, Chitwood WR, Kypson AP. Increased propensity for cell death in diabetic human heart is mediated by mitochondrial-dependent pathways. *Am J Physiol Heart Circ Physiol*. (2011) 300:H118–24. doi: 10.1152/ajpheart.00932.2010
105. Katunga LA, Gudimella P, Efird JT, Abernathy S, Mattox TA, Beatty C, et al. Obesity in a model of gpx4 haploinsufficiency uncovers a causal role for lipid-derived aldehydes in human metabolic disease and cardiomyopathy. *Mol Metab*. (2015) 4:493–506. doi: 10.1016/j.molmet.2015.04.001
106. Guo W, Kan JT, Cheng ZY, Chen JF, Shen YQ, Xu J, et al. Hydrogen sulfide as an endogenous modulator in mitochondria and mitochondria dysfunction. *Oxid Med Cell Longev*. (2012) 2012:878052. doi: 10.1155/2012/878052
107. Galloway CA, Yoon Y. Mitochondrial dynamics in diabetic cardiomyopathy. *Antioxid Redox Signal*. (2015) 22:1545–62. doi: 10.1089/ars.2015.6293
108. Jarosz J, Ghosh S, Delbridge LM, Petzer A, Hickey AJ, Crampin EJ, et al. Changes in mitochondrial morphology and organization can enhance energy supply from mitochondrial oxidative phosphorylation in diabetic cardiomyopathy. *Am J Physiol Cell Physiol*. (2017) 312:C190–7. doi: 10.1152/ajpcell.00298.2016
109. Ni R, Zheng D, Xiong S, Hill DJ, Sun T, Gardiner RB, et al. Mitochondrial calpain-1 disrupts ATP synthase and induces superoxide generation in type 1 diabetic hearts: a novel mechanism contributing to diabetic cardiomyopathy. *Diabetes*. (2016) 65:255–68. doi: 10.2337/db15-0963
110. Bernardi P, Di Lisa F, Fogolari F, Lippe G. From ATP to PTP and back: a dual function for the mitochondrial ATP Synthase. *Circ Res*. (2015) 116:1850–62. doi: 10.1161/CIRCRESAHA.115.306557
111. Makino A, Scott BT, Dillmann WH. Mitochondrial fragmentation and superoxide anion production in coronary endothelial cells from a mouse model of type 1 diabetes. *Diabetologia*. (2010) 53:1783–94. doi: 10.1007/s00125-010-1770-4
112. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxid Redox Signal*. (2010) 12:537–77. doi: 10.1089/ars.2009.2531
113. Shen X, Zheng S, Metreveli NS, Epstein PN. Protection of cardiac mitochondria by overexpression of MnSOD reduces diabetic cardiomyopathy. *Diabetes*. (2006) 55:798–805. doi: 10.2337/diabetes.55.03.06.db05-1039
114. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, et al. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes*. (2006) 55:120–7. doi: 10.2337/diabetes.55.01.06.db05-0943
115. Viglino C, Foglia B, Montessuit C. Chronic AICAR treatment prevents metabolic changes in cardiomyocytes exposed to free fatty acids. *Pflugers Arch*. (2019) 471:1219–34. doi: 10.1007/s00424-019-02285-0
116. Wu S, Lu Q, Ding Y, Wu Y, Qiu Y, Wang P, et al. Hyperglycemia-driven inhibition of AMP-activated protein kinase α2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes *in vivo*. *Circulation*. (2019) 139:1913–36. doi: 10.1161/CIRCULATIONAHA.118.033552
117. Ramsay RR. Electron carriers and energy conservation in mitochondrial respiration. *ChemTexts*. (2019) 5:9. doi: 10.1007/s40828-019-0085-4
118. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J*. (2009) 417:1–13. doi: 10.1042/BJ20081386
119. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. (2003) 552:335–44. doi: 10.1113/jphysiol.2003.049478
120. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD. Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J Biol Chem*. (2002) 277:44784–90. doi: 10.1074/jbc.M207217200
121. Scialo F, Fernandez-Ayala DJ, Sanz A. Role of mitochondrial reverse electron transport in ROS signaling: potential roles in health and disease. *Front Physiol*. (2017) 8:428. doi: 10.3389/fphys.2017.00428
122. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*. (2014) 515:431–5. doi: 10.1038/nature13909
123. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. (2000) 404:787–90. doi: 10.1038/35008121
124. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci USA*. (2000) 97:12222–6. doi: 10.1073/pnas.97.22.12222
125. Teshima Y, Takahashi N, Nishio S, Saito S, Kondo H, Fukui A, et al. Production of reactive oxygen species in the diabetic heart.

Roles of mitochondria and NADPH oxidase. *Circ J*. (2014) 78:300–6. doi: 10.1253/circj.CJ-13-1187

126. Sedlic F, Muravyeva MY, Sepac A, Sedlic M, Williams AM, Yang M, et al. Targeted modification of mitochondrial ROS production converts high glucose-induced cytotoxicity to cytoprotection: effects on anesthetic preconditioning. *J Cell Physiol*. (2017) 232:216–24. doi: 10.1002/jcp.25413
127. Turko IV, Li L, Aulak KS, Stuehr DJ, Chang JY, Murad F. Protein tyrosine nitration in the mitochondria from diabetic mouse heart. Implications to dysfunctional mitochondria in diabetes. *J Biol Chem*. (2003) 278:33972–7. doi: 10.1074/jbc.M303734200
128. Lashin OM, Szweda PA, Szweda LI, Romani AM. Decreased complex II respiration and HNE-modified SDH subunit in diabetic heart. *Free Radic Biol Med*. (2006) 40:886–96. doi: 10.1016/j.freeradbiomed.2005.10.040
129. Baseler WA, Dabkowski ER, Williamson CL, Croston TL, Thapa D, Powell MJ, et al. Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart: contribution of protein import dysfunction. *Am J Physiol Regul Integr Comp Physiol*. (2011) 300:R186–200. doi: 10.1152/ajpregu.00423.2010
130. Banerjee PS, Ma J, Hart GW. Diabetes-associated dysregulation of O-GlcNAcylation in rat cardiac mitochondria. *Proc Natl Acad Sci USA*. (2015) 112:6050–5. doi: 10.1073/pnas.1424017112
131. Hu Y, Suarez J, Fricovsky E, Wang H, Scott BT, Trauger SA, et al. Increased enzymatic O-GlcNAcylation of mitochondrial proteins impairs mitochondrial function in cardiac myocytes exposed to high glucose. *J Biol Chem*. (2009) 284:547–55. doi: 10.1074/jbc.M808518200
132. Makino A, Suarez J, Gawlowski T, Han W, Wang H, Scott BT, et al. Regulation of mitochondrial morphology and function by O-GlcNAcylation in neonatal cardiac myocytes. *Am J Physiol Regul Integr Compar Physiol*. (2011) 300:R1296–302. doi: 10.1152/ajpregu.00437.2010
133. Gawlowski T, Suarez J, Scott B, Torres-Gonzalez M, Wang H, Schwappacher R, et al. Modulation of dynamin-related protein 1 (DRP1) function by increased O-linked-β-N-acetylglucosamine modification (O-GlcNAc) in cardiac myocytes. *J Biol Chem*. (2012) 287:30024–34. doi: 10.1074/jbc.M112.390682
134. Tian C, Alomar F, Moore CJ, Shao CH, Kutty S, Singh J, et al. Reactive carbonyl species and their roles in sarcoplasmic reticulum Ca2+ cycling defect in the diabetic heart. *Heart Fail Rev*. (2014) 19:101–12. doi: 10.1007/s10741-013-9384-9
135. Shah MS, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res*. (2016) 118:1808–29. doi: 10.1161/CIRCRESAHA.116.306923
136. Ruiz-Meana M, Minguet M, Bou-Teen D, Miro-Casas E, Castans C, Castellano J, et al. Ryanodine receptor glycation favors mitochondrial damage in the senescent heart. *Circulation*. (2019) 139:949–64. doi: 10.1161/CIRCULATIONAHA.118.035869
137. Di Lisa F, Giorgio M, Ferdinandy P, Schulz R. New aspects of p66Shc in ischaemia reperfusion injury and other cardiovascular diseases. *Br J Pharmacol*. (2017) 174:1690–703. doi: 10.1111/bph.13478
138. Di Lisa F, Kaludercic N, Carpi A, Menabo R, Giorgio M. Mitochondrial pathways for ROS formation and myocardial injury: the relevance of p66(Shc) and monoamine oxidase. *Basic Res Cardiol*. (2009) 104:131–9. doi: 10.1007/s00395-009-0008-4
139. Carpi A, Menabo R, Kaludercic N, Pelicci P, Di Lisa F, Giorgio M. The cardioprotective effects elicited by p66(Shc) ablation demonstrate the crucial role of mitochondrial ROS formation in ischemia/reperfusion injury. *Biochim Biophys Acta*. (2009) 1787:774–80. doi: 10.1016/j.bbabio.2009.04.001
140. Messina E, Giacomello A. Diabetic cardiomyopathy: a "cardiac stem cell disease" involving p66Shc, an attractive novel molecular target for heart failure therapy. *Circ Res*. (2006) 99:1–2. doi: 10.1161/01.RES.0000233141.65522.3e
141. Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, et al. Protein kinase C β and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science*. (2007) 315:659–63. doi: 10.1126/science.1135380
142. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell*. (2005) 122:221–33. doi: 10.1016/j.cell.2005.05.011
143. Galimov ER. The role of p66shc in oxidative stress and apoptosis. *Acta Naturae*. (2010) 2:44–51. doi: 10.32607/20758251-2010-2-4-44-51
144. Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, et al. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene*. (2002) 21:3872–8. doi: 10.1038/sj.onc.1205513
145. Cesselli D, Jakoniuk I, Barlucchi L, Beltrami AP, Hintze TH, Nadal-Ginard B, et al. Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ Res*. (2001) 89:279–86. doi: 10.1161/hh1501.094115
146. Kaludercic N, Mialet-Perez J, Paolocci N, Parini A, Di Lisa F. Monoamine oxidases as sources of oxidants in the heart. *J Mol Cell Cardiol*. (2014) 73:34–42. doi: 10.1016/j.yjmcc.2013.12.032
147. Camici GG, Schiavoni M, Francia P, Bachschmid M, Martin-Padura I, Hersberger M, et al. Genetic deletion of p66(Shc) adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. *Proc Natl Acad Sci USA*. (2007) 104:5217–22. doi: 10.1073/pnas.0609656104
148. Napoli C, Martin-Padura I, de Nigris F, Giorgio M, Mansueto G, Somma P, et al. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. *Proc Natl Acad Sci USA*. (2003) 100:2112–6. doi: 10.1073/pnas.0336359100
149. Rota M, LeCapitaine N, Hosoda T, Boni A, De AA, Padin-Iruegas ME, et al. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. *CircRes*. (2006) 99:42–52. doi: 10.1161/01.RES.0000231289.63468.08
150. Binda C, Newton-Vinson P, Hubalek F, Edmondson DE, Mattevi A. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nat Struct Biol*. (2002) 9:22–6. doi: 10.1038/nsb732
151. De Colibus L, Li M, Binda C, Lustig A, Edmondson DE, Mattevi A. Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B. *Proc Natl Acad Sci USA*. (2005) 102:12684–9. doi: 10.1073/pnas.0505975102
152. Youdim MB, Finberg JP. New directions in monoamine oxidase A and B selective inhibitors and substrates. *BiochemPharmacol*. (1991) 41:155–62. doi: 10.1016/0006-2952(91)90471-G
153. Finberg JP, Youdim MB. Selective MAO A and B inhibitors: their mechanism of action and pharmacology. *Neuropharmacology*. (1983) 22:441–6. doi: 10.1016/0028-3908(83)90194-6
154. Edmondson DE, Mattevi A, Binda C, Li M, Hubalek F. Structure and mechanism of monoamine oxidase. *CurrMedChem*. (2004) 11:1983–93. doi: 10.2174/0929867043364784
155. Ramsay RR. Monoamine oxidases: the biochemistry of the proteins as targets in medicinal chemistry and drug discovery. *Curr Top Med Chem*. (2012) 12:2189–209. doi: 10.2174/156802612805219978
156. Santin Y, Sicard P, Vigneron F, Guilbeau-Frugier C, Dutaur M, Lairez O, et al. Oxidative stress by monoamine oxidase-a impairs transcription factor EB activation and autophagosome clearance, leading to cardiomyocyte necrosis and heart failure. *Antioxid Redox Signal*. (2016) 25:10–27. doi: 10.1089/ars.2015.6522
157. Villeneuve C, Guilbeau-Frugier C, Sicard P, Lairez O, Ordener C, Duparc T, et al. p53-PGC-1α pathway mediates oxidative mitochondrial damage and cardiomyocyte necrosis induced by monoamine oxidase-A upregulation: role in chronic left ventricular dysfunction in mice. *Antioxid Redox Signal*. (2013) 18:5–18. doi: 10.1089/ars.2011.4373
158. Kaludercic N, Carpi A, Menabo R, Di Lisa F, Paolocci N. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. *Biochim Biophys Acta*. (2011) 1813:1323–32. doi: 10.1016/j.bbamcr.2010.09.010
159. Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja D, et al. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. *Circ Res*. (2010) 106:193–202. doi: 10.1161/CIRCRESAHA.109.198366
160. Bianchi P, Kunduzova O, Masini E, Cambon C, Bani D, Raimondi L, et al. Oxidative stress by monoamine oxidase mediates

receptor-independent cardiomyocyte apoptosis by serotonin and postischemic myocardial injury. *Circulation*. (2005) 112:3297–305. doi: 10.1161/CIRCULATIONAHA.104.528133
161. Pchejetski D, Kunduzova O, Dayon A, Calise D, Seguelas MH, Leducq N, et al. Oxidative stress-dependent sphingosine kinase-1 inhibition mediates monoamine oxidase A-associated cardiac cell apoptosis. *Circ Res*. (2007) 100:41–9. doi: 10.1161/01.RES.0000253900.66640.34
162. Sturza A, Leisegang MS, Babelova A, Schroder K, Benkhoff S, Loot AE, et al. Monoamine oxidases are mediators of endothelial dysfunction in the mouse aorta. *Hypertension*. (2013) 62:140–6. doi: 10.1161/HYPERTENSIONAHA.113.01314
163. Anderson EJ, Efird JT, Davies SW, O'Neal WT, Darden TM, Thayne KA, et al. Monoamine oxidase is a major determinant of redox balance in human atrial myocardium and is associated with postoperative atrial fibrillation. *J Am Heart Assoc*. (2014) 3:e000713. doi: 10.1161/JAHA.113.000713
164. Manni ME, Rigacci S, Borchi E, Bargelli V, Miceli C, Giordano C, et al. Monoamine oxidase is overactivated in left and right ventricles from ischemic hearts: an intriguing therapeutic target. *Oxid Med Cell Longev*. (2016) 2016:4375418. doi: 10.1155/2016/4375418
165. Emory H, Mizrahi N. Glycaemic control by monoamine oxidase inhibition in a patient with type 1 diabetes. *Diab Vasc Dis Res*. (2016) 14:163–5. doi: 10.1177/1479164116675492
166. Binda C, Aldeco M, Geldenhuys WJ, Tortorici M, Mattevi A, Edmondson DE. Molecular insights into human monoamine oxidase B inhibition by the glitazone anti-diabetes drugs. *ACS Med Chem Lett*. (2011) 3:39–42. doi: 10.1021/ml200196p
167. Umbarkar P, Singh S, Arkat S, Bodhankar SL, Lohidasan S, Sitasawad SL. Monoamine oxidase-A is an important source of oxidative stress and promotes cardiac dysfunction, apoptosis, and fibrosis in diabetic cardiomyopathy. *Free Radic Biol Med*. (2015) 87:263–73. doi: 10.1016/j.freeradbiomed.2015.06.025
168. Manzella N, Santin Y, Maggiorani D, Martini H, Douin-Echinard V, Passos JF, et al. Monoamine oxidase-A is a novel driver of stress-induced premature senescence through inhibition of parkin-mediated mitophagy. *Aging Cell*. (2018) 17:e12811. doi: 10.1111/acel.12811
169. Anderson R, Lagnado A, Maggiorani D, Walaszczyk A, Dookun E, Chapman J, et al. Length-independent telomere damage drives post-mitotic cardiomyocyte senescence. *EMBO J*. (2019) 38:e100492. doi: 10.15252/embj.2018100492
170. Martini H, Iacovoni JS, Maggiorani D, Dutaur M, Marsal DJ, Roncalli J, et al. Aging induces cardiac mesenchymal stromal cell senescence and promotes endothelial cell fate of the CD90 + subset. *Aging Cell*. (2019) 18:e13015. doi: 10.1111/acel.13015
171. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*. (2008) 456:605–10. doi: 10.1038/nature07534
172. Zhang Y, Babcock SA, Hu N, Maris JR, Wang H, Ren J. Mitochondrial aldehyde dehydrogenase (ALDH2) protects against streptozotocin-induced diabetic cardiomyopathy: role of GSK3β and mitochondrial function. *BMC Med*. (2012) 10:40. doi: 10.1186/1741-7015-10-40
173. Pan G, Munukutla S, Kar A, Gardinier J, Thandavarayan RA, Palaniyandi SS. Type-2 diabetic aldehyde dehydrogenase 2 mutant mice (ALDH 2*2) exhibiting heart failure with preserved ejection fraction phenotype can be determined by exercise stress echocardiography. *PLoS ONE*. (2018) 13:e0195796. doi: 10.1371/journal.pone.0195796
174. Ni R, Cao T, Xiong S, Ma J, Fan GC, Lacefield JC, et al. Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. *Free Radic Biol Med*. (2016) 90:12–23. doi: 10.1016/j.freeradbiomed.2015.11.013
175. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med*. (2000) 192:1001–14. doi: 10.1084/jem.192.7.1001
176. Antonucci S, Mulvey JF, Burger N, Di Sante M, Hall AR, Hinchy EC, et al. Selective mitochondrial superoxide generation *in vivo* is cardioprotective through hormesis. *Free Radic Biol Med*. (2019) 134:678–87. doi: 10.1016/j.freeradbiomed.2019.01.034
177. Liang L, Shou XL, Zhao HK, Ren GQ, Wang JB, Wang XH, et al. Antioxidant catalase rescues against high fat diet-induced cardiac dysfunction via an IKKβ-AMPK-dependent regulation of autophagy. *Biochim Biophys Acta*. (2015) 1852:343–52. doi: 10.1016/j.bbadis.2014.06.027
178. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J*. (2007) 26:1749–60. doi: 10.1038/sj.emboj.7601623
179. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ*. (2015) 22:377–88. doi: 10.1038/cdd.2014.150
180. Pajares M, Cuadrado A, Engedal N, Jirsova Z, Cahova M. The role of free radicals in autophagy regulation: implications for ageing. *Oxid Med Cell Longev*. (2018) 2018:2450748. doi: 10.1155/2018/2450748
181. Pei Z, Deng Q, Babcock SA, He EY, Ren J, Zhang Y. Inhibition of advanced glycation endproduct (AGE) rescues against streptozotocin-induced diabetic cardiomyopathy: Role of autophagy and ER stress. *Toxicol Lett*. (2018) 284:10–20. doi: 10.1016/j.toxlet.2017.11.018
182. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci USA*. (2006) 103:2653–8. doi: 10.1073/pnas.0511154103
183. Kaludercic N, Maiuri MC, Kaushik S, Fernandez AF, de Bruijn J, Castoldi F, et al. Comprehensive autophagy evaluation in cardiac diseases models. *Cardiovasc Res*. (2019). doi: 10.1093/cvr/cvz233. [Epub ahead of print].
184. Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, et al. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res*. (2019) 124:1360–71. doi: 10.1161/CIRCRESAHA.118.314607
185. Sciarretta S, Zhai P, Shao D, Maejima Y, Robbins J, Volpe M, et al. Rheb is a critical regulator of autophagy during myocardial ischemia: pathophysiological implications in obesity and metabolic syndrome. *Circulation*. (2012) 125:1134–46. doi: 10.1161/CIRCULATIONAHA.111.078212
186. Xu X, Kobayashi S, Chen K, Timm D, Volden P, Huang Y, et al. Diminished autophagy limits cardiac injury in mouse models of type 1 diabetes. *J Biol Chem*. (2013) 288:18077–92. doi: 10.1074/jbc.M113.474650
187. Saito T, Nah J, Oka SI, Mukai R, Monden Y, Maejima Y, et al. An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. *J Clin Invest*. (2019) 129:802–19. doi: 10.1172/JCI122035
188. Griendling KK, Touyz RM, Zweier JL, Dikalov S, Chilian W, Chen YR, et al. Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the american heart association. *Circ Res*. (2016) 119:e39–75. doi: 10.1161/RES.0000000000000110
189. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol*. (2005) 4:5. doi: 10.1186/1475-2840-4-5
190. Berniakovich I, Trinei M, Stendardo M, Migliaccio E, Minucci S, Bernardi P, et al. p66Shc-generated oxidative signal promotes fat accumulation. *J Biol Chem*. (2008) 283:34283–93. doi: 10.1074/jbc.M804362200
191. Brandes RP, Rezende F, Schroder K. Redox Regulation Beyond ROS: why ROS should not be measured as often. *Circ Res*. (2018) 123:326–8. doi: 10.1161/CIRCRESAHA.118.313146
192. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
193. Lytvyn Y, Bjornstad P, Udell JA, Lovshin JA, Cherney DZI. Sodium glucose cotransporter-2 inhibition in heart failure: potential mechanisms, clinical applications, and summary of clinical trials. *Circulation*. (2017) 136:1643–58. doi: 10.1161/CIRCULATIONAHA.117.030012
194. Matsumura K, Sugiura T. Effect of sodium glucose cotransporter 2 inhibitors on cardiac function and cardiovascular outcome: a systematic review. *Cardiovasc Ultrasound*. (2019) 17:26. doi: 10.1186/s12947-019-0177-8
195. Di Franco A, Cantini G, Tani A, Coppini R, Zecchi-Orlandini S, Raimondi L, et al. Sodium-dependent glucose transporters (SGLT) in human ischemic heart: a new potential pharmacological target. *Int J Cardiol*. (2017) 243:86–90. doi: 10.1016/j.ijcard.2017.05.032
196. Mustroph J, Wagemann O, Lucht CM, Trum M, Hammer KP, Sag CM, et al. Empagliflozin reduces Ca/calmodulin-dependent kinase II activity

in isolated ventricular cardiomyocytes. *ESC Heart Fail*. (2018) 5:642–8. doi: 10.1002/ehf2.12336
197. Uthman L, Baartscheer A, Schumacher CA, Fiolet JWT, Kuschma MC, Hollmann MW, et al. Direct cardiac actions of sodium glucose cotransporter 2 inhibitors target pathogenic mechanisms underlying heart failure in diabetic patients. *Front Physiol*. (2018) 9:1575. doi: 10.3389/fphys.2018.01575
198. Li C, Zhang J, Xue M, Li X, Han F, Liu X, et al. SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. *Cardiovasc Diabetol*. (2019) 18:15. doi: 10.1186/s12933-019-0816-2
199. Tanaka H, Hirata KI. Potential impact of SGLT2 inhibitors on left ventricular diastolic function in patients with diabetes mellitus. *Heart Fail Rev*. (2018) 23:439–44. doi: 10.1007/s10741-018-9668-1
200. Lam CSP, Chandramouli C, Ahooja V, Verma S. SGLT-2 Inhibitors in heart failure: current management, unmet needs, and therapeutic prospects. *J Am Heart Assoc*. (2019) 8:e013389. doi: 10.1161/JAHA.119.013389
201. Pieske B, Maggioni AP, Lam CSP, Pieske-Kraigher E, Filippatos G, Butler J, et al. Vericiguat in patients with worsening chronic heart failure and preserved ejection fraction: results of the SOluble guanylate Cyclase stimulatoR in heArT failurE patientS with PRESERVED EF (SOCRATES-PRESERVED) study. *Eur Heart J*. (2017) 38:1119–27. doi: 10.1093/eurheartj/ehw593
202. Altenhofer S, Kleikers PW, Radermacher KA, Scheurer P, Rob Hermans JJ, Schiffers P, et al. The NOX toolbox: validating the role of NADPH oxidases in physiology and disease. *Cell Mol Life Sci*. (2012) 69:2327–43. doi: 10.1007/s00018-012-1010-9
203. Luo M, Guan X, Luczak ED, Lang D, Kutschke W, Gao Z, et al. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. *J Clin Invest*. (2013) 123:1262–74. doi: 10.1172/JCI65268
204. Schilling JD. The mitochondria in diabetic heart failure: from pathogenesis to therapeutic promise. *Antioxid Redox Signal*. (2015) 22:1515–26. doi: 10.1089/ars.2015.6294
205. Youdim MB, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci*. (2006) 7:295–309. doi: 10.1038/nrn1883
206. Riederer P, Lachenmayer L, Laux G. Clinical applications of MAO-inhibitors. *Curr Med Chem*. (2004) 11:2033–43. doi: 10.2174/09298670433 64775
207. Deshwal S, Di Sante M, Di Lisa F, Kaludercic N. Emerging role of monoamine oxidase as a therapeutic target for cardiovascular disease. *Curr Opin Pharmacol*. (2017) 33:64–9. doi: 10.1016/j.coph.2017.04.003

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 19 February 2020
doi: 10.3389/fcvm.2020.00018

The Aging Heart: Mitophagy at the Center of Rejuvenation

*Wenjing J. Liang and Åsa B. Gustafsson**

Department of Pharmacology, Department of Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA, United States

Aging is associated with structural and functional changes in the heart and is a major risk factor in developing cardiovascular disease. Many recent studies have focused on increasing our understanding of the basis of aging at the cellular and molecular levels in various tissues, including the heart. It is known that there is an age-related decline in cellular quality control pathways such as autophagy and mitophagy, which leads to accumulation of potentially harmful cellular components in cardiac myocytes. There is evidence that diminished autophagy and mitophagy accelerate the aging process, while enhancement preserves cardiac homeostasis and extends life span. Here, we review the current knowledge of autophagy and mitophagy in aging and discuss how age-associated alterations in these processes contribute to cardiac aging and age-related cardiovascular diseases.

Keywords: aging, autophagy, mitophagy, mitochondria, heart, PINK1, Parkin, mitophagy receptors

OPEN ACCESS

Edited by:
Junichi Sadoshima, University of Medicine and Dentistry of New Jersey, United States

Reviewed by:
Satoaki Matoba, Kyoto Prefectural University of Medicine, Japan
Qiangrong Liang, New York Institute of Technology, United States
Satoru Kobayashi, New York Institute of Technology, United States

****Correspondence:***
Åsa B. Gustafsson
abgustafsson@ucsd.edu

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *07 November 2019*
Accepted: *03 February 2020*
Published: *19 February 2020*

Citation:
Liang WJ and Gustafsson ÅB (2020) The Aging Heart: Mitophagy at the Center of Rejuvenation. Front. Cardiovasc. Med. 7:18. doi: 10.3389/fcvm.2020.00018

INTRODUCTION

Aging is a major risk factor in developing cardiovascular disease and increases exponentially with age. Cardiac aging is characterized by the presence of hypertrophy, fibrosis, accumulation of misfolded proteins, and dysfunctional mitochondria. Current efforts are dedicated to understanding the biological process of aging and to identify pathways that can be targeted to extend health and life spans. Interestingly, it has been demonstrated that many of the pathways that improve health and extend longevity in various organisms all converge on autophagy (1–8). Autophagy is a catabolic pathway that is responsible for recycling cellular proteins and organelles to maintain energy homeostasis. It participates in the elimination of pathogens and prevents activation of inflammation. It is also a key pathway in cellular quality control by eliminating dysfunctional or unwanted organelles and protein aggregates. However, there is strong evidence that autophagy is decreased with age in tissues, including the heart (5, 9–15).

The heart requires a lot of energy which is mainly generated by mitochondria via oxidative phosphorylation. However, aging is associated with altered cardiac mitochondrial metabolism and mitochondrial respiratory defects (16). The impaired fatty acid and glucose metabolism, combined with reduced mitochondrial respiration are also believed to underlie the increased susceptibility to cardiac injury in the elderly population (16). Normally, these dysfunctional mitochondria are eliminated by autophagosomes in a selective process termed mitophagy. Predictably, reduced autophagy in aging contributes to accumulation of dysfunctional mitochondria and decreased ability to adapt to stress.

Altered autophagy and mitophagy overtime are likely central contributors in the aging process. Here, we review the current knowledge of autophagy and mitophagy in aging and discuss how age-associated alterations in these processes contribute to cardiac aging and age-related cardiovascular diseases.

AUTOPHAGY

Autophagy involves the sequestration of ubiquitinated cargo into vesicles called autophagosomes and delivery of the content to lysosomes via fusion. The cargo is degraded inside lysosomes and the components are recycled to the cytoplasm. Autophagy is a highly regulated process and consists of several distinct steps; initiation, nucleation and formation of phagophore, sequestration of cargo, and fusion of autophagosome with a lysosome (**Figure 1A**). The different steps in the process are regulated by different autophagy-related proteins (Atg) (17). The mechanistic target of rapamycin (mTOR) functions as a gate keeper and prevents activation of autophagy. When mTOR is inhibited, it leads to activation of the unc-51 like autophagy activating kinase 1 (Ulk1/Atg1) which initiates the nucleation of the autophagosome via Beclin1 (18). At baseline, Beclin1 is sequestered by Bcl-2 and Rubicon to suppress autophagy but its release allows it to initiate autophagosome formation (19–21). The elongation and maturation of the growing autophagosome membrane requires two conjugation pathways. The E1-like and E2-like enzymes Atg7 and Atg10 conjugate Atg5 to Atg12. The Atg5-Atg12 complex then interacts with Atg16. Atg16 is required for the proper localization of the complex to the pre-autophagosomal membrane (22). The Atg5-12-16 complex then functions as an E3-like enzyme in the second conjugation pathway, where LC3 is covalently linked to phosphatidylethanolamine (PE). The conjugation of LC3 to PE to form LC3II is mediated by Atg7 (E1-like) and Atg3 (E2-like), respectively (17). LC3II is also involved in cargo recognition where it binds to adaptor proteins such as p62 (23). Several proteins in this pathway are altered with age which ultimately leads to diminished autophagy.

MITOPHAGY

PINK1/Parkin-Mediated Mitophagy

The PINK1/Parkin pathway contains three key elements: a mitochondrial membrane depolarization sensor (PINK1), a signal amplifier (Parkin) and a downstream signal effector (ubiquitin chains) (**Figure 1B**) (24). Under normal cellular conditions, PINK1 is partly imported into the inter mitochondrial membrane space where it is cleaved by resident proteases such as the presenilin-associated rhomboid-like protease (PARL) (25, 26). However, this process is disrupted upon loss of mitochondrial membrane potential, leading to accumulation of PINK1 on the outer mitochondrial membrane (OMM), where PINK1 in turn recruits the E3 ubiquitin ligase Parkin (25, 27, 28). PINK1 phosphorylates both ubiquitin and

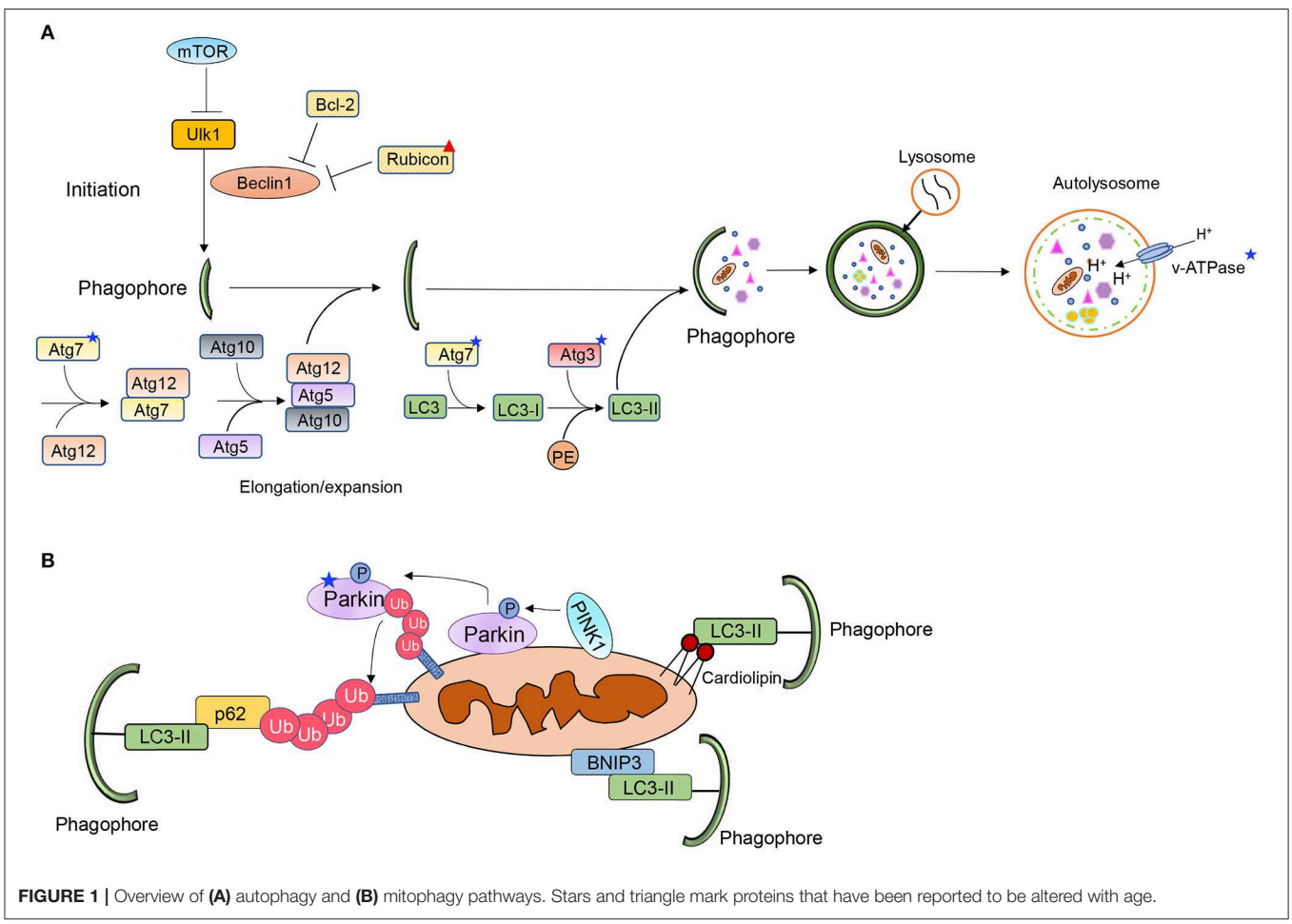


FIGURE 1 | Overview of **(A)** autophagy and **(B)** mitophagy pathways. Stars and triangle mark proteins that have been reported to be altered with age.

Parkin which contribute to both its activation and anchoring at the mitochondria (29). PINK1 has also been reported to phosphorylate MFN2 which then functions as a docking site for Parkin at mitochondria (30). This allows activated Parkin to ubiquitinate various outer mitochondrial membrane proteins (31). However, a recent study reported an alternative function for MFN2 during mitophagy where MFN2 must be degraded for mitophagy to proceed (32). MFN2 is known to tether mitochondria to ER at specific contact sites. McLelland et al. found that Parkin-mediated ubiquitination and degradation of MFN2 disrupts the contact sites and releases mitochondria from the ER. The release provides Parkin full access to its other substrates and allows for mitophagy to proceed (32). The mitochondrial proteins ubiquitinated by Parkin are recognized by various adaptor proteins, such as p62/SQSTM1 and Optineurin (33, 34). These adaptors bind to the ubiquitin-chains on proteins in the OMM via their ubiquitin-associated (UBA) domain and simultaneously directly interact with LC3 on the autophagosome via their LC3 Interacting Region (LIR) motifs (23, 33, 35).

Mitophagy Receptors

Mitochondrial proteins in the OMM can also target mitochondria to autophagosomes (**Figure 1B**). BNIP3, NIX/BNIP3L, FUNDC1, Bcl2L13, FKBP8, and Prohibitin-2 (PHB2) are some of the mitophagy receptors that have been identified to date (36–41). These proteins are integrated mitochondrial membrane proteins that are facing the cytosol. The exception is PHB2, which is localized in the inner mitochondrial membrane. PHB2 promotes removal of remaining mitochondrion after outer membrane rupture (36). The mitophagy receptors contain LIRs and can therefore bind directly to LC3 on the autophagosome membrane bypassing the need for ubiquitin and adaptor proteins. The phospholipid cardiolipin can also function as a mitophagy receptor (**Figure 1B**). Cardiolipin is localized on the inner mitochondrial membrane but is externalized on dysfunctional mitochondria where it facilitates mitophagy by interacting with LC3 (42). However, it is possible that, similar to PHB2, cardiolipin can ensure mitophagy of the inner mitochondrial compartment after outer mitochondrial membrane rupture. Although they have all been established as mitophagy receptors, it is unclear how most of them are activated to induce mitophagy of mitochondria. These proteins are also known to have alternative functions and how they switch between the two functions is not completely clear.

The physiological conditions dictating activation of the two distinct mitophagy pathways are still unclear and under intense investigation. Recently, it has been proposed that PINK1/Parkin-mediated mitophagy plays a minimal role in basal mitophagy (43, 44) and that this pathway plays a more important role in stress adaptation and repair (45, 46). Other studies have reported that mitophagy receptors are key regulators of programmed mitophagy during development or differentiation (47–49). Thus, the two different mitophagy pathways appear to have distinct functions in the cell but additional studies are clearly needed. Moreover, cross talk clearly exists between the two mitophagy pathways (50, 51). For instance, the protein phosphatase PGAM5 dephosphorylates FUNDC1 which enhances the interaction between FUNDC1 and LC3 (52). PGAM5 also coordinates with PHB2 to promote PINK1/Parkin-mediated mitophagy where PHB2 decreases PINK1 processing by inhibiting PARL while PGAM5 stabilizes PINK1 on the OMM (53). Taken together, there is clearly coordination between these two pathways, and they can compensate for each other to some extent.

AUTOPHAGY AND AGING

A growing body of data support the anti-aging effects of enhanced autophagy. Many studies have demonstrated that enhancing autophagy by limiting caloric intake, genetic manipulation or pharmacological treatments increases lifespan in various organisms (1–6). For instance, transgenic mice with systemic overexpression of Atg5 have enhanced autophagic activity in tissues which leads to health benefits such as reduced weight gain with age and extended life spans compared to wild type mice (2). Although this study did not specifically focus on the myocardium, the authors reported increased autophagic activity as well as reduced fibrosis with age in hearts of the transgenic mice. The cardioprotective effects of enhanced autophagy during the aging process were recently confirmed by the Levine group, who developed a $Becn1^{F121A/F121A}$ knock-in mouse model with constitutively increased basal autophagy due to a disruption in the Bcl-2 binding to Beclin1. They found that health and life spans are significantly increased in the knock-in mice. Moreover, aged $Becn1^{F121A/F121A}$ knock-in mice have reduced cardiac hypertrophy and interstitial fibrosis compared to aged-matched wild type mice (20), confirming that preserving autophagy in the heart delays or even prevents cardiac aging. In contrast, selective disruption of autophagy in the heart leads to accelerated cardiac aging with accumulation of ubiquitinated proteins and dysfunctional mitochondria and development of cardiac hypertrophy (54). Preserving autophagy is clearly critical in the heart to prevent biological aging.

MITOPHAGY AND AGING

Reduced mitophagy also recapitulates the age-related accumulation of dysfunctional mitochondria in tissues. Thus, the forced increase in autophagy in the above studies can also be linked to enhanced mitophagy as it would enhance elimination of dysfunctional mitochondria. Several studies have confirmed that genetic and pharmacological interventions promoting enhanced mitophagy also lead to extended life span (55, 56), while disrupting mitophagy leads to accelerated aging phenotypes (57, 58). For instance, Urolithin A is a natural compound that induces mitophagy and extends life span in *C.elegans* (56). Both systemic and neuron-specific overexpression of Parkin in flies slows aging and extends lifespan, although lifespan extension is greater with ubiquitous Parkin overexpression (59). A link also exists between Parkin-mediated mitophagy and NLRP3 inflammasome activation. The NLRP3 inflammasome is activated by the presence of mitochondrial DNA in the

cytosol that have been released from damaged mitochondria. Thus, Parkin-mediated mitophagy of damaged mitochondria functions to prevent activation of the inflammasome (60). The PINK1/Parkin pathway also diminishes STING-induced inflammation by a similar mechanism (61).

Several early studies reported that PINK1 or Parkin deficiency in Drosophila causes accumulation of dysfunctional mitochondria, flight muscle degeneration and reduced lifespan (62–64). Also, Cornelissen et al. found that mitophagic activity in flight muscle increased with aging in flies and that the age-dependent rise is abrogated by either PINK1 or Parkin deficiency (57). Parkin-deficient mice have an accelerated aging phenotype and accumulate aberrant mitochondria in aging heart (58, 65) while cardiac specific overexpression of Parkin can delay cardiac aging by enhancing mitochondrial turnover (65). These studies present evidence that enhancing mitophagy by targeting the Parkin pathway is beneficial. However, the anti-aging effect of Parkin is likely dose-dependent as aged transgenic mice with higher levels of Parkin overexpression develop cardiac fibrosis likely due to an imbalance between ubiquitination and autophagic degradation (66).

Much less is known about what happens to mitophagy receptors during aging. It was recently reported that mice deficient in both Akt2 and AMPK are predisposed to cardiac aging possible due to compromised mitophagy. These hearts have reduced levels of several mitophagy proteins including BNIP3 and FUNDC1 (15). A mouse model carrying a proofreading-defective mtDNA polymerase γ (POLG) accumulate mtDNA mutations which leads to accelerated aging (67). Unexpectedly, Parkin plays a minimal role in clearing cardiac mitochondria in POLG mice as cardiac aging is unaffected by cardiac-specific overexpression or global deletion of Parkin (66). Instead, hearts in aged POLG mice have elevated levels of the mitophagy receptor BNIP3 coupled with enhanced mitochondrial biogenesis, indicating enhanced baseline mitochondrial turnover (66). The fact that NIX/BNIP3 double knockout mice accumulate dysfunctional mitochondria in the heart at an accelerated rate with age compared to wild type mice confirms that these mitophagy receptors play a key role in baseline mitochondrial maintenance (68). Furthermore, Rana et al. recently demonstrated that promoting Drp1-mediated mitochondrial fission in midlife leads to increased mitophagy and rejuvenated mitochondria in flies. This leads to improved health span and delays the onset of pathology linked to aging (69). Together, these findings support the notion that reduced mitophagy might be a significant underlying factor in the accumulation of dysfunctional mitochondria in aged organisms contributing to their health decline and mortality. Also, the mitophagy pathway may represent a therapeutic target to counteract aging.

AGE-RELATED REDUCTION IN AUTOPHAGY AND MITOPHAGY

Although autophagy is clearly diminished with age in tissues, including the heart (5, 9–12), exactly why cardiac autophagy is reduced during aging is still unclear. Most of our current knowledge comes from studies in cell lines or other tissues. Oxidative stress can inhibit autophagy by promoting oxidation of the autophagy enzymes involved in autophagy (70). Under baseline conditions when autophagy is not activated, LC3 is covalently bound to inactive Atg3 and Atg7, which protects cysteine residues in their catalytic sites from oxidation. However, the release of LC3 upon activation of autophagy leads to exposure of the cysteines, making them available to direct oxidation during high levels of oxidative stress (70). Moreover, Parkin is also prone to oxidation of its cysteine residues which affects its E3 ubiquitin ligase activity and promotes its misfolding and aggregation (71, 72). Also, both PINK1 and Parkin can be S-nitrosylated which leads to attenuated mitophagy (73, 74). As cardiac aging is characterized by increased oxidative stress (75, 76), it is possible that this directly contributes to reduced autophagosome formation and impaired Parkin-mediated mitophagy in aged myocytes.

Low levels of chronic inflammation has also been linked to age-related diseases (77). The NLRP3 inflammasome is a cytosolic protein complex that initiates activation of inflammatory responses by inducing cell death and triggering the release of proinflammatory cytokines (77). Deregulation of the NLRP3 inflammasome has been linked to inhibition of autophagy and aging. NLRP3-deficient mice have improved health span and attenuated age-related functional decline, including reduced bone loss, improved memory and cognitive performance, and motor performance (78). Recently, it was reported that aged NLRP3-deficient mice have reduced cardiac hypertrophy and fibrosis and increased life spans compared to wild type mice (14). This study linked the NLRP3-deficiency in aged mice to reduced mTOR suppression resulting in increased autophagic activity (14).

Moreover, it is also likely that proteins involved in regulating autophagy are altered with age. For instance, Rubicon is a negative regulator of Beclin1 and it was recently reported that Rubicon expression increases in worm, fly and mouse tissues with age (5). Rubicon knockdown ameliorates age-dependent phenotypes and extends life span in both worms and flies, while Rubicon systemic-knockout mice have reduced age-associated phenotypes such as decreased kidney fibrosis (5). This suggests that Rubicon could be one of the factors contributing to the decline in autophagy during aging. However, other regulators might also be altered with age in tissues.

Finally, lysosomes function in the terminal step of autophagy (**Figure 1**) and lysosomal function is compromised with age (79). For instance, the activity of lysosomal hydrolases responsible for degrading cargo is dependent on the acidic milieu of the lysosome. After fusion with an autophagosome, the lysosome must undergo reacidification to restore the acidic pH and activate the hydrolases. The v-type ATPase is responsible for maintaining the acidic milieu by pumping proton into the lysosomal lumen and studies indicate that the v-ATPase activity and acidification are reduced with age (80). Lysosomal dysfunction has been identified in age-related neurological pathologies, such as Parkinson's and Alzheimer's disease (80). Lysosomal impairment has also been associated with decreased lifespan,

while enhancing lysosomal functional capacity can promote longevity (81, 82). In addition, the adult brain contains a pool of neural stem cells (NSCs) that can generate new neurons but the function of NSCs declines with age. Interestingly, there is an age-dependent decrease in lysosome levels in NSCs which results in fewer lysosomes available to fuse with autophagosomes (83). It is currently unclear if lysosomal function is altered in the aged heart.

CONCLUSION

In summary, declines in autophagy and mitophagy in tissues clearly play a role in the aging process and contribute to development of age-related diseases. The main questions that remain unanswered include: why are autophagy and mitophagy suppressed with age and can these pathways be restored in the aged heart? Relatively little is still known about the molecular mechanism underlying the decrease in autophagy and mitophagy and whether there are tissue specific differences. Although manipulation of autophagy and mitophagy pathways are protective in pre-clinical models, the level of activity must be carefully monitored as excessive autophagy can lead to excessive degradation of key cellular components. Increased knowledge into how these pathways are regulated as well as altered with age will allow for more specific manipulation. Further understanding will also provide important insights into how future therapies can protect the heart against age-specific functional decline.

AUTHOR CONTRIBUTIONS

Both authors contributed to the content of this article and have approved of its submission.

FUNDING

ÅG is supported by NIH R01HL138560 and R01HL132300, and TRDRP 27IR-0013 and 28IP- 0025. WL is supported by TRDRP T30FT0846.

REFERENCES

1. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med*. (2016) 22:1428–38. doi: 10.1038/nm.4222
2. Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, et al. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun*. (2013) 4:2300. doi: 10.1038/ncomms3300
3. Fernandez AF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature*. (2018) 558:136–40. doi: 10.1038/s41586-018-0162-7
4. Toth ML, Sigmond T, Borsos E, Barna J, Erdelyi P, Takacs-Vellai K, et al. Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy*. (2008) 4:330–8. doi: 10.4161/auto.5618
5. Nakamura S, Oba M, Suzuki M, Takahashi A, Yamamuro T, Fujiwara M, et al. Suppression of autophagic activity by Rubicon is a signature of aging. *Nat Commun*. (2019) 10:847. doi: 10.1038/s41467-019-08729-6
6. Simonsen, Cumming RC, Brech A, Isakson P, Schubert DR, Finley KD. Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila. *Autophagy*. (2008) 4:176–84. doi: 10.4161/auto.5269
7. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. (2009) 460:392–5. doi: 10.1038/nature08221
8. Wilkinson JE, Burmeister L, Brooks SV, Chan CC, Friedline S, Harrison DE, et al. Rapamycin slows aging in mice. *Aging Cell*. (2012) 11:675–82. doi: 10.1111/j.1474-9726.2012.00832.x
9. Inuzuka Y, Okuda J, Kawashima T, Kato T, Niizuma S, Tamaki Y, et al. Suppression of phosphoinositide 3-kinase prevents cardiac aging in mice. *Circulation*. (2009) 120:1695–703. doi: 10.1161/CIRCULATIONAHA.109.871137
10. Taneike M, Yamaguchi O, Nakai A, Hikoso S, Takeda T, Mizote I, et al. Inhibition of autophagy in the heart induces age-related cardiomyopathy. *Autophagy*. (2010) 6:600–6. doi: 10.4161/auto.6.5.11947
11. Ren X, Chen L, Xie J, Zhang Z, Dong G, Liang J, et al. Resveratrol ameliorates mitochondrial elongation via Drp1/Parkin/PINK1 signaling in senescent-like cardiomyocytes. *Oxid Med Cell Longev*. (2017) 2017:4175353. doi: 10.1155/2017/4175353
12. Zhou J, Chong SY, Lim A, Singh BK, Sinha RA, Salmon AB, et al. Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging. *Aging*. (2017) 9:583–99. doi: 10.18632/aging.101181
13. Ren J, Yang L, Zhu L, Xu X, Ceylan AF, Guo W, et al. Akt2 ablation prolongs life span and improves myocardial contractile function with adaptive cardiac remodeling: role of Sirt1-mediated autophagy regulation. *Aging Cell*. (2017) 16:976–87. doi: 10.1111/acel.12616
14. Marin-Aguilar F, Lechuga-Vieco AV, Alcocer-Gomez E, Castejon-Vega B, Lucas J, Garrido C, et al. NLRP3 inflammasome suppression improves longevity and prevents cardiac aging in male mice. *Aging Cell*. (2019) 19:e13050. doi: 10.1111/acel.13050
15. Wang S, Kandadi MR, Ren J. Double knockout of Akt2 and AMPK predisposes cardiac aging without affecting lifespan: role of autophagy and mitophagy. *Biochim Biophys Acta Mol Basis Dis*. (2019) 1865:1865–75. doi: 10.1016/j.bbadis.2018.08.011
16. Lesnefsky EJ, Chen Q, Hoppel CL. Mitochondrial metabolism in aging heart. *Circ Res*. (2016) 118:1593–611. doi: 10.1161/CIRCRESAHA.116.307505
17. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell*. (2019) 176:11–42. doi: 10.1016/j.cell.2018.09.048
18. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*. (2011) 13:132–41. doi: 10.1038/ncb2152
19. Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol*. (2009) 11:385–96. doi: 10.1038/ncb1846
20. Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol*. (2009) 11:468–76. doi: 10.1038/ncb1854
21. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell*. (2005) 122:927–39. doi: 10.1016/j.cell.2005.07.002
22. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell*. (2008) 19:2092–100. doi: 10.1091/mbc.e07-12-1257
23. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem*. (2007) 282:24131–45. doi: 10.1074/jbc.M702824200
24. Harper JW, Ordureau A, Heo JM. Building and decoding ubiquitin chains for mitophagy. *Nat Rev Mol Cell Biol*. (2018) 19:93–108. doi: 10.1038/nrm.2017.129

25. Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol*. (2010) 191:933–42. doi: 10.1083/jcb.201008084
26. Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep*. (2012) 13:378–85. doi: 10.1038/embor.2012.14
27. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol*. (2010) 189:211–21. doi: 10.1083/jcb.200910140
28. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol*. (2008) 183:795–803. doi: 10.1083/jcb.200809125
29. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol*. (2014) 205:143–53. doi: 10.1083/jcb.201402104
30. Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science*. (2013) 340:471–5. doi: 10.1126/science.1231031
31. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature*. (2013) 496:372–6. doi: 10.1038/nature12043
32. McLelland GL, Goiran T, Yi W, Dorval G, Chen CX, Lauinger ND, et al. Mfn2 ubiquitination by PINK1/parkin gates the p97-dependent release of ER from mitochondria to drive mitophagy. *Elife*. (2018) 7:e32866. doi: 10.7554/eLife.32866
33. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature*. (2015) 524:309–14. doi: 10.1038/nature14893
34. Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy*. (2010) 6:1090–106. doi: 10.4161/auto.6.8.13426
35. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol*. (2010) 12:119–31. doi: 10.1038/ncb2012
36. Wei Y, Chiang WC, Sumpter R Jr, Mishra P, Levine B. Prohibitin 2 is an inner mitochondrial membrane mitophagy receptor. *Cell*. (2017) 168:224–38 e10. doi: 10.1016/j.cell.2016.11.042
37. Bhujabal Z, Birgisdottir AB, Sjottem E, Brenne HB, Overvatn A, Habisov S, et al. FKBP8 recruits LC3A to mediate Parkin-independent mitophagy. *EMBO Rep*. (2017) 18:947–961. doi: 10.15252/embr.201643147
38. Murakawa T, Yamaguchi O, Hashimoto A, Hikoso S, Takeda T, Oka T, et al. Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nat Commun*. (2015) 6:7527. doi: 10.1038/ncomms8527
39. Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, et al. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol*. (2012) 14:177–85. doi: 10.1038/ncb2422
40. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, et al. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep*. (2010) 11:45–51. doi: 10.1038/embor.2009.256
41. Hanna RA, Quinsay MN, Orogo AM, Giang K, Rikka S, Gustafsson AB. Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy. *J Biol Chem*. (2012) 287:19094–104. doi: 10.1074/jbc.M111.322933
42. Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, et al. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol*. (2013) 15:1197–205. doi: 10.1038/ncb2837
43. McWilliams TG, Prescott AR, Montava-Garriga L, Ball G, Singh F, Barini E, et al. Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand. *Cell Metab*. (2018) 27:439–49 e5. doi: 10.1016/j.cmet.2017.12.008
44. Lee JJ, Sanchez-Martinez A, Zarate AM, Beninca C, Mayor U, Clague MJ, et al. Basal mitophagy is widespread in Drosophila but minimally affected by loss of Pink1 or parkin. *J Cell Biol*. (2018) 217:1613–22. doi: 10.1083/jcb.201801044
45. Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, et al. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem*. (2013) 288:915–26. doi: 10.1074/jbc.M112.411363
46. Song M, Gong G, Burelle Y, Gustafsson AB, Kitsis RN, Matkovich SJ, et al. Interdependence of parkin-mediated mitophagy and mitochondrial fission in adult mouse hearts. *Circ Res*. (2015) 117:346–51. doi: 10.1161/CIRCRESAHA.117.306859
47. Lampert MA, Orogo AM, Najor RH, Hammerling BC, Leon LJ, Wang BJ, et al. BNIP3L/NIX and FUNDC1-mediated mitophagy is required for mitochondrial network remodeling during cardiac progenitor cell differentiation. *Autophagy*. (2019) 15:1182–98. doi: 10.1080/15548627.2019.1580095
48. Esteban-Martinez L, Sierra-Filardi E, McGreal RS, Salazar-Roa M, Marino G, Seco E, et al. Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *EMBO J*. (2017) 36:1688–706. doi: 10.15252/embj.201695916
49. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, et al. Essential role for Nix in autophagic maturation of erythroid cells. *Nature*. (2008) 454:232–5. doi: 10.1038/nature07006
50. Zhang T, Xue L, Li L, Tang C, Wan Z, Wang R, et al. BNIP3 protein suppresses PINK1 kinase proteolytic cleavage to promote mitophagy. *J Biol Chem*. (2016) 291:21616–29. doi: 10.1074/jbc.M116.733410
51. Gao F, Chen D, Si J, Hu Q, Qin Z, Fang M, et al. The mitochondrial protein BNIP3L is the substrate of PARK2 and mediates mitophagy in PINK1/PARK2 pathway. *Hum Mol Genet*. (2015) 24:2528–38. doi: 10.1093/hmg/ddv017
52. Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H, et al. A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Mol Cell*. (2014) 54:362–77. doi: 10.1016/j.molcel.2014.02.034
53. Yan C, Gong L, Chen L, Xu M, Abou-Hamdan H, Tang M, et al. PHB2 (prohibitin 2) promotes PINK1-PRKN/Parkin-dependent mitophagy by the PARL-PGAM5-PINK1 axis. *Autophagy*. (2019) 16:419–34. doi: 10.1080/15548627.2019.1628520
54. Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, et al. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med*. (2007) 13:619–24. doi: 10.1038/nm1574
55. Schiavi, Maglioni S, Palikaras K, Shaik A, Strappazzon F, Brinkmann V, Torgovnick A, et al. Iron-starvation-induced mitophagy mediates lifespan extension upon mitochondrial stress in *C. elegans*. *Curr Biol*. (2015) 25:1810–22. doi: 10.1016/j.cub.2015.05.059
56. Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Felix AA, et al. Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. *Nat Med*. (2016) 22:879–88. doi: 10.1038/nm.4132
57. Cornelissen T, Vilain S, Vints K, Gounko N, Verstreken P, Vandenberghe W. Deficiency of parkin and PINK1 impairs age-dependent mitophagy in Drosophila. *Elife*. (2018) 7:e35878. doi: 10.7554/eLife.35878
58. Kubli DA, Quinsay MN, Gustafsson AB. Parkin deficiency results in accumulation of abnormal mitochondria in aging myocytes. *Commun Integr Biol*. (2013) 6:e24511. doi: 10.4161/cib.24511
59. Rana A, Rera M, Walker DW. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. *Proc Natl Acad Sci USA*. (2013) 110:8638–43. doi: 10.1073/pnas.1216197110
60. Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, et al. NF-κB restricts inflammasome activation via elimination of damaged mitochondria. *Cell*. (2016) 164:896–910. doi: 10.1016/j.cell.2015.12.057
61. Sliter DA, Martinez J, Hao L, Chen X, Sun N, Fischer TD, et al. Parkin and PINK1 mitigate STING-induced inflammation. *Nature*. (2018) 561:258–62. doi: 10.1038/s41586-018-0448-9
62. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA*. (2003) 100:4078–83. doi: 10.1073/pnas.0737556100

63. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*. (2006) 441:1162–6. doi: 10.1038/nature04779
64. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*. (2006) 441:1157–61. doi: 10.1038/nature04788
65. Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, et al. Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. *Nat Commun*. (2013) 4:2308. doi: 10.1038/ncomms3308
66. Woodall BP, Orogo AM, Najor RH, Cortez MQ, Moreno ER, Wang H, et al. Parkin does not prevent accelerated cardiac aging in mitochondrial DNA mutator mice. *JCI Insight*. (2019) 5:e127713. doi: 10.1172/jci.insight.127713
67. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science*. (2005) 309:481–4. doi: 10.1126/science.1112125
68. Dorn GW 2nd. Mitochondrial pruning by Nix and BNip3: an essential function for cardiac-expressed death factors. *J Cardiovasc Transl Res*. (2010) 3:374–83. doi: 10.1007/s12265-010-9174-x
69. Rana A, Oliveira MP, Khamoui AV, Aparicio R, Rera M, Rossiter HB, et al. Promoting Drp1-mediated mitochondrial fission in midlife prolongs healthy lifespan of Drosophila melanogaster. *Nat Commun*. (2017) 8:448. doi: 10.1038/s41467-017-00525-4
70. Frudd K, Burgoyne T, Burgoyne JR. Oxidation of Atg3 and Atg7 mediates inhibition of autophagy. *Nat Commun*. (2018) 9:95. doi: 10.1038/s41467-017-02352-z
71. Meng F, Yao D, Shi Y, Kabakoff J, Wu W, Reicher J, et al. Oxidation of the cysteine-rich regions of parkin perturbs its E3 ligase activity and contributes to protein aggregation. *Mol Neurodegener*. (2011) 6:34. doi: 10.1186/1750-1326-6-34
72. Wang C, Ko HS, Thomas B, Tsang F, Chew KC, Tay SP, et al. Stress-induced alterations in parkin solubility promote parkin aggregation and compromise parkin's protective function. *Hum Mol Genet*. (2005) 14:3885–97. doi: 10.1093/hmg/ddi413
73. Rizza S, Cardaci S, Montagna C, Di Giacomo G, De Zio D, Bordi M, et al. S-nitrosylation drives cell senescence and aging in mammals by controlling mitochondrial dynamics and mitophagy. *Proc Natl Acad Sci USA*. (2018) 115:E3388–97. doi: 10.1073/pnas.1722452115
74. Oh CK, Sultan A, Platzer J, Dolatabadi N, Soldner F, McClatchy DB, et al. S-nitrosylation of PINK1 attenuates PINK1/parkin-dependent mitophagy in hiPSC-based Parkinson's disease models. *Cell Rep*. (2017) 21:2171–82. doi: 10.1016/j.celrep.2017.10.068
75. Owada T, Yamauchi H, Saitoh SI, Miura S, Machii H, Takeishi Y. Resolution of mitochondrial oxidant stress improves aged-cardiovascular performance. *Coron Artery Dis*. (2017) 28:33–43. doi: 10.1097/MCA.0000000000000434
76. Dai DF, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, et al. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation*. (2009) 119:2789–97. doi: 10.1161/CIRCULATIONAHA.108.822403
77. Latz E, Duewell P. NLRP3 inflammasome activation in inflammaging. *Semin Immunol*. (2018) 40:61–73. doi: 10.1016/j.smim.2018.09.001
78. Youm YH, Grant RW, McCabe LR, Albarado DC, Nguyen KY, Ravussin A, et al. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab*. (2013) 18:519–32. doi: 10.1016/j.cmet.2013.09.010
79. Carmona-Gutierrez, Hughes AL, Madeo F, Ruckenstuhl C. The crucial impact of lysosomes in aging and longevity. *Ageing Res Rev*. (2016) 32:2–12. doi: 10.1016/j.arr.2016.04.009
80. Colacurcio DJ, Nixon RA. Disorders of lysosomal acidification-The emerging role of v-ATPase in aging and neurodegenerative disease. *Ageing Res Rev*. (2016) 32:75–88. doi: 10.1016/j.arr.2016.05.004
81. Hughes AL, Gottschling DE. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature*. (2012) 492:261–5. doi: 10.1038/nature11654
82. Folick, Oakley HD, Yu Y, Armstrong EH, Kumari M, Sanor L, et al. Aging. Lysosomal signaling molecules regulate longevity in *Caenorhabditis elegans*. *Science*. (2015) 347:83–6. doi: 10.1126/science.1258857
83. Leeman DS, Hebestreit K, Ruetz T, Webb AE, McKay A, Pollina EA, et al. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science*. (2018) 359:1277–83. doi: 10.1126/science.aag3048

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 04 March 2020
doi: 10.3389/fcvm.2020.00028

Epigenetic Control of Mitochondrial Function in the Vasculature

Shafeeq A. Mohammed[1], *Samuele Ambrosini*[1], *Thomas Lüscher*[1,2], *Francesco Paneni*[1,3,4] *and Sarah Costantino*[1]*

[1] Center for Molecular Cardiology, University of Zürich, Zurich, Switzerland, [2] Research, Education and Development, Royal Brompton and Harefield Hospital Trust and Imperial College, London, United Kingdom, [3] Department of Cardiology, University Heart Center, University Hospital Zurich, Zurich, Switzerland, [4] Department of Research and Education, University Hospital Zurich, Zurich, Switzerland

The molecular signatures of epigenetic regulation and chromatin architecture are emerging as pivotal regulators of mitochondrial function. Recent studies unveiled a complex intersection among environmental factors, epigenetic signals, and mitochondrial metabolism, ultimately leading to alterations of vascular phenotype and increased cardiovascular risk. Changing environmental conditions over the lifetime induce covalent and post-translational chemical modification of the chromatin template which sensitize the genome to establish new transcriptional programs and, hence, diverse functional states. On the other hand, metabolic alterations occurring in mitochondria affect the availability of substrates for chromatin-modifying enzymes, thus leading to maladaptive epigenetic signatures altering chromatin accessibility and gene transcription. Indeed, several components of the epigenetic machinery require intermediates of cellular metabolism (ATP, AcCoA, NADH, α-ketoglutarate) for enzymatic function. In the present review, we describe the emerging role of epigenetic modifications as fine tuners of gene transcription in mitochondrial dysfunction and vascular disease. Specifically, the following aspects are described in detail: (i) mitochondria and vascular function, (ii) mitochondrial ROS, (iii) epigenetic regulation of mitochondrial function; (iv) the role of mitochondrial metabolites as key effectors for chromatin-modifying enzymes; (v) epigenetic therapies. Understanding epigenetic routes may pave the way for new approaches to develop personalized therapies to prevent mitochondrial insufficiency and its complications.

Keywords: epigenetics, mitochondria, vascular disease, oxidative stress, endothelial function

OPEN ACCESS

Edited by:
Sebastiano Sciarretta,
Sapienza University of Rome, Italy

Reviewed by:
Shiyou Chen,
University of Missouri, United States
Michio Shimabukuro,
Fukushima Medical University, Japan

***Correspondence:**
Sarah Costantino
sarah.costantino@uzh.ch

Specialty section:
This article was submitted to Cardiovascular Metabolism, a section of the journal Frontiers in Cardiovascular Medicine

Received: *11 November 2019*
Accepted: *19 February 2020*
Published: *04 March 2020*

Citation:
Mohammed SA, Ambrosini S, Lüscher T, Paneni F and Costantino S (2020) Epigenetic Control of Mitochondrial Function in the Vasculature. Front. Cardiovasc. Med. 7:28. doi: 10.3389/fcvm.2020.00028

MITOCHONDRIA AND VASCULAR FUNCTION

Mitochondria, defined as semi-autonomous, membrane-bound organelle localized in the cytoplasm of eukaryotic cells, are emerging as a pivotal player in health, disease, and aging by regulating reactive oxygen species (ROS) production and contributing to retrograde redox signalling from the organelle to the cytosol and nucleus (**Figure 1**) (1, 2). Mitochondria play an important role in the overall cellular network formed by metabolic signalling and epigenetic pathways. Indeed, mitochondria drive catabolic and anabolic reactions supplying energy and metabolites with biosynthetic and signalling roles (3). They also maintain a bidirectional signalling crosstalk with the nucleus that generates reciprocal activation-repression patterns of gene expression (3–5). Finally, mitochondria can determine apoptotic and necrotic cell death mediated by Ca^{2+} overload and opening of the permeability transition pore (PTP) (6, 7).

Under physiological conditions, mitochondria undergo highly coordinated cycles of fission (division of a single organelle into two or more independent structures) or fusion (the opposing reaction) (8). Fission and fusion are active processes which require many specialized proteins, including mechanical enzymes that physically alter mitochondrial membranes, and adaptor proteins that regulate the interaction of these mechanical proteins with organelles. The balance between these two processes regulates the overall morphology of mitochondria within any given cell (8–10). The content of mitochondria in the cytoplasm of eukaryotic cells depend on two major processes known as mitochondrial biogenesis and mitophagy (11). Mitochondrial biogenesis is an intricate and not fully understood process which leads to an increased mitochondrial mass mainly via replication of mitochondrial DNA (mtDNA) and expression of nuclear and mitochondrial genes (12). PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator−1α) plays a prominent role in mitochondrial biogenesis by activating the nuclear respiratory factor (Nrf)-1 and −2 to promote the expression nuclear genes. PGC-1α also activates transcription factors A and B which regulate the expression of mitochondrial genes (13, 14). Following mitochondrial damage, the organelles are being selectively degraded according to a well-known biological process called mitophagy, which promotes organelle turnover while preventing accumulation of dysfunctional mitochondria (**Figure 1**) (11). In addition to the selective removal of damaged mitochondria, mitophagy is also required to adjust mitochondrial numbers to changing cellular metabolic needs, for steady-state mitochondrial turnover, and during certain cellular developmental stages, such as during cellular differentiation of red blood cells (10). Mitochondrial content may vary based on the cell type and its function. For example, in endothelial cells mitochondria occupy around 6% of cytoplasm whereas in cardiomyocytes this reaches 32% (15). Notably, the blood brain barrier which consist of highly active endothelial cells has higher mitochondrial content as compared with endothelial cells present in capillary beds (15). Mitochondria play a pivotal role in endothelial

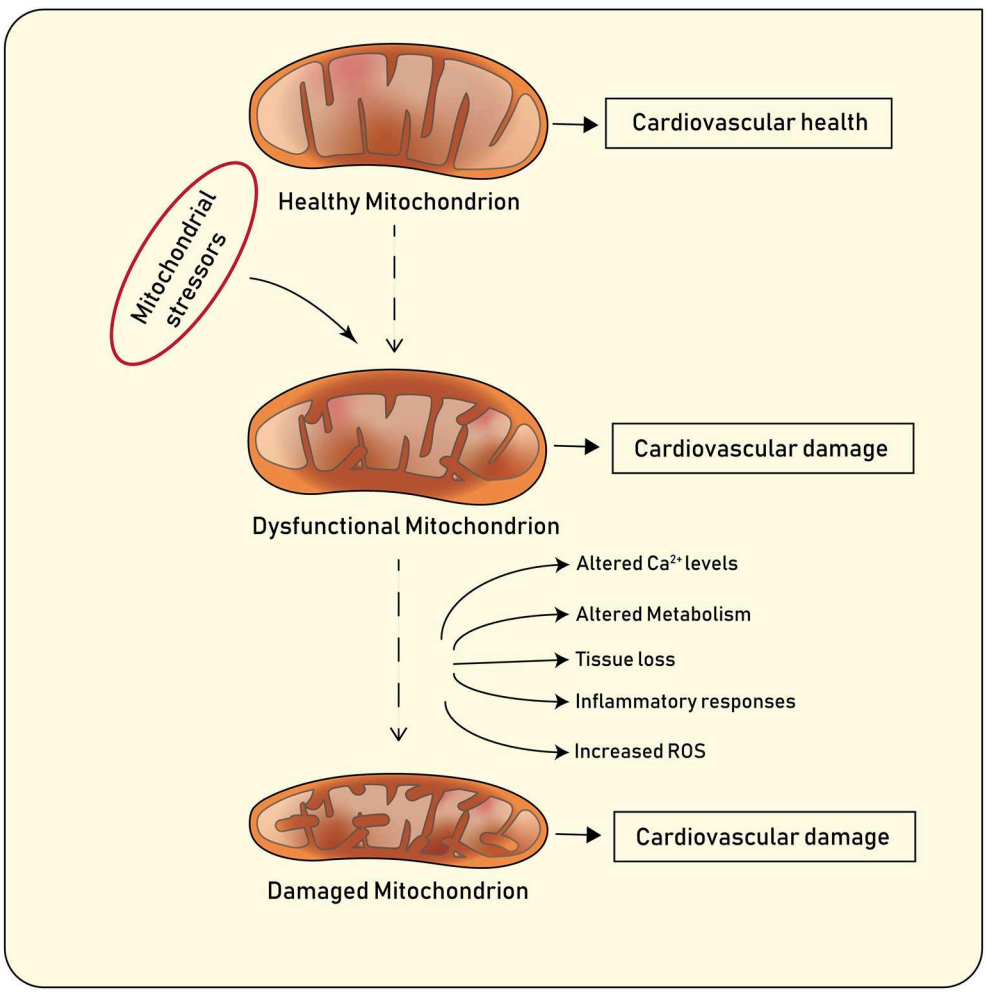


FIGURE 1 | Main features of healthy and diseased mitochondria, and implications for cardiovascular disease.

cells. Several biological processes including mitochondrial biogenesis, fission and fusion as well as mitophagy, have shown to clearly affect endothelial cell function and metabolism. Several stimuli including hypoxia, calorie restriction or exercise induce mitochondrial biogenesis in endothelial cells by increasing the expression of the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). Induction of PGC-1α is associated with a favorable transcriptional profile which protects endothelial cells from oxidative damage and apoptosis (16). In line with this notion, endothelial-specific overexpression of PGC-1α protects against angiotensin II–induced hypertension (17). By contrast, loss of endothelial PGC-1α impairs endothelial NO bioactivity eventually leading to endothelial dysfunction (18). Alterations of mitochondrial dynamics also contribute to endothelial cell phenotype. Endothelial cells from patients with diabetes display mitochondrial fragmentation and increased expression of fission-1 protein (Fis1) and dynamin-related protein-1 (Drp1). Of note, *in vitro* experiments showed that gene silencing Fis1 or Drp1 expression blunted hyperglycemia-induced alterations in mitochondrial networks, ROS production, endothelial nitric oxide synthase activation, and cGMP production (19). Alterations of mitophagy as the result of disturbed Ucp2/PTEN signaling were also associated with inadequate mitochondrial biosynthesis and increased apoptosis in endothelium (20). Altered mitochondrial clearance may also contribute to age-dependent endothelial dysfunction. Indeed, senescent cells display altered mitochondrial dynamics and loss of membrane potential (21). Interestingly enough, overexpression of proteins involved in the autophagosome formation (ATG5 and ATG12) was associated with improved mitochondrial performance, as evidenced by higher membrane potential, increased ATP production, and decreased damage to mtDNA (22, 23).

MITOCHONDRIAL ROS

Although several cytosolic enzymes (i.e., NADPH, cyclooxygenases, and xanthine oxidase) are implicated in redox balance, ROS generated from mitochondrial oxidative phosphorylation represent the most important source of oxidative stress in vascular cells (i.e., endothelial cells) (24, 25).

Mitochondrial ROS are responsible for peroxidation of polyunsaturated fatty acids (PUFAs) present in the cellular membrane as well as DNA (causing single and double strand breaks) and protein damage via oxidation of sulfhydryl and aldehyde groups, protein-protein interactions and fragmentation (26). In addition, damage of mtDNA may lead to decreased expression of electron transport chain components or expression of defective components that produce more ROS, thus creating a detrimental vicious cycle. mtDNA disruption also correlates with the extent of atherosclerosis in mouse models and human tissues. Despite the highly efficient chemical reduction of O_2 through cytochrome *c* oxidase, mitochondria still generate significant levels of ROS (27). Cellular and mitochondrial physiological levels of ROS are reached when production and scavenging are balanced (28). Mitochondrial dysfunction is believed to play an important role in a variety of diseases including diabetes, obesity, dyslipidaemia, hypertension, arrhythmias, and sudden cardiac death (29–31).

In the setting of cardiovascular risk factors, namely hyperglycemia, mitochondrial ROS can be regarded as an upstream biochemical event responsible for the activation of pro-inflammatory pathways (i.e., NF-kB), protein kinase C as well as advanced glycation end products (AGEs) (32). An increasing body of evidence has contributed to unveil different sources of mitochondrial ROS in endothelial cells. Studies in isolated mitochondria have shown that superoxide anion formation at complexes I and III accounts for 0.1-2% of the total (33). In addition to complexes I and III, the nicotinamide adenine dinucleotide phosphate oxidase (NOX) 4—a ROS-generating enzyme involved in endothelial cell senescence, migration, angiogenesis, and adaptive responses to hypoxia—is highly expressed in vascular cells and has been localized to mitochondria (34). Moreover, the monoamine oxidase (MAO) family of enzymes—which is found in the outer mitochondrial membrane—generates hydrogen peroxide (H_2O_2) during catabolism of catecholamines and has been implicated in maladaptive cellular hypertrophy and apoptosis (35). MAO-A-induced ROS are involved in serotonin-induced vasoconstriction in vascular smooth muscle cells (36). Although endothelial cells are known to express MAO, its importance for endothelial function is poorly understood (37). The mitochondrial adaptor protein $p66^{Shc}$ was recently shown to be causally involved in mitochondrial ROS generation and cellular death. In conditions of cellular stress, $p66^{Shc}$ is phosphorylated at ser36 by protein kinase C beta2 (PKCβ2) and translocates to the mitochondria where it oxidizes cytochrome *c*, leading to accumulation of H_2O_2, PTP opening, and release of solutes and proapoptotic signals (38). The causal role of $p66^{Shc}$ in vascular disease is supported by the notion that its genetic deletion or gene silencing prevents age and hyperglycemia-induced endothelial dysfunction in mice (39–41). The prolyl-isomerase 1 (Pin1), which regulates $p66^{Shc}$ translocation to the mitochondria, has also shown to be causally implicated in the regulation of mitochondrial oxidative stress and integrity in experimental models of diabetes (42, 43). The mitochondrial ATP-sensitive potassium channel ($mitoK_{ATP}$) was also recently discovered as a potential source of mitochondrial ROS in cardiac myocytes (44). Although the exact mechanism of action remains elusive, $mitoK_{ATP}$ seems to act as an uncoupling agent by reducing membrane potential and mitochondrial calcium. Pharmacological inhibition of $mitoK_{ATP}$ was found to improve endothelial function and to prevent ischemia-induced cellular apoptosis (44). Several antioxidant enzymes play a pivotal role in maintaining redox balance in mitochondria. Manganese superoxide dismutase (MnSOD) represents one of the first line defense against accumulation of mitochondrial superoxide. MnSOD is located in the mitochondrial matrix and catalyzes the conversion of superoxide anion to hydrogen peroxide (45). Loss of MnSOD in mice leads to impaired endothelium-dependent vasodilation, suggesting its role in regulating vascular function. In addition, $ApoE^{-/-}$ $MnSOD^{+/-}$ mice display early mtDNA damage and accelerated atherosclerosis when compared to control animals (46). Levels of H_2O_2 are regulated by glutathione peroxidase-1, thioredoxin-2, peroxiridoxin-3,

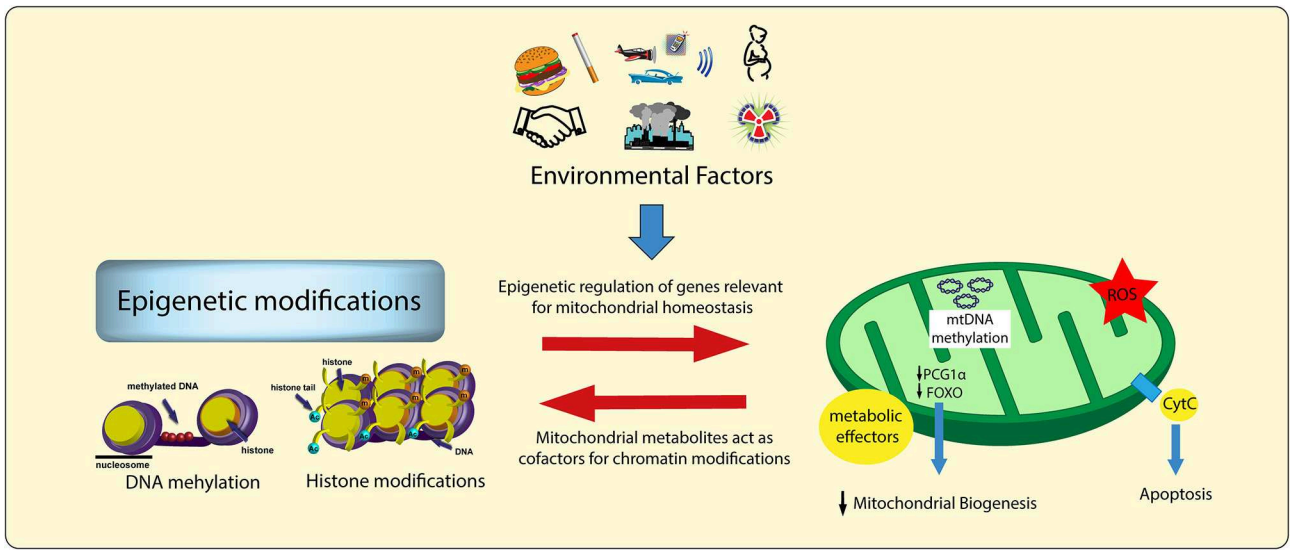


FIGURE 2 | Environmental factors, chromatin modifications, and mitochondrial damage. Environmental factors lead to specific epigenetic signatures as well as to alterations of mitochondrial intermediate metabolites (i.e., acetyl-CoA, FAD+, NAD+). These two processes influence each other, thus leading to a vicious cycle responsible for adverse chromatin modifications, maladaptive transcriptional programs, and vascular dysfunction. ROS, reactive oxygen species.

and glutaredoxin-2 (47). As noted, increased expression of these enzymes is signaled by AMPK and PGC-1α in response to H_2O_2 and other free radicals in endothelial cells (48). Studies in experimental models have shown that reduced expression of mitochondrial antioxidant enzymes can induce mitochondrial damage, endothelial dysfunction, and atherogenesis (45, 46). Conversely, overexpression of these proteins is protective against the development of vascular disease (49).

Although the role of mitochondrial ROS in vascular damage is well-established, only few studies have explored the specific contribution of mitochondria-derived ROS in the pathophysiology of endothelial dysfunction in humans. Mitochondrial ROS production and membrane hyperpolarization are significantly altered in visceral fat arteries and peripheral blood mononuclear cells isolated from patients with obesity and type 2 diabetes (50, 51). Furthermore, impaired endothelium-dependent vasodilation in freshly isolated arterioles from diabetic individuals is reversed by mild membrane depolarization or mitochondria-targeted antioxidants (50).

EPIGENETIC REGULATION OF MITOCHONDRIAL FUNCTION

Recent evidence indicates that epigenetic changes, defined as plastic modifications of DNA/histone complexes, are heavily implicated in the regulation of mitochondrial and vascular function (52, 53). Studies conducted over the last few years have unmasked a complex intersection among environmental factors, mitochondrial metabolism, epigenetic signals and transcriptional programs (54, 55). Epigenetic changes acquired during the life time may derail the expression of genes involved in mitochondrial homeostasis (52). On the other hand, metabolic alterations occurring in mitochondria may affect the availability of substrates for chromatin-modifying enzymes, thus leading to maladaptive epigenetic signatures altering chromatin accessibility and, hence, gene transcription (**Figure 2**) (54). Indeed, the availability of some intermediate mitochondrial metabolites (ATP, AcCoA, NADH, α-ketoglutarate) has shown to foster different patterns of epigenetic modifications. For examples, iron, α-ketoglutarate (α-KG) and O_2 are needed both for histone demethylation—catalysed by iron-containing jumonji-domain (jmjC) demethylases (56)—as well as for DNA demethylation of 5-methylcytosine—catalysed by the ten-eleven translocation family of dioxygenases (TET) (57). Therefore, mitochondrial sensitivity determined by environmental factors and lifestyle changes (sedentarism, physical activity, overnutrition, balanced nutrition) will favor, or prevent, the effects of metabolic disorders.

CLASSIFICATION OF EPIGENETIC CHANGES

Epigenetic mechanisms can be divided into three main categories: (i) chemical modifications of DNA (i.e., methylation); (ii) post-translational modifications of histone tails; (iii) regulation of gene expression by non-coding RNAs [i.e., microRNAs, long non-coding RNAs (lncRNAs)] (58). In the present review, we will focus on the modifications of DNA/histone complexes and their impact on mitochondrial integrity and functionality.

DNA Methylation

Methylation of DNA mainly takes place at the level of CpG regions of gene promoters through the attachment of methyl

group (CH3) from S-adenosyl methionine (SAM) to the C5 position in the cytosine-paired-with-guanine (CpG) dinucleotide sequences (59). CpG sequences are generally located into promoter regions of genes, however, they can also be located within gene bodies (58). Promoter methylation is generally associated with transcriptional repression, while gene body methylation is associated with enhanced transcription (60). Promoter methylation hampers gene expression mainly via two mechanisms: (i) by fostering transcriptional silencing, or (ii) by preventing the recruitment of transcription factors (61). Specifically, methylated cytosines are recognized by DNA methyl-binding proteins (MBPs) that repress gene transcription by preventing the interaction of transcription factors with the promoter (62). Alternatively, DNA methylation may recruit specific proteins that may also favor the recruitment of enzymes catalysing histone posttranslational modifications (PTMs) with subsequent gene repression (63, 64).

DNA methylation is a relatively stable epigenetic signature, it can be tissue-specific and, most importantly, it can be transmitted to the offspring, a phenomenon known as "epigenetic inheritance" (65). Different families of enzymes, known as methyltransferases (DNMTs), are involved in the regulation of DNA methylation: DNMT1 is responsible for the maintenance of methylation patterns in the genome by replicating the hemi-methylated CpG sites (66), whereas Dnmt3a/b are considered de novo methyltransferases (67). Methylation of DNA is a dynamic and reversible process governed by methyl-writing and -erasing enzymes (58). DNA demethylation can be achieved by either passive or active mechanisms (58). Active DNA demethylation consists in the removal of the methyl group by breaking a carbon–carbon bond. DNA demethylation may follow two main pathways: the first is dependent on cytosine deamination (AID, APOBEC3G, FTO) while the second is dependent on the oxidation of methylated cytosines (68). This latter reaction is catalysed by members of the Ten-eleven translocation (TET) proteins family (TET1-3) that convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) (69, 70). TET1 is mostly found in embryonic stem cells, whereas TET2 and TET3 are ubiquitously expressed. TET1-3 proteins could further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) that are recognized and excised by the thymine DNA glycosylase (TDG) via the base excision repair pathway (70, 71). By contrast, passive DNA demethylation is the result of DNMT1 inhibition during DNA replication (69).

Histone Modifications

DNA is packaged into repeating units called nucleosomes by wrapping around multimeric histone proteins. When nucleosomes are organized into tightly packed bundles (heterochromatin), the transcriptional machinery is hampered by a reduction of chromatin accessibility. Conversely, when chromatin is relaxed (euchromatin), DNA is more accessible to transcription factors, and gene transcription may occur (72). Histones are amenable to many posttranslational modifications (PTMs), which include methylation, acetylation, ubiquitination, phosphorylation, SUMOylation, GlcNAcylation, carbonylation, and ADP-ribosylation (73, 74). Of interest, these modifications may cluster in different patterns to regulate chromatin accessibility (59, 72, 75). Albeit the biological significance of many PTMs remains to be elucidated, considerable advances have been made in the understanding of lysine acetylation and methylation (74).

Histone acetylation, characterized by the addition of positively charged acetyl groups to amino acid residues at the level of histone tails, reduces the affinity of histones for DNA thus increasing chromatin accessibility (76). Acetylation occurs mainly on lysine residues on histones H3 and H4; this mark mainly associates with activation of transcription by enhancing chromatin accessibility (77). In this context, bromodomain and extra-terminal proteins recognize histone acetylation marks and initiate the assembly of the transcriptional machinery (78). By contrast, non-acetylated histones have been observed in transcriptionally silent genes where chromatin is compact (79). Acetylation is modulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) which are involved in addition or removal of an acetyl group, respectively (80). This modification is driven by recognition and binding of transcription factors able to recruit one of a growing family of HATs, namely CBP/p300, MYST, and GNAT (59, 73). HATs catalyse the addition of two-carbon acetyl groups to lysine residues from acetyl-CoA thus leading to gene expression (81). On the other hand, removal of acetyl groups from histone residues by HDACs represses gene transcription (82, 83). Several HDACs have been reported in humans, and they are subdivided into four classes (Class I, IIa, IIb, III, and IV) (84, 85).

In contrast to lysine acetylation, which enhances gene expression, histone methylation may result in different chromatin states according to the methylated residue and the number of added methyl groups (79). Histone methylation is defined as the transfer of methyl group from S-adenosyl-L-methionine to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs) (86). Histone methyltransferases (HMTs) have higher specificity as compared to HATs (87) and include several families of enzymes (EZH, SETD, PRDM, PRMT, METTL, and MLL) (88). Recent evidence indicates that a fine balance between histone methylation and demethylation plays a pivotal role in the regulation of chromatin accessibility.

Several lysine demethylases specific for diverse histone lysine residues have been identified (89). HDMs include members of UTX/Y, JARID1, JMJD, LSD, PHF, and FBXL enzyme families (88).

Interestingly, modifications of histones may reciprocally influence or eventually affect DNA methylation (74). In this regard, recent evidence suggests that DNA methylase (DNMTs), histone methyltransferase (HMTs), and histone acetyltransferase (HATs) are closely interconnected to regulate chromatin remodeling under specific stimuli (90). A well-described crosstalk between DNA methylation and histone H3K9 methylation, mediated by the heterochromatin

protein 1 (HP1), represents a valid example of how histone modifications may facilitate the recruitment of enzymes (DNTM3a/b) involved in DNA methylation (91). Another example is methyl-CpG binding protein 2 (MECP2), which recruits the histone methyltransferase SUV39H1 only after binding methylated DNA (92, 93). Therefore, chromatin modifications may influence each other and can propagate.

EPIGENETIC REMODELING OF MITOCHONDRIAL DNA

Increasing evidence suggests that aberrant mitochondrial DNA (mtDNA) modification play an important role in disease development and progression (94). Since the vast majority of mitochondrial proteins are encoded in the nuclear genome, appropriate communication between the nuclear, cytoplasmic and mitochondrial compartments is essential for maintaining proper mitochondrial function. The mitochondrial genome consists of roughly 1,500 genes distributed across the maternal mtDNA and nuclear DNA (nDNA) (95). Human mtDNA is a 16.5-kb circular double-stranded DNA containing a heavy (H) and a light (L) strand located in the mitochondrial matrix (96, 97). mtDNA forms an mtDNA–protein complex, known as nucleoid, with a range of proteins including prohibitins, ATPase family AAA domain-containing protein 3 (ATAD3), mitochondrial transcription factor A (TFAM) and POLG (DNA polymerase gamma, catalytic subunit) (98, 99). In contrast to nDNA, human mtDNA is maternally inherited, is intronless, and lacks histones (100). It contains 37 genes encoding 13 subunit of the oxidative phosphorylation (OXPHOS) complexes I, III, IV, and V; two rRNAs; and 22 tRNAs (2). All other mitochondrial proteins, including those required for mtDNA replication and transcription, are encoded in the nucleus and translocated to the mitochondria using specialized import systems which often involve N-terminal mitochondrial targeting sequences (101).

Emerging evidence suggests that mtDNA may also be regulated at the epigenetic level in the form of mtDNA methylation (2). While nDNA methylation is a well-established feature, mtDNA methylation has been a matter of debate (94, 102). The prevailing opinion was that mtDNA cannot be methylated for two main reasons: (i) methylase cannot access mitochondria, and (ii) mtDNA is not complexed with histones (103). Only recently, mtDNA has been reported to contain 5-methylcytosine (5mC) as well as 5-hydroxymethylcytosine (5hmC) at CpG dinucleotides. In 2011, Shock et al. have identified a mitochondrially targeted DNMT1 transcript variant (mtDNMT1) that uses an upstream alternative translation start site leading to the inclusion of a mitochondrial targeting sequence (101). mtDNMT1 binds to the mitochondrial genome in a manner proportional to the density of CpG dinucleotides. Of note, cytosine methylation in mtDNA may play different role. Indeed, mtDNA methylation represses gene expression from the light-strand promoter. However, increased or no change in transcription of genes from the heavy-strand promoter raises the possibility of a different mode of action (104). This DNMT1 variant is upregulated by the hypoxia-responsive transcription factors peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1a) and nuclear respiratory factor 1 (NRF1) suggesting a regulatory role of mtDNMT1 during vascular oxidative stress (**Figure 3**) (101).

Besides mtDNMT1, no other specific mitochondrially targeted isoforms of enzymes involved in DNA methylation or hydroxymethylation are known (100). Nevertheless, other enzymes, namely DNMT3A/B and ten–eleven translocation (TET) 1 and 2, have been detected in the mitochondrial protein fraction (105). Interestingly, the presence of these enzymes in the mitochondria seems to be tissue specific. Indeed, inside the mitochondria of 'excitable tissues' (heart, skeletal muscle, and neurons) only DNMT3A but not DNMT3b has been detected (106). Furthermore, epigenetic modifications of mtDNA can modulate the activity of nDNA, and vice versa (107). Under conditions of oxidative stress, such as exposure to hypoxia or ethanol, DNMT1 is upregulated and suppresses the expression of ND6 (101), while ND1 is upregulated. Although the significance of opposite ND1 and ND6 regulation is poorly understood, a proposed mechanism involves the interaction of MTERF1 (mitochondrial terminator factor 1) with 5-methylcytosine in the CpG dinucleotides and/or its interaction with mtDNA-bound mtDNMT1 (94).

An interesting study by Byun et al. showed a higher mtDNA methylation level in workers highly exposed to airborne pollutants compared to low airborne pollutant exposed subjects (108). In line with this finding, in a cohort of 81 individuals aged 18-91, methylation levels of the mitochondria gene 12S rRNA inversely correlated with age suggesting that mtDNA methylation may represent an epigenetic marker of ageing (109). In the retina of diabetic mice mtDNA methylation was found associated with mtDNA damage characterized by increased base mismatches and hypermethylated cytosines. Interestingly, inhibition of DNA methylation, or regulation of cytosine deamination, attenuated base mismatches at the D-loop thus preventing mitochondrial dysfunction and microvascular damage. In this study epigenetic signals of mtDNA were driven by oxidative stress as overexpression of Sod2 was able to prevent diabetes-induced D-loop hypermethylation and increase in base mismatches (110). Of clinical relevance, retinal microvasculature from human donors with diabetic retinopathy presented similar increase in D-loop methylation and decrease in mtDNA transcription (111). In another study, analysis of mtDNA methylation by bisulfite sequencing in senescent endothelial cells showed alteration in the methylation pattern of several genes regulating mitochondrial function and metabolism (112). Patients with cardiovascular disease display a significantly higher mtDNA methylation of genes encoding for cytochrome *c* oxidases (MT-CO1, MT-CO2, MT-CO3), tRNA leucine 1 (MT-TL1) and (1.67%, $P = 0.0001$) as well as genes involved in ATP synthesis (MT-ATP6 and MT-ATP8) (113). The latter study suggests that mtDNA methylation could serve as non-invasive and easy-to-obtain epigenetic biomarker and may be implicated in the etiology of CVD (**Figure 3**).

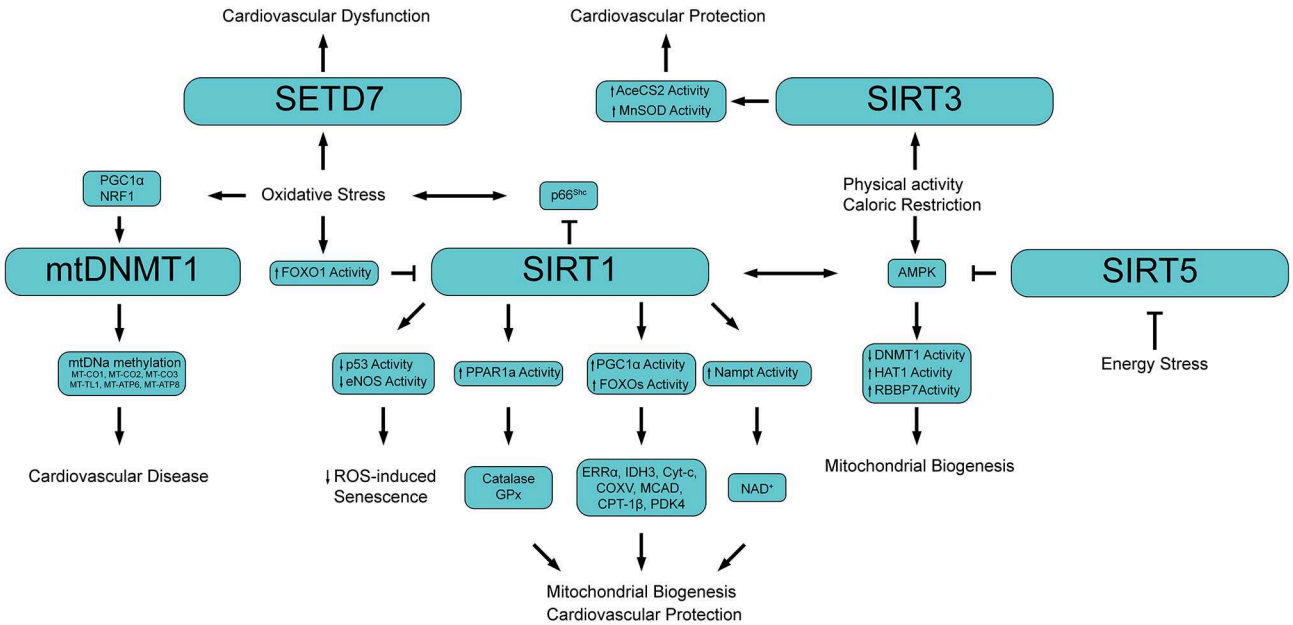


FIGURE 3 | Schematic showing the main epigenetic networks regulating mitochondrial functionality and cardiovascular disease. PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; NRF1, Nuclear respiratory factor 1; mtDNMT1, Mitochondrial DNA methyltransferase 1; MT-CO (1-3), Cytochrome c oxidase subunit (I, II, III); MT-TL1, Mitochondrially encoded tRNA-Leu 1; MT-ATP (6, 8) Mitochondrially encoded ATP synthase membrane (subunit 6, 8); FOXO1, Forkhead box O3; PPAR1a, Peroxisome proliferator-activated receptor 1 alpha; GPx, Glutathione peroxidase; ERRα, Estrogen-related receptor alpha; IDH3, isocitrate dehydrogenase 3; Cyt-c, Cytochrome c; COXV, Cytochrome c oxidase subunit 5; MCAD, Medium-chain acyl-CoA dehydrogenase; CPT-1β, Carnitine Palmitoyltransferase 1 beta; PDK4, Pyruvate dehydrogenase lipoamide kinase isozyme 4; Nampt, Nicotinamide phosphoribosyltransferase; NAD^+, Nicotinamide adenine dinucleotide; AceCS2, acetyl-CoA synthetase 2; AMPK, 5′ adenosine monophosphate-activated protein kinase; HAT1, Histone acetyltransferase 1; RBBP7, Retinoblastoma binding protein 7.

HISTONE POST-TRANSLATIONAL MODIFICATIONS AND MITOCHONDRIAL FUNCTION

Growing evidence indicates that PTMs of histones, mainly at lysine and arginine residues, significantly affect chromatin accessibility thus enabling cell-specific transcriptional programs implicated in mitochondrial dysfunction and vascular disease (**Figure 3**). Sirtuins are class III histone deacetylases (HDACs), homologs of the yeast protein Silent Information Regulatory 2 (Sir2), a deacetylase involved in yeast metabolism and lifespan (104). The sirtuin family of deacetylases include seven enzymes differentially distributed throughout the cell: SIRT1 and SIRT2 which are mainly localized in both cytoplasmic and nuclear compartments; SIRT3 SIRT4, and SIRT5, which are localized in the mitochondria, and SIRT6 and SIRT7 which are located in the cell nucleus (29, 114, 115). The deacetylation reaction catalysed by sirtuins is NAD+-dependent, and leads to the formation of *O*-acetyl-ADP ribose (AADPR) which can be used as a donor group in ADP-ribosylation reactions (116). In term of activity, all the above-mentioned sirtuins display a deacetylase activity with the exception of SIRT4 which is mostly an ADP-ribosyl transferase, and SIRT6 which exhibits both activities (104).

Available evidence indicates that sirtuins act as pivotal regulators of life span and life-extending effects of calorie restriction (2). Among the different sirtuins, SIRT3 is particularly active in the mitochondria, where it is responsible for the deacetylation of the acetyl-CoA synthase enzyme (AceCS2) (117, 118). Under appropriate nutritional conditions, AceCS2 is completely inactivated upon acetylation at Lys-642, while it is rapidly reactivated by SIRT3 deacetylation (117). Deacetylation of AceCS2 by SIRT3 increases AceCS2 activity leading to the formation of O-acetyl-ADP-ribose and nicotinamide (118), important metabolites implicated in biosynthetic and regulatory purposes (119). In line with these studies, genetic deletion of SIRT3 in mice or gene downregulation as the result of high fat diet feeding, are associated with early metabolic abnormalities which are mainly the result of mitochondrial dysfunction (120). SIRT3 also regulates mitochondrial oxidative stress levels by deacetylation of the antioxidant enzyme MnSOD (121). Although not localized in the mitochondria, SIRT1 is a major regulator of mitochondrial function via deacetylation of PGC1α and FOXOs proteins (122). SIRT1-mediated activation of these target proteins leads to increased mitochondrial respiration and lipid oxidation through regulation of several genes (i.e., ERRα, IDH3, Cyt-c, COXV, MCAD, CPT-1β, and PDK4) required in energy-depleted cell (29, 123). SIRT1 is also critically involved in a dynamic cross-talk with AMPK, a key molecular effector involved in cellular metabolism. Activation of SIRT1/AMPK by physical activity or caloric restriction is

associated with an increased usage of lipids as an energy source, mitochondrial biogenesis as well as with an increased expression of nicotinamide phosphoribosyl-transferase (Nampt), the rate-limiting enzyme in NAD+ bio-synthesis. The increase in Nampt activity leads to higher NAD+ production, which in turn activates SIRT1 (124).

Of note, activation of AMPK by SIRT1 seems to be particularly important for the phosphorylation of three main proteins involved in epigenetic remodeling: the DNA methyltransferase DNMT1, the histone acetyltransferase HAT1, and RBBP7, which inhibits DNMT1 and is a HAT1 coactivator (125). AMPK-mediated phosphorylation of these proteins triggered nucleosome remodeling thus favoring the transcription of nuclear-encoded genes involved in mitochondrial biogenesis and function (125). These results show that SIRT1-AMPK axis coordinates mitochondrial function with energy status through epigenetic regulation of nuclear gene expression. SIRT1 is also highly sensitive to the cellular redox state, and confers cardioprotection by counteracting oxidative stress through deacetylation of multiple cellular targets (126–128). In the human endothelium, SIRT1 antagonizes H_2O_2-induced premature senescence through its negative modulation of p53 by deacetylation of Lys-373, Lys-382, and Lys-320 (129). Conversely, endothelial SIRT1 overexpression reversed oxidative stress-induced premature senescence through activation of endothelial nitric oxide synthase (eNOS) (130). SIRT1 has also shown to deacetylate FOXO3 thus preventing cellular apoptosis via a mechanism involving the tumor suppressor p53 (131, 132). On the other hand, ROS-dependent acetylation of FOXO1 inhibits its transcriptional activity on SIRT1, catalase (CAT), and MnSOD target genes thus creating a detrimental vicious cycle driven by oxidative stress (133). This molecular circuitry is reinforced by the activation of the mitochondrial adaptor $p66^{Shc}$ which further amplifies ROS levels (134). Interestingly, SIRT1 controls mitochondrial oxidative stress by regulating the transcription of $p66^{Shc}$ (135–137). SIRT1-dependent deacetylation of histone 3 reduces chromatin accessibility on $p66^{Shc}$ promoter thus impeding transcription. By contrast, SIRT1 downregulation as the results of cardiovascular risk factors induces an open chromatin eventually leading to $p66^{Shc}$ expression, mitochondrial oxidative stress and endothelial dysfunction (138). It has also been shown that SIRT1 overexpression increases mitochondrial biogenesis and expression of antioxidant enzymes, namely catalase and glutathione peroxidase (GPx), via activation of the peroxisome proliferator-activated receptor coactivator (PPAR) 1-a activation (139).

SIRT5, a weak deacetylase with strong desuccinylase, demalonylase, and deglutarylase activity, has been also implicated in regulating different aspects of mitochondrial metabolism and cardiovascular function (140). SIRT5 downregulation was recently associated with mitochondrial dysfunction in endothelial progenitor cells of patients with arterial hypertension (141). Other studies reported that SIRT5 deficiency exert a protective role by suppressing mitochondrial ATP production and promoting AMPK activation in response to energy stress. Moreover, genetic deletion of SIRT5 protects against ischemic stroke via modulation of PI3K/Akt pathway (142).

Recent evidence suggests that in the diseased aorta containing atherosclerotic plaques and grafted arteriosclerosis, REF1/H3K9me3 pathway is suppressed thus leading to an increase in the mitochondrial translocation of the AIP1B isoform with subsequent generation of mitochondrial ROS and EC activation (143).

MITOCHONDRIAL ROS AND EPIGENETIC CHANGES

Mitochondrial-generated ROS have a major impact on DNA methylation. ROS can directly convert 5-methylcytosin (5mC) to 5-hydroxymethylcytosine (5hmC) which blocks the activity of DNMT1 leading to an improper methylation inheritance during mitosis and global hypomethylation (144). Moreover ROS can oxidize guanosine to 8-oxo-20-deoxyguanosine (8-oxodG) thus inhibiting methylation of adjacent cytosine and further contributing to global hypomethylation of DNA (145, 146). The formation of 8-oxodG in particular loci promotes the transcription of pro-inflammatory genes in response to TNF-α (147). Furthermore, 8-oxodG interacts with HIF1α thus affecting its ability to bind VEGF promoter with subsequent impairment of angiogenesis (148). In line with these observations, two recent meta-analyses showed that high levels of 8-oxodG are associated with atherosclerotic vascular disease and predict outcome (149, 150). High ROS levels also influence both repressive (H3K9me2/3 and H3K27me3) and active histone marks (H3K4me2/3) (151, 152).

Similarly to DNA methylation, histone methylation is dependent on SAM availability and is therefore reduced in the presence of high ROS levels (153, 154). In support of this hypothesis in a model of cardiac pressure overload the SET and MYND domain containing protein 1 (SMYD1) methyltransferase was significantly downregulated (155). On the other hand, several studies showed that hyperglycemia-induced oxidative stress increases the expression of the methyltransferase SETD7 and its epigenetic marker H3K4m eventually leading to enhanced transcription of inflammatory and oxidant genes, thus generating a vicious cycle (**Figure 3**) (156).

MITOCHONDRIAL METABOLITES AS COFACTORS FOR CHROMATIN MODIFICATIONS

By serving as essential cofactors for most chromatin-modifying enzymes, important intermediates of cell metabolism and dietary intake allow the integration of metabolic information and transcriptional control (**Figure 4**). Fluctuating metabolite concentrations are therefore proposed to provide signalling cues for continual adjustment of gene expression by modulating the epigenome to influence chromatin dynamics. Additional biochemical evidence suggests that energy metabolite concentration could affect PTMs of the chromatin-modifying machinery itself, in turn regulating enzymatic activity,

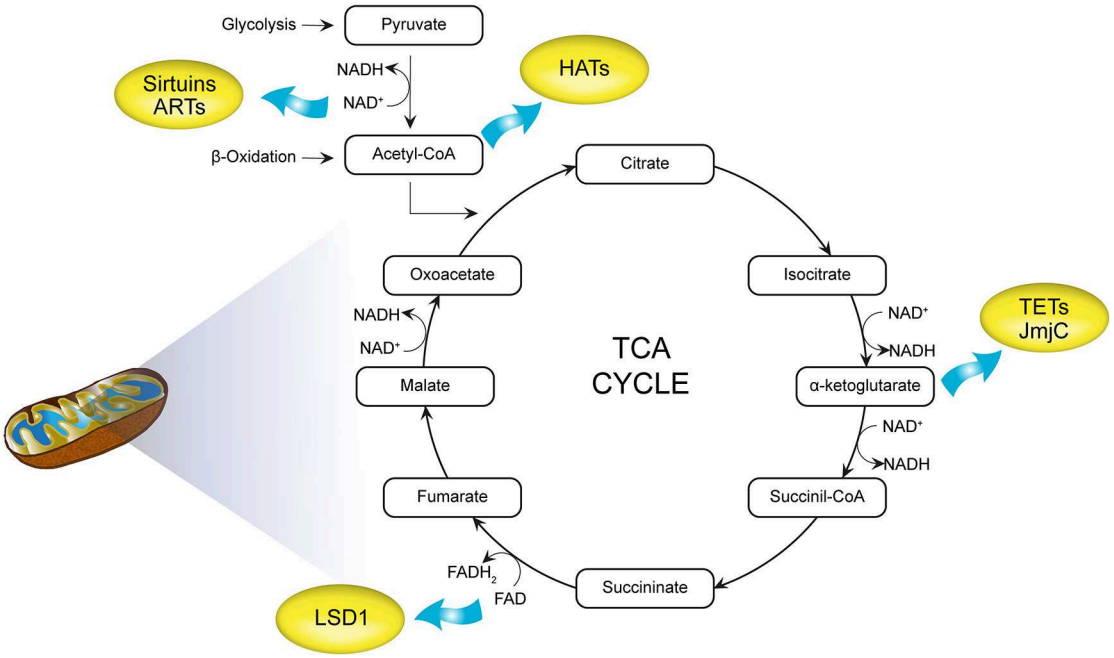


FIGURE 4 | Intermediate mitochondrial metabolites as cofactors for chromatin modifications. acetyl-CoA generated by glycolysis and β-oxidation acts as a substrate for histone acetyltransferases (HATs). Nicotinamide adenine dinucleotide (NAD^+) is required for histone deacetylases (HDACs; histone deacetylation) as well as ADP-ribosyltransferases (ARTs). α-Ketoglutarate and flavin adenine dinucleotide (FAD+) are cofactors for DNA (ten-eleven translocations, TETs) and histone demethylases [Jumonji C domain containing (JmjC), LSD1]. TCA, tricarboxylic acid cycle; HATs, histone acetyltransferases; NAD^+, nicotinamide adenine dinucleotide; HDACs, histone deacetylases; ARTs, ADP-ribosyltransferases; FAD, flavin adenine dinucleotide; TETs, ten-eleven translocations.

stability, and chromatin binding capacity associated with gene expression (54).

NAD+

NAD+ is an essential cofactor for reactions catalysed by the highly conserved SIRT HDAC family (2). Other NAD+ consuming enzymes such as ADP-ribosyltransferases have also been shown to covalently ADP-ribosylate core histones (157). PAR polymerases (PARPs) utilize NAD+ to catalyse poly(ADPribose) synthesis and are involved in the cellular stress response (158). Poly(ADP-ribose) polymerase-1 (PARP1), a major member of the PARP family, is a nuclear protein involved in chromatin remodeling and promotion of DNA repair (159). However, several studies report that in condition of oxidative stress PARP-1 also localizes to mitochondria (160–162). Mitochondrial PARP-1 is reported to actively participate in maintenance of functional integrity of the organelles (163) and to play a detrimental role when hyperactivated (160, 164). Furthermore, the potential role of PARP1 as a nuclear epigenetic regulator for the maintenance of mitochondrial DNA integrity has been suggested (159). Indeed, PARP-1 suppression reduces mtDNA integrity, as well as the expression of mitochondria-encoded respiratory complex subunits COX-1, COX-2, and ND-2 (164). Accordingly, PARP-1 localizes at promoters of nuclear genes encoding both the mtDNA repair proteins UNG1, MYH1, and APE1 and the mtDNA transcription factors TFB1M and TFB2M (164). Consistent with these findings, PARP-1 suppression impairs mitochondrial ATP production (164).

S-Adenosylmethionine

S-Adenosylmethionine (SAM) is produced by the condensation of methionine and ATP during the first of nine steps required for the conversion of methionine to succinyl-CoA, a predominantly cytoplasmic pathway that ends up in the mitochondria (29). It contains the active methyl-donor group utilized by most methyltransferase enzymes. It has been demonstrated that ROS can reduce SAM availability, thus limiting the activity of DNA and histone methyltransferases (145). This is achieved either by inhibiting methionine adenosyl-transferase and thus SAM synthesis or by inhibiting methionine synthase and thus methionine regeneration (56). Interestingly, long-term exposure to H_2O_2 decreased SAM levels leading to hypomethylation of the long interspersed nuclear element-1 (LINE-1) (165). LINE-1 hypomethylation as an indicator of global methylation status was found in blood from patients with ischaemic heart disease and stroke, and has been related to higher risk for these diseases (166).

FAD+

Derived from the vitamin riboflavin (vitamin B2), mitochondrial-generated FAD functions as the prosthetic group for certain oxidation–reduction enzymes (2). For example, LSD1 demethylase is a FAD+-dependent enzyme capable of demethylating H3K4me1/2 and H3K9me1/2 (167). LSD1

activity is regulated by redox state and it is stimulated when FAD is oxidized (168). LSD1, in turn, regulates mitochondrial respiration and energy expenditure. Specifically, LSD1 binds directly to genes such as PGC1α, PDK4, FATP1, and adipose triacylglycerol lipase (ATGL), and represses their transcription associated with loss of H3K4 methylation (169).

β-Hydroxybutyrate

The ketone body β-hydroxybutyrate (βOHB) modulates several signalling pathways with implications for metabolic disease and diabetes (170). Prolonged fasting, calorie restriction, strenuous exercise, or ketogenic diets are conditions associated with increases in serum concentrations β-OHB (171). Interestingly, βOHB is an endogenous inhibitor of many NAD+-independent HDACs (172). HDAC inhibition by βOHB might affect the pathogenesis of type 2 diabetes in at least two ways: through direct regulation of HDAC-dependent glucose metabolism, or by promoting resistance to oxidative stress (170). For examples, βOHB-mediated inhibition of HDAC1 and HDAC2 increases acetylation of histone H3K9 and H3K14 and establishes a permissive chromatin configuration for the expression of Foxo3 with subsequent transcription of its downstream antioxidant genes such as catalase and MnSOD (172). Similarly, βOHB may have similar effects on mitochondrial function, glucose homeostasis, and obesity through endogenous inhibition of HDAC3. The mechanism for these metabolic benefits of class I HDAC inhibition may be the upregulation of PGC1α in a variety of tissues (173, 174). Transcription of FGF21 is similarly upregulated via βOHB-mediated inhibition of HDAC3 which results in the activation of ketogenesis in obese mice (175). The microvascular and macrovascular complications of type 2 diabetes are thought to be due in part to increased oxidative stress brought on through several pathways including polyols, protein kinase C, hexosamine, and advanced glycosylation end products (176). In this context, the emerging role of βOHB in suppressing oxidative stress may be relevant for the management of diabetic complications. Other studies have previously suggested a role for both βOHB and HDAC inhibitors in the protection from oxidative or ischemic stress (170).

α-Ketoglutarate

Connections between metabolic cofactors and enzymes associated with the removal of epigenetic methyl modifications are also emerging (54). The TET family of dioxygenases mediate the oxidation of 5mC. The potential for the TET family (TET1/2/3) to regulate diverse physiological functions including metabolic signalling requires the TCA cycle metabolite α-KG, and this activity is inhibited by 2-hydroxyglutarate (2HG) (2) (**Figure 4**). This means that oxygen deficiency and disturbances in mitochondrial metabolism could affect the activation of TET enzymes and thus control DNA methylation (177). Hearts of mice exposed to high-fat diet (HFD) showed reduced levels of αKG and this observation was paralleled by a compromised TET1 function. Accordingly, an exogenous source of αKG restored the DNA demethylation cycle, glucose uptake, and insulin response (178).

Jumonji C domain-containing histone demethylases are α-KG-dependent (177). Although studies are yet to determine the TET-metabolism connection, mutations in isocitrate dehydrogenase genes are associated with reduced α-KG and elevated 2HG levels leading to genome-wide changes in histone and DNA methylation patterns (54).

The Jumonji C domain (JmjC) containing lysine demethylases (KDM) are the largest group, which can be divided to six subgroups (KDM2-7) depending on their chromatin interacting domains and substrate specificity (179). The activation of these enzymes is also dependent on the presence of α-KG. Therefore, disturbances in Krebs cycle function can affect histone methylation and gene expression (177).

Acetyl-CoA

Acetyl-CoA generated from glucose and fatty acid metabolism feeds into the TCA cycle to contribute to cellular energy supply. Importantly, acetyl-CoA is the essential acetyl group donor to lysine acetylation reactions and both pharmacological and genetic interventions that modify cellular acetyl-CoA concentrations directly affect acetylated proteins including histones (180). Because histone acetylation is ubiquitously associated with open chromatin and gene expression, acetyl-CoA links intermediary carbon metabolism with chromatin dynamics and transcription (54).

EPIGENETIC THERAPIES

Targeting epigenetic modifications is a highly promising approach to restore gene expression and to rescue or prevent mitochondrial insufficiency and vascular dysfunction. There are several examples of how specific interventions can be employed to modify the landscape of DNA/histone modifications in this setting.

Studies in knockout mice have shown that class I HDACs play a key role in regulating metabolism. Chronic treatment with butyrate, a broad HDAC inhibitor that is expected to phenocopy HDAC3 loss-of-function, prevents metabolic alterations in diet-induced obese as well as in aged mice, mainly by enhancing oxidative phosphorylation and beta-oxidation in mitochondria (181, 182). Butyrate treatment also improves mitochondrial biogenesis via epigenetic modulation of PGC-1α as well as induction of several microRNAs such as miR-133a-3p, miR-208b, and miR-499-5p, implicated in the regulation of mitochondrial potential and integrity (183). Similarly, the class I HDAC inhibitor SAHA, but not a class II HDAC inhibitor, increases the expression of PGC-1α thus leading to enhanced mitochondrial biogenesis, oxygen consumption in adipose tissue and skeletal muscle from mice with type 2 diabetes (174). These changes were associated with a significant improvement of insulin sensitivity, metabolic rate and oxidative metabolism (174). Moreover, treatment with SAHA was also found to reduce ischemia-reperfusion injury following myocardial infarction and to prevent apoptosis in cultured myocytes subjected to hypoxia/reoxygenation (184, 185).

Pharmacological modulation of sirtuins has also shown to impact on mitochondrial functionality and vascular function

(186). Although primarily known as a nuclear protein, SIRT1-mediated deacetylation of PGC-1α has been extensively implicated in metabolic control and mitochondrial biogenesis, which was proposed to partially underline SIRT1 role in caloric restriction and impacts on longevity. Moreover, recent evidence suggests that modulation of SIRT1 activity may also affect the turnover of defective mitochondria by mitophagy (187). In line with these evidences, SIRT1 activation by resveratrol improves vascular function while attenuating dyslipidaemia and obesity-induced metabolic alterations in human subjects (188). SIRT1-dependent improvement of flow-mediated dilation can be partially explained by increased deacetylation of $p66^{Shc}$ promoter as well as posttranslational and transcriptional regulation of endothelial NO synthase (eNOS) (137, 189). Indeed, SIRT1 inhibition significantly increases $p66^{Shc}$ transcription, mitochondrial oxidative stress and organelle disruption. Whereas, in both the diabetic vasculature and myocardium activation of SIRT1 suppresses $p66^{Shc}$ signalling thus preventing the accumulation of H_2O_2 in mitochondria and cellular death (137, 138, 190). Pharmacological activation of SIRT3 by small molecules, namely 7-hydroxy-3-(4′-methoxyphenyl) coumarin (C12), also represents a promising approach to prevent mitochondrial ROS via deacetylation and activation of MnSOD (121).

Together with SIRT1, other epigenetic modulators participate to the transcriptional regulation of the mitochondrial adaptor $p66^{Shc}$. Modulation of CpG DNA methylation by folates regulates $p66^{Shc}$ transcription (138). Consistently, a recent work found that homocysteine stimulates $p66^{Shc}$ transcription in human endothelial cells via specific CpG dinucleotides demethylation in the $p66^{Shc}$ promoter (191). Of note, $p66^{Shc}$ promoter CpG methylation was significantly reduced in peripheral blood leukocytes of patients with coronary artery disease and high plasma homocysteine levels, thus strengthening the relevance of $p66^{Shc}$-related epigenetic changes in the context of cardiovascular disease (191). Moreover, metformin, a widely used antidiabetic drug, was found to modulate SIRT1-$p66^{Shc}$ signaling in experimental models of diabetes (138, 192, 193).

Inhibitors of histone acetyltransferases have also shown to revert mitochondrial oxidative stress. The dietary compound curcumin, an inhibitor of the histone acetyltransferase CBP/p300, has shown to rescue hyperglycemia-induced endothelial dysfunction by regulating the expression of several pro-oxidant and antioxidant enzymes involved in mitochondrial oxidative stress and mitochondrial biogenesis (194). Similarly, inhibition of another acetyltransferase, GCN5, prevents angiotensin II–mediated downregulation of catalase thus fostering accumulation of mitochondrial ROS (195).

CONCLUSIONS

In conclusion, evidence discussed so far strongly suggests that specific epigenetic signals are responsible for transcriptional changes leading to mitochondrial dysfunction and cardiovascular disease. In turn, the availability of mitochondrial intermediate metabolites controls the activation of chromatin modifying enzymes. The growing understanding of chromatin modifications and their impact on transcription, will open perspective for the development of personalized biomarkers and epigenetic therapies aimed at preventing mitochondrial dysfunction and cardiovascular disease.

AUTHOR CONTRIBUTIONS

SM, SA, FP, and SC drafted the manuscript and prepared the graphical illustrations. TL revised the manuscript and figures.

ACKNOWLEDGMENTS

FP is the recipient of a H.H. Sheikh Khalifa bin Hamad Al Thani Foundation Assistant Professorship at the Faculty of Medicine, University of Zurich. This work was supported by the Zürich Heart House, the Swiss Heart Foundation, Swiss Life Foundation, Kurt und Senta-Hermann Stiftung, the EMDO Stiftung and the Schweizerische Diabetes-Stiftung (to FP); the Holcim Foundation and the Swiss Heart Foundation (to SC).

REFERENCES

1. Ogawa K, Miura T. Aphid polyphenisms: trans-generational developmental regulation through viviparity. *Front Physiol.* (2014) 5:1. doi: 10.3389/fphys.2014.00001
2. Aon MA, Cortassa S, Juhaszova M, Sollott SJ. Mitochondrial health, the epigenome and healthspan. *Clin Sci.* (2016) 130:1285–305. doi: 10.1042/CS20160002
3. Chandel NS. Mitochondria as signaling organelles. *BMC Biol.* (2014) 12:34. doi: 10.1186/1741-7007-12-34
4. Lu C, Thompson CB. Metabolic regulation of epigenetics. *Cell Metab.* (2012) 16:9–17. doi: 10.1016/j.cmet.2012.06.001
5. Wallace DC, Fan W. Energetics, epigenetics, mitochondrial genetics. *Mitochondrion.* (2010) 10:12–31. doi: 10.1016/j.mito.2009.09.006
6. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med.* (2000) 192:1001–14. doi: 10.1084/jem.192.7.1001
7. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev.* (2014) 94:909–50. doi: 10.1152/physrev.00026.2013
8. Chen Y, Liu Y, Dorn GW II. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res.* (2011) 109:1327–31. doi: 10.1161/CIRCRESAHA.111.258723
9. Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol.* (2014) 16:495–501. doi: 10.1038/ncb2979
10. Harper JW, Ordureau A, Heo JM. Building and decoding ubiquitin chains for mitophagy. *Nat Rev Mol Cell Biol.* (2018) 19:93–108. doi: 10.1038/nrm.2017.129
11. Um JH, Yun J. Emerging role of mitophagy in human diseases and physiology. *BMB Rep.* (2017) 50:299–307. doi: 10.5483/BMBRep.2017.50.6.056
12. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science.* (2003) 299:896–9. doi: 10.1126/science.1079368
13. Dominy JE Jr, Lee Y, Gerhart-Hines Z, Puigserver P. Nutrient-dependent regulation of PGC-1alpha's acetylation state and metabolic function through

the enzymatic activities of Sirt1/GCN5. *Biochim Biophys Acta.* (2010) 1804:1676–83. doi: 10.1016/j.bbapap.2009.11.023
14. Patten IS, Arany Z. PGC-1 coactivators in the cardiovascular system. *Trends Endocrinol Metab.* (2012) 23:90–7. doi: 10.1016/j.tem.2011.09.007
15. Barth E, Stammler G, Speiser B, Schaper J. Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. *J Mol Cell Cardiol.* (1992) 24:669–81. doi: 10.1016/0022-2828(92)93381-S
16. Kadlec AO, Chabowski DS, Ait-Aissa K, Gutterman DD. Role of PGC-1alpha in vascular regulation: implications for atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2016) 36:1467–74. doi: 10.1161/ATVBAHA.116.307123
17. Kluge MA, Fetterman JL, Vita JA. Mitochondria and endothelial function. *Circ Res.* (2013) 112:1171–1188. doi: 10.1161/CIRCRESAHA.111.300233
18. Craige SM, Kroller-Schon S, Li C, Kant S, Cai S, Chen K, et al. PGC-1alpha dictates endothelial function through regulation of eNOS expression. *Sci Rep.* (2016) 6:38210. doi: 10.1038/srep38210
19. Shenouda SM, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, et al. Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. *Circulation.* (2011) 124:444–53. doi: 10.1161/CIRCULATIONAHA.110.014506
20. Haslip M, Dostanic I, Huang Y, Zhang Y, Russell KS, Jurczak MJ, et al. Endothelial uncoupling protein 2 regulates mitophagy and pulmonary hypertension during intermittent hypoxia. *Arterioscler Thromb Vasc Biol.* (2015) 35:1166–78. doi: 10.1161/ATVBAHA.114.304865
21. Mai S, Klinkenberg M, Auburger G, Bereiter-Hahn J, Jendrach M. Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. *J Cell Sci.* (2010) 123:917–26. doi: 10.1242/jcs.059246
22. Mai S, Muster B, Bereiter-Hahn J, Jendrach M. Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan. *Autophagy*. (2012) 8:47–62. doi: 10.4161/auto.8.1.18174
23. Wu NN, Zhang Y, Ren J. Mitophagy, mitochondrial dynamics, and homeostasis in cardiovascular aging. *Oxid Med Cell Longev.* (2019) 2019:9825061. doi: 10.1155/2019/9825061
24. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell.* (2005) 120:483–95. doi: 10.1016/j.cell.2005.02.001
25. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc.* (1972) 20:145–7. doi: 10.1111/j.1532-5415.1972.tb00787.x
26. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. *Annu Rev Pharmacol Toxicol.* (2007) 47:143–83. doi: 10.1146/annurev.pharmtox.47.120505.105122
27. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol.* (2003) 552:335–44. doi: 10.1113/jphysiol.2003.049478
28. Aon MA, Cortassa S, O'Rourke B. Redox-optimized ROS balance: a unifying hypothesis. *Biochim Biophys Acta.* (2010) 1797:865–77. doi: 10.1016/j.bbabio.2010.02.016
29. Kaelin WG Jr, McKnight SL. Influence of metabolism on epigenetics and disease. *Cell.* (2013) 153:56–69. doi: 10.1016/j.cell.2013.03.004
30. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. *J Clin Invest.* (2005) 115:3527–35. doi: 10.1172/JCI25371
31. Yang KC, Kyle JW, Makielski JC, Dudley SC Jr. Mechanisms of sudden cardiac death: oxidants and metabolism. *Circ Res.* (2015) 116:1937–55. doi: 10.1161/CIRCRESAHA.116.304691
32. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. (2000) 404:787–90. doi: 10.1038/35008121
33. Widlansky ME, Gutterman DD. Regulation of endothelial function by mitochondrial reactive oxygen species. *Antioxid Redox Signal.* (2011) 15:1517–30. doi: 10.1089/ars.2010.3642
34. Chen F, Haigh S, Barman S, Fulton DJ. From form to function: the role of Nox4 in the cardiovascular system. *Front Physiol.* (2012) 3:412. doi: 10.3389/fphys.2012.00412
35. Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja D, et al. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. *Circ Res.* (2010) 106:193–202. doi: 10.1161/CIRCRESAHA.109.198366
36. Poon CC, Seto SW, Au AL, Zhang Q, Li RW, Lee WY, et al. Mitochondrial monoamine oxidase-A-mediated hydrogen peroxide generation enhances 5-hydroxytryptamine-induced contraction of rat basilar artery. *Br J Pharmacol.* (2010) 161:1086–98. doi: 10.1111/j.1476-5381.2010.00941.x
37. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* (2009) 417:1–13. doi: 10.1042/BJ20081386
38. Cosentino F, Francia P, Camici GG, Pelicci PG, Luscher TF, Volpe M. Final common molecular pathways of aging and cardiovascular disease: role of the $p66^{Shc}$ protein. *Arterioscler Thromb Vasc Biol.* (2008) 28:622–8. doi: 10.1161/ATVBAHA.107.156059
39. Francia P, delli Gatti C, Bachschmid M, Martin-Padura I, Savoia C, Migliaccio E, et al. Deletion of $p66^{Shc}$ gene protects against age-related endothelial dysfunction. *Circulation*. (2004) 110:2889–95. doi: 10.1161/01.CIR.0000147731.24444.4D
40. Paneni F, Mocharla P, Akhmedov A, Costantino S, Osto E, Volpe M, et al. Gene silencing of the mitochondrial adaptor p66(Shc) suppresses vascular hyperglycemic memory in diabetes. *Circ Res.* (2012) 111:278–289. doi: 10.1161/CIRCRESAHA.112.266593
41. Paneni F, Costantino S, Krankel N, Cosentino F, Luscher TF. Reprogramming ageing and longevity genes restores paracrine angiogenic properties of early outgrowth cells. *Eur Heart J.* (2016) 37:1733–7. doi: 10.1093/eurheartj/ehw073
42. Paneni F, Costantino S, Castello L, Battista R, Capretti G, Chiandotto S, et al. Targeting prolyl-isomerase Pin1 prevents mitochondrial oxidative stress and vascular dysfunction: insights in patients with diabetes. *Eur Heart J.* (2015) 36:817–28. doi: 10.1093/eurheartj/ehu179
43. Costantino S, Paneni F, Luscher TF, Cosentino F. Pin1 inhibitor Juglone prevents diabetic vascular dysfunction. *Int J Cardiol.* (2016) 203:702–7. doi: 10.1016/j.ijcard.2015.10.221
44. Queliconi BB, Wojtovich AP, Nadtochiy SM, Kowaltowski AJ, Brookes PS. Redox regulation of the mitochondrial K(ATP) channel in cardioprotection. *Biochim Biophys Acta.* (2011) 1813:1309–15. doi: 10.1016/j.bbamcr.2010.11.005
45. Brown KA, Didion SP, Andresen JJ, Faraci FM. Effect of aging, MnSOD deficiency, and genetic background on endothelial function: evidence for MnSOD haploinsufficiency. *Arterioscler Thromb Vasc Biol.* (2007) 27:1941–6. doi: 10.1161/ATVBAHA.107.146852
46. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, et al. Mitochondrial integrity and function in atherogenesis. *Circulation.* (2002) 106:544–9. doi: 10.1161/01.CIR.0000023921.93743.89
47. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and *in vivo* monitoring of ROS. *Circulation*. (2003) 108:1912–6. doi: 10.1161/01.CIR.0000093660.86242.BB
48. Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M. PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res.* (2005) 66:562–73. doi: 10.1016/j.cardiores.2005.01.026
49. Widder JD, Fraccarollo D, Galuppo P, Hansen JM, Jones DP, Ertl G, et al. Attenuation of angiotensin II-induced vascular dysfunction and hypertension by overexpression of Thioredoxin 2. *Hypertension.* (2009) 54:338–44. doi: 10.1161/HYPERTENSIONAHA.108.127928
50. Kizhakekuttu TJ, Wang J, Dharmashankar K, Ying R, Gutterman DD, Vita JA, et al. Adverse alterations in mitochondrial function contribute to type 2 diabetes mellitus-related endothelial dysfunction in humans. *Arterioscler Thromb Vasc Biol.* (2012) 32:2531–9. doi: 10.1161/ATVBAHA.112.256024
51. Costantino S, Paneni F, Virdis A, Hussain S, Mohammed SA, Capretti G, et al. Interplay among H3K9-editing enzymes SUV39H1, JMJD2C and SRC-1 drives $p66^{Shc}$ transcription and vascular oxidative stress in obesity. *Eur Heart J.* (2019) 40:383–91. doi: 10.1093/eurheartj/ehx615
52. Kowluru RA. Mitochondrial stability in diabetic retinopathy: lessons learned from epigenetics. *Diabetes*. (2019) 68:241–7. doi: 10.2337/dbi18-0016
53. Costantino S, Libby P, Kishore R, Tardif JC, El-Osta A, Paneni F. Epigenetics and precision medicine in cardiovascular patients: from basic concepts to the clinical arena. *Eur Heart J.* (2018) 39:4150–8. doi: 10.1093/eurheartj/ehx568

54. Keating ST, El-Osta A. Epigenetics and metabolism. *Circ Res*. (2015) 116:715–36. doi: 10.1161/CIRCRESAHA.116.303936
55. Costantino S, Mohammed SA, Ambrosini S, Paneni F. Epigenetic processing in cardiometabolic disease. *Atherosclerosis*. (2019) 281:150–8. doi: 10.1016/j.atherosclerosis.2018.09.029
56. Cyr AR, Domann FE. The redox basis of epigenetic modifications: from mechanisms to functional consequences. *Antioxid Redox Signal*. (2011) 15:551–89. doi: 10.1089/ars.2010.3492
57. Delatte B, Deplus R, Fuks F. Playing TETris with DNA modifications. *EMBO J*. (2014) 33:1198–211. doi: 10.15252/embj.201488290
58. Costantino S, Paneni F, Cosentino F. Targeting chromatin remodeling to prevent cardiovascular disease in diabetes. *Curr Pharm Biotechnol*. (2015) 16:531–43. doi: 10.2174/1389201016061504071136 44
59. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation*. (2011) 123:2145–56. doi: 10.1161/CIRCULATIONAHA.110.956839
60. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature*. (2010) 466:253–257. doi: 10.1038/nature09165
61. Costantino S, Ambrosini S, Paneni F. The epigenetic landscape in the cardiovascular complications of diabetes. *J Endocrinol Invest*. (2019) 42:505–11. doi: 10.1007/s40618-018-0956-3
62. Prasher D, Greenway SC, Singh RB. The impact of epigenetics on cardiovascular disease. *Biochem Cell Biol*. (2020) 98:12-22. doi: 10.1139/bcb-2019-0045
63. Miranda TB, Jones PA. DNA methylation: the nuts and bolts of repression. *J Cell Physiol*. (2007) 213:384–90. doi: 10.1002/jcp.21224
64. Matouk CC, Marsden PA. Epigenetic regulation of vascular endothelial gene expression. *Circ Res*. (2008) 102:873–87. doi: 10.1161/CIRCRESAHA.107.171025
65. Izquierdo AG, Crujeiras AB. Role of epigenomic mechanisms in the onset and management of insulin resistance. *Rev Endocr Metab Disord*. (2019) 20:89–102. doi: 10.1007/s11154-019-09485-0
66. Vilkaitis G, Suetake I, Klimasauskas S, Tajima S. Processive methylation of hemimethylated CpG sites by mouse Dnmt1 DNA methyltransferase. *J Biol Chem*. (2005) 280:64–72. doi: 10.1074/jbc.M411126200
67. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. (1999) 99:247–57. doi: 10.1016/S0092-8674(00)81656-6
68. Aavik E, Babu M, Yla-Herttuala S. DNA methylation processes in atheosclerotic plaque. *Atherosclerosis*. (2019) 281:168–79. doi: 10.1016/j.atherosclerosis.2018.12.006
69. Wu SC, Zhang Y. Active DNA demethylation: many roads lead to Rome. *Nat Rev Mol Cell Biol*. (2010) 11:607–20. doi: 10.1038/nrm2950
70. Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. *Nature*. (2013) 502:472–9. doi: 10.1038/nature 12750
71. Franchini DM, Schmitz KM, Petersen-Mahrt SK. 5-Methylcytosine DNA demethylation: more than losing a methyl group. *Annu Rev Genet*. (2012) 46:419–41. doi: 10.1146/annurev-genet-110711-155451
72. Jenuwein T, Allis CD. Translating the histone code. *Science*. (2001) 293:1074–80. doi: 10.1126/science.1063127
73. Kouzarides T. Chromatin modifications and their function. *Cell*. (2007) 128:693–705. doi: 10.1016/j.cell.2007.02.005
74. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell*. (2007) 128:669–81. doi: 10.1016/j.cell.2007.01.033
75. Martin C, Zhang Y. The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol*. (2005) 6:838–49. doi: 10.1038/nrm1761
76. Nicorescu I, Dallinga GM, de Winther MPJ, Stroes ESG, Bahjat M. Potential epigenetic therapeutics for atherosclerosis treatment. *Atherosclerosis*. (2019) 281:189–97. doi: 10.1016/j.atherosclerosis.2018.10.006
77. Gillette TG, Hill JA. Readers, writers, and erasers: chromatin as the whiteboard of heart disease. *Circ Res*. (2015) 116:1245–53. doi: 10.1161/CIRCRESAHA.116.303630
78. Filippakopoulos P, Knapp S. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat Rev Drug Discov*. (2014) 13:337–56. doi: 10.1038/nrd4286
79. Cooper ME, El-Osta A. Epigenetics: mechanisms and implications for diabetic complications. *Circ Res*. (2010) 107:1403–1413. doi: 10.1161/CIRCRESAHA.110.223552
80. Baccarelli A, Ghosh S. Environmental exposures, epigenetics and cardiovascular disease. *Curr Opin Clin Nutr Metab Care*. (2012) 15:323–9. doi: 10.1097/MCO.0b013e328354bf5c
81. Carrozza MJ, Utley RT, Workman JL, Cote J. The diverse functions of histone acetyltransferase complexes. *Trends Genet*. (2003) 19:321–9. doi: 10.1016/S0168-9525(03)00115-X
82. Lavebratt C, Almgren M, Ekstrom TJ. Epigenetic regulation in obesity. *Int J Obes*. (2012) 36:757–65. doi: 10.1038/ijo.2011.178
83. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*. (2006) 5:769–84. doi: 10.1038/nrd2133
84. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet*. (2009) 10:32–42. doi: 10.1038/nrg2485
85. Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. Histone deacetylases: unique players in shaping the epigenetic histone code. *Ann N Y Acad Sci*. (2003) 983:84–100. doi: 10.1111/j.1749-6632.2003.tb05964.x
86. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. (2011) 21:381–95. doi: 10.1038/cr.2011.22
87. Bannister AJ, Kouzarides T. Reversing histone methylation. *Nature*. (2005) 436:1103–6. doi: 10.1038/nature04048
88. Stratton MS, Farina FM, Elia L. Epigenetics and vascular diseases. *J Mol Cell Cardiol*. (2019) 133:148–63. doi: 10.1016/j.yjmcc.2019.06.010
89. Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, et al. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell*. (2006) 125:467–81. doi: 10.1016/j.cell.2006.03.028
90. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell*. (2007) 128:707–19. doi: 10.1016/j.cell.2007.01.015
91. Eskeland R, Eberharter A, Imhof A. HP1 binding to chromatin methylated at H3K9 is enhanced by auxiliary factors. *Mol Cell Biol*. (2007) 27:453–65. doi: 10.1128/MCB.01576-06
92. Thambirajah AA, Ng MK, Frehlick LJ, Li A, Serpa JJ, Petrotchenko EV, et al. MeCP2 binds to nucleosome free (linker DNA) regions and to H3K9/H3K27 methylated nucleosomes in the brain. *Nucleic Acids Res*. (2012) 40:2884–97. doi: 10.1093/nar/gkr1066
93. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res*. (2003) 31:2305–12. doi: 10.1093/nar/gkg332
94. Iacobazzi V, Castegna A, Infantino V, Andria G. Mitochondrial DNA methylation as a next-generation biomarker and diagnostic tool. *Mol Genet Metab*. (2013) 110:25–34. doi: 10.1016/j.ymgme.2013.07.012
95. Wallace DC, Fan W. The pathophysiology of mitochondrial disease as modeled in the mouse. *Genes Dev*. (2009) 23:1714–36. doi: 10.1101/gad.1784909
96. Yan C, Duanmu X, Zeng L, Liu B, Song Z. Mitochondrial DNA: distribution, mutations, and elimination. *Cells*. (2019) 8:379. doi: 10.3390/cells8040379
97. Birky CW Jr. Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc Natl Acad Sci USA*. (1995) 92:11331–8. doi: 10.1073/pnas.92.25.11331
98. Alam TI, Kanki T, Muta T, Ukaji K, Abe Y, Nakayama H, et al. Human mitochondrial DNA is packaged with TFAM. *Nucleic Acids Res*. (2003) 31:1640–5. doi: 10.1093/nar/gkg251
99. Maniura-Weber K, Goffart S, Garstka HL, Montoya J, Wiesner RJ. Transient overexpression of mitochondrial transcription factor A (TFAM) is sufficient to stimulate mitochondrial DNA transcription, but not sufficient to increase mtDNA copy number in cultured cells. *Nucleic Acids Res*. (2004) 32:6015–27. doi: 10.1093/nar/gkh921
100. van der Wijst MG, Rots MG. Mitochondrial epigenetics: an overlooked layer of regulation? *Trends Genet*. (2015) 31:353–6. doi: 10.1016/j.tig.2015.03.009
101. Shock LS, Thakkar PV, Peterson EJ, Moran RG, Taylor SM. DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. *Proc Natl Acad Sci USA*. (2011) 108:3630–5. doi: 10.1073/pnas.1012311108

102. Pollack Y, Kasir J, Shemer R, Metzger S, Szyf M. Methylation pattern of mouse mitochondrial DNA. *Nucleic Acids Res.* (1984) 12:4811–24. doi: 10.1093/nar/12.12.4811
103. Satoh M, Kuroiwa T. Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. *Exp Cell Res.* (1991) 196:137–40. doi: 10.1016/0014-4827(91)90467-9
104. Cosentino C, Mostoslavsky R. Metabolism, longevity and epigenetics. *Cell Mol Life Sci.* (2013) 70:1525–41. doi: 10.1007/s00018-013-1295-3
105. Dzitoyeva S, Chen H, Manev H. Effect of aging on 5-hydroxymethylcytosine in brain mitochondria. *Neurobiol Aging.* (2012) 33:2881–91. doi: 10.1016/j.neurobiolaging.2012.02.006
106. Wong M, Gertz B, Chestnut BA, Martin LJ. Mitochondrial DNMT3A and DNA methylation in skeletal muscle and CNS of transgenic mouse models of ALS. *Front Cell Neurosci.* (2013) 7:279. doi: 10.3389/fncel.2013.00279
107. Manev H, Dzitoyeva S. Progress in mitochondrial epigenetics. *Biomol Concepts.* (2013) 4:381–9. doi: 10.1515/bmc-2013-0005
108. Byun HM, Panni T, Motta V, Hou L, Nordio F, Apostoli P, et al. Effects of airborne pollutants on mitochondrial DNA methylation. *Part Fibre Toxicol.* (2013) 10:18. doi: 10.1186/1743-8977-10-18
109. Mawlood SK, Dennany L, Watson N, Dempster J, Pickard BS. Quantification of global mitochondrial DNA methylation levels and inverse correlation with age at two CpG sites. *Aging.* (2016) 8:636–41. doi: 10.18632/aging.100892
110. Mishra M, Kowluru RA. DNA methylation-a potential source of mitochondria DNA base mismatch in the development of diabetic retinopathy. *Mol Neurobiol.* (2019) 56:88–101. doi: 10.1007/s12035-018-1086-9
111. Mishra M, Kowluru RA. Epigenetic modification of mitochondrial DNA in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci.* (2015) 56:5133–42. doi: 10.1167/iovs.15-16937
112. Bianchessi V, Vinci MC, Nigro P, Rizzi V, Farina F, Capogrossi MC, et al. Methylation profiling by bisulfite sequencing analysis of the mtDNA non-coding region in replicative and senescent endothelial cells. *Mitochondrion.* (2016) 27:40–7. doi: 10.1016/j.mito.2016.02.004
113. Baccarelli AA, Byun HM. Platelet mitochondrial DNA methylation: a potential new marker of cardiovascular disease. *Clin Epigenetics.* (2015) 7:44. doi: 10.1186/s13148-015-0078-0
114. Verdin E, Hirschey MD, Finley LW, Haigis MC. Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. *Trends Biochem Sci.* (2010) 35:669–75. doi: 10.1016/j.tibs.2010.07.003
115. Guarente L. Franklin H. Epstein lecture: sirtuins, aging, and medicine. *N Engl J Med.* (2011) 364:2235–44. doi: 10.1056/NEJMra1100831
116. Sauve AA, Celic I, Avalos J, Deng H, Boeke JD, Schramm VL. Chemistry of gene silencing: the mechanism of NAD+-dependent deacetylation reactions. *Biochemistry.* (2001) 40:15456–63. doi: 10.1021/bi011858j
117. Hallows WC, Lee S, Denu JM. Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc Natl Acad Sci USA.* (2006) 103:10230–5. doi: 10.1073/pnas.0604392103
118. Schwer B, Bunkenborg J, Verdin RO, Andersen JS, Verdin E. Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc Natl Acad Sci USA.* (2006) 103:10224–10229. doi: 10.1073/pnas.0603968103
119. Hassa PO, Haenni SS, Elser M, Hottiger MO. Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? *Microbiol Mol Biol Rev.* (2006) 70:789–829. doi: 10.1128/MMBR.00040-05
120. Hirschey MD, Shimazu T, Huang JY, Schwer B, Verdin E. SIRT3 regulates mitochondrial protein acetylation and intermediary metabolism. *Cold Spring Harb Symp Quant Biol.* (2011) 76:267–77. doi: 10.1101/sqb.2011.76.010850
121. Lu J, Zhang H, Chen X, Zou Y, Li J, Wang L, et al. A small molecule activator of SIRT3 promotes deacetylation and activation of manganese superoxide dismutase. *Free Radic Biol Med.* (2017) 112:287–97. doi: 10.1016/j.freeradbiomed.2017.07.012
122. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature.* (2005) 434:113–8. doi: 10.1038/nature03354
123. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell.* (2006) 127:1109–22. doi: 10.1016/j.cell.2006.11.013
124. Posavec M, Timinszky G, Buschbeck M. Macro domains as metabolite sensors on chromatin. *Cell Mol Life Sci.* (2013) 70:1509–24. doi: 10.1007/s00018-013-1294-4
125. Marin TL, Gongol B, Zhang F, Martin M, Johnson DA, Xiao H, et al. AMPK promotes mitochondrial biogenesis and function by phosphorylating the epigenetic factors DNMT1, RBBP7, and HAT1. *Sci Signal.* (2017) 10. doi: 10.1126/scisignal.aaf7478
126. Nadtochiy SM, Redman E, Rahman I, Brookes PS. Lysine deacetylation in ischaemic preconditioning: the role of SIRT1. *Cardiovasc Res.* (2011) 89:643–9. doi: 10.1093/cvr/cvq287
127. Nadtochiy SM, Yao H, McBurney MW, Gu W, Guarente L, Rahman I, et al. SIRT1-mediated acute cardioprotection. *Am J Physiol Heart Circ Physiol.* (2011) 301:H1506–12. doi: 10.1152/ajpheart.00587.2011
128. Vinciguerra M, Santini MP, Martinez C, Pazienza V, Claycomb WC, Giuliani A, et al. mIGF-1/JNK1/SirT1 signaling confers protection against oxidative stress in the heart. *Aging Cell.* (2012) 11:139–49. doi: 10.1111/j.1474-9726.2011.00766.x
129. Kao CL, Chen LK, Chang YL, Yung MC, Hsu CC, Chen YC, et al. Resveratrol protects human endothelium from H(2)O(2)-induced oxidative stress and senescence via SirT1 activation. *J Atheroscler Thromb.* (2010) 17:970–9. doi: 10.5551/jat.4333
130. Ota H, Eto M, Kano MR, Ogawa S, Iijima K, Akishita M, et al. Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. *Arterioscler Thromb Vasc Biol.* (2008) 28:1634–9. doi: 10.1161/ATVBAHA.108.164368
131. Huang K, Yan ZQ, Zhao D, Chen SG, Gao LZ, Zhang P, et al. SIRT1 and FOXO mediate contractile differentiation of vascular smooth muscle cells under cyclic stretch. *Cell Physiol Biochem.* (2015) 37:1817–29. doi: 10.1159/000438544
132. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science.* (2004) 303:2011–5. doi: 10.1126/science.1094637
133. D'Onofrio N, Servillo L, Balestrieri ML. SIRT1 and SIRT6 signaling pathways in cardiovascular disease protection. *Antioxid Redox Signal.* (2018) 28:711–732. doi: 10.1089/ars.2017.7178
134. Carlomosti F, D'Agostino M, Beji S, Torcinaro A, Rizzi R, Zaccagnini G, et al. Oxidative stress-induced miR-200c disrupts the regulatory loop among SIRT1, FOXO1, and eNOS. *Antioxid Redox Signal.* (2017) 27:328–44. doi: 10.1089/ars.2016.6643
135. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, et al. The $p66^{Shc}$ adaptor protein controls oxidative stress response and life span in mammals. *Nature.* (1999) 402:309–13. doi: 10.1038/46311
136. Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, et al. Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science.* (2007) 315:659–63. doi: 10.1126/science.1135380
137. Zhou S, Chen HZ, Wan YZ, Zhang QJ, Wei YS, Huang S, et al. Repression of $p66^{Shc}$ expression by SIRT1 contributes to the prevention of hyperglycemia-induced endothelial dysfunction. *Circ Res.* (2011) 109:639–48. doi: 10.1161/CIRCRESAHA.111.243592
138. Paneni F, Volpe M, Luscher TF, Cosentino F. SIRT1, p66(Shc), and Set7/9 in vascular hyperglycemic memory: bringing all the strands together. *Diabetes.* (2013) 62:1800–7. doi: 10.2337/db12-1648
139. Tan M, Tang C, Zhang Y, Cheng Y, Cai L, Chen X, et al. SIRT1/PGC-1alpha signaling protects hepatocytes against mitochondrial oxidative stress induced by bile acids. *Free Radic Res.* (2015) 49:935–45. doi: 10.3109/10715762.2015.1016020
140. Tang X, Chen XF, Chen HZ, Liu DP. Mitochondrial Sirtuins in cardiometabolic diseases. *Clin Sci.* (2017) 131:2063–78. doi: 10.1042/CS20160685
141. Yu BB, Zhi H, Zhang XY, Liang JW, He J, Su C, et al. Mitochondrial dysfunction-mediated decline in angiogenic capacity of endothelial progenitor cells is associated with capillary rarefaction in patients with hypertension via downregulation of CXCR4/JAK2/SIRT5 signaling. *EBioMedicine.* (2019) 42:64–75. doi: 10.1016/j.ebiom.2019.03.031
142. Diaz-Canestro C, Merlini M, Bonetti NR, Liberale L, Wust P, Briand-Schumacher S, et al. Sirtuin 5 as a novel target to blunt blood-brain barrier

damage induced by cerebral ischemia/reperfusion injury. *Int J Cardiol.* (2018) 260:148–55. doi: 10.1016/j.ijcard.2017.12.060
143. Li Z, Li L, Zhang H, Zhou HJ, Ji W, Min W. Short AIP1 (ASK1-Interacting Protein-1) isoform localizes to the mitochondria and promotes vascular dysfunction. *Arterioscler Thromb Vasc Biol.* (2020) 40:112–27. doi: 10.1161/ATVBAHA.119.312976
144. Madugundu GS, Cadet J, Wagner JR. Hydroxyl-radical-induced oxidation of 5-methylcytosine in isolated and cellular DNA. *Nucleic Acids Res.* (2014) 42:7450–60. doi: 10.1093/nar/gku334
145. Kietzmann T, Petry A, Shvetsova A, Gerhold JM, Gorlach A. The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. *Br J Pharmacol.* (2017) 174:1533–54. doi: 10.1111/bph.13792
146. Le DD, Fujimori DG. Protein and nucleic acid methylating enzymes: mechanisms and regulation. *Curr Opin Chem Biol.* (2012) 16:507–15. doi: 10.1016/j.cbpa.2012.09.014
147. Pan L, Zhu B, Hao W, Zeng X, Vlahopoulos SA, Hazra TK, et al. Oxidized guanine base lesions function in 8-oxoguanine DNA glycosylase-1-mediated epigenetic regulation of nuclear factor kappaB-driven gene expression. *J Biol Chem.* (2016) 291:25553–66. doi: 10.1074/jbc.M116.751453
148. Pastukh V, Roberts JT, Clark DW, Bardwell GC, Patel M, Al-Mehdi AB, et al. An oxidative DNA "damage" and repair mechanism localized in the VEGF promoter is important for hypoxia-induced VEGF mRNA expression. *Am J Physiol Lung Cell Mol Physiol.* (2015) 309:L1367–75. doi: 10.1152/ajplung.00236.2015
149. Kroese LJ, Scheffer PG. 8-hydroxy-2'-deoxyguanosine and cardiovascular disease: a systematic review. *Curr Atheroscler Rep.* (2014) 16:452. doi: 10.1007/s11883-014-0452-y
150. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, et al. 8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxid Redox Signal.* (2016) 24:548–55. doi: 10.1089/ars.2015.6508
151. Chervona Y, Costa M. The control of histone methylation and gene expression by oxidative stress, hypoxia, and metals. *Free Radic Biol Med.* (2012) 53:1041–7. doi: 10.1016/j.freeradbiomed.2012.07.020
152. Niu Y, DesMarais TL, Tong Z, Yao Y, Costa M. Oxidative stress alters global histone modification and DNA methylation. *Free Radic Biol Med.* (2015) 82:22–8. doi: 10.1016/j.freeradbiomed.2015.01.028
153. Mentch SJ, Mehrmohamadi M, Huang L, Liu X, Gupta D, Mattocks D, et al. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell Metab.* (2015) 22:861–73. doi: 10.1016/j.cmet.2015.08.024
154. Chisholm NC, Henderson ML, Selvamani A, Park MJ, Dindot S, Miranda RC, et al. Histone methylation patterns in astrocytes are influenced by age following ischemia. *Epigenetics.* (2015) 10:142–52. doi: 10.1080/15592294.2014.1001219
155. Liu T, Wu C, Jain MR, Nagarajan N, Yan L, Dai H, et al. Master redox regulator Trx1 upregulates SMYD1 & modulates lysine methylation. *Biochim Biophys Acta.* (2015) 1854:1816–22. doi: 10.1016/j.bbapap.2015.09.006
156. Paneni F, Costantino S, Battista R, Castello L, Capretti G, Chiandotto S, et al. Adverse epigenetic signatures by histone methyltransferase Set7 contribute to vascular dysfunction in patients with type 2 diabetes mellitus. *Circ Cardiovasc Genet.* (2015) 8:150–8. doi: 10.1161/CIRCGENETICS.114.000671
157. Messner S, Altmeyer M, Zhao H, Pozivil A, Roschitzki B, Gehrig P, et al. PARP1 ADP-ribosylates lysine residues of the core histone tails. *Nucleic Acids Res.* (2010) 38:6350–62. doi: 10.1093/nar/gkq463
158. Luo X, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev.* (2012) 26:417–32. doi: 10.1101/gad.183509.111
159. Szczesny B, Brunyanszki A, Olah G, Mitra S, Szabo C. Opposing roles of mitochondrial and nuclear PARP1 in the regulation of mitochondrial and nuclear DNA integrity: implications for the regulation of mitochondrial function. *Nucleic Acids Res.* (2014) 42:13161–73. doi: 10.1093/nar/gku1089
160. Du L, Zhang X, Han YY, Burke NA, Kochanek PM, Watkins SC, et al. Intra-mitochondrial poly(ADP-ribosylation) contributes to NAD+ depletion and cell death induced by oxidative stress. *J Biol Chem.* (2003) 278:18426–433. doi: 10.1074/jbc.M301295200
161. Ivana Scovassi A, Diederich M. Modulation of poly(ADP-ribosylation) in apoptotic cells. *Biochem Pharmacol.* (2004) 68:1041–7. doi: 10.1016/j.bcp.2004.04.023
162. Pankotai E, Lacza Z, Muranyi M, Szabo C. Intra-mitochondrial poly(ADP-ribosyl)ation: potential role for alpha-ketoglutarate dehydrogenase. *Mitochondrion.* (2009) 9:159–64. doi: 10.1016/j.mito.2009.01.013
163. Rossi MN, Carbone M, Mostocotto C, Mancone C, Tripodi M, Maione R, et al. Mitochondrial localization of PARP-1 requires interaction with mitofilin and is involved in the maintenance of mitochondrial DNA integrity. *J Biol Chem.* (2009) 284:31616–24. doi: 10.1074/jbc.M109.025882
164. Lapucci A, Pittelli M, Rapizzi E, Felici R, Moroni F, Chiarugi A. Poly(ADP-ribose) polymerase-1 is a nuclear epigenetic regulator of mitochondrial DNA repair and transcription. *Mol Pharmacol.* (2011) 79:932–40. doi: 10.1124/mol.110.070110
165. Kloypan C, Srisa-art M, Mutirangura A, Boonla C. LINE-1 hypomethylation induced by reactive oxygen species is mediated via depletion of S-adenosylmethionine. *Cell Biochem Funct.* (2015) 33:375–85. doi: 10.1002/cbf.3124
166. Baccarelli A, Wright R, Bollati V, Litonjua A, Zanobetti A, Tarantini L, et al. Ischemic heart disease and stroke in relation to blood DNA methylation. *Epidemiology.* (2010) 21:819–28. doi: 10.1097/EDE.0b013e3181f20457
167. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell.* (2004) 119:941–53. doi: 10.1016/j.cell.2004.12.012
168. Donohoe DR, Bultman SJ. Metaboloepigenetics: interrelationships between energy metabolism and epigenetic control of gene expression. *J Cell Physiol.* (2012) 227:3169–77. doi: 10.1002/jcp.24054
169. Hino S, Sakamoto A, Nagaoka K, Anan K, Wang Y, Mimasu S, et al. FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure. *Nat Commun.* (2012) 3:758. doi: 10.1038/ncomms1755
170. Newman JC, Verdin E. beta-hydroxybutyrate: much more than a metabolite. *Diabetes Res Clin Pract.* (2014) 106:173–81. doi: 10.1016/j.diabres.2014.08.009
171. Gut P, Verdin E. The nexus of chromatin regulation and intermediary metabolism. *Nature.* (2013) 502:489–98. doi: 10.1038/nature12752
172. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science.* (2013) 339:211–4. doi: 10.1126/science.1227166
173. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes.* (2009) 58:1509–17. doi: 10.2337/db08-1637
174. Galmozzi A, Mitro N, Ferrari A, Gers E, Gilardi F, Godio C, et al. Inhibition of class I histone deacetylases unveils a mitochondrial signature and enhances oxidative metabolism in skeletal muscle and adipose tissue. *Diabetes.* (2013) 62:732–42. doi: 10.2337/db12-0548
175. Li H, Gao Z, Zhang J, Ye X, Xu A, Ye J, et al. Sodium butyrate stimulates expression of fibroblast growth factor 21 in liver by inhibition of histone deacetylase 3. *Diabetes.* (2012) 61:797–806. doi: 10.2337/db11-0846
176. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* (2010) 107:1058–70. doi: 10.1161/CIRCRESAHA.110.223545
177. Salminen A, Kaarniranta K, Hiltunen M, Kauppinen A. Krebs cycle dysfunction shapes epigenetic landscape of chromatin: novel insights into mitochondrial regulation of aging process. *Cell Signal.* (2014) 26:1598–603. doi: 10.1016/j.cellsig.2014.03.030
178. Spallotta F, Cencioni C, Atlante S, Garella D, Cocco M, Mori M, et al. Stable oxidative cytosine modifications accumulate in cardiac mesenchymal cells from type2 diabetes patients: rescue by alpha-ketoglutarate and TET-TDG functional reactivation. *Circ Res.* (2018) 122:31–46. doi: 10.1161/CIRCRESAHA.117.311300
179. Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell.* (2012) 48:491–507. doi: 10.1016/j.molcel.2012.11.006
180. Shi L, Tu BP. Protein acetylation as a means to regulate protein function in tune with metabolic state. *Biochem Soc Trans.* (2014) 42:1037–42. doi: 10.1042/BST20140135
181. Hong J, Jia Y, Pan S, Jia L, Li H, Han Z, et al. Butyrate alleviates high fat diet-induced obesity through activation of adiponectin-mediated pathway

and stimulation of mitochondrial function in the skeletal muscle of mice. *Oncotarget*. (2016) 7:56071–82. doi: 10.18632/oncotarget.11267
182. Walsh ME, Bhattacharya A, Sataranatarajan K, Qaisar R, Sloane L, Rahman MM, et al. The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging. *Aging Cell*. (2015) 14:957–70. doi: 10.1111/acel.12387
183. Zhang Y, Yu B, Yu J, Zheng P, Huang Z, Luo Y, et al. Butyrate promotes slow-twitch myofiber formation and mitochondrial biogenesis in finishing pigs via inducing specific microRNAs and PGC-1alpha expression1. *J Anim Sci*. (2019) 97:3180–92. doi: 10.1093/jas/skz187
184. Xie M, Kong Y, Tan W, May H, Battiprolu PK, Pedrozo Z, et al. Histone deacetylase inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy. *Circulation*. (2014) 129:1139–51. doi: 10.1161/CIRCULATIONAHA.113.002416
185. Yang J, He J, Ismail M, Tweeten S, Zeng F, Gao L, et al. HDAC inhibition induces autophagy and mitochondrial biogenesis to maintain mitochondrial homeostasis during cardiac ischemia/reperfusion injury. *J Mol Cell Cardiol*. (2019) 130:36–48. doi: 10.1016/j.yjmcc.2019.03.008
186. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: from bench to bedside. *Eur Heart J*. (2015) 36:3404–12. doi: 10.1093/eurheartj/ehv290
187. Ou X, Lee MR, Huang X, Messina-Graham S, Broxmeyer HE. SIRT1 positively regulates autophagy and mitochondria function in embryonic stem cells under oxidative stress. *Stem Cells*. (2014) 32:1183–94. doi: 10.1002/stem.1641
188. Pollack RM, Crandall JP. Resveratrol: therapeutic potential for improving cardiometabolic health. *Am J Hypertens*. (2013) 26:1260–8. doi: 10.1093/ajh/hpt165
189. Costantino S, Paneni F, Battista R, Castello L, Capretti G, Chiandotto S, et al. Impact of glycemic variability on chromatin remodeling, oxidative stress, and endothelial dysfunction in patients with type 2 diabetes and with target HbA1c levels. *Diabetes*. (2017) 66:2472–82. doi: 10.2337/db17-0294
190. Costantino S, Paneni F, Mitchell K, Mohammed SA, Hussain S, Gkolfos C, et al. Hyperglycaemia-induced epigenetic changes drive persistent cardiac dysfunction via the adaptor p66(Shc). *Int J Cardiol*. (2018) 268:179–86. doi: 10.1016/j.ijcard.2018.04.082
191. Kim CS, Kim YR, Naqvi A, Kumar S, Hoffman TA, Jung SB, et al. Homocysteine promotes human endothelial cell dysfunction via site-specific epigenetic regulation of $p66^{shc}$. *Cardiovasc Res*. (2011) 92:466–75. doi: 10.1093/cvr/cvr250
192. Arunachalam G, Samuel SM, Marei I, Ding H, Triggle CR. Metformin modulates hyperglycaemia-induced endothelial senescence and apoptosis through SIRT1. *Br J Pharmacol*. (2014) 171:523–35. doi: 10.1111/bph.12496
193. de Kreutzenberg SV, Ceolotto G, Cattelan A, Pagnin E, Mazzucato M, Garagnani P, et al. Metformin improves putative longevity effectors in peripheral mononuclear cells from subjects with prediabetes. A randomized controlled trial. *Nutr Metab Cardiovasc Dis*. (2015) 25:686–93. doi: 10.1016/j.numecd.2015.03.007
194. de Oliveira MR, Jardim FR, Setzer WN, Nabavi SM, Nabavi SF. Curcumin, mitochondrial biogenesis, and mitophagy: Exploring recent data and indicating future needs. *Biotechnol Adv*. (2016) 34:813–26. doi: 10.1016/j.biotechadv.2016.04.004
195. Xiong S, Salazar G, San Martin A, Ahmad M, Patrushev N, Hilenski L, et al. PGC-1 alpha serine 570 phosphorylation and GCN5-mediated acetylation by angiotensin II drive catalase down-regulation and vascular hypertrophy. *J Biol Chem*. (2010) 285:2474–87. doi: 10.1074/jbc.M109.065235

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past co-authorship with one of the authors FP.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 12 March 2020
doi: 10.3389/fcvm.2020.00035

Mechanisms of Anthracycline-Induced Cardiotoxicity: Is Mitochondrial Dysfunction the Answer?

*Alessandra Murabito, Emilio Hirsch and Alessandra Ghigo**

Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy

Cardiac side effects are a major drawback of anticancer therapies, often requiring the use of low and less effective doses or even discontinuation of the drug. Among all the drugs known to cause severe cardiotoxicity are anthracyclines that, though being the oldest chemotherapeutic drugs, are still a mainstay in the treatment of solid and hematological tumors. The recent expansion of the field of Cardio-Oncology, a branch of cardiology dealing with prevention or treatment of heart complications due to cancer treatment, has greatly improved our knowledge of the molecular mechanisms behind anthracycline-induced cardiotoxicity (AIC). Despite excessive generation of reactive oxygen species was originally believed to be the main cause of AIC, recent evidence points to the involvement of a plethora of different mechanisms that, interestingly, mainly converge on deregulation of mitochondrial function. In this review, we will describe how anthracyclines affect cardiac mitochondria and how these organelles contribute to AIC. Furthermore, we will discuss how drugs specifically targeting mitochondrial dysfunction and/or mitochondria-targeted drugs could be therapeutically exploited to treat AIC.

Keywords: mitochondria, anthracycline, reactive oxygen species, mitochondria-targeted drug, cardiotoxicity after chemotherapy

OPEN ACCESS

Edited by:
Rhian M. Touyz,
University of Glasgow,
United Kingdom

Reviewed by:
Steven Edward Lipshultz,
Wayne State University, United States
Gavin Oudit,
University of Alberta, Canada
Sherry-Ann Brown,
Mayo Clinic, United States
Giselle Melendez,
Wake Forest University, United States

****Correspondence:***
Alessandra Ghigo
alessandra.ghigo@unito.it

Specialty section:
This article was submitted to Cardio-Oncology, a section of the journal Frontiers in Cardiovascular Medicine

Received: *26 November 2019*
Accepted: *24 February 2020*
Published: *12 March 2020*

Citation:
Murabito A, Hirsch E and Ghigo A (2020) Mechanisms of Anthracycline-Induced Cardiotoxicity: Is Mitochondrial Dysfunction the Answer? Front. Cardiovasc. Med. 7:35. doi: 10.3389/fcvm.2020.00035

INTRODUCTION

Advances in cancer therapy resulted in marked improvements in patient survival, with anthracyclines (ANTs) probably being the most potent antineoplastic therapeutics available for the clinical practice, and still representing one of the pillars in the treatment of different tumors. In 2018 more than 3 million people were diagnosed with cancer in Europe only, and it has been estimated that currently 14.5 million people are living with a history of cancer in USA, with this number rising up to 19 million over the next 10 years (1, 2). Notably, 50% of people diagnosed with cancer today will survive at least 10 years after diagnosis, and this proportion is even higher for childhood cancer survivors. However, this improvement in survival of cancer patients has led to a greater recognition of the long-term adverse effects of antineoplastic therapies like ANTs, mostly involving the cardiovascular system. In a cohort of almost 2,000 cancer survivors monitored over 7 years, 33% of deaths were related to cardiovascular conditions while cancer-related mortality accounted for 51% of deceases. Given the concrete possibility of incurring in ANT-induced cardiotoxicity (AIC), and that the number of cancer survivors is constantly increasing, in the upcoming years there will probably be a Cardio-Oncology "epidemic." For this reason, cardiologists, oncologists, and basic scientists are combining their efforts in order to better characterize the molecular mechanisms

behind this pathology (3). In this regard, in recent years the role of mitochondria has strongly emerged, since several compounds exert their cardiotoxic effects targeting these organelles (4, 5). This is due to the fact that mitochondria are particularly important for the heart because of its high demand in energy. Since mitochondria are the organelles dedicated to ATP production, dysfunctional mitochondria are repeatedly replaced by newly synthesized ones with the purpose of sustaining the constant need for ATP, underlying the importance of mitochondria dynamics and mitophagy. Drugs that impair the proper activity of mitochondria likely cause a substantial decrease in ATP levels that, eventually, leads to myocardial dysfunction (6). For this reason, drugs preserving mitochondrial function and metabolism are receiving increasing attention in order to treat or prevent cardiotoxicity induced by several drugs, including ANTs. In this review, we will describe the crucial role in AIC of mitochondria, organelles of fundamental importance for the heart, and we will discuss about specific treatments targeting their function and metabolism.

AIC: FROM DEFINITION TO CURRENT TREATMENT

ANTs, such as doxorubicin (DOX), daunorubicin and epirubicin, are antibiotic agents highly effective as anticancer therapeutics, and for this reason they have been registered by the World Health Organization as essential medicines (7). However, it was noticed early on that their use is associated to the development of heart failure (HF) (8, 9). Already in the seventies, Von Hoff et al. analyzed retrospectively more than 4,000 DOX-treated subjects and found that the overall incidence of congestive HF caused by the treatment was 2.2%. Notably, the number of patients affected by AIC in this study is probably underestimated since it was based only on clinician-identified signs and symptoms of congestive HF. Moreover, it was already clear that the probability of incurring in AIC is strictly dependent on the total dose administered and that the use of smaller, divided doses of DOX decreases the likelihood of developing cardiotoxicity, while there is a sharp increase in the prevalence of HF occurring at increasing doses of the drug (10). Importantly, anthracyclines are rarely administered as single agents and are more often combined with radiotherapy or modern targeted therapies, like monoclonal antibodies, which importantly exacerbate toxicity (11).

AIC can manifest acutely, early after infusion, strongly compromising cancer treatment since it may require dose modification or even cessation of anticancer therapies (12). Almost 30% of patients are affected by this type of cardiotoxicity, that is characterized by electrocardiogram abnormalities, including atypical ST changes, reduced QRS voltage, tachycardia, and supraventricular premature beats. Yet, acute AIC is a rare complication and the most prevailing and significant form of AIC is the chronic one. It is characterized by left ventricular systolic dysfunction, with a reduction in left ventricular ejection fraction (LVEF), which can be very insidious since it is asymptomatic in the early stages. It can eventually progress to dilated cardiomyopathy and congestive heart failure (CHF), which is nowadays one of the main co-morbidity in childhood cancer survivors (11, 13, 14). These patients have a 12-fold increased chance of developing congestive heart failure (CHF) up to 30 years after treatment, with an occurrence of AIC up to 30% (15–17). Of notice, some cancer patients already have pre-existing cardiovascular diseases or at least cardiovascular risk factors that strongly increase the likelihood of developing cardiac issues, and specifically AIC, in these individuals.

The assessment of AIC primarily relies on evaluation of clinical symptoms and/or detection of systolic function (LVEF) by echocardiography, acquisition scans, and magnetic resonance imaging (18). In particular, cardiotoxicity is currently diagnosed when a decline of 5–55% in LVEF with HF symptoms, or an asymptomatic decline of 10 to below 55%, is observed. Nevertheless, recent studies highlight the limitations of these ejection fraction-based screenings, proposing new diagnostic strategies. In particular, strain rate imaging and troponin (Tn) leakage in the peripheral blood could be used to identify patients with early clinical signs of cardiotoxicity (19–21). From a therapeutic point of view, unfortunately there is no specific treatment targeting AIC. Efforts are being made to develop strategies to prevent AIC that, depending on their mechanism of action, are classified as primary, when focused on preventing the disease concomitantly with ANT treatment, and secondary, when prompted to prevent symptomatic progression (22). For now though all the secondary preventive strategies have limited follow up, also because of the difficulties related to monitoring cardiotoxicity in both adults and children (22). Some clinical trials have shown modest success with the usage of the standard pharmacological regimen for HF. Notably, it has been reported that the non-selective β adrenergic receptor (βAR) blocker, carvedilol, can prevent DOX-induced left ventricular dysfunction through its antioxidant properties, and can ameliorate cardiac function and survival in cancer patients under ANT therapy (23–25). More recently, it was demonstrated that early treatments with the angiotensin converting enzyme I (ACE-I) enalapril, either alone or in combination with carvedilol, are able to fully or partially recover LVEF in 82% of patients manifesting signs of cardiotoxicity within the first year after the end of ANT treatment (13). Unfortunately, these regimens are far from optimal for AIC treatment, and this is probably due to the fact that the mechanisms involved in this specific type of cardiomyopathy are different to those underlying other types of cardiac disease, like ischemic, post-infectious, and idiopathic dilated cardiomyopathies (22). This underlies the need for more specific therapeutics, and so, of a better understanding of the molecular mechanisms behind this condition.

MITOCHONDRIA: KEY PLAYERS IN AIC

If the molecular processes behind the anticancer effects of ANTs are well-known and studied, the mechanisms underlying their cardiotoxic effects are still poorly understood and controversial. It is well-established that ANTs exert their anticancer action by directly targeting and inhibiting topoisomerase 2 (Top2) in cancer cells, more specifically the 2α isoform, halting DNA transcription, and replication (26). However, the same mechanism can hardly explain the toxic effect of ANTs

on the heart, since cardiomyocytes are for definition non-dividing cells, thus leaving an open question for cardio-oncology researchers (27, 28). Recent evidence suggests that DOX cardiotoxicity is causally linked to inhibition of a Top2 isoform which is preferentially expressed by differentiated cells, like cardiomyocytes, namely Top2β, the only Top2 expressed in mitochondria (27, 29). Moreover, a number of other mechanisms of AIC, which are not necessarily linked to Top2β inhibition, have started to emerge. Interestingly, both pathways have been reported to impact on the activity of mitochondria. In the next paragraphs, we will describe Top2β-dependent (or direct) and Top2β-independent (indirect) mechanisms of DOX cardiotoxicity and how these signaling pathways converge on the dysregulation of mitochondrial activity and metabolism in cardiomyocytes.

"Direct" Mechanisms of AIC Involving Mitochondria

As mentioned above, the cellular targets of DOX are topoisomerases, more specifically of the Top2 class (30). DOX can bind both DNA and Top2 in order to form the ternary Top2-DOX-DNA cleavage complex which triggers cell death. As mentioned before, besides inhibiting Top2α in proliferating cells, ANTs can target Top2β, which is also the only known type 2 topoisomerase present in cardiac mitochondria [**Figure 1**; (27)]. In their study, Zhang et al. demonstrated that DOX treatment induces significant changes in the expression of genes controlling both mitochondrial structure and metabolism (oxidative phosphorylation pathways) in cardiomyocytes expressing Top2β ($Top2\beta^{+/+}$), but not in Top2β knockout mice ($Top2\beta^{\Delta/\Delta}$) (29). More specifically, among the genes downregulated after DOX treatment in $Top2\beta^{+/+}$, and not significantly affected in $Top2\beta^{\Delta/\Delta}$ cardiomyocytes, are Ndufa3 (encoding the NADH dehydrogenase 1-α subcomplex 3), Sdha (encoding succinate dehydrogenase complex II, subunit A), and Atp5a1 (encoding the ATP synthase subunit α). In agreement, mitochondria fail to maintain their membrane potential in DOX-treated $Top2\beta^{+/+}$ but not in $Top2\beta^{\Delta/\Delta}$ cardiomyocytes (29). In addition to modulation of genes involved in mitochondrial function and metabolism, DOX was also shown to decrease the transcription of *Ppargc1a* and *Ppargc1b*. These two genes encode for PGC-1α and PGC-1β, respectively, that by interacting with crucial transcription factors, namely NRF-1, NRF-2, and ERRα, push the expression of genes implicated in mitochondrial biogenesis (29). In keeping with their preserved mitochondrial function, cardiomyocyte-specific Top2β knockout mice are protected from DOX-induced progressive HF. Indeed, after 5 weeks of DOX treatment, $Top2\beta^{+/+}$ mice show a decrease in ejection fraction up to 50%, whereas this parameter is not altered in $Top2\beta^{\Delta/\Delta}$ mice. Zhang et al. also demonstrated that reactive oxygen species (ROS) production is reduced by 70% in the hearts of $Top2\beta^{\Delta/\Delta}$ as compared to $Top2\beta^{+/+}$ mice (29). Of note, the finding that Top2β silencing only partially reduces ROS production in cardiomyocytes treated with ANTs suggests that ROS may be generated in response to DOX by additional Top2β-independent mechanisms that will be discussed in the next paragraph.

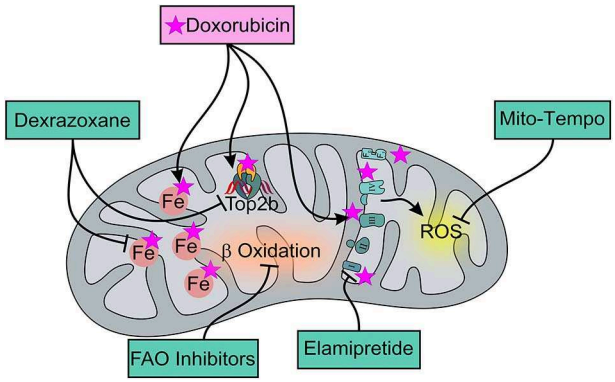


FIGURE 1 | Effects of DOX and of mitochondria-targeted drugs on mitochondrial function and metabolism. DOX preferentially accumulates within mitochondria thanks to its ability to specifically bind to the phospholipid cardiolipin, causing membrane perturbation and ETC disruption that can be limited by Elamipretide, a tetrapeptide that improves the efficiency of electron transport and restores cellular bioenergetics. ETC dysfunction mainly induces ROS production that can be though limited by the usage of the mitochondria-targeted antioxidant, Mito-Tempo, a specific scavenger of mitochondrial superoxide. Moreover, DOX can directly interact with iron to form reactive ANT-iron complexes resulting in an iron cycling between Fe^{3+} and Fe^{2+} which is associated with ROS production and altered iron homeostasis. Dexrazoxane, as an iron-chelator, can inhibit the production of ROS ensuing from the interaction between ANT and non-heme iron, ultimately alleviating DOX-induced mitochondrial oxidative stress. Moreover, Dexrazoxane can prevent DOX from binding to the Top 2β-DNA complex. For AIC treatment, FAO inhibitors can also be used for their ability to enhance glucose oxidation and prevent a decrease in intracellular ATP levels, thereby ensuring the proper maintenance of cellular homeostasis.

"Indirect" Mechanisms of AIC Involving Mitochondria

Since the initial discovery of ANT cardiotoxicity, the generation of excessive ROS has represented the most widely accepted mechanistic explanation. Even if in cardiomyocytes ROS can be produced, at least in part, as a consequence of ANT-mediated Top2β inhibition (see previous paragraph for further detail), several "indirect" or Top2β-independent mechanisms significantly contribute to ROS production and mitochondrial dysfunction. In the next paragraph, we will describe mechanisms of AIC which are unrelated to Top2β inhibition and that culminate in alterations of mitochondrial function and metabolism.

Mitochondrial ROS Production and Metabolism Dysregulation

Recent evidence suggests that ANTs, in particular DOX, preferentially accumulate in the mitochondria of cardiomyocytes, strongly impacting on both the structure and the activity of these organelles. Indeed, DOX can directly bind to the abundant phospholipid cardiolipin, located in the inner mitochondrial membrane (31, 32). This interaction hampers the electron transport chain (ETC), since it inhibits complex I and II, leading to ROS production (**Figure 1**). More specifically, a quinone moiety in the C ring of DOX can accept electrons for NADH or NADPH and is thus reduced by the respiratory

chain complex I, generating a reactive semiquinone free radical (33, 34). On one hand, this mechanism decreases the electron flow through the ETC, removing electrons normally used for ATP production; on the other hand, the reduced semiquinone can transfer the electron to O_2, generating the superoxide anion O_2^-. DOX can be generated back by this process, in a reaction known as the "redox cycling," and can be reduced again if NADH is present, producing O_2^- continuously. O_2^- can be transformed into the low-toxic hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or into other ROS (35, 36). ANT-mediated production of these reactive species in turn can activate different pathways leading to cardiomyocytes death, including apoptosis and necrosis. Intriguingly, DOX-induced cardiomyopathy has been recently linked to another form of regulated cell death, the less characterized iron-dependent cell death, also named ferroptosis, which is driven by iron-dependent lipid peroxidation. Indeed, ANTs produce ROS also because they can chelate free iron, leading to the formation of reactive iron-DOX complexes that can interact with O_2 [**Figure 1**; (37)]. Moreover, it has been shown that DOX can upregulate heme oxygenase 1, the enzyme responsible for heme degradation, and releases free iron in cardiomyocytes, leading to oxidation of lipids of the mitochondrial membrane and to a further release of free iron in cardiomyocytes, thus feeding this vicious cycle of ROS production (37). In addition, Ichikawa et al. showed that DOX specifically triggers iron accumulation in the mitochondria of isolated cardiomyocytes, without altering total cellular iron levels. Intriguingly, this preferential accumulation is also found in the hearts of DOX-treated patients. Mechanistically, the increase in mitochondrial iron levels upon ANT administration is mediated by the downregulation of the ATP-binding cassette subfamily B member 8 (ABCB8), a transporter protein mediating mitochondrial iron export. ABCB8 overexpression protects mice from DOX-induced oxidative stress and cardiomyopathy and preserves mitochondrial structure and cardiomyocyte viability. Conversely, in the absence of ABCB8, DOX-induced ROS production and mitochondrial damage are increased compared to controls, underlying the cardio-protective role of this transporter (37, 38). Notably, other aspects of mitochondrial metabolism and energy production can be disrupted by ANTs. It has been demonstrated that β-oxidation, the main process used by the healthy heart to generate energy, is inhibited upon DOX treatment through the down-modulation of carnitine palmitoyltransferase 1 (CPT-1), while glycolysis is increased by 50% within few hours as a compensatory response. However, this metabolic adaptation is reversed with time, with a strong decrease in glucose oxidation that has been demonstrated both *in vitro* and *in vivo*. This may be due to the reduction of glucose supply after the induction phase or because of the poor availability of one of the key enzymes of the process, namely phosphofructokinase (PFK) (39).

Calcium Homeostasis Dysregulation

The metabolic changes induced by DOX, and the consequent reduction in ATP levels, are known to negatively impact myocardial contractility, which may be exacerbated by an impairment of myocardial Ca^{2+} signaling. It is known that DOX affects Ca^{2+} homeostasis and signaling via several mechanisms, also involving ROS. On one hand, the lipid peroxidation elicited by DOX-mediated ROS production can alter the activity of membrane-residing proteins, such as mitochondrial calcium channels (40, 41). In addition, ANTs can impair the expression and activity of key players of myocardial contraction, namely the cardiac ryanodine receptor (RyR2) and the sarco-/endoplasmic reticulum Ca^{2+} ATPase (SERCA2) (42). In physiological conditions, the action potential mediating contraction is detected by L-type Ca^{2+} channels that activate RyR2, which are responsible for Ca^{2+} release from the sarcoplasmic reticulum (SR). This latter increase in cytoplasmic Ca^{2+} level triggers muscle contraction. Ca^{2+} levels are eventually restored to basal via the activation of SERCA2, mediating the reuptake of Ca^{2+} into the SR (42). DOX and its main metabolite, doxorubicinol (doxOL), are known to activate and increase the open probability of RyR2, though this effect is acute and detectable only right after administration of the drug and at low concentrations (42). Instead, doxOL was found to oxidize RyR2 thiols and this irreversible modification causes a significant inhibition of the channel. Interestingly, it has been shown that SERCA2 can be inhibited via the same oxidation process, which leads to a dramatic increase in cytoplasmic Ca^{2+} levels (42). In addition, this process is exacerbated by the fact that ANTs can negatively affect the transcription of the channel (42). More importantly, DOX is able to activate Calcium/Calmodulin-dependent protein kinase-II (CaMKII), which alters mitochondrial Ca^{2+} homeostasis and promotes apoptosis. CaMKII increases Ca^{2+} influx in mitochondria through mitochondrial calcium Ca^{2+} uniporter (MCU) via activation of the nuclear factor-kappa B (NF-kB) and p53. This, in turn, leads to the opening of the permeability transition pore (MTP) at lower levels of Ca^{2+} compared to normal conditions, resulting in dissipation of the mitochondrial membrane potential and in increased permeability to apoptotic factors (43, 44). Moreover, ANT-mediated ATP depletion (as described in the previous paragraph) also reduces the mitochondrial membrane potential and causes MTP opening, further dysregulating Ca^{2+} homeostasis (45).

Autophagy and Mitochondrial Dynamism Impairment

Among all mammalian cells, cardiomyocytes emerge for having the highest mitochondrial density and also the greatest respiratory capacity. This might be the reason why preserving the homeostasis of these organelles is a physiological imperative for the heart. In agreement, mitochondria damaged by DOX have to be promptly removed to maintain a healthy heart. Unfortunately, ANTs are known to disrupt the major degradative/recycling process of mitochondria, namely autophagy (46, 47). Several studies found that acute administration of high-dose ANTs can induce the accumulation of both LC3 and p62, the major autophagy markers, with a reduction in ATP levels in mouse hearts, and a significant suppression of oxygen consumption rate (OCR) in their mitochondria (46). Further analysis from Li et al. demonstrated that DOX blocks cardiomyocytes autophagic flux mediating a strong accumulation of undegraded autolysosomes. This is due to defects in lysosomal acidification caused by

DOX-mediated suppression of the activity of V-ATPase, the proton pump that generates and maintains pH gradients in this organelle (48). Furthermore, ANTs inhibit the phosphorylation of one of the positive regulators of autophagy initiation, AMPK, suggesting that ANTs dampen autophagy not only by impairing the autophagic flux but also by inhibiting its initiation. Starvation prior to ANT treatment restores AMPK signaling and autophagy, ultimately protecting the heart against cardiac dysfunction (49). Another mechanism by which DOX impairs autophagy involves the PI3Kγ pathway. Li et al. recently showed that DOX activates a PI3Kγ/Akt/mTOR cascade which ultimately converges on autophagy inhibition, while genetic or pharmacological inhibition of PI3Kγ restores the autophagic flux and protects mice against AIC (50).

Along with impaired autophagy, AIC is characterized by defective mitochondrial dynamics, which refers to organelle fusion, fission, and mitophagy, a specific autophagic mechanism targeting mitochondria. The mitochondrial fusion proteins, mitofusin1 and 2 (Mfn1 and Mfn2), and optic atrophy 1 (Opa1), as well as the mitochondrial fission protein, dynamin related protein (Drp)1, are highly expressed in the mammalian heart, wherein their genetic ablation causes dramatic cardiac dysfunction. Mfn2 levels are decreased in cardiomyocytes after treatment with DOX and this event is associated with increased mitochondrial fission, leading to mitochondrial fragmentation, mitophagy, decreased antioxidative capacity, and ultimately cell death. Accordingly, increased expression of Mfn2 in cardiomyocytes, or the use of the mitochondria-targeted antioxidant Mito-Tempo, a specific scavenger of mitochondrial superoxide, attenuate DOX-induced mitochondrial fission and prevent cardiomyocyte mitochondrial ROS production and apoptosis (51). Mito-Tempo though is not the only known compound to counteract AIC. Several others are now being investigated and will be extensively described in the following paragraphs.

TARGETING MITOCHONDRIA AND THEIR METABOLISM FOR THE TREATMENT OF AIC

In-depth study of the intertwined molecular mechanisms underlying ANT-induced mitochondrial toxicity has recently paved the way to the development of approaches potentially useful to treat AIC. However, targeting AIC in the clinical setting is still challenging, since a major requirement for these medications is that they do not interfere with the antitumor activity of ANTs. Below we will describe the most promising therapeutics for AIC, with a major focus on those targeting either ROS and their production, or mitochondrial metabolism.

Dexrazoxane

Dexrazoxane is not only one of the most studied cardioprotective adjuvant for DOX chemotherapy, but it is also the only Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved drug for AIC prevention (12, 52). Thanks to its ability to act as an iron-chelator, dexrazoxane inhibits the production of ROS ensuing from the interaction between ANTs and non-heme iron, ultimately alleviating DOX-induced mitochondrial oxidative stress [**Figure 1**; (53, 54)]. However, the concept that dexrazoxane promotes cardioprotection only by virtue of its antioxidant properties is debated, especially in view of the finding that other antioxidant drugs, such as vitamin A, vitamin E, and N-acetylcysteine, failed to provide benefits in the treatment of AIC (55–57). An additional mechanism that may account for the cardioprotective action of dexrazoxane is its ability to prevent DOX from binding to the Top2β-DNA complex. X-ray crystal structure analyses revealed that dexrazoxane can bind to the two ATP binding sites at the N terminus of Top2 and bridges two Top2 monomers in the closed-clamp configuration [**Figure 1**; (58)]. Moreover, it has also been demonstrated that dexrazoxane forms a tight complex with the ATPase domain of human Top2α and Top2β, suggesting that this compound prevents ANT from binding to Top2 (59). In addition, dexrazoxane has been shown to interact with Poly(ADP-ribose) (PAR) monomers, acting as a PAR Poly(ADP-ribose) polymerase (PARP) inhibitor (60). In agreement, inhibition of this enzyme improves cardiac function and decreases mortality without altering the anticancer activity of DOX in several animal models of DOX-induced cardiomyopathy (61). Consistent with its mechanisms of action, dexrazoxane is exploited to prevent rather than treat AIC and its use appears to be most appropriate in patients with stage A of HF, i.e., at high risk of developing the pathology. However, Ganatra et al. demonstrated that dexrazoxane exerts its cardioprotective function also in stage B HF (62). In a small cohort of patients showing pre-existing asymptomatic, systolic left ventricular (LV) dysfunction, the administration of dexrazoxane 30 min before each ANT dose was enough to allow patients to complete their planned chemotherapy, with a minimal decrease in LVEF and no elevation in HF biomarkers. On the contrary, the three patients that did not receive dexrazoxane had a marked reduction in heart function and developed HF. Of note, two of them died from cardiogenic shock and multi-organ failure (62).

Concerning the clinical efficacy of dexrazoxane, it has been shown in multiple trials that it can reduce the incidence of CHF and LVEF decline in patients treated with ANTs (63–65). These findings were also corroborated by a more recent study in which Marty et al. found that, based on both LVEF and CHF results, 164 relapsed breast cancer patients treated with dexrazoxane have significantly lower overall cardiac events in comparison with the control group treated with DOX or epirubicin only (66). Similarly, dexrazoxane has been shown to abrogate DOX-mediated mitochondrial dysfunction in childhood cancer survivors. Lipshultz et al. found that, in peripheral blood mononuclear cells (PBMCs), DOX-damaged mitochondria expand their mtDNA, which encodes for 13 polypeptides involved in oxidative phosphorylation, as an attempt to compensate for the injury and improve mitochondrial metabolism (67). Treatment with dexrazoxane, together with DOX, reduces the number of mtDNA copies per cell compared to the group treated with DOX only, suggesting preserved mitochondrial function in patients receiving the combination

therapy (67). Intriguingly, besides proving the efficacy of dexrazoxane in counteracting AIC-related mitochondrial dysfunction, this study also suggests that mitochondrial injury, and the ensuing increase of mtDNA in peripheral blood, might represent a biomarker for early detection of cardiotoxicity, which still represents an unmet clinical need.

Despite evident clinical benefits, in 2011 EMA contraindicated the usage of dexrazoxane in children since its efficacy in this subpopulation was not assessed. In addition, it was proposed that dexrazoxane could not only attenuate the anticancer effects of ANTs and increase the risk of secondary malignancies, but could also cause myelotoxicity (64–66). Nevertheless, this view has been recently refuted by a number of studies (68). A phase-III clinical trial, involving more than 500 children and adolescents affected by T-cell acute lymphoblastic leukemia (ALL) or lymphoblastic non-Hodgkin lymphoma, was conducted to investigate not only the cardio-protective effects of dexrazoxane but also its safety as well as its potential impact on the antineoplastic efficacy of ANTs (69). In addition, Lipshultz et al. found that dexrazoxane attenuates DOX-induced cardiac injury in children with acute lymphoblastic leukemia, without compromising its antileukemic efficacy (70). It was also reported that dexrazoxane alone does not increase the risk of second primary malignancies (SPMs), which are instead related to the usage of three Top2 inhibitors used in combination (doxorubicin, etoposide, and dexrazoxane) and mostly etoposide (71). For these reasons, EMA has approved the administration of dexrazoxane to children supposed to be given more than 300 mg/m^2 of ANTs (12, 52, 68).

Mito-Tempo

The novel drug named mitochondrial-targeted Tempo l (Mito-Tempo) is a well-known superoxide dismutase (SOD) mimetic. Mitochondria are the only organelles having a unique type of superoxide dismutase, the manganese-containing SOD2, which is crucial for protecting against excessive production of O_2^-, a key feature of AIC (**Figure 1**). Mice that do not express this protein develop a severe cardiomyopathy already at 10 days after birth, while mice missing one allele of SOD2 (SOD2$^{+/-}$ mice) develop hypertension with time and if challenged with an high-salt diet, suggesting a role for this enzyme in cardiac protection (72). Mito-Tempo consists of the tempol moiety bound to a triphenylphosphonium cation that allows the molecule to enter mitochondria, and this is the reason why this molecule may be highly effective in organs, such as the heart, which are rich in these organelles. Mimicking the activity of SOD, Mito-Tempo acts as an antioxidant drug in rats, and in mice it has also been shown to alleviate oxidative stress and cardiac toxicity induced by DOX (73, 74). Indeed, already in the 90's, it was demonstrated that Mito-Tempo significantly reduces the contractile impairment as well as the lipid peroxidation observed in rat heart treated acutely with DOX (75). In all these *in vivo* studies, Mito-Tempo was used in combination with ANTs in patients with no pre-existing heart disease, suggesting that it might be exploited to prevent AIC likely in patients in stage A HF. In addition, in a guinea pig model of non-ischemic HF, Mito-Tempo reversed the pathological phenotype, suggesting that this compound can also have a therapeutic effect in patients in later stages of ANT-induced HF (76). More recently, Mito-Tempo was used in combination with dexrazoxane and this combinatorial treatment ameliorates DOX-induced cardiomyopathy without altering the antitumor activity of DOX (77).

Elamipretide

Elamipretide is one of the first drugs developed to target selectively the mitochondrial ETC in order to improve the efficiency of electron transport and restore cellular bioenergetics [**Figure 1**; (78)]. More than one mechanism of action has been proposed for this tetrapeptide. It penetrates cell membranes, localizing to the inner mitochondrial membrane where it can interact with the phospholipid cardiolipin. Cardiolipin has a crucial role in maintaining the functional positioning of the ETC complexes and supercomplexes within the inner mitochondrial membrane, allowing for efficient electron transfer down the redox chain, minimizing reactive oxygen species production. This binding between cardiolipin and the tetrapeptide prevents peroxidation of the phospholipid, thereby maintaining membrane fluidity and supercomplex formation and enhancing electron transport chain function, ultimately increasing ATP synthesis and reducing mitochondrial ROS (79–82). Several studies conducted in rats showed that elamipretide can significantly improve myocardial mitochondrial ATP content, reduce myocardial infarct size and improve cardiac function (83–85). Moreover, treatment with elamipretide improves left ventricular function in animals with HF (84). Saba et al. also demonstrated a significant improvement in ejection fraction in dogs with HF treated with elamipretide for 3 months (86). In addition, this compound can ameliorate left ventricular relaxation via restoration of cardiac myosin binding protein-C (84, 86, 87). A clinical trial of elamipretide in patients with heart failure with reduced ejection fraction (HFrEF) has also been conducted to evaluate safety, efficacy, and tolerability of the compound. Daubert et al. reported that no subjects suffered any serious adverse events, and only one stopped the treatment after a single administration. Moreover, all patients had stable hemodynamic parameters of blood pressure and heart function, suggesting that elamipretide is well-tolerated also together with current standard HF medications. Most notably, patients treated with elamipretide showed a significant reduction in left ventricular volumes in comparison with placebo, despite the small sample size of the trial (88). Of course, larger studies are required to determine its safety as well as its efficacy in patients with HF, but up to now elamipretide seems to be an optimal therapeutic option for targeting mitochondrial dysfunction in the future. On note, elamipretide has not yet been tested in a specific model of AIC but all these studies suggest that this molecule can both ameliorate and prevent different aspects of mitochondrial dysfunction, leading to envisage its use in patients at different stages of the disease. Unfortunately, there is still no evidence that this drug does not alter the antineoplastic activity of ANTs, which might be a possibility because of its known ability to inhibit apoptosis (84). Further studies are needed to prove the possibility of using this molecule in Cardio-Oncology.

Autophagy-Targeting Drugs

Until now, no compounds targeting autophagy have been used in clinical trials to prevent AIC or any cardiac disease. Targeting autophagy in AIC, as well as in any disease context, is still controversial, since this process is critical to the maintenance of cellular homeostasis and it has to be finely tuned, with any perturbation being either beneficial or detrimental (47, 89). Some attempts to modulate this process have been reported in animal models and have shown promising starting results, suggesting that inhibiting this process can be protective and that can be used in the future in patients in stage A of AIC. Sciarretta et al. also demonstrated that the autophagy activator trehalose can protect from myocardial infarction-induced cardiac remodeling, suggesting the possible use of this molecule as a therapeutic agent for HF (90). Sishi et al. showed that rapamycin, a known potent activator of autophagy, is able to improve the negative effects mediated by DOX treatment when administered in combination with the anticancer therapy, leading to a decrease in ROS production, and enhanced mitochondrial function (91). Pharmacological inhibition of PI3Kγ phenocopies mTOR blockade and restores the autophagic flux, ultimately preventing AIC (50). However, boosting the autophagic process can negatively impact on the efficacy of cancer treatments since it may make the tumor resistant to chemotherapy. In agreement, autophagy inhibitors, instead of activators, have been tested in oncology so far. Several trials have been carried out inhibiting autophagy with hydroxychloroquine (HCQ), the only clinically-approved autophagy inhibitor (92), raising some concerns about the possible future usage of autophagy-activators for curing AIC.

Inhibitors of Mitochondrial Fatty Acid Beta Oxidation

Members of this category are Trimetazidine, Ranolazine, and Perhexiline and their use results in the reduction of myocardial fatty acid (FA) uptake and oxidation (**Figure 1**). In pathological conditions, such as HF, cardiac fatty acid and glucose metabolism are altered and contribute to impaired heart efficiency and function. More specifically, there is an increase in the amount of fatty acids that are oxidized by cardiac mitochondria (93–95). Since FA oxidation (FAO) consumes more energy in comparison with glucose oxidation, requiring 10% more oxygen for a given amount of ATP that is produced, an increase in the amount of FA oxidized by the mitochondria can potentially reduce cardiac efficiency and impair heart function (96). Therefore, FAO inhibitors might represent promising drugs for treating AIC in patients at more advanced stages of the disease, such as B and C, since they lead to an enhanced glucose oxidation and prevent a decrease in intracellular ATP levels, thereby ensuring the proper functioning of ionic pumps and maintenance of cellular homeostasis (97–99). Nevertheless, early and sustained inhibition of CPT-1, the crucial and limiting enzyme of FAO, was shown to prevent LV dysfunction and remodeling, as well as efficiently slowing down the development and progression of the disease, in a dog model of HF, suggesting the possible usage of FAO inhibitors also in stage A HF (100). Of note, these compounds could also provide the opportunity to target cancerous cells as well, since they depend on FAO for several aspects such as proliferation, survival and drug resistance (101).

Trimetazidine is an antiischemic agent able to specifically inhibit the long-chain mitochondrial 3-ketoacyl coenzyme A thiolase enzyme that can help cardiomyocytes to maintain proper energy metabolism. No clinical trial has been conducted using this drug for the treatment of AIC, or more generally HF, but its safety and tolerability have been proven through its use in acute coronary syndrome (102). Several studies demonstrated that trimetazidine is effective in improving LVEF, decreasing the rate of hospitalization and reducing brain natriuretic peptide (BNP) levels in subjects with HF (103–106). Moreover, it can also improve cardiac function and reduce HF symptoms when administered together with metoprolol, a βAR blocker.

Ranolazine, if used at high concentrations, is a partial inhibitor of fatty acid beta-oxidation (107). Its main mechanism of action is indeed related to its capability to inhibit late inward sodium channels. In failing myocytes, these channels are hyperactivated, leading to calcium overload and in turn contractile dysfunction and increased oxygen consumption (108). Ranolazine is approved for the treatment of chronic angina, but there is evidence suggesting its clinical effect also for HF treatment (109). Up to now, it has been demonstrated that ranolazine mediates diastolic benefits, by restoring myocyte relaxation, reducing resting tension as well as left ventricular end diastolic pressure in animal studies conducted in dogs (110, 111). Further improvements have also been reported when this drug is used in combination with βAR blockers (112). Concerning clinical trials, a small sample size study has been conducted in HF patients with preserved ejection fraction, revealing that ranolazine can provide improvement in hemodynamics, but no evidence was provided of improvement in relaxation parameters (113).

Perhexiline is another drug acting on metabolism that was originally thought as an antianginal medication and its usage was declined for several side effects, including hepatotoxicity and neurotoxicity (114). More recently, its toxicity has been found to be preventable with individualized dosing, but its clinical use remains difficult. Its activity as a fatty acid beta-oxidation inhibitor was demonstrated on rat hearts that showed a reduction of fatty acid utilization of 35%, with a concurrent increase in cardiac output of 80 mL/min/g. More specifically, it was demonstrated that perhexiline can inhibit CPT-1, known to control access of long chain fatty acids to the mitochondrial site of beta-oxidation (115). Concerning its clinical use for HF treatment, a small sample size clinical trial has been performed, particularly focused on studying its effect on oxygen consumption. A clear improvement in peak oxygen consumption was found following perhexiline treatment compared to no change in patients treated with a placebo, and improved ejection fraction was also observed, suggesting its possible and effective future employment also for AIC (116).

BEYOND CARDIOMYOCYTES

An important aspect to consider from a therapeutic perspective is that, although the majority of the studies in the field of Cardio-Oncology have focused their attention on the effects of ANTs on cardiomyocytes, these are not the unique cellular population found in the heart. The emerging view is that anticancer compounds also target cardiac fibroblasts and endothelial cells. It has been shown that, both *in vitro* and *in vivo,* DOX affects the differentiation of fibroblasts into myofibroblasts which in turn produce a huge amount of extracellular matrix components, leading to cardiac fibrosis. This process is driven by DOX-dependent ROS that activate TGF-β, the main responsible for fibroblast differentiation (117, 118). Moreover, DOX also modulates the activity of ATM, a kinase which is activated in response to DNA damage induced by oxidative stress. Interestingly, this activation occurs only in cardiac fibroblasts and not in cardiomyocytes, suggesting that this may be a cell-type specific mechanism contributing to AIC (119). How ANTs affect mitochondria in fibroblasts is still unexplored and requires additional work. Instead, more information is available on the role of these organelles in cardiac endothelial cells. Apart from increasing cell permeability and leading to edema formation, DOX can also reduce ATP levels and, in turn, mitochondrial function in these cells (120). Moreover, by means of its interaction with the nitric oxide (NO) synthase, DOX can also interfere with NO production that is essential for endothelial homeostasis (121). However, further studies are needed to further explore the role of these other cardiac cell populations in AIC, hopefully paving the way to the development of new therapeutic options.

FUTURE PERSPECTIVES

Besides the urgent need for new effective therapeutic approaches, another still unresolved issue in the field of Cardio-Oncology is how to predict who is likely to develop cardiotoxicity. Anthracycline dose, patient's age and pre-existent cardiovascular disease only partially explain the interindividual susceptibility to AIC and the prevailing hypothesis is that the sensitivity to anthracyclines has a genetic basis (122). Unveiling the genetic variants that contribute to AIC is of upmost importance since it may give the clinicians the opportunity to identify patients at risk prior the treatment, and potentially modify the therapeutic regimens by using alternative drugs or cardioprotective agents. Early candidate gene association studies (CGAS) and genome-wide association studies (GWAS) have started to reveal the first genes, that are primarily related to drug metabolism and transport, iron metabolism, DNA repair, oxidative stress, and calcium homeostasis, with no genes being directly linked to mitochondrial function regulation (123, 124). However, given the small sample sizes of these studies, additional work is warranted to conclusively validate these variants and to discover new genes implicated in AIC susceptibility. In this scenario, human-induced pluripotent stem cells (hiPSCs) represent an emerging powerful tool since they can be obtained non-invasively from blood samples, can be renewed *in vitro* and are genetically identical to the patients from whom they are derived making them the ideal experimental model for pharmacogenomics research. By exploiting hiPSCs, Knowles et al. recently discovered a number of new genetic variants which also include some genes involved in mitochondrial function regulation (125). In addition, being able to faithfully recapitulate *in vitro* the inter-individual susceptibility to AIC (126), hiPSCs offer the unique opportunity to verify *in vitro*, before the drug is administered to the patient, that the treatment does not cause toxicity, paving the way toward a personalized medicine approach in the field of Cardio-Oncology (123).

CONCLUSIONS

It is now well accepted that mitochondrial dysfunction underlies a broad spectrum of pathologies, ranging from cancer to neurodegenerative and cardiovascular disease. It is not surprising that mitochondria play a key role also in the pathogenesis of AIC, considering the ability of ANTs to bind a phospholipid of the inner mitochondrial membrane, cardiolipin, and thus to accumulate within mitochondria. A number of drugs specifically targeting mitochondrial pathways which are deregulated in pathology as well as a new class of mitochondria-targeted compounds have been developed. While most of them have already been tested in preclinical models of HF, little is still known about their therapeutic potential in the treatment of AIC. Further studies in the appropriate preclinical murine and human models of AIC are awaited to fill this gap.

AUTHOR CONTRIBUTIONS

AM and AG wrote the manuscript in consultation with EH.

FUNDING

This work was supported by a grant from Leducq Foundation (09CVD01 to EH).

REFERENCES

1. European Commission. *ECIS - European Cancer Information System* (2019). Available online at: https://ecis.jrc.ec.europa.eu/ (accessed September 01, 2019).
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* (2016) 66:7–30. doi: 10.3322/caac.21332
3. Zamorano JL, Lancellotti P, Rodriguez Munoz D, Aboyans V, Asteggiano R, Galderisi M, et al. 2016 ESC position paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC committee for practice guidelines: the task force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur Heart J.* (2016) 37:2768–2801. doi: 10.1093/eurheartj/ehw211
4. Waseem M, Parvez S. Mitochondrial dysfunction mediated cisplatin induced toxicity: modulatory role of curcumin.

Food Chem Toxicol. (2013) 53:334–42. doi: 10.1016/j.fct.2012.11.055
5. Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am J Physiol Heart Circ Physiol.* (2015) 309:H1453–67. doi: 10.1152/ajpheart.00554.2015
6. Siasos G, Tsigkou V, Kosmopoulos M, Theodosiadis D, Simantiris S, Tagkou NM, et al. Mitochondria and cardiovascular diseases-from pathophysiology to treatment. *Ann Transl Med.* (2018) 6:256. doi: 10.21037/atm.2018.06.21
7. WHO. *Model Lists of Essential Medicines* (2019). Available online at: https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf (accessed September 01, 2019).
8. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer.* (1973) 32:302–14. doi: 10.1002/1097-0142(197308)32:2<302::AID-CNCR2820320205>3.0.CO;2-2
9. Von Hoff DD, Layard MW, Basa P, Davis HL Jr, Von Hoff AL, Rozencweig M, et al. Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med.* (1979) 91:710–7. doi: 10.7326/0003-4819-91-5-710
10. Buzdar AU, Marcus C, Smith TL, Blumenschein GR. Early and delayed clinical cardiotoxicity of doxorubicin. *Cancer.* (1985) 55:2761–5. doi: 10.1002/1097-0142(19850615)55:12<2761::AID-CNCR2820551206>3.0.CO;2-P
11. Rochette L, Guenancia C, Gudjoncik A, Hachet O, Zeller M, Cottin Y, et al. Anthracyclines/trastuzumab: new aspects of cardiotoxicity and molecular mechanisms. *Trends Pharmacol Sci.* (2015) 36:326–48. doi: 10.1016/j.tips.2015.03.005
12. Cai F, Luis MAF, Lin X, Wang M, Cai L, Cen C, et al. Anthracycline-induced cardiotoxicity in the chemotherapy treatment of breast cancer: preventive strategies and treatment. *Mol Clin Oncol.* (2019) 11:15–23. doi: 10.3892/mco.2019.1854
13. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation.* (2015) 131:1981–8. doi: 10.1161/CIRCULATIONAHA.114.013777
14. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, et al. The cumulative burden of surviving childhood cancer: an initial report from the St Jude Lifetime Cohort Study (SJLIFE). *Lancet.* (2017) 390:2569–582. doi: 10.1016/S0140-6736(17)31610-0
15. Armstrong GT, Ross JD. Late cardiotoxicity in aging adult survivors of childhood cancer. *Prog Pediatr Cardiol.* (2014) 36:19–26. doi: 10.1016/j.ppedcard.2014.09.003
16. Boyd A, Stoodley P, Richards D, Hui R, Harnett P, Vo K, et al. Anthracyclines induce early changes in left ventricular systolic and diastolic function: a single centre study. *PLoS ONE.* (2017) 12:e0175544. doi: 10.1371/journal.pone.0175544
17. Hilfiker-Kleiner D, Ardehali H, Fischmeister R, Burridge P, Hirsch E, Lyon AR. Late onset heart failure after childhood chemotherapy. *Eur Heart J.* (2019) 40:798–800. doi: 10.1093/eurheartj/ehz046
18. Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. *J Am Coll Cardiol.* (2014) 64:938–45. doi: 10.1016/j.jacc.2014.06.1167
19. Armstrong GT, Joshi VM, Ness KK, Marwick TH, Zhang N, Srivastava D, et al. Comprehensive echocardiographic detection of treatment-related cardiac dysfunction in adult survivors of childhood cancer: results from the St. Jude Lifetime Cohort Study. *J Am Coll Cardiol.* (2015) 65:2511–22. doi: 10.1016/j.jacc.2015.04.013
20. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Cohen V, et al. Early detection and prediction of cardiotoxicity in chemotherapy-treated patients. *Am J Cardiol.* (2011) 107:1375–80. doi: 10.1016/j.amjcard.2011.01.006
21. Cardinale D, Sandri MT, Martinoni A, Tricca A, Civelli M, Lamantia G, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. *J Am Coll Cardiol.* (2000) 36:517–22. doi: 10.1016/S0735-1097(00)00748-8
22. Bansal N, Adams MJ, Ganatra S, Colan SD, Aggarwal S, Steiner R, et al. Strategies to prevent anthracycline-induced cardiotoxicity in cancer survivors. *Cardio-Oncol.* (2019) 5:18. doi: 10.1186/s40959-019-0054-5
23. Oliveira PJ, Bjork JA, Santos MS, Leino RL, Froberg MK, Moreno AJ, et al. Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. *Toxicol Appl Pharmacol.* (2004) 200:159–68. doi: 10.1016/j.taap.2004.04.005
24. Spallarossa P, Garibaldi S, Altieri P, Fabbi P, Manca V, Nasti S, et al. Carvedilol prevents doxorubicin-induced free radical release and apoptosis in cardiomyocytes *in vitro*. *J Mol Cell Cardiol.* (2004) 37:837–46. doi: 10.1016/j.yjmcc.2004.05.024
25. Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, et al. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *J Am Coll Cardiol.* (2006) 48:2258–62. doi: 10.1016/j.jacc.2006.07.052
26. Bodley A, Liu LF, Israel M, Seshadri R, Koseki Y, Giuliani FC, et al. DNA topoisomerase II-mediated interaction of doxorubicin and daunorubicin congeners with DNA. *Cancer Res.* (1989) 49:5969–78.
27. Capranico G, Tinelli S, Austin CA, Fisher ML, Zunino F. Different patterns of gene expression of topoisomerase II isoforms in differentiated tissues during murine development. *Biochim Biophys Acta.* (1992) 1132:43–8. doi: 10.1016/0167-4781(92)90050-A
28. Lyu YL, Lin CP, Azarova AM, Cai L, Wang JC, Liu LF. Role of topoisomerase IIbeta in the expression of developmentally regulated genes. *Mol Cell Biol.* (2006) 26:7929–41. doi: 10.1128/MCB.00617-06
29. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med.* (2012) 18:1639–42. doi: 10.1038/nm.2919
30. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science.* (1984) 226:466–8. doi: 10.1126/science.6093249
31. Sarvazyan N. Visualization of doxorubicin-induced oxidative stress in isolated cardiac myocytes. *Am J Physiol.* (1996) 271:H2079–85. doi: 10.1152/ajpheart.1996.271.5.H2079
32. Goormaghtigh E, Huart P, Praet M, Brasseur R, Ruysschaert JM. Structure of the adriamycin-cardiolipin complex. role in mitochondrial toxicity. *Biophys Chem.* (1990) 35:247–57. doi: 10.1016/0301-4622(90)80012-V
33. Davies KJ, Doroshow JH. Redox cycling of anthracyclines by cardiac mitochondria. II. anthracycline radical formation by NADH dehydrogenase. *J Biol Chem.* (1986) 261:3060–7.
34. Chen Y, Jungsuwadee P, Vore M, Butterfield DA, St Clair DK. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol Interv.* (2007) 7:147–56. doi: 10.1124/mi.7.3.6
35. Tokarska-Schlattner M, Zaugg M, Zuppinger C, Wallimann T, Schlattner U. New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics. *J Mol Cell Cardiol.* (2006) 41:389–405. doi: 10.1016/j.yjmcc.2006.06.009
36. Marcillat O, Zhang Y, Davies KJ. Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochem J.* (1989) 259:181–9. doi: 10.1042/bj2590181
37. Fang X, Wang H, Han D, Xie E, Yang X, Wei J, et al. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci USA.* (2019) 116:2672–680. doi: 10.1073/pnas.1821022116
38. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad SV, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest.* (2014) 124:617–30. doi: 10.1172/JCI72931
39. Tokarska-Schlattner M, Wallimann T, Schlattner U. Alterations in myocardial energy metabolism induced by the anti-cancer drug doxorubicin. *C R Biol.* (2006) 329:657–8. doi: 10.1016/j.crvi.2005.08.007
40. Mylonas C, Kouretas D. Lipid peroxidation and tissue damage. *In Vivo.* (1999) 13:295–309.
41. Angsutararux P, Luanpitpong S, Issaragrisil S. Chemotherapy-induced cardiotoxicity: overview of the roles of oxidative stress. *Oxid Med Cell Longev.* (2015) 2015:795602. doi: 10.1155/2015/795602
42. Hanna AD, Lam A, Tham S, Dulhunty AF, Beard NA. Adverse effects of doxorubicin and its metabolic product on cardiac RyR2 and SERCA2A. *Mol Pharmacol.* (2014) 86:438–49. doi: 10.1124/mol.114.093849
43. Dhingra R, Guberman M, Rabinovich-Nikitin I, Gerstein J, Margulets V, Gang H, et al. Impaired NF-κB signalling underlies cyclophilin D-mediated mitochondrial permeability transition pore opening in doxorubicin cardiomyopathy. *Cardiovasc Res.* (2019). doi: 10.1093/cvr/cvz240. [Epub ahead of print].

44. Zhang QL, Yang JJ, Zhang HS. Carvedilol (CAR) combined with carnosic acid (CAA) attenuates doxorubicin-induced cardiotoxicity by suppressing excessive oxidative stress, inflammation, apoptosis and autophagy. *Biomed Pharmacother.* (2019) 109:71–83. doi: 10.1016/j.biopha.2018.07.037
45. Bai P, Canto C, Oudart H, Brunyanszki A, Cen Y, Thomas C, et al. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* (2011) 13:461–8. doi: 10.1016/j.cmet.2011.03.004
46. Abdullah CS, Alam S, Aishwarya R, Miriyala SM, Bhuiyan AN, Panchatcharam M, et al. Doxorubicin-induced cardiomyopathy associated with inhibition of autophagic degradation process and defects in mitochondrial respiration. *Sci Rep.* (2019) 9:2002. doi: 10.1038/s41598-018-37862-3
47. Li M, Russo M, Pirozzi F, Tocchetti CG, Ghigo A. Autophagy and cancer therapy cardiotoxicity: from molecular mechanisms to therapeutic opportunities. *Biochim Biophys Acta Mol Cell Res.* (2019) 1867:118493. doi: 10.1016/j.bbamcr.2019.06.007
48. Li DL, Wang ZV, Ding G, Tan W, Luo X, Criollo A, et al. Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. *Circulation.* (2016) 133:1668–87. doi: 10.1161/CIRCULATIONAHA.115.017443
49. Kawaguchi T, Takemura G, Kanamori H, Takeyama T, Watanabe T, Morishita K, et al. Prior starvation mitigates acute doxorubicin cardiotoxicity through restoration of autophagy in affected cardiomyocytes. *Cardiovasc Res.* (2012) 96:456–65. doi: 10.1093/cvr/cvs282
50. Li M, Sala V, De Santis MC, Cimino J, Cappello P, Pianca N, et al. Phosphoinositide 3-Kinase gamma inhibition protects from anthracycline cardiotoxicity and reduces tumor growth. *Circulation.* (2018) 138:696–711. doi: 10.1161/CIRCULATIONAHA.117.030352
51. Tang H, Tao A, Song J, Liu Q, Wang H, Rui T. Doxorubicin-induced cardiomyocyte apoptosis: role of mitofusin 2. *Int J Biochem Cell Biol.* (2017) 88:55–9. doi: 10.1016/j.biocel.2017.05.006
52. European Commission. *Pharmaceuticals Community Register. Cardioxane Art 13* (2020). Available online at: http://ec.europa.eu/health/documents/community-register/html/ho26321.htm (accessed January 03, 2020).
53. Kwok JC, Richardson DR. The cardioprotective effect of the iron chelator dexrazoxane (ICRF-187) on anthracycline-mediated cardiotoxicity. *Redox Rep.* (2000) 5:317–24. doi: 10.1179/135100000101535898
54. Buss JL, Hasinoff BB. The one-ring open hydrolysis product intermediates of the cardioprotective agent ICRF-187 (dexrazoxane) displace iron from iron-anthracycline complexes. *Agents Actions.* (1993) 40:86–95. doi: 10.1007/BF01976756
55. Lipshultz SE, Cochran TR, Franco VI, Miller TL. Treatment-related cardiotoxicity in survivors of childhood cancer. *Nat Rev Clin Oncol.* (2013) 10:697–710. doi: 10.1038/nrclinonc.2013.195
56. Legha SS, Wang YM, Mackay B, Ewer M, Hortobagyi GN, Benjamin RS, et al. Clinical and pharmacologic investigation of the effects of alpha-tocopherol on adriamycin cardiotoxicity. *Ann N Y Acad Sci.* (1982) 393:411–8. doi: 10.1111/j.1749-6632.1982.tb31279.x
57. Myers C, Bonow R, Palmeri S, Jenkins J, Corden B, Locker G, et al. A randomized controlled trial assessing the prevention of doxorubicin cardiomyopathy by N-acetylcysteine. *Semin Oncol.* (1983) 10:53–5.
58. Classen S, Olland S, Berger JM. Structure of the topoisomerase II ATPase region and its mechanism of inhibition by the chemotherapeutic agent ICRF-187. *Proc Natl Acad Sci USA.* (2003) 100:10629–34. doi: 10.1073/pnas.1832879100
59. Roca J, Ishida R, Berger JM, Andoh T, Wang JC. Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc Natl Acad Sci USA.* (1994) 91:1781–5. doi: 10.1073/pnas.91.5.1781
60. McCormack K. The cardioprotective effect of dexrazoxane (Cardioxane) is consistent with sequestration of poly(ADP-ribose) by self-assembly and not depletion of topoisomerase 2B. *Ecancermedicalscience.* (2018) 12:889. doi: 10.3332/ecancer.2018.889
61. Pacher P, Liaudet L, Mabley JG, Cziraki A, Hasko G, Szabo C. Beneficial effects of a novel ultrapotent poly(ADP-ribose) polymerase inhibitor in murine models of heart failure. *Int J Mol Med.* (2006) 17:369–75. doi: 10.3892/ijmm.17.2.369
62. Ganatra S, Nohria A, Shah S, Groarke JD, Sharma A, Venesy D, et al. Upfront dexrazoxane for the reduction of anthracycline-induced cardiotoxicity in adults with preexisting cardiomyopathy and cancer: a consecutive case series. *Cardio-Oncol.* (2019) 5:1. doi: 10.1186/s40959-019-0036-7
63. Swain SM, Whaley FS, Gerber MC, Ewer MS, Bianchine JR, Gams RA. Delayed administration of dexrazoxane provides cardioprotection for patients with advanced breast cancer treated with doxorubicin-containing therapy. *J Clin Oncol.* (1997) 15:1333–40. doi: 10.1200/JCO.1997.15.4.1333
64. Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, et al. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *J Clin Oncol.* (1997) 15:1318–32. doi: 10.1200/JCO.1997.15.4.1318
65. Speyer JL, Green MD, Zeleniuch-Jacquotte A, Wernz JC, Rey M, Sanger J, et al. ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. *J Clin Oncol.* (1992) 10:117–27. doi: 10.1200/JCO.1992.10.1.117
66. Marty M, Espie M, Llombart A, Monnier A, Rapoport BL, Stahalova V. Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. *Ann Oncol.* (2006) 17:614–22. doi: 10.1093/annonc/mdj134
67. Lipshultz SE, Anderson LM, Miller TL, Gerschenson M, Stevenson KE, Neuberg DS, et al. Impaired mitochondrial function is abrogated by dexrazoxane in doxorubicin-treated childhood acute lymphoblastic leukemia survivors. *Cancer.* (2016) 122:946–53. doi: 10.1002/cncr.29872
68. Reichardt P, Tabone MD, Mora J, Morland B, Jones RL. Risk-benefit of dexrazoxane for preventing anthracycline-related cardiotoxicity: re-evaluating the European labeling. *Future Oncol.* (2018) 14:2663–76. doi: 10.2217/fon-2018-0210
69. Asselin BL, Devidas M, Chen L, Franco VI, Pullen J, Borowitz MJ, et al. Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed T-Cell acute lymphoblastic leukemia or advanced-stage lymphoblastic non-hodgkin lymphoma: a report of the children's oncology group randomized trial pediatric oncology group 9404. *J Clin Oncol.* (2016) 34:854–62. doi: 10.1200/JCO.2015.60.8851
70. Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, et al. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med.* (2004) 351:145–53. doi: 10.1056/NEJMoa035153
71. Seif AE, Walker DM, Li Y, Huang YS, Kavcic M, Torp K, et al. Dexrazoxane exposure and risk of secondary acute myeloid leukemia in pediatric oncology patients. *Pediatr Blood Cancer.* (2015) 62:704–9. doi: 10.1002/pbc.25043
72. Rodriguez-Iturbe B, Sepassi L, Quiroz Y, Ni Z, Wallace DC, Vaziri ND. Association of mitochondrial SOD deficiency with salt-sensitive hypertension and accelerated renal senescence. *J Appl Physiol.* (1985) 102:255–60. doi: 10.1152/japplphysiol.00513.2006
73. Zhan L, Li R, Sun Y, Dou M, Yang W, He S, et al. Effect of mito-TEMPO, a mitochondria-targeted antioxidant, in rats with neuropathic pain. *Neuroreport.* (2018) 29:1275–81. doi: 10.1097/WNR.0000000000001105
74. Rocha VC, Franca LS, de Araujo CF, Ng AM, de Andrade CM, Andrade AC, et al. Protective effects of mito-TEMPO against doxorubicin cardiotoxicity in mice. *Cancer Chemother Pharmacol.* (2016) 77:659–2. doi: 10.1007/s00280-015-2949-7
75. Monti E, Cova D, Guido E, Morelli R, Oliva C. Protective effect of the nitroxide tempol against the cardiotoxicity of adriamycin. *Free Radic Biol Med.* (1996) 21:463–70. doi: 10.1016/0891-5849(96)00124-4
76. Dey S, deMazumder D, Sidor A, Foster DB, O'Rourke B. Mitochondrial ROS drive sudden cardiac death and chronic proteome remodeling in heart failure. *Circ Res.* (2018) 123:356–71. doi: 10.1161/CIRCRESAHA.118.312708
77. Dickey JS, Gonzalez Y, Aryal B, Mog S, Nakamura AJ, Redon CE, et al. Mito-tempol and dexrazoxane exhibit cardioprotective and chemotherapeutic effects through specific protein oxidation and autophagy in a syngeneic breast tumor preclinical model. *PLoS ONE.* (2013) 8:e70575. doi: 10.1371/journal.pone.0070575
78. Szeto HH, Birk AV. Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. *Clin Pharmacol Ther.* (2014) 96:672–83. doi: 10.1038/clpt.2014.174

79. Dai DF, Chen T, Szeto H, Nieves-Cintron M, Kutyavin V, Santana LF, et al. Mitochondrial targeted antioxidant peptide ameliorates hypertensive cardiomyopathy. *J Am Coll Cardiol.* (2011) 58:73–82. doi: 10.1016/j.jacc.2010.12.044
80. Szeto HH, Liu S, Soong Y, Wu D, Darrah SF, Cheng FY, et al. Mitochondria-targeted peptide accelerates ATP recovery and reduces ischemic kidney injury. *J Am Soc Nephrol.* (2011) 22:1041–52. doi: 10.1681/ASN.2010080808
81. Birk AV, Chao WM, Bracken C, Warren JD, Szeto HH. Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. *Br J Pharmacol.* (2014) 171:2017–28. doi: 10.1111/bph.12468
82. Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br J Pharmacol.* (2014) 171:2029–50. doi: 10.1111/bph.12461
83. Cho J, Won K, Wu D, Soong Y, Liu S, Szeto HH, et al. Potent mitochondria-targeted peptides reduce myocardial infarction in rats. *Coron Artery Dis.* (2007) 18:215–20. doi: 10.1097/01.mca.0000236285.71683.b6
84. Dai W, Shi J, Gupta RC, Sabbah HN, Hale SL, Kloner RA. Bendavia, a mitochondria-targeting peptide, improves postinfarction cardiac function, prevents adverse left ventricular remodeling, and restores mitochondria-related gene expression in rats. *J Cardiovasc Pharmacol.* (2014) 64:543–53. doi: 10.1097/FJC.0000000000000155
85. Wu D, Soong Y, Zhao GM, Szeto HH. A highly potent peptide analgesic that protects against ischemia-reperfusion-induced myocardial stunning. *Am J Physiol Heart Circ Physiol.* (2002) 283:H783–91. doi: 10.1152/ajpheart.00193.2002
86. Sabbah HN, Gupta RC, Kohli S, Wang M, Hachem S, Zhang K. Chronic therapy with elamipretide (MTP-131), a novel mitochondria-targeting peptide, improves left ventricular and mitochondrial function in dogs with advanced heart failure. *Circ Heart Fail.* (2016) 9:e002206. doi: 10.1161/CIRCHEARTFAILURE.115.002206
87. Gupta RC, Sing-Gupta V, Sabbah HN. Bendavia (Elamipretide) restores phosphorylation of cardiac myosin binding protein C on serine 282 and improves left ventricular diastolic function in dogs with heart failure. *J Am Coll Cardiol.* (2016) 67:1443. doi: 10.1016/S0735-1097(16)31444-9
88. Daubert MA, Yow E, Dunn G, Marchev S, Barnhart H, Douglas PS, et al. Novel mitochondria-targeting peptide in heart failure treatment: a randomized, placebo-controlled trial of elamipretide. *Circ Heart Fail.* (2017) 10:e004389. doi: 10.1161/CIRCHEARTFAILURE.117.004389
89. Schiattarella GG, Hill JA. Therapeutic targeting of autophagy in cardiovascular disease. *J Mol Cell Cardiol.* (2016) 95:86–93. doi: 10.1016/j.yjmcc.2015.11.019
90. Sciarretta S, Yee D, Nagarajan N, Bianchi F, Saito T, Valenti V, et al. Trehalose-induced activation of autophagy improves cardiac remodeling after myocardial infarction. *J Am Coll Cardiol.* (2018) 71:1999–2010. doi: 10.1016/j.jacc.2018.02.066
91. Sishi BJ, Loos B, van Rooyen J, Engelbrecht AM. Autophagy upregulation promotes survival and attenuates doxorubicin-induced cardiotoxicity. *Biochem Pharmacol.* (2013) 85:124–34. doi: 10.1016/j.bcp.2012.10.005
92. Chude CI, Amaravadi RK. Targeting autophagy in cancer: update on clinical trials and novel inhibitors. *Int J Mol Sci.* (2017) 18:1279. doi: 10.3390/ijms18061279
93. Liu Q, Docherty JC, Rendell JC, Clanachan AS, Lopaschuk GD. High levels of fatty acids delay the recovery of intracellular pH and cardiac efficiency in post-ischemic hearts by inhibiting glucose oxidation. *J Am Coll Cardiol.* (2002) 39:718–25. doi: 10.1016/S0735-1097(01)01803-4
94. Buchanan J, Mazumder PK, Hu P, Chakrabarti G, Roberts MW, Yun UJ, et al. Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. *Endocrinology.* (2005) 146:5341–9. doi: 10.1210/en.2005-0938
95. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev.* (2010) 90:207–58. doi: 10.1152/physrev.00015.2009
96. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev.* (2005) 85:1093–129. doi: 10.1152/physrev.00006.2004
97. Dezsi CA. Trimetazidine in practice: review of the clinical and experimental evidence. *Am J Ther.* (2016) 23:e871–9. doi: 10.1097/MJT.0000000000000180
98. Lopatin YM, Rosano GM, Fragasso G, Lopaschuk GD, Seferovic PM, Gowdak LH, et al. Rationale and benefits of trimetazidine by acting on cardiac metabolism in heart failure. *Int J Cardiol.* (2016) 203:909–15. doi: 10.1016/j.ijcard.2015.11.060
99. Stanley WC, Marzilli M. Metabolic therapy in the treatment of ischaemic heart disease: the pharmacology of trimetazidine. *Fundam Clin Pharmacol.* (2003) 17:133–45. doi: 10.1046/j.1472-8206.2003.00154.x
100. Lionetti V, Linke A, Chandler MP, Young ME, Penn MS, Gupte S, et al. Carnitine palmitoyl transferase-I inhibition prevents ventricular remodeling and delays decompensation in pacing-induced heart failure. *Cardiovasc Res.* (2005) 66:454–61. doi: 10.1016/j.cardiores.2005.02.004
101. Ma Y, Temkin SM, Hawkridge AM, Guo C, Wang W, Wang XY, et al. Fatty acid oxidation: an emerging facet of metabolic transformation in cancer. *Cancer Lett.* (2018) 435:92–100. doi: 10.1016/j.canlet.2018.08.006
102. Tang WH. Metabolic approach in heart failure: rethinking how we translate from theory to clinical practice. *J Am Coll Cardiol.* (2006) 48:999–1000. doi: 10.1016/j.jacc.2006.06.024
103. Zhang L, Lu Y, Jiang H, Sun A, Zou Y, Ge J. Additional use of trimetazidine in patients with chronic heart failure: a meta-analysis. *J Am Coll Cardiol.* (2012) 59:913–22. doi: 10.1016/j.jacc.2011.11.027
104. Steggall A, Mordi IR, Lang CC. Targeting metabolic modulation and mitochondrial dysfunction in the treatment of heart failure. *Diseases.* (2017) 5:14. doi: 10.3390/diseases5020014
105. Zhou X, Chen J. Is treatment with trimetazidine beneficial in patients with chronic heart failure? *PLoS ONE.* (2014) 9:e94660. doi: 10.1371/journal.pone.0094660
106. Gao D, Ning N, Niu X, Hao G, Meng Z. Trimetazidine: a meta-analysis of randomised controlled trials in heart failure. *Heart.* (2011) 97:278–86. doi: 10.1136/hrt.2010.208751
107. Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD. Targeting fatty acid and carbohydrate oxidation–a novel therapeutic intervention in the ischemic and failing heart. *Biochim Biophys Acta.* (2011) 1813:1333–50. doi: 10.1016/j.bbamcr.2011.01.015
108. Morrow DA, Scirica BM, Sabatine MS, de Lemos JA, Murphy SA, Jarolim P, et al. B-type natriuretic peptide and the effect of ranolazine in patients with non-ST-segment elevation acute coronary syndromes: observations from the MERLIN-TIMI 36 (Metabolic efficiency with ranolazine for less ischemia in Non-ST elevation acute coronary-thrombolysis in myocardial infarction 36) trial. *J Am Coll Cardiol.* (2010) 55:1189–96. doi: 10.1016/j.jacc.2009.09.068
109. Hawwa N, Menon V. Ranolazine: clinical applications and therapeutic basis. *Am J Cardiovasc Drugs.* (2013) 13:5–16. doi: 10.1007/s40256-012-0003-2
110. Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN. Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. *J Cardiovasc Electrophysiol.* (2006) 17(Suppl. 1):S169–77. doi: 10.1111/j.1540-8167.2006.00401.x
111. Sabbah HN, Chandler MP, Mishima T, Suzuki G, Chaudhry P, Nass O, et al. Ranolazine, a partial fatty acid oxidation (pFOX) inhibitor, improves left ventricular function in dogs with chronic heart failure. *J Card Fail.* (2002) 8:416–22. doi: 10.1054/jcaf.2002.129232
112. Rastogi S, Sharov VG, Mishra S, Gupta RC, Blackburn B, Belardinelli L, et al. Ranolazine combined with enalapril or metoprolol prevents progressive LV dysfunction and remodeling in dogs with moderate heart failure. *Am J Physiol Heart Circ Physiol.* (2008) 295:H2149–55. doi: 10.1152/ajpheart.00728.2008
113. Maier LS, Layug B, Karwatowska-Prokopczuk E, Belardinelli L, Lee S, Sander J, et al. RAnoLazIne for the treatment of diastolic heart failure in patients with preserved ejection fraction: the RALI-DHF proof-of-concept study. *JACC Heart Fail.* (2013) 1:115–22. doi: 10.1016/j.jchf.2012.12.002
114. Ashrafian H, Horowitz JD, Frenneaux MP. Perhexiline. *Cardiovasc Drug Rev.* (2007) 25:76–97. doi: 10.1111/j.1527-3466.2007.00006.x

115. Kennedy JA, Unger SA, Horowitz JD. Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone. *Biochem Pharmacol.* (1996) 52:273–80. doi: 10.1016/0006-2952(96)00204-3
116. Lee L, Campbell R, Scheuermann-Freestone M, Taylor R, Gunaruwan P, Williams L, et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation.* (2005) 112:3280–8. doi: 10.1161/CIRCULATIONAHA.105.551457
117. Leask A. Getting to the heart of the matter: new insights into cardiac fibrosis. *Circ Res.* (2015) 116:1269–76. doi: 10.1161/CIRCRESAHA.116.305381
118. Cappetta D, Esposito G, Piegari E, Russo R, Ciuffreda LP, Rivellino A, et al. SIRT1 activation attenuates diastolic dysfunction by reducing cardiac fibrosis in a model of anthracycline cardiomyopathy. *Int J Cardiol.* (2016) 205:99–110. doi: 10.1016/j.ijcard.2015.12.008
119. Zhan H, Aizawa K, Sun J, Tomida S, Otsu K, Conway SJ, et al. Ataxia telangiectasia mutated in cardiac fibroblasts regulates doxorubicin-induced cardiotoxicity. *Cardiovasc Res.* (2016) 110:85–95. doi: 10.1093/cvr/cvw032
120. Wolf MB, Baynes JW. The anti-cancer drug, doxorubicin, causes oxidant stress-induced endothelial dysfunction. *Biochim Biophys Acta.* (2006) 1760:267–71. doi: 10.1016/j.bbagen.2005.10.012
121. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology.* (2010) 115:155–62. doi: 10.1159/000265166
122. Magdy T, Burridge PW. The future role of pharmacogenomics in anticancer agent-induced cardiovascular toxicity. *Pharmacogenomics.* (2018) 19:79–82. doi: 10.2217/pgs-2017-0177
123. Pinheiro EA, Fetterman KA, Burridge PW. HiPSCs in cardio-oncology: deciphering the genomics. *Cardiovasc Res.* (2019) 115:935–48. doi: 10.1093/cvr/cvz018
124. Tripaydonis A, Conyers R, Elliott DA. Pediatric anthracycline-induced cardiotoxicity: mechanisms, pharmacogenomics, and pluripotent stem-cell modeling. *Clin Pharmacol Ther.* (2019) 105:614–24. doi: 10.1002/cpt.1311
125. Knowles DA, Burrows CK, Blischak JD, Patterson KM, Serie DJ, Norton N, et al. Determining the genetic basis of anthracycline-cardiotoxicity by molecular response QTL mapping in induced cardiomyocytes. *Elife.* (2018) 7:e33480. doi: 10.7554/eLife.33480
126. Burridge PW, Li YF, Matsa E, Wu H, Ong SG, Sharma A, et al. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med.* (2016) 22:547–56. doi: 10.1038/nm.4087

Conflict of Interest: AG and EH are co-founders and board members of Kither Biotech, a startup biotech focused on the development of PI3K inhibitors.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership