Review Article

FoxO transcription factors in mitochondrial homeostasis

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Mitochondria play essential roles in cellular energetics, biosynthesis, and signaling transduction. Dysfunctional mitochondria have been implicated in different diseases such as obesity, diabetes, cardiovascular disease, nonalcoholic fatty liver disease, neurodegenerative disease, and cancer. Mitochondrial homeostasis is controlled by a triad of mitochondrial biogenesis, dynamics (fusion and fission), and autophagy (mitophagy). Studies have underscored FoxO transcription factors as key mitochondrial regulators. Specifically, FoxOs regulate mitochondrial biogenesis by dampening NRF1-Tfam and c-Myc-Tfam cascades directly, and inhibiting NAD-Sirt1-Pgc1α cascade indirectly by inducing Hmox1 or repressing Fxn and Urod. In addition, FoxOs mediate mitochondrial fusion (via Mfn1 and Mfn2) and fission (via Drp1, Fis1, and MIEF2), during which FoxOs elicit regulatory mechanisms at transcriptional, posttranscriptional (e.g. via miR-484/Fis1), and posttranslational (e.g. via Bnip3-calcineurin mediated Drp1 dephosphorylation) levels. Furthermore, FoxOs control mitochondrial autophagy in the stages of autophagosome formation and maturation (e.g. initiation, nucleation, and elongation), mitochondria connected to and engulfed by autophagosome (e.g. via PINK1 and Bnip3 pathways), and autophagosome-lysosome fusion to form autolysosome for cargo degradation (e.g. via Tfeb and cathepsin proteins). This article provides an up-to-date view of FoxOs regulating mitochondrial homeostasis and discusses the potential of targeting FoxOs for therapeutics.

Introduction

Mitochondrial homeostasis is essential to normal cell and tissue functions. Most known about mitochondria is the primary role in oxidative phosphorylation (OXPHOS) that produces energy molecule (i.e. ATP), underscoring mitochondria as the powerhouse in the cell [1–3]. Mitochondrial metabolism also produces intermediates or metabolites that serve as the chemical building blocks for biosynthesis (e.g. the synthesis of nucleotides, glucose, fatty acids, cholesterol, amino acids, and heme) [1,4]. In addition, mitochondria may release signaling molecules (e.g. reactive oxygen species, cytochrome C, and mitokines) that mediate intracellular and extracellular communications in homeostasis and stress [2,5–10]. As such, mitochondrial defects or dysfunction has been implicated in various human diseases including obesity, diabetes, cardiovascular disease, nonalcoholic fatty liver disease, neurodegenerative disease, and cancer [1,4,11–13].

Mitochondrial homeostasis is maintained primarily via a triad of mitochondrial biogenesis, mitochondrial dynamics (i.e. fusion and fission), and mitochondrial autophagy or mitophagy (i.e. autophagic removal of mitochondria) (Figure 1) [1,6,7,14–18]. Studies have shown that the family of peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1 (Pgc1) interact with energy sensors (e.g. AMPK and Sirt1) among others to switch on mitochondrial biogenesis via mitochondrial transcription factor A (Tfam) [7,12,19]. Mitochondrial network is controlled by dynamic processes of fusion, fission, and remodeling that involve mitofusin 1 (Mfn1), Mfn2, optic atrophy protein 1 (OPA1), dynamin-related protein 1 (Drp1), and mitochondrial fission protein 1 (Fis1) [6,17]. Mitochondrial dynamics not only regulates the morphology of but also facilitates content exchange.
among these organelles (including mitochondrial DNA), thereby keeping mitochondrial integrity in check [17]. Unilateral loss of fusion or fission dysregulates mitochondrial function and mitochondrial signaling pathways that mediate cell pluripotency, division, differentiation, senescence, and apoptosis [6,17]. Augmented fission promotes mitochondrial segregation and mitophagy by producing mitochondrial fragments of appropriate size for autophagosomes to engulf (non-selective mitochondrial autophagy) [6,16,17]. In addition, the dynamics proteins Drp1 and Mfn2 also participate in PINK1–Parkin mediated selective mitochondrial autophagy [6,16,17]. For instance, PINK1-mediated phosphorylation of Mfn2 facilitates Mfn2–parkin interaction, which promotes mitochondrial protein ubiquitination and recruitment of autophagosomes through the adaptor protein LC3 [6,16,20].

The family of forkhead box class O (FoxO) transcription factors include FoxO1, FoxO3, FoxO4, and FoxO6. FoxOs regulate genes that are involved in various pathways such as metabolic regulation, cell and tissue homeostasis, and immunity [21–25]. FoxO activities are controlled by a nuclear localization signal (NLS) domain, a nuclear export sequence (NES) domain, a DNA-binding (i.e. forkhead box) domain (DBD), and a C-terminal transactivation domain [21,24,26]. Emerging evidence suggests that FoxOs may localize to mitochondria and bind to mitochondrial DNA, and further studies are needed to define the role of FoxO in regulating mitochondrial genes [27,28]. Regardless, FoxO transcription factors regulate the expression of nuclear genes that mediate mitochondrial biogenesis, dynamics, and mitophagy, underscoring FoxOs as the key regulators of mitochondrial homeostasis [29–40]. This article discusses the mechanisms or pathways by which FoxOs control mitochondrial homeostasis.

**FoxO transcription factors in mitochondrial biogenesis**

FoxO proteins undergo posttranslational modifications (e.g. phosphorylation and acetylation) in response to external stimuli such as stress or altered nutrient or cellular signaling [24,26,41]. For instance, insulin signaling may silence FoxOs via protein kinase B (or Akt)-mediated phosphorylation, which controls glucose production in the liver and protein homeostasis in skeletal muscle [41–43]. Obese or diabetic individuals who are insulin resistant show metabolic derangements and mitochondrial dysfunction [44–47]. While it is under debate.
whether mitochondrial deficiency or dysfunction leads to insulin resistance [48], studies have shown that insulin sensitivity is essential to mitochondrial homeostasis by finely tuning FoxO activity [3,36,44,46,49,50].

Mitochondrial biogenesis requires Pgc1α, a transcription coactivator that can be activated by the NAD dependent deacetylase Sirt1, to trigger the cascade of NRF1-Tfam [7,12,19,29]. In line with the notion that insulin promotes mitochondrial biogenesis [46], insulin resistance activates FoxO1 and reduces mitochondrial content or compromises mitochondrial integrity [29,49,50]. Mitochondrial OXPHOS relies on a series of redox reactions (e.g. the oxidation of NADH into NAD) through respiratory chain complexes I-IV that build up an electrochemical gradient (i.e. mitochondrial membrane potential) to drive ATP production through complex V (ATP synthase) [29,51,52]. Activation of FoxO1 in the liver up-regulates heme oxygenase 1 (Hmox1), which is located in inner mitochondrial membrane and catabolizes mitochondrial heme [29,53], the essential cofactors for redox enzymes on the electron transport chain (ETC), thereby compromising the integrity and function of ETC (Figure 2A) [29,54]. Although the subcellular location of biliverdin reductase is arguable and under investigation [53,55,56], there is evidence showing that biliverdin reductase may partner with Hmox1 in inner mitochondrial membrane to facilitate heme breakdown (by Hmox1) into biliverdin and then into bilirubin (by biliverdin reductase), thereby interfering with ETC and mitochondrial respiration [53,56]. The ETC deficiency results in a lower NAD/NADH ratio and dampens the NAD-dependent deacetylase Sirt1. As a result, Pgc1α is

Figure 2. FoxO transcription factors regulate mitochondrial biogenesis.
(A) In the liver, FoxO1 may induce Hmox1, Fxn, and Urod, which disrupt mitochondrial ETC and NAD/NADH ratio, thereby suppressing NAD-dependent Sirt1-Pgc1α-NRF1-Tfam pathway in mitochondrial biogenesis. Glucagon activated FoxO1 represses NRF1 and accounts for reduced mitochondrial biogenesis in the liver. (B) In contrast with the liver, FoxO1 induces Cyb5r3 and maintains ETC activity and NAD/NADH ratio in the pancreas. It is unclear but of interest whether the FoxO1-Cyb5r3 axis regulates mitochondrial biogenesis via the known NAD-dependent Sirt1-Pgc1α-NRF1-Tfam pathway (indicated by question marks). (C) In the heart, FoxO1 activation due to diabetes causes mitochondrial abnormality by dysregulating PDK4 and CPT1 via a to-be-defined mechanism (indicated by question marks). (D) In cancer cells, FoxO3 suppresses mitochondrial biogenesis and function by inhibiting c-Myc/Tfam signaling cascade.
deactivated by high level of acetylation, which inhibits the NRF1-Tfam cascade and reduces mitochondrial biogenesis [29] (Figure 2A). In contrast, overexpression of a constitutively active Pgc1α (i.e. R13-Pgc1α that contains 13 lysine-to-arginine substitutions to mimic Pgc1α activation by deacetylation) restores mitochondrial content, suggesting that deactivation of Sirt1-Pgc1α cascade accounts for FoxO1 induced suppression of mitochondrial biogenesis [29]. Suppression of Pgc1α and mitochondrial biogenesis by FoxO1 was also observed in renal tubular epithelial cells, where FoxO1 keeps CREB from forming CREB-CBP-P300 complex, thereby down-regulating Ppargc1 (the gene encoding Pgc1α) [40]. Recent studies show that FoxO1 down-regulates NRF1-Tfam and suppresses mitochondrial biogenesis, which may account for glucagon-mediated mitochondrial alteration [50]. In addition, glucagon induces ETC deficiency through FoxO1-dependent down-regulation of Fxn and UroD, the genes involved in heme biosynthesis (Figure 2A) [50]. Interestingly, glucagon stimulates fatty acid oxidation (FAO) regardless of ETC defects in hepatocytes [50,57], and long-term exposure to high glucagon level can impair fatty acid oxidation activity [50]. The increased FAO by glucagon is attributed to inositol triphosphate receptor 1 (INSP3R1) [57]. A glutamine-dependent reductive carboxylation pathway may account for sustained FAO during ETC impairment [58], but it warrants further studies to determine whether such a mechanism underlies glucagon-induced FAO and ETC defects.

In line with the role of FoxO1–Hmox1 axis in dysregulating mitochondrial and metabolic homeostasis, Hmox1 has been associated with inflammation and insulin resistance in mouse and man [59]. Hmox1 may also contribute to hyperglycemia through catabolism of heme and release of excess free ferrous in hepatocytes, which activates FoxO1 via NF-kB mediated phosphorylation at Ser273(FoxO1) and induces gluconeogenic gene in mice [60]. The Hmox1 → Fe2+ → NF-kB → FoxO1 cascade might serve as a adaptive mechanism of selective clearance of dysfunctional mitochondria in the liver given the essential role of FoxO1 in mitochondrial autophagy (discussed in detail below). In adipose tissue and human adipocytes, Hmox1 is associated with iron excess-induced dysfunction and impaired glucose uptake and respiratory capacity [61]. Of note, induction of Hmox1 in specific immune cells may exert protective function via antioxidant and anti-inflammatory reactions, underlining cell type- or tissue-dependent roles of FoxO1 or Hmox1 [26,54]. To this end, activation of FoxO1 in pancreas was shown to promote pancreatic β-cells function and insulin secretion [62,63]. In β-cells FoxO1 can directly bind to the promoter of Cyb5r3 and transactivates the gene to encode mitochondrial membrane-bound cytochrome b5 reductase 3, the enzyme that mediates mitochondrial electron transport (Figure 2B) [63]. Ablation of FoxO1 or Cyb5r3 dysregulates mitochondrial function and NAD/NADH ratio and causes secretory granule abnormalities [63]. Nevertheless, it is unclear whether ablation of FoxO1 or Cyb5r3 impairs mitochondrial biogenesis via the known NAD-dependent Sirt1-Pgc1α pathways. In diabetic cardiomyocyte, FoxO1 induced mitochondrial alteration is associated with elevation of PDK4 and CPT1, shifting substrate from glucose to fatty acid and causing cardiac dysfunction (Figure 2C) [49]. Suppression of FoxO1 activity with a selective inhibitor (AS1842856) ameliorates mitochondrial and cardiac abnormality [49]. Of note, mitochondrial biogenesis in skeletal muscle or myoblasts may undergo a Pgc1α independent pathway to exercise or high flux of oxidative substrates (e.g. pyruvate) [64,65]. Although the Pgc1α independent pathway remains to be defined, it is of interest for future studies to determine whether and how FoxOs regulate mitochondrial biogenesis in skeletal muscle.

As another member of the FoxO family, FoxO3 plays an inhibitory role in mitochondrial biogenesis like FoxO1 [38,50]. Activation of FoxO3 results in down-regulation of mitochondrial DNA copy number, lower expression of mitochondrial proteins and mitochondrial respiratory activity in cancer cells [38]. In addition, FoxO3 induces PDK4 and reduces mitochondrial oxygen consumption rates as observed for FoxO1 [38,49]. Intriguingly, FoxO3 induced suppression of mitochondrial biogenesis appears to be independent from Pgc1 family and NRF1; instead, it depends on the inhibition of c-Myc, a transcription factor that regulates nuclear encoded mitochondrial genes by directly binding to the promoter of Tfam (Figure 2D) [38]. Overall, FoxO1 and FoxO3 appear to serve as a suppressor of mitochondrial biogenesis. In pancreas, FoxO1 was found to up-regulate mitochondrial protein and maintain mitochondrial function, and the role in mitochondrial biogenesis remains to be defined. The role of FoxO4 and FoxO6 in mitochondrial regulation is largely unexplored. During oxidative stress FoxO4 binds to the promoter of SOD2 gene and induces expression of manganese superoxide dismutase, an antioxidant enzyme located within the mitochondrial matrix [66]. FoxO4 may also interact with p53 to induce apoptosis that involves mitochondria and caspase-dependent pathway [67,68]. FoxO6 activation was associated with redox homeostasis in kidney tissues from calorie restriction rats [69]. However, in colorectal cancer cells FoxO6 seems to increase glycolysis and suppresses mitochondrial respiration, and the regulatory mechanism remains to be defined [70]. Further studies of FoxO4 and
FoxO6 in this respect are desirable and critical to paint a whole picture of FoxO transcription factors in mitochondrial regulation.

**FoxO transcription factors in mitochondrial dynamics**

Mitochondria undergo constant fusion and fission, and the balance of these dynamic processes is essential to mitochondrial hemostasis. Mitochondrial fusion is controlled by Mfn1, Mfn2 and Opa1 while mitochondrial fission is controlled by Drp1 and Fis1 among other regulators [6,17]. In overnutrition conditions (e.g. obesity), activation of FoxO1 leads to deformed mitochondria in the liver of insulin resistant mice [29,71] or glucagon treated mice [50], which is associated with dysregulated fusion (e.g. up-regulation of Mfn1 and Mfn2) and fission (e.g. down-regulation of Drp1 and Fis1) proteins (Figure 3A). Lower ATP production is reported for the deformed mitochondria compared with normal mitochondria [29]. Ablation of FoxO1 normalizes mitochondrial morphology and ATP production [29], suggesting that FoxO1 plays a central role in mitochondrial dynamics [29,50,71]. Interestingly, undernutrition conditions (e.g. nutrient depletion or starvation) activate...
cAMP-PKA pathway that leads to inhibitory phosphorylation of Drp1 and mitochondrial elongation, which serves as an important mechanism to sustain cell viability by preventing mitochondria from autophagic degradation and maintaining mitochondrial ATP production [72]. Given FoxO1 is also activated during fasting state [42], it will be of interest to investigate whether FoxO1 participates in undernutrition induced changes in mitochondrial dynamics. In addition, studies of mice lacking estrogen receptor α (ERα) in brown adipose tissue revealed a role of mtDNA polymerase γ (Polg1) in increased mitochondrial fission via Drp1 [73]. Because ERα is a potent inhibitor of FoxO1 via Akt-mediated phosphorylation [74,75], future studies are warranted to examine whether FoxO1 mediates ERα regulation of mitochondrial dynamics (Figure 3B).

FoxO3 is implicated in the regulation of mitochondrial dynamics, and the role appear to be multifaceted. In cardiomyocytes, FoxO3 inhibits mitochondrial fission by transactivating microRNA-484 (miR-484) expression [76]. FoxO3 induced miR-484 binds to the amino acid coding sequence of Fis1 mRNA and suppresses Fis1 protein expression and mitochondrial fission, which attenuates apoptosis and myocardial infarction in mice (Figure 3C) [76]. The cardioprotective effect is also associated with FoxO3 repressing mitochondrial dynamics protein of 49kDa (MiD49 or MIEF2) by directly binding to the promoter of MIEF2 gene (Figure 3C) [35]. MIEF2 protein facilitates the recruitment of Drp1 to mitochondrial membrane, where Drp1 is polymerized and rings at constriction sites to promote mitochondrial fission [77]. Overexpression of FoxO3 in cardiomyocytes suppresses mitochondrial fission and apoptosis, protecting against chemotherapeutic drug doxorubicin-induced cardiotoxicity in mice [35]. Interestingly, FoxO3 was also shown to promote mitochondrial fission, apoptosis, and cardiac stress or heart failure by up-regulating BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3) in rats [37]. Mechanistically, FoxO3 induced Bnip3 dysregulates calcium in the cytosolic and mitochondrial compartments. The increase in cytosolic calcium activates calcineurin, a phosphatase that activates Drp1 via dephosphorylation at Ser637(Drp1), thereby promoting Drp1-mediated mitochondrial fragmentation (Figure 3C) [37]. This discrepancy may arise from the different chemicals and resultant models of cardiotoxicity, i.e. phenylephrine-stressed adult cardiomyocytes versus doxorubicin-induced cardiotoxicity [35,37].

FoxO-regulation of mitochondrial dynamics plays a key role in stem cell proliferation and differentiation [32,78]. In line with FoxO dampening mitochondrial fission by transactivating miR-484 to silence Fis1 [76], ablation of FoxO1 and FoxO3 in mouse Lgr5+ intestinal stem cell or crypt-based columnar cells (CBC) promotes mitochondrial fission (Figure 3D) [32]. FoxO deficient CBC have lower mitochondrial respiration rates and undergo a metabolic transition from OXPHOS to glycolysis, which drives the differentiation of CBC into secretory Paneth cells and goblet cells [32,79]. Inhibition of mitochondrial fission by targeting Drp1 prevents the increase in secretory cell numbers [32]. Interestingly, the proliferation and differentiation of intestinal stem cells (ISC) in fruit flies requires a metabolic transition from glycolysis to OXPHOS [78]. Disruption of ETC complexes leads to up-regulated FoxO, which blocks the ISC commitment to enteroblast (EB), EB-to-absorptive enterocyte specification, and EB-to-secretory enteroendocrine cell specification [78]. The discrepancy may arise from cell type (e.g. Lgr5+ vs. Lgr5-intestinal stem cell) or species (e.g. mouse vs. fruit flies) dependent differences.

Taken together, activation of FoxOs may induce transcriptional, posttranscriptional (e.g. miR-484), and post-translational (e.g. Drp1 dephosphorylation) changes that dysregulate mitochondrial fusion and fission. FoxOs seem to play multifaceted roles in mitochondrial fission depending on experimental models or species, and further studies are warranted to identify the underlying determinants of the multifaceted roles.

**FoxO transcription factors in mitochondrial autophagy**

Selective mitochondrial clearance by autophagy may undergo receptor (e.g. Bnip3, NIX, and FUNDC1) and adaptor (e.g. NBR1 and p62/SQSTM1) dependent pathways, which facilitate mitochondria being connected to and engulfed by autophagosome (Figure 4A) [15,16,80,81]. Receptor proteins contain a COOH-terminal transmembrane domain that connects with mitochondrial membrane and an NH2-terminal LC3-interacting region (LIR) motif that binds to lipidated LC3 and facilitates connecting mitochondria to autophagosome membrane [16]. Like receptor proteins, adaptor proteins contain an LIR motif. However, a transmembrane domain is absent from adaptor proteins; instead, a ubiquitin binding domain (UBD) is present to facilitate the connection to mitochondria through the binding to polyubiquitinated proteins located on mitochondrial outer membrane [15,16]. The ubiquitin-dependent mitophagy requires PTEN induced kinase 1 (PINK1), which is accumulated during stress conditions and recruits of Parkin to mitochondria to initiate ubiquitination of mitochondrial proteins. Parkin also participates in cargo sorting, budding of mitochondrial-derived vesicles, and matrix delivery to lysosomes for degradation [82,83]. Regardless of the differences discussed above, studies have revealed cross-
talk existing between receptor-mediated pathways and adaptor-mediated ubiquitin-dependent pathways [15,81]. For instance, the mitophagy receptor protein Bnip3 interacts with PINK1 to promote PINK1 accumulation on the mitochondrial outer membrane, triggering the PINK1–Parkin mediated ubiquitin-dependent pathways [84,85]. On the other hand, Parkin induced ubiquitination of mitophagy receptor protein NIX promotes the recruitment of adaptor protein NBR1, initiating ubiquitin dependent pathway to mitochondrial clearance [86].

FoxO transcription factors regulate an array of genes involved in autophagic regulation [23,24,87]. In the process of selective mitochondrial autophagy, FoxO proteins regulate both adaptor-mediated ubiquitin-dependent pathways and receptor-mediated pathways (Figure 4A). In mouse podocyte cells, FoxO1 induces PINK1 by directly binding to the promoter of PINK1 gene and stimulates PINK/Parkin dependent mitophagy, which protects against podocyte injury and ameliorates diabetic nephropathy progression [36]. In white adipocytes, ERα signaling induces a browning phenotype by deactivating PINK1/Parkin pathways [73], presumably because ERα suppresses FoxO1 by activating Akt [74,88]. Indeed, FoxO1 occupancy on PINK1 promoter is dampened by insulin sensitization that enhances Akt-mediated inhibition of FoxO1, whereas overexpression of constitutively active FoxO1 promotes PINK1-dependent mitophagy [89]. Conditional deletion of FoxO1 and FoxO3 in cardiomyocytes down-regulates PINK1 and significantly increases the infarct area in mice subjected to myocardial infarction (MI) or acute ischemia/reperfusion (I/R) injury [90]. Interestingly, inhibition of FoxO1 prevents renal I/R injury in mice [40]. In dopamine neurons, manganese increases FoxO3 nuclear retention and activates PINK1/Parkin cascade, which is associated with reduced cell viability [91]. Mechanistically, FoxO3 stimulates mitophagy by transactivating the expression of PINK1 gene (Figure 4A) [92]. Given manganese induced neurotoxicity accounts for the loss of dopamine neurons in Parkinson’s disease (PD), future study of the FoxO3-mitophagy pathways may lead to new therapeutic options for PD [33,91].

In receptor dependent mitophagy, FoxO transcription factors control the expression Bnip3 and Bnip3L (Figure 4A) [33,37,39]. FoxO3 expression is elevated in heart failure, concurrent with up-regulation of Bnip3, mitophagy, and apoptosis in cardiomyocytes [39]. Knockdown or overexpression of FoxO3 in cardiomyocytes leads to down- or up-regulation of Bnip3, respectively [37,39]. Chromatin immunoprecipitation (ChIP) sequencing analysis suggests that FoxO3 directly binds to the promoters of Bnip3 and Bnip3L among other genes [33]. In adult neural stem and progenitor cells, ablation of FoxO3 reduces Bnip3 and Bnip3L expression and mitochondrial turnover but increases aggregate levels [33]. FoxO1 induction of Bnip3 was also observed in neurons lacking JNK [93] and in skeletal muscle [94]. Overexpression of Sirt1 deactivates FoxO1 and FoxO3 through deacetylation, thereby suppressing Bnip3 [94].

With the assistance of adaptor or receptor proteins, mitochondria are connected with and engulfed by autophagosomes (Figure 4A,B), which in turn fuse with lysosome to form autolysosomes for mitochondrial degradation (Figure 4C) [16]. FoxOs regulate not only adaptor- and receptor-dependent engulfing of mitochondria (as discussed above) but also gene expression that are involved in autophagosome [23,24] and lysosome regulation [31,95]. Specifically, FoxOs regulate genes involved in the stages of initiation (e.g. Ulk1 and Ulk2),
nucleation (e.g. Becn1, Atg14, and Pi3kIII), elongation (e.g. Map1lc3b, Gabarapl, Atg4, Atg5, and Atg12), and fusion (e.g. Tfeb and Rab7) (Figure 4A), which has been discussed in recent reviews [23,24]. As a target of FoxOs, Tfeb regulates autophagosomal and lysosomal genes as well as the fusion of autophagosome with lysosome [96–98]. Tfeb activity is controlled by posttranslational modification, such as mTORC1 mediated phosphorylation that excludes Tfeb from the nucleus [99–101]. At transcriptional level, Tfeb gene was transactivated by FoxO1, which might account for mitophagy regulation and white-beige adipose tissue conversion [95]. FoxO1 induces Tfeb by directly binding to the promoter of Tfeb gene [95], and inhibition of FoxO1 down-regulates Tfeb and its target genes (e.g. CTSB, CTSD, CTSH, and CTSS) (Figure 4C) [31]. In aged T cells, FoxO1 deficiency increases cell mass and secretion of cytoprotective exosomes due to impairment of TFEB-mediated lysosomal activity and proteostasis [31].

Together, FoxOs induce mitophagy in three major aspects, (i) expression of autophagosome machinery proteins, (ii) expression of adaptor and receptor proteins that facilitate mitochondria connected to and engulfed by autophagosome, and (iii) expression of lysosome proteins essential to autolysosome formation and cargo degradation.

Conclusions
Mitochondrial quality is controlled through a triad of biogenesis, dynamics, and mitophagy, which underpins metabolic health and tissue homeostasis. Accumulated evidence has underscored FoxO transcription factors as the key regulators of mitochondrial homeostasis. FoxO activation may suppress mitochondrial biogenesis, dysregulate mitochondrial fusion and fission, and induces mitophagy through adaptor- and receptor-dependent pathways. Dysregulation of FoxOs is associated with mitochondrial alterations and metabolic derangements, and pharmacological modulation of FoxO activity has been one of the top candidates for drug discovery [21]. Regardless, caution should be exercised with the following complexity in order to develop effective therapeutics in the future: first, FoxOs may regulate mitochondria in a cell type- and tissue-dependent manner. For instance, inactivation of FoxO1 improves mitochondrial homeostasis in the liver [29,50,71] and kidney [40] but the opposite was observed in the pancreatic β-cells [62,63]. Likewise, ablation of FoxO in cardiomyocytes dampens PINK1/Parkin dependent mitophagy and increases cardiomyocyte injury [90], while inhibition of FoxO1 prevents renal I/R injury in mice [40]. Secondly, the interplays among mitochondrial biogenesis, dynamics, and mitophagy may complicate the outcome of FoxO modulation. In addition to mediating mitochondrial dynamics, Mfn2 and Drp1 also regulate mitophagy by interacting with PINK1, Parkin, and Bnip3 in cardiomyocytes and dopamine neurons [20,37,102–104]. Moreover, Mfn2 also regulates Pgc1α-mediated mitochondrial adaptation in response to increased energy demand in skeletal muscle and brown adipose tissue [105]. As such, targeting FoxOs for mitochondrial biogenesis (e.g. via Pgc1α cascade) could impose undesired effects on mitochondrial dynamics and mitophagy, or vice versa. Future studies designed to precisely target FoxOs and mitochondrial alterations are critical for the development of effective therapeutics, such as selective organ targeting approaches and nanotechnology [106,107].

Competing Interests
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Abbreviations
Akt or PKB, protein kinase B; AMPK, AMP-activated protein kinase; Atg12, autophagy related 12; Atg14, autophagy related 14; Atg4, autophagy related 4; Atg5, autophagy related 5; ATP, adenosine triphosphate; Becn1, Beclin 1; Bnip3, BCL2 interacting protein 3; Bnip3L, BCL2 interacting protein 3 like; CBC, crypt-based columnar cell; ChIP, chromatin immunoprecipitation; CPT1, carnitine palmitoyltransferase 1; CREB, cAMP

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response element-binding protein; CTSB, cathepsin B; CTSD, cathepsin D; CTSK, cathepsin H; CTSS, cathepsin S; Cyb5r3, cytochrome b5 reductase 3; DBD, DNA-binding domain; Drp1, dynamin-related protein 1; EB, enteroblast; ERα, estrogen receptor α; ETC, electron transport chain; FAO, fatty acid oxidation; Fis1, fission protein 1; FoxO, forkhead box class O; FUNDC1, FUN14 Domain Containing 1; Fxn, frataxin; Hmox1, heme oxygenase 1; I/R, ischemia/reperfusion; ISC, intestinal stem cell; LC3 or Map1lc3, microtubule-associated protein 1A/1B-light chain 3; LIR, LC3-interacting region; Mfn1, mitofusin 1; Mfn2, mitofusin 2; Mi, myocardial infarction; MIEF2 or MiD49, mitochondrial dynamics protein of 49 kDa; miR-484, microRNA-484; NBR1, neighbor of BRCA1 gene 1; NES, nuclear export sequence; NF-kB, nuclear factor-kB; NIX, Nip3-like protein X; NLS, nuclear localization signal; NRF1, nuclear respiratory factor 1; OPA1, optic atrophy protein 1; OXPHOS, oxidative phosphorylation; P53, tumor protein p53; PD, Parkinson’s disease; PDK4, pyruvate dehydrogenase kinase 4; Pgc1, peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1; Pi3k, the class III phosphatidylinositol 3-kinase; PINK1, PTEN induced kinase 1; Polg1, mitochondrial DNA polymerase γ; PGC1α, PPAR-γ coactivator 1; Pi3kIII, the class III phosphatidylinositol 3-kinase; PINK1, PTEN induced kinase 1; Polg1, mitochondrial DNA polymerase γ; SOD2, manganese superoxide dismutase; Tfam, mitochondrial transcription factor A; TFEB, transcription factor EB; UBD, ubiquitin binding domain; Ulk1, Unc-51 like autophagy activating kinase 1; Ulk2, Unc-51 like autophagy activating kinase 2; Uroq, uroporphyrinogen decarboxylase.

References

1 Suomalainen, A. and Battersby, B.J. (2018) Mitochondrial diseases: the contribution of organelle stress responses to pathology. Nat. Rev. Mol. Cell Biol. 19, 77–82 https://doi.org/10.1038/nrm.2017.66
2 McBride, H.M., Neuspiel, M. and Wasiak, S. (2006) Mitochondria: more than just a powerhouse. Curr. Biol. 16, R551–R560 https://doi.org/10.1016/j.cub.2006.06.054
3 Cheng, Z., Tseng, Y. and White, M.F. (2010) Insulin signaling meets mitochondria in metabolism. Trends Endocrinol. Metab. 21, 589–598 https://doi.org/10.1016/j.tem.2010.06.005
4 Spinelli, J.B. and Haigis, M.C. (2018) The multifaceted contributions of mitochondria to cellular metabolism. Cell Stem Cell 28, 394–408 https://doi.org/10.1016/j.stem.2018.02.011
5 Cheng, Z. and Ristow, M. (2013) Mitochondria and metabolic homeostasis. Antioxid. Redox Signal. 19, 240–242 https://doi.org/10.1089/ars.2013.5255
6 Giacomelli, M., Pyakaré, A., Glytsou, C. and Scorrano, L. (2020) The cell biology of mitochondrial membrane dynamics. Nat. Rev. Mol. Cell Biol. 21, 204–224 https://doi.org/10.1038/s41580-020-0210-7
7 Quiros, P.M., Mottis, A. and Auwerx, J. (2016) Mitonuclear communication in homeostasis and stress. Nat. Rev. Mol. Cell Biol. 17, 213–226 https://doi.org/10.1038/nrm.2016.23
8 Chandelier, N.S. (2015) Evolution of mitochondria as signaling organelles. Cell Metab. 22, 204–206 https://doi.org/10.1016/j.cmet.2015.05.013
9 Chandelier, N.S. (2014) Mitochondria as signaling organelles. BMC Biol. 12, 34 https://doi.org/10.1186/1741-7007-12-34
10 Chakrabarty, R.P. and Chandelier, N.S. (2021) Mitochondria as signaling organelles control mammalian stem cell fate. Cell Stem Cell 28, 394–408 https://doi.org/10.1016/j.stem.2021.02.011
11 Cheng, Z., Zheng, L. and Almeida, F.A. (2018) Epigenetic reprogramming in metabolic disorders: nutritional factors and beyond. J. Nutr. Biochem. 54, 1–10 https://doi.org/10.1016/j.jnutbio.2017.10.004
12 Cheng, Z., Schmelz, E.M., Liu, D. and Huber, M.W. (2014) Targeting mitochondrial alterations to prevent type 2 diabetes—Evidence from studies of dietary redox-active compounds. Mol. Nutr. Food Res. 58, 1739–1749 https://doi.org/10.1002/mnr.21030747
13 Cheng, Z. and Almeida, F.A. (2014) Mitochondrial alteration in type 2 diabetes and obesity: an epigenetic link. Cell Cycle 13, 890–897 https://doi.org/10.1089/cc.2014.28189
14 Ploumi, C., Daskalaki, I. and Tavernarakis, N. (2017) Mitochondrial biogenesis and clearance: a balancing act. FEBS J. 284, 183–195 https://doi.org/10.1111/febs.13820
15 Palkaras, K., Lionaki, E. and Tavernarakis, N. (2018) Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat. Cell Biol. 20, 1013–1022 https://doi.org/10.1038/s41566-018-0176-2
16 Gustafsson, A.B. and Dorn, G.W. (2019) Evolving and expanding the roles of mitophagy as a homeostatic and pathogenic process. Physiol. Rev. 99, 853–892 https://doi.org/10.1152/physrev.00005.2018
17 Chan, D.C. (2020) Mitochondrial dynamics and its involvement in disease. Annu. Rev. Pathol. 15, 235–259 https://doi.org/10.1146/annurev-pathmedchds-012419-032711
18 Romanello, V. and Sandri, M. (2021) The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass. Cell. Mol. Life Sci. 78, 1305–1328 https://doi.org/10.1007/s00018-020-03662-0
19 Piccinini, E., Villani, G. and Moschetta, A. (2019) Metabolic aspects in NAFLD, NASH and hepatocellular carcinoma: the role of PGC1 activators. Nat. Rev. Gastroenterol. Hepatol. 16, 160–174 https://doi.org/10.1038/s41575-018-0089-3
20 Chen, Y. and Dorn, G.W. (2013) PINK1-Phosphorylated mitofusin 2 is a parkin receptor for culling damaged mitochondria. Science 340, 471–475 https://doi.org/10.1126/science.1231031
21 Calisli, G., Lam, E.W. and Link, W. (2021) Therapeutic strategies targeting FOXO transcription factors. Nat. Rev. Drug Discov. 20, 21–38 https://doi.org/10.1038/s41573-020-0088-2
22 Martins, R., Ligthow, G.J. and Link, W. (2016) Long live FOXO: unravelling the role of FOXO proteins in aging and longevity. Aging Cell 15, 196–207 https://doi.org/10.1111/ace.12427
23 Webb, A.E. and Brunet, A. (2014) FOXO transcription factors: key regulators of cellular quality control. Trends Biochem. Sci. 39, 159–169 https://doi.org/10.1016/j.tibs.2014.02.003
Lettelier-Barbato, D., Ioannilli, L., Aquilano, K., Ciccarone, F., Dunleavy, M. and Ciriolo, M.R. (2019) Foxo1 localizes to mitochondria of adipose tissue and is affected by nutrient stress. *Metabolism* **95**, 64–92. https://doi.org/10.1016/j.metabol.2019.04.006

Cheng, Z., Guo, S., Coppes, K., Dong, X., Kollipara, R., Rodrigs, J.T. et al. (2009) Foxo1 integrates insulin signaling with mitochondrial function in the liver. *Nat. Med.* **15**, 1307–1311. https://doi.org/10.1038/nm.2049

Ludhikuze, M.C. and Colman, M.J.R. (2021) Metabolic regulation of stem cell differentiation: a Forkhead Box O transcription factor perspective. *Antioxid. Redox Signal.* **34**, 1004–1012. https://doi.org/10.1089/ars.ab1808

Jin, J., Li, X.Y., Hu, B., Kim, C., Cao, W.Q., Zhang, H.M. et al. (2020) FoxO1 deficiency impairs proteostasis in aged T cells. *Sci. Adv.* **6**, eaab1808. https://doi.org/10.1126/sciadv.ab1808

Chen, Z. (2019) Foxo1: mute for a tuned metabolism? *Sci. Transl. Med.* **11**(459), 19–23. https://doi.org/10.1126/scitranslmed.aat2158

Neill, B.T. and Nair, K.S. (2017) Insulin regulation of proteostasis and clinical implications. *Cell Metab.* **26**, 424–436. https://doi.org/10.1016/j.cmet.2017.05.017

Ruegsegger, G.N., Vanderboom, P.M., Dasari, S., Klaus, K.A., Kabiraj, P., McCarthy, C.B. et al. (2019) Exercise and metformin counteract altered insulin-deficient states. *J. Clin. Invest.* **129**, 3671–3681. https://doi.org/10.1172/jci120943

Zheng, L.D., Linarelli, L.E., Liu, L., Wall, S.S., Greenawald, M.H., Seidel, R.W. et al. (2015) Insulin resistance is associated with epigenetic and genetic modification of mitochondrial DNA in obese humans. *Clin. Epigenetics* **7**, 60. https://doi.org/10.1007/s13148-015-0093-1

Morrow, R.M., Piccard, M., Derbeneva, O., Leipzig, J., McManus, M.J., Gouspillou, G. et al. (2017) Mitochondrial energy deficiency leads to hyperproliferation of skeletal muscle mitochondria and enhanced insulin sensitivity. *Proc. Natl Acad. Sci. U.S.A.* **114**, 2705–2710. https://doi.org/10.1073/pnas.1700997114

Yan, D., Cai, Y., Luo, J.R., Liu, J.J., Li, X., Ying, F. et al. (2020) FoxO1 contributes to diabetic cardiomyopathy via inducing imbalanced oxidative metabolism in type 1 diabetes. *J. Cell. Mol. Med.* **24**, 7850–7861. https://doi.org/10.1111/jcmm.15418

Yang, W.B., Yan, H., Pan, Q., Shen, J.Z., Zhou, F.H., Wu, C.D. et al. (2019) Guacino regulates hepatic mitochondrial function and biogenesis through FoxO1. *J. Endocrinol.* **241**, 265–278. https://doi.org/10.1530/JOE-19-0081

Shum, M., Shintre, C.A., Althoff, T., Gutierrez, V., Segawa, M., Saxberg, A.D. et al. (2021) ABCB10 exports mitochondrial biliverdin, driving metabolic maladaptation in obesity. *Sci. Transl. Med.* **13**, eabl1869. https://doi.org/10.1126/scitranslmed.abl1869

Chaban, Y., Boekema, E.J. and Dudkina, N.V. (2014) Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. *Biochim. Biophys. Acta* **1837**, 418–426. https://doi.org/10.1016/j.bbadis.2013.10.004

Converso, D.F., Taille, C., Carreiras, M.C., Jaltovich, A., Poderoso, J.J. and Boczko, J.J. (2006) HO-1 is located in liver mitochondria and regulates mitochondrial iron content and metabolism. *FASEB J.* **20**, 1236–1238. https://doi.org/10.1096/fj.05-4204fe

Campbell, N.K., Fitzgerald, H.K. and Dunne, A. (2021) Regulation of inflammation by the antioxidant haem oxygenase 1. *Nat. Rev. Immunol.* **21**, 411–425. https://doi.org/10.1038/s41577-020-00491-x
Tao, Z., Asham, H., Parke, J., Sanchez, M. and Cheng, Z. (2022) Mechanisms of autophagic responses to altered nutritional status. J. Nutr. Biochem., 108955 https://doi.org/10.1016/j.jnutbio.2022.108955

Tao, Z., Zheng, L.D., Smith, C., Luo, J., Robinson, A., Almeida, F.A. et al. (2018) Estradiol signaling mediates gender difference in visceral adiposity via autophagy. Cell Death Dis. 9, 309 https://doi.org/10.1038/s41419-018-0372-9

Bartolome, A., Garcia-Aguilar, A., Asahara, S.I., Kiddy, Y., Guillon, C., Pajvari, U.B. et al. (2017) MTORC1 regulates both general autophagy and mitophagy after oxidative phosphorylation uncoupling. Mol. Cell. Biol. 37, e00441-17 https://doi.org/10.1128/MCB.00441-17

Sengupta, A., Molkenst, J.D., Paik, J.H., DePinho, R.A. and Yutzey, K.E. (2011) Foxo transcription factors promote cardiomyocyte survival upon induction of oxidative stress. J. Biol. Chem. 286, 7468–7478 https://doi.org/10.1074/jbc.M111.179242

Song, D.M., Ma, J.X., Chen, L., Guo, C.X., Zhang, Y.Y., Chen, T. et al. (2017) FOXO3 promoted mitophagy via nuclear retention induced by manganese chloride in SH-SYSY cells. Metallomics 9, 1251–1259 https://doi.org/10.1039/C7MT00085E

Mei, Y., Zhang, Y., Yamamoto, K., Xie, W., Mak, T.W. and You, H. (2009) FOXO3a-dependent regulation of Pink1 (Park6) mediates survival signaling in response to cytokine deprivation. Proc. Natl Acad. Sci. U.S.A. 106, 5153–5158 https://doi.org/10.1073/pnas.0901104106

Xu, P., Das, M., Reilly, J. and Davis, R.J. (2011) JNK regulates FoxO-dependent autophagy in neurons. Genes Dev. 25, 310–322 https://doi.org/10.1101/gad.1984311

Lee, D. and Goldberg, A.L. (2013) SIRT1 protein, by blocking the activities of transcription factors FoxO1 and FoxO3, inhibits muscle atrophy and promotes muscle growth. J. Biol. Chem. 288, 30515–30526 https://doi.org/10.1074/jbc.M113.489716

Liu, L., Tao, Z., Zheng, L.D., Broke, J.P., Smith, C.M., Liu, D. et al. (2016) Foxo1 interacts with transcription factor EB and differentially regulates mitochondrial uncoupling proteins via autophagy in adipocytes. Cell Death Dis. 2, 16066 https://doi.org/10.1038/cddiscovery.2016.66

Settembre, C., Di Malta, C., Polito, V.A., Garcia Arenicibia, M., Vetrini, F., Erdin, S. et al. (2011) TFEB links autophagy to lysosomal biogenesis. Science 332, 1429–1433 https://doi.org/10.1126/science.1204592

Settembre, C. (2012) Transcription factor EB: a central regulator of both the autophagosome and lysosome (Reprinted from science, vol 332, pg 1429–1433, 2011). Hepatology 55, 1632–1634 https://doi.org/10.1002/hep.25619

Zhao, Y.G., Codogno, P. and Zhang, H. (2021) Machinery, regulation and pathophysiological implications of autophagosome maturation. Nat. Rev. Mol. Cell Biol. 22, 733–750 https://doi.org/10.1038/s41580-021-00392-4

Deleito-Seldas, N. and Elyyan, A. (2021) The mTOR-autophagy axis and the control of metabolism. Front. Cell Dev. Biol. 9, 655731 https://doi.org/10.3389/fcell.2021.655731

Rocznik-Ferguson, A., Petit, C.S., Froehlich, F., Oian, S., Ky, J., Angarala, B. et al. (2012) The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. J. Signal. 5, ra42 https://doi.org/10.1126/scisignal.2002790

Settembre, C., Zoncu, R., Medina, D.L., Vetrini, F., Erdin, S. et al. (2012) A lysosome-to-nucleus signalling mechanism senses and regulates the lysosomes via mTOR and TFEB. EMBO J. 31, 1095–1108 https://doi.org/10.1038/embj.2012.32.22

Wang, H.X., Song, P.P., Du, L., Tian, W.L., Yue, W., Liu, M. et al. (2011) Parkin ubiquitinates Drp1 for proteasome-dependent degradation implication of dysregulated mitochondrial dynamics in Parkinson disease. J. Biol. Chem. 286, 11649–11658 https://doi.org/10.1074/jbc.M111.144238

Quinay, M.N., Thomas, R.L., Lee, Y. and Gustafsson, A.B. (2010) Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore. Autophagy 6, 855–862 https://doi.org/10.4161/auto.6.7.13005

Quinay, M.N., Lee, Y., Rikka, S., Sayen, M.R., Molkenst, J.D., Gottlieb, R.A. et al. (2010) Bnip3 mediates permeabilization of mitochondria and release of cytochrome c via a novel mechanism. J. Mol. Cell. Cardiol. 48, 1146–1156 https://doi.org/10.1016/j.yjcc.2009.12.004

Soriano, F.X., Liesa, M., Bach, D., Chan, D.C., Palacin, M. and Zorzano, A. (2006) Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, estrogen-related receptor-alpha, and mitofusin 2. Diabetes 55, 1783–1791 https://doi.org/10.2337/db05-0509

Cheng, Q., Wei, T., Farbiak, L., Johnson, L.T., Dilliar, S.A. and Siegwart, D.J. (2020) Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nat. Nanotechnol. 15, 313 https://doi.org/10.1038/s41565-020-0669-6

Zhao, Z.M., Ukew, A., Kim, J. and Mitragotri, S. (2020) Targeting strategies for tissue-specific drug delivery. Cell 181, 151–167 https://doi.org/10.1016/j.cell.2020.02.001