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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 exocellular 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

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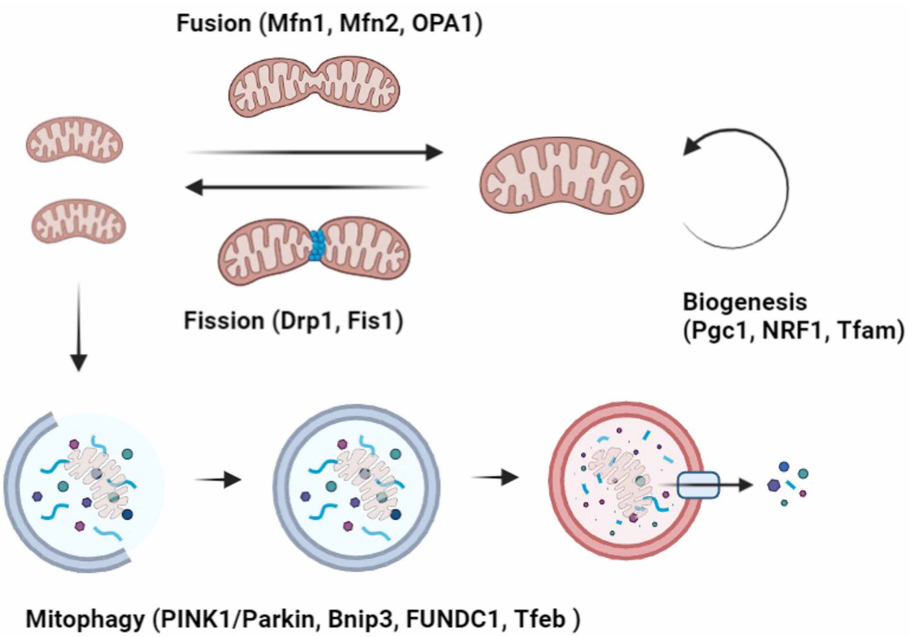

Figure 1. A schematic view of the triad in mitochondrial homeostasis.

Finely tuned mitochondrial biogenesis, dynamics (fusion and fission), and mitophagy contribute to the homeostasis of these organelles. Studies have established key regulators of mitochondrial biogenesis (e.g. Pgc1, NRF1, and Tfam), mitochondrial fusion (e.g. Mfn1, Mfn2, and OPA1), fission (e.g. Drp1 and Fis1), and mitophagy (e.g. PINK1/Parkin, FUNDC1, and Tfeb).

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

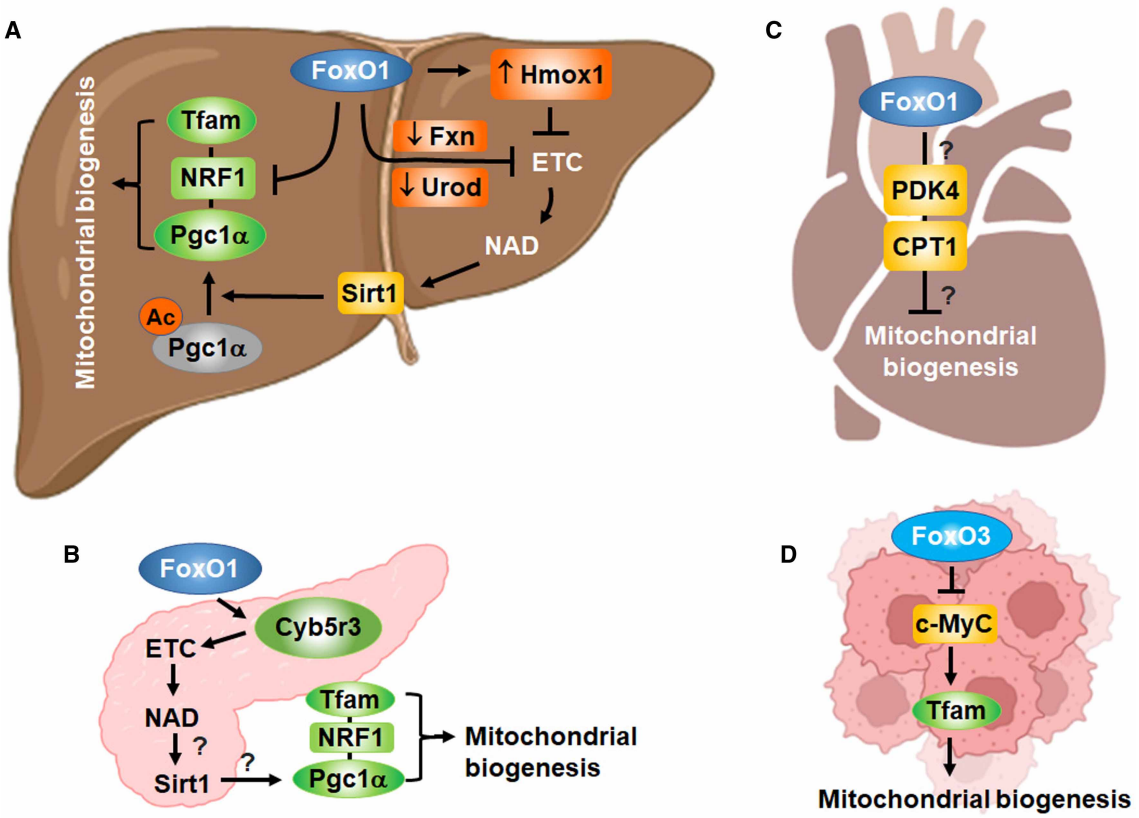

Figure 2. FoxO transcription factors regulate mitochondrial biogenesis.

(**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.

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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 metaflammation and insulin resistance in mouse and man [59]. Hmox1 may also contribute to hyperglycemia through catabolism of heme and release of excessive free ferrous in hepatocytes, which activates FoxO1 via NF-kB mediated phosphorylation at Ser273(FoxO1) and induces gluconeogenic gene in mice [60]. The Hmox1 → Fe^{2+} → NF-kB → FoxO1 cascade might serve as an 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 in response 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

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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

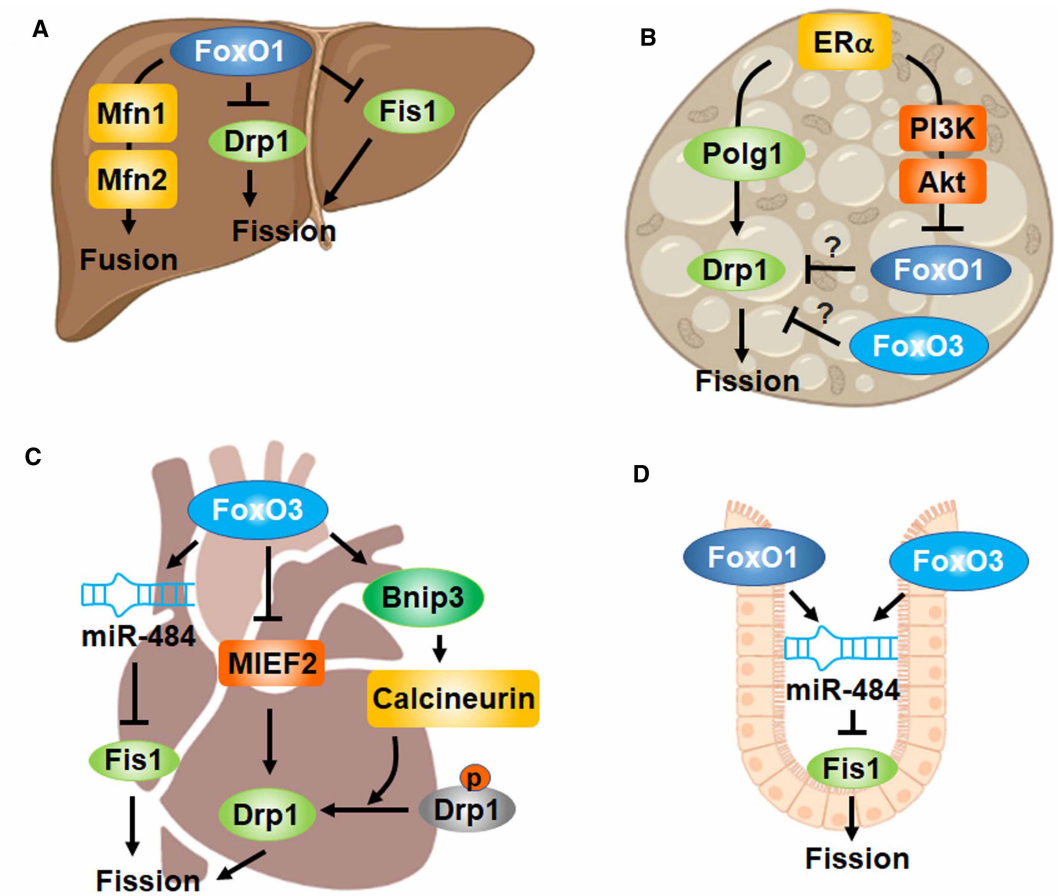

Figure 3. FoxO transcription factors regulate mitochondrial dynamics.

(**A**) In the liver or hepatocytes, FoxO1 up-regulates fusion proteins (Mfn1 and Mfn2) but down-regulates fission proteins (Drp1 and Fis1), leading to enlarged mitochondria. (**B**) Estrogen receptor (ERα) signaling induces Drp1 via Polg1 in brown adipocytes. It is known that ERα signaling deactivates FoxO via PI3K/Akt in adipose tissue and the liver, raising the question whether FoxOs may account for ERα induced mitochondrial fission (indicated by question marks). (**C**) FoxO3 inhibits mitochondrial fission by repressing MIEF2 or inducing miR-484, which are cardioprotective in doxorubicin (DOX)-induced mouse cardiotoxicity. Notably, FoxO3 was shown to stimulate mitochondrial fission via Bnip3-calcineurin mediated dephosphorylation (activation) of Drp1 in phenylephrine (PE)-stressed adult cardiomyocytes or heart from rats. The discrepancy may arise from different models of cardio stress induced by DOX vs PE. (**D**) In intestinal crypt-based columnar cells, FoxO1 and FoxO3 dampens mitochondrial fission by transactivating miR-484 that in turn silences Fis1.

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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 function 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-

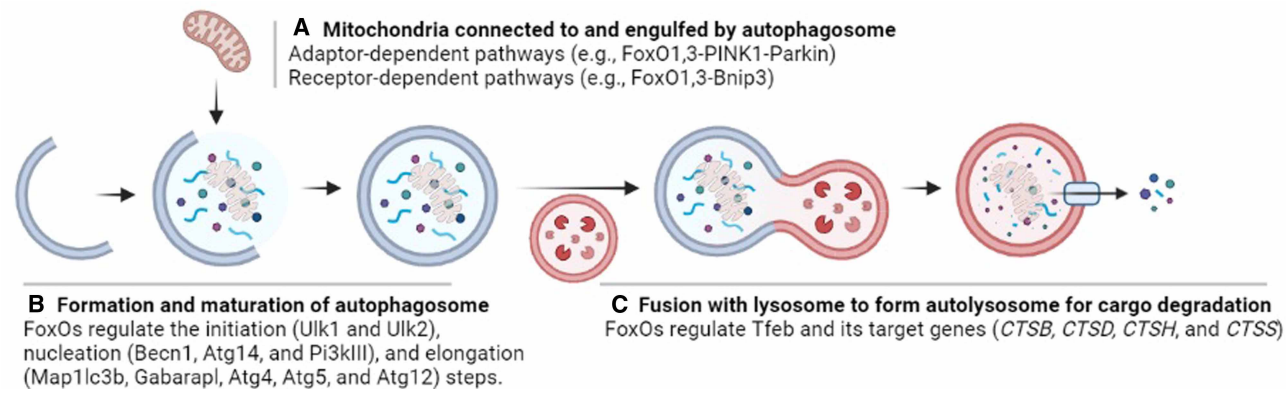

Figure 4. FoxO transcription factors regulate mitochondrial autophagy (mitophagy).

FoxOs mediate mitophagy in three aspects: (**A**) mitochondria connected to and engulfed by autophagosome via adaptor- and receptor-dependent pathways; (**B**) the formation and maturation of autophagosome (e.g. the initiation, nucleation, and elongation steps), and (**C**) fusion with lysosome to form autophagosome for cargo degradation.

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*),

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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 downregulates 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 cytotoxic 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 cardiac ischemia/reperfusion (I/R) 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

The author declares that there are no competing interests associated with this manuscript.

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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

response element-binding protein; CTSB, cathepsin B; CTSD, cathepsin D; CTSH, 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-*k*B, nuclear factor-*k*B; 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; Pi3kIII, the class III phosphatidylinositol 3-kinase; PINK1, PTEN induced kinase 1; Polg1, mitochondrial DNA polymerase γ; Sirt1, Sirtuin 1; 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; Urod, uroporphyrinogen decarboxylase.

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