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Review

The FoxO–Autophagy Axis in Health and Disease

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Autophagy controls cellular remodeling and quality control. Dysregulated autophagy has been implicated in several human diseases including obesity, diabetes, cardiovascular disease, neurodegenerative diseases, and cancer. Current evidence has revealed that FoxO (forkhead box class O) transcription factors have a multifaceted role in autophagy regulation and dysregulation. Nuclear FoxOs transactivate genes that control the formation of autophagosomes and their fusion with lysosomes. Independently of transactivation, cytosolic FoxO proteins induce autophagy by directly interacting with autophagy proteins. Autophagy is also controlled by FoxOs through epigenetic mechanisms. Moreover, FoxO proteins can be degraded directly or indirectly by autophagy. Cutting-edge evidence is reviewed that the FoxO–autophagy axis plays a crucial role in health and disease.

Introduction

Autophagy (see Glossary) is a self-degradative process that serves as an important adaptive mechanism in response to altered cellular signaling or cellular stresses (e.g., nutrient stress, infection, hypoxia, and toxicity) [1–3]. The term 'autophagy' derives from the Greek for 'self-eating' [4]. To date three types of autophagy have been defined, namely **macroautophagy**, **microautophagy**, and **chaperone-mediated autophagy**. By removing toxic protein aggregates, lipids, organelles, and intracellular pathogens, autophagy plays a key role in cellular renovation and quality control [5,6]. As such, autophagy has been found to protect against aging, metabolic disorders, infection, and neurodegenerative diseases [6–8]. Of note, autophagy may also promote disease progression or drug resistance, for example via prosurvival effects in cancer cells [6–8]. In addition, increased autophagy is associated with obesity and metabolic disorders, whereas downregulating autophagy activity can enhance metabolic homeostasis [7–12]. The multifaceted roles of autophagy in human disease strongly suggest that modulation of autophagy may lead to new therapeutics targeting key regulators of autophagy, such as FoxO transcription factors [13,14].

FoxO family members (or FoxO orthologs) have been identified across species such as worm (daf-16), fly (dFoxO), zebrafish, rodent, and human [15,16]. In mammals, the FoxO family includes FoxO1, FoxO3, FoxO4, and FoxO6. The transactivation activities of FoxOs are tuned by a conserved 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 [16–18]. In response to stress or external stimuli (e.g., oxidative stress and altered nutrient status or growth factor signaling), FoxO proteins undergo post-translational modifications (PTMs) in the NLS and NES domains, and translocate from the cytoplasm to the nucleus, or vice versa, to regulate the expression of an array of genes across tissues [16,19]. FoxO transcription factors have been shown to regulate metabolic homeostasis [14,16,20–29], neurogenesis and neuroprotection [30–32], cardiac remodeling [33–35], skeletal muscle homeostasis [36], immunity [37–40], endocytosis [41], stem cell homeostasis [42,43], and cancer cell growth and invasion [44–47].

Highlights

Autophagy plays a key role in cellular remodeling and quality control. Dysregulated autophagy has been observed in several human diseases including obesity, diabetes, cardiovascular disease, and cancer.

A FoxO–autophagy axis has been demonstrated by current evidence, which has revealed both health- and disease-promoting effects depending on the tissue and the cell type, underscoring the importance of the physiological and pathophysiological context in interpreting the roles of the FoxO–autophagy axis in health and disease.

FoxO proteins induce autophagy not only through transactivation of autophagy genes but also by interacting with autophagy proteins and by epigenetically (e.g., via histone modifications and miRNA) regulating autophagy activity. Post-translational modification and epigenetic modulation of FoxOs may induce or downregulate autophagy.

Autophagy can directly or indirectly regulate FoxO protein turnover. Autophagy-mediated degradation of FoxO proteins accounts for circadian control of glucose and diet-induced metabolic syndrome. It also sheds light on drug resistance in cancer chemotherapy.

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Increasing evidence has linked FoxOs to macroautophagy (hereafter referred to as autophagy) in human health and diseases. First, FoxOs bind to the promoter regions and transactivate the expression of autophagy genes to induce autophagy, which represents the typical function of FoxOs as transcription factors [5,6,48,49]. Second, FoxOs may function independently of transcriptional regulation by interacting directly with autophagy proteins (e.g., Atg7) in the cytoplasm to regulate autophagy [40,50–53]. Third, it has been recognized that FoxOs employ **epigenetic** mechanisms (e.g., **histone modifications** and microRNAs) to control autophagy activity and, likewise, epigenetic modulation of FoxOs may affect autophagy [39,54–60]. Epigenetic changes reflect altered interactions between environmental factors and genes, adding to the notion that autophagy serves as an adaptive process in response to external stimuli [39,54–60]. Last, emerging studies show that FoxO protein turnover is controlled by autophagy, and that these regulatory pathways are crucial for metabolic homeostasis and anticancer therapy [13,14]. Evidence from cutting-edge research has painted a new picture of the FoxO–autophagy axis which casts light on the crucial role of FoxOs in health and disease from an autophagy perspective. Targeting the FoxO–autophagy axis has shown promise in preventing or reversing disease progression in animal models (e.g., diabetic muscle atrophy [61]). In the following sections new evidence is reviewed to provide an updated view of the FoxO–autophagy axis and its regulatory circuits; outstanding questions and controversial issues in the literature that warrant future study are also discussed.

The Transcriptional View of the FoxO–Autophagy Axis

FoxO transcription factors bind to the promoters of autophagy genes and induce gene expression, and this requires FoxO translocation from the cytosolic compartment into the nucleus (Figure 1A). Activating PTMs such as AMPK-induced FoxO phosphorylation and PRMT6-induced methylation promotes FoxO translocation to the nucleus [55,62]. Inhibitory PTMs (e.g., AKT-induced phosphorylation), however, facilitate exclusion of FoxO from the nucleus [63–67] (an in-depth discussion of PTMs is presented in the section 'The Post-Translational

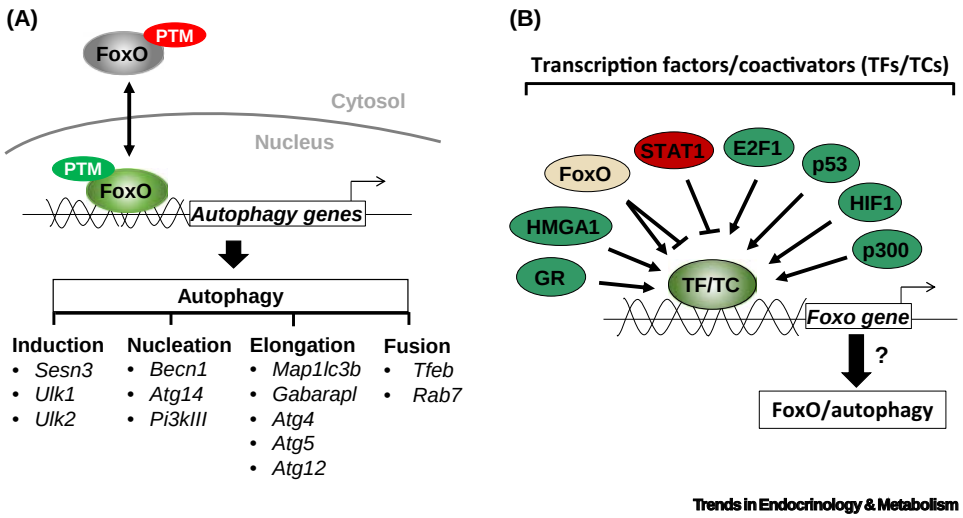

Figure 1. Transcriptional Regulation of the FoxO–Autophagy Axis. (A) FoxO proteins induce autophagy by transactivating the expression of genes encoding autophagy proteins involved in multiple stages (e.g., induction, nucleation, elongation, and fusion) of the autophagic process. Post-translational modifications (PTMs) can promote or prevent the transactivation activity of FoxO proteins by mediating their nuclear translocation or exclusion. Inhibitory PTMs (red) induce nuclear exclusion (e.g., Akt-induced phosphorylation) whereas activating PTMs (green) promote the nuclear accumulation (e.g., AMPK-induced phosphorylation) of FoxOs. (B) Several transcription factors or coactivators (TFs/TCs) have been shown to regulate FoxO gene expression, and some of the TFs/TCs are also implicated in autopagy (text for detailed discussion). Whether FoxOs are the major mediator of these TFs/TCs in regulating autophagy remains to be defined.

Glossary

Autophagy: a self-digestion process that cells use for proteolytic degradation of cellular components (e.g., protein aggregates, lipid droplets, and dysfunctional organelles) in lysosomes. Three types of autophagy have been identified, namely macroautophagy, microautophagy, and chaperone-mediated autophagy.

Chaperone-mediated autophagy: an autophagic process where cargoes or substrates form a complex with chaperone proteins (such as Hsc-70), followed by translocation across the lysosomal membrane with the assistance of lysosomal membrane receptor LAMP-2A (lysosome-associated membrane protein 2A) to enter lysosomes for degradation. The complex of cargo with Hsc-70 may also be delivered to the membrane of late endosomes, where the cargos are internalized through multivesicular bodies and undergo degradation in the endosomal lumen when endosomes fuse with lysosomes.

DNA methylation: a process of adding a methyl group to the DNA molecule at the carbon-5 position of the cytosine ring to form 5-methylcytosine, thereby modifying the function or expression profile of a gene. DNA methylation represents an important epigenetic mechanism, and it either suppresses or promotes gene expression depending on the location of methylation (i.e., promoter region vs gene body). DNA methyltransferases are required for *de novo* synthesis (e.g., DNMT3a, and DNMT3b) and maintenance (e.g., DNMT1) of DNA methylation.

Endoplasmic reticulum (ER) stress: a state where the protein folding and secretory capacity of the ER is compromised, thereby overloading the ER by unfolded proteins in its lumen, and augmenting the unfolded protein response.

Epigenetics: the study of molecular events or factors that affect gene expression beyond the genomic sequence, including DNA modifications, histone modifications, and non-coding RNAs. Epigenetic changes reflect the interactions between genes and environmental cues.

Gluconeogenesis: a multiple-step process in the liver and kidneys that produces glucose using glycerol, lactate, propionate, and some amino acids as substrates. Gluconeogenesis

View of the FoxO–Autophagy Axis'). Genetic modulation of FoxO is associated with significantly altered expression of several autophagy genes, including those regulating autophagy induction (e.g., *Ulk1* and *Ulk2*), nucleation (e.g., *Becn1* and *Atg14*), elongation (e.g., *Map1lc3b*, *Gabarapl*, and *Atg4*), and autophagosome–lysosome fusion (e.g., *Tfeb*, *Rab7*) (Figure 1A) [48,49,61,68–72]. Although sestrin 3 (Sesn3) does not directly induce autophagy, it activates AMPK and deactivates mTORC1, which leads to activation of autophagy via the mTORC1–ULK1 pathway [73–75]. By transcriptionally inducing *Sesn3*, FoxO proteins act on mTORC1 to remove the brake on autophagy initiation [73–75].

FoxO-induced autophagy has been implicated in muscle atrophy [61,76,77] as well as in cardiac remodeling and atrophy [35,78]. FoxOs also induce muscle-specific E3 ubiquitin ligases (e.g., MAFbx and MuRF1) that contribute to muscle atrophy by promoting proteasomal protein degradation and inhibiting protein synthesis via eIF3f [61,76,79,80]. Muscle-specific ablation of FoxOs completely rescued muscle mass in diabetic mice and in mice with insulin receptor or IGF-1 receptor deficiency [61]. Interestingly, FoxO-induced autophagy is indispensable and plays a protective role in other tissues such as liver [69,70,81], brain [30,74,82], and kidney [68], as well as in intervertebral disk and cartilage homeostasis [83,84]. Deletion of hepatic *Foxo1*, *Foxo3*, and *Foxo4* dysregulated lipid metabolism, which was associated with downregulated Atg14 and autophagy [70]. However, overexpression of Atg14 protected mice from developing hypertriglyceridemia induced by a high-fat diet (HFD) [70]. The cytoprotective role of the FoxO–autophagy axis was also identified in alcohol-induced hepatotoxicity and liver **ischemia**/reperfusion injury [69,81]. In aging brains, FoxO expression progressively increases; however, FoxO-induced autophagy acts as a guardian of neuronal integrity by inhibiting age-progressive axonal degeneration [74]. During adult neurogenesis FoxOs maintain robust autophagic flux and neuronal morphogenesis, whereas ablation of FoxOs dysregulated dendritic morphology as well as spine density and positioning [30]. These findings highlight the crucial roles of FoxO-induced autophagy in tissue homeostasis.

Although it is well established that FoxOs can directly induce the expression of autophagy genes through their transactivation functions (Figure 1A), the transcription factors that regulate the expression of *Foxo* genes remain largely unknown. FoxO3 protein may act on its own promoter [85], and on the promoter of *Foxo1* [67], to induce *Foxo1* and *Foxo3* gene expression (Figure 1B). In particular, FoxO3-induced *Foxo1* upregulation is required for FoxO3-mediated autophagy in human embryonic kidney cells (HEK293T) and mouse embryonic fibroblast (MEF) cells [67]. Ablation of FoxO1 using siRNA dampened the induction of autophagy in cells overexpressing wild-type FoxO3 or constitutively active FoxO3 (i.e., transcriptionally active FoxO3 that carries alanine mutations at residues Thr32, Ser253, and Ser315, sites of inhibitory phosphorylation) [67]. By contrast, FoxO3 was found to have no effect on *Foxo1* transcripts in primary myotubes [62] or to transcriptionally suppress *Foxo1* and inhibit autophagy in different human cancer cells, including prostate cancer PC3 cells, colon cancer HCT116 cells, and breast cancer MDA-MB-231 cells [86]. This suggests that FoxO regulation of autophagy in cancer differs from that in normal or benign cells (e.g., fibroblasts and muscle cells), underlining the importance of the cell type and physiological context when interpreting autophagy data (Box 1). Indeed, in embryonic stem cells (ESCs) that maintain high autophagic flux, FoxO1 was found to play a key role in ESC self-renewal, pluripotency, and differentiation [42]. Furthermore, FoxO1-mediated autophagy is required for adipocyte differentiation and transdifferentiation [49,87]. Thus, it would be premature to generalize the roles of FoxO in autophagy when developing FoxO-targeting strategies for disease treatment.

Previous studies have shown that the *Foxo1* gene is transcriptionally regulated by the chromatin factor high-mobility group A1 (HMGA1) [88]. HMGA1 can bind to the promoter of the

involves hormonal regulation of enzymatic reactions to sustain glucose supplies during the postabsorptive state.

Histones: a family of proteins that are positively charged and associate with negatively charged DNA molecules to form complexes in which the DNA is wrapped around the histones and condenses into chromatin in chromosomes. Five types of histones have been identified: the core histones (H2A, H2B, H3, and H4) and the linker histones (H1 and H5).

Histone modification: an enzymatic process in which chemical groups are added to histones; modifications include acetylation, methylation, phosphorylation, sumoylation, and ubiquitylation. Histone modifications affect histone–DNA interactions and chromatin structure, resulting in transcriptional activation or inactivation.

Ischemia: a condition where blood flow and oxygen supply are restricted, thereby compromising metabolism and cell or tissue function. Restricted blood flow and oxygen supply to the heart causes cardiac ischemia.

Macroautophagy: an autophagic process in which double-membrane vesicles (i.e., autophagosomes) sequester and deliver targeted cellular components (i.e., cargoes or substrates) to lysosomes, where lysosomes and autophagosomes fuse to form autolysosome to degrade the sequestered cargoes.

Metabolic syndrome: a group of risk factors or conditions including high blood pressure, hyperglycemia (or high blood glucose), abdominal obesity (or increased waist circumference), and dysregulated cholesterol or triglyceride levels. An individual with metabolic syndrome has a higher risk of developing heart disease, stroke, diabetes, and even neurological (cerebrovascular) complications.

Microautophagy: an autophagic process where cargoes or substrates are engulfed directly by the lysosome for degradation through invagination of the lysosomal membrane.

Non-coding RNA: a class of RNA molecules that are not translated into proteins. Among thousands of non-coding RNAs identified, some may alter mRNA stability or translational efficiency, including microRNA (miRNA or miR), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), and long non-coding RNA (lncRNA). Some may

Box 1. Physiological and Pathological Significance of the FoxO–Autophagy Axis

The FoxO–autophagy axis is crucial for maintaining tissue homeostasis in liver [69,70,81], brain [30,74,82], kidney [68], and intervertebral disk and cartilage [83,84]. For instance, ablation of the hepatic FoxO–autophagy axis dysregulates lipid metabolism, whereas restoration of autophagy protects against HFD-induced hypertriglyceridemia [70] as well as against alcohol-induced hepatotoxicity and liver ischemia/reperfusion injury [69,81]. In the brain, the FoxO–autophagy axis downregulates age-progressive axonal degeneration and enhances neuronal integrity [74]. In line with FoxOs maintaining a robust autophagic flux and neuronal morphogenesis during adult neurogenesis, ablation of FoxOs dysregulates dendritic morphology as well as spine density and positioning [30]. However, increased activity of FoxO–autophagy axis can be pathogenic. For instance, FoxO-induced autophagy results in cardiac [35,78] and skeletal muscle atrophy [61,76,77,101], and this might account for Duchenne muscular dystrophy or Becker muscular dystrophy [108]. The mechanisms of muscle atrophy include post-translational and epigenetic regulation of FoxOs and autophagy [101,108]. As such, muscle-specific ablation of FoxOs downregulated autophagy and restored muscle mass in diabetic mice or in mice with insulin receptor or IGF-1 receptor deficiency [61]. Of note, FoxO protein turnover is regulated by autophagy, and thus modulates the sensitivity of tumor cells to chemotherapeutic drugs [13]; ablation of autophagy upregulates FoxO protein and downstream proapoptotic factors, thereby potentiating anticancer effects. The physiological/pathogenic roles of the FoxO–autophagy axis can be more complex depending on changes in the cellular microenvironment. For instance, activation of the FoxO–autophagy axis by myocardial ischemia compromises mitochondrial metabolism and stimulates cardiomyocyte death, whereas blocking the FoxO–autophagy axis prevents cardiac apoptosis and fibrosis [102]; however, resveratrol induces FoxO autophagy via Sirt1 activation and reduces myocardial injury in diabetic hearts [94]. Moreover, the FoxO–autophagy axis has been shown to prevent or promote cancer and obesity [10,13,46,49,87,117,118], likely because of differences in the cellular microenvironments that determine disease progression [116].

endogenous *FOXO1* gene in human hepatocellular carcinoma HepG2 cells and human embryonic kidney 293 cells, increasing *FOXO1* mRNA and protein levels, which induces FoxO1 target genes such as those involved in **gluconeogenesis** [88]. In terms of autophagy regulation, however, HMGA1 was reported to downregulate autophagy in MEFs, human squamous carcinoma SCC-13 cells (skin cancer), and cervical cancer-derived HeLa cells [89]. HMGA1 knockdown transcriptionally upregulated the autophagy-initiating kinase *Ulk1* gene [89], which was also shown to be a FoxO target gene (Figure 1A). Chromatin immunoprecipitation indicated that HMGA1 binds to the promoter of the *Ulk1* gene, suggesting that HMGA1 is a repressor of *Ulk1* [89]. Whether FoxO1 is involved in HMGA1-mediated autophagy via ULK1 is unclear, but the current literature suggests that additional mechanisms beyond HMGA1 may account for FoxO–autophagy regulation and dysregulation, and these warrant further investigation (Figure 1B). To this end, signal transducer and activator of transcription 1 (STAT1) has been implicated in autophagy as a repressor of *Foxo1* transcription in diabetic kidney disease [90]. Other transcription factors participating in the regulation of autophagy include E2F1, HIF-1α, p53, and the glucocorticoid receptor (GR) [17]. Because these transcription factors have been shown to regulate *Foxo* transcripts in different contexts (e.g., cardiac ischemia/reperfusion injury and tumorigenesis), it is tempting to speculate that they may regulate autophagy via FoxOs (Figure 1B).

The Post-translational View of the FoxO–Autophagy Axis

FoxO proteins may regulate autophagy independently of transactivation activity, in other words by mechanisms other than binding to the promoters of autophagy genes and gene expression upregulation [40,50,67,72,91]. In fact, FoxO proteins can be excluded from the nucleus, and cytoplasmic FoxO protein binds to Atg7, an E1-like enzyme, to promote autophagy activity [40,50,91] (Figure 2A). It was shown that acetylation (e.g., Lys262, Lys265, and Lys274 in *Homo sapiens* FOXO1) [50,72,91] and Akt-induced phosphorylation (e.g., Thr24, Ser256, and Ser319 for *Mus musculus* Foxo1, and Thr32, Ser253, and Ser315 for *H. sapiens* FOXO1) [40,67,91] play an important role in FoxO1 translocation into the cytoplasm and the interaction with Atg7 for autophagy induction (Figure 2A). Stress conditions (e.g., starvation or oxidative stress) promoted the dissociation of deacetylases Sirt1 or Sirt2 from their substrate FoxO1 and upregulated the acetylation of FoxO1, which is required for FoxO1 binding to Atg7 and stimulating autophagy [50,91]. FoxO1 acetylation can also be elevated by the

facilitate protein synthesis (e.g., tRNA and rRNA). Many other identified noncoding RNAs either show no function or remain to be functionally characterized.

Oxidative stress: a state of imbalance between oxidants and antioxidants that favors oxidative reactions, causing oxidative damage to biomolecules and dysregulating redox signaling pathways.

Proteasomal degradation: a pathway that involves the addition of ubiquitin (Ub) to intracellular proteins that are then targeted to the proteasome for degradation. Ub modification of targeted proteins is catalyzed by the E3 Ub-protein ligase, with the assistance of E1 Ub-activating enzyme and E2 Ub-carrier or conjugating proteins. Once enzymatically labeled with Ub chains, targeted proteins are recognized by the 26S proteasome (multiprotease complex) for degradation.

Transcription factor: a protein that controls the rate of gene expression, namely the transcription of genetic information in DNA into mRNA, by binding to DNA regulatory sequences (enhancers and silencers) of target genes. Transcription factors may also form complexes with other proteins to activate or repress gene expression.

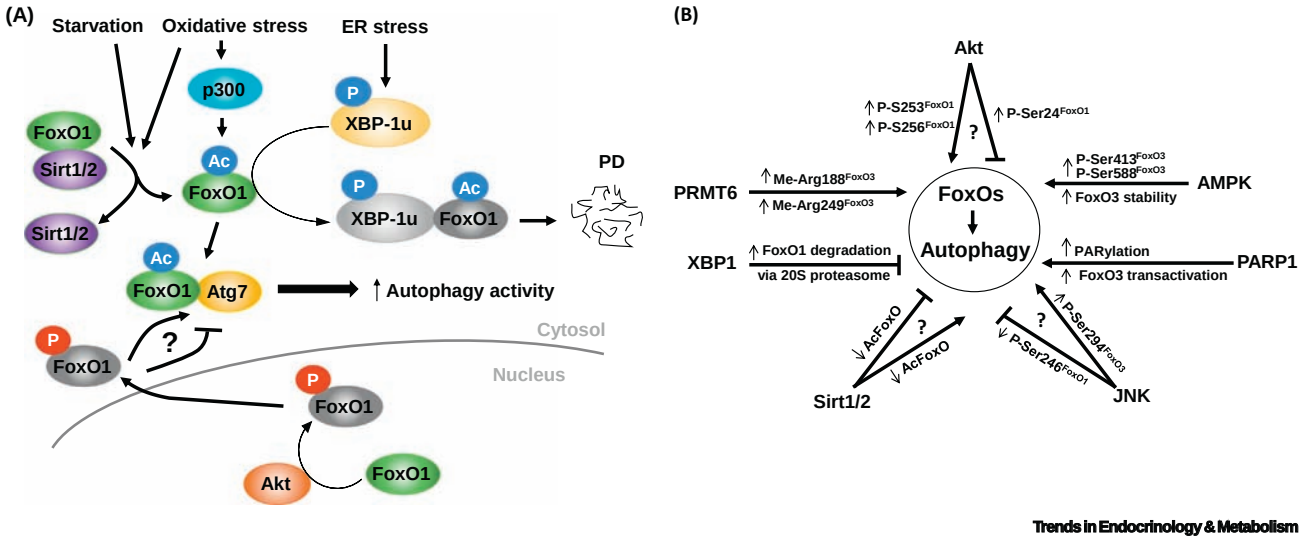

Figure 2. Post-translational Regulation of the FoxO–Autophagy Axis. (A) FoxO regulates autophagy by interacting with autophagy protein Atg7 in the cytoplasm, and this process is enhanced by post-translational modifications (PTMs) such as acetylation (Ac) by p300 (or as a result of dissociation from Sirt1 and Sirt2) and phosphorylation (P) by Akt. Acetylated FoxO protein may bind to XBP-1u and undergo proteasomal degradation (PD). Interactions of FoxO proteins with Atg7 and XBP-1u were shown to play an important role in autophagy under conditions of oxidative stress, starvation, and ER stress. Questions remain regarding how autophagy is sustained or enhanced regardless of loss of FoxO transactivation of autophagy genes, and how FoxO protein is stabilized versus Akt-mediated phosphorylation that promotes proteasomal degradation of FoxO protein. The green and grey colors indicate active and inactive forms of FoxO protein, and Akt-induced inhibitory phosphorylation of FoxO is indicated in red. Acetylation (in blue) may promote FoxO interaction with Atg7 to induce autophagy, and with XBP-1u to induce PD of FoxO. (B) Additional PTMs regulating autophagy include phosphorylation, acetylation, methylation (Me), and the addition of poly(ADP-ribose) chains to FoxO proteins (PARylation), which control autophagy gene expression by affecting FoxO turnover, nuclear localization, or transactivation activity. Future studies will be necessary to better understand the roles of Akt-, JNK-, and Sirt1/2-induced PTMs in FoxO-mediated autophagy, and how PTM interactions may affect autophagy, as many questions or controversies remain (see text for detailed discussion).

CREB-binding protein paralog p300 during oxidative stress [91], and this mechanism was shared by aldosterone-induced autophagy in podocytes, where p300 facilitates FoxO1–Atg7 binding [72]. These findings provide a new view of FoxO1 regulation of autophagy in the cytoplasm. This also raises an outstanding question about how FoxO1 coordinates its nuclear (transcriptional) versus cytosolic (post-translational) roles in autophagy that are modulated by acetylation and deacetylation [63,81,92,93]. Specifically, FoxO1 deacetylation by Sirt1 and nuclear translocation are required for autophagy induction in vascular endothelial cells, and this could explain how biomechanical stimuli or laminar blood flow can protect against vascular disease [92]. In diabetic mice, treatment with resveratrol activates Sirt1 and leads to deacetylated FoxO1, which in turn binds to the *Rab7* promoter to induce Rab7 and autophagy in diabetic hearts, thereby reducing myocardial injury [94]. In the liver, Sirt1 deficiency increased FoxO3 acetylation and suppressed the expression of autophagy genes, including those involved in initiation (*Atg101*), vesicle nucleation (*Atg14*), elongation (*Lc3b* and *Atg3*), and mitophagy (*Bnip3*), and the resulting inhibition of autophagy was linked to glycogen storage disease [93]. Sirt1-mediated deacetylation of FoxO3 was also found to protect against alcohol-induced liver injury via nuclear translocation and transcriptional regulation of autophagy genes [81]. Therefore, both acetylation (nuclear exclusion) and deacetylation (nuclear translocation) appear to be crucial for autophagy induction. It is unclear how FoxO1–Atg7 (protein–protein) interaction in the cytoplasm compensates for the dampened expression of several autophagy genes (Figure 1A) as a result of acetylation or nuclear exclusion of FoxO1. What determines whether and when to acetylate or deacetylate FoxOs for cytosolic or nuclear regulation of autophagy remains largely unknown (Figure 2B).

A similar scenario has been observed in the case of phosphorylation of FoxO1 by Akt. It was suggested that Akt-induced FoxO1 phosphorylation excludes FoxO1 from the nucleus, thus enhancing FoxO1–Atg7 (protein–protein) interaction in the cytoplasm and increasing autophagy activity [40,67], which is crucial for natural killer cell development and innate immunity [40] (Figure 2A). Nevertheless, it is unclear how FoxO1 might be stabilized in the cytoplasm given that Akt-mediated phosphorylation promotes nuclear exclusion of FoxO1 and facilitates its proteasomal degradation [16]. In fact, stearoyl-CoA desaturase 1 (SCD1)-induced lipogenesis and lipid raft-coupled Akt activation were found to increase FoxO1 phosphorylation and nuclear exclusion, leading to lower autophagy activity in MEFs [66]. A similar phenotype was reported in mouse granulosa cells treated with melatonin or follicle-stimulating hormone (FSH) to protect against oxidative damage, where melatonin or FSH induced the PI3K–Akt cascade, leading to phosphorylation of FoxO1 and abolition of autophagic activity [63,91]. These studies suggest that the mechanism of Akt-induced phosphorylation in regulating FoxO1 and autophagy is more complex than expected, particularly because two studies on MEFs gave distinct phenotypes [66,67]. Future studies are warranted to determine how FoxO1 may circumvent proteasomal degradation upon Akt-induced phosphorylation, and how its cytosolic versus nuclear roles in autophagy regulation are coordinated (Figure 2B).

An important question to ask is whether Akt-mediated FoxO1 phosphorylation facilitates FoxO1 acetylation and increases autophagy. In mouse ovarian granulosa cells, treatment with FSH or melatonin induced both Akt-mediated phosphorylation and Sirt1-mediated deacetylation of FoxO1, which prevented FoxO1 from binding to Atg7 protein and downregulated autophagy [63,91]. These findings suggest that, in the absence of acetylation, phosphorylation of FoxO1 alone is insufficient for cytosolic FoxO1 to bind to Atg7 to induce autophagy. Interestingly, acetylated FoxO1 may bind to the X-box-binding protein 1u (XBP-1u) which is activated by extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) via phosphorylation of Ser61 and Ser176, and then undergoes proteasomal degradation [95]. As a result, XBP-1u activation leads to suppressed autophagy in cancer cells [95] or under conditions where the unfolded protein response or **endoplasmic reticulum (ER) stress** is triggered (Figure 2A,B) [53,96]. In a mouse model of Huntington's disease that is characterized by ER stress, ablation of XBP1 upregulated FoxO1 and enhanced autophagy, improving neuronal survival and motor performance [96]. Thus, the XBP1–FoxO1 interaction may serve as an important mechanism by which ER stress leads to pathogenic changes via autophagy (Figure 2A,B).

In addition to Akt, other kinases such as AMPK and JNK have been implicated in the regulation of autophagy via FoxO (Figure 2B) [55,62,82,97–99]. AMPK can phosphorylate FoxO3 at Ser413 or Ser588, which promotes nuclear accumulation of FoxO3 and triggers autophagy via transcriptional or epigenetic mechanisms [55,97]. Activation of AMPK also stabilizes FoxO3 under conditions of nutrient depletion or hypoxia, increasing nuclear FoxO3 and autophagy gene transcription [62,100]. The AMPK-regulated FoxO–autophagy axis provides an important mechanism of adaptation to environmental stresses such as cadmium-mediated toxicity, oxidative stress, hypoxia, nutritional stress, and exercise [62,97,100]. Similarly, oxidative stress may activate c-Jun N-terminal kinase (JNK1) that induces FoxO3 phosphorylation at Ser294 and nuclear translocation [99]. During bone remodeling, the differentiation of mesenchymal stem cells into osteoblasts leads to increased mitochondrial metabolism and reactive oxygen species, and the JNK1-regulated FoxO3–autophagy axis plays a central role in bone homeostasis [99]. JNK was shown to mediate indirect phosphorylation of FoxO1 at Ser246 through cyclin-dependent protein kinase (CDK) in neurons, but how JNKs interact with CDKs remains elusive [82] (Figure 2B). Of interest, JNK activation of FoxO may lead to AMPK activation via SESN, thus potentiating autophagy activity [73–75,98].

Emerging evidence have revealed further PTMs that regulate FoxO activity in autophagy, including methylation [101] and the addition of poly(ADP-ribose) (PAR) chains (PARylation) (Figure 2B) [102]. Hyperactivity of Foxo3 was reported in mice in response to activation of PRMT6 (protein arginine methyltransferase 6) that induced methylation of Foxo3 at Arg188 and Arg249, leading to increased autophagy and muscle atrophy [101]. PRMT1 (another member of the arginine methyltransferase family) serves as an endogenous suppressor of the PRMT6–Foxo3 cascade to maintain skeletal muscle metabolism and regeneration. Intriguingly, PRMT1 was shown to induce FoxO1 methylation at Arg248 and Arg250, which activated FoxO1 by blocking Akt-mediated inhibitory phosphorylation [103]. As a result, ablation of PRMT1 promotes Akt-mediated FoxO1 phosphorylation at Ser253, resulting in nuclear exclusion, polyubiquitination, and proteasomal degradation of FoxO1 [103]. Whether and how PRMT1-mediated FoxO1 activation regulates autophagy is unknown, and this would be of interest to investigate in future studies. The other new PTM is mediated by poly(ADP-ribose) polymerase-1 (PARP1), which PARylates FoxO3 and exerts profound effects on autophagy via altering FoxO3 localization and transactivation activity [102]. In addition, PARP1 can induce epigenetic changes that contribute to augmented autophagy, leading to altered mitochondrial metabolism and promoting cardiomyocyte death [102].

The Epigenetic View of the FoxO–Autophagy Axis

Environmental factors (e.g., nutritional status) interact with cells or organisms to elicit epigenetic changes that have profound effects on gene expression, development, and metabolism [104–106]. Under starvation conditions, autophagy is activated to break down cellular components (e.g., protein aggregates and organelles) for use in energy generation and biosynthesis to maintain homeostasis and viability [107]. Starvation-induced autophagy was recently linked to epigenetic changes, where FoxO plays a key role [55,102,108]. Glucose starvation activates the energy sensor AMPK, which phosphorylates FoxO3 and stimulates its nuclear translocation [55]. Acting as a repressor, FoxO3 binds to the promoter of *SKP2* gene and downregulates *SKP2* transcription and SKP2 protein, the key component of the SCF E3 ubiquitin ligase complex that degrades CARM1 (coactivator-associated arginine methyltransferase 1). As a result of FoxO3-mediated suppression of SKP2 and the SCF E3 ubiquitin ligase complex, CARM1 is upregulated to modify **histone** H3 (Arg17 dimethylation) and to function as a transcriptional coactivator, leading to upregulation of autophagosomal and lysosomal genes through TFEB [55] (Figure 3A). In addition to histone modification, fasting and starvation were shown to regulate microRNAs (e.g., miR-378 and miR-205-5p) via FoxO [108,109]. FoxOs can up- or downregulate various miRNAs that are involved in MAPK, Wnt, and insulin signaling [109]. Fasting induced miR-378 and autophagy in skeletal muscle, whereas ablation of miR-378 led to defective autophagy and excessive apoptosis, a phenotype relevant to Duchenne muscular dystrophy and Becker muscular dystrophy [108]. These results suggest that miR-378-mediated autophagy is crucial for muscle health and homeostasis. The role of FoxO in miR-378 regulation of autophagy is related to the kinase Akt, which is known to suppress FoxO activity via phosphorylation (discussed earlier). However, miR-378 can dampen Akt kinase activity by silencing the expression of the Akt activator, PDK1 (phosphoinositide-dependent protein kinase 1). FoxO1 and FoxO3 are therefore activated by miR-378, which induces upregulation of autophagy genes as well as of miR-378 (a positive feedback regulator of FoxO) (Figure 3A). In addition, miR-378 deactivates mTORC1 by dampening the PDK1–Akt cascade, which initiates autophagy via ULK1, and also dampens the caspase 9–caspase 3 cascade to suppress apoptosis. The coordinated activity of autophagy versus apoptosis integrates metabolism with the adaptive response of myocytes to maintain normal muscle mass [108]. Of note, a miR–Akt–FoxO axis has also been discovered in the liver, where miR-205-5p activates Akt and inhibits FoxO in primary hepatocytes, thereby suppressing hepatic glucose production [109].

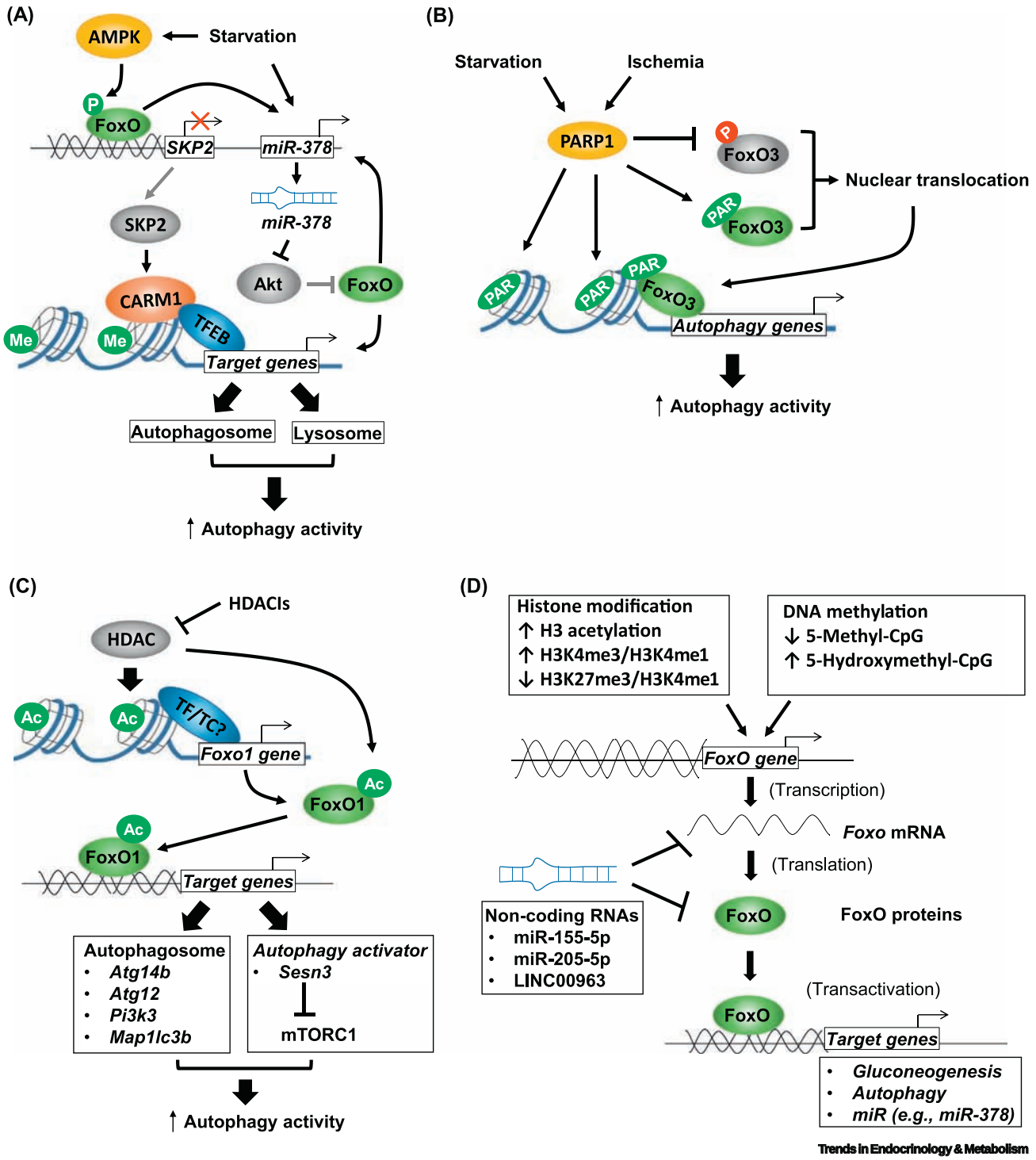

Figure 3. Epigenetic Regulation of the FoxO–Autophagy Axis. (A) FoxOs control autophagy gene expression by regulating chromatin structure [histone methylation (Me) via suppression of SKP2 to upregulate CARM1] and microRNA expression. The arrow and inhibitory bar symbols in black indicate active functions, and those in grey indicate inactive functions. (B) FoxO3 induces autophagy through a mechanism synergizing poly(ADP-ribose)-induced histone modification (PARylation), chromatin

(Figure legend continued at the bottom of the next page.)

Activation of poly(ADP-ribose) polymerase 1 (PARP1) is commonly observed in myocardial ischemia/reperfusion injury, where PARP1 functions as a DNA damage sensor to regulate the negative charge of histones and modulate histone–DNA interactions for chromatin remodeling, DNA repair, and transcriptional regulation [110]. Using NAD^+ as a substrate, PARP1 produces ADP-ribose to form long branches of poly(ADP-ribose) on glutamic acid residues of target proteins (PARylation), including histones and FoxO3 (Figure 3B) [102]. PARylation of FoxO3 promotes its nuclear localization, which is associated with decreased inhibitory phosphorylation of FoxO3. In addition, modification of histone H1 through PARylation led to dissociation of histone H1 from DNA, which exposes the promoter regions of autophagy genes and promotes FoxO3 binding to the promoters of target genes (e.g., *Lc3*, *Gabarapl1*, and *Atg12*) [102]. Therefore, autophagy is augmented in myocardial ischemia through epigenetic reprogramming of FoxO3 transactivation of autophagy genes, leading to impaired mitochondrial metabolism and cardiomyocyte death (Figure 3B). Activation of PARP1 also accounts for starvation-induced myocardial autophagy, and, importantly, silencing of PARP1 prevented FoxO3 nuclear translocation and transactivation activity upon starvation or myocardial ischemia, thereby inhibiting cardiac apoptosis and fibrosis (Figure 3B) [102].

Given the crucial role of FoxO in autophagy regulation, it is conceivable that epigenetic modulation of FoxOs results in altered autophagy activity [73]. Targeting histone deacetylases (HDACs) pharmacologically (i.e., with small-molecule inhibitors such as suberoylanilide hydroxamic acid and trichostatin A) could effectively induce autophagy, and this was associated with FoxO1 upregulation at the mRNA and protein levels. HDAC inhibitors (HDACIs) also increased FoxO1 acetylation, leading to nuclear accumulation of FoxO1 protein and elevated transactivation of autophagy genes and *Sesn3* (Figure 3C) [73]. Sesn3 promotes autophagy by suppressing the mTOR–ULK1 pathway [73–75]. Presumably, histone acetylation is increased as a result of inhibition of HDACs, causing chromatin relaxation and facilitating access to *Foxo1* promoters by unknown transcription factors or coactivators that trigger transactivation (Figure 3C) [111–113]. It is known that histone acetylation removes the positive charge of lysine and releases histone from destabilized nucleosomes to expose DNA, thus increasing the accessibility of the promoter regions in chromatin [113]. Indeed, histone modifications (e.g., histone H3 acetylation and histone H3K4 trimethylation) increased *Foxo1* gene expression, resulting in dysregulation of FoxO1 target genes and gluconeogenesis, which accounted for the **metabolic syndrome** in offspring of mice subjected to gestational sleep disturbance (Figure 3D) [54]. In addition, other epigenetic mechanisms have been identified in the regulation of *Foxo* genes, including **DNA methylation** (5-hydroxymethyl-CpG and 5-methyl-CpG) [39,54] and non-coding RNAs (e.g., miR-155-5p and long intergenic non-coding RNA LINC00963) [59,60]. LINC00963 suppresses *Foxo3* gene expression and contributes to renal interstitial fibrosis and oxidative stress during chronic renal failure [59]. In vulvar lichen sclerosis, miR-155-5p was found to enhance fibroblast proliferation by downregulating *Foxo3* gene expression [60]. These epigenetic mechanisms may eventually affect FoxO protein expression and subsequent transactivation of downstream targets such as gluconeogenic genes, autophagy genes, and even miRNAs (Figure 3D). Overall, epigenetic regulation of the FoxO–autophagy axis is new and largely unexplored. Future studies are warranted

relaxation, FoxO3 nuclear localization, and augmented transactivation of autophagy genes. PARylation increases FoxO3 nuclear accumulation by blocking the inhibitory phosphorylation (P) that excludes FoxO3 from the nucleus. (C) FoxO1 also augments autophagy activity through epigenetic mechanisms that increase histone acetylation and increase *Foxo1* gene expression, presumably because acetylation-induced chromatin relaxation promotes the accessibility of *Foxo1* promoter to its transcription factors or coactivators (TFs/TCs). Coupled with FoxO1 acetylation, epigenetic mechanisms induce the expression of autophagy and autophagy inducer genes (i.e., *Sesn3*). Grey coloration indicates inactive histone deacetylases (HDACs) as a result of inhibition by HDAC inhibitors (HDACIs). (D) Additional epigenetic mechanisms impacting on FoxO gene and protein expression include DNA methylation, non-coding RNA, and histone modifications that may regulate *Foxo* promoter and enhancer activities. Non-coding RNAs (e.g., miR-378 and miR-205-5p) may also inhibit FoxO protein activity via Akt. Altered expression of FoxO genes or protein activity due to epigenetic reprogramming eventually affects FoxO target genes, including gluconeogenic genes, autophagy genes, and miRNAs.

to establish whether and how the epigenetic memories of FoxOs may affect transgenerational health and disease risk via autophagy.

Autophagy Regulation of FoxO Protein Turnover

FoxO proteins undergo proteasomal degradation following Akt-mediated phosphorylation [16], and this has been linked to autophagy dysregulation and disease progression (e.g., cancer) [66,67,114]. Protein–protein interactions, such as FoxO interacting with XBP1, also promote FoxO turnover via proteasomal degradation [53,95,96]. Recently, the DDB1–CUL4A ubiquitin E3 ligase complex was found to regulate FoxO1 turnover via circadian protein cryptochrome 1 (CRY1) [28]. A substrate for the DDB1 E3 ligase, CRY1 can be ubiquitinated by the DDB1–CUL4A–CDT2 E3 ligase complex and targeted for proteasomal degradation. However, deletion of hepatic *Ddb1* stabilizes CRY1, which induces constant degradation of Foxo1 and suppresses Foxo1-dependent gluconeogenesis [28]. Intriguingly, autophagy can also regulate FoxO1 protein turnover through CRY1, which acts as an autophagy substrate [14]. Specifically, autophagy targets the circadian clock and glucose production by selectively degrading CRY1, which stabilizes and upregulates Foxo1 to induce gluconeogenesis genes in mice during the diurnal window (Figure 4A) [14]. Further study identified several light chain 3 (LC3)-interacting motifs in CRY1 that facilitated autophagosome engulfment of CRY1 for autophagic degradation [14]. The autophagy–CRY1–Foxo1 axis may account for the hyperglycemia and prediabetic phenotype in diet-induced obesity because HFD feeding promotes autophagic degradation of CRY1 and upregulates Foxo1, the key driver of hepatic glucose production (Figure 4A) [14,20,21].

Autophagy inhibitors (e.g., chloroquine or hydroxychloroquine) have been tested in combination with anticancer drugs in clinical studies of cancer therapy [115]. Importantly, autophagy-mediated

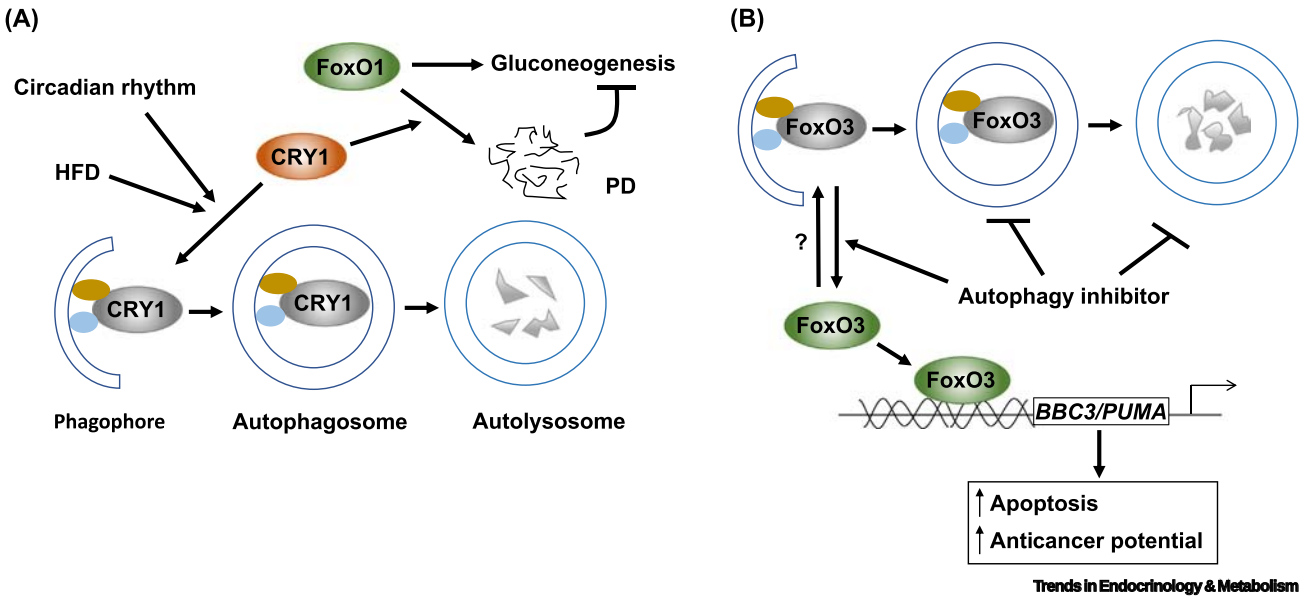

Figure 4. Autophagy Regulates FoxO Protein Turnover. (A) Autophagy prevents proteasomal degradation (PD) of FoxO1 by removing CRY1. Because CRY1-mediated degradation of FoxO1 suppresses gluconeogenesis, circadian removal of CRY1 via autophagy is crucial for maintaining a normal diurnal glucose profile in mice. A high-fat diet (HFD) promotes autophagic degradation of CRY1, contributing to the upregulation of FoxO1-mediated gluconeogenesis and hyperglycemia. The light blue and brown ovals stand for the molecules (e.g., LC3 or other unknown mediators) that facilitate autophagosome engulfment of the target proteins for autophagic degradation. (B) Autophagy promotes FoxO3 degradation in autolysosomes. Inhibition of autophagy leads to accumulation of FoxO3 and induces FoxO3 transactivation of proapoptotic *PUMA* gene, sensitizing cancer cells to chemotherapeutic drugs. What triggers autophagic degradation of FoxO3 and how it is regulated remain to be defined.

FoxO3 protein turnover plays a central role in sensitizing tumor cells to chemotherapeutic drugs [13]. It was found that Foxo3 protein was degraded by basal autophagy, and autophagy inhibition therefore upregulated FoxO3, leading to transactivation of the proapoptotic *BBC3/PUMA* gene and increasing apoptosis sensitization (Figure 4B) [13]. Specifically, the reversal of FoxO3 turnover by an autophagy inhibitor can switch the function of drugs from inhibiting tumor cell growth to promoting tumor cell death (apoptosis), thereby potentiating their anticancer effects. Blockade of FoxO3 binding to the *PUMA* promoter or *PUMA* gene knockdown eliminated the sensitizing effects of autophagy inhibition on tumor cells in response to drug-induced apoptosis [13]. This is the first study showing that FoxO3 protein acts as the cargo (substrate) of autophagy (Figure 4B), and it opens a new window to explore the mechanisms of FoxO protein turnover. For instance, it raises crucial questions regarding the mechanism that directs FoxO3 protein to the pathway of autophagic degradation. Specifically, what are the factors that initiate FoxO3 protein transport and engulfment by autophagosomes (Figure 4B)? Do all the FoxO proteins (FoxO1, FoxO3, FoxO4, and FoxO6) share the same mechanism of autophagy-mediated degradation? If not, what determines the selectivity of autophagic degradation of specific FoxO proteins? What are the physiological and pathophysiological roles of basal autophagy-mediated FoxO turnover in nontumorigenic settings?

Concluding Remarks and Future Perspectives

The FoxO transcription factors (particularly FoxO1 and FoxO3) are well established as the crucial inducers of autophagy. Depending on the tissue type and the physiological context, FoxO-induced autophagy can promote both health and disease. For instance, FoxO-mediated autophagy is crucial for lipid metabolism in the liver and protects against steatosis [70], and it also underlies recovery from alcohol-induced hepatotoxicity and liver ischemia/reperfusion injury [69,81]. In skeletal muscle, however, FoxO-augmented autophagy promotes muscle atrophy [61,76,77,101] and may even contribute to Duchenne muscular dystrophy or Becker muscular dystrophy [108]. Consistent with the notion that FoxO regulates cardiac remodeling [33,34], evidence has emerged to support the role of FoxO-induced autophagy in cardiac atrophy [35,78]. Complexity arises when various physiological contexts are taken into consideration. Activation of the FoxO–autophagy axis during myocardial ischemia compromises mitochondrial metabolism and stimulates cardiomyocyte death, whereas blocking the FoxO–autophagy axis prevents cardiac apoptosis and fibrosis [102]. By contrast, resveratrol induces FoxO autophagy via Sirt1 activation and lessens myocardial injury in diabetic hearts [94]. In diseases (e.g., cancer and obesity) where the cellular microenvironments play a key role [116], profound evidence supports an either preventive or pathogenic role of FoxO/autophagy [10,13,46,49,87,117,118]. In addition, measurements of autophagy using different methods may confound data interpretation, highlighting the importance of examining autophagy flux (rather than the steady-state levels of autophagy proteins) wherever possible [119,120]. Therefore, extra care should be exercised in interpreting the role of FoxO/autophagy in different tissue types, physiological contexts, disease stages, and microenvironments.

Mechanistically, FoxOs can regulate autophagy via transcriptional, post-translational, and epigenetic pathways. As transcription factors, FoxOs transactivate autophagy genes by binding to their promoters to induce autophagy. However, future studies are warranted to determine whether autophagy underpins the physiological or pathophysiological roles of the upstream regulators of FoxOs, including the transcription factors that control *Foxo* transcripts (Figure 1B) and the corepressors that counteract FoxO activity (e.g., CK1α, DGKζ, FCoR, SCP4, and SIN3A) [23,29,43,114,121,122]. At the post-translational level, FoxO may interact with Atg7 protein in the cytoplasm to induce autophagy, but little is known about (i) the mechanism compensating for the loss of nuclear transactivation of autophagy genes cause by cytosolic translocation of FoxOs, (ii) how FoxOs circumvent PTM-induced proteasomal degradation, and (iii) whether FoxO proteins interact with other autophagy proteins, and whether this is a common mechanism for

Outstanding Questions

How do epigenetic mechanisms (DNA methylation, histone modification, and non-coding RNAs) affect the physiological and pathophysiological roles of the FoxO–autophagy axis? Are the effects transgenerational and reversible through interventions?

What triggers FoxO turnover via autophagy? How is this process regulated? Is the mechanism of protein turnover common to all FoxOs? What roles does turnover play in the progression or prevention of diseases other than cancer?

Are FoxOs the major mediators linking HMGA1, STAT1, E2F1, HIF-1α, p53, and GR to autophagy, and what mechanisms are involved?

In addition to FoxO1 and Atg7, do other FoxOs and autophagy proteins bind to each other to induce autophagy in the cytoplasm? Does this represent a common mechanism across different tissues? If not, why not?

To induce autophagy in the cytoplasm, what keeps cytosolic FoxO proteins from proteasomal degradation after Akt-induced phosphorylation and nuclear exclusion? How do cells compensate for downregulation of FoxO-targeted autophagy genes as a result of FoxO nuclear exclusion so as to sustain or induce autophagy activity?

cytosolic FoxOs to induce autophagy. Epigenetic regulation of FoxOs and autophagy has been identified at the level of DNA methylation, histone modification, and non-coding RNAs. However, epigenetic analysis of the FoxO–autophagy axis is still in its infancy, and whether and how epigenetic changes in FoxOs and autophagy affect transgenerational health and disease risks remains elusive. Although the evidence that autophagy regulates FoxO turnover is limited, it opens a new window to study the role of the FoxO–autophagy axis in metabolic syndrome and cancer. Given the multifaceted role of the FoxOs in autophagy, further studies on the underlying mechanisms will be crucial for the development of new therapeutics targeting the FoxO–autophagy axis.

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