How does estrogen work on autophagy?

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ABSTRACT

Macroautophagy/autophagy is vital for intracellular quality control and homeostasis. Therefore, careful regulation of autophagy is very important. In the past 10 years, a number of studies have reported that estrogenic effectors affect autophagy. However, some results, especially those regarding the modulatory effect of 17β-estradiol (E2) on autophagy seem inconsistent. Moreover, several clinical trials are already in place combining both autophagy inducers and autophagy inhibitors with endocrine therapies for breast cancer. Not all patients experience benefit, which further confuses and complicates our understanding of the main effects of autophagy in estrogen-related cancer. In view of the importance of the crosstalk between estrogen signaling and autophagy, this review summarizes the estrogenic effectors reported to affect autophagy, subcellular distribution and translocation of estrogen receptors, autophagy-targeted transcription factors (TFs), miRNAs, and histone modifications regulated by E2. Upon stimulation with estrogen, there will always be opposing functional actions, which might occur between different receptors, receptors on TFs, TFs on autophagy genes, or even histone modifications on transcription. The huge signaling network downstream of estrogen can promote autophagy and reduce overstimulated autophagy at the same time, which allows autophagy to be regulated by estrogen in a restricted range. To help understand how the estrogenic regulation of autophagy affects cell fate, a hypothetical model is presented here. Finally, we discuss some exciting new directions in the field. We hope this might help to better understand the multiple associations between estrogen and autophagy, the pathogenic mechanisms of many estrogen-related diseases, and to design novel and efficacious therapeutics.

Abbreviations: AP-1, activator protein-1; HATs, histone acetyltransferases; HDAC, histone deacetylases; HOTAIR, HOX transcript antisense RNA

Introduction

Macroautophagy/autophagy is a biochemical process necessary for the maintenance of intracellular homeostasis, and its failure can lead to pathological problems such as metabolic diseases, tumors, and developmental defects. Considering that interfering with autophagy is a potential therapy for several diseases, autophagy has become a promising and attractive target for the pharmaceutical industry [1,2]. However, the regulatory mechanisms and pathophysiological states of autophagy have not been fully elucidated, especially for tumors [2,3]. Estrogen is a sex hormone that plays an important role not only in the development of female secondary sexual characteristics, but also in bone development, cardiovascular system protection, and homeostasis [4]. In addition, the occurrence and development of many tumors have been shown to be estrogen dependent [4]. Increasingly, ESR (estrogen receptor) ligands are being designed, synthesized, and used to treat related diseases, and some have already been used clinically [5]. As early as a few decades ago, it was reported that estrogen affects the autophagy of renal tubules [6,7]. In the past 10 years, a number of studies have reported that ESR ligands affect autophagy-related proteins and morphologies in different tissues [8–40]. However, these reports are quite scattered (Table 1, 2). Some results, especially those regarding the modulatory effect on autophagy of 17β-estradiol (E2), the major endogenous estrogen in mammals, seem inconsistent (Table 1) [8–20]. In view of the importance of the crosstalk between estrogen signaling and autophagy, this review summarizes the ESR ligands reported to affect autophagy (characteristic autophagy proteins and key morphologies). In addition, tissue and subcellular distribution of the ESRs, autophagy-targeted TFs, miRNAs, and histone modifications are discussed as well. Finally, we examine the potential crosstalk between estrogen signaling and the autophagy pathway and summarize the molecular mechanisms involved in the regulation of autophagy by estrogen. We hope this will not only enrich our understanding of the known estrogenic regulation, but also promote more insight into the molecular mechanisms of estrogen’s action on autophagy.

Regulation of E2 on autophagy

As early as the 1970s, an association between castration and altered ultrastructural patterns of autophagosomes and lysosomes in different tissues was reported [6,7]. In early reports, castration leads to a higher rate of autophagy with an increased number of lysosomes and degrading organelles, which suggested for the first time that the systemic deprivation of steroid sex hormones could be linked to autophagy [6,7]. However,
both the molecular mechanisms and signaling mediators of autophagy were poorly known then. In recent years, increasing research has shown that autophagy is involved in the effects of estrogen on various diseases (Table 1).

The process of autophagy requires the coordination of numerous proteins. To determine whether autophagy is inhibited or promoted not only depends on the single index of biomarker proteins such as MAP1LC3, but also on changes of autophagy flux, autophagy substrates, and key autophagy-related genes, directly or indirectly through TFs and gene transcription via GPER1 [41]. For example, chloroquine inhibits autophagy by blocking the fusion of lysosomes with autophagosomes, resulting in the accumulation of MAP1LC3-II and autophagosomes. Therefore, some of the actions on autophagy shown in Table 1 need further confirmation. However, it is clear that E2 has multiple effects on autophagy. In many cases, E2 plays a role in promoting autophagy [8–16], but at other times, when cellular autophagy has been stimulated by hypoxia, lipopolysaccharides (LPS), spinal cord injury, or ovariectomy, E2 shows a restrictive effect on gene expression of some autophagy proteins [17–20]. Similar phenomena have also been observed for resveratrol, which can prevent the upregulation of autophagy induced by rapamycin (Table 2).

Endogenous and exogenous ESR ligands perform biological functions with specific receptors, classical nuclear receptors (ESR1 and ESR2), and a membrane receptor, GPER1 (G protein-coupled estrogen receptor 1) [5]. In the classical pathway, activated ESRs undergo conformational changes after binding with estrogen, release from the heat shock proteins (HSPs), and then transfer to the nucleus and form homologous or heterologous dimers. In the nucleus, ESRs interact with the estrogen responsive element (ERE) and regulate the expression of target genes, including some autophagy-related genes, directly or indirectly through TFs [42]. Different from ESRs, GPER1 is expressed only in the cytoplasm and mainly interacts with non-classical EREs in the cytoplasm, which signals through the G protein-coupled receptor (GPCR) pathway (Table 2).

### Table 1. The regulation of E2 on autophagy.

| Ligand | Action | Disease | Cell Line or Animal | Tissue | Dose | Effector proteins | Autophagy morphology | Ref |
|--------|--------|---------|---------------------|--------|------|-------------------|----------------------|-----|
| E2     | Induce | Breast cancer | MCF-7 | breast | 10 nM | MAP1LC3-II/MAP1LC3-I, SQSTM1, BCL2, ER11 | autophagosome | [8] |
|        |        | Hypoxia-induced pulmonary hypertension | PAEC | lung | 10 nM | MAP1LC3-II, ER-independent | NA | [9] |
|        |        | Nephrototoxicity | mProx24 | proximal renal tubule | 10 nM | MAP1LC3-II, MAP1LC3-I, ATG5 | autophagosome | [10] |
|        |        | Osteoporosis | MC3T3-E1 | bone | 0.2 mg/kg/day | MAP1LC3-II, BCL2, BECN1, AKT1, UK1 | NA | [11] |
|        |        | Ovarian cancer | Skov-3, Ovarc-3(HTB-161), A2780 (ESR1^+^), A2780CP (ESR1^-^) | ovary | 10 µM | MAP1LC3-II, MAP1LC3-I, BCL2, BECN1, AKT1, UK1, GAPDH | acidic vesicular organelles | [12] |
|        |        | Parkinson disease | / | CNS | 1 mg/kg/day | MAP1LC3-II, MAPK1 | autolysosomes/autophagosomes | [13] |
|        |        | Renal cell carcinoma | RCC cell lines | kidney | 7 µM | MAP1LC3-II, MAP1LC3-I, SQSTM1, AMBRA1, PIK3C3 | autophagic vesicles | [14] |
|        |        | Testicular germ cell tumors | TCAM2 | testis | 28 µM | MAP1LC3-II, MAP1LC3-I, SQSTM1, BECN1, AMBRA1, PIK3C3, UVRAG, PIK3CA, ER11 | autophagic vesicles | [15] |
|        |        | Heart disease | H9c2 cells | heart | 10 nM | MAP1LC3-II, MAP1LC3-I, ESR2, AKT1, PIK3C3 | NA | [16] |
|        |        | Myocardial injury | cardiomyocytes | heart | 10 nM | MAP1LC3-II, MAP1LC3-I, ATG5, BECN1 | NA | [17] |
|        |        | Spinal cord injury | PC12 | medulla | 20 nM | MAP1LC3-II, MAP1LC3-I, BECN1, ATG5, ATG7 | NA | [18] |
|        |        | Ovariectomy | / | proximal tibia | 10 µg/kg/day | MAP1LC3-II, MAP1LC3-I, SQSTM1, ATG5, BECN1 | acidic vesicular organelles | [19] |

Both endogenous and exogenous ESR ligands perform biological functions with specific receptors, classical nuclear receptors (ESR1 and ESR2), and a membrane receptor, GPER1 (G protein-coupled estrogen receptor 1) [5]. In the classical pathway, activated ESRs undergo conformational changes after binding with estrogen, release from the heat shock proteins (HSPs), and then transfer to the nucleus and form homologous or heterologous dimers. In the nucleus, ESRs interact with the estrogen responsive element (ERE) and regulate the expression of target genes, including some autophagy-related genes, directly or indirectly through TFs [42]. Different from ESRs, GPER1 is expressed only in the cytoplasm and mainly interacts with non-classical EREs in the cytoplasm, which signals through the G protein-coupled receptor (GPCR) pathway (Table 2).

#### ESR distribution in organs and subcellular locations

As shown in Figure 1, compared to ESRs, GPER1 is more widely expressed in human tissues. GPER1 is predominately expressed in the thyroid gland, skeletal muscle, adrenal gland, and kidney [43]. In some organs, both ESR1 and ESR2 are expressed with GPER1 [44], whereas in others, only one subtype of ESR is mainly expressed. It is worth mentioning that only ESR2 has ever been reported to be expressed in the stomach [45].

The expression of these receptors and their intracellular distribution are different in different cell types, and the effects of estrogen on their distribution are also different [46–49]. The subcellular localization of the receptors is closely related to their functions, and it changes in different conditions.
Table 2. The regulation of autophagy by ESR ligands.a

| Disease                  | Tissue                  | Cell line | Ligand               | Type | Dose  | Effector proteins                                      | Autophagy morphology                                      | Ref |
|--------------------------|-------------------------|-----------|----------------------|------|-------|---------------------------------------------------------|-----------------------------------------------------------|-----|
| Breast cancer            | breast                  | MCF-7     | Fulv                 | ER antagonist | 1 μM   | MAP1LC3-II, MAP1LC3-I, BCL2L1, BECN1, MAPK/ERK, AKT1    | autophagosomes                                             | [21,22] |
|                          |                         | T47D, MCF-7, BT-474, tam | 4-OH TAM           | ER antagonist | 0.5, 1, 5 μM | MAP1LC3-II, NO, MAPK1, PLIN2, BCL2 | autophagosomes, lysosomes/autolysosomes increased autophagic vacuoles, increases autophagosomes /MDC-labeled vesicles autophagic flux | [23,24] |
|                          |                         | MCF-7, MCF-7 tamR, LCC2, MDA-MB-231 | TAM       | SERM          | 1, 3, 5 μM | MAP1LC3-II/MAP1LC3-I, SQSTM1, BECN1, MAPK1/3, BAX-BCL2, ATG12–ATG5 | autophagosomes, lysosomes/autolysosomes increased autophagic vacuoles, increases autophagosomes /MDC-labeled vesicles autophagic flux | [21,22,25–28] |
| Diabetic                 | heart                   | H9c2      | resveratrol          | mixed ER antagonist/ antagonist | 100 μM | MAP1LC3/MAP1LC3B, BAX-BCL2 | autophagosome | [31] |
| Cardiac function I       | pituitary tumor         | GH3       | resveratrol          | mixed ER antagonist/ antagonist | 25, 50 μM | MAP1LC3-II/MAP1LC3-I, BECN1, BCL2, | autophagosome formation | [36] |
| Pituitary gland          |                         |           |                      | | | | | |
| Glioblastoma             | glioblastoma            | U87, X1016, JX6 | TAM |               | 9, 12 μM | MAP1LC3-II, MAP1LC3-I, ATG51, MAP1LC3B-II, MAP1LC3B-I, BCL2, ATK1, MAPK1, GPER1 | induces autophagic vacuole formation | [35] |
| Oral squamous cell       | mouth                   | SCC4, SCC9, HSC-3 | G15       | GPER1 antagonist | 0–20 μM | MAP1LC3-II, MAP1LC3-I, IFI27, PARP1, ESR1 | induces autophagy (not alone) | [38,39] |
| Carcinoma                | bone                    | MG63      | 2-ME | GPER1 antagonist | 10 μM | MAP1LC3-II/MAP1LC3-I | autophagosome | [37] |
| Ovarian cancer           | ovary                   | PEO1, BG-1, SKOV-3 | Fulv | | 1 μM | MAP1LC3-II, MAP1LC3-I, IFI27, PARP1, ESR1 | autophagosome | [38,39] |
| Toxoplasmosis            | breast                  | MCF-7     | TAM               | SERM | 5, 10 μM | MAP1LC3-II/MAP1LC3-I | autophagosome, lysosomes/autolysosomes increased autophagic vacuoles, increases autophagosomes /MDC-labeled vesicles autophagic flux | [21,22,25–28] |

a Abbreviations: 2-ME, 2-methoxyestradiol; 4-OH TAM, 4-hydroxytamoxifen; BAX, BCL2 associated X, apoptosis regulator; BCL2, BCL2, apoptosis regulator; Fulv, fulvestrant; MTOR, mechanistic target of rapamycin kinase; MDC, monodansylcadaverine; RICTOR, RPTOR independent companion of MTOR complex 2; SERM, selective estrogen receptor modulator; TAM, tamoxifen. 1, genes are upregulated by ESR ligands unless so marked, which indicates that ESR ligands downregulate them.

cell types and physiological states. For example, upon binding with E2, ESRs located in endothelial cytosolic/nuclear and endothelial surface membranes are upregulated and activate NOS3 (nitric oxide synthase 3) via genomic and nongenomic pathways, respectively [50]. The nitric oxide (NO) synthesized in this manner can suppress MTOR expression and induce autophagy [51]. Mitochondrial ESRs (mtESRs) are expressed in many human cells including cardiac muscle, lens epithelium, and osteosarcoma, hepatoma, and breast cancer cells [52–55], and mediate the synthesis of mitochondrial respiratory chain proteins (MRCs) via induction with E2 [55–57]. The expression levels of mtESRs also increase with exposure to E2 [55–57]. However, the fate of mtESRs and their role during selective mitochondrial autophagy (mitophagy) is not yet clear. The subcellular localization of GPER1 might be related to different tumor characteristics. GPER1, being localized in the cytoplasm in breast cancer, is correlated with non-ductal carcinoma with better tissue differentiation and lower tumor grade, which is more common in luminal A and B breast cancers [58]. In contrast, when GPER1 is localized in the nucleus, tumors are poorly differentiated or there is triple-negative breast cancer (TNBC), and the prognosis is even worse [58]. Both ESR1 and GPER1 are distributed in lysosomes in an E2-dependent manner, revealing the existence of a lysosome-dependent degradation pathway for these receptors [59,60]. Whether ESR1 and GPER1 distributed in lysosomes participate in autophagy upon fusion with autophagosomes, and their corresponding pathophysiological effects, is unclear.

In general, the intracellular translocation of these receptors remains controversial and appears to be frequently determined by estrogen, though they are clearly involved in the association between estrogen and autophagy (Figure 2).

TFs involved

As shown in Figure 3 and Table 3, all of the receptors that might mediate the regulation of estrogen on core autophagy proteins via TFs. These TFs are involved in the regulation of all autophagic processes, including autophagy induction, vesicle nucleation and elongation, and retrieval.

CEBPB

CEBPB links autophagy to the biological clock and maintains nutrient homeostasis by stimulating autophagic gene expression [61]. The increase of nuclear CEBPB protein is dependent on MAPK14. The phosphorylation of MAPK14 is in turn dependent on PLA2G1B (phospholipase A2 group IB), PRKC/PKC activation, and oxidative stress [62]. Expression levels of ESRs and PRKCA are inversely related [63]. CREBBP/CREB
CREB binding protein) controls CEBPB expression by interacting with 2 sites near the TATA box [64]. Recent research has shown that the breast tumor cell-activated phosphoinositide 3-kinase-AKT1 signaling pathway induces cytoplasmic GPER1 translocation of carcinoma-associated fibroblasts in an XPO1 (exportin 1)-dependent pattern, and leads to the activation of the estrogen-GPER1-cAMP-PRKA/PKA-CREB signaling axis that triggers the aerobic glycolysis switch in carcinoma-associated fibroblasts [65].

**FOXO3**

The *Atg14* gene is a direct target of FOXOs, and FOXOs positively regulate *Atg14* gene expression [66]. In skeletal muscle, both the ubiquitin-proteasomal and autophagic-lysosomal pathways, the 2 major systems of protein breakdown, are under control of FOXO3. BNIP3 appears to mediate the effect of FOXO3 on autophagy [67]. The activation of FOXO3 by PRKAA2 (protein kinase AMP-associated catalytic subunit alpha 2) induces the expression of MAP1LC3-II, ATG8, BECN1, and BNIP3 in
mouse skeletal muscle [67,68]. Constitutive activation of FOXO3 induces MAP1LC3-II and BNIP3 expression and leads to reversible heart atrophy in active Foxo3 transgene mice by constitutive tetracycline regulation [69]. E2 strongly enhances the effects of FOXO3 via ESR1 [70]. In addition, estrogen mediates the inactivation of FOXO3 by GPER1 [71]. FOXO3 proteins in human and mouse have the same AKT1 phosphorylation sites and similar regulatory properties [71].

**TP53**

TP53 is present in both the nucleus and cytoplasm, and its regulation in autophagy depends on its subcellular localization. The target autophagy genes of TP53 include ULK1, ULK2, ATG2B, ATG4A, ATG7, ATG10, VMP1 (vacuole membrane protein 1), and UVRAG [72]. Initially, ESR1 was reported to be a suppressor of TP53 that can recruit nuclear receptor corepressors (NCOR1 and NCOR2) and HDAC1 (histone deacetylase 1) [73]. However, recent research has shown that ESR2 can attenuate the crosstalk between ESR1 and TP53 by reducing the recruitment of both NCOR1 and NCOR2 by ESR1. ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation by reducing ESR1-TP53 binding via physical interaction [74]. In addition, the MDM2 proto-oncogene, which is both a TP53 target gene and a negative feedback regulator of TP53, has bidirectional effects on ESR1 by direct interaction [75].

**ZKSCAN3**

ZKSCAN3 and TFEB regulate lysosomal biogenesis and autophagy in an opposing manner [76]. ZKSCAN3 is a repressor of several autophagy-related genes, including MAP1LC3 and WIPI2 (human homologs of yeast Atg18). WIPI2 has a positive role in the cycling of ATG9. ZKSCAN3 and TFEB are regulated in an opposing manner [76]. For instance, PRKC leads to reduced phosphorylation, nuclear translocation, and activation of TFEB by inactivating GSK3B with a MTORC1-independent mechanism; however, PRKC activates MAPK8/JUN NH2-terminal kinase (mitogen-activated protein kinase 8) and MAPK14, which phosphorylate ZKSCAN3, leading to its translocation and inactivation. ZKSCAN3 phosphorylation at Thr153 by MAPK9 or MAPK14 is required for ZKSCAN3 cytoplasmic translocation.
| Table 3. Autophagy proteins regulated by E2 via TFs. |
|---------------------------------------------------|
| **Autophagy proteins** | **TFs** | **Effect of TFs on** | **Tissue or Cell Line** | **Ref** | **Receptor** | **Effect of E2 on TFs** | **Tissue or Cell Line** | **Ref** |
|------------------------|---------|----------------------|-------------------------|---------|-------------|-------------------------|-------------------------|---------|
| **Regulation of autophagy induction** | | | | | | | | |
| ULK1 | CEBPB | Enhance | Mouse liver | [61] | ESR1 | PRKCA enhances CEBPB by MAPK14 | HL-60 | [62] |
| | | | | | | PRKCA and ESR1 are inversely related | Breast cancer | [63] |
| | GPER1 | E2 activates GPER1-CAMP-PRKCA-CREB | | | | Rat liver | [65] |
| FOXO3 | Enhance | Mouse skeletal muscle | | [68] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | GPER1 | E2 inactivates FOXO3 | | | | MCF-7 | [71] |
| TP53 | Enhance | Human HCT116 cells | | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| ZKSCAN3 | Suppress | Human HeLa cells | | [145] | ESR1 | ESR1 is inversely related to PRKCA | Mouse | [77] |
| | GPER1 | Estrogen inactivates ZKSCAN3 by MAPK14 | | | | HeLa, HKB293, HepG2 | MDA-MB-231 | [83] |
| Vesicle nucleation | | | | | | | | |
| ATG14 | FOXO3 | Enhance | Mouse liver | [66] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | GPER1 | Estrogen inactivates FOXO3 | | | | MCF-7 | [71] |
| BCL2 | NFKB1 | Enhance | Rat hippocampal neurons | [78] | ESR1 | ESR1 inhibits NFKB1 activity | HL-60, HeLa | [82] |
| | | | | | | MCF-7, HeLa, HKB293, HepG2 | MDA-MB-231 | [83] |
| STAT3 | Suppress | B cell lymphoma | | [84] | ESR1 | ESR1 increases STAT3 transcription | MCF-7 | [86] |
| | GPER1 | Activation of GPER1 decreases the transcriptional activities of NFKB1 | | | | MDA-MB-231 | [83] |
| TPS3 | Suppress | Human HCT116 cells | | [72,146] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| BECN1 | FOXO3 | Enhance | Mouse skeletal muscle | [68,147] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| JUN | Enhance | Human cancer cells CNE2 and Hep3B | | [92] | ESR1 | E2 induces the MAPK1/3, JUN, and MAPK14-dependent mitochondrial apoptotic pathway | Mouse spermatocyte-derived cell | [94] |
| | STAT3 | Suppress | | | | MCF-7 | [95] |
| | GPER1 | GPER1 decreases STAT3 | | | | HeLa, U937, HCA58, COV434 | MDA-MB-231 | [83] |
| PIK3C3 | FOXO3 | Enhance | Mouse skeletal muscle | [67] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | GPER1 | GPER1 decreases NFKB1 | | | | MCF-7 | [71] |
| UVRAG | TPS3 | Enhance | Human HCT116 cells | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| TFEB | Enhance | Mouse liver | | [87] | ESR1 | MTORC1 inhibits TFE | MCF-7 | [91] |
| | | | | | | (p)-MTORC1 expression is mainly related to ER | GC-2 | [94] |
| NFKB1 | Enhance | Human T-cells | | [79] | ESR1 | ESR1 inhibits NFKB1 | MCF-7 | [95] |
| | | | | | | MCF-7, HeLa, HKB293, HepG2 | HeLa, U937, HCA58, COV434 | [82] |
| PIK3C3 | FOXO3 | Enhance | Mouse skeletal muscle | [67] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | GPER1 | GPER1 decreases NFKB1 | | | | MCF-7 | [71] |
| UVRAG | TPS3 | Enhance | Human HCT116 cells | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| TFEB | Enhance | Mouse liver | | [87] | ESR1 | MTORC1 inhibits TFE | MCF-7 | [91] |
| | | | | | | (p)-MTORC1 expression is mainly related to ER | GC-2 | [94] |
| Vesicle elongation | | | | | | | | |
| ATG4 | FOXO3 | Enhance | Mouse skeletal muscle | [147] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | TFEB | Enhance | Mouse liver | [87] | ESR1 | MTORC1 inhibits TFE | MCF-7 | [71] |
| | | | | | | (p)-MTORC1 expression is mainly related to ER | GC-2 | [94] |
| TP53 | Enhance | Human HCT116 cells | | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| | | | | | | MCF-7 | [74] |
| ATG7 | FOXO3 | Enhance | Mouse skeletal muscle | [147] | ESR1 | E2 enhances FOXO3 | MCF-7 | [70] |
| | TFEB | Enhance | Mouse liver | [87] | ESR1 | MTORC1 inhibits TFE | MCF-7 | [71] |
| | | | | | | (p)-MTORC1 expression is mainly related to ER | GC-2 | [94] |
| | TP53 | Enhance | Human HCT116 cells | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| | | | | | | MCF-7 | [74] |
| | ATG10 | TP53 | Enhance | Human HCT116 cells | [72] | ESR1 | ESR1 represses TP53-mediated transcription | MCF-7 | [73] |
| | ESR2 | ESR2 antagonizes ESR1-TP53-mediated transcriptional regulation | | | | MCF-7 | [74] |
| | | | | | | MCF-7 | [74] |
| (Continued)
| Table 3. (Continued). |
|-----------------------|
| **Autophagy** |
| **Proteins** | **Effect of TFs on** | **Tissue or Cell Line** | **Ref** |
| CEBPB | Enhance | MCF-7, HeLa, HEK293, HepG2 | [61] |
| ERα | Enhance | MCF-7, HepG2, COV434, COV34A | [62] |
| PRKCA | Enhance | MCF-7, HeLa, HEK293, HepG2 | [63] |
| ESR1 | Enhance | MCF-7, HeLa, HEK293, HepG2 | [64] |
| GPER1 | Enhance | MCF-7, HEK293, COV434 | [65] |
| TFEB | Enhance | MCF-7, HeLa, HEK293, HepG2 | [66] |

**Abbreviations:** A549, human lung adenocarcinoma cells; CEBPB, CCAAT enhancer binding protein beta; COV434, Human ovarian granulosa tumor cells; COV34A, human granulosa cell tumors; GC-2, a mouse spermatocyte cell line; GC-2, mouse spermatocyte cell line; GCT, granulosa cell tumors; HCASMC, human coronary artery smooth muscle cells; HCT116, human colon carcinoma cells; HEK293, human embryonic kidney 293 cells; HepG2, human liver cancer cell line; HIF1A, hypoxia inducible factor 1 alpha; HL-60, human promyelocytic cells; HUVEC, human umbilical vein endothelial cells; IMR90, normal human lung fibroblasts; JNK2, mitogen-activated protein kinase 9; MAPK14/p38, mitogen-activated protein kinase 14; MTORC1, mechanistic target of rapamycin kinase complex 1; NFKB1, nuclear factor kappa B subunit 1; PRKC/PKC, protein kinase C; SMCs, human coronary artery smooth muscle cells; STAT3, signal transducer and activator of transcription 3; SW480, a p53 double-mutant cell line; TFE3, transcription factor E3; U87, human primary glioblastoma cells; U937, human macrophage cells.
and promotes lysosome biogenesis [77]. The expression levels of ESRs and PRKC are inversely related [63].

**NFKB1**

NFKB1 upregulates the expression of both BCL2 and BECN1 [78,79]. However, NFKB1 silences Bnip3 gene transcription by competing with E2F1 (E2F transcription factor 1) for binding to the Bnip3 promoter [80]. The ubiquitin-binding protein SQSTM1 is required for RAS-induced NFKB1 activation in human tumors [81]. ESRs mediate the inhibition of NFKB1 activity at several levels [82]. Activation of GPER1 by the specific agonist G-1 leads to significant inhibition of the phosphorylation, nuclear localization, and transcriptional activities of NFKB1 [83]. Both IL6 (interleukin 6) and VEGFA (vascular endothelial growth factor A) are inhibited accordingly [83].

**MiRNAs involved**

In addition to the transcription factors listed above, E2 can regulate the expression of some autophagy-related proteins by suppressing or stimulating miRNA expression.

**HIF1A**

The expression of BNIP3, BECN1, and ATG5 required for selective mitochondrial autophagy depends on HIF1A [96]. ESR2 inhibits HIF1A activity by decreasing the binding of HIF1A to the promoter of the VEGFA gene, which attenuates VEGFA transcription induced by hypoxia [97]. Activation of GPER1 by both E2 and G-1 increases VEGFA via upregulation of HIF1A via the GPER1-EGFR-MAPK1-FOS signaling pathway [98]. The HIF1A gene itself contains an estrogen response element, and its expression is directly regulated by ESR1 [99].

**TFEB**

As a master regulator of lysosomal biogenesis, TFEB promotes the transcription of several lysosomal genes by directly binding to their promoters. Autophagy-related genes Map1lc3, Sqstm1, and Atg9 are also upregulated by TFEB [76,87]. Under well-nourished conditions, MTORC1 inhibits TFEB by its post-translational modification of phosphorylation. In contrast, under starvation conditions, lysosomal disruption and pharmacological inhibition of MTORC1 activates TFEB [88,89]. Upon estrogen stimulation, ESR1 binds to RPTOR (regulatory associated protein of MTOR complex 1) [90]. The MTOR kinase can phosphorylate Ser104/106 of ESR1 and promote the transcription of ER target genes [90,91].

**JUN**

JUN is involved in the regulation of both BECN1 and MAP1LC3 transcription [92,93]. In ESR- or GPER1-positive GC-2 cells, E2 induces the MAPK1/3, JUN, and MAPK14-dependent mitochondrial apoptotic pathway [94]. In ESR-negative GPER1 or CYP19A1/ aromatase-positive SKBR3 cells, tamoxifen acts as a GPER1 agonist and recruits the FOS-JUN complex to AP-1-responsive elements that are located within the CYP19A1/ aromatase promoter [95].

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**STAT3**

Functional crosstalk between BCL2, RAC1, and activated STAT3 exists and promotes a permissive redox milieu for cell survival [84]. STAT3 signaling events occur downstream of MMP14/MT1-MMP (matrix metalloproteinase 14). Gene silencing of MMP14 and STAT3 abrogates both STAT3 phosphorylation and Bnip3 expression [85]. Activation of GPER1 inhibits HIF1A and STAT3 signals in TNBC cells [83]. Upon LEP (leptin) treatment, ESR1 increases the transactivation and target gene expression of STAT3 by direct binding. In addition, the enhancement of LEP-mediated activation of STAT3 is independent of ESR1 ligands [86].

**MiR214**

Estrogen treatment downregulates the MiR214 family [100]. MiR214 directly targets mitochondrial uncoupling proteins (UCPs) [101]. UCPs are members of the larger family of mitochondrial anion carrier proteins (MACPs), and UCP2 is involved in uncoupling oxidative phosphorylation and facilitating energy dissipation as heat [102]. UCP2 overexpression not only decreases nonspecific destructive intracellular reactive oxygen species (ROS), but also induces autophagy and endocrine resistance by the phosphorylation of AKT1 and MTOR [21].

**MiR21**

Regulation of MiR21 transcription by E2 varies depending on experimental conditions, cell line, and control genes used in the analysis. In HepG2 human hepatoma cells, E2 inhibits MiR21 expression via ESR1 [103]. In MCF-7 cells, MiR21 is upregulated by the ESR2 selective ligand diarylpropionitrile (DPN) [104]. In systemic lupus erythematosus, estrogen-regulated STAT1 activates and promotes TLR8 expression via MiR21 [105]. Both the expression and functional loss of STAT1 are related to the development of mammary adenocarcinomas [106]. STAT1 suppresses ATG12 and BECN1 [107]. MiR21 also targets BCL2 mRNA in rat and human beta cells [108].

**Long noncoding RNA HOTAIR**

E2-GPER1 induces HOTAIR through the suppression of MiR148A [109]. In hepatocellular carcinoma, HOTAIR activates autophagy by upregulating ATG3 and ATG7 [110]. In endometrial cancer cells, HOTAIR regulates autophagy and contributes to cisplatin-induced resistance [110]. In addition, BECN1, multidrug resistance (MDR), and ABCB1 (ATP binding cassette subfamily B member 1) are all regulated by HOTAIR [111].
**MIRLET7G**

In MCF-7 cells, E2 suppresses the expression of MIRLET7G in a time- and MAP2K/MEK-MAPK-dependent manner [104]. Mirlet7g regulates autophagy in mouse granulosa cells by targeting IGFR1 (insulin like growth factor 1 receptor) and downregulates AKT-MTOR [112]. Direct regulation of MIRLET7G by E2 via ESR1 has been reported [104,113].

In addition, MIR30D increases with hormone deprivation in MCF-7 cells. MIR30D regulates autophagy genes including BECN1, ATG5, ATG12, and ATG2 [76]. However, dextran-coated charcoal stripping to treat serum in the original report could eliminate many hormones from the serum, which leaves the effect of estrogens on MIR30D unclear [104].

**Histone modifications involved**

Histones are the chief protein components of chromatin. Various posttranslational modifications on histones are mainly produced by histone-modifying enzyme complexes in a dynamic manner [76]. These post-translational modifications, including acetylation and methylation, can influence the overall chromatin structure and have clear functional consequences [76]. Acetylation is a modulator of the ligand-dependent gene regulatory activity of ESRs [114]. Estrogen can interfere with the nuclear regulation of autophagy by regulating proteins in histone modifier complexes. Such regulation of histone modification by estrogen is likely to play a role in estrogen-dependent signaling pathways in a variety of estrogen target tissues in both normal and pathological states.

**HDACs**

In skeletal muscle, HDAC1 and HDAC2 regulate autophagy by inducing autophagic gene expression and autophagosome formation. Loss of these HDACs leads to the accumulation of toxic autophagic intermediates in the myofibers of mice [115]. In cardiomyocytes, HDAC inhibitors suppress autophagy. HDAC1 and HDAC2 are required for the autophagic response, and the overexpression of HDAC2 alone increases autophagy [116]. E2 or dipropyltinrile and β-LGND2 (ESR2 agonists) comparably suppress HDAC2 production, phosphorylation, and the resulting prohypertrophic mRNA expression induced by angiotensin II [117]. The expression of ESR1 can be reactivated by destabilization or reduction of the corepressor complex, formed by MTA1 (metastasis associated 1), IFI16 (interferon gamma inducible protein 16), and HDACs, on the ESR1 promoter [118].

**HATs**

Several HATs have ubiquitination-linked enzymatic activity. As a HAT, KAT2B (lysine acetyltransferase 2B) acetylates both histone and non-histone proteins and promotes autophagy by inhibiting the AKT1-MTOR signaling pathway [119]. DNA binding and transactivation activities of ESR1 are enhanced by acetylation at lysines 266 and 268 by EP300, which can be reversed by native deacetylases such as SIRT1 (sirtuin 1) [114]. KAT7, another HAT, promotes the degradation of ESR1 through ubiquitination in a proteasome-dependent manner. KAT7 knockdown promotes ESR1 expression [120]. In vitro, E2 inhibits the E3 ubiquitin ligase activity of KAT7 on ESR1 [120].

**Post-translational status of histone H3**

ESR1 silences TP53-activated transcription by directing the assembly of SUV39H1/histone-lysine N-methyltransferase (suppressor of variegation 3–9 homolog 1) and histone H3 lys9 trimethylation (H3K9me3) at estrogen-repressed genes [74]. ESR2 downregulates SUV39H1/H2 and releases the ESR1-induced transcriptional block by abrogating the repressive heterochromatin conformation of H3K9me3 [74]. Furthermore, ESR2 stimulates the accumulation of trimethylated histone H3 lys4 (H3K4me3) and POLR2A/RNA polymerase II on ESR1-repressed genes, which then induces the transcription of the repressed genes involved in TP53-based tumor suppression [74].

**Balancing acetylation at H4K16**

Dynamic histone modifications play a pivotal role in cell-regulatory events [121]. The acetylation of histone H4 at lysine 16 (H4K16) can influence higher-order chromatin structure, which plays an important role in transcription [76]. Acetylated H4K16 is a primary histone substrate of native deacetylase SIRT1 [76]. In addition, SIRT1 has several non-histone targets, including ATG5, ATG7, MAP1LC3, FOXOs, E2F1, TP73, PPARC1A (PPARG coactivator 1 alpha), NFKB1, and TP53, all of which are involved in the regulation of autophagy [76]. In oxygen-glucose deprived neurons, estrogen promotes PRKAA2 activation through ESR1 [122]. Estrogen increases SIRT1 expression and activation [123]. In sirt1-knockout neurons, estrogen-induced PRKAA2 activation disappears, which prevents the neuroprotection of estrogen [122].

**Concluding remarks and future perspectives**

The modulation of estrogenic effects relating to autophagy could contribute to the development of potential strategies to treat numerous human diseases (Table 1, 2). To clarify the estrogenic function of autophagy between normal physiological and pathological conditions, including tumors, there have been several studies on the estrogenic regulation of autophagy in ESR-positive breast cancers [124–128]. Arguably, much of what is known has come from this area, reflecting the fact that breast cancer is the most common cancer in women, and 70% are treated with drugs that target ESR action. The roles of autophagy in cancer have been confounded by numerous laboratory studies showing that both the enhancement and inhibition of autophagy seem to improve cancer treatment outcomes [129,130]. A similar situation is also seen in other diseases, such as clinically relevant murine models of ischemic stroke [131]. Moreover, several clinical trials are already in place combining both autophagy inducers (rapamycin, everolimus) and autophagy inhibitors (chloroquine, 3-methyladenine) with endocrine therapies [132,133]. Not all patients experience benefit, which further confuses and complicates our understanding of the main effects of autophagy in breast cancer.
Based on previously reported models [134,135], a hypothetical model (Figure 4) is presented here to help understand how the estrogenic regulation of autophagy affects cell fate. Autophagy is vital for intracellular quality control and homeostasis. Therefore, careful regulation of autophagy is very important because either excessive or insufficient autophagy can be destructive to cells [136]. It has been proven that estrogen can delay the apoptosis of breast cancer cells via ESRs [137]. For the treatment of estrogen-related cancer cells, estrogen needs to be antagonized, which might be because of the protective effect of estrogen on cell balance via regulation of autophagy as shown here.

As shown in this review, upon stimulation with estrogen, most autophagy-related genes are under the regulation of more than one TF, and most TFs are under the regulation of 2 or 3 receptors of estrogen. In addition, estrogen can interfere with autophagy via nuclear regulation by histone modifications. Thus, there will always be opposing functional actions, which might occur between different receptors, receptors on TFs, TFs on autophagy genes, or even histone modifications on transcription. In addition to those mentioned above, such as ESR1 and ESR2 [74], TFEB and ZKSCAN3 [76], and TP53 and MDM2 [75], need to be further explored in the future. The huge signaling network downstream of estrogen can promote autophagy and reduce overstimulated autophagy at the same time, which allows autophagy to be regulated by estrogen in a restricted range. Estrogen's mechanism of balancing autophagy is obviously more

![Figure 4](image-url)

**Figure 4.** Model of how estrogenic regulation of autophagy affects cell fate. E2 helps maintain moderate autophagy and cellular homeostasis. Both deficient and excessive autophagy are abnormal. Deficient autophagy can lead to unfolded protein response (UPR) stress, which may reestablish homeostasis through the induction of autophagy. However, the UPR can further lead to carcinogenesis. The proliferation of cancer may induce a status of hypoxia and starvation, both of which can induce autophagy. Here, if a new balance is achieved, cells still have a chance to survive, which is bad for the treatment of cancer. Only when excessive autophagy releases enough calcium from the endoplasmic reticulum to the cytoplasm can apoptosis be triggered. Furthermore, activated caspases will cleave BECN1 and turn off autophagy. Autophagy inducers may prevent carcinogenesis when autophagy in non-cancer cells is deficient, or they may promote excessive autophagy in cancer cells and lead to apoptosis. Autophagy inhibitors seem to block the survival of cancer cells during starvation; however, the inhibition of autophagy cannot persist. The persistent stimulation of UPR stress also promotes autophagy.
complicated than this. For instance, estrogen in the blood can cause negative feedback to reduce levels of hormones in the body. In addition to estrogen, other hormones, such as androgens, also play a role in autophagy [138]. Some estrogen-regulated TFs and miRNAs can target ESRs, thereby forming another negative feedback loop to fine-tune estrogen-mediated cellular responses, including autophagy [104,139].

Because autophagy is tightly controlled by estrogen (Figure 5), it would make sense to determine the fluctuation range of autophagy corresponding to estrogenic regulation under various physiological and pathological conditions. This leads to a major problem for autophagy research – autophagy is a dynamic process that is hard to assess in living organisms (especially humans) by existing methods in vitro [129]. Considering the correlation between autophagy and cell substance balance, if we indirectly characterize autophagy by assessing the material and energy balance in cells instead of focusing solely on the process of autophagy itself, it will be easier to choose between autophagy inducers and inhibitors in the future. Of course, the optimal choice of markers remains to be determined. However, it is foreseeable that developing dynamic markers of cellular balance will be conducive to studying autophagy manipulation and clinical therapeutics.

So far, studies of estrogen function on autophagy have mainly focused on breast tissue, however there are still many issues that need to be clarified. Take for example the TFs: although the effects of estrogen on TFs have mostly focused on breast cancer cells, the effects of TFs on autophagy are rarely seen in breast cancer cells (Table 3). In other tissues such as brain and bone, the role of autophagy has also been studied for its importance [140,141]. With regard to estrogenic regulation of autophagy in these tissues, there are also some related studies (Tables 1 and 2). Except for the issues mentioned above for breast cancer, more issues need to be solved for the diseases of these tissues. Take for example gliomas: there have been no clinical studies combining both autophagy inducers and autophagy inhibitors with ESR ligands, though they all have independent clinical trials [142,143]. The specificity of drug distribution in these tissues also need to be addressed [143].

![Figure 5. Association between estrogen and autophagy. E2 balances the expression of core autophagy proteins through diverse transcription factors, miRNAs, and histone modifications via signaling pathways downstream of the receptors. The autophagic proteins controlled by E2 are involved in the entire process of autophagy. Lipids released by autophagy are the major source of cholesterol, the precursor of estrogen biosynthesis. E2 in the blood causes a negative feedback to reduce circulating levels of hormones. E2 activates NOS3 and initiates the synthesis of NO via membrane ESRs. NO induces autophagy by suppressing MTOR expression. In addition, some estrogen-regulated TFs and miRNAs can target ESRs. As the major mechanism for ESR degradation in eukaryotic cells, ESRs dissociate from complexes with HSPs upon binding of E2, are ubiquitinated by ubiquitin ligases (ULs), and are targeted for degradation. The fate of mtESRs and lysosomal ESR1 and GPER1 during autophagy is not yet clear. AC, acetylation; GF, growth factor; HSPs, heat-shock proteins; HRAS, HRas proto-oncogene, GTPase; Me, methylation; RAF1, Raf-1 proto-oncogene, serine/threonine kinase; RTKs, receptor tyrosine kinases; SRC, SRC proto-oncogene, non-receptor tyrosine kinase; ULs, ubiquitin ligases.](image-url)
In addition, although GPER1 is more widely distributed in human tissues than ESRs and is expressed in TNBC cells and associated with drug resistance of ESR antagonists in endocrine therapy, GPER1 antagonists are very few and have not been developed to work in humans [5]. Whether ESR1 and GPER1 distributed in lysosomes participate in autophagy upon fusion with autophagosomes, and the corresponding pathophysiological effects, are not clear. High levels of some autophagy biomarkers such as MAP1LC3, or autophagy morphologies such as autophagosomes triggered by the induction or accumulation caused by autophagy inhibition, need stricter distinction in future research. These directions might allow us to better understand the multiple associations between estrogen and autophagy, the pathogenic mechanisms of many estrogen-related diseases, and to design novel and efficacious therapeutics.

Acknowledgments

We thank Dr. Wei Liu and Dr. Eugene Chun, School of Molecular Sciences and Biodesign Institute, Arizona State University, USA, for critical reading of the manuscript. We thank Dr. Nian-Hong Chen, Laboratory of Signal Transduction, Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, USA, for helpful discussion of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81502311].

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