Chapter

Endoplasmic Reticulum Stress during Mammalian Follicular Atresia

Nayeli Torres-Ramírez, Rosario Ortiz-Hernández, M. Luisa Escobar-Sánchez, Olga M. Echeverría-Martínez and Gerardo H. Vázquez-Nin

Abstract

Follicles are ovarian structures that contain a single germ cell. During the mammalian reproductive lifetime, ovarian follicles mature through the process of follicular development, with the aim of selecting oocytes for ovulation. As part of this process, several follicles are eliminated by means of follicular atresia, a mechanism that mainly involves apoptosis. Nevertheless, it has been shown that there are other routes of programmed cell death in the ovary including autophagy, paraptosis, and necroptosis. Surprisingly, the endoplasmic reticulum is involved in these different programmed cell death pathways. Moreover, there are several evidences for the pathways triggered by intra- and extracellular signals in endoplasmic reticulum-induced cell death. Thus, it is important to analyze the participation of endoplasmic reticulum in follicular atresia.

Keywords: ovary, follicular atresia, endoplasmic reticulum, apoptosis, autophagy, paraptosis, necroptosis

1. Introduction

The endoplasmic reticulum plays several important roles in normal cellular physiology. Some functions include protein synthesis, folding, and distribution to the Golgi apparatus. Alterations in protein synthesis inside the endoplasmic reticulum have been related to the trigger of different programmed cell death routes such as necroptosis, apoptosis, autophagy, and paraptosis, with apoptosis being the most studied process.

The mammalian ovary is an excellent model to study the mechanisms of programmed cell death because 99% of the follicles, the functional units of the ovary, undergo degeneration through follicular atresia, which maintains intraovarian homeostasis. Follicular atresia involves the physiological elimination of most germinal cells (oocytes) before they are ovulated, both in fetal and reproductive lives.

The presence of different programmed cell death pathways in follicular atresia have recently been shown, and these can be directly related to endoplasmic reticulum signaling. In this chapter we describe evidences of the linkage between endoplasmic reticulum alterations and programmed cell death, with special emphasis on follicular atresia.

2. Follicular development and atresia

The mammalian ovary is a paired organ that is responsible for generating competent oocytes for successful fertilization and early embryonic development. To do this, these germinal cells need to mature within transient functional complexes called follicles. Follicles form for an oocyte surrounded by somatic cells. During reproductive life, follicles are continuously recruited into the pool of growing follicles and change their size, morphology, and physiology, leading to different stage classifications including primordial, primary, secondary, and antral (**Figure 1**).

At birth, the ovaries contain a fixed number of nongrowing primordial follicles, characterized by an oocyte enclosed by flattened pre-granulosa cells. In primary follicles, the oocyte is surrounded by a monolayer of cubical granulosa cells. Secondary follicles are formed by two or more layers of granulosa cells. Antral follicles accumulate fluid and develop an antral cavity. The accumulation of fluid is useful for transporting nutrients and waste products.

Follicular growth is a continuous process that is under strict control by hormones, growth factors, cytokines, and environmental factors. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), insulin-like growth factor (IGF)-I, and estradiol are the principal regulators of follicular growth. FSH, a gonadotropin secreted by the pituitary gland, together with estradiol and IGF-I, is responsible for stimulating follicular growth and maturation. Moreover, FSH, LH, and estradiol enhance IGF-I secretion [1]. Additionally, FSH stimulates granulosa cells to develop LH receptor sites. The main function of LH is stimulating ovulation.

Several follicles grow and undergo ovulation, releasing an oocyte that is available for fertilization, but the principal destiny of ovarian follicles is follicular atresia, which is a physiological process that eliminates more than 99% of the follicles. Follicular atresia can occur in all stages of follicular development and ensures that only healthy follicles that contain optimal quality oocytes will be ovulated. Follicular degeneration occurs by programmed cell death (PCD). Apoptosis is the main route of follicular atresia, but may not be the only process involved (**Figure 2**). Other forms of PCD such as autophagy and paraptosis may also participate in this process [2–4].

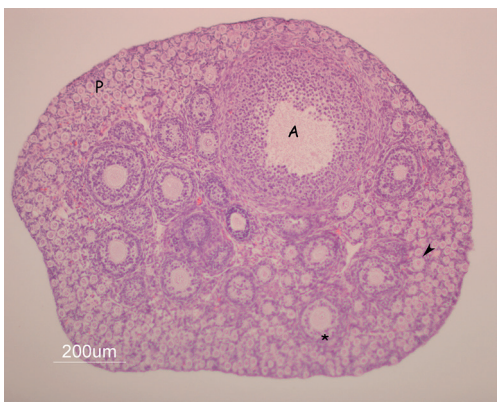

Figure 1.
Ovary of mouse. Follicles are in different stages of growth. Primordial (P), primary (head arrow), secondary (asterisk), and antral (A) follicles.

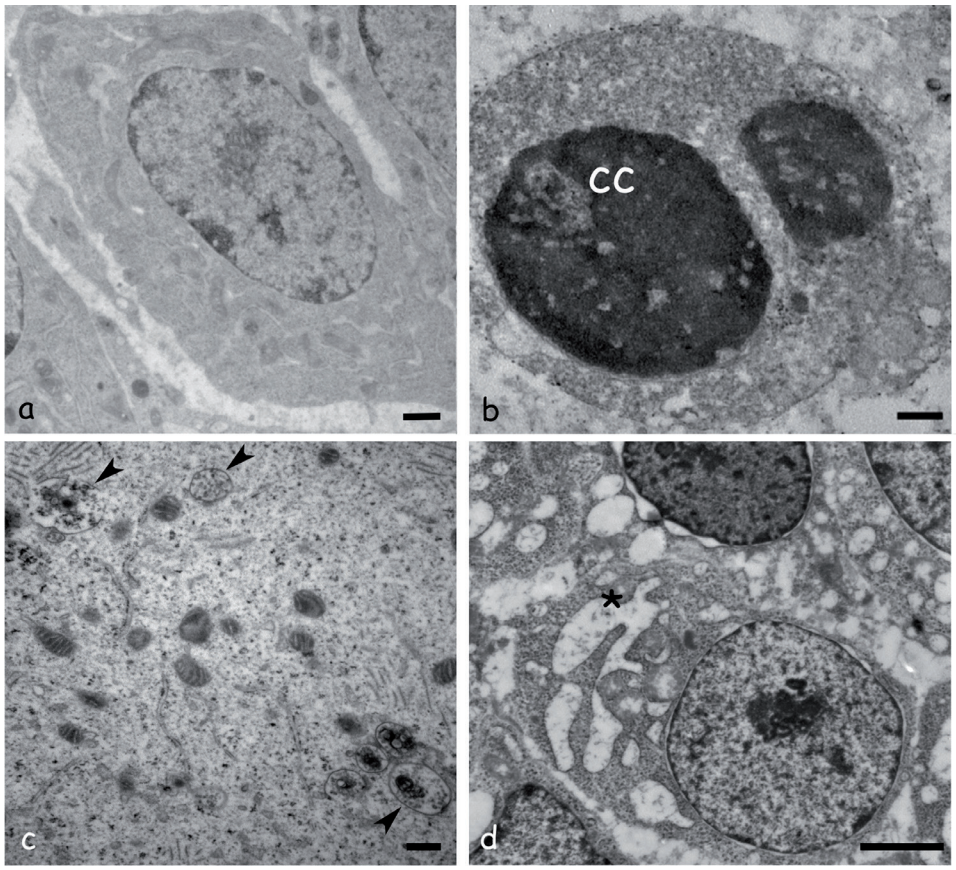

Figure 2.
Transmission electron microscope images of granulosa cells in different programmed cell death pathways. (a) healthy granulosa cell, (b) apoptotic body with highly condensed chromatin (cc), (c) autophagic cell with autophagic vesicles (head arrow), and (d) paraptotic granulosa cell with endoplasmic reticulum swelling (asterisk). Bars (a–c) 500 nm, and (d) 2 μm.

3. The endoplasmic reticulum and cell death

The endoplasmic reticulum (ER) is the organelle that is responsible for the folding and maturation of both transmembrane proteins and proteins that follow the route of secretion. Protein folding is facilitated by chaperones and oxidoreductases including binding immunoglobulin protein/glucose-regulated protein 78-kDa (BiP/GRP78), calnexin, calreticulin, and protein disulfide isomerase (PDI). An increase of cellular translational activity is possible under both normal and altered conditions, causing an overload of accumulating misfolding or unfolded proteins inside the ER. During ER stress, damaged proteins need to be degraded, but there is a limited number of proteases in the ER, and thus misfolded proteins are ejected from the ER and returned to the cytoplasm to be ubiquitinated and degraded by the 26S proteasome. These events are collectively referred to as ER-associated degradation (ERAD) [5]. Also, ER stress triggers the unfolded protein response (UPR), which is orchestrated by three ER-resident UPR sensors, inositol-requiring kinase 1 (IRE1), protein kinase R-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) [6, 7].

The UPR establishes an adaptive program aimed at re-establishing ER homeostasis by increasing the folding capacity of the cell, reducing protein synthesis, and enhancing the clearance of abnormally folded proteins and damaged organelles.

The proteins PERK and IRE1α and β are important players during UPR because they undergo oligomerization and autophosphorylation due to their interactions with peptides and unfolded proteins [8, 9]. Additionally, IRE1 promotes the unconventional splicing of X-box binding protein 1 (XBP-1) mRNA and an unspecific decrease of mRNAs better known as regulated IRE1-dependent decay (RIDD) [10, 11]. Afterward, the protein XBP-1 is translocated to the nucleus to activate the transcription of chaperones and ERAD factors [12]. RIDD suppresses protein inflow by degrading the mRNA of proteins with signal peptides or proteins with transmembrane domains, and in this manner RIDD permits proteins that are incorrectly folded inside the ER to be folded correctly [10]. PERK phosphorylates eukaryotic translation initiation factor (eIF2a), which then accumulates on the cytosolic side and leads to the downregulation of translation and enhances the translation of Grp78 and the transcription factor ATF4 [13, 14]. It has been shown that during early mouse embryonic development, Grp78 suppresses ER stress and pro-apoptotic pathways via ER signaling [15]. ATF6 is regulated by proteolysis in the Golgi apparatus, allowing the N-terminal fragments to be translocated into the nucleus where they function as a transcription factor [16, 17]. The processing of both ATF6- and IRE1α-mediated splicing of XBP1 mRNA is required for the full activation of the UPR [18].

UPR works like a protection mechanism. For example, in pancreatic beta cell line INS-1E, glucosamine and high glucose induce UPR activation and generate a feedback loop at the level of insulin transcription [19]. However, chronic or irreversible UPR can trigger cell death pathways, mainly apoptosis, but ER stress can induce other programmed cell death mechanisms including autophagy, necroptosis, and paraptosis.

3.1 The ER and follicular atresia

Morphological ER disturbances during follicular atresia have been observed for a long time. Henderson et al. [20] observed a higher surface area of endoplasmic reticulum in granulosa cells cultured from atretic follicles. Moreover, researchers have used electron microscopy to observe the dilation and disintegration of RER cisterns and the swelling of mitochondria [21].

These morphological disturbances in ovaries are associated with ER stress and UPR activation under both physiological and pathological conditions [22]. UPR is present during follicular growth and maturation and follicular atresia and in the corpus luteum. ER stress during follicular growth and maturation has been evidenced by means of the expression of XPB1 and heat shock 70 kDa protein 5 (HSPA5) accompanied by the activation of IRE1 and PERK [23]. The ER stress level and cellular response depend on the signal and its intensity. It has been shown that a lipid-rich intrafollicular environment induces ER stress and impaired oocyte nuclear maturation [24]. Likewise, in the ovary a moderate activation of ER stress depends upon PERK and p38 signaling [25], evidencing a UPR response in the cells of this organ.

4. Apoptosis

Apoptosis, the term proposed by Kerr et al. [26], describes an intrinsic suicide mechanism that involves cell shrinkage and the loss of cell contacts, chromatin condensation, and cleavage [27]. This process is better known as programmed cell death type 1 (PCD type 1). The biochemical activation of apoptosis can be directed through extrinsic and intrinsic pathways. The extrinsic pathway is initiated by the activation of cell surface death receptors to their ligands, like the Fas Ligand and TNF. After binding, apoptotic signals are transmitted through dead effector domains

and caspase recruitment domains. The intrinsic pathway is governed by a variety of cellular stresses including DNA damage, endoplasmic reticulum stress, and nutrient deprivation, which culminates in mitochondrial outer membrane permeabilization (MOMP), resulting in the release of mitochondrial proteins including cytochrome c and Smac/DIABLO. Apoptosis pathways converge on a common machinery of cell destruction that is activated by caspases, a family of cysteine proteases that cleave after an aspartate residue [28, 29]. The caspases implicated in apoptosis are divided into initiators and executioners, where initiator caspases (caspase-8 and caspase-9) activate the executor caspases (caspase-3, caspase-6, and caspase-7).

The Bcl-2 family, which are central regulators of MOMP, are a large class of both pro- and anti-apoptotic proteins. The Bcl-2 family is divided into three subfamilies: multidomain anti-apoptotic such as BCL-2, BCL-XL (BCL2L1), MCL-1, BCL-W (BCL2L2), and A1 (BCL2A1), multidomain pro-apoptotic such as BAX and BAK, and pro-apoptotic BH3-only molecules that include BID, BIM, PUMA (p53 upregulated modulator of apoptosis), and NOXA [30]. BH3-only proteins antagonize anti-apoptotic BCL-2 proteins to release and activate Bak/Bax [31]. Bax and Bak induce external membrane mitochondrial permeabilization and cytochrome c release [32]. Nevertheless, some death stimuli can trigger caspase-independent cell death pathways where other organelles such as the endoplasmic reticulum and the mitochondria have an important function in the release and activation of death factors [33].

In atretic follicles, this PCD was thoroughly described by Tilly et al. [34] and can be conducted through the intrinsic or the extrinsic pathway [35]. In ovaries, apoptosis can be triggered by deprivation of various signal molecules, survival factors, growth factors (IGF and EGF), and gonadotropins (FSH and LH). Apoptosis can occur in both oocytes and somatic cells. Cell elimination has been observed in follicles in different stages of development, from fetal to adult organisms [3, 36–38]. Although different routes of PCD can occur during follicular atresia, apoptosis plays a major role (**Figure 2b**).

4.1 The role of the ER in apoptosis

Apoptosis is triggered by chronic or irreversible ER stress and UPR and occurs through either the extrinsic or intrinsic pathway. Further, apoptosis can be carried out by two pathways, a classical Bax-/Bak-dependent apoptotic response that can be inhibited by ERK1/2 signaling and an alternative ERK1-/2- and Bax-/Bak-independent pathway [39]. No single component is entirely necessary, but the interaction of many different mechanisms results in apoptosis during ER stress [40]. Under ER stress Bax and Bak interact with the cytosolic region of IRE1α, which is required for the modulation of IRE1α signaling [41].

The activity of the BH3-only protein Bim is induced through different pathways. The first one involves protein phosphatase 2A-mediated dephosphorylation, which prevents its ubiquitination and the proteasomal degradation of Bim. A second pathway is direct transcriptional induction that is C/EBP homologous protein (CHOP)-C/EBPalpha-mediated, and a third comprises a repression of miRNAs led by PERK [42, 43]. On the other hand, PUMA, p53, and NOXA contribute to ER stress-induced apoptosis [44].

It has been reported that CHOP (a transcription factor of pro-apoptotic proteins such as Bim) increases during ER stress [45]. ATF4 and CHOP increase a generalized protein synthesis, provoking ATP depletion, oxidative stress, and cell death [46]. Also, IRE1α degrades the miRNA that represses caspase-2 mRNA translation, which causes an increase in the protein levels of this initiator protease of the mitochondrial apoptotic pathway [47].

4.2 The role of the ER in apoptosis during follicular atresia

ER stress and UPR during follicular atresia are not fully understood; however, there are several evidences of these processes in the ovary. For example, cisplatin, a widely used chemotherapeutic agent, can induce ER stress, which promotes apoptosis and autophagy in granulosa cells, causing excessive follicle loss and endocrine disorders [48].

In goat ovaries, ER stress is involved in follicular atresia through ATF6 and PERK/eIF2α/ATF4 signaling. Furthermore CHOP, caspase-12, and Grp78 proteins are upregulated in apoptotic granulosa cells during follicular atresia [49, 50]. ATF6 is a protein that is extensively distributed in the granulosa cells of ovarian follicles and oocytes in adult mice, and the amount of ATF6 increases in the presence of FSH and LH. ATF6 regulates apoptosis, the cell cycle, steroid hormone synthesis, and other modulators of folliculogenesis in granulosa cells, which may impact the development, ovulation, and atresia of ovarian follicles [51].

The presence of apoptosis-inducing factor (AIF) has been identified in granulosa cells. This protein mediates caspase-independent apoptosis and causes chromatin condensation and DNA fragmentation. AIF expression increases during follicular atresia, and AIF depletion protects ER stress-mediated goat granulosa cell apoptosis [52].

Reactive oxygen species (ROS) generation and oxidative stress can be upstream or downstream UPR targets. That is, UPR is interconnected with different enzymatic mechanisms of ROS generation, and they may depend on Ca^{2+} levels, ROS themselves, and PDI, which associates with NADPH oxidase and regulates its function [53]. ROS are pro-apoptotic factors in antral follicles. During oxidative stress, JNK activates FoxO1, which increases PUMA and induces apoptosis in granulosa cells [54]. Furthermore, pentosidine, a biomarker for advanced glycation end products, is accumulated in apoptotic human oocytes and increases with age [55].

UPR and ER stresses also have important roles in the regulation of corpus luteum (CL) regression. The overexpression of p-JNK, CHOP, caspase-12, and active caspase-3 during CL regression points to ER stress-dependent apoptosis [56, 57].

5. Autophagy

Autophagy is a catabolic pathway of cell constituents that contributes to cell survival in response to stress. Autophagy does not cause a loss of cell chemical components because the cell reutilizes them. There are three major types of autophagy, microautophagy, chaperon-mediated autophagy, and macroautophagy.

In microautophagy, vesicles bud into the lysosomal lumen by direct invagination of the boundary membrane, resulting in degradation of both cytoplasmic components and the lysosomal membrane by lysosomal hydrolases. This process involves sequential stages of vacuole invagination and vesicle scission [58].

Chaperon-mediated autophagy is the selective transport of proteins into lysosomes. The first step is protein recognition and lysosomal targeting. Protein recognition takes place in the cytosol through the binding of hsc70 to a KFERQ-like motif present in all chaperon-mediated autophagy substrates [59]. In the second step, proteins bind to receptors at the lysosomal membrane, Lamp2A, or a similar protein receptor for subsequent translocation and lysosomal degradation [60]. Receptors are subcompartmentalized in lipid microdomains to engage the processes of degradation, multimerization, and membrane retrieval [61].

Macroautophagy, also referred to as autophagy, involves the engulfment of cytoplasmic portions in a nonselective manner, as well as the degradation of specific proteins, organelles, and invading bacteria by a selective autophagy. Autophagy begins with the formation of an isolation membrane, the phagophore, which is a disk-like structure where the Atg machinery assembles. An isolation membrane grows to generate a double-membrane autophagosome, followed by elongation to form a mature autophagosome that captures cytosolic cargo. The fusion of mature autophagosomes with endosomes or lysosomes results in a single-membrane autolysosome where cargo is degraded by acid hydrolases [62].

Autophagy (Atg)-related proteins are the core machinery for autophagosome biogenesis and consist of several functional units: the ULK1-Atg13-FIP200-Atg101 protein kinase complex; the PI3K class III complex containing the core proteins VPS34, VPS15, and beclin 1; the PI3P-binding WIPI/Atg18-Atg2 complex; Atg9A; and the ubiquitin-like Atg5/Atg12 and Atg8/LC3 conjugation systems [63].

Autophagosome maturation involves the clearance of PI3P by Ymr1, a PI3P phosphatase, triggering the dissociation of the Atg machinery. Mature autophagosomes are transported to lysosomes through the microtubule cytoskeleton. The FYVE and coiled-coil domain containing 1 (FYCO1) protein binds to LC3, PI3P, and the small GTPase Rab7 and acts as an adaptor between autophagosomes and microtubules [64, 65]. Finally, the autolysosome is generated by autophagosome and lysosome fusion, where sequestered cargos are digested.

5.1 The role of the ER in autophagy

Autophagy and ER stress can be physiological processes in organisms. For example, they regulate endometrial function by modulating the mTOR pathway [66]. Also, autophagy contributes to the recovery of cell homeostasis after ER stress. During ER stress, damaged proteins are degraded by ERAD. However, some misfolded proteins are resistant, so autophagy is a final cell protection strategy deployed against ER-accumulated cytotoxic aggregates that cannot be removed by ERAD [67]. Additionally, ubiquitin is a common signal for both the ubiquitin-proteasome system and autophagy. In the mouse neuroblastoma cell line neuro-2a treated with tunicamycin, an ER stress inductor, the proteins involved in proteasomal degradation were downregulated, while proteins involved in ubiquitination were upregulated. Moreover, tunicamycin triggered autophagy, suggesting that it may serve as a compensatory effect to proteasomal degradation [68]. Also, ER-resident chaperones and enzymes that reduce the overload of misfolded proteins need to be removed by autophagy.

The structure or phagophore assembly site (PAS) localizes proximal to the ER. Autophagosome formation and transport to the vacuole are stimulated in an Atg protein-dependent manner. ER stress can induce an autophagic response because it increases Atg1 kinase activity and reflects both the nutritional status and autophagic state of the cell [69]. ER exit sites are essential for autophagy and are proximal to the PAS. Sec62, a constituent of the translocon complex that regulates protein import into the mammalian ER, intervenes during recovery from ER stress to selectively deliver ER components to the autolysosomal system for clearance and therefore is a critical molecular component in the maintenance and recovery of ER homeostasis [70].

The eIF2α/ATF4 pathway directs an autophagy gene transcriptional program in response to amino acid starvation or ER stress. The eIF2α kinase and the transcriptional factors ATF4 and CHOP are required to increase the transcription of a set of genes implicated in the formation, elongation, and function of the autophagosome,

including Atgs and beclin 1, increasing the capacity to maintain autophagy in stressed cells. These autophagy genes exhibit different dependencies on ATF4 and CHOP, which means that they have a differential transcriptional response according to the stress intensity [71]. In human heart failure, the overexpression of the ER stress markers Grp78, PERK, CHOP, and ATF3 correlates with the expression of autophagy genes [72].

IRE1, a UPR sensor, has two isoforms, IRE1α and IRE1β, which both have RNase and kinase activities. However, in *Arabidopsis thaliana*, RNase activity of IRE1β, but not its protein kinase activity, is required for ER stress-mediated autophagy [73]. In *Dictyostelium*, the response to ER stress involves the combined activation of an IRE1α-dependent gene expression program and the autophagy pathway [74]. In mammalian cells, the spliced form of XBP 1 upregulates Nedd4-2, an E3 ubiquitin ligase involved in targeting proteins for subsequent degradation, in response to ER stress. It is also important for the induction of an appropriate autophagic response [75].

Different cancer cell models have allowed a better understanding of the mechanisms involved in autophagy triggered by ER stress. In cervical tumor cells, ER stress and UPR induced by X-ray exposition led to the activation of the NF-κB signaling pathway, autophagy, and apoptosis [76]. NF-κB is important for the proliferation, invasion, and metastasis of cervical cancer cells. Furthermore, in a model of breast cancer, autophagy and apoptosis were triggered through ER stress, UPR, and a high expression of CHOP and JNK [77].

Moreover, ERK and JNK activation is associated with cross talk between autophagy and another PCD. In L929 fibrosarcoma cells, ERK and JNK can link a signal from caspase-8 inhibition to autophagy, which in turn induce ROS production and PARP activation, leading to ATP depletion and necroptosis [78].

Ca^{2+} exchange between the ER and mitochondria is mediated through domains called mitochondria-associated membranes (MAMs). The interruption of Ca^{2+} flux between these organelles generates metabolic stress where AMPK present in MAMs triggers autophagy via beclin-1 phosphorylation [79, 80]. Autophagy activation might prevent proper interorganelle communication that would maintain mitochondrial function and cellular homeostasis [79].

In ER stress, some miRNAs promote the survival of the cells, while others promote cell death. In HeLa cells under RE stress, miR-346 positively regulates the expression of glycogen synthase kinase 3 beta (GSK3B) which reduces the interaction of beclin-1 and BCL2 to induce autophagy, ROS reduction, and cell death [81].

5.2 The role of the ER in autophagy during follicular atresia

Autophagy is mainly induced in granulosa cells (**Figure 2c**) during folliculogenesis and shows a high correlation with apoptosis, and furthermore, both routes of PCD could play active roles in oocyte depletion [82]. According to Meng et al. [83], antral follicular degeneration is initiated by granulosa cell apoptosis, while preantral follicular atresia occurs mainly via enhanced granulosa cell autophagy. Surprisingly, apoptosis and autophagy can be present in the same cell at the same time, just as cells can show caspase-3 active, DNA fragmentation, and immunodetection of LC3 and Lamp 1 [2, 3].

The signals that establish autophagy or apoptosis as the route of cell death are not fully understood. Consistent with Zhang et al. [84], atresia initiation is associated with a cross talk of different PCDs including apoptosis and autophagy, a dramatic shift of steroidogenic enzymes, deficient glutathione metabolism, and vascular degeneration. In a rat model, FSH, a survival factor, decreased autophagy through LC3-II inhibition and Akt-mTOR pathway activation [85]. Shen et al. [86]

assessed the mechanism involved in autophagy inhibition by the Akt-mTOR pathway in granulosa cells exposed to FSH and oxidative stress because mTOR, a negative regulator of autophagy, inhibits FOXO1, which promotes the expression of several autophagy genes. They found that FSH induced granulosa cell survival via FOXO1 inhibition by the PI3K-Akt-mTOR pathway [86]. Nevertheless, in mouse granulosa cells, FSH was related to follicle development and atresia because FSH induces autophagy signaling via HIF-1α [87].

Despite the studies on the role of the ER in autophagy, its specific participation in follicular atresia is still unknown.

6. Necroptosis

Necroptosis is a subtype of regulated necrosis and shares the same morphological changes, including organelle swelling and membrane rupture. Necroptosis is a caspase-independent cell death, and its execution involves the active disintegration of mitochondrial, lysosomal, and plasma membranes. This PCD is triggered by various stimuli, such as TNF, Fas ligand, and TRAIL and depends on the serine/threonine kinase activity of RIP1. Additionally, a set of 432 genes regulates necroptosis and cellular sensitivity to this PCD by a signaling network that mediates innate immunity [88]. Moreover, Bmf, a BH3-only protein, is required for death receptor-induced necroptosis [88].

Moreover, environmental toxicants like cadmium can activate necroptosis. Intermediate levels of cadmium are associated with lost plasma membrane integrity, a decrease of ATP levels, and mitochondrial membrane potential and cell swelling, which are features associated with necroptotic cell death [89].

The core pathway of necroptosis relies on the assembly of an amyloid-like structure termed the necrosome. The necrosome is a multiprotein complex formed by receptor-interacting protein kinase 3 (RIPK3), RIPK1, and mixed lineage kinase domain-like (MLKL). Oligomerization and intramolecular autophosphorylation of RIPK3 lead to the recruitment and phosphorylation of MLKL. RIPK3 and MLKL continuously shuttle between the nucleus and the cytoplasm, whereas RIPK1 is constitutively present in both compartments [90]. Nuclear RIPK1 becomes ubiquitinated, and then nuclear MLKL becomes phosphorylated and oligomerized [90]. MLKL mediates plasma membrane rupture. MLKL forms cation channels that are preferentially permeable to Mg^{2+} in the presence of Na^{+} and K^{+} [91]. MLKL-induced membrane depolarization and cell death exhibit a positive correlation to channel activity.

6.1 The role of ER in necroptosis

The role of the ER in necroptosis has been evidenced using necrostatin-1, an inhibitor of necroptosis, which has a protective effect on the endoplasmic reticulum and mitochondria and alleviates ER stress after spinal cord injury [92]. Furthermore, Grp78 promotes an inflammatory response through the upregulation of necroptosis and subsequent activation of NF-κB and AP-1 pathways [93]. The depletion of reticulocalbin 1, an ER-resident Ca^{2+}-binding protein, induces Grp78, activates PERK, and phosphorylates eIF2α. Moreover, the activation of CaMKII and the inactivation of Akt are important for necroptosis in response to reticulocalbin 1 depletion [94].

The function of MLKL and RIPK in necroptosis has been widely studied. The signal transducer and activator of transcription 3 (STAT3) was demonstrated to be downstream of calpain and regulates RIPK3 expression and MLKL phosphorylation and induces ER stress and mitochondrial calcium dysregulation [95]. Moreover, in cardiomyocytes upregulated RIPK1 and RIPK3 evoke ER stress, accompanied by an

increase in intracellular Ca^{2+} levels and xanthine oxidase expression, which raised cellular ROS that mediated the mitochondrial permeability transition pore opening and necroptosis [96, 97]. In addition, the activation of JNK1/2 is regulated by RIPK3 [96].

Moreover, there are proteins that can participate in necroptosis and other types of PCD such as AIF and MLKL. Apoptosis-inducing factor (AIF), a protein normally located within the intermembrane space of mitochondria, is linked to apoptosis and necrosis. However, it has been shown that mitochondrial depolarization induced by ER stress promotes AIF release and nuclear condensation, which is consistent with necroptotic cell death [98–100]. MLKL, a member of the necrosome, also participates in chelerythrine (CHE)-promoted apoptosis through nuclear MLKL translocation and a special band of MLKL, which is promoted by a mutual regulation between the MLKL and PERK-eIF2α pathways in response to ROS formation [101].

6.2 The role of the RE in necroptosis during follicular atresia

Necroptosis has been widely researched, but there is still much to investigate, including the mechanism that mediates its execution. Nevertheless, necroptosis studies have been carried out under pathological conditions, and thus it is important to use physiological models like follicular atresia.

Necroptosis contributes to follicular atresia and luteolysis [102]. The factors involved in granulosa cell necroptosis can be regulated by acetylcholinesterase (AChE), cytokines, starvation, and oxidative stress via TNFα [103]. Also, an ovarian AChE variant, the read-through isoform AChE-R, has a nonenzymatic function that stimulates RIPK1-/MLKL-dependent necroptosis [103]. Therefore, although the participation of the ER in necroptosis and the contribution of this PCD in follicular atresia have been shown, the interrelation between ER stress-induced necroptosis and follicular atresia is completely unknown.

7. Paraptosis

Sperandio et al. [104] introduced the term paraptosis to describe a route of caspase-independent PCD that has morphological, biochemical, and transcriptional features that are different from apoptosis [104]. Endoplasmic reticulum swelling, mitochondrial swelling, and resistance to apoptosis inhibitors without nuclear shrinkage or pyknosis characterize paraptosis. Although paraptosis is a caspase-independent cell death, participation of caspase-9 has been shown under experimental conditions [104].

Paraptosis can be triggered by different stimuli including insulin-like growth factor I receptor (IGFIR), JAY/TROY, and ROS. IGF-I is a regulator of multiple cell signaling pathways including PI3K-Akt1-RPS6 and ERK1/2 MAPK that are critical for cell proliferation, migration, and survival [105]. IGFIR-induced paraptosis is mediated by caspase-9, and at least two signal transduction pathways participate in the execution of paraptosis, the MAPK and JNK pathways [104, 106].

TAJ/TROY, a member of the tumor necrosis factor receptor superfamily, induces morphological features of paraptosis accompanied by phosphatidylserine externalization, the loss of the mitochondrial transmembrane potential, and independent caspase activation [105]. Moreover, programmed cell death 5 (PDCD5), an apoptosis-promoting protein, enhances TAJ-/TROY-induced paraptotic cell death [107].

ROS production can trigger paraptosis through PINK and mitophagy activation [108, 109]. Covalent modifications of free sulfhydryl groups on proteins cause protein misfolding and the accumulation of misfolded proteins, leading to ER stress, CHOP activation, and paraptosis [110, 111]. In malignant hepatoma cells with

Bcl-xL-mediated apoptotic defects, the disruption of thiol homeostasis and treatment with doxorubicin and pyrrolidine dithiocarbamate induced paraptotic cell death [112].

The full signal transduction pathway and identification of specific markers for paraptosis are still unclear. Nevertheless, phosphatidylethanolamine-binding protein (PEBP-1), a suppressor of the MAPK pathway, has been identified, and prohibitin, a mitochondrial protein, is a mediator of paraptosis [113]. Furthermore, the redistribution of α- and β-tubulin and tropomyosin has been observed in the early stages of paraptosis. Other characteristics of the paraptotic pathway involve alterations mainly in signal transduction proteins, mitochondrial proteins, and some metabolic proteins [113].

7.1 The role of RE in paraptosis

Cancer cells are the best model to study paraptosis because there can be apoptosis and/or autophagy resistance. In melanoma cells, the sustained activation of the IRE1α and ATF6 pathways driven by the MEK/ERK pathway avoids ER stress-induced apoptosis [114].

Different compounds for cancer treatment have shown paraptosis induction. For example, HeLa, A549, and PC-3 cells treated with celastrol induced vacuoles derived from the dilation of ER, a feature of apoptotic cell death; moreover, this was accompanied by autophagy and apoptosis. Furthermore, the ER swelling triggered by celastrol induced ER stress markers including Grp78, PERK, IRE, and CHOP and alterations to proteasome function that resulted in the accumulation of ubiquitinated protein [115, 116]. Moreover, paraptosis can be accelerated by pre-treatment with the proteasome inhibitor MG132 [117]. On the other hand, cyclosporine A treatment of cervical cancerous SiHa cells showed ER stress and UPR preceded by massive cytoplasmic vacuole formation that culminated in a paraptosis-like cell death [118]. Moreover, murine hepatoma 1c1c7 cells and the human non-small cell lung cancer A549 cell line exposed to a combination of photodamage and benzoporphyrin derivative result in ER swelling and paraptotic cell death [119].

For the pathways involved in paraptosis, ER vacuoles can be dependent on the PI3K/Akt signaling pathway [120]. Moreover, in BC3H1 myoblast cell lines exposed to yessotoxin, paraptosis was accompanied by cytoskeletal alterations and the activation of JNK/SAPK1 [121]. However, in acute lymphoblastic leukemia cells, everolimus, a mTOR inhibitor, showed that JNK signaling was not required for paraptotic cell death [122]. Paraptosis in epithelial ovarian cancer (EOC) cells treated with morusin was characterized by VDAC-mediated Ca^{2+} influx into mitochondria, and subsequent mitochondrial Ca^{2+} overload contributes to mitochondrial swelling and dysfunction, leading to the accumulation of ER stress markers, the generation of ROS, and the loss of mitochondrial membrane potential ($\Delta\psi m$) in EOC cells [123].

7.2 The role of the RE in paraptosis during follicular atresia

Knowledge of the role of paraptosis during follicular atresia is still limited. In *Bombyx mori*, apoptosis, autophagy, and paraptosis occur in the ovarian nurse cell cluster during late vitellogenesis, whereas middle vitellogenesis is exclusively characterized by the presence of paraptosis, preceding both apoptosis and autophagy [124]. In mammals, paraptosis was evidenced by ER swelling (**Figure 2d**) and CHOP immunodetection in granulosa cells during follicular atresia in adult Wistar rats [4].

The mechanisms involved in paraptosis during follicular atresia are still unknown. The paraptotic inductor IGFR might be related because it is implicated in follicular growth and selection [104, 125]. Moreover, IGF2R and the binding protein genes IGFBP5 and IGFBP6 are overexpressed in atretic follicles [126]. However, more studies on paraptosis during follicular atresia are necessary.

8. Conclusions

Endoplasmic reticulum stress is a strong signal that triggers different programmed cell death pathways. Interestingly, programmed cell death via endoplasmic reticulum stress is not exclusive to pathological or experimental conditions but is present in physiological processes like follicular atresia. However, the specific mechanisms and signals for choosing a particular cell death pathway are still unknown. In this way, research on the pathways and mechanisms involved in programmed cell death activated by endoplasmic reticulum stress are fundamental, particularly for follicular atresia, as this process ensures the ovulation of competent oocytes for fertilization.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Nayeli Torres-Ramírez, Rosario Ortiz-Hernández, M. Luisa Escobar-Sánchez, Olga M. Echeverría-Martínez and Gerardo H. Vázquez-Nin*
Laboratory of Electron Microscopy, Department of Cellular Biology, Faculty of Sciences, National Autonomous University of Mexico, Mexico City, Mexico

*Address all correspondence to: vazqueznin@ciencias.unam.mx

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