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REVIEW

Autophagy in hypoxic ovary

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Abstract

Oxygen deprivation affects human health by modulating system as well as cellular physiology. Hypoxia generates reactive oxygen species (ROS), causes oxidative stress and affects female reproductive health by altering ovarian as well as oocyte physiology in mammals. Hypoxic conditions lead to several degenerative changes by inducing various cell death pathways like autophagy, apoptosis and necrosis in the follicle of mammalian ovary. The encircling somatic cell death interrupts supply of nutrients to the oocyte and nutrient deprivation may result in the generation of ROS. Increased level of ROS could induce granulosa cells as well as oocyte autophagy. Although autophagy removes damaged proteins and subcellular organelles to maintain the cell survival, irreparable damages could induce cell death within intra-follicular microenvironment. Hypoxia-induced autophagy is operated through 5′ AMP activated protein kinase–mammalian target of rapamycin, endoplasmic reticulum stress/unfolded protein response and protein kinase C delta–c-junN terminal kinase 1 pathways in a wide variety of somatic cell types. Similar to somatic cells, we propose that hypoxia may induce granulosa cell as well as oocyte autophagy and it could be responsible at least in part for germ cell elimination from mammalian ovary. Hypoxia-mediated germ cell depletion may cause several reproductive impairments including early menopause in mammals.

Keywords Granulosa cells · Oocyte · HIF-1α · Follicular atresia · Beclin 1

Introduction

The reduced level of pO_2 generates hypoxic condition, which is characterized by insufficient supply of oxygen to meet the physiological requirement of tissue/cells in the body. The increase of harmful gases and particulate matters in air due to rapid industrialisation, deforestation and motor vehicles affects oxygen delivery through blood and utilization capacity of a cell causing cellular/physiological hypoxia [1–4]. Further, the presence of these pollutants in air may generate inflammation in pulmonary and vascular systems [4, 4] thereby decreasing blood oxygen saturation level [4, 5, 6]. For instance, hypoxia has been observed in the placenta of women who used firewood/kerosene for cooking purpose [7].

Hypoxia affects various aspects of cell functions including metabolism, growth, cell division and cell death [8, 9]. The hypoxia-mediated changes in cellular physiology modulate cardiovascular, neuronal and reproductive physiology [10–13]. Ovary is a primary female reproductive organ responsible for generation of competent oocyte required for successful fertilization and early embryonic development (Fig. 1a). Mammalian ovary contains almost 5–6 million germ cells during 20th week of embryonic development. Majority of these germ cells are eliminated by follicular atresia, while only 1 million germ cells remain available for selective recruitment during entire life span after birth [14]. These germ cells form oogonia and migrate to gonadal ridges, enter into 1st meiotic division and remains arrested at diplotene stage of prophase I for a long period of time [15]. Those primordial follicles in response to pituitary gonadotropins may enter into process of folliculogenesis to form graffian follicle just prior to ovulation of competent oocyte [16]. The primary oocytes in mammalian ovary possess zona pellucida (ZP) encircled

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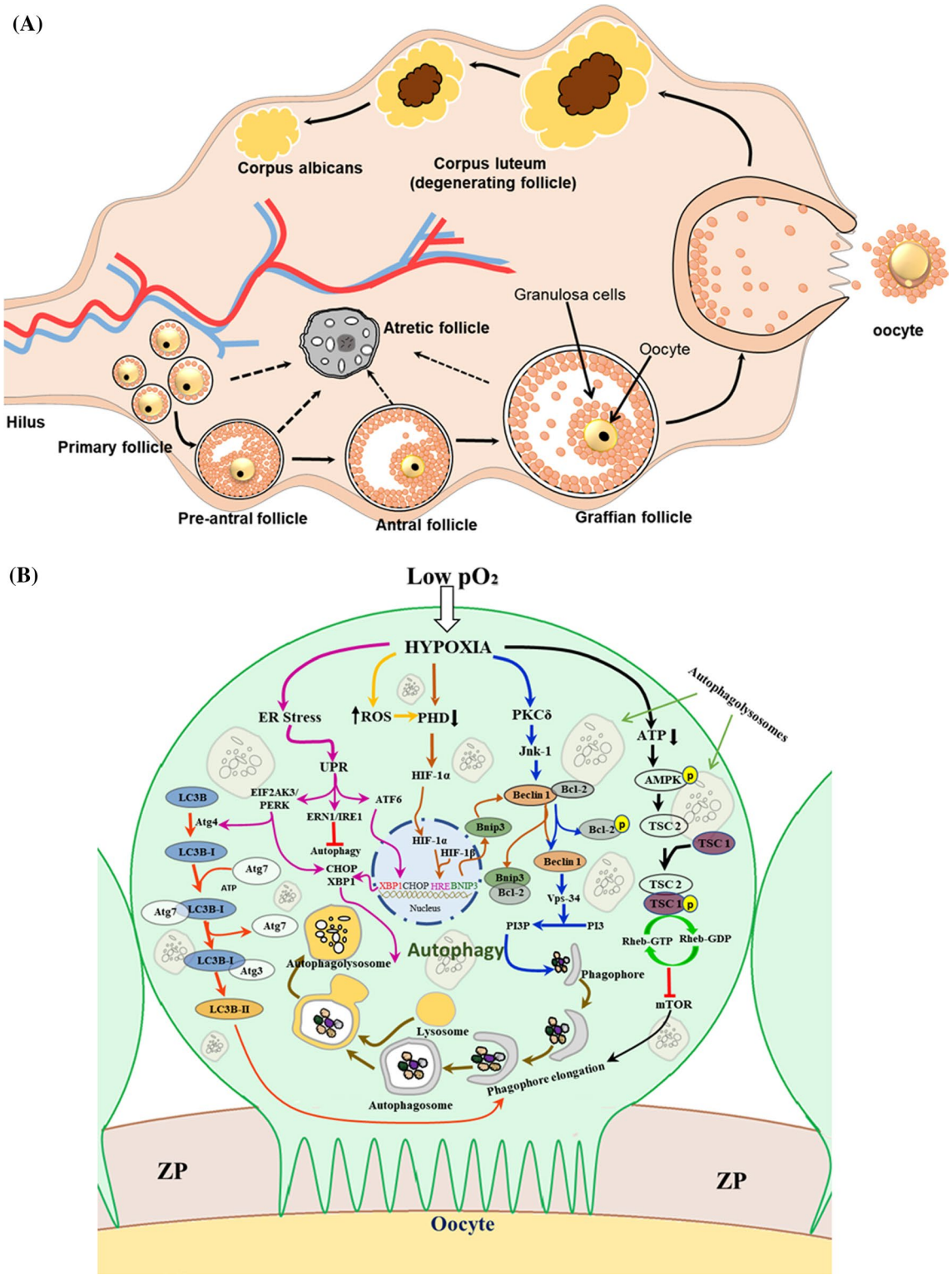
(A)
Corpus albicans
Corpus luteum
(degenerating follicle)
Granulosa cells
Atretic follicle
Oocyte
oocyte
Hilus
Primary follicle
Pre-antral follicle
Antral follicle
Graffian follicle
(B)
Low pO2
HYPOXIA
ER Stress
ROS
PHD
PKCδ
ATP
Autophagolysosomes
UPR
HIF-1α
Jnk-1
AMPK
LC3B
EIF2AK3/
PERK
ATF6
ERN1/IRE1
Beclin 1
Bcl-2
Atg4
Bnip3
Bcl-2
TSC 2
TSC 1
LC3B-I
Autophagy
HIF-1α
HIF-1β
Atg7
CHOP
XBP1
XBP1 CHOP HRE BNIP3
Nucleus
Beclin 1
Bnip3
Bcl-2
Vps-34
PI3P
PI3
Rheb-GTP
Rheb-GDP
Autophagolysosome
Autophagy
Phagophore
mTOR
LC3B-II
Lysosome
Autophagosome
Phagophore elongation
ZP
ZP
Oocyte

◀ **Fig. 1** **a** Schematic diagram of mammalian ovary showing follicles at different stages of development. **b**, **c** Magnified schematic diagram showing proposed mechanism of hypoxia-induced autophagy in follicular cells of mammalian ovary. **b** In Granulosa cells, reduction of pO_2 level causes cellular hypoxia. Hypoxia may induce ER stress, ROS generation, PKDδ activation and decreases PHD activity as well as ATP production in a cell. Increase of ROS level decreases PHD activity that stabilizes HIF-1α and its accumulation. HIF-1α is then translocated to nucleus, where it forms a heterodimer with HIF-1β and binds to HRE inducing transcription of BNIP3 gene. Bnip3 protein binds with Bcl-2 and disrupts its interaction with Beclin 1 making Beclin 1 free. The Beclin 1–Bcl-2 interaction can also be disrupted through phosphorylation of Bcl-2 by Jnk-1 activated in response to PKCδ. Free Beclin 1 increases catalytic efficiency of Vps-34 which then coverts PI3 into PIP3 required for phagophore formation. ATP depletion in response to hypoxia triggers phosphorylation of AMPK. The phosphorylated AMPK activates TSC2 which binds with TSC1 leading to its phosphorylation by Rheb-GTP. The phosphorylated TSC2-TSC1 inhibits mTOR and induces autophagy. ER stress induces UPR which is sensed by three different UPR sensors, EIF2AK3/PERK, ERN1/IRE1 and ATF 6. ATF 6 enters into nucleus, promotes transcription of chaperone CHOP and XBP1 and induces autophagy induction. CHOP is also upregulated in response to EIF2AK3/PERK that activates Atg 4 to cleave LCB and generates LCB-I. LC3B-I is then activated by binding of Atg7 in a ATP-dependent manner and then transferred to Atg3. Atg3 promotes conjugation of PE to LC3B-I to generate processed LC3B-II. Activated LCB-II is recruited to growing phagophore and plays important role in fusion of its edges and cargo selection in a cell. **c** In follicular oocyte, hypoxia may trigger all four major pathways to induce autophagy as described in **b**. In addition, granulosa cell death may deprive oocyte from nutrients, growth factors and survival factors that result in the activation of starvation-induced AMPK–mTOR-mediated pathway and promote phagophore formation. Once the phagophore is formed, all these pathways promote its elongation and autophagolysosome formation, which finally engulfs most of the cytoplasmic machinery that probably results in autophagic cell death

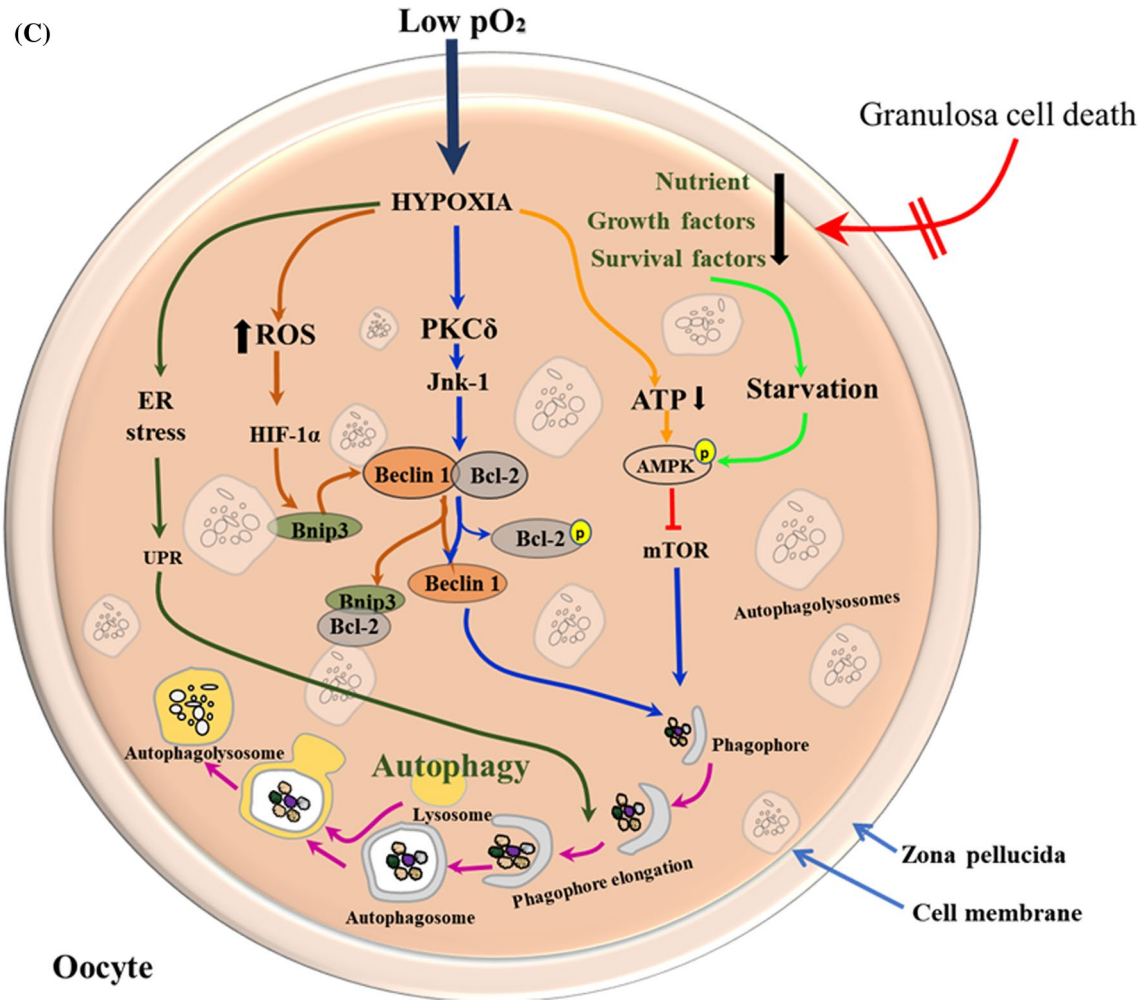

Fig. 1 (continued)

by layers of two major somatic cell types namely theca and granulosa cells. Theca cells are steriodogenic in nature and responsible for synthesis of estradiol-17β required for follicular growth and development. Theca cells are further differentiated into theca interna and theca externa; while granulosa cells are differentiated into mural and cumulus granulosa cells. The cumulus granulosa cells are the immediate somatic cells encircling primary oocyte that is morphologically characterized by germinal vesicle and nucleolus in center.

Growing body of evidences suggests the adverse impact of hypoxia on ovarian function in several ways. For instance, it triggers the depletion of follicular reserve in spiny mouse [17], reduces luteal growth in sheep ovary [13], decreases follicular development in hamster [18], promotes reactive oxygen species (ROS) generation and follicular aging in human granulosa cells [19, 20]. Although hypoxic cell employs survival strategy during mild and initial phase, sustained hypoxia may trigger various cell death pathways including autophagy, apoptosis, necrosis and necroptosis depending on duration and severity of hypoxia [21–23].

Autophagy is a highly regulated process of self-degradation that eliminates damaged, unwanted or surplus subcellular proteins and organelles with the help of lysosomal activity [24]. Various factors including starvation, hormones, stress and other pathological conditions may induce autophagy to maintain homeostasis and longevity of a cell [25] through the turnover of damaged proteins and organelles [24, 26]. Mammalian target of rapamycin (mTOR) is a central stress sensor and master regulator of the autophagy [27]. However, hypoxia could also trigger autophagy through various mTOR-independent pathways including protein kinase C delta–c-jun-N terminal kinase 1 (PKCδ–JNK-1) [28], endoplasmic reticulum (ER) stress or unfolded proteins response (UPR) [29] and generation of ROS [30]. Although autophagy is a protective mechanism to maintain cellular homeostasis [31, 32], excessive accumulation of indigestible materials due to autophagic degradation of damaged proteins and organelles like mitochondria, ER and ribosome could lead to autophagic cell death [33, 34]. Recent studies suggest the involvement of autophagy in the regulation of follicular development, granulosa cell as well as oocyte death leading to follicular atresia [35, 36], corpus luteum regression [37] and oocyte aging [38] and pathogenesis of metabolic disorder like Polycystic ovarian syndrome (PCOS) [39, 40].

Although hypoxia-mediated cell death has been studied in greater detail in various cell types, hypoxia-mediated autophagy remains poorly understood in the follicular cells of mammalian ovary. This review article updates the information on the involvement of autophagy in granulosa cell as well as oocyte and proposes the possible mechanism of hypoxia-mediated autophagy in the follicular cells of mammalian ovary.

Hypoxia-mediated physiological changes in ovary

The presence of certain harmful gases in air may compromise the oxygen delivery capacity of blood or alter the ability of a cell to utilize the available oxygen [1, 2]. Several pathological conditions of pulmonary as well as cardio-vascular systems may also cause reduced pO_2 level in blood [41, 42]. The specialized chemoreceptor cells in arterial circulation and neuroepithelial bodies present in the airway sense hypoxic conditions and accordingly modulate pulmonary ventilation as well as perfusion to optimize the supply of O_2 to the metabolizing cells/tissue. Under hypoxic conditions, peripheral blood vessels are dilated, whereas pulmonary vasculatures are constricted to shunt the blood away from poorly ventilated region for optimizing the oxygen supply to tissues [43, 44]. Most of the nucleated cells sense changes in O_2 concentration and respond quickly through the activation of pre-existing proteins and in long term through the regulation of gene transcription [45]. One of the most important transcription factors induced in response to hypoxia is hypoxia-inducible factor (HIF) [46]. It regulates gene transcription to maintain oxygen homeostasis for adaption to low oxygen tension [47].

The HIF is a heterodimeric protein consisting of an oxygen-dependent α-subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed aryl hydrocarbon receptor nuclear translocator/β (ARNT/β) subunit located in the nucleus. On the other hand, HIF-1α mRNA level does not alter in normoxia as well as hypoxia [46]. However, protein is polyubiquitinylated and rapidly degraded in normoxia but gets accumulated in hypoxia [46]. In normoxia, prolyl hydroxylases (PHD1-3) hydroxylate two proline residue of HIF-1α [48–50]. The hydroxylated HIF-1α is then recognized by von Hippel–Lindau (VHL) protein that ubiquitinates HIF-1α and helps in proteasomal degradation [51]. In hypoxic condition, PHD activity decreases and HIF-1α proline residues are not hydroxylated, resulting in the accumulation of stabilized protein [52]. Once stabilized, HIF-1α enters into the nucleus, joins with HIF-1β to generate heterodimer transcription factor and by binding to hypoxia response elements (HRE) present in their regulatory region promotes the expression of target genes like luteinizing hormone receptor, inhibin-α VEGF, Endothelin 2, BNIP3, PDE4D, NRF2F2, disintegrin and metalloproteinase with thrombospondin-like motifs-1, etc. [53–59]. HIF-1α target genes affect almost all aspect of the cellular functions including metabolism [8], growth, proliferation, secretion of cytokines as well as mitogen, [60] and cell death [9]. Hypoxia-mediated changes in cellular

functions affect cardio-vascular, central nervous system and reproductive physiology [10–13].

Mammalian ovary is a metabolically active organ and generates ROS at an extraordinary scale. Due to large size of follicle, follicular oocyte is more susceptible toward hypoxia [61, 62]. The chronic hypoxia may result in ovarian dysfunction and altered hormonal profile [63, 64]. Development of ovarian follicle is a dynamic process that involves proliferation, differentiation and death of somatic cells encircling oocyte [65, 66]. Follicles are recruited and selected dominant follicle ruptures to release meiotically competent oocyte, while non-selected follicle undergoes atresia [67]. Within the follicle, a bidirectional talk is important for survival and several functions of both encircling granulosa cells as well as oocyte [68]. The encircling cumulus granulosa cells nourish the oocyte by transferring nutrients, growth factors and survival factors [69]. In turn, oocyte modulates cumulus cell functions by secreting paracrine factors that include growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) [70]. The GDF9 and BMP15 modulate cell proliferation [71], metabolism [72], expansion [73], luteinisation [74] and apoptosis [75] of encircling granulosa cells within the follicular microenvironment.

The follicular oocyte is encircled by several layers of granulosa cells and thecal cells, and between both lies a tight barrier of basement membrane that blocks the infiltration of blood vessels to region of granulosa cells and oocyte separating them from blood supply. The O_2 as well as nutrients have to pass through these layers of somatic cell before it becomes available to oocyte; hence, pO_2 is compromised within the follicular microenvironment in mammals as the size of follicle grows larger [76]. Hypoxia induces polycystic ovaries, estradiol biosynthesis, alters estrous cycle and decreases fertility in female rat suggesting its negative impact on folliculogenesis in ovary [64]. A brief exposure of hypoxia (7–8 min) resulted in decreased number as well as diameter of primordial and primary follicle, reduced follicular reserve and ovarian volume in spiny mouse fetuses [17]. Hypoxia caused at high altitude changes the morphology and function of antral follicle and corpora lutea in sheep [13]. Further, it also increases HIF-1α and vascular endothelial growth factor (VEGF) expression level in luteal cells of sheep. These sheep also had reduced number of preovulatory follicles as well as growth of corpora lutea [13]. Reduced blood flow causes hypoxic condition in follicular microenvironment, induces generation of ROS and activation of HIF-1α [77]. The active HIF-1α binds to hypoxia response elements region of VEGF gene promoter in ovarian cells and induces upregulation of VEGF [56, 57]. Increased level of ROS due to pathological conditions or drug treatment induce granulosa cell death [57, 78–82] inhibit follicular growth, development and induces meiotic arrest [83, 84] as well as apoptosis in rat oocytes [78, 83]. The elevated level of ROS has been reported in patients of Primary ovarian insufficiency (POI) [85], and could be used as a promising indicator for risk of POI [86]. The increased level of ROS may also be attributed to increase mutation in ATPase6 gene [85] and mitochondrial cytochrome *c* oxidase 1 gene in POI patients [87]. Further, high level of ROS is associated with pathogenesis of polycystic ovarian syndrome (PCOS) [88, 89]. However, the exact role of ROS in pathogenesis of PCOS is ill-understood.

The hypoxia-specific genes are upregulated in granulosa cells of aged women suggesting hypoxia as main mechanism underlying ovarian senescence and deterioration of oocyte quality [58]. Further, hypoxia induces HIF-1α and its downstream targets like phosphodiesterase 4D (PDE4D), neuron-derived orphan receptor-1 (NOR-1 or NR4A3), nuclear receptor subfamily-1 (NR2F2), neovascularization by VEGF and ATP synthesis through glycolysis [58].

The presence of air pollutant 7, 12-dimethylbenz (a) anthracene (DMBA), a polycyclic aromatic hydrocarbon affects follicular growth and development and deteriorates oocyte quality [90]. It destroys follicles, reduces ovarian volume and alters mRNA expression of number of genes involved in the cell survival, proliferation and primordial follicle activation in mouse as well as rat ovary [90, 91] resulting in the decrease of ovarian volume [92, 93]. Studies suggest the involvement of phosphatidylinositol-3 kinase (PI3K) pathway that converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3), leading to phosphorylation of protein kinase B (PKB/Akt) [94]. Increased Akt phosphorylation with a decrease of forkhead box O3 A (FOXO3A) phosphorylation and activation of mTOR have been observed in DMBA-treated primordial follicle as well as in oocyte of mice [95] suggesting the role of PI3K signaling and PI3K/Akt/mTOR-mediated autophagy in ovary.

Hypoxia-induced autophagy

Low pO_2 is one of the major causes for the induction of autophagy [96, 97]. Depending upon the degree of severity and duration of oxygen deprivation, hypoxia triggers different pathways of autophagy. For instance, chronic and moderate hypoxia triggers HIF-1α [98] as well as PKCδ–JNK1-mediated pathways to induce autophagy [28]. On the other hand, a rapid and severe oxygen fluctuation induce autophagy via HIF-1α independent as mTOR-mediated pathway [99] and UPR [29]. Autophagy may also promote

survival by removing damaged mitochondria and hypoxia-mediated ROS production [26].

HIF-1α-dependent autophagy

The BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) gene is a specific target of HIF-1α that gets fully expressed during moderate hypoxia [100]. BNIP3 and its homologue BNIP3L are prosurvival proteins, [101] that are involved in hypoxia-induced autophagy. In moderate hypoxia, HIF induces BNIP3 and disrupts the interaction between Beclin 1 and Bcl-2 [59]. The free Beclin1 induces autophagy [96] and mitophagy [59] instead of apoptosis [102] (Fig. 1b).

During hypoxia, cell is capable of supporting oxidative production of ATP through tricarboxylic acid (TCA) cycle and electron transport chain (ETC) up to some extent [103]. Leakage of electron from ETC generates ROS. On the other hand, reoxygenation following hypoxia leads to uncontrolled superoxide generation that causes increased oxidative stress [103]. The reduced pO_2 as well as nitric oxide levels result in the generation of ROS and decreased PHD activity [104, 105]. The decreased PHD activity stabilizes HIF-1α and induces autophagy through BNIP/BNIP3L-mediated disruption of Beclin1 and Bcl-2 interaction [48]. Studies suggest that stabilization and/or synthesis of HIF-1α under hypoxia is dependent on the PI3K/Akt pathway [106]. In cases of severe hypoxia or anoxia, additional pathways such as platelet-derived growth factor receptor (PDGFR), which is HIF-1α dependent [107], and protein deglycase or Parkinson disease protein 7 (DJ-1/PARK7) may also regulate autophagy [29].

HIF-1α-independent autophagy

The serine/threonine kinase (mTOR) is a principal inhibitory regulator of autophagy [108, 109]. It induces autophagy during severe hypoxia (Fig. 1b). The long-term hypoxia and ATP depletion could result in the phosphorylation of 5′ AMP activated protein kinase (AMPK) that activates Tuberous sclerosis complex 2 (TSC2) proteins [110]. The activated TSC2 forms a complex with TSC1 through a combination of GTP-binding protein Rheb and inhibits mTOR function [111]. The inhibition of mTOR activity also occurs through two independent pathways, the DNA damage response 1 (REDD1) protein [111, 112] and activation of stress sensor protein, ataxia telangiectasia mutated (ATM) [113].

Under severe hypoxic conditions, autophagy is induced through UPR pathway [114]. It has been reported that UPR activates stress sensors [115] and these sensors could activate autophagy [29, 116–118]. During initial stage of hypoxia, PKCδ activates autophagy by promoting JNK1-mediated Bcl-2 phosphorylation that dissociates Beclin 1 from Bcl-2 proteins [119]. As the hypoxia prolongs, PKCδ and Beclin 1 proteins are cleaved by caspase-3 protein, which is associated with the apoptosis [120, 121]. On the other hand, carbobenzoxy-valyl-alanyl-aspartyl-[*O*-methyl]—fluoromethylketone (Z-VAD-fmk), a caspase inhibitor, induces autophagy [122]. Indeed, PKCδ–JNK1 signaling plays an important role to protect cells from hypoxic stress by inducing autophagy.

Players and pathways involved in hypoxia-mediated autophagy have been well studied in a wide variety of somatic cell types [28, 101, 114]; however, involvement of hypoxia-mediated autophagy in mammalian ovary remains poorly understood. Few studies suggest that hypoxia induces HIF-1α and VEGF expression in luteal cells, reduces number of antral follicle and the growth of corpora lutea in sheep ovary [13]. HIF-1α-mediated mTOR signaling pathway has been reported to induce mouse granulosa cells autophagy in response to follicle-stimulating hormone (FSH) [123]. The increased expression of HIF-1α is associated with mouse granulosa cells autophagy [124]. The Cobalt chloride ($CoCl_2$)-induced hypoxia increases expression of autophagy-related genes like LC3, Atg5, Beclin 1, Atg7 and BNIP3 in mouse granulosa cells [123, 124]. Based on these studies, we propose that granulosa cell proliferation may compromise the pO_2 in follicular microenvironment that may trigger HIF-1α-mediated autophagy. Autophagy has also been reported in follicle loss from the ovarian of rat and murine exposed to cigarette smoke [125, 126] probably by inducing hypoxia.

Autophagy in mammalian ovary

Involvement of autophagy has been reported in mouse [127], rat [35, 128, 129], porcine [130] goose and quail ovary [131, 132]. Autophagy plays an important role in the maintenance and regulation of ovarian primordial follicle reserve; knock-out of autophagy-related genes result in the decrease of primordial follicle pool leading to the POI [133, 134]. Germ cell-specific knock-out of ATG7 gene leads to POI with the decrease of follicle as well as oocyte number in the ovary [134]. In addition, the presence of loss of function variant of ATG7 and ATG9A genes results in the impairment of autophagy which suggests the important role of defective autophagic machinery in POI patients [135]. The POI is also associated with mutation in autophagy regulatory Tsc1 or Tsc2 genes and elevated mTOR activity that leads to premature activation and early depletion of primordial follicle pool in the ovary [136, 137]. However, rapamycin (an inhibitor of mTOR) limits the conversion of primordial follicle into

developing follicle and inhibits follicular atresia, thus preventing depletion of ovarian reserve [138]. Autophagy in ovary prevents granulosa cell apoptosis in younger women, while in aged women, a decline of autophagy augments the expression of apoptotic marker, ROS and higher percentage cell death [139]. Inhibition of autophagy leads to massive accumulation of age-related catabolic waste during folliculogenesis in IL-33(−/−) mice [140]. Another protective role of autophagy can be seen in pig ovaries subjected to heat stress [141]. Heat stress increases abundance of autophagosome and expressions of beclin 1 and LC3B-II in interstitial as well as follicular cells. Abundance of BCL2L1 and phosphorylated BCL2 was also increased with no caspase 3 cleavages, suggesting the suppression of apoptotic signaling in the ovary [141]. Milk deprivation in female neonates for 12–36 h induces autophagy-mediated differentiation or the formation of primordial follicles from naked oocytes that prevents depletion of germ cells from ovarian pool. Further, starvation resulted in higher number of primordial follicle. Oocyte cytoplasm showed abundance of autophagy-related proteins and suppressed expression of apoptotic proteins such as caspase 9 and caspase 3 [142].

Autophagy has also been reported in granulosa cells of obese women due to high level of oxidized low-density lipoprotein (oxLDL) and oxidative stress [143, 144]. H_2O_2-induced oxidative stress also causes granulosa cell death in mouse ovary via autophagy [145, 146]. Melatonin and FSH suppress autophagy-mediated granulosa cells deaths by inhibiting JNK-mediated dissociation of BCL2/BECN1 complex [147] and activating PI3K-Akt-mTOR signaling cascade with suppressing FOXO1 transcriptional activity, respectively [146]. Autophagy, with or without apoptosis is also involved in oocyte and granulosa cell death during follicular atresia in rat [139, 143, 148]. The autophagy is mainly induced in granulosa cells during various phases of ovarian cycle in rat [35, 149], and both autophagy and apoptosis have been reported in granulosa cells of mouse ovary [150]. The primordial follicle and theca cells show weak LC3-II expression, while granulosa cells at all the stage of folliculogenesis showed high level of LC3-II expression [35]. On the other hand, LC3-II expression was not reported in follicular oocyte [35]. The granulosa cells of atretic follicle also showed intense expression of caspase-3 and LC3 immunoreactivity as compared to that of healthy follicle [35]. Gonadotropin treatment suppresses autophagy by activating PI3K-Akt-dependent or independent mTOR signaling in granulosa cells of rat [35, 151–154].

Studies suggest that autophagy is actively involved in the depletion of oocyte from rat ovary [37]. Follicular cells show simultaneous presence of both autophagic and apoptotic markers in same cell at the same time during all phases of estrous cycle in rat [37, 155]. A large number of oocytes are removed by a process sharing features of apoptosis and autophagy [156]. Most of the oocyte in early stage of death are simultaneously positive to active caspase-3, DNA breaks (apoptosis), increase of lamp1 and acid phosphatase a characteristic of autophagy [156]. A similar mechanism of cell death has been reported in oocytes of pre-pubertal rat cultured in vitro [156]. Thus, process of cell death in oocyte probably begins with the degradation of cytoplasmic components including mitochondria. During initial phase, caspase-3 is activated and oocyte undergoes apoptotic cell death [156]. Autophagy cell death accounts for massive depletion of germ cells from the ovary of Lim homeobox 8 (Lhx8) (a protein involved in patterning and differentiation) ablated mouse probably due to disrupted DNA repair mechanism. It leads to dramatic reduction of ovarian reserve and generation of sterile fibrotic ovaries [157]. Age-dependent changes in the type of cell death have been reported in oocyte where it uses different combinations of apoptosis and autophagy. For instance, oocytes are mostly eliminated by apoptosis, autophagy and even mixed events of both death pathways during prepubertal age [129]. However, autophagy can be observed at all the age group of rat [129]. The estrous cycle-dependent cell death events have also been reported in rat oocyte [37]. Apoptosis is predominant during estrous phase and autophagy is more common during proestrous stage. Both apoptosis and autophagy are observed during diestrous and metaestrous phase in rat ovary [37].

Autophagy plays a preventive role in post-maturation aging of mouse oocyte. The p62 protein expression decreased, while LC3-II puncta, autophagosome content are increased after 12 h of oocyte aging [38]. Induction of autophagy either by rapamycin or LiCl corrected the aging parameters by decreasing cytoplasmic calcium, ROS, caspase level and cytoplasmic fragmentation along with reduction in proportion of oocyte with barrel shaped spindle and congressed Autophagy was induced as natural stress response during vitrification-warming of mouse oocyte [158], but enhancing autophagy by rapamycin had negative effects on fertilization and development of oocyte [159] and inhibition activated apoptosis via caspase-9 and 12 activation [160]. Inhibition of autophagy during in vitro maturation of porcine oocyte also induced DNA damage, apoptosis and disrupted mitochondrial membrane potential exerting detrimental effects on polar body extrusion and oocyte competency [161]. These studies suggest that the increase of autophagy prevents caspases activation and apoptosis. Autophagy is reported during luteal cell death in rat [128], marmoset monkey [162] and human [163, 164]. Studies suggest that the autophagy promotes luteal cell death by regulating apoptotic cell death in non-primates species [128, 165] chromosome [38]. On the other hand, decreased autophagy accelerates the aging in mouse [38].

Recent studies suggest the role of autophagy in pathogenesis of metabolic disorder like PCOS. Autophagy-related

ultra-structural changes, consistent with increased expression of LC3B and decreased SQSTM1/p62 are observed in cortex of PCOS ovary in rat [40]. Increased autophagy was also evidenced in ovarian tissue of PCOS patients along with differential expression of autophagy-related genes [40]. The granulosa cells of PCOS patients show high level of LC3-II proteins and mRNA expression with increased autophagosome formation. The increased expression of SUMO-specific protease (SENP3) induces GC autophagy in the ovary of PCOS patients [166]. Further, the elevated level of mTOR and P-mTOR has been observed in of DHEA induced PCOS mice ovary [167]. In addition, autophagy-inducing gene and transcription factor FOXO1 are reduced in endometrial tissue of PCOS patients [39]. These studies suggest the involvement of autophagy in pathogenesis of PCOS.

Conclusion

The presence of apoptosis and autophagy markers in same cells of ovary suggests the onset of autophagy and apoptosis from the beginning and only one of these processes may induce final disposal of oocyte in rat [129]. Autophagy may also be initiated when the process of apoptosis cannot be achieved [168]. Another possibility is that both processes of cell death are activated at the same time from the beginning itself and both actively participate in the disposal of oocyte. Since volume of the oocytes is significantly larger than somatic cells, it is possible that the combined degradation process may be sufficient in the elimination of a large cytoplasmic content of only one cell [129]. The role of hypoxia in modulating ovarian physiology and the role of autophagy in various physiological processes have separately been studied. However hypoxia-mediated autophagy and its impact on physiological/pathological changes in mammalian ovary remains ill-understood. From existing literature, we propose that hypoxia could be involved in the induction of autophagy within the follicular microenvironment of ovary. However, further studies are required to find out the pathways governing hypoxia-induced autophagy and the possible role of autophagy during hypoxic stress in mammalian ovary. Once the players and pathways of hypoxia-mediated autophagy are known, the therapeutic strategies could be developed to prevent the hypoxia-mediated loss of germ cell from the ovary. Studies on hypoxia-mediated autophagy in mammalian ovary could also be helpful in the management of problems like PCOS and POI in patients staying in hypoxic conditions.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were disclosured.

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