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ORIGINAL PAPER

Apoptosis in mammalian oocytes: a review

Meenakshi Tiwari[1] · Shilpa Prasad[1] · Anima Tripathi[1] · Ashutosh N. Pandey[1] · Irfan Ali[1] · Arvind K. Singh[2] · Tulsidas G. Shrivastav[3] · Shail K. Chaube[1]

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Abstract Apoptosis causes elimination of more than 99 % of germ cells from cohort of ovary through follicular atresia. Less than 1 % of germ cells, which are culminated in oocytes further undergo apoptosis during last phases of oogenesis and depletes ovarian reserve in most of the mammalian species including human. There are several players that induce apoptosis directly or indirectly in oocytes at various stages of meiotic cell cycle. Premature removal of encircling granulosa cells from immature oocytes, reduced levels of adenosine 3′,5′-cyclic monophosphate and guanosine 3′,5′-cyclic monophosphate, increased levels of calcium (Ca^{2+}) and oxidants, sustained reduced level of maturation promoting factor, depletion of survival factors, nutrients and cell cycle proteins, reduced meiotic competency, increased levels of proapoptotic as well as apoptotic factors lead to oocyte apoptosis. The BH3-only proteins also act as key regulators of apoptosis in oocyte within the ovary. Both intrinsic (mitochondria-mediated) as well as extrinsic (cell surface death receptor-mediated) pathways are involved in oocyte apoptosis. BID, a BH3-only protein act as a bridge between both apoptotic pathways and its cleavage activates cell death machinery of both the pathways inside the follicular microenvironment. Oocyte apoptosis leads to the depletion of ovarian reserve that directly affects reproductive outcome of various mammals including human. In this review article, we highlight some of the important players and describe the pathways involved during oocyte apoptosis in mammals.

Keywords Ovary · Oocyte · Granulosa cells · Signal molecules · Apoptotic pathways

Introduction

Mammalian ovary is responsible for generating competent oocytes required for the successful fertilization and early embryonic development. Apoptosis, a programmed cell death, plays a major role in the elimination of germ cells at all the stages of oogenesis and even after ovulation [1, 2]. More than 99 % of germ cells are eliminated from ovary via apoptosis through follicular atresia, while less than 1 % are culminated into oogonia [1, 3]. These oogonia enter into meiosis to give rise to primary oocytes [4, 5]. Primary oocytes are arrested at diplotene stage for several months to several years depending upon the mammalian species [6, 7]. These diplotene-arrested oocytes are encircled by several layers of granulosa cells inside the follicular microenvironment.

A cross-talk between encircling granulosa cells and diplotene-arrested oocytes is important for the survival of both cell types [8, 9]. The granulosa cell apoptosis and/or premature removal of encircling granulosa cells deprive oocyte from growth factors, nutrients and survival factors that may lead to apoptosis in diplotene-arrested oocytes cultured in vitro [10–14]. Our studies suggest that granulosa cell apoptosis inside the follicular microenvironment leads to oocyte apoptosis in rat [14–16]. The granulosa cell intactness protects oocytes from oxidative stress damage

✉ Shail K. Chaube
shailchaubey@gmail.com

[1] Cell Physiology Laboratory, Department of Zoology, Banaras Hindu University, Varanasi- 221005, U.P., India

[2] Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221005, U.P., India

[3] Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Baba Gang Nath Marg, Munirka, New Delhi 110067, India

in vitro [17–19]. Generation of reactive oxygen species (ROS) or depletion of antioxidant system leads to oocyte apoptosis [12–14, 20]. However, granulosa cell apoptosis in ovulated cumulus oocyte complexes can be used as predictors of oocyte quality [21–23].

A small number of follicles containing primary oocytes are selectively recruited during entire reproductive life of mammalian female including human. Follicular oocytes resume meiosis in response to pituitary gonadotropins surge or if removed from ovary and cultured in vitro [6, 7, 10, 11, 24, 25]. Although these diplotene-arrested oocytes (Fig. 1a) frequently undergo spontaneous meiotic resumption from diplotene arrest and further get arrested at metaphase-I (M-I) stage (Fig. 1b) but they are more susceptible to in vitro culture conditions and frequently die via apoptosis [15, 16, 18, 19].

At the time of ovulation, graafian follicles rupture and give rise to competent oocytes arrested at metaphase-II (M-II) stage. Once ovulated, these oocytes possess first polar body and waits for fertilization (Fig. 1c). If the fertilization does not occur, postovulatory aging results either spontaneous activation followed by metaphase-III (M-III) like arrest (Fig. 1d) [7, 26–29] or apoptosis (Fig. 1e) in oocytes [15, 16, 20, 30–34]. Studies suggest that good quality oocytes are ovulated first during early reproductive life. As the maternal aging occurs, oocyte becomes more susceptible towards apoptosis and limits reproductive outcome in human [35–37]. Thus, apoptosis plays a major role in eliminating majority of germ cells at all the stages of oogenesis and depletes ovarian reserve in various mammalian species including human.

Players of oocyte apoptosis

There are several players responsible for oocyte apoptosis in mammals (Fig. 2). Encircling granulosa cells decide the fate of an oocyte inside the follicular microenvironment [8]. Deprivation of oocytes from various signal molecules, survival factors and growth factors from encircling granulosa cells trigger susceptibility of oocytes towards apoptosis [15, 16]. This is supported by the observations that premature removal of granulosa cells from oocyte or granulosa cell apoptosis reduce meiotic as well as developmental competence [38–41] and increase susceptibility of follicular oocyte towards apoptosis [12, 13, 33, 34, 42].

Reduced granulosa cell-oocyte communication interrupts the transfer of adenosine 3′,5′-cyclic monophosphate

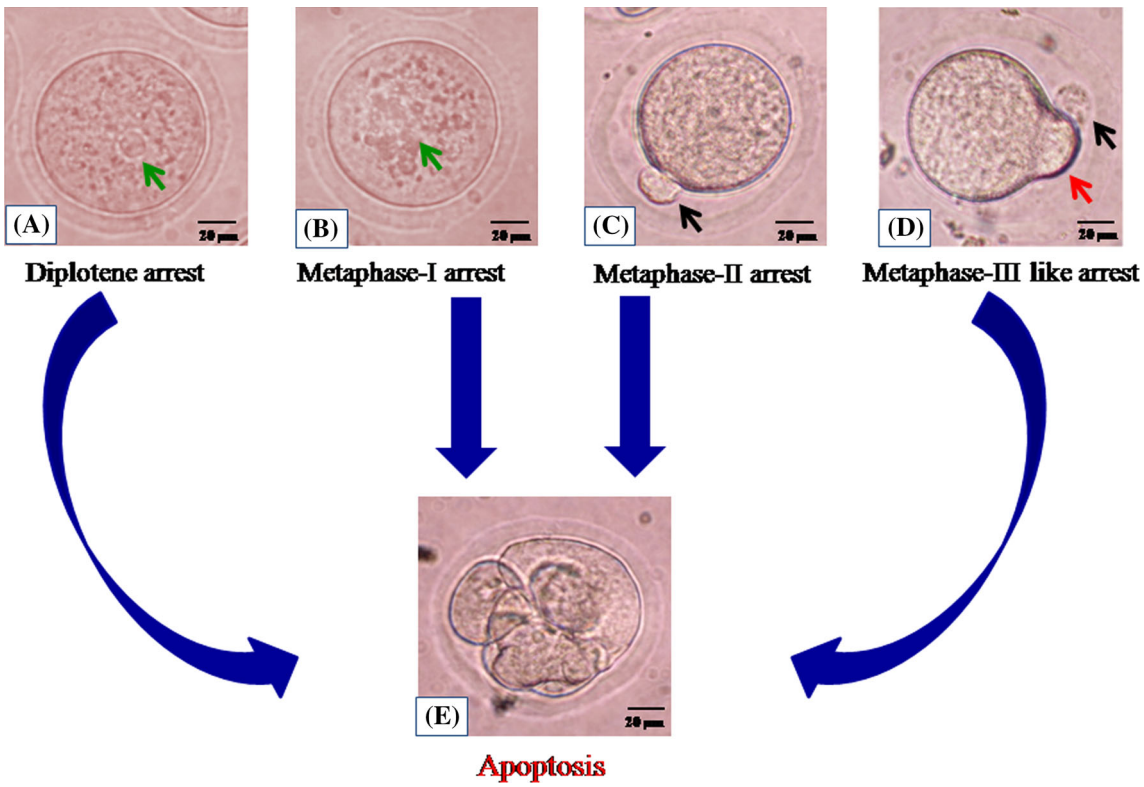

Fig. 1 Representative photograph showing morphological features characteristics of **a** diplotene arrest (*green arrow* showing germinal vesicle), **b** M-I arrest (*green arrow* showing germinal vesicle breakdown), **c** M-II arrest (*black arrow* showing first polar body), **d** M-III like arrest (*black arrow* showing first polar body *red arrow* showing second polar body extrusion) and **e** apoptosis in mammalian oocytes. Several factors could induce apoptosis in oocytes at various stages of meiotic cell cycle and reduces ovarian reserve (Color figure online)

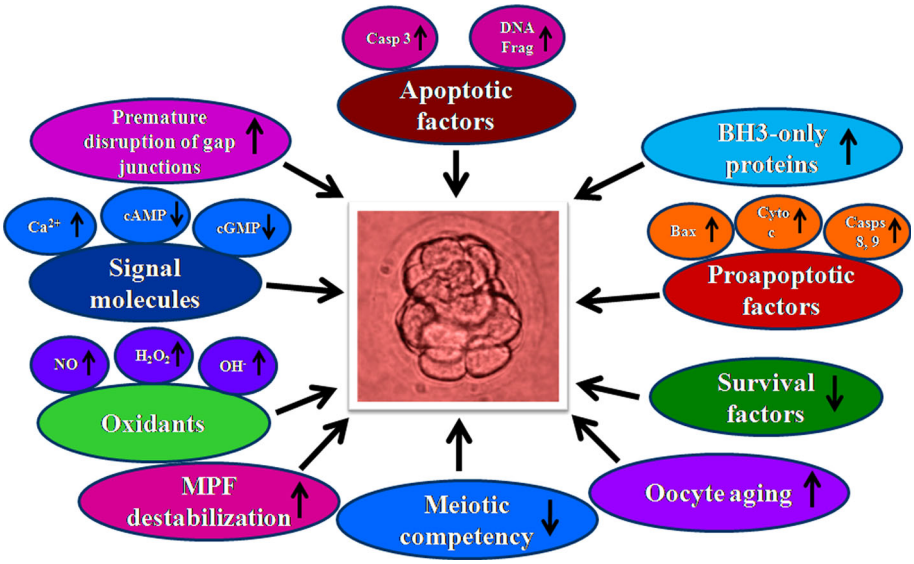

Fig. 2 Schematic representation showing various players of oocyte apoptosis such as premature disruption of gap junctions, Signal molecules (Ca^{2+}, cAMP and cGMP), Oxidants (NO, H_2O_2 and OH^-), MPF Destabilization, Meiotic competency, Oocyte aging, Survival factors, Proapoptotic factors (Bax, cytochrome c, caspases 8 and 9), BH3-only proteins and apoptotic factors (caspase 3 and DNA fragmentation). *Casp 3* Caspase 3, *DNA Frag* DNA Fragmentation, *Cyto c* Cytochrome c, *Casps 8, 9* Caspases 8 and 9

(cAMP) [43], guanosine 3′,5′-cyclic monophosphate (cGMP) [44, 45] and nitric oxide (NO) [46] levels to the follicular oocyte. Reduction of these signal molecules may trigger the generation of reactive oxygen species (ROS) in diplotene-arrested oocytes [11, 24, 25, 47]. These findings are further supported by our previous studies that diplotene-arrested oocytes are more susceptible to hydrogen peroxide (H_2O_2)-induced apoptosis as compare to M-II arrested oocytes [12–14, 30]. Increased inducible nitric oxide synthase expression and thereby NO level induce oocyte apoptosis [16, 18, 19, 32].

Calcium (Ca^{2+}) is one of the major signal molecules that regulate oocyte physiology [48–50]. The high sustained level of intracellular calcium ([Ca^{2+}]i) induces meiotic cell cycle arrest and apoptosis [26, 51, 52]. On the other hand, abnormally high ([Ca^{2+}]i) results in cell death [53, 54]. Calcium ionophore (CI) increases cytosolic free Ca^{2+} possibly by mitochondrial remodelling [55] and mitochondria membrane depolarization [56] leading to apoptosis in rat [30], pig [57] and bovine oocytes [58, 59] cultured in vitro. Studies from our laboratory suggest that CI increases cytosolic free Ca^{2+} level, induces generation of ROS and thereby apoptosis in rat oocytes cultured in vitro [30, 32, 60]. Sustained reduced levels of cAMP and cGMP as well as increased level of Ca^{2+} induce generation of ROS [24, 25]. This notion is further supported by our studies that increased levels of ROS with reduced catalase activity induce morphological apoptotic features in rat oocytes [15, 16, 18, 19].

Increased generation of ROS may lead to oxidative stress [6, 24, 25, 61, 62]. The oxidative stress reduces survival factors and induces destabilization of maturation promoting factor (MPF) in diplotene as well as M-II arrested oocytes. MPF stabilisation requires a series of phosphorylation/dephosphorylation of Cdk1, dissociation and degradation of cyclin B1 in oocytes [7]. Studies from our laboratory suggest that the inhibition of Cdk1 activity using roscovitine induces meiotic cell cycle arrest and apoptosis [33, 34, 63–65] probably by modulating the level of MPF heterodimer. Although MPF destabilization triggers spontaneous exit from M-II arrest [27–29], sustained decrease of destabilised MPF level triggers oocyte apoptosis [33, 34].

Oocyte after ovulation, either in vivo or under in vitro culture conditions, has limited number of adenosine triphosphate (ATP) [66] that results in the generation of ROS and thereby downregulation of anti-apoptotic factor such as Bcl2 [16]. The reduced anti-apoptotic factor leads to increased proapoptotic as well as apoptotic factors results in oocyte apoptosis [12–16, 33, 34, 60]. Factors that push oocytes to initiate apoptotic cell death indirectly are termed as proapoptotic factors. Apoptotic factors are directly involved in the disruption of histoarchitecture of a cell leading to appearance of morphological apoptotic features. BH3-only proteins act as proapoptotic factors and are essential mediators of apoptosis within ovary in several mammalian species [2, 5]. Apoptosis in the follicular oocytes results in the depletion of germ cells from ovarian reserve [67]. The ratio of apoptotic promoter (such as Bax expression) to suppressor (such as Bcl2 expression) within a cell determines whether cell will undergo apoptosis or survive [12–16, 68]. The involvement of Bax protein and caspase-3 activation during oocytes apoptosis has been reported in mouse and rat oocytes [12, 13, 69].

It has been generally accepted that an increased level of cytochrome *c* initiates apoptosis in oocytes [34, 60, 70]. The release of cytochrome *c* from internal stores activates upstream and downstream caspases in a cell leading to oocyte

apoptosis [12–16, 32, 34, 60]. Caspases are a family of cysteine-dependent aspartate-directed proteases that cleave intracellular polypeptides resulting into disruption of cellular architecture that leads to morphological changes characteristics of apoptosis [69]. Caspase-3 has substrate specificity for destruction of structural and specific proteins that leads to DNA damage in multiples of 180–200 base-pair, a hallmark feature of oocyte apoptosis [12–16, 18, 19, 30, 33, 34, 57, 60, 69, 71]. Although we have described major players responsible for depleting ovarian reserve by inducing oocyte apoptosis, there are several other equally important players involved in this process, which are not discussed herewith.

Underlying pathways in oocyte apoptosis

Oocyte apoptosis in mammals involves both mitochondria-mediated (intrinsic) [72] as well as cell surface death receptors-mediated (extrinsic) pathways (Fig. 3). Increased oxidative stress is one of the major factors that induce oocyte apoptosis [18, 19]. Various players, as described above, follow either mitochondria-mediated or death receptors-mediated pathways and some of them links these two pathways to induce oocyte apoptosis [2]. Players that induce generation of ROS follow mitochondria-mediated oocyte apoptosis [18, 19]. The proapoptotic ligands (FASL and TNFα) bind to their respective receptor and activate cell surface death receptors. Activation of death-receptors followed by caspases leads to death-receptor mediated apoptosis [73].

Increased levels of ROS due to decreased levels of cAMP as well as cGMP in oocytes [24, 47] and increased level of cytosolic free Ca^{2+} drive mitochondria-mediated apoptosis in follicular oocytes in mammals [60]. Studies suggest that increased cytosolic free Ca^{2+} level in response to CI induces generation of H_2O_2 [74]. The increased level of ROS can modulate expressions of Bax/Bcl2 ratio in mitochondria membrane and thereby membrane potential [69, 74]. Change in the mitochondria membrane potential triggers cytochrome *c* release in the cytoplasm of a cell [34, 60, 70], which activate upstream and downstream caspases in oocytes [74].

The proapoptotic BH3-only proteins act as key regulators of apoptosis within the ovary [2]. BID, a BH3-only protein, acts as a bridge between mitochondria-mediated and death receptor-mediated pathways. A truncated BID (tBID) induces overexpression of Bax, which then modulates mitochondria membrane potential that results in the release of cytochrome *c*. Cytochrome *c* binds to apoptotic protease activating factor 1 in the cytoplasm that activates caspase-9 as well as caspase-3. The caspase-3 cleaves key structural and regulatory proteins leading to several biochemical and morphological changes associated with oocyte apoptosis [60, 69, 75–77].

The extrinsic apoptotic pathway is initiated by activation of tumour necrosis factor receptor family (FAS and TNFR1), which bind to their ligands (FASL and TNFα) [2, 33]. Recent studies from our laboratory suggest that reduced Thr-161 phosphorylated Cdk1 as well as cyclin B1 levels destabilize MPF and push FasL-mediated oocyte apoptosis [33]. The increased FasL concentration results in Fas receptor trimerization and recruitment of the adaptor

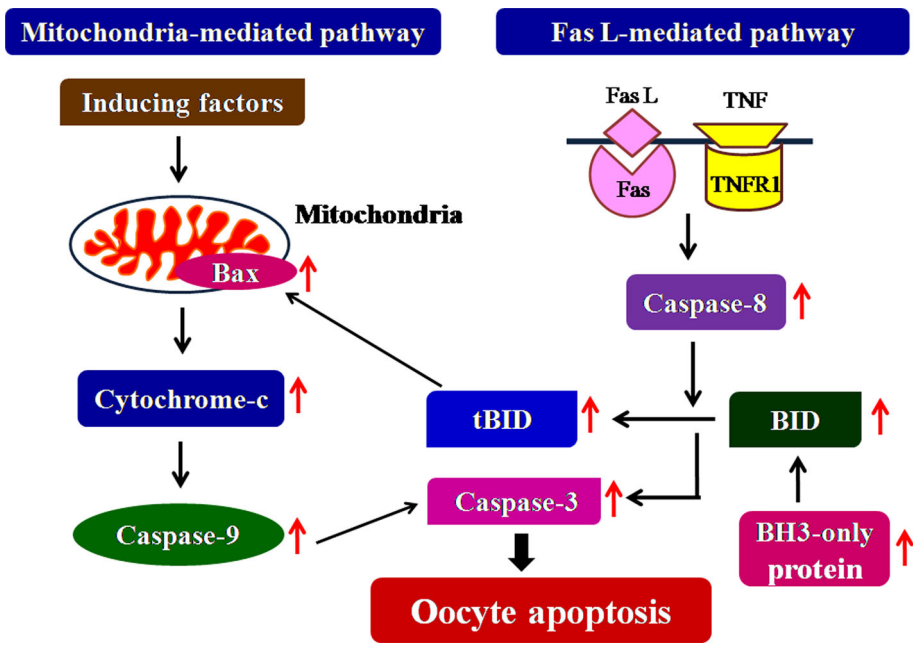

Fig. 3 Schematic hypothetical diagram showing involvement of mitochondria- and death receptor-mediated pathways in apoptosis of mammalian oocytes

molecule Fas-associated death domain-containing protein (FADD) through interaction between its own and clustered receptor death domains [33, 78]. On recruitment by FADD, procaspase-8 gets oligomerized and activated via autocatalysis. Active caspase-8 stimulates apoptosis by cleaving and activating caspase-3 [73]. This notion is further strengthened by our observations that roscovitine increases caspases-8 as well as caspases-3 activities in treated oocytes that showed cytoplasmic fragmentation (Fig. 1e). The activated caspase-3 cleaves key structural and regulatory proteins that result in DNA fragmentation, a hallmark feature of apoptosis [69]. These fragmented DNA are detected in a single oocyte using TUNEL assay [12–16, 33, 34, 69, 78–80].

Future prospectus

Several players are involved in inducing oocyte apoptosis either following mitochondria- or death-receptor mediated pathway or both. Based on the available literature, we propose major players and pathways involved in oocyte apoptosis. However, furthermore studies are required to delineate the stage specific involvement of these players and pathways inducing apoptosis in diplotene-, M-I, M-II and M-III arrested oocytes in mammals. Oocyte apoptosis is one of the major causes for the depletion of germ cells from ovary and has direct negative impact on female fertility in various mammalian species including human. Discovery of very small embryonic-like cells opens exciting new perspectives for neo-oogenesis but the source of new oocytes is still unclear and under debate [81, 82]. Although a ray of light is coming from ovarian stem cells to increase the number of oocytes, emphasis must be given to prevent oocyte loss via apoptosis from the ovary due to environmental changes, pathological conditions or drugs treatment so that the early depletion of ovarian reserve can be protected. The availability of good quality and number of oocytes could improve reproductive outcome in several mammalian species including human.

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Conflict of interests The authors declare that they have no competing interests.

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