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THE MOLECULAR BASIS OF OVARIAN CELL DEATH DURING GERM CELL ATTRITION, FOLLICULAR ATRESIA, AND LUTEOLYSIS

Jonathan L. Tilly, Ph.D.[1]

The Vincent Center for Reproductive Biology, Department of Obstetrics and Gynecology, Massachusetts General Hospital/ Harvard Medical School,Boston, MA 02114 USA

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1. ABSTRACT

Physiological cell death mechanisms (termed apoptosis, programmed cell death, active cell death, and biological cell death) play a fundamental role in the cyclic function of the ovary as it strives in each cycle to ovulate a viable egg for fertilization. Healthy ovarian follicles and corpora lutea are also required for the secretion of steroids to prepare the female reproductive tract for embryo implantation and gestation. Using molecular biological approaches combined with classic histological examinations, several recent studies have confirmed the occurrence of apoptosis in female germ cells (oogonia and oocytes) during fetal ovarian development, granulosa cells during follicular atresia, and cells of the corpus luteum during luteolysis.

Additionally, new light has been shed on the potential gene products which function to precisely coordinate the balance of life and death in the ovary. It is the purpose of this review to discuss the concepts of physiological cell death as they relate to ovarian function, and to offer testable hypotheses concerning the intracellular effector pathways responsible for directing ovarian cell fate in response to changes in hormonal stimuli.

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[1] To whom correspondence should be addressed, at The Vincent Center for Reproductive Biology, VBK137F-GYN, Massachusetts General Hospital/Harvard Medical School, Fruit Street, Boston, MA 02114 USA Tel: (617) 724-2182; Fax: (617) 726-7548.Email: tilly.jonathan@mgh.harvard.edu

2. PHYSIOLOGICAL CELL DEATH: MECHANISMS AND GENES

2.1. Introduction.

The concept of apoptosis suggests that at some point in the destiny of any given cell, an irreversible cascade of gene-directed events is set in motion which permits efficient removal of that cell from its neighboring counterparts without a disruption in normal tissue function (reviewed in 1-3). Many of the features of physiological cell death have been remarkably conserved through evolution such that the existence of a universal pathway for execution of the cell suicide command has been proposed (1-3). Unlike the random process of pathological cell death (most often referred to as necrosis) which occurs in contiguous tracts of cells following exposure to highly noxious stimuli, apoptosis proceeds in an orderly fashion and in general does not elicit an immune response at the site of cell loss (4,5). At its most

Table 1. Reported Regulators of Physiological Cell Death[1]

Name	Role in Cell Death	Mechanism of Action?[2]	Reference
BCL-2	Inhibition	Cellular Redox State	31,32,33
		Calcium Homeostasis	34
		Ras Interaction	35
		BAX Interaction	14,18,28,29
BAX	Activation	BCL-2/BCL-X_L Interaction	14,18,28,29
BCL-X_L	Inhibition	BAX Interaction	19,27,28
BCL-X_S	Activation	BCL-2 Interaction	19,27,28
MCL-1	Inhibition	BAX Interaction	20,28
BAD	Activation	BCL-2/BCL-X_L Interaction	22
BAG-1	Inhibition	BCL-2 Interaction	21
BAK	Activation	BCL-2/BCL-X_L Interaction	24,25,26
CED-9	Inhibition	Unknown (BCL-2 homolog)	17
SOD	Inhibition	Anti-Oxidant Enzyme	81
GSHPx	Inhibition	Anti-Oxidant Enzyme	31,81
Catalase	Inhibition	Anti-Oxidant Enzyme	81
p53	Activation	*bcl-2*/*bax* Gene Transcription	40,43,44,45
c-myc	Activation	Gene Transcription	41,42
ICE	Activation	Proteolysis	48
ICH-1_L	Activation	Proteolysis	49
ICH-1_S	Inhibition	ICH-1_L Antagonism	49
ICE_{rel}II[3]	Activation	Proteolysis	51,52,53
ICE_{rel}III	Activation	Proteolysis	53
MCH-2	Activation	Proteolysis	54
CED-3	Activation	Unknown (ICE homolog)	47
Calpain	Activation	Proteolysis	55

[1]This is only a partial listing of proteins which have been implicated in activating or inhibiting the cell suicide pathway.
[2]The precise mechanisms of action of the majority of cell death regulators indicated are unknown. The examples provided are derived from studies currently available in the literature, and probably do not represent the full spectrum of biological functions.
[3]The protein referred to as ICE_{rel}II (ref. 53) has also been termed ICH-2 (ref. 51) and TX (ref. 52).

basic level, a cell dying by apoptosis exhibits a marked reduction in cytoplasmic volume coincident with or immediately followed by nuclear pyknosis resulting from high and low molecular weight genomic DNA cleavage. Intracellular organelles and nuclear remnants are then neatly packaged into plasma membrane-bound vesicles, referred to as apoptotic bodies, and phagocytized by neighboring cells or resident macrophages (4,5). The time course for apoptosis, from the first discernible morphological change to phagocytic removal, can be remarkably short (6). Thus, much of our knowledge of the features of apoptosis have been derived from *in vitro* studies of isolated cells as the detection of apoptosis *in vivo* is hindered by the efficiency and rapidity of phagocytic clean-up.

Physiological cell death occurs in essentially all multicellular organisms. Functions of cell death include embryonic pattern formation (7), development of the male and female reproductive tracts (8,9), establishment of balanced pre- and post-synaptic neuronal junctions in the developing brain (reviewed in 10), removal of autoreactive T-cells from the thymocyte cell repertoire (reviewed in 11), regression of post-lactational breast tissue (12), and a general homeostatic maintenance of proper cell numbers in most tissues (1,2,4). Of equal importance, disruptions in the normal course of cell death have been linked to many pathological disorders ranging from tumorigenesis (abnormally low cell death rates; (13)) to Alzheimer's disease (excessive cell death; (10)). This has served to fuel the fire for research by a large number of investigators on the underlying events that can be manipulated to regain control of normal cell death in the affected tissue. The search for these clues to life and death has primarily centered on identification of proteins that either promote or prevent activation of the cell suicide pathway in a variety of tissues (Table 1) (reviewed in 2,3,14,15). The number of genes which encode proteins involved in the regulation of apoptosis has grown at an almost exponential rate, albeit much less is known of their mechanisms of action.

For the sake of clarity and brevity in this review, a sampling of the genes identified thus far will be grouped into four classes for subsequent discussion: 1) proteins encoded by members of the *bcl-2* gene family; 2) oxidative stress response factors; 3) transcriptional regulators; and 4) cytoplasmic proteases including calpain and members of the interleukin-1β-converting enzyme (ICE) gene family.

2.2. The *bcl-2* Gene Family.

Proteins encoded by *bcl-2* and related genes are probably the most well-studied cell death regulatory factors (reviewed in 14,16). The *bcl-2* (B-cell leukemia/lymphoma-2) proto-oncogene was the first negative regulator of apoptosis reported. This gene was originally identified through analysis of the human t(14;18) (q32;q21) chromosomal translocation which predisposes affected individuals to the development of B-cell lymphomas. This translocation juxtaposes the *bcl-2* gene with the immunoglobulin G heavy chain locus, leading to deregulated over-expression of *bcl-2* (14). Following reports of this initial correlation between high levels of *bcl-2* expression and tumorigenesis, numerous studies have confirmed the death repressor role of the BCL-2 protein in a variety of cell types and under a spectrum of conditions known to trigger cell death (14,16). These data are more intriguing when taken with the fact that BCL-2 was found to prevent apoptosis without altering proliferation rates, a function not attributed to any other protein at the time. Of additional note, a homolog of *bcl-2* (termed *ced-9*) has been characterized in the nematode, *Caenorhabditis elegans*. Through elegant gene mutation studies, the protein encoded by the *ced-9* gene has been shown to play a fundamental role in cell death inhibition in the worm during development, analogous to the death repressor activity of BCL-2 in vertebrates (17).

The proposed function of BCL-2 as a regulator of apoptosis also served as the catalyst for subsequent investigations by increasing numbers of laboratories to characterize other proteins involved in the cell death cascade. Two of these initial efforts led to simultaneous reports of the first additional members of the *bcl-2* gene family, namely the *bax* (*bcl-2*-associated-x gene; (18)) and *bcl-x* (*bcl-2*-related gene-x; (19)) genes. The BAX protein was isolated via its ability to bind to, and thus co-immunoprecipitate with, BCL-2. Analysis of the function of BAX through gene transfer experiments revealed its actions to be that of a death susceptibility factor, initially believed to act by countering the death repressor activity of BCL-2 (18). However, it is now thought that BAX directly leads to cell death following the formation of BAX:BAX homodimers, a process that can be disrupted by the presence of BCL-2 (14). At the same time that *bax* was reported, another *bcl-2* homolog termed *bcl-x* was identified in chicken lymphoid cells by low stringency hybridization cDNA cloning. Interestingly, the human cDNA was also cloned in this study; however, unlike its avian counterpart, the human *bcl-x* gene appears to undergo alternative splicing to yield two messenger RNA variants: a long isoform (encoding BCL-X_L) which functions like BCL-2 to suppress apoptosis, and a truncated or short isoform (encoding BCL-X_S) which can mimic the actions of BAX by antagonizing BCL-2-promoted cellular survival (19). Subsequent to these findings, several additional members of the *bcl-2* gene family have been isolated (20-26), and the proteins encoded by these genes have been shown to interact with each other to direct cell fate (24-28). Additionally, precise functional domains present within the sequences of BCL-2 and related factors have been characterized and appear prerequisite for protein:protein interaction and the regulation of cell death (29).

The function of any member of the BCL-2 family remains an area of intensive investigation as very little data exist regarding the actions of these cell death regulatory proteins within the cell. The localization of BCL-2 within intracellular membranes, particularly those of the mitochondria, initially suggested that this factor may regulate the reduction-oxidation (redox) state of the cell (30). Assuming this to be the case, two independent studies subsequently reported that over-expression of *bcl-2* protects cells from death induced by reactive oxygen species (31,32). On the other hand, a follow-up study suggested that BCL-2 may actually function as a pro-oxidant factor, thus conveying its protective effects indirectly through induction of the cell's normal oxidative stress response repertoire of defense enzymes (33). In any case, it should be noted that the regulation of cellular redox is probably not the only mechanism by which BCL-2 acts, as additional studies have implicated this protein in calcium homeostasis (34) and growth factor-associated signaling events (35).

2.3. Reactive Oxygen Species.

The link of BCL-2 to reactive oxygen species provides an interesting association between this cell death regulator and the second category of genes to be discussed, namely members of the oxidative stress response gene family. Reactive oxygen species are generated in all cells as a consequence of normal metabolic function, or as a result of ligand-activated receptors tied to membrane phospholipid metabolism (reviewed in 36). Members of this gene family include three forms of superoxide dismutase enzyme (secreted, mitochondrial and cytosolic) which convert superoxide anion radical to peroxide intermediates, as well as catalase and glutathione peroxidase which are responsible for metabolism of peroxides to water. Extensive cellular damage occurs in response to accumulation of reactive oxygen species or their intermediates, and recent data indicate that the end-result of this damage in most cases is the induction of apoptosis (reviewed in 37). Additionally, disruptions in nuclear DNA integrity (*e.g.* formation of 8-hydroxydeoxyguanosine moieties) elicited by free radicals provide an important link to the next family of cell death genes to be discussed, the transcriptional regulators of cell death.

2.4 Transcriptional Regulators.

Transcriptional regulators represent an interesting family of proteins as these factors, unlike BCL-2, can modulate both mitosis and apoptosis. Two of these proteins in particular, p53 and c-myc, have been directly linked to the induction of apoptosis (38-42). Of the target genes affected by nuclear accumulation of p53 or c-myc, members of the *bcl-2* gene family have emerged as primary targets for transcriptional regulation. Using both *in vivo* and *in vitro* approaches, the p53 protein has been reported to bind sequence-specific enhancer regions in the *bax* gene promoter and repressor elements in the *bcl-2* gene (43-45). Thus, accumulation of p53 in the nucleus of a compromised cell is

thought to trigger a disrupted balance between *bax* (increased) and *bcl-2* (decreased) expression, a scenario known to predispose a cell to apoptosis activation (18). A primary stimulus for stabilization and nuclear translocation of p53 is DNA damage (46), such as that resulting from attack by reactive oxygen species (36). If this is the case, cellular damage induced by uncontrolled oxidative stress may lead to apoptosis as a consequence of p53-mediated alterations in expression of cell death-associated genes. The role of c-myc in regulation of the expression of *bcl-2* and related genes is not as well characterized; however, the *bax* gene contains four consensus sequences for c-myc binding which suggests that this transcription factor may as well act, at least in part, via altered expression of these target cell death genes (45).

2.5. Cytoplasmic Proteases.

The fourth family of genes, composed primarily of cytoplasmic proteases, are among the most recently identified components of the cell death pathway. Similar to the *ced-9*/*bcl-2* homology discussed earlier, our knowledge of the role of many of these proteases in cell death stemmed from initial studies of the protease homolog in *C. elegans*, CED-3 (47). Genetic mutation of the *ced-3* gene leads to a persistence of unwanted cells in the worm due to a loss of normal cell death during development. Sequence homology analysis of *ced-3* with known vertebrate genes revealed a striking level of similarity with the vertebrate cysteine protease, interleukin-1β-converting enzyme (ICE; (47)). Following the initial observation that CED-3 and ICE are likely homologs, numerous reports have described a rapidly growing family of ICE-related proteases that possess two important conserved features: a pentapeptide motif (QACRG) which likely serves as the catalytic domain, as well as cleavage specificity at aspartate residues (reviewed in 15). To date, members of this family include ICE, ICE-and-*ced-3*-homolog-1 (Ich-1), cysteine-protease-P32 (CPP32), ICE-relative-II (ICE_{rel}II; also called Tx or Ich-2), ICE_{rel}III, and Mch-2 (48-54). Various approaches leading to enhanced or reduced activity of ICE-related proteases in cells have provided compelling evidence that members of this family of cell death regulators play an important, if not prerequisite, role in apoptosis in vertebrate species (15), analogous to that described for the *ced-3* gene product in *C. elegans* (47).

Not all cytoplasmic proteases implicated in cell death, however, belong to the ICE gene family. In many cell types, calpain has also been proposed as a mediator of apoptosis (55). This calcium-activated protease is ubiquitously expressed in most cells, and exists in two forms within the cell. These two forms are composed of a common large subunit derived from a single gene bound to isoform-specific small subunits encoded by separate genes. Depending upon the level of cytoplasmic calcium required to activate each form of the enzyme, the two forms of calpain can be easily segregated (reviewed in 56). Interestingly, the regulation of calpain activity does not reside solely at the level of gene expression as most cells also express a specific inhibitor of calpain termed calpastatin (56). Therefore, reminiscent of the BCL-2:BAX rheostat described earlier, the balance of calpain to calpastatin is likely an important determinant of whether active calpain is available for execution of the cell death command.

The targets of ICE-related proteases and calpain during apoptosis are diverse, but turn out to be very logical when evaluated in hindsight (reviewed in 15). In addition to activating each other, enzymes encoded by members of the ICE gene family have been reported to cleave proteins involved in DNA repair (57,58) and messenger RNA processing (59), as well as structural components of the nuclear matrix (60). Along these same lines, targets for calpain include cytoskeletal proteins (actin, fodrin) and gap junction proteins (56,61). Thus, a scan of this representative list of proteins which are targets for protease attack during cell death reveals a common or underlying theme, namely that all of these proteins are required for the maintenance of normal cell structure, function and homeostasis.

3. MECHANISMS AND EFFECTORS OF CELL DEATH IN THE OVARY

3.1. Peri-Natal Germ Cell Attrition

3.1.1. Identification of Physiological Cell Death in Germ Cells.

Despite the fact that the regulation of cell death has been extensively studied in several extragonadal tissues, only recently have reproductive biologists realized that the cyclic nature of germ cell development in the ovary and testis is also very tightly linked to the process of apoptosis. At one of the earliest points during embryonic ovarian development, when oogonia are undergoing mitosis to increase the total germ cell pool, large numbers of these cells are lost through degeneration (62-64). Additionally, oogonia that escape death during mitotic division may still be lost via apoptosis during the first phases of meiosis when the diploid oogonia mature into haploid oocytes (62,64-66). Although the reasons for the massive destruction of oogonia and oocytes remains a mystery, it has been estimated that the fate of up to two-thirds of the ovarian germ cell pool present during embryogenesis is degeneration. Moreover, it is now known that oogonia and oocytes which degenerate *in vivo* or *in vitro* exhibit many of the morphological and biochemical criteria associated with an apoptotic event. These features include chromatin margination and fragmentation with the resultant nuclear pyknosis, cellular condensation, and induction of cell death markers such as tissue transglutaminase (67-69).

3.1.2. Hormonal Control of Germ Cell Survival.

From a regulatory standpoint, somatic cell-derived growth factors, most notably the product of the *Steel* gene (stem cell growth factor/SCF or kit ligand), appear to be critically important for the maintenance of germ cell survival. Data which strongly support this concept come from both *in vivo* (naturally-occurring mutations in the genes encoding SCF or the SCF receptor, *c-kit*) and *in vitro* (cultures of primordial germ cells) analyses. It has been known for almost forty years that mutations in the SCF and *c-kit* genes lead to gonadal dysgenesis and sterility (70). The fundamental role of SCF in germ cell survival has been

further substantiated by studies of SCF effects on primordial germ cells cultured *in vitro*. As anticipated from the genetic mutation studies, the presence of SCF prevents apoptosis which occurs in germ cells deprived of tropic hormone support (68). Interestingly, SCF, although critical for germ cell survival, does not by itself markedly alter germ cell proliferation, suggesting that the actions of SCF in the developing ovary must be complemented by the presence of additional growth factors.

3.1.3. Intracellular Effectors of Survival Signals in Germ Cells.

The intracellular pathways which mediate the anti-apoptotic effects of SCF (and other factors?) in germ cells are not well-understood. A recent report has linked activation of the retinoic acid receptor pathway to enhanced germ cell proliferation (71), suggesting that downstream genes which are targets for transcriptional regulation by the ligand-activated retinoic acid receptor may be important components of oogonium and oocyte survival. The identity of these genes have yet to be elucidated. However, recent findings obtained from analysis of ovaries collected from mice possessing a targeted disruption of the *bcl-2* gene have revealed that a loss of BCL-2 bioactivity is associated with a compromised endowment of oocytes and primordial follicles (72). Based on the similar phenotypes observed in ovaries of mice which lack SCF, c-kit or BCL-2, it has been proposed that SCF mediates germ cell survival, at least in part, via enhanced expression of *bcl-2* (72). Concrete proof of this hypothesis, however, awaits further testing.

3.2. Post-Natal Follicular Atresia

3.2.1. Occurrence and Hormonal Regulation of Apoptosis in Follicles.

Once follicles are established in the ovary during the peri-natal period, germ cell loss occurs primarily as an indirect consequence of atresia of follicles not selected for ovulation. In all species studied thus far, the initiation of apoptosis in granulosa cells is one of the earliest signs of follicular demise (reviewed in 73). The occurrence of apoptosis in granulosa cells of atretic follicles has been documented by both morphological (74,75) and biochemical criteria (76,77), and the use of DNA oligonucleosomes to identify apoptotic cells has recently paved the way for a large number of studies concerning the regulation of atresia (see below). Data derived from these efforts have demonstrated that, *in vivo*, pituitary-derived gonadotropins (follicle-stimulating hormone or FSH, luteinizing hormone or LH) are the primary endocrine factors responsible for inhibiting apoptosis in granulosa cells of developing follicles (78,79). These findings have been confirmed and extended through analysis of apoptosis in granulosa cells of ovarian follicles incubated *in vitro* as a model for elucidating the events associated with atresia (80,81). Moreover, the ability of FSH and LH to suppress apoptosis and atresia likely involves augmented signaling through intrafollicular growth factor pathways (78,80), which in turn may be tied to progesterone as a final autocrine mediator (82). It should also be pointed out that gonadotropin-independent pathways probably contribute to the maintenance of granulosa cell survival (83,84), collectively demonstrating the complexity of events which surround the fate of any given follicle. The involvement of multiple hormonal signaling pathways in apoptosis regulation has been reported for granulosa cells of the avian ovary as well (83,85). These findings suggest that, regardless of species, the final step which either activates or represses widespread apoptosis during atresia is dependent upon the prior interpretation of many extracellular signals.

3.2.2. The *bcl-2* Gene Family and Follicular Atresia.

The intracellular effectors responsible for the regulation of apoptosis in granulosa cells are no less complex. Studies towards this endpoint have, however, been greatly facilitated by the wealth of information available in the literature concerning the genes involved in apoptosis in cells of extragonadal tissues (see previous section and Table 1). Using these data as a foundation, initial investigations centered on the potential role of BCL-2 and related factors in deciding the fate of rat granulosa cells during follicular development (79). Results from these studies indicated that increased expression of the *bax* death susceptibility gene coincides with the induction of apoptosis in granulosa cells during atresia both *in vivo* and *in vitro* (79). The role of BAX in mediating granulosa cell demise has been reinforced by histological analysis of ovaries collected from mice deficient in functional BAX protein (86). These data have indicated that “knock-out” of the *bax* gene renders granulosa cells resistant to the normal induction of apoptosis in follicles destined for atresia (*e.g.* possessing degenerative oocytes) (86). These findings provide concrete proof that this cell death factor plays a fundamental role in regulating apoptosis in granulosa cells. Expression of the *bcl-2* and *bcl-x* genes in granulosa cells of rodent and avian ovaries has also been reported (79,85,87), albeit the role of the proteins encoded by these genes in determining the fate of granulosa cells remains to be established.

3.2.3. Oxidative Stress During Atresia.

In keeping with the proposal that apoptosis proceeds in many tissues via a universal pathway, a follow-up study was conducted to complement the data derived from analysis of *bcl-2*-related genes in the ovary. This series of experiments reported on the potential role of reactive oxygen species as a trigger for apoptosis in granulosa cells during atresia (81). Data derived from these investigations demonstrated that the ability of FSH to suppress apoptosis in granulosa cells of rat follicles *in vitro* could be mimicked by addition of anti-oxidant enzymes to the culture medium. Although these data provide the first evidence that reactive oxygen species may activate the apoptotic pathway in granulosa cells, it remained unclear how large enzymes such as superoxide dismutase and catalase, when added exogenously, could convey protection from reactive oxygen species within the cell. However, recent experiments with cultured cells have demonstrated that addition of exogenous catalase to the culture medium does in fact dramatically raise the intracellular levels of catalase activity (88), thus supporting the concept that prolonged oxidative stress may be one determinant of granulosa cell demise (81). Moreover, expression of factors such as superoxide dismutase (secreted and mitochondrial) in the immature rat ovary is markedly increased in response to exogenous gonadotropin treatment

in vivo (81). Collectively, these data suggest that not only do gonadotropins enhance expression of anti-oxidant genes in the ovary, but that the defense enzymes encoded by anti-oxidant genes (*e.g.* superoxide dismutase, catalase) are as effective as gonadotropins in the suppression of granulosa cell apoptosis. However, more work is needed to characterize the pathways activated by reactive oxygen species in granulosa cells, and to elucidate how these pathways then converge with other signaling events to activate apoptosis during atresia.

3.2.4. Transcriptional Regulators in Granulosa Cells.

The role that transcriptional regulators play in the process of granulosa cell apoptosis has primarily centered on the p53 tumor suppressor protein. Two reports are currently available in the literature, one of which documents by immunocytochemical procedures the nuclear accumulation of p53 in rat granulosa cells destined for apoptosis (89). By comparison, p53 is absent in ovaries collected from gonadotropin-stimulated rats, consistent with the fact that this cell death inducer is only present in ovarian cells during episodes of apoptosis (89). In support of these data, over-expression of p53 in rat granulosa cells has been reported to trigger a rapid onset of apoptosis (90), confirming that the actions of p53 in the ovary are indeed tied to the regulation of cell death. Although nothing is known of how nuclear accumulation of p53 in granulosa cells leads to apoptosis, p53 is detectable only in follicle populations that concomitantly express high levels of *bax* (*e.g.* atretic follicles) (see 79 and 89). These data therefore suggest that the *bax* death gene may in fact be a target for p53 transactivation in the ovary, as has been reported for other cell types (43,45). To date there exists only a single preliminary observation describing the relationship between c-myc expression and apoptosis in granulosa cells (87). These data indicate that the levels of c-myc are highest in populations of avian granulosa cells in follicles that still retain the capacity to undergo atresia (87). Although these findings are consistent with the proposed role of c-myc in apoptosis induction (41,42), the precise functions of c-myc in granulosa cells during atresia require further investigation.

3.2.5. Protease Activation During Apoptosis in Granulosa Cells.

Only recently has the role of cytoplasmic proteases, specifically members of the ICE gene family, in the demise of granulosa cells been assessed (91). Following isolation of cDNAs encoding rat ICE, Ich-1 and CPP32, it was determined that levels of Ich-1 and CPP32 mRNA in the ovary are reduced following *in vivo* gonadotropin stimulation (91). By comparison, extremely low levels of ICE mRNA are detectable in ovarian homogenates and expression of this protease is not altered by gonadotropin treatment. Along these same lines, ICE activity is undetectable in either healthy or atretic ovarian antral follicles of the rat, collectively indicating that ICE *per se* is probably not involved in the final life-and-death decision making process in granulosa cells (91). It should also be noted that one of the primary products of ICE activity, namely active interleukin-1β (IL1β), has been reported to slightly but non-significantly increase basal rates of apoptosis in rat follicles cultured *in vitro*. Moreover, this cytokine does not antagonize (nor potentiate) tropic hormone-supported survival of granulosa cells in follicles *in vitro* (91), further substantiating that ICE itself is not likely involved in the survival or death of granulosa cells.

Although another recent report has suggested that IL1β prevents apoptosis in granulosa cells of cultured rat follicles (92), interpretation of these findings is difficult for several reasons. First, the ICE/IL1β pathway is primarily linked to the induction of cell death (15). Moreover, the source of ICE activity required for generation of IL1β from the precursor proenzyme is unknown as ICE activity is below detectable limits in rat follicles at the stage of development used for those studies (91). Lastly, previous work documents a cytotoxic effect of IL1β in rat ovarian cell dispersates (93), and these data are similar to the slight but non-significant increase in apoptosis induced by IL1β in rat ovarian follicles reported in a separate study (91). In any case, enhanced intrafollicular activity of other members of the ICE protease family (*e.g.* ICH-1, CPP32) may be responsible for such endpoints as endonuclease activation (91) and disrupted messenger RNA processing (94) in granulosa cells during the initiation and progression of apoptosis.

3.3. Regression of the Corpus Luteum (CL)

3.3.1. Cell Death in the CL.

Lastly, the development of a CL following ovulation of a follicle is required for the maintenance of pregnancy (95,96). The CL functions primarily as a site for the massive synthesis of progesterone which is required for the maintenance of the uterine endometrial lining. If pregnancy does not occur, the CL predictably regresses at a specific point in the estrous or menstrual cycle (95,96), and the ensuing loss of steroid hormone support to the uterus leads to apoptosis in endometrial cells (97). Although several studies concerning cell death during luteal regression have been published, the precise role of apoptosis in the fate of the CL remains unclear. Regression of the CL occurs in two phases, the first of which is associated with the loss of progesterone synthesizing capacity. This process, known as functional regression, occurs prior to any discernible morphological changes in luteal cell integrity and is likely a reversible step if sufficient luteotropic support is provided (95,96). In contrast, the second phase of luteolysis, termed structural regression, is probably not reversible. Structural regression of the CL occurs well after the initial decline in steroid output, and is most likely the point at which apoptosis comes into play (98-101). Data to support this contention are derived from analysis of both natural and induced luteolysis in many species, with the common link being the identification of apoptosis in structurally regressing luteal tissue (98-101).

3.3.2. Control of Cell Death During Luteolysis.

To date, two key hormones appear to directly regulate the process of cell death during luteal regression: the luteolysin prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and the luteotropin

chorionic gonadotropin (CG). Consistent with the themes presented throughout this review, ample evidence exists which link the actions of both $PGF_{2\alpha}$ and CG to the regulation of reactive oxygen species in the CL. For example, the immediate cellular response to $PGF_{2\alpha}$ following interaction of the hormone with its receptor in luteal cells is believed to be the generation of free radical species (102). Luteal cells exposed to an environment rich in reactive oxygen species *in vitro* exhibit a markedly impaired ability to synthesize progesterone (103; reviewed in 104, 105), one of the hallmark features of functional luteolysis *in vivo*. Furthermore, prolonged oxidative stress has been suggested as a trigger for apoptosis during structural luteolysis, a hypothesis supported by several lines of investigation (101). On the other hand, treatment of rats with human CG has been reported to increase the expression of anti-oxidant enzymes in the CL (106), whereas a lack of CG support in the human CL may predispose the luteal cells to increased oxidative stress (107). These data, taken with the fact that human CG can directly suppress the occurrence of apoptosis in luteal tissue (108), strongly support that changes in the redox state of luteal cells may be one of the primary determinants of the lifespan of the CL (104,105). Although activation of other intracellular effector pathways, such as those involving changes in *bcl-2* and *c-myc* expression, have been proposed as potential mechanisms underlying luteolysis (109-111), additional studies are required to clarify the role of these signaling events in determining the fate of luteal cells.

4. CONCLUSIONS

The recent use of sophisticated molecular biological techniques to study the female reproductive system has provided unequivocal proof that the process of apoptosis is a fundamental event in normal ovarian function. The occurrence of apoptosis in germ cells, granulosa cells, and cells of the CL is most likely regulated by cell type-specific hormonal signals. However, death of all ovarian cells may share a final common pathway of intracellular effectors involving members of the *bcl-2* gene family, oxidative stress response factors, transcriptional regulators, and cytoplasmic proteases. Characterization of the role of these conserved cell death regulators will provide exciting new data regarding the molecular basis of ovarian development and function. Furthermore, this knowledge may allow development of novel approaches to overcome normal and pathophysiological senescence of the ovary, and to improve the pregnancy success rates of Assisted Reproductive Technology Programs.

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