Germ Cell Apoptosis: Clinical Implications

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Abstract

Apoptosis, a genetically regulated cell suicide, is an energy-requiring process that can be observed throughout the whole of the animal and plant kingdoms. It is morphologically distinct from the degenerate process of necrosis. Apoptosis permits the safe disposal of cells at the point in time when they have fulfilled their biological functions. Apoptosis plays an important role in cellular homeostasis and organ developmental processes in general and in gonadal development in particular. This process is known to be accelerated or diminished, in many pathologic states. Therefore, the understanding of apoptotic regulation has significant clinical ramifications.

This article reviews the basic understanding of programmed cell death with respect to germ cell apoptosis (male and female). This review attempts to summarize data gathered over the last few years, from both experimental and patient settings, which not only documents the presence of apoptosis, but begins to define its contribution to the pathogenesis of a number of genital diseases with respect to areas of interest to pediatric surgeons, urologists and gynecologists. Pro or antiapoptotic interventions may become the future target for cell and organ protection in patients suffering from gonadal diseases.

Keywords Apoptosis; Cell death; Testis; Ovary

Apoptosis: General

Definition of Apoptosis

In the multicellular organism, a dynamic process of cell turnover is maintained through a complex balance between cell proliferation, cell differentiation as well as cell death. Apoptosis, an ancient cell suicide program that is essential for development, tissue homeostasis and immunity. It is an evolutionarily conserved and highly regulated process of nonfunctional cells death [1,2]. Through this process, the body disposes of unwanted cells by self-destruction and is our utmost defense against damaged cells to maintain the healthy balance between cell survival and cell death in metazoan [3]. Cell death is advantageous for the organism because it removes terminally injured or unwanted cells that utilize valuable substrates and nutrients. In the last decades, many of the essential pathways that control this phenomenon have been elucidated.

There are two main kinds of cell death: "accidental cell death" and "programmed cell death". "Accidental cell death" implies cell death caused by accidental external agents, such such as ischemia, heat or toxic agent. Necrosis (from the Greek νεκρός, "dead") is the premature death of cells and living tissue. Cells which die due to necrosis do not usually send the same chemical signals to the immune system that cells undergoing apoptosis do. This prevents nearby **phagocytes** from locating and engulfing the dead cells, leading to a build-up of dead tissue and cell debris at or near the site of the cell death. For this reason, it is often necessary to remove necrotic tissue surgically. Necrosis begins with an impairment of the cell's ability to maintain homeostasis, leading to an influx of water and extracellular ions. Intracellular organelles, most notably the mitochondria, and the entire cell swell and rupture (cell lysis). Due to the ultimate breakdown of the plasma membrane, the cytoplasmic contents including lysosomal enzymes are released into the extracellular fluid. Therefore, in vivo, necrotic cell death is often associated with extensive tissue damage resulting in an intense inflammatory response. Morphologically, accidental cell death is characterized by cellular swelling and was defined by Majno and Joris by the term "oncosis" (from the Greek word ónkos meaning swelling) [4]. "Programmed cell death" refers to a situation where a cell is programmed to die at a specific point in its life time. The term apoptosis is derived from the Greek words apó, meaning from and ptósis, meaning a fall. It is a programmed death in the sense that a cellular genetic clock selects a specific time for the cell to die. In 1962-1964 Kerr induced ischemic liver injury by tying off a large branch of the portal vein and observed two types of cell death: classical necrosis, and a process involving conversion of scattered cells into small round masses of cytoplasm that often contained specks of condensed nuclear chromatin. At first the author called this kind of cell death shrinkage necrosis, a year later he called it apoptosis [5,6].

Apoptosis or programmed cell death is an active, genetically controlled process of cell suicide. It is a physiologic process whereby the body disposes of unwanted cells by self-destruction and is our utmost defense against damaged cells. In contrast to necrosis, apoptosis is a mode of cell death that occurs under normal physiological conditions and the cell is an active participant in its own demise. It is most often found during normal cell turnover and tissue homeostasis, embryogenesis, induction and maintenance of immune tolerance, development of the nervous system and endocrine-dependent tissue atrophy.

The cell undergoing apoptosis is characterized by cell shrinkage, nuclear condensation, and DNA fragmentation, with the cytoplasmic membrane remaining intact during the early stage. Therefore, apoptosis is morphologically and biochemically different from necrosis, and in contrast to necrosis, does not release its contents to damage surrounding tissues or provoke an inflammatory response.

In describing apoptosis, Majno and Joris [4] reported the following key features of apoptosis: 1) apoptosis is a form of cell death characterized by morphological and biochemical criteria; 2) morphologically the cells shrinks, become denser, the chromatin becomes pyknotic and packed into smooth masses; 3) there is little or no swelling of mitochondria or other organelles (in contrast to cell death via oncosis); 4) biochemically, the DNA is broken into segments (of approximately 185 bp) between nucleosomes, resulting in the ladder appearance of DNA on agarose gels; 5) the process is under genetic control and can be initiated by an internal clock, or by extracellular agents such as hormones, cytokines, killer cells and other agents; 6) apoptosis can run its course very fast, even in minutes (in contrast to necrosis which is full-blown only after 12 to 24 hours after total ischemia); 7) the rapid development of apoptosis warns us that generalization are dangerous.

Recently, interest in apoptotic cell death has gained considerable momentum. Alteration in cell survival has been shown to contribute to pathogenesis of a number of human diseases. Evidence indicates that insufficient apoptosis and concomitant accumulation of cell mass can manifest as cancer or autoimmunity, while accelerated cell death is evident in acute and chronic degenerative diseases, immunodeficiency, and infertility [7].

Regulation of Apoptosis

In contrast to necrosis, which is more of an accidental death process, apoptosis comprises of highly regulated and reproducible events that lead ultimately to cell death. Several regulatory genes affecting apoptosis have been identified and divided into pro-apoptotic genes (bax, bik, bak, bcl-xs, bad, p53, c-jun, hrk) and anti-apoptotic genes (bcl-2, bcl-xL, rb, mcl-1, a1, brag-1, bfl-1). Many reports on apoptosis focused on the role of the executioners, Cysteinyl-asparate-acid-proteinases, termed "caspases" which are triggered in response to pro-apoptotic signals. Caspases cleave numerous substrates at the carboxyl side of an aspirate residue upon induction of apoptosis. Classification of human caspases is based either on their function, the size of their pro-domain or cleavage specificity. Considering the first criteria, caspase-1, -4 and -5 belong to the group I (inflammatory) caspases that are involved in cytokine maturation. Regulation of apoptosis, on the other hand, is controlled by group II caspases, which are divided into two classes: initiator (apical) caspases (caspase-2, -8, -9 and -10) and effector (executioner) caspases (caspase-3, -6 and 7) [8]. Emerging evidence suggests that many apoptotic signaling pathways converge at the mitochondria, where signals are processed through a series of molecular events culminating in the release of potent death factors that trigger either caspase-dependent or independent apoptosis [9]. Mitochondrial proteins that cause caspase-dependent cell death include cytochrome c, which triggers caspase-9 activation by binding and activating the apoptotic protease activating factor-1 (Apaf-1), and Smac/Diablo that potentiates caspase activation by binding Inhibitor of Apoptosis Proteins (IAP) and blocking their caspase-inhibitory activity [10]. A key caspase involved in the apoptotic pathway is caspase-3 (also known as Yama, CPP32, and Apopain). Inhibition of caspase-3 has

been linked to prevention of apoptotic death in vitro [11], although certain stimuli can induce apoptosis by a caspase-3-independent pathway.

Mitochondria have been reported to release caspase-independent death effectors, such as apoptosis-inducing factor (AIF) [12] and endonuclease G [13], which translocates to the nucleus where it causes ligonucleosomal DNA fragmentation. The release of these death factors is regulated by the Bcl-2 family of proteins [14]. Members of the Bcl-2 family are characterized by the presence of distinct conserved sequence motifs known as Bcl-2 homology (BH) domains designated BH1, BH2, BH3 and BH4. The multi-BH domain family members are either anti- or pro-apoptotic. In general, the anti-apoptotic members (e.g. Bcl-2, BclXL, Mcl-1, Bcl-w from mammals and Ced-9 from Caenorhabditis elegans) display sequence homology in all four BH domains, whereas the pro-apoptotic members (e.g. Bax, Bak and Bok) have homologous BH1-3 domains. Bcl-2 is expressed in a variety of embryonic and postnatal tissues which suggests a critical role for bcl-2 in organogenesis and tissue homeostasis. It has been reported that the Bcl-2 gene product protects cells from programmed cell death, and its over-expression has been proposed to be tumorogenic and to mediate resistance of tumors to therapy. Bcl-2 seems to have particular significance in lymphocyte development and function of the immune system. At least two family members, Bcl-xs and Bax, act in opposition to Bcl-2 [14].

In conclusion, Apoptosis, or programmed cell death, is an evolutionarily conserved and highly regulated process of nonfunctional cells death. Through this process, the body disposes of unwanted cells by self-destruction and is our utmost defense against damaged cells. Despite intense investigation into the role of apoptosis in many human diseases, little information is presently available concerning altered patterns of apoptosis in germ cell.

Here, we provide an overview of mechanisms by which the main regulatory molecules govern apoptosis in normal germ cells and describe models of apoptotic dysregulation based on alterations in their function that facilitate the evasion of apoptosis in germ cells.

Testicular Germ Cell Apoptosis

Apoptosis and Testicular Development

Germ cell apoptosis is a common event occurring during development of human gonads. Apoptosis of germ cells plays critical roles in a wide variety of physiological processes during development of the fetal and adult testicular tissue. It is required for normal spermatogenesis and is believed to ensure cellular homeostasis and maintain the fine balance between germ cells and Sertoli cells [15]. Modi et al. have examined the occurrence of programmed cell death in normal and chromosomally aneuploid testis during the second trimester of human development and had shown that germ cell apoptosis is a common event, occurring during development of human gonads. A chromosomal defect, by some means, accelerates apoptosis that probably leads to gonadal dysgenesis later in life [16]. The vigorous growth of the testis during the newborn period with subsequent stabilization during first years of prepuberty seems to be mainly mediated by decreased apoptosis.

The factors that modulate apoptosis of testicular cells are not known, but it is remarkable that this change takes place before the testosterone peak of the post natal gonadal activation in the first trimester of life [17]. Apoptosis control in adult testis is crucial to

achieve normal spermatogenesis. It has been reported that accelerated apoptosis of primary spermatocytes might account for germ cell loss in aging men [18]. Growing evidence suggests that germ cell apoptosis is responsible for sperm malformation causing human infertility in patients affected by severe teratospermia concomitant with andrological pathologies such as testicular ischemia following testicular torsion, varicocele, cryptorchidism and infection.

Testicular Ischemia-Reperfusion and Apoptosis

Despite improvement in early diagnosis and changes in clinical management, infertility remains the most common long term complication of testicular torsion [19]. The degree of fertility loss in an individual with testicular torsion depends on the extent of the ischemia and the subsequent damage to the contralateral testis. Although necrosis has been assumed to be synonymous with germ cell death after an ischemic insult, extensive studies in various experimental models of testicular Ischemia-Reperfusion (IR) have established that apoptosis is a significant, and perhaps the principal contributor to cell death. The first evidence for torsion-induced apoptosis was observed by Turner and coworkers [20] in ischemic testis at 4 hours after reperfusion of 1-hour of torsion, and was accompanied by leukocyte margination and diapedesis. The authors hypothesized that apoptosis was induced by reactive oxygen species arising from reperfusing leukocytes. In evaluating the molecular mechanisms of the induction of germ cell-specific apoptosis in ischemic testis, the same research team has demonstrated that induction of germ cell apoptosis is initiated through the mitochondria-associated molecule Bax as well as Fas-FasL interactions [21]. Lysiak and coworkers measured the effect of ischemia-reperfusion of the testis on germ cell specific apoptosis and have demonstrated that neutrophil recruitment to the testis after IR following with stimulation of proinflammatory cytokines, TNF-alpha and IL-1 beta, may be responsible for germ cell apoptosis in this model [22]. In evaluating the role of chemical anoxia in germ cell death, Erkkila K and coworkers had shown that in most of the testicular cells, mitochondrial respiration appears to play a crucial role in controlling primary cell death cascades [22]. Zini et al. observed a threefold increase in apoptotic germ cells per cross-sectional area following 3 hours testicular ischemia compared to sham testes [23]. Concomitant increase in nitric oxide synthase eNOS in Leydig, Sertoli, and vascular endothelial cells was detected, suggesting co-localization of eNOS protein and germ cell apoptosis. Although increased germ cell apoptosis in ischemic testis was described in many experiments, the role of apoptosis in aspermatogenesis of the contralateral testis following testicular ischemia is unknown. Further experiments are required to determine the role of apoptosis in germ cell loss in contralateral testis following testicular ischemia.

Cryptorchidism and Apoptosis

Undescended testis is one of the most important congenital anomalies in male urogenital organs that may cause male infertility. The mechanisms of infertility in patients suffering from cryptorchidism are poorly understood. Apoptosis of germ cells has been reported recently as a mechanism responsible for infertility. Hikim et al. have shown that exposure of the rat testis to heat results in stage- and cell-specific activation of germ cell apoptosis [24].

Tomomasa et al. observed an increased germ cell apoptosis and impaired spermatogenesis in rats with experimental cryptorchidism [25]. Most apoptotic germ cells were considered spermatocytes.

Electron microscopy revealed apoptosis of spermatocytes with condensation of chromatins and agglutination of cytoplasmic contents [25]. Wang et al. have investigated the possible involvement of apoptosis in the increased germ-cell degeneration in undescended testes in rats. The authors conclude suggest that spermatogenesis decreases not only in the undescended testis but also in the contralateral testis [26].

Varicocele and Apoptosis

Varicocele is a common cause of male infertility. Despite data obtained from many experimental studies, the pathophysiology of varicocele remains unclear. New studies on testicular tissue of men with varicocele have demonstrated increased apoptosis among developing germ cells, which may be the cause of oligospermia [27,28]. Baccetti et al. observed increased apoptosis in the sperm cells of the ejaculate of sterile men suffering from varicocele. The authors concluded that natural presence of apoptosis, which starts in the testis and is revealed in the ejaculate, may explain many abnormal ultra-structural sperm patterns hitherto unexplained in fertile and infertile individuals [29]. Tanaka and coworkers measured the expression of Bcl-2, Bax, caspase-1 and caspase-3 in bilateral testicular specimens from infertile men with varicocele to determine which of these proteins were related to hypospermatogenesis. The authors showed that reduced expression of caspase-3 participates in the regulation of apoptosis in testes of infertile men with varicocele [30].

In conclusion, the understanding of testicular physiology and pathology requires knowledge of the regulation of cell death. Apoptosis control in testis is crucial to achieve normal spermatogenesis. Growing evidence suggests that germ cell apoptosis is responsible for sperm malformation causing human infertility in patients affected by severe teratospermia concomitant with andrological pathologies such as testicular ischemia following testicular torsion, varicocele, cryptorchidism and infection.

Ovarian Germ Cell Apoptosis

Apoptosis and Ovarian Development

The biological state of the ovum remains the key element in normal reproduction. Apoptosis seems to be the mechanism that makes the female biological clock tick. Apoptosis plays a major role during folliculogenesis and dominant follicle selection and also plays part in corpus luteum regression. In addition, it has been shown that programmed cell death plays important roles in the mammary gland development and ductal morphogenesis. During puberty, lumen formation is associated with the selective apoptosis of centrally located cells. In turn, postlactational involution of the mammary gland is characterized by the secretory epithelial cells undergoing programmed cell death. The human ovary is an extremely dynamic organ in which excessive or defective follicles are rapidly and effectively eliminated early in ontogeny and thereafter continuously throughout reproductive life. Numerous studies have confirmed that the loss of germ cells from the fetal and neonatal ovaries occurs via apoptosis [31,32]. More than 99% of follicles disappear, primarily due to apoptosis of granulosa cells, and only a minute fraction of the surviving follicles successfully complete the path to ovulation [33]. For the majority of those germ cells that survive the pre- and peri-natal waves of apoptosis, now enclosed by somatic cells as follicles, their fate is not any better as apoptosis continues to decimate the oocyte pool

through a process referred to as follicular atresia. The balance between signals for cell death and survival determines the destiny of the follicles. Follicular growth during the preantral early antral transition is mainly regulated by intraovarian oocyte-granulosa-theca cell interactions and regulators, such as growth factors, cytokines, and steroids. Age-related decrease in the number of oocytes, as well as disturbed neuroendocrine function of the ovary and lesions in the uterus, contribute to reduced fertility. Decreasing number of ovarian follicles is accompanied by reduction of their quality including mainly abnormalities of the nucleus (dispersed chromatin, decondensation of chromosomes and abnormalities connected with the spindle apparatus). This results in failed reproduction due to abnormal gametogenesis, fertilization process, early development of the embryo and abnormal implantation [34]. The signaling molecules involved in the apoptotic pathway of oocytes, particularly during meiotic prophase progression, remain to be identified.

Two main apoptotic pathways, intrinsic and extrinsic pathways, are known to operate in mammalian germ cells [35]. The intrinsic pathway is triggered by various extracellular and intracellular stresses, such as growth factor withdrawal and DNA damage, and involves the release of cytochrome c from the mitochondria into the cytosol. The free cytochrome c binds to Apoptotic Protease-Activating Factor-1 (APAF-1), which then binds to an initiator caspase 9. Members of the Bcl-2 family of proteins play a major role in governing the mitochondrial apoptotic pathway, with proteins such as Bad and Bax functioning as apoptosis inducers and Bcl-2 and Bcl-x as suppressors. Bcl-2 (pro-survival), Bax (pro-apoptotic) and c-Myc are expressed in granulosa cells of both fetal and adult ovaries, suggesting their possible role in atresia. The extrinsic pathway is activated by the binding of ligands such as Fas to death receptors on the cell membrane. This binding recruits a ligand-specific death domain, which in turn activates initiator caspase 8 or 10 and subsequent apoptotic events. The intrinsic and extrinsic pathways may cross talk to amplify apoptotic signals. For example, activated caspase 8 can initiate the mitochondrial apoptotic pathway by the cleavage of Bid. On the other hand, caspase 8 can also be activated downstream of caspase 9 [36,37]. Many researchers have proposed that oocyte cell death during meiotic prophase I is stage-specific, affecting the pachytene stage in particular, and linked to the theme that oocytes that cannot complete the pachytene stage consequently arrest and undergo cell death [16]. Recent experiment has shown that factor associated suicide (Fas) and its ligand (FasL) signaling are involved in regulation of bovine oocyte apoptosis, perhaps related to B cell lymphoma/leukemia-2 associated X upregulation [38].

Ovarian Ischemia-Reperfusion and Apoptosis

Strong evidence suggests that reactive oxygen species are involved in initiation of apoptosis in antral follicles caused by several chemical and physical agents [39]. Several experiments have shown protective effects of antioxidants and/or evidence of oxidative damage, suggesting that reactive oxygen species may play a role in these smaller follicles as well. Oxidative damage to lipids in the oocyte has been implicated as a cause of persistently poor oocyte quality after early life exposure to several toxicants. Developing germ cells in the fetal ovary have also been shown to be sensitive to toxicants and ionizing radiation, which induce oxidative stress. The correlation between ischemic tissue damage and the duration of ischemia was verified [40]. It appeared that stromal cells were more vulnerable to ischemia compared to primordial follicles [40]. In a porcine model of warm ovarian ischemia, Hussein et al. have shown that apoptosis is involved

in follicular atresia, that Bcl-2 is induced by warm ischemia; and that cryopreservation insult does not alter the apoptotic signals with short tissue preparation time [41]. Vitamin E has been recently shown to ameliorate IR-induced histological alterations in ovary. It also decreased serum levels of Malondialdehyde (MDA) and Myeloperoxidase (MPO), reduced the activity of p-JNK in the ovaries, and reduced numbers of apoptotic follicular cells [42].

Gonadal Germ Cell Tumors and Apoptosis

The ability of tumor cells to evade apoptosis and to proliferate excessively is a general property of cancer. Control of malignant ovarian tumours as the induction of apoptosis in these malignancies would be of therapeutic benefit.

Ovarian teratoma is most common ovarian germ cell tumor [43]. Benign gonadal teratomas are derived from nontransformed germ cells (these include the usual mature ovarian teratoma and dermoid and epidermoid cysts) as well as malignant gonadal teratomas that are malignant because of their derivation from a malignant germ cell through the intermediary forms of an invasive germ cell tumor, such as yolk sac tumor or embryonal carcinoma ('preteratomatous' malignant transformation). Signal Transducer and Activator of Transcription 1 (STAT1) serves in the protection of the organism against pathogens and other harmful insults. Recent experiment has demonstrated a significant role for STAT1 in protecting from teratoma formation in a later step of tumorigenesis, e.g. by inducing apoptosis and eliminating premature or aberrantly formed follicles which have the potential to transform into teratomas [44]. Overexpression of Bcl-2 protein in the ovary leads to decreased ovarian somatic cell apoptosis, enhanced folliculogenesis, and increased susceptibility to ovarian germ cell tumorigenesis in transgenic animals [45].

Dysgerminoma the second most common ovarian germ cell tumor, although it represents only about 2% of them because of the marked predominance of teratomas in the ovary compared to their low frequency in the testis [46]. The role of germ cell apoptosis in development of dysgerminoma has never been discussed. A yolk sac tumor is the third most common form of ovarian germ cell tumor (1% of cases). Most of the ovarian yolk sac tumors occur as pure neoplasms, whereas pure yolk sac tumors of the testis are rare in adults but the most common testicular germ cell tumor in children, peaking at 1.5 years of age and representing about 70% of pediatric testicular germ cell tumors [47]. Islam MS et al. have investigated recently cell susceptibility to hyperthermia-induced apoptosis in two Rat Yolk Sac Tumor cell lines (RYSTs) and attempted to correlate this with the known potentially relevant molecular determinants of apoptosis, p53 protein status, Bcl-2 family of proteins and heat shock proteins [48]. The authors have shown that hyperthermia induced apoptosis in parent cell line, NMT-1 (carrying wild-type p53 gene) and apoptosis in RYSTs may be independent of p53-dependent signaling pathway.

Although progress has been made in understanding its etiology and progression of ovarian cancer, it remains the most deadly reproductive cancer. For this reason, novel approaches are required to make headway in this challenging disease. The prognostic role of key components of apoptotic and prosurvival pathways such as p53, EGFR, and HER2 has been extensively studied because resistance to chemotherapy is often caused by failure of tumor cells to go into apoptosis. However, it is more than likely that different ovarian cancer subtypes with extensive molecular heterogeneity exist. Therefore,

exploration of the potential of specific tumor-targeted therapy, based on expression of a prognostic tumor profile, may be of interest [49].

Conclusions

Apoptosis plays an important role in developmental processes, as well as cellular homeostasis. This process is known to be accelerated or diminished, in many pathologic states. Therefore, the understanding of apoptotic regulation has significant clinical ramifications. The ability to modify germ cell apoptosis, either by gene deletion or insertion (of pro-apoptotic and pro-survival genes) has considerable impact on our understanding of the role of individual genes in germ cell death. This understanding would be beneficial in several aspects. First, control of malignant gonadal tumors as the induction of apoptosis in these malignancies would be of therapeutic benefit. Second, prevention or delay of premature gonadal failure, would improve the reproductive outcome, when compared to third treatment or prevention of gonadal transplant rejection. In this regard, the prevention of apoptosis in the parenchymal cells and the induction of immunological tolerance in the immunocytes would be desirable. Finally, the control of inflammatory diseases of the gonads, where induction of apoptosis and phagocytosis is involved, would require a suppression of the inflammatory response. Germ cells to undergo apoptosis could form the basis for treatment of many gonadal disorders.

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