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REVIEW

Physiological and Pathological Cell Deaths in the Reproductive Organs

Kazuhiro Sakamaki

Laboratory of Developmental Cell Biology, Division of Systemic Life Science, Graduate School of Biostudies, Kyoto University, Kyoto 606-8507, Japan

ABSTRACT. **Apoptosis of testicular germ cells and oocytes and their supporting cells in the gonads occurs at physiological and normal conditions or after exposure to pathological stimuli. Cell-death regulators, including Bcl-2 family members, caspases, Fas and p53 are thought to be involved in these processes. This article reviews the details of the apoptotic machinery in the reproductive organs by describing briefly the abnormal phenotypes observed in transgenic and gene-ablated mice.**

Key words: apoptosis/testis/ovary/Fas/Bcl-2/caspase/p53

1. Introduction

In multicellular organisms, apoptosis or programmed cell death is an important physiological process to remove superfluous cells including those that are generated in excess, have already completed their specific functions, or are harmful to the host. These processes are detected during embryonic development and in homeostasis in adulthood. Apoptosis is also critical for the establishment of the immune systems and nervous systems. Dysregulation of apoptosis often causes tissue degeneration or malformation leading to pathogenesis accompanied with serious diseases (Thompson, 1995).

In humans, seven million fetal germ cells at 20 weeks are reduced to two million oocytes at birth, and eventually to 300,000 at puberty in females (Baker, 1963). Furthermore, more than 90% of ovarian follicles containing oocytes undergo a degenerative process during reproductive life (Hsueh *et al*., 1994). Theoretically, an undifferentiated germ cell expands the cell number to 4,096 by proliferation at the mitotic phase and by division into four haploid spermatids through the meiotic phase. In normal testis, however, up to 75% of the sperm number is lost due to germ cell degeneration during spermatogenesis (Huckins, 1978; Allan *et al.*, 1987). Thus, naturally occurring cell death, or apoptosis, is physiologically observed in both sexes. In addition, gonads are organs that are very sensitive to exogenous stimuli such as drugs, X-ray irradiation and heating. In both testis and ovary, germ cells and oocytes are degenerated by these stimuli, resulting in infertility.

It is known that androgen depletion induced by castration results in cell death in the male prostate. In females, cell death occurs in the uterine epithelium during the estrous cycle. This review focuses on cell death observed in the testis and the ovary in mammals, and discusses the molecular mechanisms leading to physiological and pathological cell deaths in these reproductive organs. To this end, cell death observed in oocyte attrition (Morita and Tilly, 1999; Reynaud and Driancourt, 2000), follicular atresia (Hsueh *et al.*, 1994; Kaipia and Hsueh, 1997), ovulation (Murdoch, 2000) and luteolysis (Tilly, 1996; Davis and Rueda, 2002) and ovarian apoptosis (Hsu and Hsueh, 2000; Pru and Tilly, 2001; Tilly, 2001; Johnson and Bridgham, 2002) in females will be considered. Similarly, testicular germ cell death observed in males (Sinha Hikim and Swerdloff, 1999; Print and Loveland, 2000; Young and Nelson, 2001; Koji, 2001) will be described. To understand these physiological and pathological events in both gonads, the details of cell death regulators as revealed by transgenic and gene-ablated mice, will be reviewed. In particular, similarities and differences in the functions of these regulators in each sex will be explored.

2. Physiological cell death in the male gonads

Testicular germ cell degeneration is very common in

Kazuhiro Sakamaki, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan.
Tel: +81–75–751–4786, Fax: +81–75–751–4784
E-mail: ksakamak@virus.kyoto-u.ac.jp

Abbreviations: GnRH, gonadotropin-releasing hormone; EDS, ethane 1,2-dimethanesulfonate; VCD, 4-vinylcyclohexene diepoxide; PAHs, polycyclic aromatic hydrocarbons; DXR, doxorubicin; Bok, Bcl-2-related ovarian killer; FasL, Fas ligand; TRAIL, TNFα-related apoptosis-inducing ligand; *lpr, lymphproliferation*; *W, White spotting*.

mammalian testes during development and adult life. It is thought that degeneration accompanied with cell death is provoked and regulated by the balance between survival and apoptotic signals.

2.1 Loss of primordial germ cells in embryos and germ cells during development

In rodent testis, primordial germ cells are known to undergo degeneration during the fetal period (De Rooij and Lok, 1987; Coucouvanis *et al.*, 1993). Furthermore, about half of germ cells in the rat testis die around the time of birth (Roosen-Runge and Leik, 1968). During pubertal development, a prominent loss of germ cells also occurs at the first wave of the seminiferous epithelial cycle (Billig *et al.*, 1995; Packer *et al.*, 1995). Thus, before growing up, animals lose excessive numbers of germ cells by cell death at several specific stages of development.

2.2 Degeneration of germ cells during normal spermatogenesis

Even in adults, a significant number of germ cells degenerate spontaneously during life. Indeed, 25–75% of the expected sperm yield is thought to be lost during mature spermatogenesis in mammals (Huckins, 1978; Johnson *et al.*, 1983; Allan *et al.*, 1987; Blanco-Rodriguez and Martinez-Garcia, 1996). Interestingly, it seems that germ cell degeneration is synchronized at the specific stage of the seminiferous epithelial cycle (Billig *et al.*, 1995). Furthermore, it has been reported that testicular regression accompanied with cell death in non-domestic animals occurs during the seasonal cycle (Young and Nelson, 2001).

3. Physiological cell death in the female gonads

In females, cell death is observed in primordial germ cells of embryos, in oogonia and in oocytes during development. In addition, oocyte death occurs during maturation. Furthermore, in adults, regressive events called “atresia” and “luteolysis” accompany cell death of oocytes and granulose cells within follicles and luteal cells in the corpus luteum.

3.1 Loss of primordial germ cells and degeneration of oogonia and oocytes in the embryonic ovary

Concomitant with mitotic division, primordial germ cells migrate to and colonize in the developing genital ridge. During migration, some cells are removed by cell death. In the newly formed ovary, after repeated mitotic divisions, germ cells (oogonia) exit from the mitotic cycle and enter meiotic division (Peters, 1970). Then, germ cells named as oocytes arrest at the first prophase. During both mitosis and meiosis, large numbers of oogonia and oocytes are also depleted by cell death, resulting in less than one-third of the total number of potential germ cells being provided to primordial follicles (Coucouvanis *et al.*, 1993; Tilly, 1996). In the human fetal ovary, similar events occur as described above (Baker, 1963). There are no clear answers as to why drastic degeneration of germ cells occurs during oogenesis.

3.2 Cell death of oocytes in primordial and preantral follicles

Oocyte death occurs within the primordial and the preantral follicles. Morita *et al.* clearly demonstrated this event by showing that exogenous expression in mouse oocytes of Bcl-2, an anti-apoptotic molecule, leads to an increased number of healthy preantral follicles and fewer atretic preantral follicles (Morita *et al.*, 1999).

3.3 Cell death during folliculogenesis and ovulation

Once the primordial follicle pool is established, more than 90% of the follicles undergo a degenerative process called atresia and never reach ovulation in adults. Death of granulosa cells and oocytes occurs during the late preantral to the early antral stage. The hormonal regulation of cell death in granulosa cells during atresia appears to be very complex, and probably many factors are involved in this process. As atretogenic factors, TNFα, gonadotropin-releasing hormone (GnRH), androgens, IL-6 and free radicals are under investigation (Hsueh *et al.*, 1994; Kaipia and Hsueh, 1997). Why few percent of mature follicles within the ovary survive and proceed to ovulation, and what differences occur in the apoptotic potential of cells within follicles at various developmental stages, will be clarified. Interestingly, apoptosis is required for normal ovulation in cells within the follicle-associated ovarian surface epithelium (Murdoch and McDonnel, 2002).

3.5 Cell death at the end of lutenization

The corpus luteum is essential for establishing and maintaining pregnancy. In the failure or at the end of pregnancy, however, the corpus luteum will cease to produce progesterone and the structure itself will regress in size. This regressive process, called luteolysis, is accompanied by the cell death of luteal cells (Shikone *et al.*, 1996; Tilly, 1996; Davis and Rueda, 2002). A number of factors have been implicated in the luteolytic process including prostaglandin F2α, prolactin, reactive oxygen species, cytokines, nitric oxide and endothelin-1 (Davis and Rueda, 2002). However, not all factors have the capacity to directly regulate apoptosis. In this review, the focus is on the involvement of cytokines such as TNFα and Fas ligand as mediators in luteal regression, which is described below.

4. Pathological cell death in the reproductive organs

Germ cells in both types of gonads are very sensitive to

pathological stimuli. It has been reported that testicular germ cell degeneration occurs during various stages of spermatogenesis under experimentally manipulated conditions, such as cryptorchidism (Shikone *et al.*, 1994; Yin *et al.*, 1998; Watts *et al.*, 2000; Xu *et al.*, 2000), ischemia-reperfusion (Turner *et al.*, 1997; Shiraishi *et al.*, 2000; Koji *et al.*, 2001), hypophysectomy (Russell and Clermont, 1977), treatment with GnRH antagonists (Billig *et al.*, 1995; Hikim *et al.*, 1995), heating (Yamamoto *et al.*, 2000; Miura *et al.*, 2002), and drug treatment (Nonclercq *et al.*, 1996; Lee *et al.*, 1997; Nandi *et al.*, 1999; Franca *et al.*, 2000; Zhu *et al.*, 2000; Yu *et al.*, 2001). In addition, Nandi *et al.* reported that reduction of intratesticular testosterone due to Leydig cells degeneration by treatment with ethane 1,2-dimethanesulfonate (EDS) indirectly induces germ cell apoptosis (Nandi *et al.*, 1999).

It has been shown that ovotoxicity induced in rats by treatment with 4-vinylcyclohexene diepoxide (VCD) accelerates the normal rate of atresia in small ovarian follicles of rats, resulting in the activation of a caspase-mediated cascade (Hu *et al.*, 2001). Another toxic chemical, polycyclic aromatic hydrocarbons (PAHs) released into the environment by fossil fuel combustion and also present in tobacco smoke, have been shown to induce oocyte destruction and ovarian failure in exposed mice, and to cause early menopause in women. Recently, it has been shown that exposure of mice to PAHs induces the expression of Bax in oocytes, followed by apoptosis (Matikainen *et al.*, 2001a). Furthermore, oocytes exposed to therapeutic levels of the antitumor drug, doxorubicin (DXR) undergo apoptosis (Perez *et al.*, 1997). Interestingly, oocytes from Bax-deficient, but not p53-null, female mice displayed resistance to DXR-induced apoptosis. These reports suggest the involvement of Bax in ovarian cell death by pathological stimuli.

5. Molecular mechanisms of apoptosis in the reproductive organs

At least 60 apoptosis-related molecules have been identified in mammals. Among them, this section focuses mainly on members of the Bcl-2 family, caspases, death receptors and p53 of the core apoptotic machinery. With reference to studies of mutant mice lacking or overexpressing those molecules, a brief overview of observations that have been well characterized with respect to phenotypes in the testis and ovary will be given.

5.1 Members of the Bcl-2 family

At least 19 members of the Bcl-2 family have been identified in vertebrate and subclassified based on their function in cell death regulation as anti-apoptotic and pro-apoptotic molecules (Gross *et al.*, 1999). They are important sensors that receive signals to induce or suppress apoptosis via intra- and extracellular signaling pathways. In both gonads, it seems that a balance between the anti-apoptotic members Bcl-2, Bcl-X and Bcl-w, and the pro-apoptotic members, including Bax, Bok and Bad, is critical in the regulation of germ cell, oocyte and supporting cell survival prenatally and postnatally.

(1) The anti-apoptotic Bcl-2 members

Transgenic mice expressing Bcl-2 show an irregular spermatogenesis accompanied by sterility. This appears to result from the prevention of an early and massive wave of apoptosis in the testis (Rodriguez *et al.*, 1997; Sugiyama *et al.*, 2001). Similarly, in the ovary of transgenic mice overexpressing Bcl-2, a suppression of follicular cell apoptosis and an enhancement of folliculogenesis were observed (Hsu *et al.*, 1996). In contrast, the ovaries of Bcl-2-deficent mice exhibited numerous primordial follicle-like structures formed aberrantly, which contained a single layer of granulosa cells devoid of oocytes (Ratts *et al.*, 1995). These results suggest that expression of Bcl-2 is critical for the endowment of a normal complement of germ cells and primordial follicles in the ovary. No abnormal phenotypes were reported in Bcl-$2^{-/-}$ males because Bcl-2 is not expressed in the testis. Secondly, anti-apoptotic Bcl-X has been investigated in gonads. Hennighausen and colleagues showed that E12.5-E15.5 Bcl-$X^{-/-}$ embryos have a decreased number of germ cells due to apoptosis, suggesting the involvement of Bcl-X in maintaining the survival of mouse germ cells during gonadogenesis (Rucker *et al.*, 2000). On the contrary, Bcl-X-expressing transgenic mice showed highly abnormal spermatogenesis similar to phenotypes observed in Bcl-2 transgenic mice (Rodriguez *et al.*, 1997). Third, the anti-apoptotic molecule Bcl-w is expressed in the gonads. Bcl-w-deficient mice exhibit sterility associated with progressive testicular degeneration. Germ-cell defects are first observed during pubertal development and spermatogenesis is blocked during late spermiogenesis. Gradual depletion of all stages of germ cells and Sertoli cells continues until six months of age (Ross *et al.*, 1998; Print *et al.*, 1998; Russell *et al.*, 2001). As no abnormalities in Bcl-$w^{-/-}$ females have been reported, Bcl-w may not function in the female gonad same as Bcl-2 in the male gonad.

(2) The pro-apoptotic Bcl-2 members

Bax, Bok/Mtd and Bak belong to the subfamily of pro-apoptotic Bcl-2 members that have multiple domains. Bax plays an important role in the regulation of apoptosis in both types of gonads. Bax-deficient males were infertile as a result of disordered seminiferous tubules with an accumulation of atypical premeiotic germ cells, but no mature haploid sperm (Knudson *et al.*, 1995). Further, Knudson and coworkers demonstrated that germ cell degeneration in Bax$^{-/-}$ mice occurs in the first wave of spermatogenesis during late pubertal development (Russell *et al.*, 2002). They proposed that massive hyperplasia occurring in Bax$^{-/-}$ mice resulted in Bax-independent cell death, triggered by the

crowding of the seminiferous epithelium. Similarly, Bax-deficient ovaries contained unusual atretic follicles with excess granulosa cells in females. Young adult $Bax^{-/-}$ mice possess three times more primordial follicles in their ovarian reserve than their wild-type sisters (Perez *et al.*, 1999). These phenotypes observed in the ovary are similar the testis. In human, Bax expression was significantly higher in the regressing corpus luteum than in the midluteal phase, suggesting the involvement of Bax in luteolysis (Sugino *et al.*, 2000). Furthermore, Hsu *et al*. have isolated a pro-apoptotic Bcl-2 gene, Bcl-2-related ovarian killer (Bok) from a rat ovarian fusion cDNA library. They also determined the localization of Bok mRNAs in granulosa cells that underwent apoptosis during follicle atresia (Hsu *et al.*, 1997). In males, Bok is also detected in spermatogonia, pachytene spermatocytes and Sertoli cells (Suominen *et al.*, 2001). Interestingly, FSH-protecting germ cells from apoptosis inhibited Bok gene expression, suggesting the involvement of Bok in germ cell degeneration. Another pro-apoptotic molecule, Bak, is critical for mitochondria-mediated intrinsic apoptotic signal transduction and was seen in spermatogonia in human testis (Oldereid *et al.*, 2001). However, Bak-deficient mice did not develop any reproducible abnormalities (Lindsten *et al.*, 2000).

(3) The BH3-only Bcl-2 members

It has been shown that pro-apoptotic Bcl-2 family proteins with only the BH3 domain including Bad heterodimerize with pro-apoptotic Bax and promote release of cytochrome c from mitochondria leading to cell death. By in situ hybridization analysis, it has been reported that expression of BAD mRNA in granulosa cells of different sizes of follicles (Kaipia *et al.*, 1997). In testis, Bad was expressed in spermatids and spermatozoa (Oldereid *et al.*, 2001). However, the apoptotic mechanism regulated by BH3-only molecules including Bad is still unknown in both sexes.

5.2 Death receptors

TNFα, Fas ligand (FasL) and TNFα-related apoptosis-inducing ligand (TRAIL) belonging to the TNFα family of cytokines are known to induce apoptosis upon binding to their death domain-containing receptors, TNF-R1, Fas, TRAIL-R1 and TRAIL-R2, respectively. These receptors are now called “death receptors” and activate the downstream caspase cascade leading to suicide (Ashkenazi and Dixit, 1998).

(1) Fas

Fas-mediated apoptotic signaling pathway is now well characterized as shown in Fig. 1. The physiological role of Fas has been investigated in the immune system whose function is in the deletion of activated T-cells as well as in T cell-mediated cytotoxicity (Nagata and Golstein, 1995). However, Fas transcripts are also detected in reproductive organs, suggesting that Fas may play a role in the regulation of homeostasis within these tissues. In human testis, it has been shown that Fas expression correlated with germ cell degeneration in meiotic and post-meiotic arrest of spermatogenesis, suggesting the involvement of Fas in the apoptotic elimination of defective germ cells (Francavilla *et al.*, 2002). In rodents, however, the expression level of Fas protein on the surface of germ cells is quietly very low (Sakata *et al.*, in press). In addition, testes of both Fas-deficient *lpr* (*lymphproliferation*) spontaneous mutant mice and Fas knockout mice displayed normal development (Sakata *et al.*, in press). Therefore, we assume that in nonseasonal animals, Fas may be dispensable for germ cell degeneration during spermatogenesis. Although it was thought that Fas was not essential in germ cell homeostasis, recent reports still insist on the involvement of Fas in physiological death of germ cells (Wang *et al.*, 1998; Tres and Kierszenbaum, 1999; Koji, 2001; Francavilla *et al.*, 2002). It is well known that many small mammals stop breeding during the winter and their testes regress. Young *et al.* assessed the possible role of apoptosis during naturally occurring, short day-induced gonadal regression in white-footed mice (Young *et al.*, 1999). In their report, up-regulation of the Fas protein in the testes of short day-treated males was demonstrated.

In females, on the other hand, Fas and its ligand FasL are detected in the ovary and appear to be involved in physiological process accompanied by cell death. They are expressed in granulosa cells and oocytes during folliculogenesis (Guo *et al.*, 1994; Quirk *et al.*, 1995; Hakuno *et al.*, 1996; Kondo *et al.*, 1996; Guo *et al.*, 1997; Sakamaki *et al.*, 1997; Xu *et al.*, 1997; Kim *et al.*, 1998; Vickers *et al.*, 2000; Bridgham and Johnson, 2001; Porter *et al.*, 2001) and in luteal cells occurring luteolysis (Sakamaki *et al.*, 1997; Roughton *et al.*, 1999; Kuranaga *et al.*, 2000; Quirk *et al.*, 2000; Sapi *et al.*, 2002; Taniguchi *et al.*, 2002).

In pathological cell death in both reproductive organs, several reports suggest the involvement of Fas. For example, Fas expression was shown to increase in the testes by drug treatment, ischemia-reperfusion or experimental cryptorchidism (Lee *et al.*, 1997; Koji *et al.*, 2001; Shikone *et al.*, 1994; Ohta *et al.*, 1996; Ogi *et al.*, 1998; Yin *et al.*, 1998). Further, Miura *et al.* proved that germ cell apoptosis induced by heat exposure is mainly mediated by Fas (Miura *et al.*, 2002). Although Fas is likely involved in pathological germ cell death, these lines of evidence are not conclusive. Recently, it has been shown that Fas gene mutations are involved in the pathogenesis of testicular germ cell tumors (Takayama *et al.*, 2002). In abnormal ovaries of the so-called polycystic ovary syndrome, Fas has been reported to promote ovarian vascular remodeling (Cataldo *et al.*, 2000). In Fas-deficient *lpr* mutant females, ovarian adenopathy was also observed (Xu *et al.*, 1998). Taken together these reports suggest that pathological abnormality of both gonads may be caused by dysfunction of Fas.

c-Kit-mediated survival signals are essential in spermatogenesis and oogenesis. In the gonads of the c-Kit-deficient *W* (*White spotting*) mutant mice, degeneration of tes-

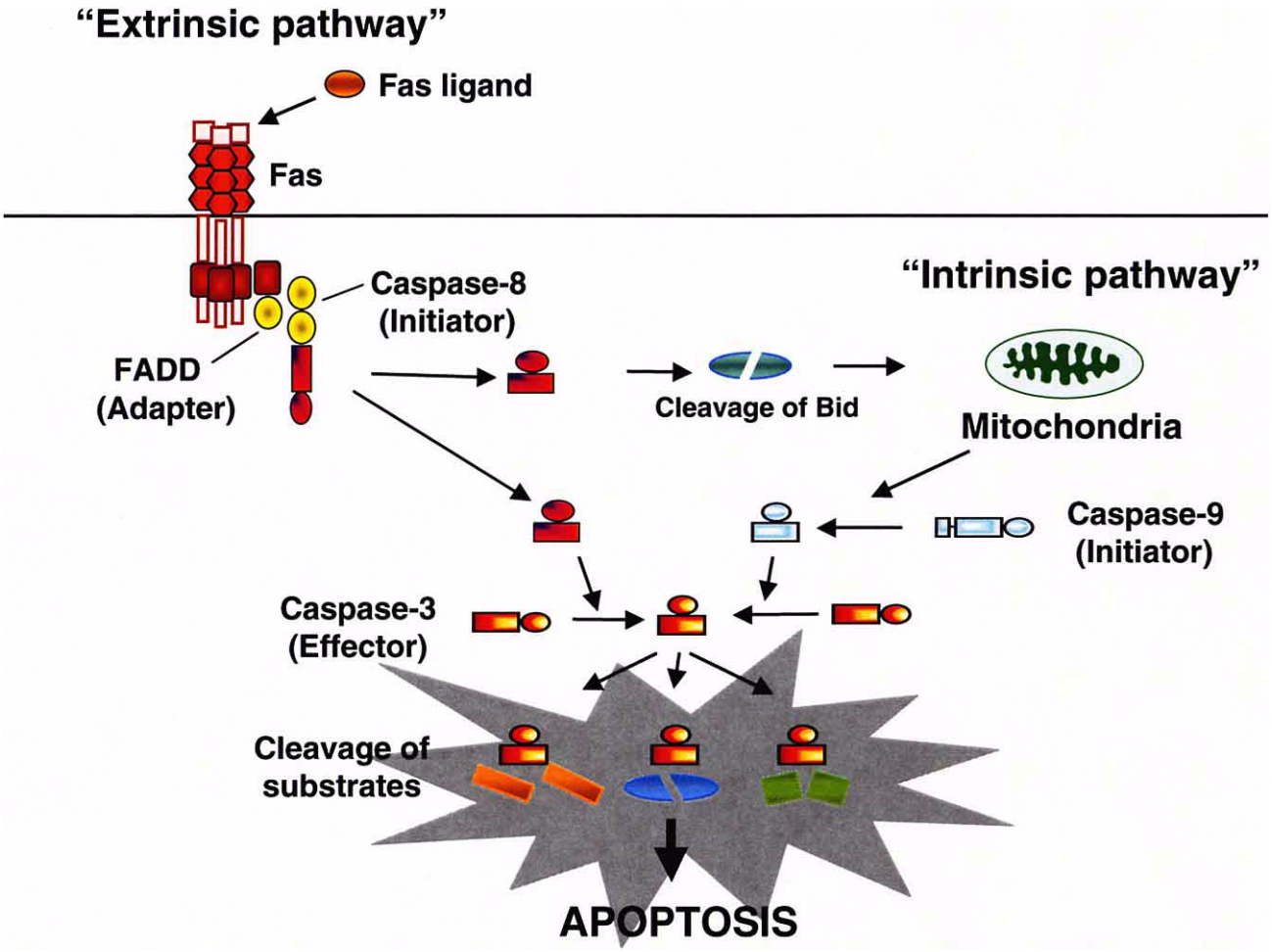

Fig. 1. A schematic representation of the Fas-mediated apoptotic signaling pathway.

ticular germ cells and oocytes are accompanied by cell death, resulting in infertility (Besmer *et al.*, 1993). To clarify the relationship between c-Kit-mediated survival signals and Fas-mediated apoptotic signals in gonads, we generated double-mutant mice (W^v/W^v:Fas$^{-/-}$) by intercrossing the c-Kit-deficient W^v mice and Fas knockout mice (Sakata *et al.*, in press). We examined their reproductive organs and found that Fas-deficiency rescues abnormal phenotypes by loss of c-Kit signal. As shown in Fig. 2A, the regression of ovary was recovered in double-mutant mice. In addition, we observed a partial recovery of spermatogenesis in double-mutant mice. In some seminiferous tubules, differentiated germ cells were detected in double-mutant mice (Fig. 2B). These results suggest that Fas is involved in the degeneration of oocytes and testicular germ cells when survival signals are defective.

(2) Other death receptors

It has been reported that the TNF receptor was localized on oocytes, granulose cells and interstitial cells and was involved in the apoptosis of oocytes in the ovary (Bridgham and Johnson, 2001; Marcinkiewicz *et al.*, 2002). Grataroli *et al.* have investigated TRAIL and its receptors, TRAIL-R1 and TRAIL-R2 in the rat testis during development (Grataroli *et al.*, 2002). They have demonstrated that TRAIL is expressed in the different germ cell types and its receptors are predominantly detected in the postmeiotic germ cells during normal development.

5.3 Caspases

Caspases are key effector components of apoptosis (Thornberry and Lazebnik, 1998). In mammals, 14 caspases constituting a family of cysteine proteases have been identified. These caspases are classified into two groups involved in apoptosis and inflammation. Based on their positions in the apoptotic signaling cascade, apoptosis-related caspases are further divided into two subgroups. One group called the initiator caspase includes caspase-2, -8, -9 and -10 and another group called the effector caspase consists of

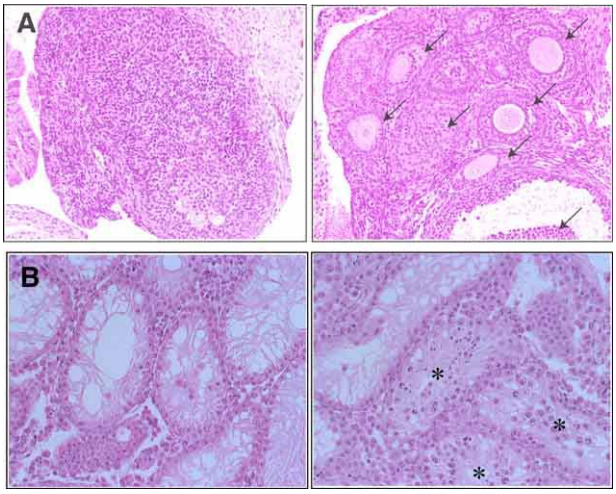

Fig. 2. Histological analyses of ovaries and testes from mutant mice. Sections prepared from ovaries (A) and testes (B) of W^v/W^v:Fas$^{+/+}$ (left panels) and W^v/W^v:Fas$^{-/-}$ (right panels) mice were stained with hematoxylin and eosin. Arrows show the developing follicles in the ovary and asterisks indicate the seminiferous tubules containing the differentiated spermatocytes, spermatids and sperms in the testis.

caspase-3, -6 and -7. Initiator caspases are activated by upstream apoptotic signals mediated through death receptors or mitochondria, followed by the activation of the downstream caspases. Then, activated effector caspases cleave more than two hundred cellular components leading to cell death.

(1) Caspase-3

In females, the presence of caspase-3 was shown in granulosa cells of atretic, but not healthy, follicles suggesting that the expression of this enzyme is up-regulated as part of the apoptotic process in granulosa cells (Boone and Tsang, 1998). Although loss of caspase-3 function did not affect developmental oocytes apoptosis, caspase-3$^{-/-}$ female mice had aberrant atretic follicles containing granulosa cells that failed to be eliminated by apoptosis (Matikainen *et al.*, 2001b). Therefore, it was indicated that caspase-3 was functionally required for granulosa cell apoptosis during follicular atresia, but is dispensable for germ cell apoptosis in the female. By examining the corpus luteum of caspase-3$^{-/-}$ mice, it was shown that caspase-3 was also required for apoptosis to proceed normally during luteal regression (Carambula *et al.*, 2002). Exposure of pathological stimuli such as ethanol stimulates germ cell degeneration in the testis (Eid *et al.*, 2002). In this apoptotic cell death, it has been shown that caspase-3 and Fas are highly expressed. Similarly, caspase-3, detected in oocytes and granulosa cells of preantral follicles at various stages of development, was selectively increased by treatment with VCD as described above (Hu *et al.*, 2001). Thus, the effector caspase-3 may be induced in pathological status in the gonads.

(2) Caspase-2 and caspase-9

The initiator caspase-2 is expressed in multiple cell lineages of the ovary including oocytes. In ovaries of caspase-2$^{-/-}$ mice, excess numbers of germ cells were endowed and the oocytes were resistant to cell death following exposure to chemotherapeutic drugs (Bergeron *et al.*, 1998). These reports suggest that caspase-2 may be involved in oocyte attrition. It has been reported that another initiator caspase-9 was activated in human luteinized granulosa cell apoptosis (Khan *et al.*, 2000). Additional data indicated that Apaf-1, which forms a complex named “apoptosome” with caspase-9 and leads to the conversion of the latent procaspase-9 to its active form, is located in granulose cells (Robles *et al.*, 1999). In Apaf-1-deficient males, degeneration of spermatogonia occurred and resulted in the quasi-absence of sperm (Honarpour *et al.*, 2000). These lines of evidence show the involvement of caspase-9 activated by mitochondria-mediated intrinsic apoptosis signals in both gonads.

(3) Caspase-8

Caspase-8 is an initiator caspase that transmits apoptotic signals mediated through death receptors such as Fas and TNF-R1. Therefore, caspase-8 is essential for the extrinsic signaling pathways. As apoptosis triggered by death receptors is reported in both the testis and the ovary, caspase-8 appears to function in them. In the previous study, we have identified caspase-8 transcripts in both gonads by RT-PCR analysis (Sakamaki and Yonehara, 1998).

(4) Caspase-11

Caspase-11 is characterized as a mediator of cytokine processing. In caspase-11-deficient mice, however, the proportion of fetal ovarian germ cells is increased (Morita *et al.*, 2001). This evidence led us to think that cytokines processed by caspase-11 might affect gametogenesis as secondary effect.

5.4 p53

p53 is a tumor suppressor molecule involved in growth arrest and apoptosis of various types of cells (Liebermann *et al.*, 1995). During normal spermatogenesis in the mouse, spermatogonia do not express high levels of p53. In testes of p53-deficient mice, however, the number of spermatogonia was increased to 50%. In addition, p53 protein was increased in spermatogonia after X-ray irradiation. Based on these data, Beumer *et al.* proposed that p53 was an important factor in normal spermatogonial cell proliferation as well as in the regulation of apoptosis after DNA damage (Beumer *et al.*, 1998). In females, p53 was detected in granulosa cells in the follicles and luteal cells in the corpus luteum of the ovary (Trott *et al.*, 1997; Makrigiannakis *et al.*, 2000). In large antral and preovulatory follicles occurring atresia, expression of p53 and Fas was increased, suggesting involvement of p53 as well as Fas in this process (Kim *et al.*, 1999). Thus, it is likely that p53 plays a role in

the removal of lethally damaged germ cells and oocytes.

Jordan *et al.* generated W^v/W^v:p53$^{-/-}$ double-mutant mice and examined their testes and ovaries (Jordan *et al.*, 1999). In double-mutant testes, an increase was detected in sperm viability in contrast to those of W^v/W^v single-mutant littermates. The appearance of this germ cell rescue is male-specific, as female ovaries were similar in mice homozygous for the c-Kit mutation with or without p53. Interestingly, our W^v/W^v:Fas$^{-/-}$ double-mutant females displayed a recovery of oocyte and follicles in the ovary (Fig. 2A). Therefore, it is thought that two apoptotic signaling pathways exist in the reproductive organs. In the male's case, cell death of testicular germ cells in the absence of c-Kit survival signals may be regulated by p53- and/or Fas-mediated apoptotic signaling pathways. Further examination of Fas expression by regulation of p53 using p53$^{-/-}$ mice showed that Fas expression was reduced in spleen and liver from p53$^{-/-}$ mice compared to wild-type mice, while similar expression levels were observed in several tissues including the testis between the two groups (Lin *et al.*, 2002). This report suggested that p53 regulated Fas expression in a tissue-specific manner, and that Fas was expressed in gonads independently from p53.

Testicular germ cell apoptosis in the cryptorchid testis is caused by abdominal heat stress as described above. p53 was thought to be one of the cell death regulators responsible for germ cell loss in experimental cryptorchidism. However, germ cell death still occurred with a 3-day delay even in p53$^{-/-}$ mice. Fas is another candidate responsible for the p53-independent phase of apoptosis in the cryptorchid testis of p53$^{-/-}$ mice (Yin *et al.*, 2002). In this report, p53$^{-/-}$ and *lpr/lpr* double-mutant mice were generated resulting in germ cell apoptosis, which was delayed by an additional 3 days delay. From this evidence, it is proposed that abdominal heat stress to testes induces germ cell loss through two apoptotic pathways: a p53-dependent pathway responsible for the initial phase of germ cell apoptosis, and a p53-independent and Fas-mediated pathway that accounts for subsequent apoptosis.

6. Conclusion

This review attempted to briefly overview the apoptosis observed physiologically and pathologically in the testis and the ovary, and provides some examples of recent work showing how the cell death machinery is activated and when cell death regulators work during these events. Apoptotic processes are not always coincident between both gonads and some differences occur between them. More work is needed in basic research to answer the question, why cell death occurs in the gonads, which would develop clinical application for patients who suffer from serious diseases of the reproductive organs.

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