Ubiquitin C-Terminal Hydrolase L-1 Is Essential for the Early Apoptotic Wave of Germinal Cells and for Sperm Quality Control During Spermatogenesis


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ABSTRACT

Ubiquitination is required throughout all developmental stages of mammalian spermatogenesis. Ubiquitin C-terminal hydrolase (UCH) L1 is thought to associate with monoubiquitin to control ubiquitin levels. Previously, we found that UCHL1-deficient testes of gad mice have reduced ubiquitin levels and are resistant to cryptorchid stress-related injury. Here, we analyzed the function of UCHL1 during the first round of spermatogenesis and during sperm maturation, both of which are known to require ubiquitin-mediated proteolysis. Testicular germ cells in the immature testes of gad mice were resistant to the early apoptotic wave that occurs during the first round of spermatogenesis. TUNEL staining and cell quantitation demonstrated decreased germ cell apoptosis and increased numbers of premeiotic germ cells in gad mice between Postnatal Days 7 and 14. Expression of the apoptotic proteins TP53, Bax, and caspase-3 was also significantly lower in the immature testes of gad mice. In adult gad mice, cauda epididymis weight, sperm number in the epididymis, and sperm motility were reduced. Moreover, the number of defective spermatozoa was significantly increased; however, complete infertility was not detected. These data indicate that UCHL1 is required for normal spermatogenesis and sperm quality control and demonstrate the importance of UCHL1-dependent apoptosis in spermatogonial cell and sperm maturation.

INTRODUCTION

Ubiquitin and ubiquitin-dependent proteolysis are involved in a variety of cellular processes, such as cell cycle progression, degradation of intracellular proteins, programmed cell death, and membrane receptor endocytosis [1–5]. In spermatogenesis, the ubiquitin-proteasome system is required for the degradation of numerous proteins throughout the mitotic, meiotic, and postmeiotic developmental phases [4, 6, 7]. Ubiquitin C-terminal hydrolases (UCHs) control the cellular ubiquitin balance by releasing ubiquitin from tandemly conjugated ubiquitin monomers (Ubb, Ubc) and small adducts or unfolded polypeptides [4, 8–10]. UCHL1 is expressed at high levels in both testis and epididymis and may play an important role in the regulation of spermatogenesis [11–14]. In addition to its hydrolase activity [15], UCHL1 has a variety of functions, including dimerization-dependent ubiquitin ligase activity, and association with and stabilization of monoubiquitin in neuronal cells [16–18]. Furthermore, it has been suggested that UCHL1 also functions as a regulator of apoptosis [19]. The gracile axonal dystrophy (gad) mouse is an autosomal recessive spontaneous mutant carrying an intragenic deletion of the gene encoding Uch1 [21]. We recently found that testes of gad mice, which lack UCHL1 expression [18, 20, 21], have reduced ubiquitin levels and are resistant to cryptorchid injury-mediated germ cell apoptosis [22].

During prepubertal development, an early and massive wave of germinal cell apoptosis occurs in mouse testis [23, 24]. This early germ cell apoptotic wave affects mainly spermatogonia and spermatocytes and appears to be essential for functional spermatogenesis in adulthood. Decreased apoptosis has been reported in the early phase of spermatogenesis in transgenic mice overexpressing the antiapoptotic proteins Bcl2 or Bcl-xL [23, 25] and in mice deficient in the apoptotic protein Bax [26]. This reduction in apoptosis is associated with the disruption of normal spermatogenesis and infertility. Our previous work demonstrated that gad mice exhibit pathological changes such as progressively decreasing spermatogonial stem cell proliferation [13] and increased expression of the antiapoptotic proteins Bcl2 and Bcl-xL in response to apoptotic stress [19, 22]. Furthermore, we showed that UCHL1 functions during prepubertal development to effect normal spermatogenesis and to modulate germ cell apoptosis [22]. However, the mechanism by which UCHL1 regulates apoptosis during prepubertal development remains unclear. To further investigate the role of UCHL1 in immature testes, we evaluated the function of UCHL1 during early spermatogenesis. Here, we show that immature testes of gad mice accumulate premeiotic germ cells and are resistant to the massive wave of germinal cell apoptosis during the first round of spermatogenesis, eventually leading to alterations in sperm produc-
tion, motility, and morphology in adult mice. Our data suggest that UCHL1-dependent apoptosis is essential for normal spermatogenesis.

MATERIALS AND METHODS

Animals

We used male gad (CBA/RFM) mice [21] at 7, 14, 21, 28, and 35 days and 10 wk of age. The gad mouse is an autosomal-recessive mutant that was produced by crossing CBA and RFM mice. The gad line was maintained by intercrossing for more than 20 generations. This strain was maintained at our institute. Animal care and handling were in accordance with institutional regulations and were approved by the Animal Investigation Committee of the National Institute of Neuroscience, National Center of Neurology and Psychiatry.

Histological and Immunohistochemical Assessment of Testes

Testes were embedded in paraffin wax after fixation in 4% paraformaldehyde, sectioned at 4-μm thickness, and stained with hematoxylin for counting [13]. Light microscopy was used for routine observations. For immunohistochemical staining, the sections were incubated with 10% goat serum for 1 h at room temperature followed by incubation overnight at 4°C with a rabbit polyclonal antibody against UCHL1 (1:1000 dilution; peptide antibody) [20] in PBS containing 1% BSA. Sections were then incubated for 1 h with biotin-conjugated anti-rabbit IgG diluted 1:200 in PBS, followed by Vectorstain ABC-PO (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Sections were washed in PBS and avidin-biotin complex for 30 min (Vector Laboratories, Burlingame, CA). The sections were counterstained with hematoxylin.

Quantitative Analysis of Testicular Cell Number

The total number of cells was determined by counting the testicular cells including Sertoli cells of seminiferous tubules. Quantitative determinations were made using four each of wild-type and gad mice at 7 and 14 days of age. Five sections from each mouse were processed in parallel for counting with hematoxylin. Twenty circular seminiferous tubules in each section were then selected by randomly from those tubules, and 400 circular seminiferous tubules were measured using the 400× lens of a Zeiss Axioplan microscope. The total cell number was not determined by dividing cell types such as testicular germ cells and Sertoli cells because it was difficult to distinguish the difference of cell types [26]. There were no significant differences in nuclear size in either of the group studies. Thus, the total number of cells reflected all cell types of seminiferous tubules.

Quantitative Analysis of Apoptotic Germ Cells

Quantification was performed using four each of wild-type and gad mice at 7, 14, 21, 28, and 35 days of age. The total number of apoptotic cells was determined by counting the positively stained nuclei in 20 circular seminiferous tubules in each section [22]. Five sections from each mouse and a total 400 circular seminiferous tubules per each group were processed.

Germ Cell Isolation, Culture, and Viability Measurement

Germ cells from wild-type and gad mice were prepared using a modification of the procedure described by Kwon et al. [20]. Briefly, testes from three 2-wk-old mice were incubated twice for 30 min at 25°C in Dulbecco Modified Eagle medium (DMEM)-F12 medium containing 0.5 mg/ml collagenase IV-S (Sigma-Aldrich, St. Louis, MO) and then digested for 60 min at 25°C in DMEM-F12 medium containing 1 mg/ml trypsin (Sigma-Aldrich). The cell suspension was digested and washed several times to eliminate testicular somatic cells. The cells were then counted and cultured at 2.0 × 10⁵ cells/ml in DMEM-F12 medium containing 10% fetal bovine serum (FBS). The cells were harvested at each day for 5 days, and viability was assessed using the Vi-Cell XR cell viability analyzer (Beckman Coulter, Fullerton, CA).

Quantitative mRNA Analysis of Uchl1 and Uchl3 Genes by Real-Time PCR

SYBR Green-based real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR: PRISM 7700 Sequence detection system, ABI, Columbia, MD) was performed [20] in SYBR Green Master mix using the following primers: Uchl1, 5′-TTCTGTTCACAACAGTGGCAGG-3′ and 5′-TCAGCTGGAAGGGCATTGC-3′; Uchl3, 5′-TGAAGGTCAGCTGAGCACC-3′ and 5′-AATGGGAATGGTTCCGTCC-3′; β-actin, 5′-CGTCCTGTCATCAAAAAGAGAA-3′ and 5′-CAATAGTGACGCCTGGCGCT-3′. To compare Uchl1 and Uchl3 gene expression in the first round of spermatogenesis, the formula 2⁻ΔΔCt was used to calculate relative expression compared with testes of 7-day-old mice.

Western Blotting

Western blots were performed as previously reported [19, 22]. Total protein (5 μg/lane) was subjected to SDS-polyacrylamide gel electrophoresis using 15% gels (Perfect NT Gel, DRC, Japan). Proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) and blocked with 5% nonfat milk in TBS-T (50 mM Tris base, pH 7.5, 150 mM NaCl, 0.1% [w/v] Tween-20). The membranes were incubated individually with one or more primary antibodies to UCHL1 and UCHL3 (1:1000 dilution; peptide antibodies) [20], Bcl-xl, Bax, TRP53, and inactive caspase-3 (1:1000 dilution; all from Cell Signaling Technology, Beverly, MA). Blots were further incubated with peroxidase-conjugated goat anti-mouse IgG or goat anti-rabbit IgG (1:5000 dilution; Pierce, Rockford, IL) for 1 h at room temperature. Immunoreactions were visualized using the SuperSignal West Dura Extended Duration Substrate (Pierce) and analyzed using a ChemiImager (Alpha Innotech, San Leandro, CA).

Sperm Motility, Morphology, and Immunohistochemical Assessments

Sperm were collected from the right cauda epididymis [27] of 10-wk-old wild-type and gad mice in 400 μl human tubal fluid medium containing 0.5% bovine serum albumin and then incubated at 37°C under 5% CO₂ in air for 1–2 h. Using a computer-assisted semen analysis system (TOX IVOS, Hamilton Throne Research, Beverly, MA) [28], sperm were analyzed for the following motion parameters: percentage of motile sperm (MSM), percentage of progressively motile sperm (PMP), average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), lateral head displacement (ALH), linearity (VSL/VCL × 100), and straightness (VSL/VAP × 100). All procedures were performed at 37°C. To study the spermatozoa morphology, sperm were smeared and then evaluated for defects in the head, midpiece, and principal piece and for head detachment. For immunohistochemical staining, the sections were incubated with antibodies against UCHL1 (1:1000 dilution; peptide antibody) [20] and ubiquitin (1:500 dilution; DakoCytomation, Glostrup, Denmark) overnight at 4°C in PBS containing 1% BSA.

Statistical Analysis

The mean and standard deviation were calculated for all data (presented as mean ± SD). One-way analysis of variance (ANOVA) was used for all statistical analyses.

RESULTS

Expression of UCHL1 During the First Round of Spermatogenesis

We used Western blotting to characterize the level of UCHL1 and UCHL3 expression in testes from immature wild-type and gad mice (Fig. 1, B and C). In agreement with previous data [20], UCHL1 expression was significantly elevated on Day 14 in testicular lysates obtained from 7-, 14-, 21-, 28-, and 35-day-old wild-type mice. The level of UCHL3 expression increased with age and did not differ between gad and wild-type mice (Fig. 1B), suggesting that UCHL3 expression is regulated independently of UCHL1 during the first round of spermatogenesis [20]. We also assessed the expression pattern of Uchl1 and Uchl3 genes during juvenile spermatogenesis using SYBR Green-
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FIG. 1. Expression of UCHL1 and UCHL3 during the first round of spermatogenesis. A) Comparison of Uchl1 and Uchl3 gene expression levels (2−ΔΔCt) by SYBR Green-based real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). The value for gene expression from the testes of 7-day-old mice was set to 1.0. B) Comparison of UCHL1 and UCHL3 expression by Western blotting of testicular lysates from wild-type and gad mice. Blots were reprobed for α-tubulin, which was used to normalize the protein load. Representative images from four independent experiments are shown. C) Quantitative analysis of changes in UCHL1 and UCHL3 levels by Western blotting. Relative protein expression (optical density) of the bands in panel B, normalized to α-tubulin. Each data point represents the mean ± SD (n = 4; * P < 0.05).

Immunohistochemistry of UCHL1 and Quantitative Morphometric Assessment

Immunohistochemical analysis revealed UCHL1 expression in spermatogonia from wild-type mice but not gad mice (Fig. 2A). Preliminary examination of tubules from immature testes revealed an overproduction of germ cells in gad mice at 7 and 14 days of age, the number of spermatogonia and preleptotene spermatocytes was significantly increased in gad mice compared with wild-type mice (Fig. 2A). The increase in the number of these cell types was further confirmed by quantitative analysis, which showed that the total number of testicular cells, including Sertoli cells, was significantly higher in 7- and 14-day-old gad mice (Fig. 2B).

TUNEL Staining of Apoptotic Germ Cells During the First Round of Spermatogenesis

To further investigate the mechanism underlying the observed differences in testicular cell numbers between wild-type and gad mice during the first round of spermatogenesis, we examined germ cell apoptosis in tissue sections from mice at 7, 14, 21, 28, and 35 days of age by TUNEL assay. During the first round of spermatogenesis, the total number of apoptotic cells in 20 circular seminiferous tubules decreased significantly (n = 4; P < 0.05) in gad mouse testes as compared with wild-type mice (Fig. 3A). Although germ cell apoptosis significantly increased at Day 14 in the testes of both wild-type and gad mice, gad mice had significantly fewer apoptotic germ cells (n = 4; P < 0.05) in seminiferous tubules (Fig. 3B).

Testicular Germ Cells of gad Mice Are Resistant to Apoptosis-Inducing Conditions In Vitro

Sertoli cells, which support germ cells, express UCHL1 [12]. To explore the viability of germ cells independently of the effect of Sertoli cells, testicular germ cells from 2-wk-old wild-type and gad mice were cultured in suspension for 5 days in the presence of 10% FBS. We then examined the resistance of these in vitro cell culture to apoptosis-inducing conditions. Although both wild-type and gad mouse cells were sensitive to apoptosis-inducing conditions, the gad mouse cells had comparatively greater viability (Fig. 4). Overall results clearly show that the absence of UCHL1 increase germ cell survival.
FIG. 3. A) The total TUNEL-positive germinal cells per 20 circular seminiferous tubules in wild-type and gad mice on various postnatal days. In each group, the data represent the mean ± SD (n = 4; * P < 0.05). B) The extent of apoptosis in 2-wk-old mice. i, wild-type mice; ii, gad mice. Green fluorescence, TUNEL-positive cells; red fluorescence, nuclei stained with propidium iodide. Magnification ×100. Bar = 30 μm.

Levels of Apoptotic Proteins During the First Round of Spermatogenesis

Germ cell apoptosis involves genes encoding various factors, such as Trp53, the Bcl2 family, and caspase, which are targets for ubiquitination [29–31]. Our previous work demonstrated that the expression of antiapoptotic proteins (Bcl2 family and XIAP) is significantly elevated following cryptorchid stress in gad mice [22]. To explore whether the germ cell apoptotic wave is associated with changes in the levels of proteins known to be associated with cell death or survival, Western blot analysis was performed on testicular lysates obtained from 7-, 14-, 21-, 28-, and 35-day-old wild-type and gad mice (Fig. 5). Levels of TRP53 and Bax proteins were strikingly elevated in 7-day-old mice but barely detectable on Day 35. Caspase-3 was also strikingly elevated in 7-day-old mice. Since TRP53 modulates Bax expression [22, 32], the observed up-regulation of Bax is consistent with elevated TRP53 levels during the early apoptotic wave. Expression of the antiapoptotic protein Bcl-xL was weaker in immature compared with mature testes. Levels of TRP53, Bax, and caspase-3 proteins were significantly decreased in 7- and 14-day-old gad mice relative to the levels observed in wild-type testes (Fig. 5B). By contrast, the level of Bcl-xL protein appeared to be up-regulated earlier in gad mice (at 28 days) than in wild-type mice (at 35 days) (Fig. 5B).

Assessment of Cauda Epididymidis and Spermatozoa Morphology in gad Mice

The cauda epididymidis from wild-type and gad mice were weighed, and the sperm were collected and analyzed. The cauda epididymidis from gad mice weighed significantly less, likely resulting from the lower sperm concentration measured in gad mice (19.5 × 10^6/ml) compared with wild-type mice (23.6 × 10^6/ml) (Table 1). Furthermore, abnormal sperm morphology, including head and midpiece defects or a detached head, occurred significantly more often in gad mice (Table 1 and Fig. 6A). Immunocytochemical analysis showed that UCHL1 and ubiquitin were expressed in defective spermatozoa but not in normal spermatozoa (Fig. 6B). Ubiquitin, a marker for sperm abnormalities [33], was detected mainly in defective spermatozoa. However, despite a significantly elevated number of defective spermatozoa, ubiquitin expression in gad mouse spermatozoa was similar to that in wild-type mice (data not shown).

FIG. 4. In vitro survival of testicular germ cells. Testicular germ cells were isolated from wild-type and gad mice at 14 days of age. After culture, viability was determined using a Vi-Cell XR cell viability analyzer (Beckman Coulter). Viability at each time point was normalized to that at Day 0. Each data point represents the mean ± SD (n = 4; * P < 0.05).

FIG. 5. A) Western blot analyses showing TRP53, Bax, caspase-3, and Bcl-xL levels in wild-type and gad mice during the first round of spermatogenesis. Protein (5 μg/lane) was prepared from whole testes at 7, 14, 21, 28, and 35 days of age. Blots were reprobed for α-tubulin to normalize for differences in the amount of protein loaded. Representative images of four independent experiments are shown. B) Quantitative Western blot analysis of changes in TRP53, Bax, caspase-3, and Bcl-xL levels. Relative protein expression (optical density) of the bands in panel A, normalized to α-tubulin. Each data point represents the mean ± SD (n = 4; * P < 0.05).
however, if damaged proteins are not degraded as easily as such lesions are incompatible with the ultimate function of these cells [23, 24, 37]. The early apoptotic wave may result in early elimination of defective germ cells in which DNA alterations have occurred through chromosomal crossing over during the first meiotic division [23, 24, 37].

Several lines of evidence indicate that UCHL1 associates with monoubiquitin and that the monoubiquitin pool is reduced in gad mice relative to wild-type mice [18, 19, 22]. Furthermore, testes from UCHL1-deficient gad mice [22] and mice carrying the K48R mutation in ubiquitin [38] show resistance to cryptorchid-induced apoptosis, suggesting that ubiquitin is critical for modulating testicular germ cell death. Normally, damaged proteins are polyubiquitinated and degraded via the ubiquitin-proteasome system; however, if damaged proteins are not degraded as easily when monoubiquitin is either mutated or reduced [22, 38], then germinal cells may become resistant to programmed cell death. Our results with the gad mouse suggest that ubiquitin induction is important for regulating programmed germinal cell death that is normally observed during the first round of spermatogenesis. We have now shown that immature testes from gad mice are resistant to the massive wave of germinal cell apoptosis during the first round of spermatogenesis. The increased resistance of UCHL1-deficient germ cells to apoptosis-inducing conditions in vivo and in vitro suggests that UCHL1 is involved in spermatogenesis (Figs. 3 and 4). The activity of the ubiquitin-proteasome system may be required for specific transitions between multiple developmental cellular processes and sequential apoptosis during spermatogenesis [6, 7, 39]. In addition, the ubiquitin-proteasome system is required for the degradation or modification of numerous germ cell-specific proteins during different phases of spermatogenesis [39–41].

Early apoptosis in testicular germ cells is regulated by a complicated signal transduction pathway. The testes contain high levels of TRP53, Bcl2 family, and caspase-3 proteins, which are targets for ubiquitination [29–31, 42–45]. However, the involvement of the ubiquitin system in the regu-

**Spermatozoa Motility in gad Mice**

We measured sperm motility parameters in wild-type and gad mice. Of the parameters assessed, MSP, PMP, VAP (μm/sec), VSL (μm/sec), and VCL (μm/sec) were significantly lower in gad mice. ALH (μm), linearity (%), and straightness (%) did not differ significantly between gad and wild-type mice (Fig. 7). Of the parameters we measured, the number of PMP differed most significantly between gad mice (24.4%) and wild-type mice (34.3%) (Fig. 7A).

**DISCUSSION**

Spermatogenesis is a highly complex process involving male germ cell proliferation and maturation from spermatogonia to spermatozoa [34]. Apoptosis is common during this process and is believed to play an important role in controlling germ cell numbers and eliminating defective germ cells that carry DNA mutations, thus ensuring the production of intact, functional spermatoozoa [35–37]. Normally, germ cells are extremely sensitive to DNA damage, as such lesions are incompatible with the ultimate function of these cells [23, 24, 37]. The early apoptotic wave may result in early elimination of defective germ cells in which DNA alterations have occurred through chromosomal crossing over during the first meiotic division [23, 24, 37].

**TABLE 1.** Analysis of epididymal tail weight and sperm morphology (mean ± SD) in 10-week-old wild-type and gad mice.

<table>
<thead>
<tr>
<th></th>
<th>Tail weight (mg)</th>
<th>Sperm concentration (10^6/ml)</th>
<th>Head</th>
<th>Midpiece</th>
<th>Principal piece</th>
<th>Detached head</th>
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<tr>
<td>wild-type</td>
<td>30.0 ± 0.8</td>
<td>23.6 ± 3.7</td>
<td>7.2 ± 1.5</td>
<td>2.4 ± 1.3</td>
<td>1.1 ± 0.2</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>gad/gad</td>
<td>24.7 ± 1.1*</td>
<td>19.5 ± 3.3*</td>
<td>14.1 ± 2.8*</td>
<td>4.7 ± 1.5*</td>
<td>1.7 ± 0.6</td>
<td>3.7 ± 1.2*</td>
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* Significantly different from wild-type mice (n = 7; P < 0.05).

**FIG. 6.** A) Abnormal morphology of spermatoozoa from gad mice. Spermatoozoa were collected from the cauda epididymidis of 10-wk-old gad mice. Head defects (open arrows) and midpiece defects (closed arrows) are indicated. Magnification ×400. B) Immunocytochemistry of UCH-L1 and ubiquitin in wild-type and gad mice. UCH-L1- and ubiquitin-positive spermatoozoa (closed arrows) and normal spermatoozoa (both negative, arrowheads) in wild-type mice are indicated. The inset shows an image of spermatoozoa from gad mice. Magnification ×1000.

**FIG. 7.** Kinematic analysis of spermatoozoa from the cauda epididymidis of 10-week-old wild-type and gad mice. A) Sperm motility. MSP, Percentage of motile sperm; PMP, Percentage of progressively motile sperm (n = 7; * P < 0.05). Data represent the mean ± SD. B) Movement characterization. VAP, Average path velocity (μm/sec); VSL, Straight-line velocity (μm/sec); VCL, Curvilinear velocity (μm/sec); ALH, Lateral head displacement (μm); LIN, Linearity (VSL/VCL × 100); STR, Straightness (VSL/VAP × 100). Data are expressed as a percentage of the values obtained for each parameter in wild-type mice (n = 7; * P < 0.05).
latory mechanisms of germ cell apoptosis has not been identified. A previous study showed that UCHL1-deficient gad mice express high levels of antiapoptotic proteins (Bcl2 family and XIAP) in the testis following cryptorchid-induced stress [22]. Alterations in the carefully maintained balance between the expression of apoptosis-inducing and apoptosis-protecting proteins may constitute one mechanism underlying the suppression of germ cell apoptosis observed in gad mice [46]. The decreased levels of TRP53, Bax, and caspase-3 observed in gad mice in this study are consistent with the suppression of germ cell apoptosis. In addition, the expression of the antiapoptotic protein Bcl-xL increased earlier in gad mice compared with wild-type mice. Therefore, the control of the apoptotic wave probably depends on variations in the balance between Bax and Bcl-xL [23, 47]. Analysis of the first round of spermatogenesis over time demonstrated a striking and massive wave of apoptotic germinal cells in 14-day-old mice (Fig. 3). High levels of UCHL1 protein were also observed at this age (Fig. 1) [20]. This early apoptotic wave was suppressed in the testes of gad mice, which had an abundance of germ cells compared with wild-type mice (Fig. 2). Moreover, the suppression of germ cell death is consistent with our previous report on cryptorchid stress injury in gad mice [22]. The testes of gad mice showed a phenotype similar to that of Bax-deficient mice or those overexpressing Bcl2 or Bcl-xL [23, 25, 26]. Also, the testes of Trp53−/− mice exhibited a similar phenotype involving decreased germ cell apoptosis and an increased number of germ cells [48].

In the present study, we also characterized spermatzoa in gad mice with regard to the following reproductive endpoints: 1) the weight of reproductive organs, 2) the concentration of sperm cells, and 3) the motility and morphology of spermatzoa collected from the cauda epididymis. The weight of cauda epididymids from gad mice was significantly lower compared with that from wild-type mice. The concentration of sperm cells was also significantly lower, and most motility parameters of spermatzoa collected from the cauda epididymids were affected in gad mice (Fig. 7). The significant decline in progressive forward motility, VAP, VSL, and VCL indicates that UCHL1 deficiency affects not only the ability of spermatzoa to move in the forward direction but also their vigor. In addition, the percentage of morphologically abnormal spermatozoas was significantly higher in gad mice (Table 1 and Fig. 6A).

Sperm production in the testis is a regulated balance between germ cell division and germ cell loss [26, 49], and there is emerging evidence that the ubiquitin-proteasome system may be central to the coordination of this process. For example, during spermatogenesis, the general activity of the ubiquitin-proteasome system is high, probably reflecting the requirement for massive degradation of cytoplasmic and nuclear proteins [6, 7, 50, 51]. Additionally, mutation of the ubiquitin-conjugating enzyme HR6B results in impaired spermatogenesis during nuclear condensation in spermatids [39, 41]. We found the fact that UCH-L1 associates with monoubiquitin in several lines of gad mice [18, 19, 22]. Furthermore, both proteins are expressed abundantly and at comparable levels in testis and the epididymis [11, 13, 14], suggesting that the functions of two proteins are important during spermatogenesis. Ubiquitin is present in defective spermatozoas, and proteins in these cells become ubiquitinated during epididymal passage (Fig. 6B) [11, 14, 33, 52, 53]. Furthermore, ubiquitination in the epididymis may trigger apoptotic mechanisms that recognizes and eliminate abnormal spermatozoas [49, 54, 55].

Further study is required to elucidate the functional significance of the association between UCHL1 and ubiquitin during spermatzoa maturation in the epididymis. However, our observations suggest that UCHL1 may function to regulate sperm production and to ubiquitinate proteins in defective spermatozoas. Our present study demonstrates that UCHL1-deficient gad mice are resistant to the wave of germinal cell apoptosis that occurs during the first round of spermatogenesis and that these mice have defects in sperm production, motility, and morphology. These results suggest that UCHL1 functions in the early apoptotic wave during the first round of spermatogenesis and in the control of sperm quality during sperm maturation.

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REFERENCES

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