The New Function of Two Ubiquitin C-Terminal Hydrolase Isozymes as Reciprocal Modulators of Germ Cell Apoptosis

Jungkee KWON1, 2)

1)Department of Laboratory Animal Medicine, College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea, and 2)Department of Degenerative Neurological Diseases, National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan

Abstract: Ubiquitination is required throughout all developmental stages of mammalian spermatogenesis. The two ubiquitin C-terminal hydrolase (UCH) enzymes, UCH-L1 and UCH-L3, deubiquitinate ubiquitin-protein conjugates and control the cellular balance of ubiquitin. These two UCH isozymes have 52% amino acid identity and share significant structural similarity. A new function of these two closely related UCH enzymes during spermatogenesis which is associated with germ cell apoptosis has been analyzed. Apoptosis, in general, is thought to be partly regulated by the ubiquitin-proteasome system. During spermatogenesis, apoptosis controls germ cell numbers and eliminates defective germ cells to facilitate testicular homeostasis. In this paper, I review the distinct function of the two UCH isozymes in the testis of gad and Uchl3 knockout mice, which are strongly but reciprocally expressed during spermatogenesis. In addition, the importance of UCHL1-dependent apoptosis for normal spermatogenesis and sperm quality control is discussed.

Key words: apoptosis, spermatogenesis, UCH-L1, UCH-L3, ubiquitin

Introduction

Numerous studies have demonstrated that ubiquitination and the proteasome system play important roles in controlling the levels of various cellular proteins, thereby regulating basic cellular processes such as cell cycle progression, signal transduction, and cell transformation [1, 4, 5, 10, 11]. Recent studies have shown that ubiquitination-mediated degradation and change in activity regulate many molecules of the cell death machinery, such as p53, caspase and Bcl-2 family members [34].

In the ubiquitin-proteasome system, the levels of ubiquitin are strictly controlled by the balance of two groups of specific enzymes: ubiquitinating enzymes (E1, E2 and E3) and deubiquitinating enzymes (DUBs) [4]. DUBs are subdivided into ubiquitin C-terminal hydrolases (UCHs) and ubiquitin specific proteases (UBPs) [26]. The genes for at least four UCHs, UCH-L1 and UCH-L3–5, have been identified in mice. Among them, UCH-L1 and UCH-L3 predominate; these isozymes have 52% amino acid identity and share significant structural similarity [15]. Despite their high sequence homology, the in vitro hydrolytic activities of these
two enzymes differ significantly. The activity (Kcat) of UCH-L3 is more than 200-fold higher than UCH-L1 when a fluorogenic ubiquitin substrate is used [22]. In addition, it has been suggested that UCH-L1 associates with monoubiquitin [25], and that UCH-L3 binds to Nedd8, subsequently processing its C-terminus [37]. Nedd8 is a small ubiquitin-like protein that, like ubiquitin, is conjugated to a lysine residue in a substrate protein.

Mammalian spermatogenesis is a complex process of cellular differentiation. Ubiquitin-mediated proteolysis is involved in different steps and processes during spermatogenesis. To elucidate the functional differences of UCH-L1 and UCH-L3, a pathophysiological analysis using the testes of gad and Uchl3 knockout mice was performed. In this paper, I review the apparent reciprocal functions for the two deubiquitinating enzymes, UCH-L1 and UCH-L3, with respect to mediating injury following apoptotic stress in spermatogenesis.

**gad mouse, Uchl3 knockout mouse and Uchl1 transgenic mouse**

The gad mouse is an autosomal recessive spontaneous mutant that was identified in 1984 [35]. This mouse has an intragenic deletion of the UCH-L1 gene and does not express UCH-L1, making it comparable to a Uchl1 null mutant [16, 30]. The Uchl3 knockout mouse was generated by a standard method using homologously recombinant ES cells from 129SV mice, followed by back-crossing of the knockout line several times to C57BL/6J mice [15]. The transgenic (Tg) Uchl1 mouse was carrying a 0.7-kb FLAG-tagged mouse Uchl1 cDNA with the human translation elongation factor-1 (EF-1) promoter [25]. To corroborate fertility disturbances in Uchl1 Tg mice, a subset of the mice was continuously mated with wild-type C57BL/6J mice. All Uchl1 Tg mice were identified as infertile.

**UCH-L1 and UCH-L3 function as reciprocal modulators of germ cell apoptosis**

UCH-L1 and UCH-L3 showed distinct expression during spermatogenesis

Ubiquitin C-terminal hydrolases catalyze the hydrolysis of C-terminal esters and amides of ubiquitin. The predominant mice isoymes, UCH-L1 and UCH-L3, are highly similar in sequence; however, UCH-L3 mRNA is expressed ubiquitiously, and UCH-L1 mRNA is selectively expressed in neurons and testes/ovaries [15]. To address this difference in characteristics, Kwon et al. generated polyclonal antibodies that specifically react with mice UCH-L1 and UCH-L3. Using western blotting and immunohistochemistry, they detected high levels of UCH-L3 in meiotic pachytene spermatocytes and post-meiotic spermatids contrary to UCH-L1 expression (Fig. 1). These results suggest that UCH-L1 and UCH-L3 may play distinct roles in spermatogenesis, and that UCH-L1 functions in mitotic proliferation, whereas UCH-L3 functions in the meiotic differentiation of spermatocytes into spermatids. The expression of UCH-L3 in meiotic spermatocytes and post-meiotic spermatids during spermatogenesis was then demonstrated [18]. Although UCH-L3 shares considerable sequence homology with UCH-L1, the hydrolytic activity of UCH-L3 in vitro differs substantially from that of UCH-L1. The high activity of UCH-L3 is consistent with its expression during the post-mitotic phase of spermatogenesis, in which maturation from spermatocytes to spermatids may be critically dependent on the ubiquitin pathway. These results demonstrate that the expression of the UCH isoymes, UCH-L1 and UCH-L3, is differentially and developmentally regulated during spermatogenesis, and that UCH-L1 and UCH-L3 likely have distinct functions during different developmental phases [18].

**UCH-L1 and UCH-L3 are reciprocal modulators of germ cell apoptosis**

During spermatogenesis, apoptosis controls germ cell numbers and eliminates abnormal germ cells to facilitate testicular homeostasis [27]. Recent studies have indicated that ubiquitination targets proteins for degradation and modulates the turnover of various classes of short-lived signaling proteins [21, 34]. Germ cell apoptosis following cryptorchid stress involves genes for various factors, such as Bcl-2 family proteins, p53 and caspases [7, 24]. To understand the pathophysiological roles of UCH-L1 and UCH-L3 in vivo, two mutant mice, gad and Uchl3 knockout mice, were examined after cryptorchid injury. The cryptorchid testes of the two mutant mice had fundamental differences after injury, i.e., testes of gad mice were relatively re-
sistant to injury, whereas Uchl3 knockout mice showed profound apoptosis-mediated germ cell loss.

There are several proposed mechanisms for germ cell loss following experimental cryptorchidism [28, 36]. With regard to cryptorchid injury, the balance between the expression of apoptosis-protecting and apoptosis-inducing proteins constitutes one possible mechanism underlying the observed germ cell protection and apoptosis from apoptosis in gad and Uchl3 knockout mice, respectively. In gad mice, cryptorchid injury caused a large increase in the antiapoptotic proteins Bcl-2, Bcl-xL and XIAP, a result which was consistent with a previous report using retina [9]. In addition, the expression levels of the prosurvival proteins pCREB and BDNF also increased in gad mice. Cryptorchid testes of Uchl3 knockout mice showed slightly increased expression of the apoptotic proteins p53, Bax and caspase-3 after injury. In total, these results suggest that UCH-L1 plays a role in balancing the expression of apoptosis-inducing and apoptosis-protecting proteins. In contrast, UCH-L3 seems to resist germ cell apoptosis following cryptorchid injury. Recent studies demonstrate that many molecules in the cellular apoptosis machinery, such as p53, Bcl-2 family, XIAP and caspase members, are targets for ubiquitination [34]. This suggests that ubiquitination is one of the major mechanisms by which apoptotic cell death is regulated. It has been suggested that UCH-L1 has been suggested to associates with monoubiquitin [25], and that the monoubiquitin level is reduced in gad mice relative to wild-type mice. In addition, it has been shown that UCH-L3 binds and cleaves the C-terminus of the ubiquitin-like protein, Nedd8. The cryptorchid testes of Uchl3 knockout mice showed high levels of Nedd8 expression following cryptorchid injury. These observations suggest that UCH-L3 may function as a deneddylating enzyme in vivo, although further studies are necessary to clarify whether UCH-L3 interacts with Nedd8 during spermatogenesis [19]. These results demonstrate apparent reciprocal functions for the two deubiquitinating enzymes, UCH-L1 and UCH-L3, with respect to mediating injury following experimental cryptorchidism (Fig. 2).

**UCH-L1 and UCH-L3 have region-specific expression along the epididymis**

The mammalian epididymis is a highly convoluted...
tubule that connects the efferent ducts of the testis to the vas deferens. The epididymis is composed of three distinct compartments, caput (head), corpus (body) and cauda (tail), each having a specific role in sperm maturation, sustenance, transport, and storage [2, 6]. The functional regionalization of the epididymis is represented at the molecular level by regional differences in gene expression [13, 14]. Regional differences along the epididymis might be essential characteristics of the environment required for sperm quality control.

Two UCH isozymes, UCH-L1 and UCH-L3, have region-specific expression along the epididymis. The level of UCH-L1 expression is significantly higher in the caput epididymidis, the main maturation organ, whereas that of UCH-L3 is significantly higher in the cauda epididymidis, the main storage organ (Fig. 3). These region-specific variations in UCH-L1 and UCH-L3 expression suggest that they have distinct functions during epididymal passage. The regional differentiation of the epididymis, as suggested by region-specific gene expression, reflects the different luminal environments between regions [13, 14]. The expression pattern of monoubiquitin showed region-specific patterns similar to UCH-L1. Under specific circumstances, the caput epididymidis contains high levels of monoubiquitin, which may serve to maintain apoptotic mechanisms that eliminate abnormal spermatozoa [33]. This concept is consistent with the high levels of apoptotic p53 and Bax observed in the caput epididymidis compared with the corpus and cauda epididymidis.

To characterize the apoptotic functions of UCH-L1 and UCH-L3 in the epididymis, gad and Uchl3 knockout mice were examined after efferent duct ligation [3]. After duct ligation, the number of apoptotic cells increased in the caput epididymidis of Uchl3 knockout mice compared with wild-type mice, whereas gad mice were relatively resistant in this regard. In gad mice, the levels of the antiapoptotic proteins Bcl-2/Bcl-xL were significantly elevated in the caput epididymidis regardless of efferent duct ligation. These results demonstrate that the two UCH isozymes, UCHL1 and UCHL3, have distinct expression levels along the epididymis and apparent reciprocal functions in response to apoptotic stress [20], as was shown with cryptorchid stress [19].

UCH-L1-dependent apoptosis for normal spermatogenesis and sperm quality control

UCH-L1 overexpression induces massive apoptosis during spermatogenesis

To confirm the function of UCH-L1 as an inducer of apoptosis during spermatogenesis, Uchl1 transgenic (Tg) mice were constructed that showed excess UCH-L1 expression [25]. The testes of Uchl1 Tg mice showed the arrest of spermatogenesis by massive germ cell death, leading to male sterility. The first description of a proapoptotic role for UCH-L1 in Uchl1 Tg mice was presented by Fig. 4, which clearly reveals the morphological and TUNEL results. Apoptosis was observed mainly in primary spermatocytes, which had weak or negative UCH-L1 expression. In Uchl1 Tg mice, constitutive expression of UCH-L1 in the testis results in a blockade of spermatogenesis at the

![Fig. 3](image_url) Comparison of UCH-L1, monoubiquitin and UCHL3 expression by western blotting of caput, corpus and cauda epididymidis lysates from two wild-type (CBA/RFM and C57BL/6J), gad and Uchl3 knockout mice. Blots were reprobed for α-tubulin, which was used to normalize the protein load (from Kwon et al., 2006 [20]).
UCH-L1 is essential for the early apoptotic wave and for sperm quality

Apoptosis is common during spermatogenesis 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 spermatozoa [27]. The early apoptotic wave may result in early elimination of defective germ cells in which DNA alterations have occurred through chromosomal crossover during the first meiotic division [12, 29]. It was shown that immature testes from gad mice are resistant to the massive wave of germ cell apoptosis during the first round of spermatogenesis. Early apoptosis in testicular germ cells is regulated by a complicated signal transduction pathway. The decreased levels of p53, Bax and caspase-3 observed in gad mice in these studies are consistent with the suppression of germ cell apoptosis.

Furthermore, it has shown that the concentration of sperm cells was significantly lower, and most motility parameters of spermatozoa collected from the cauda epididymis were affected in gad mice. In addition, the percentage of morphologically abnormal spermatozoa was significantly higher in gad mice. Sperm production in the testis is a regulated balance between germ cell division and germ cell loss [31], and there is emerging evidence that the ubiquitin-proteasome system may be central to the coordination of this process. Ubiquitin is present in defective spermatozoa (Fig. 5), and proteins in these cells become ubiquitinated during epididymal passage [8, 23, 33]. Ubiquitination in the epididymis may trigger apoptotic mechanisms that recognize and eliminate abnormal spermatozoa [31]. These results demonstrate that UCH-L1-deficient gad mice are resistant to apoptotic stress during spermatogenesis, and this apoptotic resistance leads to alterations in sperm production, motility and morphology [17, 32]. These results suggest that UCH-L1 functions to control quality during sperm maturation.

Conclusion

In this paper, I have reviewed the apparent reciprocal functions of the two deubiquitinating enzymes, UCH-L1 and UCH-L3, with respect to mediating apoptotic...
stress during spermatogenesis. I also propose an apoptotic function for UCH-L1 in the testis through the ubiquitin-proteasome pathway. Further biochemical analyses of UCH family members will help to elucidate the role of UCH isozenes in the complex molecular mechanisms involved in spermatogenesis.

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References


