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Expression of Fas-associated Factor 1 in the Developing Testis

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Abstract : FAS associated factor 1 (FAF1) is a Fas-associating molecule, which enhances Fas mediated apoptosis. FAF1 gene is expressed most abundantly in the testis among the mouse organs. The aim of this study was to reveal the expression and the role of FAF1 in the developing testis.

H-E stain and FAF1 immunohistochemistry were performed in the testis and epididymis of the E15.5 embryo, and 1, 2, and 8 week-old C57/BL6 mice.

FAF1 was expressed in the testis from E 15.5 embryo to 8 week-old mice. Cell type of FAF1 positive cells was different among the developmental stage. Furthermore, cellular (cytoplasmic or nuclear) localization of FAF1 in the male germ cells was different during the developmental stage. FAF1 was expressed mainly in the nuclei of the germ cells 1 and 8 weeks after birth, when cell differentiation occurs actively in the testis. However, FAF1 was expressed in the cytoplasms of germ cells 2 weeks after birth, when apoptosis occurs maximally in the testis.

Taken together, it can be suggested FAF1 expressed in male germ cells in the testis. FAF1 might be involved in regulation of the cellular function during spermatogenic cell differentiation and apoptosis in the testis.

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Key words : FAF1, Testis, Spermatogenesis, Cell differentiation, Apoptosis

Introduction

Fas associated factor 1 (FAF1) is a Fas-associating molecule, which enhances Fas mediated apoptosis (Chu et al. 1995, Becker et al. 1997, Kim et al. 2005). Although FAF1 contains domains found in the proteins of ubiquitination pathway, the function of FAF1 in relation to ubiquitin is largely unknown. FAF1 localizes in the nucleus, the perinuclear cytoplasm, and the nucleoli depending on the cell type (Frohlich et al. 1998, Ryu and Kim 2001).

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Human FAF1 was detected in various tissues, however, the most abundant expression was seen in the testis (Ryu et al. 1999, Adham et al. 2008). In Sertoli and Leydig cells of male mice, FAF1 immuno-positive staining is barely detectable. Immuno-positive reactions are observed in all germ cells, but the most intense immunoreaction is found in elongated spermatids. In preparations of germ cell suspension, a high level of FAF1 immunostaining was found in cytoplasm of elongated spermatids and in giant cells containing multiple nuclei in 9 week-old mouse. No FAF1 expression is discernable in the mature spermatid (Adham et al. 2008).

Spermatogenesis in mammals takes place in seminiferous tubules, all starting and ending in the rete testis

through which the spermatozoa leave the testis (de Rooij, 2001). The seminiferous tubules are lined by peritubular myoid cells and contain both Sertoli cells as well as germ cells at all stages of their development into spermatozoa. The process of spermatogenesis starts with a series of mitotic divisions carried out by a cell type called spermatogonia that are situated on the basal membrane of the seminiferous tubules. The last mitotic division renders the spermatocytes, that go through the S phase, then pass through the lengthy prophase of the first meiotic division and subsequently carry out the two meiotic divisions to give rise to haploid spermatids (Shinohara et al. 2000, Cooke and Saunders 2002).

The 49-kDa FAF1 protein is found in testes of qk/qk mutant mice (in which spermatogenesis is arrested at the spermatid stage), whereas 49-kDa FAF1 protein can not be detected in the testes of W/Wv mutant mice (which lack all germ cells) or in the cryptorchid testes of *Insl3*$^{-/-}$ mutant mice (in which spermatogenesis is arrested at the stage of pachytene spermatocytes) (Adham et al. 2008).

However, there was no report about expression of FAF1 in the developing testis. The aim of the study was to investigate expression and role of FAF1 in the testis during the developmental stage in mice.

Materials and Methods

1. Animals

The E15.5 embryo, and 1, 2 and 8 week-old C57/BL6 male mice were used in this experiment. All animals were maintained under standard laboratory conditions on a 12-h light/dark cycle, with free access to food and water in 8 week-old mice.

2. H-E stain and immunohistochemistry

1) Tissue preparation

Perfusion fixation was performed transcardially in the 8 week-old mice, first with 50 mL of phosphate-buffered saline (0.05 M, pH 7.4) containing heparin (1 IU/mL) at 4˚C and then with 50 mL of ice-cold 10% neutral buffered formalin for 10 min at a flow rate of 30～40 mL/min. Whole body of the E15.5 embryo and 1, 2 week-old mice removed immediately and then fixed in the ice-cold 10% neutral buffered formalin overnight. The fixed tissues were embedded in paraffin.

2) H-E stain

The deparaffinized sections were stained with routine H-E stain method.

3) Immunohistochemistry for FAF1

Immunohistochemistry for FAF1 was performed in the sections containing testis. Briefly, deparaffinized sections were heated for 4 min in a pressure cooker containing 10 mM citrate buffer (pH 6.0) for antigen retrieval. Subsequent procedures were conducted at room temperature (RT). The sections were pretreated with 3% H_2O_2 in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 30 min to quench endogenous peroxidase. Sections were incubated 1 hour at RT in the polyclonal FAF1 goat antibody (Santa Cruz, USA) in 0.1 M PBS, pH 7.4, containing 0.1% Triton X-100, 1.5 % bovine serum albumin (BSA), and 1 : 200 normal rabbit serum (NRS), followed by incubation for 1 hour at RT in 1 : 200 biotinylated rabbit anti-goat IgG (Vector, USA) and 1 : 200 NRS in PBS. Immnunoreactions were visualized by incubation for 1 hour at RT in avidin-biotin-peroxidase complex (1 : 100, ABC kit, Vector, USA) in PBS and 5～10 min in 0.05% 3,3′-diaminobenzidine (DAB) and 0.01% H_2O_2 in 0.1 M PBS. Immunolabeled sections were dehydrated in a graded ethanol series, defatted in xylene, and mounted. A similar procedure was used in control experiments, except the sections were processed in the absence of the primary FAF1 antibody.

Results

1. FAF1 immunoreactivity in the testis of E15.5 embryo

There were a few gonocytes in the primordial seminiferous tubule of E15.5 embryo (Fig. 1a). The size of the primordial Sertoli cells was relatively small, which was located in the peripheral part of the tubules, but the size of the gonocytes were relative large, which was localized in the central part of the tubules (Fig. 1a). Only the gonocytes in the central part of the tubules were FAF1 immunoreactive, FAF1 immunoreactivity was not found in the Sertoli cells. Immunoreactivity of the gonocytes was strong in the cytoplasm, and relatively weak in the nuclei of the gonocytes (Fig. 1b).

2. FAF1 immunoreactivity in the testis 1 week after birth

The seminiferous tubules revealed tubular structure (Fig. 2a). FAF1 immunoreactivity was found in some spermatogonia in the peripheral part of the tubules. Some of the spermatogonia were FAF1 negative. FAF1 immunoreacivity was mainly localized in the nuclei of the spermatogonia (Fig. 2b).

3. FAF1 immunoreactivity in the testis 2 weeks after birth

Fragmentation of nuclei, a marker of apoptosis, occurred in the germ cells in the seminiferous tubules 2 weeks after birth (Fig. 3a). FAF1 immunoreactivity was found in the cytoplasm of the germ cells and in the nuclei of some Sertoli cells (Fig. 3b).

4. FAF1 immunoreactivity in the testis 8 weeks after birth

Spermatids were found in the seminiferous tubules 8 weeks after birth (Fig. 4a). FAF1 immunoreactivity was seen in mot spermatogonia in the seminiferous tubules. FAF1 immunoreactivity was localized mainly in the nucleus of the spematogonia (Fig. 4b)

5. FAF1 immunoreactivity in the epididymis 8 weeks after birth

A large number of spermatozoa were present in the lumen of the epididymis (Fig. 5a). FAF1 immunoreactivity was not found in the sperms located in the epididymis lumen. However, FAF1 immunoreactivity was seen in the nuclei of the epithelial cells of the epididymis (Fig. 5b).

Discussion

This study provides the first description of FAF1 expression in the developing testis. FAF1 was expressed in the testis from embryo to adult mice. Type of FAF1 positive cells was different among the developmental stage. Furthermore, localization of FAF1 in the male germ cells was different during developmental stage.

Complex function for FAF1, which may be executed during FAS signalling and/or in the ubiquitination pathway, may be essential for cell differentiation and survival (Sommer et al. 1991, Siomi et al. 1995, Forhlich et al. 1998). FAF1 gene has a nuclear localization signal. Therefore, FAF1 seems to function as a nuclear factor. It is possible that FAF1 could bind to basic nuclear proteins such as histones via its hydrophilic a-helical domain 2 based on its high homology with a chromatin assembly factor like domain (Ryu et al. 1999)

The development of germ cells in mice testes follows a well-known sequence of events. The proliferating primordial germ cells colonize the genital ridge by 11.5 days post-coitum (dpc) (Ginsburg et al. 1990, Yoshimizu et al. 2001). From 14.5 to 19.5 dpc, the

gonocytes enter a quiescent period during which mitosis and apoptosis no longer occur (Vergouwen et al. 1991, Nagano et al. 2000). After birth, the gonocytes resume mitosis at the same time as a second wave of apoptosis and start to differentiate into spermatogonia (Mori et al. 1997, Rodriguez et al. 1997, Boulogne et al. 1999). Our data showed that FAF1 was expressed in the gonocytes of E 15.5 (15.5 dpc) embryos. However, the function of FA1 expression in the gonocytes of E 15.5 embryos is not clear.

It was found that during postnatal development of the mouse testis apoptotic cell death is higher from days 8 to 22 than either before or after this period, the degenerative cell death of spermatogonia and primary spermatocytes involves apoptosis with fragmentation of nuclear DNA. Previous studies indicated that germ cell degeneration in adult male rats occurs spontaneously and in response to endocrine disruption, various environmental agents (e.g., heat, irradiation, and ischemia), and exposure to cytotoxic drugs (Allan et al. 1992, Billig et al. 1995, Brinkworth et al. 1995).

In our study, FAF1 was expressed in the nuclei of the spermatogonia 1 and 8 weeks after birth. On the contrary, FAF1 was expressed in the cytoplasms of the germ cells (some of which showed apoptotic appearance) 2 weeks after birth, when apoptosis occurs maximally in the testis (Mori et al. 1997). These data reveal that nuclear localization in the spermatogonia 1 week after birth may be related to cell differentiation, but cytoplasmic localization in the germ cells might be related to apoptosis 2 weeks after birth (Mori et al. 1997, Rodriguez et al. 1997, Boulogne et al. 1999). Apoptosis is rare in the testis 8 weeks after birth in mice (Mori et al. 1997). Therefore, FAF1 expression in the spermatogonia may be involved in regulation of the cellular function during spermatogenic cell differentiation 8 weeks after birth.

FAF1 immunoreactivity was not found in sperms in the epididymis in this study. However, FAF1 immunoreactivity was seen in the nuclei of the epithelial cells of the epididymis. These results suggest that fully differentiated sperms in the epididymis, do not express FAF1, and FAF1 expression in the epithelial cells of the epididymis may be related to regulate cell differentiation or other novel function as a transcription factor (Schwoebel and Moore 2000, Wilkinson et al. 2001, Stein et al. 2003).

In conclusion, FAF1 was mainly expressed in male germ cells in the testis. FAF1 may be involved in regulation of the cellular function during spermatogenic cell differentiation and apoptosis in the testis.

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Legends for Figures

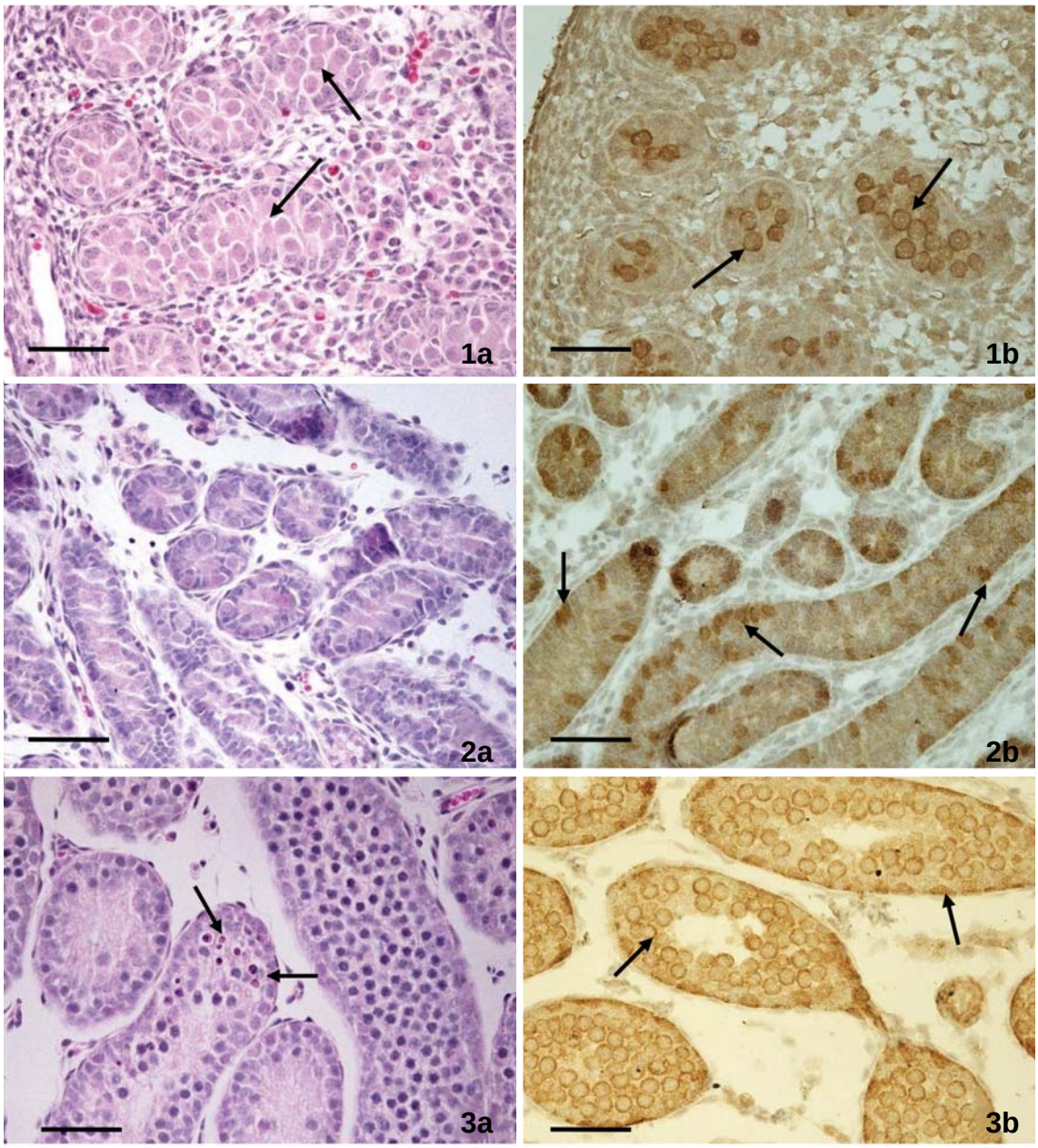
Fig. 1. General morphology (1a, H-E stain) and FAF1 immunoreactivity (1b) in the E15.5 embryo testis. FAF1 immunoreactivity was found in the gonocytes of the testis. Arrows, gonocytes; Scale bars=50 μm.

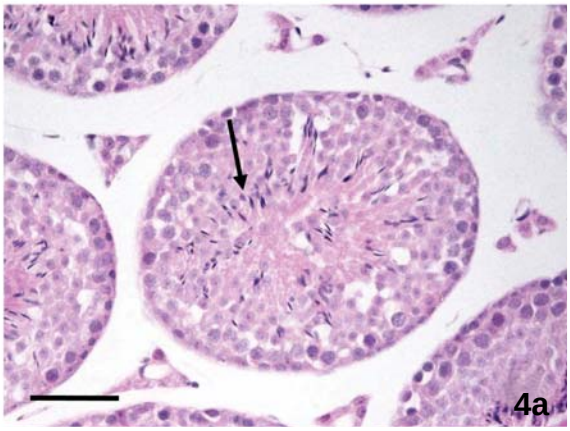
Fig. 2. General morphology (2a, H-E stain) and FAF1 immunoreactivity (2b) in the testis 1 week after birth. FAF1 immunoreactivity was found in the spermatogonia in the peripheral part of the tubule. Arrows, spermatogonia; Scale bars=50 μm.

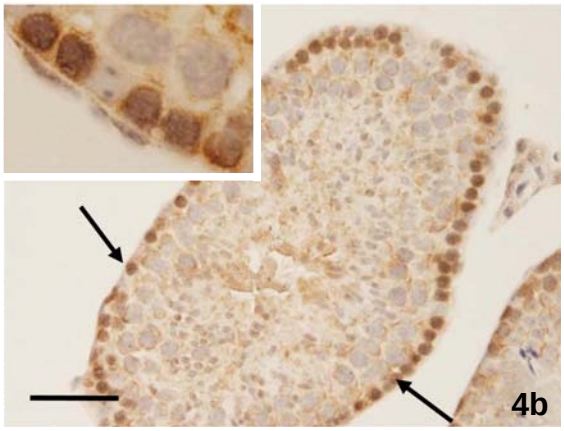
Fig. 3. General morphology (3a, H-E stain) and FAF1 immunoreactivity (3b) in the testis 2 weeks after birth. Fragmentation of nuclei (arrows in 3a) occurred in the germ cells in the seminiferous tubule. FAF1 immunoreactivity was mainly found in the cytoplasm of the spermatogonia and spermatocytes (left arrow in 3b), and probably in the nuclei of Sertoli cells (right arrow in 3b) in the testis. Scale bars=50 μm.

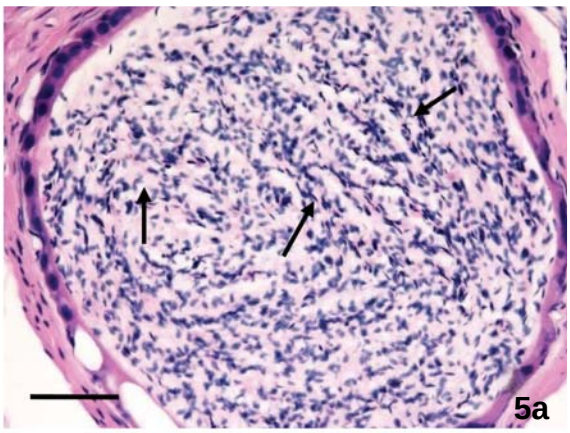
Fig. 4. General morphology (4a, H-E stain) and FAF1 immunoreactivity (4b) in the testis 8 weeks after birth. Spermatids (arrows in 4a) were found in the seminiferous tubules. FAF1 immunoreactivity was seen mainly in the nuclei of the spermatogonia (arrows in 4b) in the seminiferous tubules. Upper rectangle is high magnification view of FAF1 immunoreactive cells. Scale bars=50 μm.

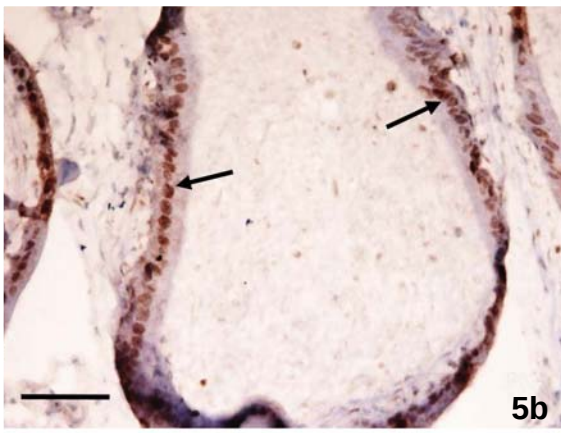
Fig. 5. General morphology (5a, H-E stain) and FAF1 immunoreactivity (5b) in the epididymis 8 weeks after birth. A lot of spermatozoa (arrows in 5a) were presented in the epididymis. FAF1 immunoreactive spermatozoa was not found in the epididymis. However, FAF1 immunoreactivity was seen in the nuclei of the epithelial cells (arrows in 5b) of the epididymis. Scale bars=50 μm.











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발생중인 고환에서 Fas-associated factor 1의 발현

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간추림 : Fas-associated factor 1 (FAF1)은 Fas 관련 물질로서, Fas 매개 세포사멸을 유도한다. FAF1 유전자는 생쥐의 여러 기관 중 고환에서 가장 많이 발현된다. 본 연구의 목적은 생쥐의 발생 또는 발달 중인 고환에서 FAF1의 발현과 그 역할을 규명하고자 하였다.

C59/BL6 15.5일과 생후 1, 2 및 8주령의 C57/BL6 생쥐 고환에서 H-E 염색과 FAF1에 대한 면역조직화학염색을 시행하였다.

FAF1은 생쥐 15.5 태자부터 8주령의 생쥐까지 모두 발현되었다. 고환에서 FAF1 양성세포는 발생 및 발육단계별로 달랐다. 더욱이 FAF1의 세포 내 (세포질 또는 핵) 발현양상은 고환의 발생 또는 발달 단계별로 달랐다. 특히, 세포분화가 활발히 진행되는 1주령 및 8주령 생쥐의 생식세포에서는 FAF1이 주로 핵에 발현되었으며, 세포사멸이 최대로 일어나는 2주령의 생식세포에서는 주로 세포질에서 FAF1이 발현되었다.

이를 종합하면, FAF1은 생쥐 고환의 생식세포에서 주로 발현되며, 정자형성 시 세포기능을 조절하여 세포분화와 세포사멸에 관여하고 있음을 보여주었다.

찾아보기 낱말 : FAF1, 고환, 정자형성, 세포분화, 세포사멸

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