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Title	Morphological analysis of spermatogenesis in sterile mice caused by the Hybrid sterility 3 gene and finding of a novel gene associated with spermiogenesis by the subtraction method
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Citation	Japanese Journal of Veterinary Research, 46(2-3), 141-142
Issue Date	1998-11-30
Doc URL	https://hdl.handle.net/2115/2691
Type	departmental bulletin paper
File Information	KJ00003408024.pdf

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Morphological analysis of spermatogenesis in sterile mice caused by the *Hybrid sterility 3* gene and finding of a novel gene associated with spermiogenesis by the subtraction method

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The sterility of interspecific hybrids, like mules and leopons, is a well-known phenomenon. In mice, interspecific or intersubspecific hybrid sterility induced by *Hybrid sterility* (*Hst*) genes has been occasionally found in matings between laboratory mice and wild mice. One of these genes, *Hst3*, which controls the sterility of interspecific hybrids between laboratory mice (*Mus musculus domesticus*) and *Mus spretus* originating from the Spanish wild mouse, is associated with X-Y chromosome dissociation accompanied by spermatogenic breakdown after metaphase I (MI). For detailed analysis of hybrid sterility caused by *Hst3*, C57BL/6 congenic mice carrying *Hst3* derived from *M. spretus* were created in order to exclude the influence of other *Hst* genes. The aim of this study was to analyze the developmental breakdown during spermatogenesis in *Hst3*-caused sterile mice. Additionally, we attempted to find a novel gene associated with spermatogenesis by the subtraction method using these congenic mice.

Up to postnatal day 12, spermatogenesis progressed relatively normally in both types of testis. At day 14, when early pachytene spermatocytes appeared in the normal testis, much degeneration of these cells was detected in the sterile testis by the TUNEL method, used to detect a markers for apoptosis. At day 20, many round spermatids appeared in the normal testis, while no round spermatids were found in the stertile testis. At day 60 of sexual maturity, all kinds of germ cells were observed in the normal testis. However, only a few spermatids and spermatozoa, showing abnormal contours, were found in histological sections of the sterile testis. In the sterile testis, many degenerated spermatocytes showing nuclear condensation, eosinophilic cytoplasm in H.E staining and TUNEL reactivity were demonstrated at the MI stage.

According to morphologial differences between normal and sterile testes, the subtraction (tester: normal testis cDNA, driver: sterile testis cDNA) method was performed using congenic mouse testis to analyze the normal spermatogenesis-associated genes. Transcripts of some subtracted cDNA clones were expressed at higher levels in normal testis than in sterile testis by Northern blot analysis. One of them, clone F77, showing significant non-homology with entries in the database, demonstrated remarkable expression of an approximately 4kb transcript in the normal testis, compared to the sterile testis. Additionally, the transcript was not detected in any other organs except the testis. Northern blot analysis using developing normal testis and the germ cell-deficient W/W^V mutant testis, showed that the transcript of cDNA clone F77 was expressed only in normal adult testis, in which it was detected in elongated spermatids and partially round spermatids by in situ hybridization. cDNA clone F77 was mapped at a proximal position between *Mt-1* (45.0) and *Ctrb* (55.0) on mouse chromosome 8, in which no candidate genes related to its nucleotide sequ-

ence were found.

These results suggested that there were at least two time-specific checkpoints in spermatogenesis of the sterile mice caused by *Hst3*, resulting in apoptotic cell death. Furthermore, it was noted that cDNA clone F77 was detected as a novel gene in the present study. It was expressed in a relatively late stage of spermiogenesis (mainly in elongated spermatids), and appeared to be involved in specific spermiogenetic cell differentiation.

Transfer of Fas antigen cDNA constructed with an expression vector in cationic liposome into MRL-*lpr* mice

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Gene delivery systems using recombinant viral vectors or nonviral vectors such as liposomes have been reported. Vectors from retroviruses are known to transfer genes effectively into cells. However, the therapeutic use of viruses as gene delivery vehicles entails some problems, because retroviruses might disrupt DNA of the host cells they infect, and could cause potentially harmful results. On the other hand, nonviral methods of gene transfer would be suitable for repeated use, since they do not result in immune responses. The liposome, which is a small fatty sphere, is a nonviral vector and has been studied as well as retrovirus vectors.

It is considered that gene therapy using Fas antigen in vivo is an important way to eliminate tumours or cause them to regress through apoptosis. Mouse lymphoproliferation gene (*lpr*), which is a nonfunctional mutation of Fas antigen, has been assigned to chromosome 19. Few transcripts of Fas antigen in MRL-*lpr* mice are expressed in the thymus and liver compared to normal mice because an insertion by an early transposable element occurs in intron 2. The clinical symptoms of MRL-*lpr* mice are lymphoadenopathy, splenomegaly, hypergammaglobulinaemia and arthritis, which resemble human systemic lupus erythematosus. In this study the author designed expression vectors carrying Fas antigen cDNA in the correct orientation, named pEF-Fas and pCMV-Fas. First, it became apparent in vitro experiment and through Western blot analysis that Fas antigen protein is expressed in simian COS-1 cells transfected with lipofectin and pEF-Fas or pCMV-Fas complexes. Then the author used MRL-*lpr* mice to investigate the effect of Fas antigen expression by means of transfection of the expression vectors enclosed in cationic liposomes. After injection through the tail vein, the vectors carrying Fas antigen cDNA-liposome complex were successfully transferred mainly in the lung, liver, spleen, kidney and pancreas as detected by Southern blot analysis. Furthermore, RNA transcripts were found in lung and liver by Northern blot analysis, but were not observed in any other organ. Fas antigen protein, however, was not observed even in lung and liver by Western blot analysis. Finally the author attempted 66% partial hepatectomy to find Fas antigen protein effectively, but could not detect it. Further study is necessary to investigate the reason why the