doi:10.1093/jmcb/mjt031
Published online August 22, 2013

Letter to the Editor

Tex101 is essential for male fertility by affecting sperm migration into the oviduct in mice

Dear Editor,

Sperm transport in the female genital tract is physiologically important for mammalian fertilization. The female reproductive system contains multiple natural selective barriers, such as successful uterotubal junction (UTJ) migration and zona pellucida (ZP) binding, to ensure sperm with normal motility and morphology to transmit into oviduct for fertilization (Yanagimachi, 1994; Ikawa et al., 2010). Tex101 is a glycosylphosphatidyl inositol (GPI)-anchored glycoprotein identified as a molecular marker of germ cells (Kurita et al., 2001). Although there have been indications that the malfunction of *Tex101* may affect male fertility (Yin et al., 2009), little is known about its exact physiological function and the underlying molecular mechanisms. Recently, a study showed that *Tex101* gene knockout sperm were unable to pass through UTJ or bind to ZP, which led to male infertility (Fujihara et al., 2013). Here, we independently generated *Tex101* knockout mice and confirmed the infertile phenotype caused by UTJ migration defect. We also found that *Tex101* knockout sperm lost the adhesive ability to the surface of female genital tract. Several members of a disintegrin and metalloprotease (ADAM) transmembrane protein family with cell adhesion ability, including ADAM3, ADAM4, ADAM5, and ADAM6, were lost in *Tex101* knockout epididymal sperm. These observations may shed new light on the diagnosis of male infertility and development of contraceptive methods in human.

High abundant Tex101 protein was only detected in the testis of male mice (Supplementary Figure S1A). To investigate the function of Tex101 *in vivo*, we generated *Tex101* gene knockout mice (Supplementary Figure S1). During the 2-year observation period, neither *Tex101* heterozygous mutant (*Tex101*$^{+/-}$) nor *Tex101* homozygous mutant (*Tex101*$^{-/-}$) mice (over 30 mice per group) showed any overt developmental abnormalities. However, although with normal mating ability, male *Tex101*$^{-/-}$ mice could not produce offspring, which confirmed the infertile defect of *Tex101* deletion (Supplementary Table S1) (Fujihara et al., 2013).

We next characterized the defects of *Tex101*$^{-/-}$ sperm causing male infertility. The histology and weight of testis from wild-type (*Tex101*$^{+/+}$) and *Tex101*$^{-/-}$ male mice exhibited no identifiable difference (Supplementary Figure S2). In addition, no difference in sperm count, sperm viability, or motility parameters was observed (Supplementary Table S2). However, none of oocytes from females mated with *Tex101*$^{-/-}$ mice was fertilized at 18 h after mating plug formation (Supplementary Figure S3), suggesting that sperm from *Tex101*$^{-/-}$ mice were either unable to reach the fertilization place or unable to fertilize the oocytes. We then counted sperm collected from the oviducts of mated females. Large amounts of sperm were found in female mice mated with *Tex101*$^{+/+}$ males (323 ± 84, $n = 8$), yet no sperm (0, $n = 24$) was recovered from females mated with *Tex101*$^{-/-}$ males (Figure 1A). Similarly, sperm were only observed in the UTJ lumen of female mice mated with *Tex101*$^{+/+}$ males but not those mated with *Tex101*$^{-/-}$ males (Supplementary Figure S4). These results demonstrated that *Tex101*$^{-/-}$ sperm were unable to pass through the UTJ of female genital tract. However, *Tex101*$^{-/-}$ sperm still fertilized oocytes (Figure 1B) at a lower rate compared with *Tex101*$^{+/+}$ sperm (Figure 1C, 40% vs. 58%, $P = 0.048$) in *in vitro* fertilization (IVF) assays. Moreover, among 24 in-tubal inseminated (ITI) female mice, four were successfully pregnant and produced 12 healthy offspring, indicating that *Tex101*$^{-/-}$ sperm were still capable to fertilize oocytes *in vivo* when the UTJ transportation was avoided (Figure 1D, E, and Supplementary Table S3). In contrast, in intra-uterine insemination (IUI) assays, no offspring was produced in the *Tex101*$^{-/-}$ group (Supplementary Table S3), further confirming that the male infertility defect of *Tex101*$^{-/-}$ mice was primarily caused by the UTJ migration defect of sperm.

We noticed that *Tex101*$^{-/-}$ sperm seldom bound to dissected epithelium and ZP in the computer-assisted sperm analysis and IVF experiments. To further assess the membrane adhesive ability of *Tex101*$^{-/-}$ sperm, different cells inside the female genital tract, including the epithelium of UTJ and isthmus oviduct, cumulus cells, and oocytes, were dissected out and incubated separately *in vitro* with *Tex101*$^{+/+}$ and *Tex101*$^{-/-}$ sperm. After incubation for 30 min, *Tex101*$^{+/+}$ sperm adhered to all types of epithelium cells robustly, whereas *Tex101*$^{-/-}$ sperm were rarely attached (Figure 1F and G). These results demonstrated that sperm of *Tex101*$^{-/-}$ mice had lost their adhesive ability, thus failed to bind to the surface of cells in female genital tract.

To investigate the functional mechanisms of *Tex101*, we used mass spectrometry to characterize the differentially expressed proteins between *Tex101*$^{+/+}$ and *Tex101*$^{-/-}$ cauda epididymal sperm. A total of 30 proteins were identified with >1.5-fold expression changes, including two ADAM protein family members, ADAM5 and ADAM6 (Supplementary Table S4). Previous studies showed that ADAM3 but not other ADAM proteins played a key role in causing the infertile phenotypes (Ikawa et al., 2010; Fujihara et al., 2013); therefore, we detected the expression of all ADAM family proteins with predominant expression in testis by western blot. All examined proteins had no observable expression difference in testicular sperm between *Tex101*$^{+/+}$ and *Tex101*$^{-/-}$ mice. However, in cauda

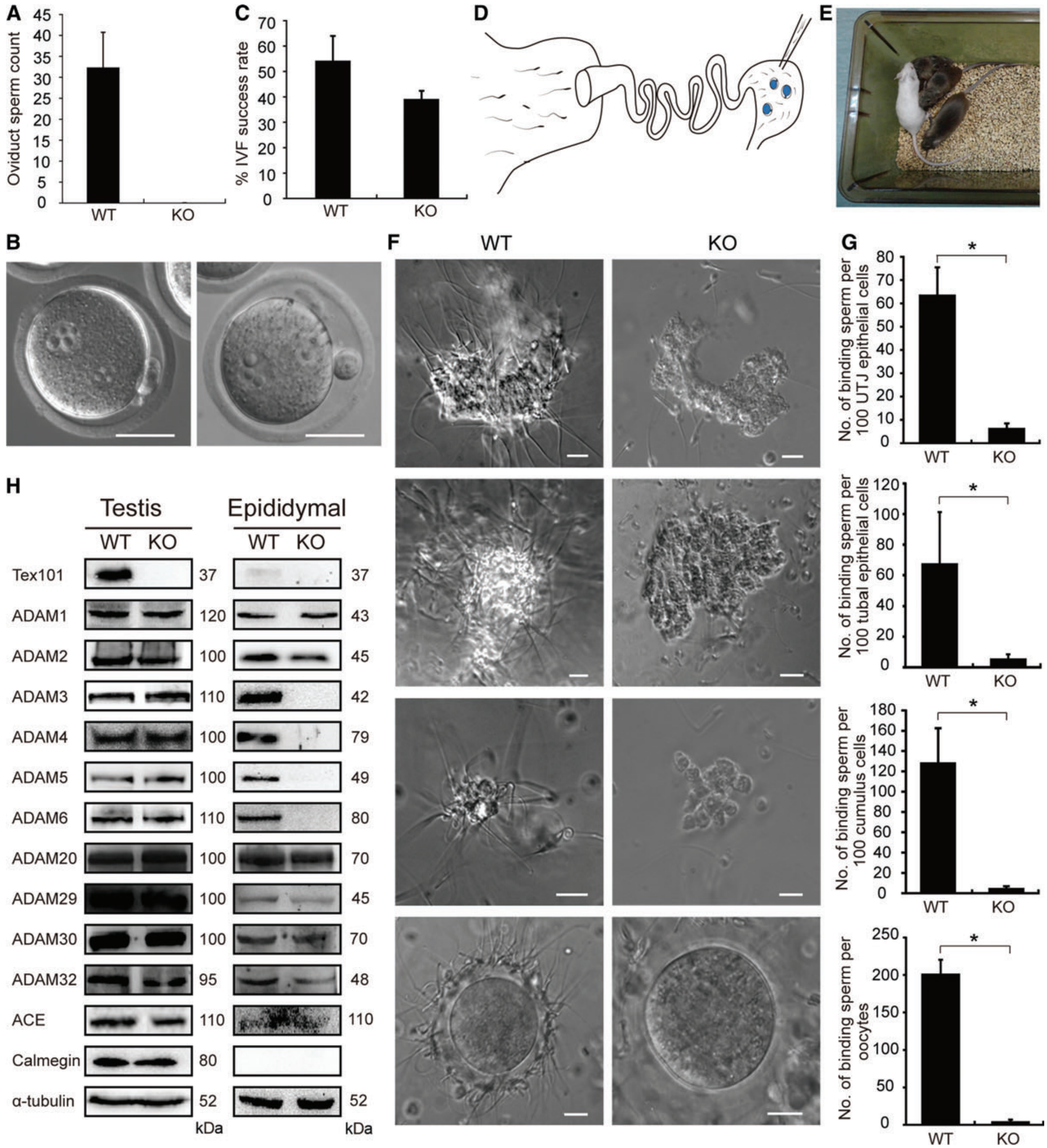

Figure 1 Functional studies of *Tex101*$^{-/-}$ sperm. (**A**) Count of sperm collected from the oviduct of females mated with *Tex101*$^{+/+}$ (WT) and *Tex101*$^{-/-}$ mice (KO), respectively. (**B**) *In vitro* fertilized oocytes with *Tex101*$^{+/+}$ (left) and *Tex101*$^{-/-}$ sperm (right). The presence of the pronuclei in both panels indicates successful fertilization. Scale bar, 50 μm. (**C**) *In vitro* fertilization (IVF) rate of *Tex101*$^{+/+}$ and *Tex101*$^{-/-}$ sperm. (**D**) Schematic overview of the intra-tubal insemination (ITI) procedure. (**E**) Live healthy offspring mice produced by ITI with *Tex101*$^{-/-}$ sperm. (**F**) The adhesion of *Tex101*$^{+/+}$ (WT, left panel) and *Tex101*$^{-/-}$ (KO, right panel) sperm to different types of cells shown by phase-contrast photos. Panels from top to bottom are: UTJ epithelium, oviduct epithelium, cumulus cells, and oocytes. The adhered sperm can be identified by their long tails. Scale bar, 20 μm. (**G**) Count of adhered sperm on different cell types. WT, *Tex101*$^{+/+}$ sperm; KO, *Tex101*$^{-/-}$ sperm. *$P < 0.001$, Student's *t*-test. (**H**) Western blot analysis of testis-specific ADAM proteins, tACE, and Calmegin in *Tex101*$^{+/+}$ (WT) and *Tex101*$^{-/-}$ (KO) testicular and epididymal sperm.

epididymal sperm, ADAM3, ADAM4, ADAM5, and ADAM6 were all lost in *Tex101*$^{-/-}$ mice (Figure 1H).

The ADAM proteins gradually mature during their passage into epididymis (Seals and Courtneidge, 2003). However, mature forms of ADAM3, ADAM4, ADAM5, and ADAM6 could not be detected in *Tex101*$^{-/-}$ sperm in any region (caput, corpus, and cauda) of the epididymis, whereas the accumulations of pro-proteins were observed, indicating that these ADAM proteins failed to mature during the transition process of sperm to epididymis (Supplementary Figure S5A and B). The maturation of ADAM protein requires the formation of multiple complex among ADAM proteins or ADAM proteins with other proteins (Nishimura et al., 2007). In addition to known interactions, we also found that Tex101 co-precipitated with ADAM3 and ADAM5 in wild-type sperm, whereas in *Tex101*$^{-/-}$ testicular sperm, these interactions as well as those among ADAM proteins were completely abolished or largely reduced (Supplementary Figure S5C). Taken together, our results suggested that the deletion of *Tex101* affected the interactions among ADAM3, ADAM4, ADAM5, and ADAM6 in the testis, and interfered their epididymal maturation.

In the present study, we independently generated *Tex101* knockout mice and confirmed their infertile defect caused by inability of sperm to migrate into oviduct. ZP-binding is thought as an important step for successful fertilization (Primakoff and Myles, 2002), which was always co-identified with UTJ migration defect in a serial of knockout mice, leading to male infertility (Ikawa et al., 2010). Here, we demonstrate that the UTJ migration defect is the primary cause of infertility in *Tex101*$^{-/-}$ mice as *Tex101*$^{-/-}$ sperm could fertilize oocytes both *in vitro* and *in vivo* via assisted reproduction. Our results call for more research on the UTJ migration and ZP-binding process to clarify their roles in fertilization in physiological circumstances. Of note, we found that *Tex101*$^{-/-}$ sperm were not only defective in binding to ZP, but also unable to bind to the surface of multiple cell types in female genital tract. Previous studies showed that adhesion to genital tract epithelium might be as essential as motility for sperm to enter the oviduct (Suarez, 1987; Suarez and Pacey, 2006; Talevi and Gualtieri, 2010). Our finding proposed that the loss of adhesive ability of *Tex101*$^{-/-}$ sperm might be the physiological basis of UTJ migration defect, which also resulted in the ZP-binding defect. We also identified simultaneously loss of four ADAM proteins in *Tex101*$^{-/-}$ knockout mice, indicating that uncharacterized ADAM proteins other than ADAM3 may also play important roles in regulating sperm functions. As human ADAM3 is a pseudo-gene, our results suggest that other testis-specific ADAM proteins may exist and function in humans. Taken together, our results may shed new light on both human infertility diagnosis and contraceptive drug development.

[Supplementary material is available at Journal of Molecular Cell Biology online. This study was independently conducted and completed in parallel with a similar study that was published (Fujihara et al., 2013) while this manuscript was under review of another journal before submitting to Journal of Molecular Cell Biology. This study was supported by grants from the National Basic Research Program of China 2012CBA01300 (to Q.Z.), 2013CB947903 (to X.Y.Z.), and 2011CB944304 (to J.H.S.), and grant from the National Natural Science Foundation of China (81222006).]

Wei Li[1,†], Xue-Jiang Guo[2,†], Fei Teng[1,3], Xiao-Jun Hou[1], Zhuo Lv[1], Shu-Ya Zhou[1], Ye Bi[2], Hai-Feng Wan[1], Chun-Jing Feng[1,3], Yan Yuan[2], Xiao-Yang Zhao[1], Liu Wang[1], Jia-Hao Sha[2,*], and Qi Zhou[1,*]

[1]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[2]State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 210029, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China
[†]These authors contributed equally to this work.
[*]Correspondence to: Qi Zhou, E-mail: qzhou@ioz.ac.cn; Jia-Hao Sha, E-mail: shajh@njmu.edu.cn

References

Fujihara, Y., Tokuhiro, K., Muro, Y., et al. (2013). Expression of TEX101, regulated by ACE, is essential for the production of fertile mouse spermatozoa. Proc. Natl Acad. Sci. USA *110*, 8111–8116.

Ikawa, M., Inoue, N., Benham, A.M., et al. (2010). Fertilization: a sperm's journey to and interaction with the oocyte. J. Clin. Invest. *120*, 984–994.

Kurita, A., Takizawa, T., Takayama, T., et al. (2001). Identification, cloning, and initial characterization of a novel mouse testicular germ cell-specific antigen. Biol. Reprod. *64*, 935–945.

Nishimura, H., Myles, D.G., and Primakoff, P. (2007). Identification of an ADAM2-ADAM3 complex on the surface of mouse testicular germ cells and cauda epididymal sperm. J. Biol. Chem. *282*, 17900–17907.

Primakoff, P., and Myles, D.G. (2002). Penetration, adhesion, and fusion in mammalian sperm-egg interaction. Science *296*, 2183–2185.

Seals, D.F., and Courtneidge, S.A. (2003). The ADAMs family of metalloproteases: multidomain proteins with multiple functions. Genes Dev. *17*, 7–30.

Suarez, S.S. (1987). Sperm transport and motility in the mouse oviduct: observations in situ. Biol. Reprod. *36*, 203–210.

Suarez, S.S., and Pacey, A.A. (2006). Sperm transport in the female reproductive tract. Hum. Reprod. Update *12*, 23–37.

Talevi, R., and Gualtieri, R. (2010). Molecules involved in sperm-oviduct adhesion and release. Theriogenology *73*, 796–801.

Yanagimachi, R. (1994). Mammalian fertilization. In: Knobil, E., and Neil, J.D. (eds). The Physiology of Reproduction (2nd edn). New York: Raven Press, 189–317.

Yin, L., Chung, C.M., Huo, R., et al. (2009). A sperm GPI-anchored protein elicits sperm-cumulus cross-talk leading to the acrosome reaction. Cell. Mol. Life Sci. *66*, 900–908.