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 transit into oviduct for fertilization (Yanagimachi, 1994; Ikawa et al., 2010). Tex101 is a glycosylphosphatidylinositol (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...
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 Guaitieri, 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.

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