Roles of Testis-enriched Proteins in Spermatogenesis and Their Applications.

スルタナ, タスリン

Name ：スルタナ　タスリン (SULTANA TASRIN)
Title ： Roles of Testis-enriched Proteins in Spermatogenesis and Their Applications.
(精巣高発現タンパク質の精子形成における機能と応用)

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Thesis Summary

Spermatogenesis is the intricate process in which spermatogonial stem cells differentiate into mature spermatozoa within the seminiferous tubules of testis. Multiple stages including mitosis, meiosis, and spermiogenesis are precisely regulated during this whole process (1). Different cell junctions including intercellular bridges (ICB), ectoplasmic specialization (ES), and blood-tesis barrier (BTB) are known to assist the process. This thesis consists of three chapters. In chapter 1, the research aimed to inhibit cancer cell proliferation via induction of ICB. In chapters 2 and 3, the impacts of two different testis-enriched proteins on sperm morphology and male mice fertility were investigated.

In **chapter 1**, partial TEX14 peptides were used to induce ICBs in HeLa cells. ICBs are stable junctions that interconnect daughter cells derived from single spermatogonial stem cells. TEX14, the first reported intercellular bridge protein, is essential for formation and maintenance of intercellular bridge and crucial for male fertility. TEX14 blocks germ cell abscission prior to cytokinesis. GPPX3Y motif of TEX14 plays vital role in this process by interfering with CEP55-ALIX or CEP55-TSG101 interaction, since this motif binds strongly with CEP55 hinge region. In this study, three partial TEX14 peptides; made of 7, 13 and 27 amino acids and each having GPPX3Y region, were used to induce ICBs in HeLa cells. Partial TEX14 peptides expressing cells showed lower proliferation rate due to the presence of intercellular bridges and eventually led to apoptosis. Our findings suggest that these TEX14 short peptides might be used in suppression of abscission in continuously proliferating cells like cancer cells.

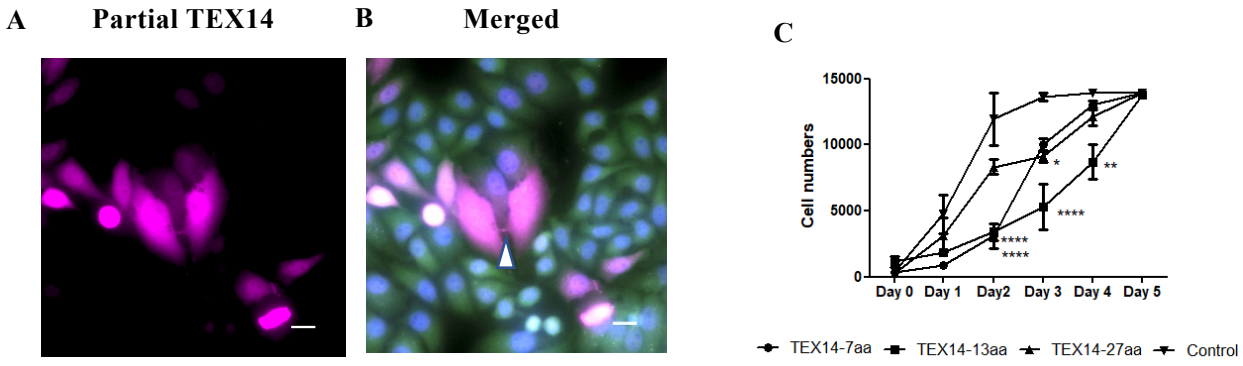

Fig.1: Partial TEX14 peptides induced ICBs and reduced cell proliferation. A. Cells expressing TEX14-27aa in pink. B. Merged with ICB marker MKLP. Scalebar 20μm. Arrowhead indicates ICB. C. Reduction in cell proliferation in TEX14 short peptides expressing cells. Asterisks indicate significance.

In **chapter 2**, TSNAXIP1 (Translin-associated factor X (TSNAX)-interacting protein 1) was found to express predominantly in testis. Immunostaining revealed that TSNAXIP1 localized in the perinuclear region of spermatids. TSNAXIP1 KO mice were generated using CRISPR-Cas9 genome editing. Male mice lacking TSNAXIP1 were sub-fertile with smaller testis size and reduced sperm count, though spermatogenesis

was not aberrant in KO. Importantly, about 23% of KO sperms had unique flower-shaped head and about 35% sperms had abnormal head-neck junction.

Moreover, KO sperm motility was affected significantly. IVF procedure couldn't increase the fertility percentage. This finding underscores the importance of TSNAXIP1 in proper development and function of sperms.

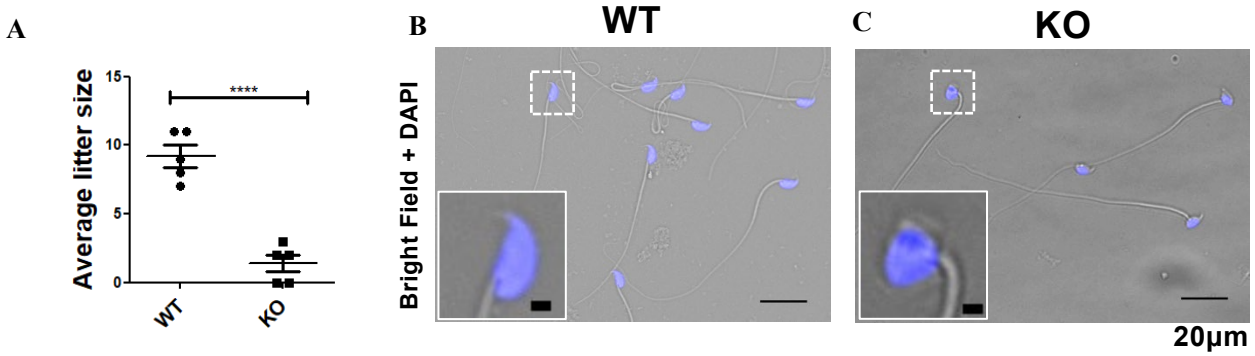

Fig.2: TSNAXIP1 KO mice are sub-fertile with abnormal sperm morphology. A. KO male mice had reduced litter size than WT. B. WT sperm morphology. C. Flower-shaped sperm in KO. Insets show magnification.

In **Chapter 3**, Centrosomal protein 112 (CEP112) was investigated. CEP112 is predominantly expressed in the testis with a molecular weight of 112kDa. By utilizing CEP112 KO mice, the study revealed that the male mice lacking CEP112 are infertile. Notably, there are no significant alterations in testis weight, sperm count, and spermatogenesis in CEP112 deficient mice. However, about 90% of KO sperms displayed abnormal sperm head morphology, with bent heads, or complete lack of head or coiled sperms. IVF showed limited success in increasing fertility rates in the absence of CEP112. Linearity of KO sperm motility was significantly reduced. Our findings suggest that CEP112 is crucial for male fertility.

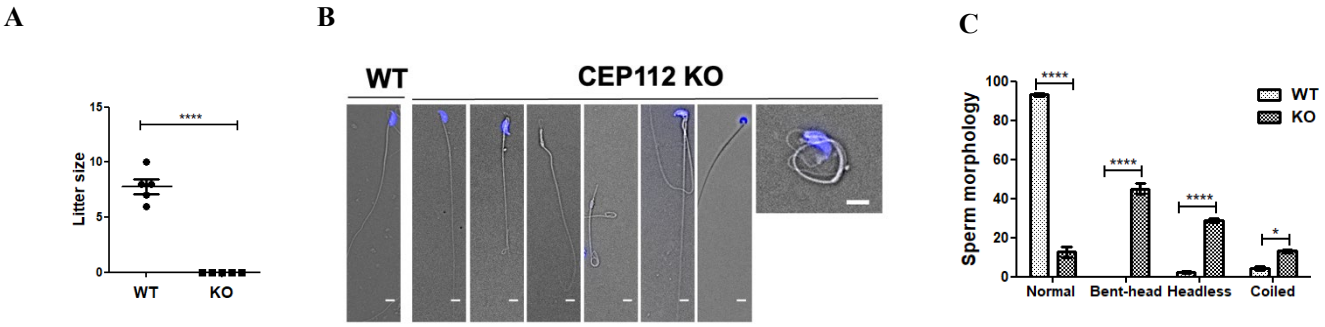

Fig.3: CEP112 deficiency affects male mice fertility. A. CEP112 KO male are mice infertile. B. Different abnormal morphologies of CEP112 KO sperms. Scalebar 5µm. C. Abnormal sperm morphology percentage in WT and CEP112 KO.

Reference:

1. Kuchakulla M, Narasimman M, Khodamoradi K, Khosravizadeh Z, Ramasamy R. How defective spermatogenesis affects sperm DNA integrity. Andrologia. 2021;53(1).