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Original Article

Role of tripartite motif protein 27 as a gametogenesis-related protein in human germ cells

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Abstract: Background: The distribution and functional integrity of members of the tripartite motif (TRIM) protein family are essential for cell proliferation, development and apoptosis, and TRIM proteins have been linked to various cancers. To explore the diagnostic potential and mechanisms of TRIM27 in human spermatogenesis and oogenesis, we analyzed its localization pattern and putative roles in human testes and ovaries. Methods: TRIM27 mRNA and protein levels in human testes and ovaries were investigated using RT-PCR and western blotting, respectively. TRIM27 was abundantly transcribed in human testes and ovaries, particularly during the early stages of spermatogenesis, and localized in the nuclei of primary spermatocytes. Immunofluorescence also revealed a diffuse distribution in the cytoplasm of round spermatids, and the protein was abundant in ovary tissue during various stages of oogenesis development. Results: TRIM27 mRNA and protein was abundantly transcribed in male and female human germ cells by RT-PCR and western blotting in the human testes followed by the ovary. Immunohistochemical results revealed TRIM27 protein was abundant in the sex body of primary spermatocytes undergoing meiotic prophase during the first cycle of spermatogenesis. Moreover, Trim27 was diffusely localized in the cytoplasm of spermatids and round spermatids. Furthermore, TRIM27 was localized to both the nucleus and cytoplasm of human ovary cells. Conclusions: TRIM27 as a gametogenesis-related protein could play multiple roles in the regulation of sex body formation and germ cell proliferation during spermatogenesis and oogenesis. The identification and characterization of TRIM27 enhances our understanding of the molecular mechanisms underpinning its functions, and provides insight into its potential role in the pathogenesis of germ cell differentiation and infertility.

Keywords: Trim27, spermatogenesis, oogenesis, germ cells, human

Introduction

Male infertility is a serious medical and social issue across the world. Gametes are produced during gametogenesis, which is a complex biological process involving a multitude of genes whose expression must be carefully coordinated, and the products of which engage in numerous specific and tightly regulated protein-protein interactions. During gametogenesis, these proteins are essential for sperm/oocyte function, which directly determines germ cell proliferation and fertilization parameters such as sperm motility, sperm concentration and/or the fusion of sperm and egg membranes during fertilization [1-3]. Due to their important role in male reproduction, the abnormal expression of gametogenesis-related genes can result in immune system disorders and ultimately infertility.

Tripartite motif (TRIM) proteins constitute a family of ~74 members in humans and mice that are known to play multiple roles in a wide range of processes including cell growth, apoptosis, tumour suppression, DNA damage signalling, stem cell differentiation, senescence, and immune responses against viruses [4-10]. TRIM27 (also known as the Ret finger protein, RFP) is a TRIM family protein originally identified as fused to the ret proto-oncogene in transformed NIH3T3 cells [11]. TRIM27 is highly expressed in various tumours such as endometrial cancer, breast cancer, lung cancer and

ovarian cancer [12-17], and it has been linked to tumour progression, cell development and apoptosis [18-21]. Moreover, TRIM27 is a novel E3 ubiquitin ligase that binds to Ub-conjugating enzyme 2 (E2) [22], and also interacts with PTEN to catalyse a non-canonical form of PTEN ubiquitination in AKT signalling [23]. Furthermore, TRIM27 binds to and activates USP7, resulting in positive regulation of TNF-α-induced apoptosis [19]. TRIM27 is highly expressed in mouse testes, and differentially expressed during spermatogenesis [18, 24]. Moreover, TRIM27 interacts with SLX2 and is localized in the nuclei of primary spermatocytes, especially at the sex body during meiosis [24]. Although TRIM27 is highly expressed in various cell types and in mouse testes [25, 26], the exact expression pattern, cellular localization and function of TRIM27 in human testes and ovaries remain unclear.

In the present study, TRIM27 was found to be abundantly expressed in human testis and ovary tissue using RT-PCR and western blotting, and differentially expressed during spermatogenesis and oogenesis. TRIM27 was localized to the nuclei of primary spermatocytes, and to the cytoplasm of round spermatids. Moreover, TRIM27 and γ-H2AX were co-localised at the sex body of the synaptonemal complex. Trim27 appeared to be both a nuclear and cytoplasmic protein in the ovaries. These results indicate that TRIM27 is a gametogenesis-related protein that may play multiple roles in the regulation of sex body formation and germ cell proliferation during spermatogenesis and oogenesis.

Materials and methods

Sample collection and chemicals

The study was approved by the ethics commission of the medical faculty of the Peking university third hospital, Beijing, China. All participants gave their written informed consent, and written informed consent forms were obtained from all subjects. This research was performed of recruiting 32 patients with obstructive azoospermia (OA) or/and sperm extraction failure who underwent TESA to obtain testicular tissue to the intracytoplasmic sperm injection (ICSI) cycle, carry out testicular biopsy and explore the Johnsen score of bilateral testicular histopathology from January, 2016 through December, 2016. Serum endocrine profile, karyotype and Y chromosomal microdeletion analysis were performed and these data were normal on all patients.

Testicular tissue samples were obtained from patients suffering from infertility at the centre of reproduction medicine, Peking university third hospital. All experiments were performed in accordance with the NIH guide for the care by the Peking university third hospital in Beijing. Unless otherwise stated, all reagents for cell culture were purchased from Invitrogen (Carlsbad, CA, USA) and Sigma-Aldrich (St. Louis, MO, USA). The Matchmaker library construction and screening kit were purchased from Clontech (BD Biosciences. San Jose, CA, USA).

Preparation of RNA and RT-PCR

Total RNA was treated with DNase I to avoid DNA contamination, and the quantity of extracted RNA was confirmed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA was purified from the aqueous phase using the miRNeasy Mini Kit (Qiagen, Venlo, the Netherlands). An A260/A280 ratio in the range of 1.8 to 2.0 indicated acceptable purity. RNA samples were denatured for 2 min at 70°C prior to cDNA synthesis. RNA from human ovaries and testes at different stages of development were extracted for RT-PCR analysis. Briefly, total RNA (5 μg) was used as template for reverse transcription with Superscript III (Invitrogen) according to the manufacturer's protocol and as previously described [27, 28]. The products of RT-PCR were separated by agarose gel electrophoresis and stained with ethidium bromide (EB). Relative amounts of cDNA were normalized against actin. Trim27 was amplified using primers 5'-TGCCAACATCTCCCACCTCAG-3' and 5'-CCAAGACACAGGGAAACAGAT TG-3'.

Extract preparation and western blotting

Human testis and ovary samples were homogenized in RIPA lysis buffer (50 mM Tris HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, protease inhibitors) as previously described [29, 30]. After centrifugation, supernatants were diluted with sample buffer and boiled. An equal amount of protein (20-50 μg total protein/lane) was loaded, separated by SDS-PAGE,

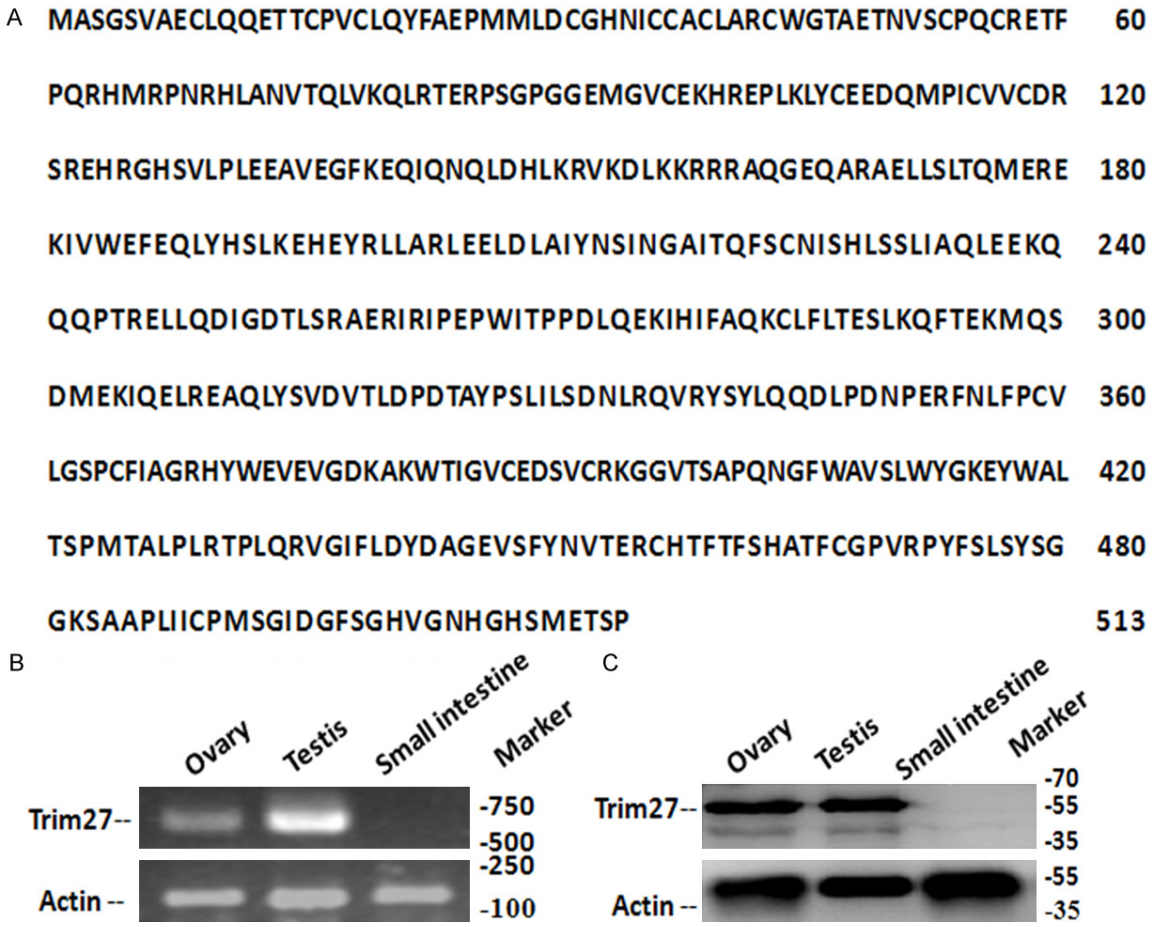

Figure 1. Protein sequence of TRIM27 and its expression were determined by RT-PCR and western blotting. A. The human TRIM27 cDNA encodes a protein of 513 amino acids; B. TRIM27 mRNAs are highly expressed in testis and ovary tissues. C. Western blotting shows TRIM27 expressed in both the testes and ovaries.

and proteins were transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked and incubated with primary antibodies, followed by HRP-conjugated secondary antibody. Proteins were visualized using an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA).

Immunohistochemistry and immunocytochemistry

Immunohistochemistry was performed using standard procedures as previously described [24]. Briefly, 8 μm frozen ovary and testis sections were fixed immediately in 4% paraformaldehyde for 15 min at room temperature. After blocking, sections were incubated with affinity-purified Trim27 antibody (diluted at 1: 400 in blocking buffer) or pre-immune rabbit serum (negative control) for 1 h at room temperature. FITC-conjugated anti-rabbit (1:500; Jackson Laboratories, Cambridgeshire, UK) were used as the secondary antibody. Nuclei were stained with hematoxylin or DAPI. Appropriate controls were included in each staining step. All results were repeated for three times.

The spreading human primary spermatocyte chromosome was performed as previously described [31, 32]. Briefly, human seminiferous tubules were isolated and kept in a hypotonic extraction buffer for 30 min. Subsequently, tubules in sucrose solution were cut into pieces using fine forceps and the resulting cell suspension was mixed with 3.7% PFA solution and spread on to a clean glass slide. Slides were washed in 0.4% Photoflo (Kodak), dried at room temperature, and stained using anti-SYCP3 (1:200, Abcam), anti-γH2AX (1:200, Up-

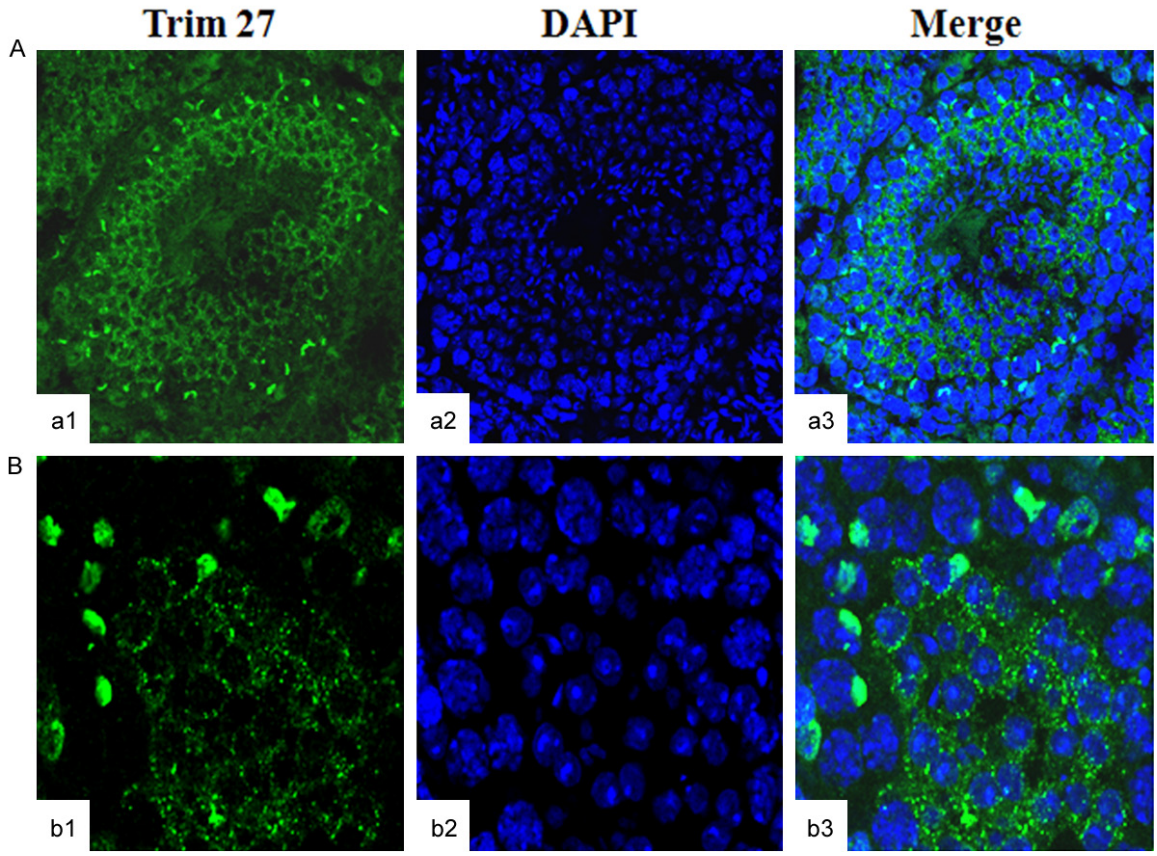

Figure 2. TRIM27 expression determined of human testis sections by immunostaining. TRIM27 (green signals) is clearly present in both the nucleus (A) and cytoplasm (B) of testis cells, and was localized in the sex body of primary spermatocytes (B), and also evident in the cytoplasm Sertoli cells. Legend as in Scale bars = 10 μm.

state), and anti-Trim27 (1:200, NOVUS). FITC-conjugated or TRITC-conjugated anti-rabbit (1:500; Jackson Laboratories) was used as a secondary antibody. DAPI (Sigma-Aldrich) was added at a final concentration of 1 μg/ml for 10 min to stain nuclei, and samples were mounted on microscope slides. Protein subcellular localization was determined using a laser confocal microscope (Zeiss).

Results

Trim27 belongs to the zinc finger protein superfamily

The longest Trim27 cDNA has a predicted 513 amino acid open reading frame, encoding a putative 58.3 kDa protein (**Figure 1A**). According to the PANTHER classification system, the Trim27 protein belongs to the TRIM family that includes ~74 members whose RNA transcripts have been identified. TRIM family members contain a TRIM/RBCC motif that consists of a RING finger motif, one or two B-box domains, and a predicted coiled-coil region. Trim27 is located on human chromosome 6, and consists of a RING domain, three coiled-coil regions, a B-box domain and one SPRY domain. The RING finger is a zinc-binding domain which plays an important role in ubiquitin (Ub) E3 ligase binding to Ub-conjugating enzymes (E2). Like other TRIM proteins, TRIM27 has an N-terminal Zn-binding RING domain, suggesting it may function as an E3 ligase.

Expression of Trim27 mRNA and protein in human testes and ovaries

Trim27 mRNA and protein was detected in human testis and ovary tissue by RT-PCR (**Figure 1B**) and western blotting (**Figure 1C**), respectively. Trim27 expression in testes during different developmental stages was investi-

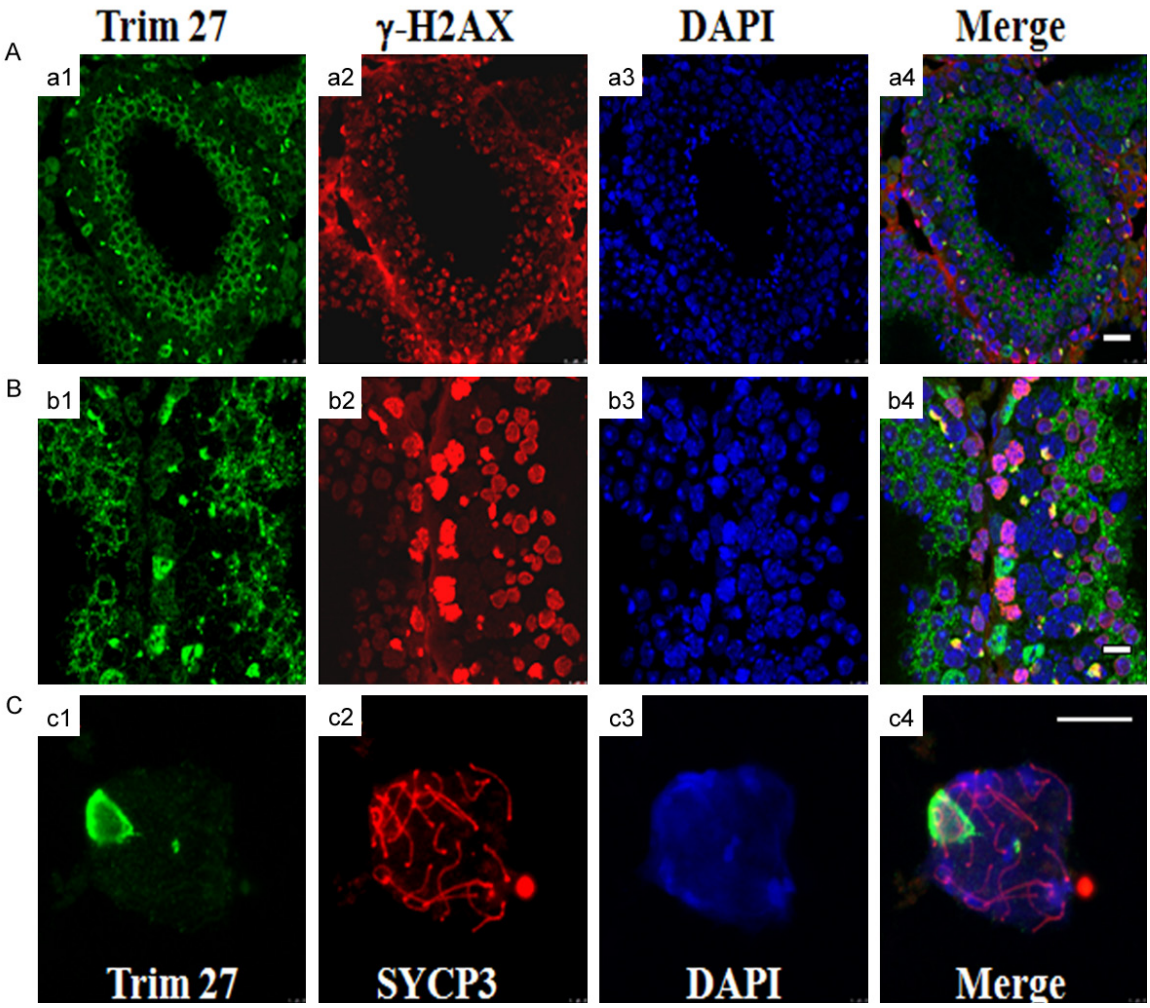

Figure 3. Co-localization of TRIM27 and γ-H2AX protein was detected in the testis and chromosome sections. A: TRIM27 (green) and nucleus (blue) signals at low magnification. TRIM27 protein (green) was localized in primary spermatocytes undergoing meiotic prophase during the first cycle of spermatogenesis. B: TRIM27 (green) and nucleus (blue) signals at high magnification. TRIM27 (green) was clearly present in the cytoplasm of round spermatids and nuclear (blue) in the primary spermatocytes. Interestingly, TRIM27 (green) and γ-H2AX (red) were co-localized in the sex body of primary spermatocytes. C: TRIM27 (green) and SYCP3 (red) co-localized in the primary spermatocytes of chromosome sections, suggesting TRIM27 is a meiosis-associated protein. Legend as in Scale bars = 10 μm.

gated, and expression was consistently high, consistent with previous findings [25, 26]. Trim27 protein was also detected in human testis tissue (**Figure 1C**), and the protein expression pattern matched the RNA expression pattern (**Figure 1B**). TRIM27 appeared to have two based on western blotting in ovaries and testis samples.

The immunohistochemistry results revealed a strong and specific staining for Trim27 in the seminiferous tubules. TRIM27 protein was abundant in primary spermatocytes undergoing meiotic prophase during the first cycle of spermatogenesis (**Figure 2A**), suggesting it may be involved in male germ cell development. Moreover, TRIM27 was diffusely localised in the cytoplasm of spermatids and round spermatids (**Figure 2B**). The results showed that Trim-27 mRNA was highly expressed in human testis tissue. Furthermore, although TRIM27 was generally localized within nuclei, its specific localization in the cytoplasm of round spermatids and Sertoli cells suggests a possible involvement of cell proliferation in gametogenesis.

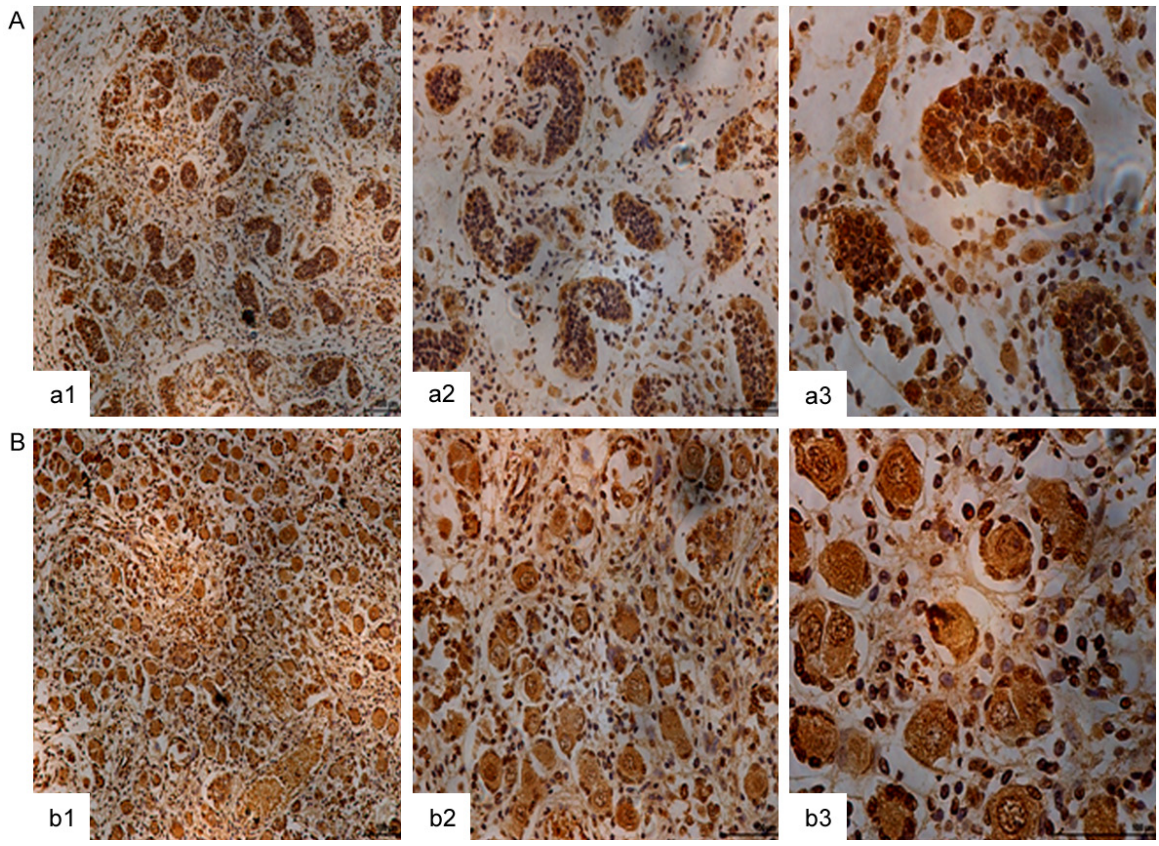

Figure 4. Localization of TRIM27 protein was detected in developing female gonads. A: TRIM27 was clearly present in the adult ovaries section. B: TRIM27 was also evident in the nucleus of follicle cells, and is diffusely dispersed in the cytoplasm of granule cells. Legend as in Scale bars = 10 μm.

Trim27 is preferentially localized to the sex body

When meiosis reaches the late pachytene/diplotene stage, more intense and smaller stained spots became visible, in addition to the diffused staining patterns observed in the earlier stages. These spots were also observed |on some of the tubules of adult testis (**Figure 3A**), and were very likely sex bodies, given their size and morphology (co-localization of TRIM27 and γ-H2AX was confirmed and is shown in **Figure 3B**).

Given its localization, it is likely that TRIM27 is a component of the sex body after analysing the distribution of more than 200 germ cells from 7 case of human testis section. In zygotene spermatocytes, TRIM27 was distributed uniformly within nuclei, but staining was more intense in the sex body, and exactly matched the staining pattern of γ-H2AX. In pachytene spermatocytes, TRIM27 was exclusively localized to the sex body, as was γ-H2AX. TRIM27 and γ-H2AX were also co-localized at sex bodies in primary meiotic spermatocytes (**Figure 3C**), but were separated in round spermatids, in which γ-H2AX remained restricted to sex bodies, while TRIM27 was diffusely, localised in the cytoplasm of differentiating spermatids. These results suggest complexes of TRIM27 and γ-H2AX could play important roles in the formation of sex bodies during meiosis.

Trim27 is also present in ovaries

TRIM27 was also distributed on the adult and baby ovaries (**Figure 4**). Immunostaining was performed on human ovary sections to investigate a potential role in oogenesis at different developmental stages (**Figure 4A**). TRIM27 was found to be present in the primordial follicle (**Figure 4B**) on the ovary sections, and this was confirmed by immunostaining and western blotting. In addition, TRIM27 was diffusely located in the cytoplasm of granule cells. The immuno-

histochemistry results indicated that TRIM27 was present during various developmental stages of oogenesis. The subcellular localization of TRIM27 at different stages of meiotic maturation was examined by immunofluorescence staining, and TRIM27 was localised in the nuclei of oocytes, consistent with a role in oocyte maturation.

Discussion

TRIM family proteins comprise ~74 family members that are divided into 11 subgroups based on their domains [33-36]. The distribution and functional integrity of TRIM family members are essential for cell proliferation, development, apoptosis, the cell cycle and antiviral activity, and they act as novel E3 ubiquitin ligases, but have been implicated in cancers [36-41]. The exact biological functions of most TRIM proteins remain unclear. Recent evidence suggests Trim27 proteins are co-localized with SLX2 and γ-H2AX in the sex body, suggesting SLX2-TRIM27 complexes could play multiple roles in the regulation of sex body formation, sex inactivation and germ cell proliferation during spermatogenesis in mice [24]. The N-terminal region harbours a RING finger, two B-boxes, and a predicted α-helical coiled-coil domain, which together form the RBCC/TRIM motif found in many family members, while the C-terminal region contains a filamin-type immunoglobulin domain. TRIM27 was found to be abundantly transcribed in human testes, particularly during the early stages of spermatogenesis. However, the spatial and temporal expression and function of TRIM27 remains obscure. In this study, Trim27 was abundantly transcribed in human testes and ovaries. In addition, Trim27 expression was detected in meiotic spermatocytes, which are cells that support the spermatogenic process. Although Trim27 was generally localized within nuclei, its specific localization in the cytoplasm of round spermatids and Sertoli cells indicates a possible involvement in gametogenesis in both sexes. Furthermore, the presence of the Trim-27 protein in the primordial follicle of ovary sections determined by RT-PCR and immunostaining strongly suggests an involvement in human spermatogenesis and oogenesis.

The recent discovery of specific proteins located within the sex body has aided our understanding of its structure and function [8, 11, 13, 28]. The phosphorylated form of H2AX (γ-H2AX) is globally distributed during the leptotene and zygotene stages, but becomes concentrated at the asynapsed sex body during the pachytene and diplotene stages [42-45]. However, its expression and precise underlying mechanisms in testes during meiosis have not been determined. In this study, we report the identification and characterization of TRIM27 that appears to participate in the development of gametogenesis. The Trim27 protein was found to be expressed in both the cytoplasm and cell nucleus. Furthermore, immunohistochemical examination revealed its localization in neurons in the human testis, consistent with a role in regulating spermatogenesis. Our results showed that TRIM27 is localized to sex bodies where it interacts with SLX2. It is possible that TRIM27 may act as a meiosis-associated protein that influences meiosis progression. Its presence in round spermatids also indicates an involvement in germ cell proliferation.

Conclusions

We cloned human Trim27 and investigated its expression and localization in germ cells. Its abundant expression in germ cells and its cellular localization suggests Trim27 is a novel spermatogenesis-associated protein that may play an important role in this process by influencing sex body formation and germ cell proliferation during spermatogenesis. Identification and characterization of this novel testis protein may offer a new perspective for understanding the molecular mechanisms involved in germ cell differentiation. These results provide additional support for understanding male infertility. Moreover, future studies on spermatogenesis in individuals with mutations in Trim27 may reveal further details of the mechanisms underlying germ cell differentiation and infertility.

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All the involved patients provided written informed consent.

Disclosure of conflict of interest

None.

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References

[1] Dhanoa JK, Mukhopadhyay CS and Arora JS. Y-chromosomal genes affecting male fertility: a review. Vet World 2016; 9: 783-91.

[2] Rosario R, Adams IR and Anderson RA. Is there a role for DAZL in human female fertility? Mol Hum Reprod 2016; 22: 377-83.

[3] de la Noval BD. Potential implications on female fertility and reproductive lifespan in BRCA germline mutation women. Arch Gynecol Obstet 2016; 294: 1099-1103.

[4] Kang WS, Park JK, Kim YJ, Cho AR, Park HJ, Kim SK, Paik JW, Lee KJ, Na HR, Kim YY, Lim HK, Jeong HG and Kim JW. Association of tripartite motif family-like 2 (TRIML2) polymorphisms with late-onset Alzheimer's disease risk in a Korean population. Neurosci Lett 2016; 630: 127-31.

[5] Xu W, Xu B, Yao Y, Yu X, Cao H, Zhang J, Liu J and Sheng H. RNA interference against TRIM29 inhibits migration and invasion of colorectal cancer cells. Oncol Rep 2016; 36: 1411-8.

[6] Tomar D, Prajapati P, Lavie J, Singh K, Lakshmi S, Bhatelia K, Roy M, Singh R and Benard G. TRIM4; a novel mitochondrial interacting RING E3 ligase, sensitizes the cells to hydrogen peroxide (H2O2) induced cell death. Free Radic Biol Med 2015; 89: 1036-48.

[7] Zhan W, Han T, Zhang C, Xie C, Gan M, Deng K, Fu M and Wang JB. TRIM59 promotes the proliferation and migration of non-small cell lung cancer cells by upregulating cell cycle related proteins. PLoS One 2015; 10: e0142596.

[8] Scherer M, Klingl S, Sevvana M, Otto V, Schilling EM, Stump JD, Muller R, Reuter N, Sticht H, Muller YA and Stamminger T. Crystal structure of cytomegalovirus IE1 protein reveals targeting of TRIM family member PML via coiled-coil interactions. PLoS Pathog 2014; 10: e1004512.

[9] Fujimura T, Inoue S, Urano T, Takayama K, Yamada Y, Ikeda K, Obinata D, Ashikari D, Takahashi S and Homma Y. Increased expression of tripartite motif (TRIM) 47 is a negative prognostic predictor in human prostate cancer. Clin Genitourin Cancer 2016; 14: 298-303.

[10] Liu C, Huang X, Hou S, Hu B and Li H. Silencing of tripartite motif (TRIM) 29 inhibits proliferation and invasion and increases chemosensitivity to cisplatin in human lung squamous cancer NCI-H520 cells. Thorac Cancer 2015; 6: 31-7.

[11] Takahashi M, Ritz J and Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell 1985; 42: 581-8.

[12] Tezel GG, Uner A, Yildiz I, Guler G and Takahashi M. RET finger protein expression in invasive breast carcinoma: relationship between RFP and ErbB2 expression. Pathol Res Pract 2009; 205: 403-8.

[13] Zoumpoulidou G, Broceno C, Li H, Bird D, Thomas G and Mittnacht S. Role of the tripartite motif protein 27 in cancer development. J Natl Cancer Inst 2012; 104: 941-52.

[14] Iwakoshi A, Murakumo Y, Kato T, Kitamura A, Mii S, Saito S, Yatabe Y and Takahashi M. RET finger protein expression is associated with prognosis in lung cancer with epidermal growth factor receptor mutations. Pathol Int 2012; 62: 324-30.

[15] Horio M, Kato T, Mii S, Enomoto A, Asai M, Asai N, Murakumo Y, Shibata K, Kikkawa F and Takahashi M. Expression of RET finger protein predicts chemoresistance in epithelial ovarian cancer. Cancer Med 2012; 1: 218-29.

[16] Tsukamoto H, Kato T, Enomoto A, Nakamura N, Shimono Y, Jijiwa M, Asai N, Murakumo Y, Shibata K, Kikkawa F and Takahashi M. Expression of Ret finger protein correlates with outcomes in endometrial cancer. Cancer Sci 2009; 100: 1895-901.

[17] Chen W, Lu J, Qin Y, Wang J, Tian Y, Shi D, Wang S, Xiao Y, Dai M, Liu L, Wei G, Wu T, Jin B, Xiao X, Kang TB, Huang W and Deng W. Ret finger protein-like 3 promotes tumor cell growth by activating telomerase reverse transcriptase expression in human lung cancer cells. Oncotarget 2014; 5: 11909-23.

[18] Tezel G, Nagasaka T, Shimono Y and Takahashi M. Differential expression of RET finger protein in testicular germ cell tumors. Pathol Int 2002; 52: 623-7.

[19] Zaman MM, Nomura T, Takagi T, Okamura T, Jin W, Shinagawa T, Tanaka Y and Ishii S. Ubiquitination-deubiquitination by the TRIM27-USP7 complex regulates tumor necrosis factor alpha-induced apoptosis. Mol Cell Biol 2013; 33: 4971-84.

[20] Kato T, Enomoto A, Watanabe T, Haga H, Ishida S, Kondo Y, Furukawa K, Urano T, Mii S, Weng L, Ishida-Takagishi M, Asai M, Asai N, Kaibuchi K, Murakumo Y and Takahashi M. TRIM27/MRTF-B-dependent integrin beta1 expression defines leading cells in cancer cell collectives. Cell Rep 2014; 7: 1156-67.

[21] Nie D, Zhang D, Dai J, Zhang M, Zhao X, Xu W, Chen Z, Wang L, Wang Z and Qiao Z. Nicotine induced murine spermatozoa apoptosis via up-regulation of deubiquitinated RIP1 by Trim27 promoter hypomethylation. Biol Reprod 2016; 94: 31.
[22] Joazeiro CA and Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. Cell 2000; 102: 549-52.
[23] Lee JT, Shan J, Zhong J, Li M, Zhou B, Zhou A, Parsons R and Gu W. RFP-mediated ubiquitination of PTEN modulates its effect on AKT activation. Cell Res 2013; 23: 552-64.
[24] Zhuang XJ, Tang WH, Feng X, Liu CY, Zhu JL, Yan J, Liu DF, Liu P and Qiao J. Trim27 interacts with Slx2, is associated with meiotic processes during spermatogenesis. Cell Cycle 2016; 15: 2576-2584.
[25] Cao T, Shannon M, Handel MA and Etkin LD. Mouse ret finger protein (rfp) proto-oncogene is expressed at specific stages of mouse spermatogenesis. Dev Genet 1996; 19: 309-20.
[26] Tezel G, Nagasaka T, Iwahashi N, Asai N, Iwashita T, Sakata K and Takahashi M. Different nuclear/cytoplasmic distributions of RET finger protein in different cell types. Pathol Int 1999; 49: 881-6.
[27] Zhuang XJ, Lu YQ, Zhang M, Lu SS and Lu KH. Microisolation and microcloning of bovine X-chromosomes for identification of sorted buffalo (Bubalus bubalis) spermatozoa. Anim Reprod Sci 2011; 126: 32-6.
[28] Shi YQ, Zhuang XJ, Xu B, Hua J, Liao SY, Shi Q, Cooke HJ and Han C. SYCP3-like X-linked 2 is expressed in meiotic germ cells and interacts with synaptonemal complex central element protein 2 and histone acetyltransferase TIP60. Gene 2013; 527: 352-9.
[29] Zhuang XJ, Tang WH, Liu CY, Zhu JL, Feng X, Yan J, Lian Y, Liu P and Qiao J. Identification and characterization of Xlr5c as a novel nuclear localization protein in mouse germ cells. PLoS One 2015; 10: e0130087.
[30] Zhuang XJ, Shi YQ, Xu B, Chen L, Tang WH, Huang J, Lian Y, Liu P and Qiao J. SLX2 interacting with BLOS2 is differentially expressed during mouse oocyte meiotic maturation. Cell Cycle 2014; 13: 2231-7.
[31] Zhuang XJ, Hou XJ, Liao SY, Wang XX, Cooke HJ, Zhang M and Han C. SLXL1, a novel acrosomal protein, interacts with DKKL1 and is involved in fertilization in mice. PLoS One 2011; 6: e20866.
[32] Shi YQ, Liao SY, Zhuang XJ and Han CS. Mouse Fem1b interacts with and induces ubiquitin-mediated degradation of Ankrd37. Gene 2011; 485: 153-9.
[33] Meroni G and Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 2005; 27: 1147-57.
[34] Ozato K, Shin DM, Chang TH and Morse HC 3rd. TRIM family proteins and their emerging roles in innate immunity. Nat Rev Immunol 2008; 8: 849-60.
[35] Nisole S, Stoye JP and Saib A. TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 2005; 3: 799-808.
[36] Tomar D and Singh R. TRIM family proteins: emerging class of RING E3 ligases as regulator of NF-kappaB pathway. Biol Cell 2015; 107: 22-40.
[37] Tocchini C and Ciosk R. TRIM-NHL proteins in development and disease. Semin Cell Dev Biol 2015; 47-48: 52-9.
[38] Alloush J and Weisleder N. TRIM proteins in therapeutic membrane repair of muscular dystrophy. JAMA Neurol 2013; 70: 928-31.
[39] Cambiaghi V, Giuliani V, Lombardi S, Marinelli C, Toffalorio F and Pelicci PG. TRIM proteins in cancer. Adv Exp Med Biol 2012; 770: 77-91.
[40] Ikeda K and Inoue S. TRIM proteins as RING finger E3 ubiquitin ligases. Adv Exp Med Biol 2012; 770: 27-37.
[41] Yap MW and Stoye JP. TRIM proteins and the innate immune response to viruses. Adv Exp Med Biol 2012; 770: 93-104.
[42] Turner JM, Aprelikova O, Xu X, Wang R, Kim S, Chandramouli GV, Barrett JC, Burgoyne PS and Deng CX. BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr Biol 2004; 14: 2135-42.
[43] Luo M, Zhou J, Leu NA, Abreu CM, Wang J, Anguera MC, de Rooij DG, Jasin M and Wang PJ. Polycomb protein SCML2 associates with USP7 and counteracts histone H2A ubiquitination in the XY chromatin during male meiosis. PLoS Genet 2015; 11: e1004954.
[44] Vigodner M. Sumoylation precedes accumulation of phosphorylated H2AX on sex chromosomes during their meiotic inactivation. Chromosome Res 2009; 17: 37-45.
[45] Wang L, Liu W, Zhao W, Song G, Wang G, Wang X and Sun F. Phosphorylation of CDK2 on threonine 160 influences silencing of sex chromosome during male meiosis. Biol Reprod 2014; 90: 138.