

Cancer/Testis genes in relation to sperm biology and function

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ABSTRACT

Cancer testis antigens (CTAs), a large family of tumor-associated and immunogenic antigens expressed in human tumors of various histological origins, are highly restricted to the testis and trophoblast. CTAs have been identified as potent targets for tumor-specific immunotherapeutic advances and have immensely lead to the development of different clinical trials of CTA-based vaccine therapy because of their resilient *in vivo* immunogenicity and tumor-restricted expression pattern. Bladder cancer, non-small cell lung carcinoma, and melanoma are grouped as high CT gene expressors. Prostate and breast cancer as moderate, and colon and renal cancers are considered as low CT gene expressors. Large percentages of these identified CT genes are expressed during spermatogenesis but their function is still vaguely unknown. Researchers have taken a keen interest in CT genes as pertaining to their role in tumor growth and spermatogenesis. Testis has many similarities with cancerous tissues like cell division, immigration, and immortalization. The aim is to give a concise in-depth review on the role of some specific CT genes in spermatogenesis.

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Introduction

Cancer/Testis (CT) genes are a diverse group of testis specific genes, atypically expressed in about 40% of different types of cancers (1). Blood-testis barrier creates an immune privileged site for the germ cell antigens. Restricted expression profile of CTAs in normal testis, makes them not accessible to cytotoxic lymphocytes, so it does not express the major histocompatibility complex molecule (1). A subfamily of the CT genes encode immunogenic antigens and stimulate immune responses in cancer patients (2).

Testis has many similarities with cancerous tissues like cell division, immigration, and immortalization. Various CT genes have similar action with different types of cancers and oocyte maturation; example been WWP2 N-terminal-like, a testis-specific signaling protein, induces meiotic resumption and oocyte activation events, and is also expressed in actively dividing cancerous cell lines (3). The CT genes, OIP5, TAF7L (4), and AURKC (5) have been demonstrated as promising and potent candidates for therapeutic cancer vaccines and biomarkers for breast cancer.

However, the immunohistochemical analysis of MAGE-1 in lung neoplasms has suggested that mRNA expression of a CT antigen in a tumor doesn't mean it could be used as an immunotherapeutic target because homogenous protein expression in the target tissues is essential (6) (Figure 1). CT genes are not only expressed in solid tumors but have also been implicated in hematopoietic neoplasms like acute lymphoblastic leukemia (7).

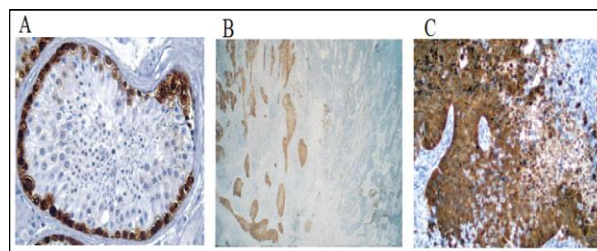


Figure 1. CTAs expression in normal and tumor tissues; A: Immunohistochemical staining displays the expression of MAGE4 by testicular germ cells B: Squamous cell carcinoma. An area of MA454 reactivity next to an immunonegative tumor area (heterogeneous expression of the CTA) C: Expression of NY-ESO-1 in urinary bladder carcinoma. The brown staining shows antibody binding. This Figure was reproduced from references (2, 6)

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Table 1. Characteristics of CT-X genes

CTA gene family	Number of genes in the family	CT family	Chromosome location	Expression during germline maturation	Functions in spermatogenesis
CAGE	1	CT26	Xp22.13	spermatids, spermatozoa	possible helicase
CSAGE	2	CT24	Xq28	ND	unknown
CTp11/SPANX	4	CT11	Xq27.1	spermatids	unknown
E2F-like/ HCA661	1	CT30	Xq26.2	ND	transcription factor
FATE1	1	CT43	Xq28	ND	unknown
FTHL17	1	CT38	Xp21	spermatogonia	possible ferritin heavy polypeptide-like protein
GAGE1	8	CT4	Xp11.4–p11.2	ND	unknown
HOM-TES-85	1	CT28	Xq23	ND	possible transcriptional regulatory protein
IL13RA1	1	CT19	Xq24	ND	receptor for interleukin-13
MAGEA	12	CT1	Xq28	spermatogonia	translational co-repressor and male infertility
MAGEB	4	CT3	Xp21–p22	migration PGCs	unknown
MAGEC1	2	CT7	Xq26, Xq27.2	ND	unknown
MAGEC2	1	CT10	Xq27	ND	unknown
NA88	1	CT18	Xp22.12	ND	unknown
NXF2	1	CT39	Xq22.1	spermatogonia	mrna export to the cytoplasm
NY-ESO-1	3	CT6	Xq28	spermatogonia	unknown
NY-SAR-35	1	CT37	Xq28	ND	enhances spermatogonial cell migration and proliferation
PAGE5	2	CT16	Xp11.22	ND	unknown
SAGE1	1	CT14	Xq26	ND	sage1 supports the origin of spermatocytic seminoma from spermatogonia and provides new evidence for heterogeneity spermatocytic seminoma
SSX	5	CT5	Xp11.23–p11.22	ND	translational co-repressor
XAGE1/GAGED	8	CT12	Xp11.22–p11.21	ND	unknown

CAGE, cancer antigen 1; CSAGE, chondrosarcoma-associated gene 1; FATE1, fetal and adult testis expressed 1; FTHL17, ferritin, heavy polypeptide-like 17; GAGE1, G antigen 1; IL13RA1, interleukin-13 receptor- α 1; MAGE, melanoma antigen; ND, not determined; NXF2, nuclear RNA export factor 2; NY-SAR-35, New York sarcoma 35; PAGE5, P antigen family, member 5; PGCs, primordial germ cells; SAGE1, sarcoma antigen 1; SPANX, sperm associated with the nucleus, X chromosome; SSX, synovial sarcoma; TAF7L, TAF7-like RNA polymerase II, XAGE1, X antigen family, member

The nomenclature of CT genes depends on any gene that demonstrates an mRNA expression profile limited to the testis and neoplastic cells. Several characteristic features and definitions of CTAs have emerged in different researches varying from genes exhibited solely in adult testis germ cells and tumors (8), to dominant testicular expression (9) and also possibly expressed in placenta and ovary, regulated by epigenetic mechanisms (10) and mapping to the X chromosome (11) (Table 1). The CT database has listed more than 250 RefSeq nucleotide identifiers belonging to about 150 gene families (www.cta.lncc.br). The human X chromosome houses about 10% of CT genes (12).

Classification of cancer/testis genes

A classification system of CTAs suggests differential expression profiles of CTAs as (i) testis restricted CTAs, (ii) testis-brain restricted CTAs that express in the testis and central nervous system, or (iii) testis selective, expressed in the testis and at

lower levels in 1 to 2 additional tissues, when compared to the testes (13). Out of the 153 CT genes, 39 had transcripts presented solely in the adult testis (placenta is also classified as testis-restricted) while 14 CT genes had additional expression in the brain, though classified as testis-brain restricted and 85 genes had their main expression in testis and classified as testis-selective (13). Strangely, X chromosome is the location for almost half of the explained CT genes and these are known as CT-X genes. CT-X genes characteristically exhibit a testis-restricted expression profile and they possess more antigenic properties than non-CT-X CTAs that are located all over the autosomes. Many CT-X genes are sub-family members of larger families like the MAGEs, which are made up of genes like MAGEA1–12, MAGEB1–18, and MAGEC1–7 (14). MAGE proteins from all sub-families at amino level exhibit 46% conservation and most of the subfamilies consist of a MAGE homology domain. Group 1 subfamily consists of MAGEs in the A, B, and C classes consisting of more than 45 genes and is

restricted to the testis and to tumors. Classes D to I and NECDIN are within the Group II and they are expressed in a broad range of tissues (14). There are other notable families of CT-X genes like GAGE, PAGE, XAGE, SPANX, SSX, NY-ESO-1, PIWIL2, and CT47A (15).

It seems that expression of CTAs like MAGE, BAGE, and GAGE gene families in cancer cells is not randomly distributed among different individuals and family members of these genes tend to be clustered in a different subset of tumors, signifying a common mechanism for transcriptional activation (e.g. methylation) (16). Several researchers have carried out numerous studies to investigate the frequency of expression of CTAs in tumors though most of it has been truncated by sample size. However, with large and wide-ranging data sets like that from The Cancer Genome Atlas (TCGA), an extensive analysis of CT genes expression level, state of mutation, and copy number throughout many diverse tumor types is accomplishable. The number of occurrence of reactivation of specific CTAs and the degree of activation of the whole group of CTAs can be investigated via this distinct type of analysis. In addition, any prognostic implication of CTA expression can be investigated and determined via the analysis of the reactivation patterns (17).

Roles of cancer/testis genes in gamete formation

The roles of CT genes in gamete formation remain comparatively unclear; however, the knowledge of gene expression and knockout studies has thrown more light on the multi-functions of CT genes. CT genes are responsible for some processes during the initial stages of spermatogenesis and their expression (CTAs, such as *MAGEA1* and *NY-ESO-1*) is evident in the early stages of spermatogenesis (6). The association of MAGE-A1 and NY-ESO-1 with highly proliferating germ cells and a more general function for GAGE proteins in germ cells is suggested after studying the expression of these CTAs in fetal gonads by immunohistochemistry (18). Several research studies have reported that a subset of CTAs plays essential roles in male fertility, as mice deficient of single CTAs commonly demonstrate decreased fertility (Table 2). CTAs also could provide important roles to a various set of processes in their local setting, suggesting that they can be efficient in providing various tumorigenic features. Normal and healthy development of the majority of knockout animals for CT genes suggests that they are not involved in processes of somatic tissues (19, 20). The primary functions of more than 140 identified members of the CT antigens still remains unknown (21, 22). However the roles of CT genes have been implicated during the process of spermatogenesis and some specific CT genes have been identified via their relationship

with spermatogonia, spermatocytes, spermatids, and spermatozoa.

Primordial germ cells (PGCs) are the common origins of the germline (Figure 2). PGCs migrate via the dorsal mesentery of the embryo and finally enter the region of developing fetal gonad in the genital ridge around the 10th thoracic level. Within the genital ridge, if the cells are carrying a Y chromosome, the PGCs become enclosed by the Sertoli cells and ultimately differentiate into spermatogonia type A, which proliferate and differentiate mitotically and get arrested at G1 phase until birth. However, proliferation is resumed at puberty, which in-turn initiates the process of spermatogenesis. There are two types of spermatogonia A germline, namely spermatogonia A pale and dark. Spermatogonia A dark serve as the pluripotent stem cells that can renew themselves to maintain the pool of stem cells while spermatogonia A pale can undergo differentiation and proliferation to produce more spermatozoa via two meiotic divisions. Firstly, meiosis I involving the reduction of the tetraploid primary spermatocytes to diploid secondary spermatocytes and secondly, equatorial meiotic II division to form haploid spermatids which through the process of spermiogenesis form mature spermatozoa (Figure 2).

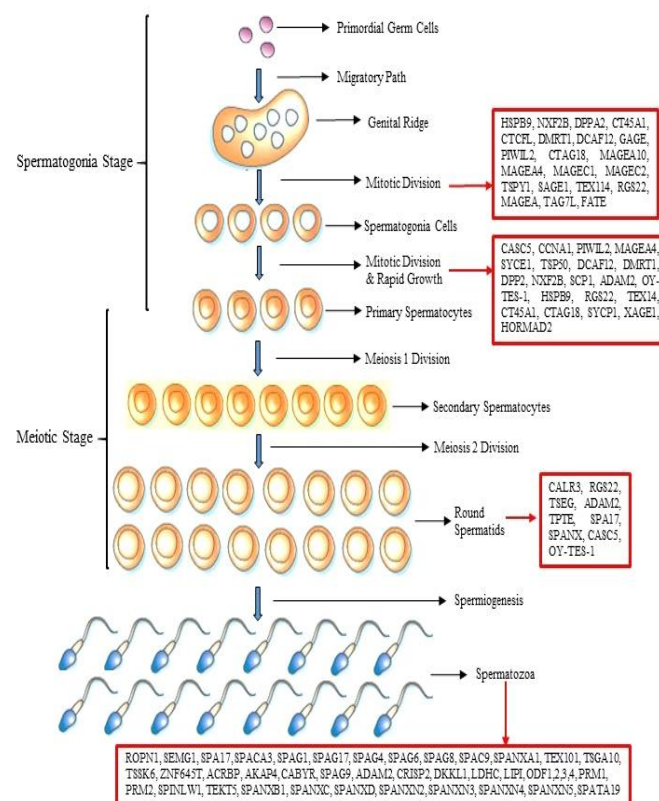


Figure 2. Summary of CT antigens implicated in the process of spermatogenesis

Most CT-X antigens like MAGE and NY-ESO-1 are implicated in the process of spermatogenesis, while CT-X antigens like SCP1, associated with meiosis, have been expressed in spermatocytes.

Furthermore, CT-X antigens like OY-TES-1/ACRBP (acrosin-binding protein), are expressed by spermatids (23, 24).

Table 2. Cancer/Testis genes roles in reproduction

	CT Family	CT genes	Ch-Location	Status	Male phenotype	Female phenotype	References
RNA Regulation	CT80.2	PIWIL2	8p21.3	testis-selective	viable, infertile; microrchidia, meiotic prophase arrest	infertile	(25, 26)
	CT41.1	TDRD1	10q25.3	testis-selective	viable, infertile; microrchidia, meiotic prophase arrest	infertile	(27, 28)
	CT41.2	TDRD6	6p12.3	testis-selective	viable, infertile; microrchidia, meiotic prophase arrest	none	(20)
	CT128	MAEL	1q24.1	testis-selective	viable, infertile; microrchidia, meiotic prophase arrest	NA	(29)
Fertilization/Implantation	CT93	CALR3	19p13.11	testis-selective	viable, infertile; sperm unable to penetrate zona pellucida	none	(30)
	CT92	PLAC1	Xq26	testis-selective	viable, fertile; placentomegaly, intrauterine growth retardation	same as male	(31)
	CT99	AKAP4	Xp11.2	testis-restricted	viable, infertile; fibrous sheath defect	none	(32)
	CT91	ROPN1	3q21.1	testis-selective	viable, reduced fertility; defects in the principal piece	NA	(33, 34)
	CT15	ADAM2	8p11.2	testis-selective	viable, reduced fertility; defects in migration, adhesion to zona pellucida, sperm-egg fusion	none	(35)
	CT34	DKKL1	19q13.33	testis-restricted	viable, impaired fertility; defects in zona pellucida penetration	none	(36)
	CT8	SYCP1	1p13-p12	testis-selective	viable, infertile; microrchidia due to defects in homologous recombination	infertile	(37)
Homologous Recombination	CT42	TEX15	8p12	testis-selective	viable, infertile; microrchidia due to chromosome synapse failure	none	(38)
	CT76	SYCE1	10q26.3	testis-selective	viable, infertile; microrchidia due to chromosome synapse failure	infertile	(39)
	CT35	SPO11	20q13.2-q13.3	testis-selective	viable, infertile; microrchidia due to chromosome synapse failure	infertile	(40)
	CT46	HORMAD1	1q21.2	testis/brain-restricted	viable, infertile; microrchidia due to meiotic arrest at pachytene stage	infertile	(41)
	Transcription	CT33	MORC1	3q13	testis-selective	viable, infertile; microrchidia due to meiotic arrest at zygotene/leptotene stage	none
CT27		CTCF	20q13.31	testis-selective	viable, fertile; microrchidia, multinucleated sperm cells, increased sperm death	none	(42)
CT9		BRDT	1p22.1	testis-selective	viable, fertile; oligoasthenozoospermia	none	(43)
CT41.3		TDRD4	13q12.12	not mentioned	viable, infertile; sperm arrest at round spermatid stage	none	(44)
Other	CT 121	ARX	Xp21	not mentioned	nonviable; small olfactory bulb, small testes	NA	(45)
	CT56	THEG	19pter-p13	testis-selective	viable, fertile; deletion of c-terminal domain leading to small testes	none	
	CT 120.2	TMEFF2	2q32.3	testis-selective	viable, growth retardation; die after weaning	same as male	(46)
	CT39	NXF2	Xq22.1	testis-selective	viable, subfertile; meiotic arrest		(47)
	CT32	LDHC	11p15.5-p15.3	testis-selective	Viable, Infertile; Low ATP, Diminished hyperactive motility	none	(48)
	CT134	ODF2	9q34.11	not mentioned	Not viable; Preimplantation defect	not viable	(49)
	CT40	TAF7L	Xq22.1	testis-selective	Viable, Fertile but produce small litters; Folded/Angulated sperm tails	none	(50)

NA: Not Available

Synaptonemal complex protein 1 (SCP-1)

SCP-1, also called SYCP1, HOM-TES-14, and CT8 is selectively expressed during meiotic division I (reduction division) of spermatocytes prophase specifically from zygotene to diplotene in the human testis because it's responsible for the formation of the synaptonemal complexes (24). SCP-1 is involved in pairing and recombination of homologous chromosomes during meiotic division I (24). Most CT antigens are located on chromosome X, but the SCP-1 is localized on chromosome 1 (15). The restricted expression of this CTA in pre-meiotic spermatocytes for chromosomal pairing demonstrates its importance in meiosis (Table 2).

ACRBP

ACRBP, also known as OY-TES-1, SP32, and CT23, is an acrosin binding protein located on chromosome 12 (51). OY-TES-1 is the human homolog of pro-acrosin binding protein sp32 precursor first described in pig and mouse testes (51). It is only and highly expressed in the testis and localized at the acrosomal region of mature spermatozoa but not on any somatic cells. Interestingly, in a recent study it was found that while in human there is only one mRNA of the gene, in mice, the alternative splicing of the pre-mRNA of this gene is responsible for regulating the biogenesis of sperm acrosome (52).

Piwi2

P-element induced wimpy testis (Piwi) is a class of genes involved in processes like maintaining differentiation and self-renewal in stem cells and also RNA silencing (53, 54), which was first identified in *Drosophila* (55). Miwi, a murine homolog of the Piwi, is specifically expressed in spermatocytes and spermatids and encodes a cytoplasmic protein essential for spermatogenesis (56). In addition, a stem-cell protein Piwil2 (Piwi-like 2) (Table 2) has been reported to be precisely expressed in the testes of humans and mice, restricted to spermatogonia and early spermatocytes (57). It has critical roles in early prophase of meiosis and germline stem cell self-renewal in testis (25, 58). It also binds to piRNAs and mRNAs and in this way is implicated in translational regulation of many genes during early spermatogenesis (59, 60). Epigenetic studies in infertile men have shown that this disorder is associated with promoter hypermethylation associated silencing of PIWIL2 and another CTA named TDRD1 (61).

Role of cancer/testis genes in sperm metabolism

Several CT genes are involved in the regulation of energy production in sperm. COX6B2, a sperm-specific factor of complex IV of the electron transport chain, is a promoter of efficient oxidative phosphorylation by stimulating complex IV dimerization (62). The

CT gene, SPATA19, was isolated and identified as a mitochondrial adhesion protein that efficiently stimulates the packing of mitochondria into the neck region of the sperm (63). Conclusively the two above-mentioned CT genes could be critical in sperm to satisfy the increased energy needs for motility. Furthermore, the inter-conversion between lactate and pyruvate is catalyzed by the first testis-specific isozyme discovered, lactate dehydrogenase C (LDHC) (63). While LDHA and LDHB are also found in the sperm, more than 80% of the LDH activity in the spermatozoa is related to LDHC (63). The *ldhc* null mice are infertile with impaired motility of the sperm and inability to fertilize oocytes (63). Odet *et al.* found inability of the sperm to consume glucose, rapid decreases in the progressive motility and ATP levels in the sperm of these knockout mice and also disruption of homeostasis (48, 64).

RNA regulation

Several research studies have investigated the role of CTAs in sustaining and regulating mRNA expression in sperm cells. The mouse orthologues of the CTAs PIWIL2 and TDRD1, in association with piwi-interacting RNAs (piRNAs), may be responsible for silencing transposons during spermatogenesis. Deletion of either PIWIL2 or TDRD1 results in prophase stage arrest of spermatogenesis thus increasing transposon LINE-1 expression (27, 65). Interestingly, the chromatoid body, which houses mRNAs and RNA-binding proteins, promotes regulation of gene expression during spermiogenesis during inactive transcription (66) and houses numerous CT genes. Furthermore, several CT genes like TDRD6, TDRD1, MAEL, and MILI have also been identified within the chromatoid body and research studies have reported that mice lacking CT genes like TDRD6 and MAEL have arrested spermatogenesis at the round spermatid stage, thereby leading to infertility in male mice (19, 20, 67). It should be noted that mice lacking MAEL, MILI, TDRD6, and TDRD1 have been reported viable and healthy, suggesting that these CT genes are non-essential for somatic cells (19, 20).

Ct genes functions in relation to sperm motility

Sperm motility is mediated by the flagellum via its undulatory movement which moves the sperm forward. The sperm flagellum is made of the axoneme, the outer dense fibers, and the fibrous sheath. The axoneme is in turn made up of dynein-and-tubulin radial-spoke structure that specifically mediates its movement. The axoneme has an outer protective fibrous sheath covering it that regulates its structure. The structure of the flagella is made up of at least 13 known proteins and 50% of the flagella structure is made up of a CT gene called AKAP4. It should be noted that the CTAs; AKAP3, CABYR, SPA17, ROPN1, and

TSGA10 also constitute the fibrous sheath (68-70). Double knockout mice for *ROPN* and *ROPN1L* have infertility due to defects in flagellum structure and immotile sperm (33). The molecular mechanisms that coordinate and regulate the movement of the flagellum are still not lucid; however CT genes like *AKAP4*, *rhophilin-1*, and *ropporin* have been demonstrated to be present in flagella structures thereby suggesting active signaling cascades in sperm.

To study genes behavior during mouse spermatogenesis, the *in vitro* derived germ cells could be a credible model (71). In this way, the expression pattern of TSGA10 as a known Cancer/Testis gene (72, 73), is similar during *in vitro* and *in vivo* germ cell generation (71). TSGA10 has a critical function during spermatogenesis (74).

Conclusion

Most of the physiological functions for some of the CT antigens during spermatogenesis still remain unknown, however, some of these CT antigens have been investigated and identified as proteins closely related to: (a) Spermatogonial stem cells via mitosis like *Piwil2*, (b) Transcription regulation like *SS18-SSX* proteins, (c) *SCP-1* functions in the crossing over process in spermatocytes to during prophase 1 meiotic division I, (d) Germ cell apoptosis like *MAGE* proteins and *Piwil2*, and the function of *OY-TES-1* and *SP-17* in spermiogenesis and sperm motility, respectively. Conclusively Figure 2 gives a summarized illustration about CT antigens involved in the various phases of cellular events related to spermatogenesis.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Scanlan MJ, Simpson AJ, Old LJ. The cancer/testis genes: review, standardization, and commentary. *Cancer Immunol* 2004; 4:1-15.
2. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer* 2005; 5:615-625.
3. Nourashrafeddin S, Dianatpour M, Aarabi M, Mobasher MB, Kazemi-Oula G, Modarressi MH. Elevated Expression of the Testis-specific Gene *WBP2NL* in Breast Cancer. *Biomark Cancer* 2015; 7:19-24.
4. Mobasher MB, Shirkoohi R, Modarressi MH. Cancer/testis *OIP5* and *TAF7L* genes are Up-regulated in breast cancer. *Asian Pac J Cancer Prev* 2015; 16:4623-4628.
5. Mobasher MB, Shirkoohi R, Zendehdel K, Jahanzad I, Talebi S, Afsharpad M, Modarressi MH. Transcriptome

analysis of the cancer/testis genes, *DAZ1*, *AURKC*, and *TEX101*, in breast tumors and six breast cancer cell lines. *Tumour Biol* 2015; 36:8201-8206.

6. Jungbluth AA, Stockert E, Chen YT, Kolb D, Iversen K, Coplan K, et al. Monoclonal antibody MA454 reveals a heterogeneous expression pattern of *MAGE-1* antigen in formalin-fixed paraffin embedded lung tumours. *Br J Cancer* 2000; 83:493-497.

7. Mobasher MB, Modarressi MH, Shabani M, Asgarian H, Sharifian RA, Vossough P, Shokri F. Expression of the testis-specific gene, *TSGA10*, in Iranian patients with acute lymphoblastic leukemia (ALL). *Leuk Res* 2006; 30:883-889.

8. Scanlan MJ, Gordon CM, Williamson B, Lee SY, Chen YT, Stockert E, et al. Identification of cancer/testis genes by database mining and mRNA expression analysis. *Int J Cancer* 2002; 98:485-492.

9. Zendman AJ, Ruiter DJ, Van Muijen GN. Cancer/testis-associated genes: identification, expression profile, and putative function. *J Cell Physiol* 2003; 194:272-288.

10. Costa FF, Le Blanc K, Brodin B. Concise review: cancer/testis antigens, stem cells, and cancer. *Stem Cells* 2007; 25:707-711.

11. Kalejs M, Erenpreisa J. Cancer/testis antigens and gametogenesis: a review and "brain-storming" session. *Cancer Cell Int* 2005; 5:4.

12. Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, et al. The DNA sequence of the human X chromosome. *Nature* 2005; 434:325-337.

13. Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, et al. Genome-wide analysis of cancer/testis gene expression. *Proc Natl Acad Sci U S A* 2008; 105:20422-20427.

14. Doyle JM, Gao J, Wang J, Yang M, Potts PR. *MAGE-RING* protein complexes comprise a family of E3 ubiquitin ligases. *Mol Cell* 2010; 39:963-974.

15. Cheng YH, Wong EW, Cheng CY. Cancer/testis (CT) antigens, carcinogenesis and spermatogenesis. *Spermatogenesis* 2011; 1:209-220.

16. Dalerba P, Frascella E, Macino B, Mandruzzato S, Zamboni A, Rosolen A, et al. *MAGE*, *BAGE* and *GAGE* gene expression in human rhabdomyosarcomas. *Int J Cancer* 2001; 93:85-90.

17. von Boehmer L, Keller L, Mortezaei A, Provenzano M, Sais G, Hermanns T, et al. *MAGE-C2/CT10* protein expression is an independent predictor of recurrence in prostate cancer. *PLoS One* 2011; 6:e21366.

18. Gjerstorff MF, Kock K, Nielsen O, Ditzel HJ. *MAGE-A1*, *GAGE* and *NY-ESO-1* cancer/testis antigen expression during human gonadal development. *Hum Reprod* 2007; 22:953-960.

19. Takebe M, Onohara Y, Yokota S. Expression of *MAEL* in nuage and non-nuage compartments of rat spermatogenic cells and colocalization with *DDX4*, *DDX25* and *MIWI*. *Histochem Cell Biol* 2013; 140:169-181.

20. Vasileva A, Tiedau D, Firooznia A, Muller-Reichert T, Jessberger R. *Tdrd6* is required for spermiogenesis, chromatoid body architecture, and regulation of miRNA expression. *Curr Biol* 2009; 19:630-639.

21. Fratta E, Coral S, Covre A, Parisi G, Colizzi F, Danielli R, Nicolay HJ, Sigalotti L, Maio M. The biology of cancer testis antigens: putative function, regulation and therapeutic potential. *Mol Oncol* 2011; 5:164-182.

22. Mirandola L, M JC, Cobos E, Bernardini G, Jenkins MR, Kast WM, Chiriva-Internati M. Cancer testis antigens: novel biomarkers and targetable proteins for ovarian cancer. *Int Rev Immunol* 2011; 30:127-137.

23. Shen Y, Xu J, Yang X, Liu Y, Ma Y, Yang D, *et al.* Evidence for the involvement of the proximal copy of the MAGEA9 gene in Xq28-linked CNV67 specific to spermatogenic faileddagger. *Biol Reprod* 2017; 96:610-616.
24. Meuwissen RL, Offenbergh HH, Dietrich AJ, Riesewijk A, van Iersel M, Heyting C. A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J* 1992; 11:5091-5100.
25. Kuramochi-Miyagawa S, Kimura T, Ijiri TW, Isobe T, Asada N, Fujita Y, *et al.* Mili, a mammalian member of piwi family gene, is essential for spermatogenesis. *Development* 2004; 131:839-849.
26. Lim AK, Knowles BB. Controlling endogenous retroviruses and their chimeric transcripts during natural reprogramming in the Oocyte. *J Infect Dis* 2015; 212:S47-51.
27. Reuter M, Chuma S, Tanaka T, Franz T, Stark A, Pillai RS. Loss of the Mili-interacting Tudor domain-containing protein-1 activates transposons and alters the Mili-associated small RNA profile. *Nat Struct Mol Biol* 2009; 16:639-646.
28. Fereydouni B, Salinas-Riester G, Heistermann M, Dressel R, Lewerich L, Drummer C, *et al.* Long-term oocyte-like cell development in cultures derived from neonatal marmoset monkey ovary. *Stem Cells Int* 2016; 2016:2480298.
29. Soper SF, van der Heijden GW, Hardiman TC, Goodheart M, Martin SL, de Boer P, *et al.* Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. *Dev Cell* 2008; 15:285-297.
30. Ikawa M, Tokuhira K, Yamaguchi R, Benham AM, Tamura T, Wada I, *et al.* Calsperin is a testis-specific chaperone required for sperm fertility. *J Biol Chem* 2011; 286:5639-5646.
31. Jackman SM, Kong X, Fant ME. Plac1 (placenta-specific 1) is essential for normal placental and embryonic development. *Mol Reprod Dev* 2012; 79:564-572.
32. Miki K, Willis WD, Brown PR, Goulding EH, Fulcher KD, Eddy EM. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. *Dev Biol* 2002; 248:331-342.
33. Fiedler SE, Dudiki T, Vijayaraghavan S, Carr DW. Loss of R2D2 proteins ROPN1 and ROPN1L causes defects in murine sperm motility, phosphorylation, and fibrous sheath integrity. *Biol Reprod* 2013; 88:41.
34. Fiedler SE, Sisson JH, Wyatt TA, Pavlik JA, Gambling TM, Carson JL, Carr DW. Loss of ASP but not ROPN1 reduces mammalian ciliary motility. *Cytoskeleton (Hoboken)* 2012; 69:22-32.
35. Han C, Kwon JT, Park I, Lee B, Jin S, Choi H, *et al.* Impaired sperm aggregation in Adam2 and Adam3 null mice. *Fertil Steril* 2010; 93:2754-2756.
36. Kohn MJ, Szein J, Yagi R, DePamphilis ML, Kaneko KJ. The acrosomal protein Dickkopf-like 1 (DKKL1) facilitates sperm penetration of the zona pellucida. *Fertil Steril* 2010; 93:1533-1537.
37. de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, *et al.* Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. *Genes Dev* 2005; 19:1376-1389.
38. Yang F, Eckardt S, Leu NA, McLaughlin KJ, Wang PJ. Mouse TEX15 is essential for DNA double-strand break repair and chromosomal synapsis during male meiosis. *J Cell Biol* 2008; 180:673-679.
39. Bolcun-Filas E, Hall E, Speed R, Taggart M, Grey C, de Massy B, *et al.* Mutation of the mouse Syce1 gene disrupts synapsis and suggests a link between synaptonemal complex structural components and DNA repair. *PLoS Genet* 2009; 5:e1000393.
40. Romanienco PJ, Camerini-Otero RD. The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol Cell* 2000; 6:975-987.
41. Shin YH, Choi Y, Erdin SU, Yatsenko SA, Kloc M, Yang F, *et al.* Hormad1 mutation disrupts synaptonemal complex formation, recombination, and chromosome segregation in mammalian meiosis. *PLoS Genet* 2010; 6:e1001190.
42. Suzuki T, Kosaka-Suzuki N, Pack S, Shin DM, Yoon J, Abdullaev Z, *et al.* Expression of a testis-specific form of Gal3st1 (CST), a gene essential for spermatogenesis, is regulated by the CTCF paralogous gene BORIS. *Mol Cell Biol* 2010; 30:2473-2484.
43. Shang E, Nickerson HD, Wen D, Wang X, Wolgemuth DJ. The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing proteins, is essential for male germ cell differentiation. *Development* 2007; 134:3507-3515.
44. Pan J, Goodheart M, Chuma S, Nakatsuji N, Page DC, Wang PJ. RNF17, a component of the mammalian germ cell nuage, is essential for spermiogenesis. *Development* 2005; 132:4029-4039.
45. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, *et al.* Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet* 2002; 32:359-369.
46. Chen TR, Wang P, Carroll LK, Zhang YJ, Han BX, Wang F. Generation and characterization of Tmeff2 mutant mice. *Biochem Biophys Res Commun* 2012; 425:189-194.
47. Pan J, Eckardt S, Leu NA, Buffone MG, Zhou J, Gerton GL, *et al.* Inactivation of Nxf2 causes defects in male meiosis and age-dependent depletion of spermatogonia. *Dev Biol* 2009; 330:167-174.
48. Odet F, Duan C, Willis WD, Goulding EH, Kung A, Eddy EM, Goldberg E. Expression of the gene for mouse lactate dehydrogenase C (Ldhc) is required for male fertility. *Biol Reprod* 2008; 79:26-34.
49. Salmon NA, Reijo Pera RA, Xu EY. A gene trap knockout of the abundant sperm tail protein, outer dense fiber 2, results in preimplantation lethality. *Genesis* 2006; 44:515-522.
50. Cheng Y, Buffone MG, Kouadio M, Goodheart M, Page DC, Gerton GL, *et al.* Abnormal sperm in mice lacking the Taf11 gene. *Mol Cell Biol* 2007; 27:2582-2589.
51. Ono T, Kurashige T, Harada N, Noguchi Y, Saika T, Niikawa N, *et al.* Identification of proacrosin binding protein sp32 precursor as a human cancer/testis antigen. *Proc Natl Acad Sci U S A* 2001; 98:3282-3287.
52. Kanemori Y, Koga Y, Sudo M, Kang W, Kashiwabara S, Ikawa M, *et al.* Biogenesis of sperm acrosome is regulated by pre-mRNA alternative splicing of Acrbp in the mouse. *Proc Natl Acad Sci U S A* 2016; 113:E3696-3705.
53. Katahira J, Yoneda Y. Nucleocytoplasmic transport of microRNAs and related small RNAs. *Traffic* 2011; 12:1468-1474.
54. Thomson T, Lin H. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. *Annu Rev Cell Dev Biol* 2009; 25:355-376.
55. Saito K, Nishida KM, Mori T, Kawamura Y, Miyoshi K, Nagami T, *et al.* Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. *Genes Dev* 2006; 20:2214-2222.

56. Deng W, Lin H. miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. *Dev Cell* 2002; 2:819-830.
57. Lee JH, Schutte D, Wulf G, Fuzesi L, Radzun HJ, Schweyer S, et al. Stem-cell protein Piwil2 is widely expressed in tumors and inhibits apoptosis through activation of Stat3/Bcl-XL pathway. *Hum Mol Genet* 2006; 15:201-211.
58. Unhavaithaya Y, Hao Y, Beyret E, Yin H, Kuramochi-Miyagawa S, Nakano T, et al. MILI, a PIWI-interacting RNA-binding protein, is required for germ line stem cell self-renewal and appears to positively regulate translation. *J Biol Chem* 2009; 284:6507-6519.
59. Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, et al. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* 2006; 442:203-207.
60. Heyn H, Ferreira HJ, Bassas L, Bonache S, Sayols S, Sandoval J, et al. Epigenetic disruption of the PIWI pathway in human spermatogenic disorders. *PLoS One* 2012; 7:e47892.
61. Huttemann M, Jaradat S, Grossman LI. Cytochrome c oxidase of mammals contains a testes-specific isoform of subunit VIb--the counterpart to testes-specific cytochrome c? *Mol Reprod Dev* 2003; 66:8-16.
62. Suzuki-Toyota F, Ito C, Toyama Y, Maekawa M, Yao R, Noda T, et al. Factors maintaining normal sperm tail structure during epididymal maturation studied in Gopc-/- mice. *Biol Reprod* 2007; 77:71-82.
63. Goldberg E, Eddy EM, Duan C, Odet F. LDHC: the ultimate testis-specific gene. *J Androl* 2010; 31:86-94.
64. Odet F, Gabel SA, Williams J, London RE, Goldberg E, Eddy EM. Lactate dehydrogenase C and energy metabolism in mouse sperm. *Biol Reprod* 2011; 85:556-564.
65. Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ. Developmentally regulated piRNA clusters implicate MILI in transposon control. *Science* 2007; 316:744-747.
66. Meikar O, Da Ros M, Korhonen H, Kotaja N. Chromatoid body and small RNAs in male germ cells. *Reproduction* 2011; 142:195-209.
67. Wang J, Saxe JP, Tanaka T, Chuma S, Lin H. Mili interacts with tudor domain-containing protein 1 in regulating spermatogenesis. *Curr Biol* 2009; 19:640-644.
68. Modarressi MH, Behnam B, Cheng M, Taylor KE, Wolfe J, van der Hoorn FA. Tsga10 encodes a 65-kilodalton protein that is processed to the 27-kilodalton fibrous sheath protein. *Biol Reprod* 2004; 70:608-615.
69. Chiriva-Internati M, Cobos E, Da Silva DM, Kast WM. Sperm fibrous sheath proteins: a potential new class of target antigens for use in human therapeutic cancer vaccines. *Cancer Immun* 2008; 8:8.
70. Fiedler SE, Bajpai M, Carr DW. Identification and characterization of RHOA-interacting proteins in bovine spermatozoa. *Biol Reprod* 2008; 78:184-192.
71. Miryounesi M, Nayernia K, Mobasheri MB, Dianatpour M, Oko R, Savad S, et al. Evaluation of *in vitro* spermatogenesis system effectiveness to study genes behavior: monitoring the expression of the testis specific 10 (Tsga10) gene as a model. *Arch Iran Med* 2014; 17:692-7.
72. Dianatpour M, Mehdipour P, Nayernia K, Mobasheri MB, Ghafouri-Fard S, Savad S, et al. Expression of Testis Specific Genes TSGA10, TEX101 and ODF3 in Breast Cancer. *Iran Red Crescent Med J* 2012; 14:722-726.
73. Salehipour P, Nematzadeh M, Mobasheri MB, Afsharpad M, Mansouri K, Modarressi MH. Identification of new TSGA10 transcript variants in human testis with conserved regulatory RNA elements in 5'untranslated region and distinct expression in breast cancer. *Biochim Biophys Acta*. 2017; 9:973-982.
74. Rahmani Rad F, Mobasheri M, Modarressi MH. TSGA10, as a Cancer/Testis gene. *Tehran Univ Med J TUMS Pub* 2015; 73:231-242.