Iranian Journal of Basic Medical Sciences

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IJBMS

Cancer/Testis genes in relation to sperm biology and function

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ARTICLE INFO

Article type:
Mini-Review

Article history:
Received: Oct 17, 2016
Accepted: May 25, 2017

Keywords:
Cancer testis genes
Carcinogenesis
Spermatogenesis
Sperm biology
Sperm genes functions
Testis genes

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.

► *Please cite this article as:*
Babatunde KA, Najafi A, Salehipour P, Modarressi MH, Mobasheri MB. Cancer/Testis genes in relation to sperm biology and function. Iran J Basic Med Sci 2017; 20:967-974. doi: 10.22038/IJBMS.2017.9259

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 immortal-lization. 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 resump- tion 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).

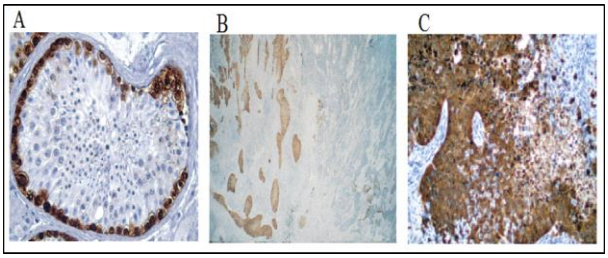

Figure 1. CTAs expression in normal and tumor tissues; A: Immunohistochemical staining displays the expression of MAGE4 by testicular germ cells B: Squamous cell carcinoma. An area of MA454 reactivity next to an immunonegative tumor area (heterogeneous expression of the CTA) C: Expression of NY-ESO-1 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- α1; 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 te 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, sperma- tocytes, 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 chromo-some, 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 spermiation form mature sperma-tozoa (Figure 2).

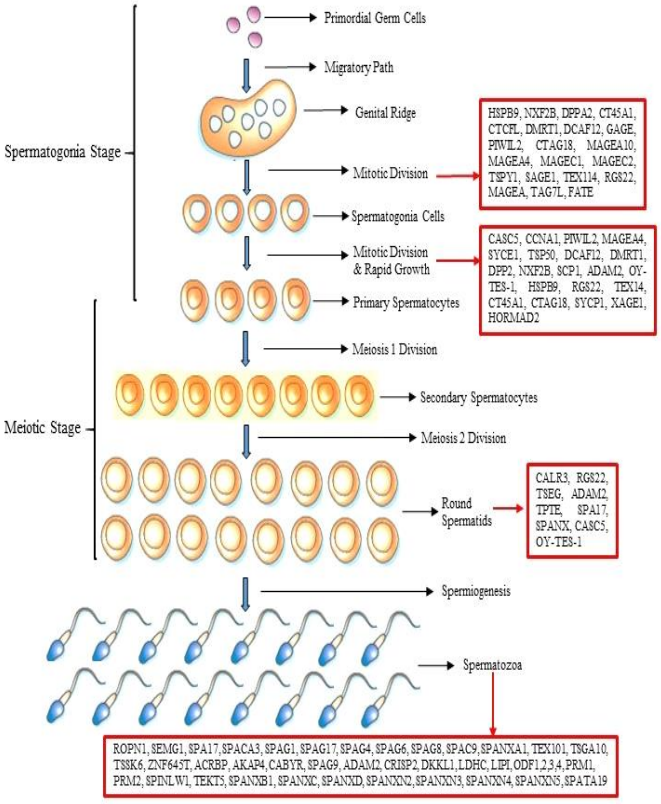

Figure 2. Summary of CT antigens implicated in the process of spermatogenesis

Most CT-X antigens like MAGE and NY-ESO-1 are implicated in the process of spermatogenesis, while CT-X antigens like SCPI, 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)
Homologous Recombination	CT8	SYCP1	1p13-p12	testis-selective	viable, infertile; microrchidia due to defects in homologous recombination	infertile	(37)
	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	CTCFL	20q13.31	testis-selective	viable, fertile; microrchidia, multinucleated sperm cells, increased sperm death	none	(42)
	CT9	BRDT	1p22.1	testis-selective	viable, fertile; oligoasthenoteratozoospermia	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 TDRD 1 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 theses 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.

Acknowledgment

We acknowledge the deputy of research, Tehran University of Medical Sciences, Tehran, Iran and the Swiss government scholarship committee for the funding.

Conflict of interest

The authors declare that they have no conflicts of interest.

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