

An emerging role of TEX101 protein as a male infertility biomarker

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ABSTRACT

Infertility is an important aspect of human reproduction. It affects up to 15% of couples, with the male factor contributing to approximately 50% of all cases. Azoospermia is one of the most severe forms of male infertility, which is characterized by the absence of sperm in semen. The mechanisms underlying male infertility remain unknown. Currently, clinicians rely on semen analysis to predict the reproductive potential of a male, and testicular biopsy is the only reliable method to diagnose different subtypes of azoospermia. Recently, advances in proteomics encouraged the search for novel male infertility biomarkers in seminal plasma. In this review, we focus on TEX101, a testicular germ cell-specific protein, one of the most promising male infertility biomarkers. We discuss its role in spermatogenesis and fertilization and summarize our current knowledge about this new potential biomarker.

Introduction

Infertility, the inability to conceive a child within one year of regular unprotected intercourse, shows a high prevalence (up to 15% of couples) and is associated with both men and women (1). Male factor (exclusive or combined with female abnormalities) contributes to approximately 50% of infertility cases (2). In attempts to overcome this problem, infertile couples seek medical advice and pursue fertility treatments such as hormonal and drug therapy or assisted reproductive technologies (ART) (3).

The impact of male factor infertility was traditionally overlooked despite its significance for men's health. However, over the last decade, this topic has received the required attention and there has been a notable progress in the field of male infertility. Cellular and molecular mechanisms of reproduction and their impact on the clinical outcome are still not fully understood due to the complexity of the male reproductive system. With 3-4% of couples remaining childless (4), male infertility remains an important clinical and societal issue.

Forms and subtypes of male infertility

Clinical forms of male infertility result in reduced sperm counts (oligospermia), reduced sperm motility (asthenospermia), abnormal sperm morphology (teratospermia) and in most severe cases, a complete lack of spermatozoa in semen, known as azoospermia. Azoospermia accounts for 5-20% of infertile men and could be further categorized as pre-testicular or non-obstructive (NOA), and obstructive azoospermia (OA) (5). In most situations pre-testicular azoospermia is linked to hypothalamic or pituitary dysfunction, resulting in low serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This can cause low testosterone levels and failure of the testes to produce sperm. This group of infertile men can be easily diagnosed utilizing current clinical methods (blood test to determine reproductive hormones levels) (6). NOA may be sub-classified as maturation arrest (MA), Sertoli cell-only syndrome (SCO), and hypospermatogenesis (HS) (7). OA results from physical obstruction in the male genital tract. It resembles vasectomy, a surgical procedure in which vas deferens is severed and sealed, used for male sterilization (8).

Development of sperm antibodies in semen or blood can be an additional cause of male infertility, affecting 5-6% of infertile men. Their presence results in reduction of sperm motility and prevention of sperm-egg binding during fertilization.(9)

Challenges in male infertility diagnosis

If male factor infertility is suspected, a variety of diagnostic approaches are used to identify infertility forms and subtypes and for considering treatment options. Initially, physical examination followed by semen analysis and endocrine profiling are performed. Semen analysis, the most common way to

diagnose male infertility, includes examination of macroscopic (coagulation, color, viscosity, pH, and volume) and microscopic (sperm count, concentration, motility, morphology and viability) parameters of seminal fluid (10). Since the composition of seminal plasma can be affected by environmental factors, infections and other pathologies, the results of semen analysis are frequently either normal or ambiguous, leading to inconclusive diagnosis (11). In these cases, patients are diagnosed with idiopathic infertility and specific treatment is not provided (12).

In an attempt to diagnose forms and subtypes of azoospermia, a diagnostic testicular biopsy is often used as the only reliable method to distinguish between OA and NOA (7;13). Testicular biopsy is an invasive surgical procedure with possible complications such as tissue damage, bleeding and development of chronic pain. Thus, there is an urgent need for alternative, non-invasive approaches for identification of categories of male infertility. Recent developments in the -omics technologies should aid in discovering novel infertility treatments (14) and for developing non-invasive tests to eliminate diagnostic testicular biopsies, classify infertility forms and suggest options for treatment.

Search for male infertility biomarkers

Different types of molecules, such as genes, messenger RNA, microRNA, proteins, metabolites or combination of these, can serve as biomarkers (15). Although approaches for biomarker discovery and development are rapidly maturing and the number of biomarker discovery projects are steadily increasing, translation of biomarkers from bench to bedside is still slow. In a recent review, Kovac et al.(16) summarized the most significant genomic, proteomic and metabolomics approaches to identify male infertility biomarkers (Table 1).

Proteins are the most promising molecules to develop disease biomarkers. Alternations in protein abundance and activity in different physiological states reflect dynamic alternations which may hardly be predicted at the genome level (16). Proteomic analysis of seminal plasma (SP) or spermatozoa can provide information regarding the presence of a protein, its abundance, and post-translational modifications. Several studies conducted in the 1980's resulted in discovering roles of transferrin, heparin-binding proteins (HBPs), prolactin inducible protein (PIP), and human cationic antimicrobial protein (hCAP18) in spermatogenesis and fertilization (16). Recently, by comparing the SP proteome of healthy fertile men before and after vasectomy, Batruch et al. identified a list of testis and epididymis-expressed proteins. Among these proteins, TEX101, PGK2, HIST1H2BA, SLC2A14, SPACA3, GAPDHS, and AKAP4 were the top candidates for developing biomarkers of vasectomy success (17). In the follow-up study, 30 of those biomarker candidates were verified in pre- and post-vasectomy SP samples as well as SP from patients with NOA. Several testis-specific proteins, such as TEX101, LDHC and ECM1, were identified as key male infertility biomarkers (18). Recently, TEX101 and ECM1 were used to develop an algorithm for non-invasive differential diagnosis of azoospermia forms (OA versus NOA) (19). SP levels of TEX101 could also distinguish different subtypes of NOA. TEX101 levels of

120ng/mL or higher denote normal spermatogenesis, while levels of 5-120ng/mL are associated with HS or MA, and levels below 5ng/ml (theoretically zero) indicate SCO syndrome (Figure 1). Apart from azoospermia diagnosis, TEX101 levels in SP may predict the outcome of sperm retrieval procedures used for assisted reproduction. Men diagnosed with OA, NOA-HS, and NOA-MA are recommended for testicular sperm extraction (TESE), while for men with NOA-SCO, the success rate of TESE is negligible. In that case, TESE, a full-scale several hour surgery under general anaesthesia, could be avoided. In this article, we will focus on TEX101 protein, discuss its role in spermatogenesis and fertilization and summarize our knowledge about this new potential infertility biomarker.

TEX101, a novel testicular germ cell-specific protein

Mouse TEX101 protein (encoded by testis expressed 101 gene, *Tex101*) was originally identified in mice by Kurita et al. (20). Mouse TEX101 is a testicular germ cell-specific protein predominantly located on the plasma membrane of germ cells during gametogenesis. According to The Human Protein Atlas (www.proteinatlas.org), human TEX101 is not expressed in any other human tissue or cell type, including Sertoli and Leydig cells of the testicular tissue.

The TEX101 gene is located on the long arm of chromosome 19 at position 19q13.31 in humans and chromosome 7 in mouse, with homology of sequence approximately 55% (21). It should be mentioned that most of our knowledge on TEX101 derives from studies conducted using mouse models. However, taking into consideration that the positions of the cysteine residues and the potential glycosylphosphatidylinositol (GPI)-anchoring site within the sequence are highly conserved in mammalian species, we can assume that most of the data generated with mouse models may be valid for human TEX101.

Studying TEX101 at a genomic level, Tsukamoto et al. (22) found that mouse TEX101 gene is regulated by two distinct promoters and forms three major transcripts. Based on nucleotide and amino acid sequence, it is predicted that the human ortholog exists in two isoforms; the cytosolic form and the extracellular GPI-anchored form, under the regulation of two promoters (Figure 2A). However, by performing immunohistochemistry (IHC) experiments Kurita et al. (20) demonstrated that TEX101 is located mainly on the plasma membrane of germ cells during the whole process of gametogenesis, but it is only weakly present in the cytoplasm of the cells. Jin et al. (23) showed that TEX101 is highly glycosylated at all potential sites and that it is expressed as a GPI-anchored protein on the cell surface, as it was speculated by Kurita et al. (20) (Figure 2B). Furthermore, they presented evidence that TEX101 is present in the lipid rafts on sperm surface, as it was also demonstrated by Sleight et al. (24).

Given that TEX101 is present on the cell surface during all stages of spermatogenesis, Takayama et al. (25) intended to examine the fate of mouse TEX101 during sperm transport through the male reproductive tract. They reported that mouse TEX101 is eventually cleaved and released from the cell surface of epididymal sperm while it passes through the caput epididymis.

One of the missing parts regarding TEX101 is its 3-dimensional structure. Considering that it possesses a Ly-6/uPAR (Lymphocyte Antigen 6/ urokinase Plasminogen Activator Receptor) domain defined by 8-10 distinct cystein residues, it can be predicted that the structure of TEX101 might resemble that of uPAR. A number of proteins, comprising the Ly-6 family, share this single Ly-6/uPAR domain, and they are mapped to chromosome 8. However, the uPAR locus, *PLAUR*, encoding three tandem Ly-6/uPAR domains is found at 19q13.3 (26). Surprisingly, a few members of the Ly-6 family are mapped at the same locus, among which is TEX101 (19q13.31) and SAMP14 (19q.31.33). Based on this fact, we can speculate that these proteins are more closely related to uPAR, and may share the same or similar functions (27).

Functional roles of membrane-bound and soluble forms of TEX101

The information that is already gained regarding TEX101 function stems from studies that were based on mouse models (28;29). Nevertheless, the ultimate goal is to translate this knowledge into the human male reproductive system.

Spermatozoa are, inarguably, the most highly differentiated cell type of the human body. Numerous distinct processes need to be completed for generating mature and functional spermatozoa that have the ability to fertilize the oocyte. In the following section we describe step-by-step the events that occur, having as starting point the spermatogenesis in testes, and as terminal point the sperm-egg interaction in the female reproductive tract. Based on the fact that TEX101 accompanies sperm, either anchored to its membrane, or shed into seminal plasma, we are going to highlight all the events in which TEX101 is involved.

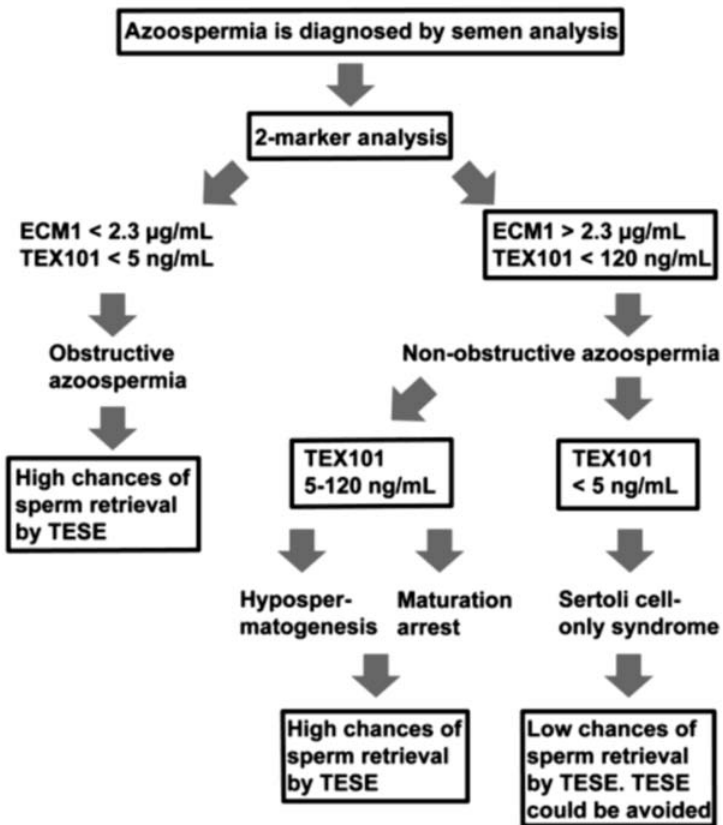
Spermatogenesis

Spermatogenesis is a highly ordered process that occurs in the male testis and is responsible for the production of a large number of spermatozoa, controlled by a complex system of paracrine and endocrine activity within the seminiferous tubules of the testis. The spermatogonia (the stem cells of the testis) are located in the basal compartment of the seminiferous tubule, between the basement membrane and the Sertoli cells.

Serial cross-sections of a seminiferous tubule show that sperm cells differentiate in spermatogenic cycles. LH-regulated release of testosterone from Leydig cells marks the initiation of spermatogenesis and development of germ cells into spermatozoa (30). Spermatogonial mitosis leads to a sufficient population of cells that will become spermatozoa. Following cell proliferation, diploid spermatogonial cells differentiate into spermatocytes, which in turn undergo meiosis and produce haploid spermatids (31). Finally, during the last stage of spermatogenesis (spermiogenesis), round haploid spermatids transform into mature spermatozoa undergoing a series of morphological and biochemical changes.

Figure 1. Differential diagnosis of azoospermia and prediction of subtypes of non-obstructive zoospermia with seminal plasma proteins ECM1 and TEX101.

Figure 1



When azoospermia is diagnosed by semen analysis, low SP levels of ECM1 (<2.3 µg/mL) and TEX101 (<5 ng/mL) proteins suggest obstructive azoospermia, while high SP level of ECM1 (>2.3 µg/mL) suggests non-obstructive azoospermia. SP concentration of TEX101 protein may also discriminate between non-obstructive azoospermia subtypes of Sertoli cell-only (< 5 ng/mL) and hypospermatogenesis or maturation arrest (5-120 ng/mL). Men with obstructive azoospermia have good chances of sperm retrieval by testicular sperm extraction (TESE), while for men with Sertoli cell-only, sperm retrieval is unlikely and TESE can be avoided. From Drabovich, A.P. et al. Differential Diagnosis of Azoospermia with Proteomic Biomarkers ECM1 and TEX101 Quantified in Seminal Plasma. *Sci. Transl. Med.* 5, 212ra160 (2013) (19). Reprinted with permission from AAAS.

During spermiogenesis, the mature spermatozoa acquire all the essential and unique regions: formation of the acrosome, development of flagellum (tail), elimination of cytoplasm and condensation of the nucleus (32). It is estimated that one spermatogonium is able to differentiate into approximately 250 spermatozoa in ~35 days, depending on the species (33). By the end of spermatogenesis, morphologically complete spermatozoa detach from the Sertoli cell microenvironment and they are delivered into the lumen of the seminiferous tubule (34). Released spermatozoa passively migrate to the epididymis for further maturation.

TEX101, as previously mentioned, is a testicular germ cell-specific protein and is located on plasma membrane of spermatocytes, round and elongated spermatids, and testicular spermatozoa. The question that rises is whether TEX101 affects the outcome of spermatogenesis, or it remains on the cells to play its role later in the forthcoming processes.

Recently, two different groups (28;29) independently generated *Tex101*^{-/-} mice in an attempt to

investigate *in vivo* the effects of TEX101 knock-out on the production of functional spermatozoa. In both studies, heterozygous (Tex101^{+/-}) and homozygous (Tex101^{-/-}) mutant mice were generated, and disruption of TEX101 caused no deleterious effect. Although Tex101^{-/-} mice had normal mating ability, they were not able to produce offsprings, confirming the infertile phenotype. Interestingly, there was no significant difference in the weight and the histology of the testes between Tex101^{-/-} and Tex101 wild type mice. Furthermore, sperm count, acrosome reaction efficiency, and sperm motility and viability parameters were examined and no significant differences were found. To conclude, although TEX101 is found on the sperm cell membrane during spermatogenesis, there is still no evidence supporting its essential role for normal spermatogenesis.

Epididymal maturation

Development of germ cells into testicular spermatozoa is followed by their transit to epididymis for post-testicular maturation. Although the morphology of testicular spermatozoa is similar to sperm cells, they are not fully mature and lack both the motility and the ability required to bind to zona pellucida (ZP) and interact with egg cells (35).

By the time testicular spermatozoa enter the epididymal tubule, post-testicular maturation starts, accompanied by transcriptional and translational silencing (36). Given this translational dormancy, it seems clear that epididymal maturation of sperm is driven mainly by post-translational modifications (PTMs) (37;38). Modifications to sperm surface occur while spermatozoa reside in the epididymal fluid (secreted by epithelial cells) (39). The milieu surrounding spermatozoa is changing along the three regions of the epididymis (caput, corpus, cauda), in terms of protein content and chemical composition. Due to these changes, there is a progressive modification of most of the testicular proteins that are bound to the sperm membrane. These modifications include: (i) sperm surface protein removal by proteolysis, (ii) sperm surface protein processing and maturation, (iii) redistribution of proteins on the surface of the membrane, and finally (iv) transient or permanent integration of epididymal proteins into sperm membrane (40).

It is well-known that GPI-anchored proteins are a major component of the specialized membrane microdomains, called lipid rafts (41). Proteins that are found to be localized in lipid rafts are implicated in the recognition of ZP, the outer layer of the oocyte, by spermatozoa (42). Shedding of some proteins from the sperm surface is crucial for the sperm binding ability, and is attributed to two possible scenarios: (i) GPI-anchored proteins are activated after they are released from the sperm membrane; (ii) GPI-anchored proteins are cleaved to facilitate the sperm-ZP binding (43).

TEX101 is one of these GPI-anchored proteins and is cleaved from the sperm surface and released into the seminal fluid. Although it was proven that TEX101 is enzymatically shed from the surface of epididymal sperm (25), the enzyme involved in that process has not been known until recently.

Interestingly, Kondoh et al. (43) demonstrated that another GPI-anchored protein, testicular angiotensin-converting enzyme (tACE), is also expressed on the cell surface of testicular sperm and is shed from the sperm membrane during epididymal maturation (44). Furthermore, tACE, catalyzed shedding of GPI-anchored proteins from the sperm surface and affected the egg-sperm binding ability during fertilization. Combination of these two findings led to the assumption that tACE was responsible for the release of TEX101 during the epididymal transit. Kondoh's discovery regarding the novel GPI-ase activity of tACE came along with a number of 'debate articles' (45-48), that questioned the significance of the GPI-ase activity in reproduction, arguing that the well-known dipeptidase activity of ACE is crucial for sperm maturation and fertilization. A few years later, the same group (49) concluded that tACE had both GPI-ase and dipeptidase activities (43;47) which are required for sperm-ZP interaction. The dipeptidase activity takes place in the epididymis (50), and angiotensin II, the product of dipeptidase action, maintains pH homeostasis of the epididymal fluid (51). All in all, the dipeptidase activity of ACE indirectly supports its GPI-ase activity by maintaining an optimal pH (6.5) in the epididymis (49). All remaining doubts were unraveled recently by Fujihara et al. (28),

who proved with *Ace*^{-/-} mouse model that TEX101 is indeed cleaved from epididymal sperm by ACE. Furthermore, the effect of ACE (both isoforms, somatic and testicular) on TEX101 was also examined *in vitro*, and a good correlation between *in vitro* and *in vivo* data was found. Surprisingly, the removal of TEX101 by ACE occurred in a substrate-specific manner, while other testis-specific GPI-anchored proteins, like SPACA4 (27), remained unaffected both *in vivo* and *in vitro*. Failure in epididymal maturation of TEX101 is associated with infertility, as it was demonstrated by *in vivo* experiments with *Ace*^{-/-} and *TEX101*^{-/-} mouse models (28;29).

Role of soluble TEX101 in sperm-egg interaction

Following the first stage of maturation in the epididymis, sperm undergoes additional remodeling events by the accessory fluids secreted before and at the time of ejaculation. When sperm enters the female genital tract, it has to pass the cervix and the uterus, and eventually reach the oviduct. Three major regions comprise the oviduct: the uterotubular junction (UTJ), the isthmus, and the ampulla. Each one of these regions seems to play a distinct role, and all together they contribute to a successful fertilization. During this transit, sperm interacts with a number of components of the uterine and oviduct secretions, and in this way complete maturation is achieved (52). Although a large amount of ejaculated spermatozoa is initially released in the female tract, only a very low percentage will manage to migrate up to the UTJ, and enter the oviduct, while most sperm is eliminated from the female tract by other mechanisms (53). The significance of sperm migration through the UTJ during sperm transport has been recently demonstrated by Tokuhira et al. (54).

The UTJ connects the uterus with the oviduct and forms a natural barrier through which only

spermatozoa can pass (54;55). UTJ migration defect was always co-identified with failure of fertilization, associated with male infertility (55). There is evidence that sperm migration through the UTJ is under hormonal regulation, although the exact molecular basis of this transit is not well defined. Sperm from eight different knockout mice was analysed and showed UTJ transport deficiency. Null mice models for *Ace*, *Adam1a*, *Adam2*, *Adam3*, *Calr3*, *Clgn*, *Tpst2*, and *Pdilt* (56) revealed infertile phenotypes associated with sperm transport deficiencies. Surprisingly, proteomic analysis of all the mutant mouse lines mentioned, demonstrated that ADAM3, a sperm surface protein, is always absent or located incorrectly in the detergent-rich membrane domains (57). Although, initially, Shamsadin et al (58) reported that *Adam3*^{-/-} mice had no effect in sperm migration, a subsequent study by Yamaguchi et al (59), revealed the importance of ADAM3 for the oviductal migration. ADAM3 is now recognized as an essential factor for sperm transport through the UTJ.

TEX101 was recently added to the list of molecules which regulate ADAM3 function. Fujihara et al (28), proved that lack of TEX101 was associated with infertile phenotype. They moved one step further and confirmed that infertile phenotype resulted from the defect of sperm migration into the oviduct. Interestingly, spermatozoa directly delivered into the oviduct were capable to fertilize the oocyte. *Tex101*^{-/-} phenotype closely resembled the *Adam3*^{-/-} phenotype (59). By investigating ADAM3 in *Tex101*^{-/-} mice, it was shown that TEX101 interacts with ADAM3 on the surface of the testicular germ cells. Unlike the other proteins associated with ADAM3, TEX101 did not mediate the delivery of ADAM3 on sperm surface, since their interaction took place on sperm surface rather than in the endoplasmic reticulum (ER). During epididymal maturation of *Tex101*^{-/-} spermatozoa, ADAM3 was processed normally, but it was not resistant to degradation, suggesting that TEX101 was responsible to protect ADAM3 from proteases present in the epididymal fluid. In the cases of *Ace* deficiency, both proteins remained on sperm surface, but they were dissociated, as it was indicated by their distribution. All these findings were independently confirmed by Li et al. (29), who also found that the absence of TEX101 during maturation in the epididymis affected ADAM4, ADAM5, and ADAM6 proteins. ADAM proteins, known for their adhesion ability (60), accumulated on the testicular sperm, but failed to mature in *Tex101*^{-/-} sperm, leading to UTJ migration defect. All these facts allow us to conclude that cell adhesion properties of spermatozoa are as essential for sperm migration as their motility.

After passing through the UTJ, spermatozoa are binding to the epithelial cells of the isthmus, the second segment of the oviduct. It was proposed that such interaction mediated by the lectin-like proteins present on the sperm head and carbohydrate residues present on the oviductal epithelial cells (61), formed a reservoir of spermatozoa. Release of sperm occurs gradually (to allow only few spermatozoa to reach the oocyte), and is associated with sperm membrane remodeling, loss of binding molecules (capacitation), and hyperactivation of sperm motility (56).

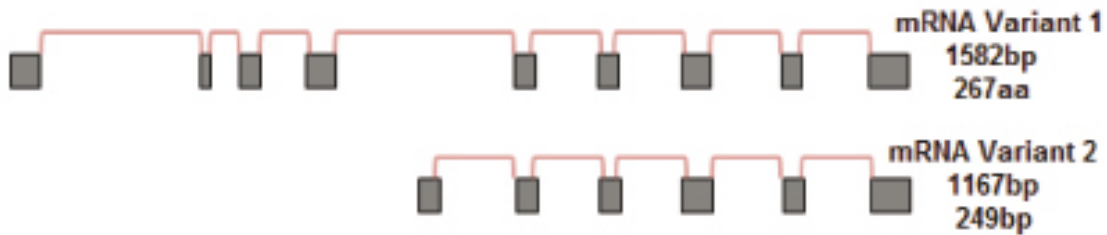
Figure 2

A

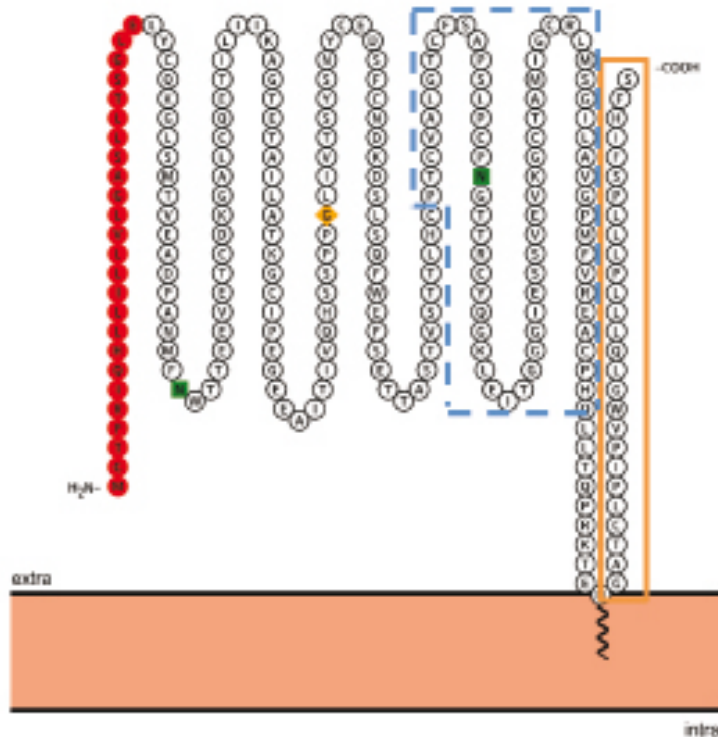
Location: 19q13.31
Sequence: Chromosome: 19; NC_000019.9 (43892763..43922767)



B



C



(Fig. 2 - cont'd) **Figure 2. Genomic, transcriptomic and proteomic organization of TEX101.**

(A) Chromosomal location, orientation and position on chromosome of TEX101 gene [<http://www.ncbi.nlm.nih.gov/gene/83639>]

(B) Structure of human TEX101 gene and its splice variants. Exons are represented with gray boxes.

(C) TEX101 protein structure. Signal peptide, post-translational modification sites and uPAR domain are highlighted in red, green and blue, respectively. GPI-anchored signal peptide is highlighted in yellow. SNV variants are marked with orange color.

The sperm release from the isthmus and its passage to the ampulla, the last region of the oviduct where fertilization takes place, is modulated by signaling between the cumulus-oocyte complex (COC), that is already located in the ampulla, and the epithelial cells of the oviduct (62). The cumulus is a layer of cells that surrounds and protects the oocyte. It is very important for the oocyte maturation, being the mediator of various signals. These cells interact not only with one another, but also with the oocyte (63). The ZP is a glycoprotein-enriched membrane surrounding the plasma membrane of the oocyte. It hosts a number of membrane glycoproteins responsible for sperm-binding upon fusion. It is of essential importance for successful fertilization, since failure of sperm-ZP binding is directly associated with infertility (64).

Gradient of temperature and chemoattractants such as progesterone guide the sperm towards the oocyte. Once spermatozoa are delivered, they have to cross the cumulus cells surrounding the oocyte (55;56). It was previously assumed that the acrosome reaction (AR), the release of hydrolytic enzymes and ZP penetration, is induced when sperm meets with ZP. However, it was recently demonstrated by Yin et al. (65;66), that the sperm-cumulus cross-talk modulates the AR while sperm is passing through the cumulus layer. Sun et al. (67), summarized recent data on the acrosome reaction, and the essential role of cumulus cells. Two potential models of AR with regard to its initiation were proposed. The first model suggests that AR occurs in the cumulus layer, and the hydrolytic enzymes released from spermatozoa facilitate the cumulus mass dispersion. This model, however, cannot explain the presence of acrosome-intact spermatozoa close to ZP. According to the alternative model, AR is initiated at the surface of ZP, and the “false-reacted” spermatozoa in the cumulus layer never reach it. Combining the information of all recent reports, it seems that ZP is not the only site of AR, but cumulus cells may also induce this process. A reasonable explanation could be that the acrosome reacted sperm may disperse the cumulus layer, so the intact sperm can pass through and bind to ZP. This means that a large number of sperm have to be sacrificed to facilitate the passage of a small population, which will participate in the fertilization process.

TEX101 has been recently identified in the cumulus-sperm interaction during the AR (65). The authors support that TEX101 was detected on mature spermatozoa, in contrast to most of the reports demonstrating that it disappears after epididymal maturation (25;28;29). To our understanding, this implication of TEX101 in sperm-cumulus cross-talk, could occur by the involvement either of soluble TEX101 that is present in the seminal plasma, and accompanies sperm while they travel in the female tract, or by a low level of protein that may remain bound to spermatozoa. In a study by Yin et al. (65), TEX101 role in sperm-cumulus interaction was suggested. TEX101 cleavage and binding to the surface of cumulus cells leads to Ca^{+2} mobilization and progesterone production by the cumulus, events that are known to induce AR and facilitate the penetration of the cumulus layer. In an attempt to study the role of cumulus cells in AR, the same group focused on identifying candidate molecules that mediated TEX101 binding on cumulus cells and induced Ca^{+2} mobilization (68). Based on their previous study of cancer cells (69), they have proved that TEX101 could bind to uPA/uPAR complex and interfere with uPA activity. The uPA system is known to be involved in signal transduction, apart from its serine protease activity, and is associated with Ca^{+2} mobilization (68). Cumulus cells express uPA/uPAR (70) and it is possible that TEX101 could interact with this complex on cumulus surface, triggering Ca^{+2} mobilization and progesterone release required for AR.

Concluding remarks

During the past decade there have been intense efforts not only to characterize novel germ cell specific proteins, such as TEX101, but also to elucidate their functions. Certain observations related to TEX101 role and its interactions with other molecules both in male and female reproductive tract still need to be confirmed and validated. For example, reliable and accurate translation of mouse data into humans should be made. Since TEX101 may emerge as a powerful male infertility biomarker, understanding its functional role will be essential.

Accumulating evidence based on animal models indicates that TEX101 interacts with various molecules during post-testicular maturation of spermatozoa, and interaction with oocytes. Some members of the ADAM family, ACE, and the components of uPA system are potential interacting partners of TEX101, but there is still much research to be done for shedding light in all these processes, and their contribution in fertilization. Since testicular and epididymal ADAMs play a crucial role in fertilization (71), further studies on their interaction with TEX101 should provide information about relevant molecular mechanisms. Phylogenetic analysis of ADAM proteins shows their crucial roles in reproductive biology (72). Identification of the human ADAM orthologs would be the next step in this field of research. The interaction of TEX101 with the uPA/uPAR complex is a completely independent field of research that needs further investigation. It seems that TEX101 and the uPA system components are not only co-expressed by sperm cells, but also co-exist in the oviduct and

are present during sperm-cumulus and sperm-ZP interaction. It has been reviewed (73) that uPA system components are secreted by Sertoli cells, and are involved in tissue remodeling during the last stages of spermatogenesis, facilitating the passage and release of spermatozoa from the Sertoli cell microenvironment within the seminiferous tubules. Different components of the uPA system are also present on the oocyte, cumulus cells, spermatozoa, and in the oviductal fluid where all the aforementioned meet and interact. Recently, Mondejar et al. (74), reviewed the regulation of fertilization by the uPA system components. They report that plasminogen/plasmin system is involved in sperm motility and AR, and its dysfunction leads to infertility in humans. It is also activated upon gamete interaction and it seems to regulate initially, the degradation and penetration of cumulus layer and ZP, through its localized proteolytic activity, and subsequently, the sperm entry in the oocyte, preventing polyspermy. If TEX101 indeed interacts with uPA, this would lead us to the conclusion that TEX101 is implicated in all the processes mentioned, and could mediate their regulation.

Bioinformatic analysis could be an alternative pathway to study TEX101 function and regulation. Apart from the proteomic level, modulation of TEX101 expression and regulation could be studied at the genomic, epigenetic, or transcriptomic level, using publically available databases of gene expression, such as COSMIC. This approach could lead to the identification of Single Nucleotide Variants (SNVs) that affect the expression and activity of TEX101. Additionally, the existence of testicular tissue-specific transcription factors (TF), or microRNAs would be of great importance in the regulation of TEX101 expression at the genomic and transcriptomic levels, respectively. All this information could eventually be useful in developing approaches for male infertility treatments, and development of male contraceptives.

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Review criteria:

The PubMed database was searched for full-text English-language articles published from 1942 to 2013 using the search terms "TEX101", "NYD-SP8", "ADAMs", "male infertility and biomarkers", "spermatogenesis", "epididymal maturation", "acrosome reaction", "cumulus-oocyte complex"

“fertilization”. The majority of articles included were published after 2005. The reference lists of selected articles were searched for further relevant publications.

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Table 1. Potential male infertility biomarkers. OA, obstructive azoospermia; NOA, non-obstructive azoospermia

Gene	Protein	Protein name	Clinical application	Reference
ECM1	ECM1_HUMAN	Extracellular matrix protein 1	OA vs. NOA	Drabovich et al.(19)
TKTL1	TKTL1_HUMAN	Transketolase-like protein 1	Azoospermia	Rolland et al. (75)
LDHC	LDHC_HUMAN	L-Lactate dehydrogenase C chain	OA/NOA vs. NS	Drabovich et al.(19)
PTGDS	PTGDS_HUMAN	Prostaglandin-H2 D-isomerase	OA diagnosis	Heshmat et al. (76)
PIP	PIP_HUMAN	Prolactin-inducible protein	Azoospermia	Davalieva et al. (77)
SPACA1	SPACA1_HUMAN	Sperm acrosome membrane-associated protein 1	Globozoospermia	Fujihara et al. (78)
SPAG11B	SG11B_HUMAN	Sperm associated antigen 11B	OA vs. NS	Drabovich et al.(19)
TEX101	TEX101_HUMAN	Testis-expressed sequence 101 protein	OA vs. NOA NOA subtypes	Drabovich et al.(19)
CAMP	CAMP_HUMAN	Cathelicidin antimicrobial peptide	OA vs. NOA	Drabovich et al.(19)
AZU1	CAP7_HUMAN	Azurocidin	Infertility	Kumar et al.(79)