

UCSF

UC San Francisco Previously Published Works

Title

Gonadoblastoma locus and the TSPY gene on the human Y chromosome

Permalink

<https://escholarship.org/uc/item/1tw451dw>

Journal

Birth Defects Research Part C Embryo Today Reviews, 87(1)

ISSN

1542-975X

Authors

Lau, Yun-Fai Chris

Li, Yunmin

Kido, Tatsuo

Publication Date

2009-03-01

DOI

10.1002/bdrc.20144

Peer reviewed

Gonadoblastoma Locus and the TSPY Gene on the Human Y Chromosome

Yun-Fai Chris Lau,* Yunmin Li, and Tatsuo Kido

The gonadoblastoma (GBY) locus is the only oncogenic locus on the human Y chromosome. It is postulated to serve a normal function in the testis, but could exert oncogenic effects in dysgenetic gonads of individuals with intersex and/or dysfunctional testicular phenotypes. Recent studies establish the testis-specific protein Y-encoded (TSPY) gene to be the putative gene for GBY. TSPY serves normal functions in male stem germ cell proliferation and differentiation, but is ectopically expressed in early and late stages of gonadoblastomas, testicular carcinoma in situ (the premalignant precursor for all testicular germ cell tumors), seminomas, and selected nonseminomas. Aberrant TSPY expression stimulates protein synthetic activities, accelerates cell proliferation, and promotes tumorigenicity in athymic mice. TSPY binds to type B cyclins, enhances an activated cyclin B-CDK1 kinase activity, and propels a rapid G₂/M transition in the cell cycle. TSPY also counteracts the normal functions of its X-homologue, TSPX, which also binds to cyclin B and modulates the cyclin B-CDK1 activity to insure a proper G₂/M transition in the cell cycle. Hence, ectopic expression and actions of the Y-located TSPY gene in incompatible germ cells, such as those in dysgenetic or ovarian environments and dysfunctional testis, disrupt the normal cell cycle regulation and predispose the host cells to tumorigenesis. The contrasting properties of TSPY and TSPX suggest that somatic cancers, such as intracranial germ cell tumors, melanoma, and hepatocellular carcinoma, with detectable TSPY expression could exhibit sexual dimorphisms in the initiation and/or progression of the respective oncogenesis. **Birth Defects Research (Part C) 87:114–122, 2009. © 2009 Wiley-Liss, Inc.**

Key words: TSPY; TSPX; gonadoblastoma gene; Y chromosome; cell cycle regulation; sexual dimorphism

Y CHROMOSOME AND HUMAN ONCOGENESIS

The Y chromosome is a male-specific chromosome in the human genome. It contains genes that serve vital functions in sex determination and physiology of male-specific organs, such as the testis

and prostate (Skaletsky et al., 2003). The involvement of this chromosome in oncogenesis has long been observed among intersex patients, including 46,XY sex-reversed females, 46,XY pseudohermaphrodites, mixed gonadal dysgenesis (45,X/46,XY mosaic), and a subpopulation of 45,XO

Turner patients (Verp and Simpson, 1987; Bianco et al., 2006; Looijenga et al., 2007; Schoemaker et al., 2008). These individuals harbor a whole or part of the Y chromosome, but either lack a functional sex determining gene, SRY, in their genome or suffer from mutations affecting other genes in the sex determination pathway. There is a prevalence of gonadoblastoma among these patients with a frequency as high as 30%–66% (Scully, 1953, 1970; Verp and Simpson, 1987). About 80% of these gonadoblastoma patients are phenotypic females while the remainder are males. Gonadoblastomas are premalignant forms of germ cell tumors consisting of aggregates of large, round germ cells, and immature Sertoli/granulosa cells within an ovarian type stroma, and if untreated could develop into more aggressive germ cell tumors, such as dysgerminoma in females and seminoma and nonseminoma in males. In 1987, David Page (1987) hypothesized that there is a locus, termed gonadoblastoma on the Y chromosome (GBY), which predisposes the dysgenetic gonads of the intersex patients to develop such in situ tumors. He further predicted that the GBY gene has a normal function in the

Yun-Fai Chris Lau, Yunmin Li, and Tatsuo Kido are from the Division of Cell and Developmental Genetics, Department of Medicine, VA Medical Center, University of California, San Francisco, San Francisco, California 94121.

Grant sponsors: Congressionally Directed Biomedical Research in Prostate Cancer of the Department of Defense, Research Enhancement Award in Prostate Cancer, Merit Award of the Department of Veterans Affairs.

*Correspondence to: Chris Lau, Department of Medicine, VA Medical Center, 111C5, University of California, San Francisco, 4150 Clement Street, San Francisco, CA 94121. E-mail: chris.lau@ucsf.edu

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bdrc.20144

testis and acts as an oncogene only in the dysgenetic gonads. Deletion mapping narrowed the location of the GBY locus to a small region on the short arm and proximal region on the long arm of the Y chromosome (Page, 1987; Salo et al., 1995; Tsuchiya et al., 1995). Recent studies in our laboratory and others (Gallagher et al., 2005; Yin et al., 2005; Hoei-Hansen et al., 2006) suggest that the testis-specific protein Y-encoded (TSPY) repeated gene is the putative gene for this oncogenic locus and could potentially be involved in other human cancers, including brain tumors, hepatocellular carcinoma, and melanoma.

PROPERTIES OF TSPY

The TSPY gene is one of the early genes identified from the human Y chromosome. Initial studies demonstrated that the predominant 1.3-kb transcripts were derived from transcriptional units of 2.8-kb in size consisting of 6 exons and 5 introns (Arneemann et al., 1987, 1991; Zhang et al., 1992; Schnieders et al., 1996). The completion of the sequencing of the male-specific region of the human Y chromosome (MSY) shows that the TSPY structural gene is embedded in a 20.4-kb unit that is tandemly repeated ~35 times on GBY critical region (Skaletsky et al., 2003). These repeat units show >98% sequence homology, both among TSPY transcriptional units and their flanking sequences, and constitute the largest and most homologous protein-coding tandem array, so far identified in the human genome. The TSPY repeat cluster shows copy number variation (CNV) among the general populations, ranging from 23 to 64 repeat units and encompassing 0.47 to 1.3 Mb of DNA, and ranks top on length changes among various key segments of the human Y chromosome (Repping et al., 2006). Significantly, CNVs are also observed among prostate cancer patients (Vijayakumar et al., 2006), suggesting that the TSPY repeat

blocks could potentially be hotspots of genomic instability under diseased conditions.

The TSPY gene is located on the Y chromosome of all placental mammals, such as the primates and artiodactyls (Vogel et al., 1997). Similar to the human situation, they are repetitive genes on this male-specific chromosome. The rat, however, has only one functional Tspy gene on its Y chromosome (Dechend et al., 1998; Mazeyrat and Mitchell, 1998). In the murines, several *Apodemus* species and *Mus platythrix* possess a functional Tspy gene while other species in the *Mus* subgenus, including the laboratory mouse (*Mus musculus*), harbor an apparently nonfunctional (with in-frame and splice junction mutations) Tspy gene on their Y chromosomes (Schubert et al., 2000a,b). Several TSPY-like (TSPYL) genes have been identified on the autosomes and X chromosome of human and various mammalian genomes (Vogel et al., 1998; Dechend et al., 2000). It is conceivable that some of these Tspy-like genes might serve similar functions in place of the nonfunctional Tspy gene on the mouse Y chromosome.

The predominant TSPY isoform is a 38-kDa phosphoprotein containing 308 amino acids (Schnieders et al., 1996; Lau et al., 2003). It harbors a conserved domain, designated as SET/NAP domain consisting of ~160 amino acids, initially identified in the product of the SET oncogene and nucleosome assembly protein-1 (NAP-1) (von Lindern et al., 1992; Adachi et al., 1994; Tsuchiya et al., 1995; Lau, 1999; Ozbun et al., 2001). TSPY, SET, and NAP1 are the founding members of a protein family designated as TSPY/SET/NAP (Vogel et al., 1998; Lau, 1999; Ozbun et al., 2001). The TSPY transcriptional units are capable of producing a variety of transcripts through alternative splicing events (Lau et al., 2003). All variant TSPY transcripts are in-frame with the predominant transcript and encode a panel of polymorphic

TSPY proteins, which harbor the conserved SET/NAP domain at their carboxyl termini. Members of the TSPY/SET/NAP protein family serve a diverse spectrum of functions, including DNA replication, transcription modulation, chromatin modeling, and cell cycle regulation (Chai et al., 2001; Ozbun et al., 2001; Seo et al., 2001; Ayyanathan et al., 2003; Canela et al., 2003; Kandilci et al., 2004; Vera et al., 2005; Oram et al., 2006; Muto et al., 2007). Mutations and/or dysregulation of members of this gene family have been associated with various forms of human cancers, such as leukemia (von Lindern et al., 1992; Adachi et al., 1994; Nagata et al., 1995), lung cancer (Ozbun et al., 2001), melanoma (Gallagher et al., 2005), hepatocellular carcinoma (Yin et al., 2005), intracranial germ cell tumor (Hoei-Hansen et al., 2006), gonadoblastoma, testicular germ cell tumors, and prostate cancer (Schnieders et al., 1996; Hildenbrand et al., 1999; Lau, 1999; Lau and Zhang, 2000; Dasari et al., 2001; Lau et al., 2003; Li et al., 2007a,b).

Six TSPY-like genes have been identified among human and various mammalian genomes. In particular, one, called TSPY-Like2 (TSPYL2), is localized on the X chromosome of all mammals examined (Lau et al., 2007). It has been mapped to a syntenic region of the X chromosome harboring other Y-homologous sequences/genes and shares significant homology with TSPY including exon/intron junctions and gene organization. Based on these properties, TSPYL2 has recently been designated as the TSPY X-homologue (TSPX) gene (Delbridge et al., 2004; Lau et al., 2007). The single-copy TSPX gene is about 6.3-kb in size and consists of 7 exons. It encodes two protein isoforms of 667 and 693 amino acids, respectively. Both isoforms harbor the SET/NAP domain, but different at their N-termini due to utilization of different in-frame initiation codons. TSPX shares high homology with TSPY at their SET/NAP and flanking

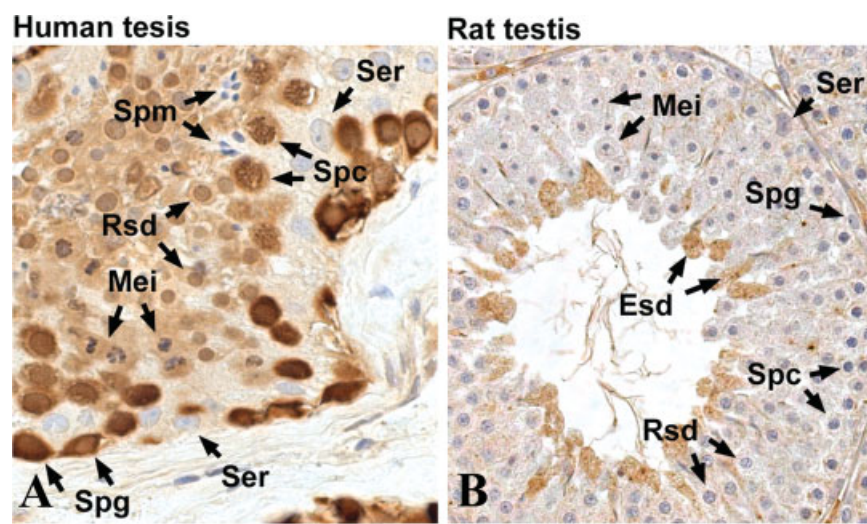


Figure 1. Immunostaining patterns of human TSPY and rat Tspy in testis using specific antibodies against the respective protein (Kido and Lau, 2005, 2006). (A) Human TSPY is primarily expressed in the spermatogonia, spermatocytes, and to a lesser extent, spermatids in the human testis. (B) Rat Tspy is mainly expressed in elongated spermatids in the rat testis. Spg, spermatogonia; Spc, spermatocytes; Ser, Sertoli cells; Rsd, round spermatids; Spm, sperm; Mei, meiotic cells; Esd, elongated spermatids. Bar represents 30 and 50 microns in A and B, respectively.

domains, but harbors a N-terminal proline-rich domain and a carboxyl bipartite acidic domain, which are absent in TSPY. We hypothesize that TSPY and TSPX were derived from a common ancestral gene. They diverged from each other during the evolution of the mammalian sex chromosomes, in which TSPX maintained similar structure and organization of the primordial gene while TSPY lost its N-terminal proline-rich domain and carboxyl acidic domain before it was amplified on the Y chromosome. The autosomal TSPY-like genes are single-exon genes and could be derived from retrotransposition events involving TSPY transcripts (Delbridge et al., 2004; Lau et al., 2007). Significantly, mutations of TSPY-L1 on chromosome 6 result in truncation of the SET/NAP domain and have been demonstrated to be responsible for the sudden infant death with dysgenesis of the testis syndrome (Hering et al., 2006). Hence, the TSPY-like genes might have acquired important biological functions and were evolutionarily retained as functional genes in the human genome. TSPY, TSPX, and TSPY-like genes possess unique gene structures,

genomic organization, and protein properties, and hence could be considered as a subfamily of the TSPY/SET/NAP protein family (Lau et al., 2007).

FUNCTIONS OF TSPY AND TSPX GENES

Currently, the exact functions of TSPY and TSPY-like genes are uncertain. The human TSPY is primarily expressed in prespermatogonia/gonocytes of embryonic testis and spermatogonia, spermatocytes, and to some extent, round spermatids in adult testis (Hon-ecker et al., 2004; Kido and Lau, 2005) (Fig. 1A). It is postulated to serve important functions on the proliferation of both embryonic prespermatogonia/gonocytes and adult spermatogonial cells, the pluripotent stem cells of the male reproductive system (Lau, 1999). It binds both mitotic and meiotic cyclin Bs and might serve additional functions in mitotic and meiotic divisions (Lau, 1999). Interestingly, the single-copy rat Tspy gene is primarily expressed in elongated spermatids, rather than spermatogonia (Kido and Lau, 2006) (Fig. 1B), suggesting it

might serve separate function(s) apart from those ascribed to the human TSPY. Indeed, additional studies showed that the rat Tspy is capable of binding to histones in the cytoplasm of spermatids. These observations suggest that Tspy could serve as a chaperone for histones that are being displaced by protamines during spermiogenesis (Mezquita, 1985; Ivanovska and Orr-Weaver, 2006) and are sequestered by Tspy in the cytoplasm of elongated spermatids (Kido and Lau, 2006). The human TSPY is potentially capable of similar chaperone function, because its protein could also be localized in spermatids, albeit at reduced levels (Kido and Lau, 2005). Hence, if the human TSPY serves multiple functions in both the propagation and differentiation of the stem germ cells of the male reproductive system, these observations suggest that some of these TSPY functions have been replaced by other genes, probably the Tspy-like genes, in the rodents.

TSPX is an evolutionarily conserved gene on the X chromosomes of most mammals (Lau et al., 2007). It has been isolated by several laboratories using various strategies, and has been designated with different names, including cell division autoantigen 1 (CDA1) (Chai et al., 2001), differentially expressed nucleolar TGF- β 1 target (DENTT) (Ozbun et al., 2001), and CASK interacting nucleosome assembly protein (CINAP) (Wang et al., 2004) by three laboratories that studied this gene in detail. Collectively, these studies supported the notation that TSPX is an important and broadly expressed gene that serves vital and multiple functions in modulating cell cycle progression and transcriptional regulation. TspX/Dentt is expressed early in the heart and the primitive brain in the mouse E8 embryos, particularly in the choroids plexus, spinal cord, and dorsal root ganglia (Ozbun et al., 2003). Such expression is later extended to other embryonic organs. In adult, TspX/Dentt is expressed intensely in the

pituitary gland; islets of Langerhans in the pancreas; choroid plexus, cortical motoneurons, and Purkinje cells in the brain; Leydig and Sertoli cells and spermatocytes in the testis; and oocytes, thecal cells, and corpora lutea in the ovary, among other tissues (Martinez et al., 2002). Significantly, Tsp/Cinap is widely expressed in various regions of the mouse brain, including cerebral cortex, hippocampus, striatum, hypothalamus, cerebellum, and the ventricular and subventricular zones, two regions with actively dividing neuronal progenitor cells (Lin et al., 2006). The mouse Tsp/Cinap protein is associated with the synapse and is capable of translocating to the nucleus, where it modulates gene regulation by interacting with CASK, a coactivator for Tbr-1, an essential transcription factor in cerebral development. Tsp/Cinap binds with CASK, forms a transcriptional complex with Tbr-1, and participates in the chromatin dynamics involved in the regulation of Tbr-1 target genes (Wang et al., 2004).

Overexpression of TSPX/CDA1 in HeLa cells retards cell cycle progression, primarily arresting cells at the G₂/M stage (Chai et al., 2001). TSPX induces p53- and MEK/ERK1/2 MAPK-dependent expression of the growth inhibitor gene, p21^{Waf1/Cip1}, by acting through the p53 response element on the promoter of the p21^{Waf1/Cip1} gene, thereby exerting its inhibitory effects on cell proliferation (Tu et al., 2007). Ectopic expression of TSPX/DENTT in human lung and breast cancer cells diminishes cell growth, migratory potential, and capacity to form colonies under anchorage-independent culture conditions (Kandalaf et al., 2008). Hence, TSPX/DENTT has been postulated to be a tumor suppressor gene (Kandalaf et al., 2008). Together with its expression patterns in normal tissues, these observations suggest that TSPX could play normal and multi-functional roles in modulating an orderly progression of the cell cycle in proliferating cells,

maintaining the nonproliferative states of terminally differentiated cells, and participating in gene regulatory mechanisms through partnering with other transcription factors and cofactors.

TSPY AS THE GENE FOR GBY

As predicted by Page (1987), the GBY gene(s) serves a normal function in the testis, but acts as an oncogene only when it is present and expressed in dysgenetic gonads. Recent studies in our laboratory and those of others have clearly established the candidacy of TSPY as the gene for GBY. First, it is expressed in fetal prespermatogonia/gonocytes and adult spermatogonia, and is likely to serve vital normal functions in male stem germ cell proliferation and meiotic division (Schnieders et al., 1996; Honecker et al., 2004; Kido and Lau, 2005). This fulfills the normal function requirement, as suggested for GBY. Second, it is mapped to the critical region for GBY on the short arm of the Y chromosome (Salo et al., 1995; Tsuchiya et al., 1995). This critical region harbors primarily the tandem and functional repeat units of the TSPY gene (Skaletsky et al., 2003). Third, it is ectopically and abundantly expressed not only in gonadoblastoma tissues, but also testicular germ cell tumors (TGCTs), including the premalignant precursor (CIS), seminoma, and selected nonseminomas (Kersemaekers et al., 2005a; Li et al., 2007a,b). These observations suggest that TSPY could play a key role(s) in the early development of germ cell tumors. Indeed, various stages of gonadoblastoma development could be reconstituted morphologically from some gonadoblastoma specimens, which paint a picture of TSPY-positive tumor germ cells arising from the periphery of follicular structures in a dysgenetic gonad (Li et al., 2007b). The TSPY-positive cells take on a proliferative growth that eventually fills up the entire follicular structures and forms nests of tumor germ cells, characteristic of

a gonadoblastoma. Fourth, TSPY expression is tightly correlated with those of established tumor germ cell markers, such as placental alkaline phosphatase, OCT3/4 and c-KIT, in gonadoblastomas, CIS, and seminomas (Kersemaekers et al., 2005b; Li et al., 2007a,b). As CIS is the premalignant precursor for all TGCTs (Rajpert-de Meyts and Hoei-Hansen, 2007; Hersmus et al., 2008; Kristensen et al., 2008), the abundant expression of TSPY in CIS suggests that it could also play a key role(s) in early stages of the tumorigenesis of these types of germ cell tumors.

TRANSGENIC MOUSE STUDIES OF TSPY

As gonadoblastoma occurs mostly in the dysgenetic gonads of phenotypic females and, as the GBY gene, TSPY must be functional in germ cells in female environments at or before tumorigenesis occurs in order to exert its oncogenic actions in the process(es). Establishing a transgenic model of gonadoblastoma in mice with TSPY transgene has been a difficult, if not daunting, task. Despite repeated efforts by us and others, only one transgenic mouse line has been successfully established in the laboratory of Stephanie Schubert and Jörg Schmidtke (Schubert et al., 2003), in which ~50 copies of an 8.5-kb human genomic DNA consisting of a functional TSPY gene were integrated in the Y chromosome of the host animal. This transgenic mouse line resembles the human TSPY gene cluster on the Y chromosome. The TSPY transgenes are properly regulated and primarily expressed in the spermatogonial cells in adult testis. The transgenic mice, however, are phenotypically normal and do not develop any tumors at high incidence (Schubert et al., 2003, 2008). Currently, it is uncertain why transgenic mouse lines harboring the human structural gene (promoter, gene and flanking sequences) are difficult to establish using the conventional microinjection of fertilized mouse

eggs. It is conceivable that TSPY transgene integrated in the non-Y chromosomes could be dysregulated in the host and ectopic TSPY expression might be detrimental to normal development. Nevertheless, limited success has been achieved to support the notion that TSPY is the GBY gene.

To address the issue on whether TSPY is capable of expression in ovary, we had established a Cre-LoxP transgene activation system and demonstrated that a 2.4-kb promoter of human TSPY was capable of directing the expression of a reporter transgene in the ovarian follicular oocytes and testicular germ cells of transgenic mice (Kido and Lau, 2005), confirming the functionality of its promoter in testicular and ovarian environments. In addition, we had also generated 8 founding transgenic mice (5 males and 2 females) harboring a 12.5-kb region of human Y-DNA containing the 2.8-kb TSPY structural gene. All founders were fertile, but could not pass on their transgene to their offspring, despite analysis of ~200 pups from various crosses. On the other hand, transgenic mice harboring a modular TSPY gene directed by the human kallikrein 2 (HK2) promoter (Xie et al., 2001; Seethammagari et al., 2006) are capable of transmitting the transgenes to their offspring and expressing their transgenes specifically in the epithelial cells of their prostates (Kido and Lau, unpublished observations). Studies of our founders harboring the 12.5-kb TSPY transgene demonstrated that the human gene was expressed in spermatogonia and spermatocytes in the testes of males. Selected areas showed signs of atrophy or vacuolarization morphology of their testes. Significantly, one female founder (~1.5 yr old) showed aggregates or nests of abnormal cells in her ovaries, many of which expressed the TSPY transgene at high levels (Fig. 2A–D), similar in morphology and TSPY expression to tumor cells in gonadoblastoma of human patients (Fig. 2E–F). To the best of our knowledge, gonadoblasto-

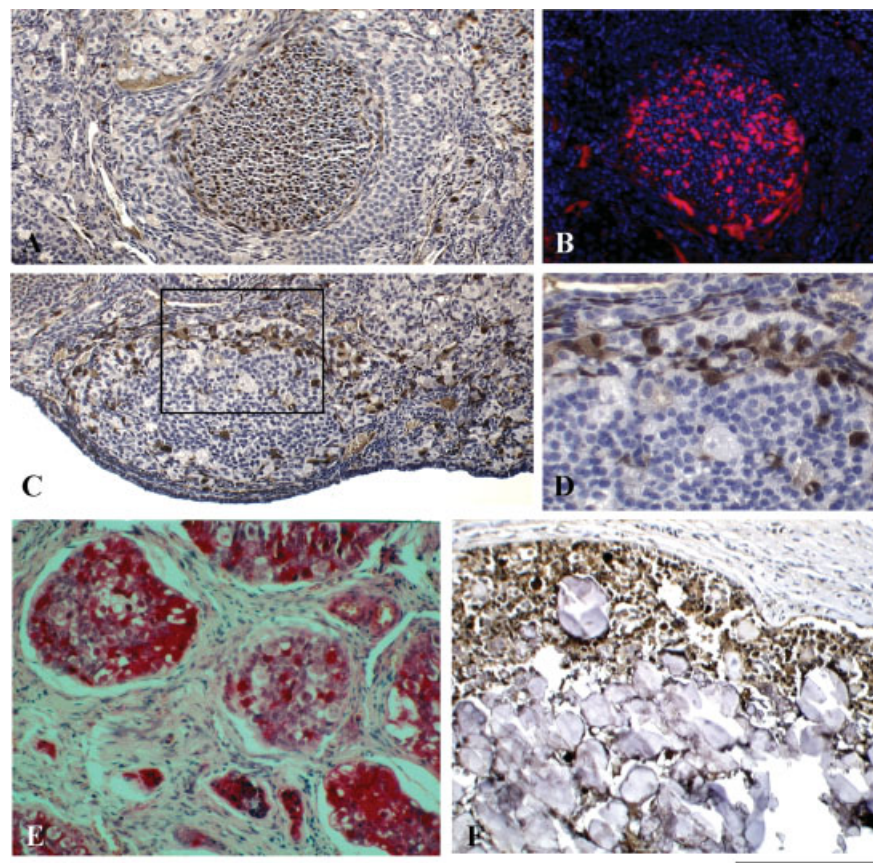


Figure 2. Gonadoblastoma-like structures in ovaries of a female transgenic mouse harboring a 12.5-kb human TSPY transgene. Nested structures that expressed human TSPY transgene products (A,B) could be detected with immunostaining (A) and immunofluorescence (B) with human TSPY antibodies, resembling the immunostaining pattern of a gonadoblastoma specimen (E) (Lau et al., 2000). Another follicular structure showed TSPY expression along the periphery (C,D), resembling the expression pattern of TSPY in another gonadoblastoma specimen (F) (Li et al., 2007b). Boxed area in C is enlarged in D. Bar represents 200 microns in A–C, and E,F; 100 microns in D.

mas or type II germ cell tumors have never been reported for the laboratory mouse (Oosterhuis and Looijenga, 2005; Looijenga and Oosterhuis, 2007). Most mouse testicular cancers are of teratoma and/or embryonal carcinoma origins (Matin et al., 1998; Cools et al., 2005; Youngren et al., 2005). The present results, although preliminary in nature, are significant. First, they confirm that the human TSPY promoter and the structural TSPY gene are functional in ovarian germ cells. This is a new finding because the Y-located transgenes in the Schubert-Schmidtke line transmits only paternally; the transgene never transmits to females (Schubert et al., 2003). Second, the human TSPY transgene in one of

our founders could be epigenetically dysregulated specifically in ovarian cells and develop structures resembling gonadoblastoma. These observations, therefore, support the hypothesis that ectopic expression of TSPY in ovaries promotes germ cell tumorigenesis and gonadoblastoma development.

POSSIBLE MECHANISMS OF TSPY ONCOGENIC FUNCTIONS

We hypothesize that TSPY and TSPX are evolved from the same ancestral gene, but have diverged substantially in their respective functions (Lau et al., 2007). TSPX serves essential and normal func-

tions in modulating cell cycle progression and transcription regulation while TSPY has acquired specialized functions in male stem germ cell proliferation and meiotic division, among others. Because of their structural differences, apart from their conserved SET/NAP domain, ectopic expression of TSPY in incompatible cells, such as susceptible germ cells in dysgenetic/female gonad and/or dysfunctional testis, could counteract TSPX functions, thereby exerting proliferative and oncogenic actions on these germ cells. Alternatively, TSPY could act independently and induces proliferation of susceptible cells.

To support this hypothesis, we have demonstrated that TSPY interacts with the eukaryotic translation elongation factor 1A, eEF1A, and enhances protein synthesis in the host cells (Kido and Lau, 2008). Since elevated levels of protein synthesis are characteristics of actively growing and/or replicating cells, such TSPY enhancement of protein synthesis could exacerbate the proliferative properties and predispose host cells to oncogenic actions. Further, we have shown that overexpression of TSPY accelerates cell cycle progression and shortens the G₂/M phase in transfected cells (Oram et al., 2006). This is in contrast to the observations on TSPX, where overexpression of TSPX retards cell cycle progression at the G₂/M stage (Chai et al., 2001; Kandalaft et al., 2008). Microarray analysis of gene expression between cells overexpressing and repressing TSPY shows that various oncogenes and progrowth genes, including some of those located on chromosome 12p, are up-regulated in TSPY overexpressing cells (Oram et al., 2006; Li et al., 2007a). As selected genes on chromosome 12p are either amplified or up-regulated in some TGCTs, particularly on seminomas (Looijenga et al., 2003; Oosterhuis and Looijenga, 2005), such up-regulation of progrowth genes by TSPY supports the postulation that ectopic expression of TSPY could contribute to germ cell tu-

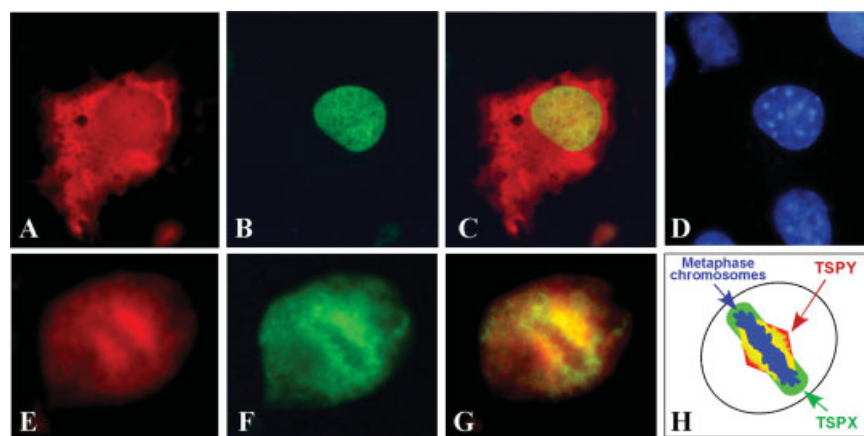


Figure 3. Distribution of TSPY and TSPX in interphase and metaphase cells. COS7 cells were cotransfected with constitutively expressed TSPY and FLAG-tagged TSPX constructs, and analyzed by immunofluorescence with specific antibodies against TSPY (A, red) and FLAG-TSPX (B, green). TSPY was localized in both cytoplasm and nucleus while TSPX was primarily localized in the nucleus of interphase cells. Other studies suggested that TSPX could be distributed in both cytoplasm and nucleus in some cells and tissues (Chai et al., 2001; Martinez et al., 2002; Ozbun et al., 2003; Lin et al., 2006). During metaphase, TSPY (E) and TSPX (F) were mainly distributed around the metaphase plate. TSPX seemed to occupy a more extensive location. The merged image (G) showed that both proteins overlapped extensively around the metaphase plate. Previously, we demonstrated that TSPY was co-localized with tubulin on the mitotic spindles (Li and Lau, 2008); its overlapping distribution with TSPX suggested that these two proteins might exert yet-to-be defined effects on this organelle during mitosis. C, merged image of A and B; D, DAPI staining; H, diagrammatic illustration of TSPY and TSPX distribution in the metaphase cell in E-G.

morigenesis. Indeed, cells overexpressing TSPY are capable of forming tumors in nude mice at accelerated rates than those without TSPY expression (Oram et al., 2006). Therefore, these results support the notation that ectopic expression of TSPY favors cell proliferation and growth, and predisposition to carcinogenesis.

The contrasting effects of TSPY and TSPX on the G₂/M stage of the cell cycle are intriguing. As SET has been demonstrated to interact with cyclin Bs (Canela et al., 2003), we have conducted a series of studies designed to evaluate if TSPY and/or TSPX indeed could interact with this type of cyclins and what effects such interactions might have on the functions of cyclin B-cyclin dependent kinase 1. Our results demonstrated that both TSPY and TSPX are capable of binding to cyclin B1, B2, and B3, but not cyclin A, via their SET/NAP domain in vitro and in vivo (Li and Lau, 2008). TSPY enhances while TSPX represses the phosphorylation activities of an activated cyclin B1-CDK1 complex in

cultured cells in vitro. The inhibitory domain of TSPX has been mapped to its carboxyl acidic domain, which is absent in TSPY. Retrofitting this acidic domain to the carboxyl terminus of TSPY renders the chimera molecule to be inhibitory on cyclin B1-CDK1 activities (Li and Lau, 2008). Immunofluorescence shows that TSPY is preferentially localized to the mitotic spindles of the metaphase cells (Li and Lau, 2008). While TSPX takes on a similar, but relatively more disperse, location around the metaphase plate (see Fig. 3). Currently, we are uncertain on what functions TSPY and TSPX might have on the mitotic spindles. Their contrasting effects on the cyclin B1-CDK1 kinase activities suggest that TSPY and TSPX might exert opposite functions on this mitotic organelle, such as phosphorylation of the spindle or kinetochore components, resulting in accelerating and retarding the G₂/M transition, respectively, in the cell cycle when they are ectopically expressed in the host cells (see Fig. 4). We

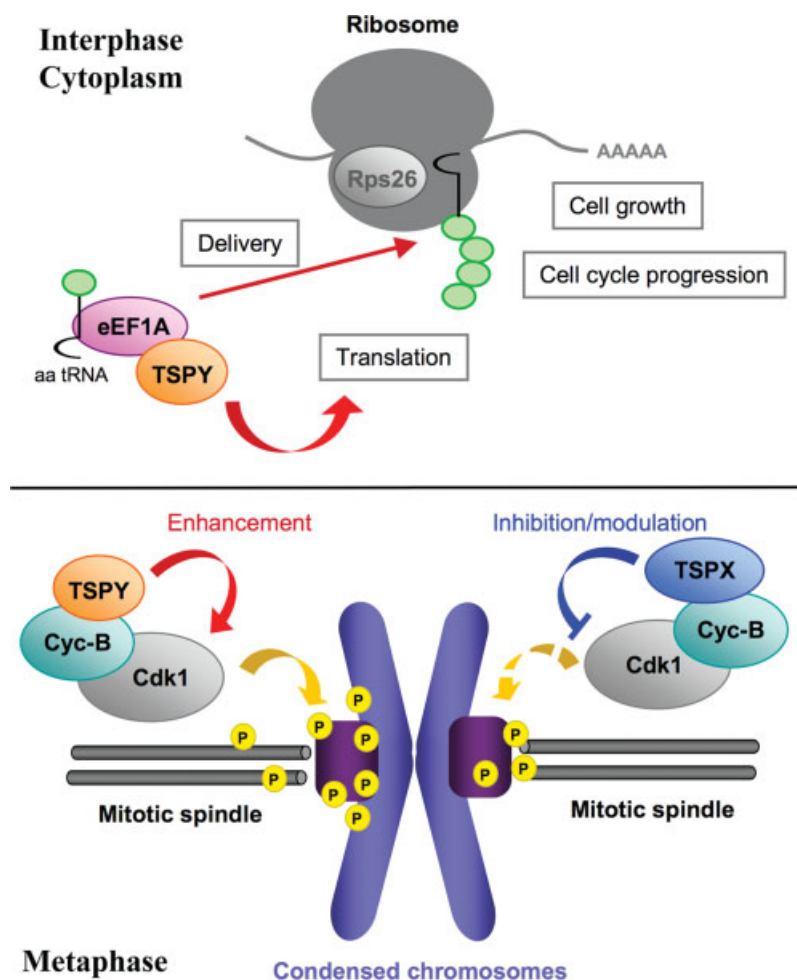


Figure 4. Postulated functions of TSPY on cell growth and proliferation. TSPY interacts with the eukaryotic elongation factor 1A (eEF1A) and enhances the protein synthetic activities, thereby promoting cell growth and cell replication (Top). During mitosis, TSPX regulates the proper cell cycle progression through metaphase by binding to cyclin B and modulating/inhibiting the Cdk1 phosphorylation of components of the mitotic spindle and kinetochore (Bottom). Ectopically expressed TSPY competitively binds to cyclin B and enhances the Cdk1 kinase activities, thereby accelerating a rapid transition of the G₂/M phase and shortening the duration of the cell cycle.

surmise that TSPX could be important in modulating an orderly transition of the G₂/M stage by properly regulating the checkpoints, while TSPY could abolish or circumvent the same checkpoints, thereby accelerating a quick transition in this phase of the cell cycle. Since G₂/M checkpoints are critical for the cells to properly prepare for mitotic division after DNA replication in the S phase, any abolishment of G₂/M checkpoint(s) could result in chromosome nondisjunction in the subsequent mitosis, thereby increasing the potential for chromosome rearrangements and

genomic instability, and tumorigenesis (Smits and Medema, 2001; Pietsenpol and Stewart, 2002; Kastan and Bartek, 2004; Houtgraaf et al., 2006; Knauf et al., 2006; Lobrich and Jeggo, 2007; Schmit and Ahmad, 2007; Decordier et al., 2008).

Y CHROMOSOME GENES AND SEXUAL DIMORPHISMS IN HEALTH AND DISEASES

The contrasting properties of TSPY and TSPX have raised an interest-

ing question on the roles of Y chromosome genes in human physiology and diseases (McBride et al., 2002; Fairbairn and Roff, 2006; Gutierrez-Adan et al., 2006; Lleo et al., 2008). TSPY, like many of the genes residing on the male-specific region on the Y chromosome (MSY), plays important roles for male germ cell development and differentiation. Ectopic or even normal expression of MSY genes (Skaletsky et al., 2003) could exert sexually dimorphic effects on the host cells or tissues in both spatial and temporal manners during embryonic development and postnatal life. The peculiar structural arrangement of the TSPY gene array (Skaletsky et al., 2003) has greatly exaggerated its potential in contributing to such sexual dimorphisms in human diseases. Indeed, TSPY had been demonstrated to express in not only male-specific cancers, i.e., TGCTs and prostate cancer (Lau et al., 2000, 2003; Li et al., 2007a,b), but also intracranial germ cell tumors, melanoma, and hepatocellular carcinoma (Gallagher et al., 2005; Yin et al., 2005; Hoei-Hansen et al., 2006). These studies showed that TSPY expression was most frequently observed among cancer samples at early stages than those at late stages of the respective oncogenic processes (Gallagher et al., 2005; Yin et al., 2005). Since TSPY exerts contrasting effects on TSPX, an essential housekeeping gene (Li and Lau, 2008), such ectopic TSPY expression could bring about certain aspects of sexual dimorphisms on respective tumorigenesis of these somatic cancers. Similarly, epigenetic dysregulation of any of the MSY genes could potentially disrupt the normal functions of respective autosomal or X-located homologues/analogues, thereby contributing to a diverse spectrum of sexual dimorphic and quantitative traits in health and diseases.

ACKNOWLEDGMENTS

Y-FC Lau is a Research Career Scientist of the Department of Veterans Affairs.

REFERENCES

- Adachi Y, Pavlakakis GN, Copeland TD. 1994. Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. *J Biol Chem* 269:2258–2262.
- Arnemann J, Epplen JT, Cooke HJ, et al. 1987. A human Y-chromosomal DNA sequence expressed in testicular tissue. *Nucleic Acids Res* 15:8713–8724.
- Arnemann J, Jakubiczka S, Thuring S, et al. 1991. Cloning and sequence analysis of a human Y-chromosome-derived, testicular cDNA, TSPY. *Genomics* 11:108–114.
- Ayyanathan K, Lechner MS, Bell P, et al. 2003. Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: A mammalian cell culture model of gene variegation. *Genes Dev* 17:1855–1869.
- Bianco B, Lipay MV, Melaragno MI, et al. 2006. Detection of hidden Y mosaicism in Turner's syndrome: Importance in the prevention of gonadoblastoma. *J Pediatr Endocrinol Metab* 19:1113–1117.
- Canela N, Rodriguez-Vilarrupla A, Estanyol JM, et al. 2003. The SET protein regulates G2/M transition by modulating cyclin B-cyclin-dependent kinase 1 activity. *J Biol Chem* 278:1158–1164.
- Chai Z, Sarcevic B, Mawson A, et al. 2001. SET-related cell division autoantigen-1 (CDA1) arrests cell growth. *J Biol Chem* 276:33665–33674.
- Cools M, van Aerde K, Kersemaekers AM, et al. 2005. Morphological and immunohistochemical differences between gonadal maturation delay and early germ cell neoplasia in patients with undervirilization syndromes. *J Clin Endocrinol Metab* 90:5295–5303.
- Dasari VK, Goharderakhshan RZ, Perinchery G, et al. 2001. Expression analysis of Y chromosome genes in human prostate cancer. *J Urol* 165:1335–1341.
- Dechend F, Schubert S, Nanda I, et al. 1998. Organization and expression of rat Tspy. *Cytogenet Cell Genet* 83:270–274.
- Dechend F, Williams G, Skawran B, et al. 2000. TSPY variants in six loci on the human Y chromosome. *Cytogenet Cell Genet* 91:67–71.
- Decordier I, Cundari E, Kirsch-Volders M. 2008. Mitotic checkpoints and the maintenance of the chromosome karyotype. *Mutat Res* 651:3–13.
- Delbridge ML, Longepied G, Depetris D, et al. 2004. TSPY, the candidate gonadoblastoma gene on the human Y chromosome, has a widely expressed homologue on the X—Implications for Y chromosome evolution. *Chromosome Res* 12:345–356.
- Fairbairn DJ, Roff DA. 2006. The quantitative genetics of sexual dimorphism: Assessing the importance of sex-linkage. *Heredity* 97:319–328.
- Gallagher WM, Bergin OE, Rafferty M, et al. 2005. Multiple markers for melanoma progression regulated by DNA methylation: Insights from transcriptomic studies. *Carcinogenesis* 26:1856–1867.
- Gutierrez-Adan A, Perez-Crespo M, Fernandez-Gonzalez R, et al. 2006. Developmental consequences of sexual dimorphism during pre-implantation embryonic development. *Reproduction in domestic animals = Zuchthygiene* 41Suppl 2:54–62.
- Hering R, Frade-Martinez R, Bajanowski T, et al. 2006. Genetic investigation of the TSPYL1 gene in sudden infant death syndrome. *Genet Med* 8:55–58.
- Hersmus R, de Leeuw BH, Wolffenbuttel KP, et al. 2008. New insights into type II germ cell tumor pathogenesis based on studies of patients with various forms of disorders of sex development (DSD). *Mol Cell Endocrinol* 291:1–10.
- Hildenbrand R, Schroder W, Brude E, et al. 1999. Detection of TSPY protein in a unilateral microscopic gonadoblastoma of a Turner mosaic patient with a Y-derived marker chromosome. *J Pathol* 189:623–626.
- Hoei-Hansen CE, Sehested A, Juhler M, et al. 2006. New evidence for the origin of intracranial germ cell tumours from primordial germ cells: Expression of pluripotency and cell differentiation markers. *J Pathol* 209:25–33.
- Honecker F, Stoop H, de Krijger RR, et al. 2004. Pathobiological implications of the expression of markers of testicular carcinoma *in situ* for fetal germ cells. *J Pathol* 203:849–857.
- Houtgraaf JH, Versmissen J, van der Giessen WJ. 2006. A concise review of DNA damage checkpoints and repair in mammalian cells. *Cardiovasc Res* 7:165–172.
- Ivanovska I, Orr-Weaver TL. 2006. Histone modifications and the chromatin scaffold for meiotic chromosome architecture. *Cell Cycle* 5:2064–2071.
- Kandalaf LE, Zudaire E, Portal-Nunez S, et al. 2008. Differentially expressed nucleolar transforming growth factor-beta1 target (DENT1) exhibits an inhibitory role on tumorigenesis. *Carcinogenesis* 29:1282–1289.
- Kandilci A, Mientjes E, Grosveld G. 2004. Effects of SET and SET-CAN on the differentiation of the human promonocytic cell line U937. *Leukemia* 18:337–340.
- Kastan MB, Bartek J. 2004. Cell-cycle checkpoints and cancer. *Nature* 432:316–323.
- Kersemaekers AM, Honecker F, Stoop H, et al. 2005a. Identification of germ cells at risk for neoplastic transformation in gonadoblastoma: An immunohistochemical study for OCT3/4 and TSPY. *Hum Pathol* 36:512–521.
- Kersemaekers AM, Honecker F, Stoop H, et al. 2005b. Identification of germ cells at risk for neoplastic transformation in gonadoblastoma: An immunohistochemical study for OCT3/4 and TSPY. *Hum Pathol* 36:512–521.
- Kido T, Lau YF. 2005. A Cre gene directed by a human TSPY promoter is specific for germ cells and neurons. *Genesis* 42:263–275.
- Kido T, Lau YF. 2006. The rat Tspy is preferentially expressed in elongated spermatids and interacts with the core histones. *Biochem Biophys Res Commun* 350:56–67.
- Kido T, Lau YF. 2008. The human Y-encoded testis-specific protein interacts functionally with eukaryotic translation elongation factor eEF1A, a putative oncoprotein. *Int J Cancer* 123:1573–1585.
- Knauf JA, Ouyang B, Knudsen ES, et al. 2006. Oncogenic RAS induces accelerated transition through G2/M and promotes defects in the G2 DNA damage and mitotic spindle checkpoints. *J Biol Chem* 281:3800–3809.
- Kristensen DM, Sonne SB, Ottesen AM, et al. 2008. Origin of pluripotent germ cell tumours: The role of micro-environment during embryonic development. *Mol Cell Endocrinol* 288:111–118.
- Lau YF. 1999. Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. *Am J Hum Genet* 64:921–927.
- Lau YF, Zhang J. 2000. Expression analysis of thirty one Y chromosome genes in human prostate cancer. *Mol Carcinog* 27:308–321.
- Lau Y, Chou P, Iezzoni J, et al. 2000. Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. *Cytogenet Cell Genet* 91:160–164.
- Lau YF, Lau HW, Komuves LG. 2003. Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. *Cytogenet Genome Res* 101:250–260.
- Lau YF, Kido T, Li YM. 2007. The TSPY Gene Family. In: CW Lau YF, editor. *The Y chromosome and male germ cell biology*. New Jersey: World Scientific Press. pp. 73–90.
- Li Y, Lau YF. 2008. TSPY and its X-encoded homologue interact with cyclin B but exert contrasting functions on cyclin-dependent kinase 1 activities. *Oncogene* 27:6141–6150.
- Li Y, Tabatabai ZL, Lee TL, et al. 2007a. The Y-encoded TSPY protein: A significant marker potentially plays a role in the pathogenesis of testicular germ cell tumors. *Hum Pathol* 38:1470–1481.
- Li Y, Vilain E, Conte F, et al. 2007b. Testis-specific protein Y-encoded gene is expressed in early and late stages of gonadoblastoma and testicular carcinoma *in situ*. *Urol Oncol* 25:141–146.
- Lin CW, Huang TN, Wang GS, et al. 2006. Neural activity- and develop-

- ment-dependent expression and distribution of CASK interacting nucleosome assembly protein in mouse brain. *J Comp Neurol* 494:606–619.
- Lleo A, Battezzati PM, Selmi C, et al. 2008. Is autoimmunity a matter of sex? *Autoimmun Rev* 7:626–630.
- Lobrich M, Jeggo PA. 2007. The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat Rev Cancer* 7:861–869.
- Looijenga LH, Oosterhuis JW. 2007. Are all testicular seminomas of animals in fact spermatocytic seminomas? *Vet Pathol* 44:126.
- Looijenga LH, Zafarana G, Grygalewicz B, et al. 2003. Role of gain of 12p in germ cell tumour development. *APMIS* 111:161–171; discussion 172–163.
- Looijenga LH, Hersmus R, Oosterhuis JW, et al. 2007. Tumor risk in disorders of sex development (DSD). *Best Pract Res Clin Endocrinol Metab* 21:480–495.
- Martinez A, Ozbun LL, Angdisen J, et al. 2002. Expression of differentially expressed nucleolar transforming growth factor-beta1 target (DENTT) in adult mouse tissues. *Dev Dyn* 224:186–199.
- Martin A, Collin GB, Varnum DS, et al. 1998. Testicular teratocarcinogenesis in mice—A review. *APMIS* 106:174–182.
- Mazeyrat S, Mitchell MJ. 1998. Rodent Y chromosome TSPY gene is functional in rat and non-functional in mouse. *Hum Mol Genet* 7:557–562.
- McBride MW, Carswell HV, Graham D, et al. 2002. Genetic and gender determinants of cerebrovascular disease. *Sem Nephrol* 22:127–134.
- Mezquita C. 1985. Chromatin composition, structure and function in spermatogenesis. *Revis Biol Celular* 5: V-XIV, 1–124.
- Muto S, Senda M, Akai Y, et al. 2007. Relationship between the structure of SET/TAF-Ibeta/INHAT and its histone chaperone activity. *Proc Natl Acad Sci USA* 104:4285–4290.
- Nagata K, Kawase H, Handa H, et al. 1995. Replication factor encoded by a putative oncogene, set, associated with myeloid leukemogenesis. *Proc Natl Acad Sci USA* 92:4279–4283.
- Oosterhuis JW, Looijenga LH. 2005. Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 5:210–222.
- Oram SW, Liu XX, Lee TL, et al. 2006. TSPY potentiates cell proliferation and tumorigenesis by promoting cell cycle progression in HeLa and NIH3T3 cells. *BMC Cancer* 6:154.
- Ozbun LL, Martinez A, Angdisen J, et al. 2003. Differentially expressed nucleolar TGF-beta1 target (DENTT) in mouse development. *Dev Dyn* 226:491–511.
- Ozbun LL, You L, Kiang S, et al. 2001. Identification of differentially expressed nucleolar TGF-beta1 target (DENTT) in human lung cancer cells that is a new member of the TSPY/SET/NAP-1 superfamily. *Genomics* 73:179–193.
- Page DC. 1987. Hypothesis: A Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads. *Development* 101 (Suppl):151–155.
- Pietenpol JA, Stewart ZA. 2002. Cell cycle checkpoint signaling: Cell cycle arrest versus apoptosis. *Toxicology* 181/182:475–481.
- Rajpert-de Meyts E, Hoei-Hansen CE. 2007. From gonocytes to testicular cancer: The role of impaired gonadal development. *Ann N Y Acad Sci* 1120:168–180.
- Repping S, van Daalen SK, Brown LG, et al. 2006. High mutation rates have driven extensive structural polymorphism among human Y chromosomes. *Nat Genet* 38:463–467.
- Salo P, Kaariainen H, Petrovic V, et al. 1995. Molecular mapping of the putative gonadoblastoma locus on the Y chromosome. *Genes Chromosomes Cancer* 14:210–214.
- Schmit TL, Ahmad N. 2007. Regulation of mitosis via mitotic kinases: New opportunities for cancer management. *Mol Cancer Ther* 6:1920–1931.
- Schnieders F, Dork T, Arnemann J, et al. 1996. Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. *Hum Mol Genet* 5:1801–1807.
- Schoemaker MJ, Swerdlow AJ, Higgins CD, et al. 2008. Cancer incidence in women with Turner syndrome in Great Britain: A national cohort study. *Lancet Oncol* 9:239–246.
- Schubert S, Dechend F, Skawran B, et al. 2000a. Molecular evolution of the murine tspy genes. *Cytogenet Cell Genet* 91:239–242.
- Schubert S, Dechend F, Skawran B, et al. 2000b. Silencing of the Y-chromosomal gene tspy during murine evolution. *Mamm Genome* 11:288–291.
- Schubert S, Skawran B, Dechend F, et al. 2003. Generation and characterization of a transgenic mouse with a functional human TSPY. *Biol Reprod* 69:968–975.
- Schubert S, Kamino K, Bohm D, et al. 2008. TSPY expression is variably altered in transgenic mice with testicular feminization. *Biol Reprod* 79:125–133.
- Scully RE. 1953. Gonadoblastoma; a gonadal tumor related to the dysgerminoma (seminoma) and capable of sex-hormone production. *Cancer* 6:455–463.
- Scully RE. 1970. Gonadoblastoma. A review of 74 cases. *Cancer* 25:1340–1356.
- Seethammagari MR, Xie X, Greenberg NM, et al. 2006. EZC-prostate models offer high sensitivity and specificity for noninvasive imaging of prostate cancer progression and androgen receptor action. *Cancer Res* 66:6199–6209.
- Seo SB, McNamara P, Heo S, et al. 2001. Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell* 104:119–130.
- Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. 2003. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423:825–837.
- Smits VA, Medema RH. 2001. Checking out the G(2)/M transition. *Biochim Biophys Acta* 1519:1–12.
- Tsuchiya K, Reijo R, Page DC, et al. 1995. Gonadoblastoma: Molecular definition of the susceptibility region on the Y chromosome. *Am J Hum Genet* 57:1400–1407.
- Tu Y, Wu W, Wu T, et al. 2007. Anti-proliferative autoantigen CDA1 transcriptionally up-regulates p21(Waf1/Cip1) by activating p53 and MEK/ERK1/2 MAPK pathways. *J Biol Chem* 282:11722–11731.
- Vera J, Jaumot M, Estanyol JM, et al. 2005. Heterogeneous nuclear ribonucleoprotein A2 is a SET-binding protein and a PP2A inhibitor. *Oncogene* 25:260–270.
- Verp MS, Simpson JL. 1987. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet* 25:191–218.
- Vijayakumar S, Hall DC, Reveles XT, et al. 2006. Detection of recurrent copy number loss at Yp11.2 involving TSPY gene cluster in prostate cancer using array-based comparative genomic hybridization. *Cancer Res* 66:4055–4064.
- Vogel T, Dechend F, Manz E, et al. 1997. Organization and expression of bovine TSPY. *Mamm Genome* 8:491–496.
- Vogel T, Ditttrich O, Mehraein Y, et al. 1998. Murine and human TSPYL genes: novel members of the TSPY-SET-NAP1L1 family. *Cytogenet Cell Genet* 81:265–270.
- von Lindern M, van Baal S, Wiegant J, et al. 1992. Can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3' half to different genes: characterization of the set gene. *Mol Cell Biol* 12:3346–3355.
- Wang GS, Hong CJ, Yen TY, et al. 2004. Transcriptional modification by a CASK-interacting nucleosome assembly protein. *Neuron* 42:113–128.
- Xie X, Zhao X, Liu Y, et al. 2001. Robust prostate-specific expression for targeted gene therapy based on the human kallikrein 2 promoter. *Hum Gene Ther* 12:549–561.
- Yin YH, Li YY, Qiao H, et al. 2005. TSPY is a cancer testis antigen expressed in human hepatocellular carcinoma. *Br J Cancer* 93:458–463.
- Youngren KK, Coveney D, Peng X, et al. 2005. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature* 435:360–364.
- Zhang JS, Yang-Feng TL, Muller U, et al. 1992. Molecular isolation and characterization of an expressed gene from the human Y chromosome. *Hum Mol Genet* 1:717–726.