INVITED REVIEW

Male Health

Battle of the sexes: contrasting roles of testis-specific protein Y-encoded (TSPY) and TSPX in human oncogenesis

Yun-Fai Chris Lau, Yunmin Li, Tatsuo Kido

The Y-located testis-specific protein Y-encoded (*TSPY*) and its X-homologue *TSPX* originated from the same ancestral gene, but act as a proto-oncogene and a tumor suppressor gene, respectively. TSPY has specialized in male-specific functions, while TSPX has assumed the functions of the ancestral gene. Both TSPY and TSPX harbor a conserved SET/NAP domain, but are divergent at flanking structures. Specifically, TSPX contains a C-terminal acidic domain, absent in TSPY. They possess contrasting properties, in which TSPY and TSPX, respectively, accelerate and arrest cell proliferation, stimulate and inhibit cyclin B-CDK1 phosphorylation activities, have no effect and promote proteosomal degradation of the viral HBx oncoprotein, and exacerbate and repress androgen receptor (AR) and constitutively active AR variant, such as AR-V7, gene transactivation. The inhibitory domain has been mapped to the carboxyl acidic domain in TSPX, truncation of which results in an abbreviated TSPX exerting positive actions as TSPY. Transposition of the acidic domain to the C-terminus of TSPY results in an inhibitory protein as intact TSPX. Hence, genomic mutations/aberrant splicing events could generate TSPX proteins with truncated acidic domain and oncogenic properties as those for TSPY. Further, TSPY is upregulated by AR and AR-V7 in ligand-dependent and ligand-independent manners, respectively, suggesting the existence of a positive feedback loop between a Y-located proto-oncogene and male sex hormone/receptors, thereby amplifying the respective male oncogenic actions in human cancers and diseases. TSPX counteracts such positive feedback loop. Hence, TSPY and TSPX are homologues on the sex chromosomes that function at the two extremes of the human oncogenic spectrum.
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INTRODUCTION

The human sex chromosomes, *i.e.*, the X and Y chromosome, evolved from a pair of identical chromosomes about 300 million years ago.[1] One homologue had acquired a sex-determining gene and became the Y chromosome, while the other one became the X chromosome, resulting in a XY and XX sex chromosome constitution for males and females, respectively. The Y chromosome had undergone various chromosomal rearrangements, reduction in gene content, specialization in testis determination and differentiation, and other male-specific functions, such as sperm production.[1–3] Parallel to such genetic evolution, the female with two X chromosomes had adopted a dosage compensation process, in which one of the two X chromosomes is inactivated early during embryogenesis, thereby in general balancing the gene dosage between the sexes.[4] The modern Y chromosome harbors identical genes on the pseudoautosomal regions (PARs) shared with the X chromosome; and X-degenerate, ampliconic and unique genes on the male-specific region of the Y chromosome (MSY).[2] Recent complete sequencing of the Y chromosome of different mammalian species, including humans, suggests that most of the genes on the MSY region have relatively conserved homologues on the X chromosome, which escape X-inactivation.[5] They are widely expressed in various tissues and serve various regulatory functions in transcription, translation, chromatin modification, RNA splicing, and protein ubiquitination. There are a few exceptions, including the sex-determining region Y gene (*SRY*),[6] the RNA-binding motif protein Y-linked (*RBMY*),[7] and the testis-specific protein Y-encoded (*TSPY*),[8] that had diverged significantly from their X-homologues, *i.e.*, SRY-box 3 (*SOX3*),[9] RNA-binding motif protein X-linked (*RBMX*),[10,11] and TSPY homologue on the X chromosome (*TSPX*),[12–15] respectively, and serve male-specific functions, such as sex determination, male germ cell renewal, and spermatogenesis. The Y homologues are primarily expressed in the testis, while the respective X homologues could have diverse expression patterns and are subjected to X-inactivation in a variety of tissues.[16] Various studies showed that *TSPY* and *TSPX* had evolved to be a proto-oncogene and a tumor suppressor gene, respectively. Thus, an abnormal activation of a proto-oncogene, *i.e.*, *TSPY*, on the MSY region of the Y chromosome will have a positive male-specific effect(s) on oncogenesis in males, while inactivation/deletion of an X-located tumor suppressor gene, *i.e.*, *TSPX*, will predispose males to oncogenesis, since males have

Division of Cell and Developmental Genetics, Department of Medicine, VA Medical Center and Institute for Human Genetics, University of California, San Francisco, CA 94121, USA.
Correspondence: Dr. YFC Lau (chris.lau@ucsf.edu)

only one X chromosome. Accordingly, *TSPY* and *TSPX* represent a pair of homologues on the sex chromosomes that function at the two extremes of the human oncogenic spectrum.

THE *TSPY* AND *TSPX* GENES ON THE SEX CHROMOSOMES

TSPY was one of the earliest genes isolated from the human Y chromosome.[8,17] *TSPX* was cloned by various laboratories and had been termed as TSPY-Like 2 or *TSPYL2*,[18] cell division antigen 1 or *CDA1*,[12] differentially expressed nucleolar-transforming growth factor-β1 target or *DENTT*,[14] and CASK-interacting nucleosome assembly protein or *CINAP*.[19] The TSPY homologue on the X chromosome (*TSPX*) has been adopted to indicate that *TSPY* and *TSPX* originated from the same ancestral gene on the proto-X and proto-Y chromosomes (**Figure 1**) and possess similar exon-intron organization.[13,15] Sex chromosome evolution resulted in *TSPY* being specialized in male-specific function(s) on the Y chromosome and *TSPX* maintaining likely the functions of the ancestral gene on the X chromosome. Although both genes still harbor a conserved SET/NAP domain, initially identified in the nuclear oncoprotein SET and the nucleosome assembly protein 1 (NAP1),[18] they diverged significantly in other parts of their encoded proteins. Principally, TSPX harbors an acidic domain of ~290 amino acids at its carboxyl terminus, while TSPY lacks such domain (**Supplementary Figure 1**). The truncation of the acidic domain could be essential for the specialization of TSPY functions in male spermatogonial stem cells and spermatogenesis, while the retention of the acidic domain could be important for TSPX housekeeping functions.[15] Several TSPY-like intronless genes have been identified on the autosomes[15,18] and could be results of retrotransposition events involving TSPY since they do not harbor the carboxyl acidic domain in their encoded proteins. Collectively,
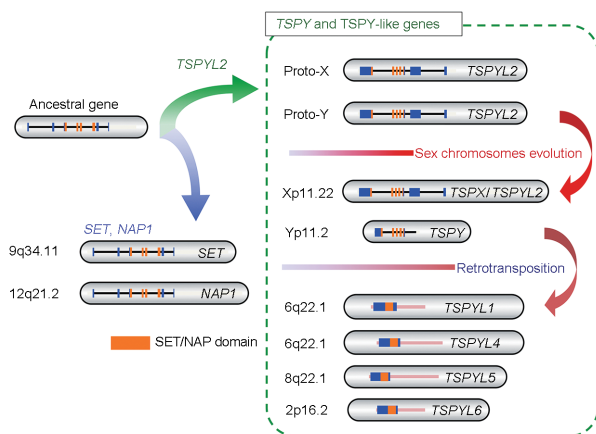
these genes have been designated as the SET/NAP/TSPY superfamily. Comparative analysis of the members of this superfamily with the Conserved Domain Database at the NCBI[20] identified the respective SET/NAP domain within each protein (**Supplementary Figure 1**). Based on the expected values (E-values) of domain homology, TSPY, TSPX, and other TSPYL proteins possess similar E-values and could constitute a subfamily of TSPY-like proteins harboring NAP domains, somewhat distinct from those of SET/NAP proteins (**Supplementary Figure 1**).

TSPY is an ampliconic gene and repeated 30–60 times on the MSY region of the Y chromosome.[2] Most TSPY transcriptional units are 2.8 kb in size[8] and embedded in 20.3-kb tandem repeats with 98% sequence homology.[2] They span about 0.6–1.2 Mb of DNA and constitute likely the largest block of functional and protein-coding repetitive sequences in the human genome. It has 6 exons but undergoes alternative splicing events, resulting in multiple in-frame transcript coding for various protein isoforms with 156–308 amino acids, all of which harbor the SET/NAP domain.[21] *TSPX* is a 6.2-kb single-copy gene, located on p11.22 region on the short arm of the X chromosome.[13,15] It encodes a protein of 693 amino acids. Both genes maintain similar genomic organization with a unique exon 1 and conserved exons 2–5 coding for the SET/NAP domain, but diverge at the 3' ends with the last *TSPY* exon lacking the exons 6 and 7 coding sequence for the acidic domain of TSPX (**Figure 2a** and **2b**). The SET nuclear oncogene is a major member of the SET/NAP/TSPY family. It also harbors a short acidic stretch of ~52 amino acids at the C-terminus of its encoded protein. The crystal structure of the human SET protein with a truncated acidic domain (SETΔC) has been elucidated,[22] which
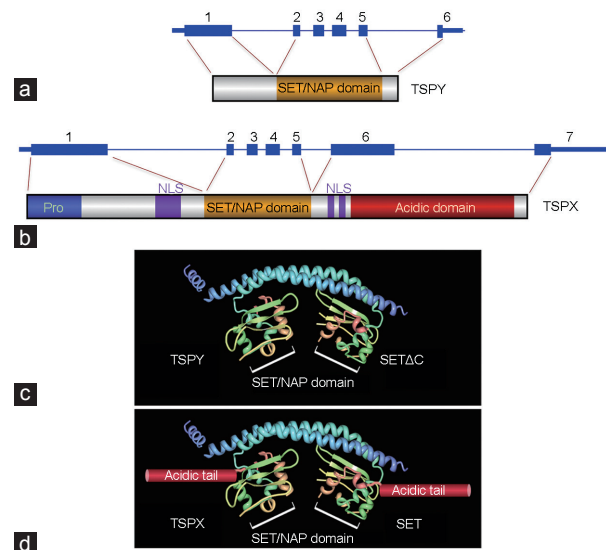

Figure 1: Diagrammatic illustration on the evolution of the SET/NAP/TSPY gene family. An ancestral gene gave rise to autosomal-located *SET* and *NAP1* genes while one (*TSPYL2*) integrated onto the proto-X and proto-Y chromosomes. During the evolution of the sex chromosomes, the proto-Y chromosome had acquired a sex-determining gene and evolved into the modern Y chromosome, while the proto-X chromosome evolved into the X chromosome. The *TSPYL2* gene on the Y chromosome had specialized to serve male-specific functions, such as spermatogenesis, amplified itself tandemly and became the ampliconic *TSPY* gene. The *TSPYL2* gene on the X chromosome maintained likely the structure and functions of the ancestral gene and became the *TSPX* gene. Additional retrotransposition events, likely from TSPY transcripts, generated other intronless TSPY-like genes on the autosomes. The respective chromosomal locations are labeled on the left of each member of the gene family. *NAP1*: nucleosome assembly protein 1; *SET*: SET nuclear proto-oncogene; *TSPX*: TSPY homologue on the X chromosome; *TSPY*: testis-specific protein Y-encoded.

Figure 2: *TSPY* and *TSPX* genomic organization and postulated atomic models deduced from the SETΔC structure. (**a**) *TSPY* and (**b**) *TSPX* maintain similar genomic organizations, with a unique exon 1, but conserved exons 2–5, encoding the SET/NAP domain. *TSPX* contains exons 6 and 7 encoding the acidic domain at its C-terminus, while *TSPY* lacks such protein-coding exons and the carboxyl acidic domain. Accordingly, TSPY could assume the dimerized structure of SET protein without the acidic domain, *i.e.*, (**c**) SETΔC, with the SET/NAP domains as a pair of "earmuffs of a headphone" (PDB ID: 2E50),[22] (**d**) while intact TSPX and SET with C-terminal acidic domain would have the acidic domain protruding from the "earmuffs." NAP1: nucleosome assembly protein 1; NLS: nuclear localization signal; SET: SET nuclear proto-oncogene; SETΔC: SET protein with a truncated acidic domain; TSPX: TSPY homologue on the X chromosome; TSPY: testis-specific protein Y-encoded.

suggests a configuration with an N-terminal backbone dimerized structure and the SET/NAP domains as a pair of "earmuffs of a headphone" (**Figure 2c**). Sequence alignment suggests that SET, TSPY, and TSPX are highly conserved at the SET/NAP domain.[15,23] Hence, if both the TSPY and TSPX assume similar structures, one could envision TSPY to possess similar structural organization as that of SETΔC, while the full-length SET and TSPX could have a set of its acidic domains protruding from the "earmuffs" of the dimerized structure (**Figure 2d**). At present, the organization and effects of the acidic domain on the overall structure of TSPX as well as that for the intact SET protein are unknown. Further, it is uncertain if TSPY, TSPX, and/or other TSPYL proteins could form heterodimers with the same or different functions.

THE NORMAL FUNCTIONS OF *TSPY* AND *TSPX* GENES

As a specialized gene on the Y chromosome for male-specific functions, *TSPY* is primarily expressed in the prespermatogonia and gonocytes in embryonic testis[24] and spermatogonia and spermatocytes in adult testis.[25] The *TSPY* gene array possesses the highest length variation and mutation rate from father-to-son transmission on the Y chromosome.[26] Copy number variation and recombination of exon 1 of *TSPY* have been reported to be associated with infertility in men,[27,28] suggesting that it serves important functions in spermatogonial stem cell renewal and spermatogenesis.[29,30] Currently, the exact functions of TSPY in the spermatogenic processes have not been defined. We showed that TSPY is capable of binding to the type B mitotic cyclins and exacerbates the activities of the cyclin B-cyclin dependent kinase 1 (CDK1) phosphorylation enzymatic activities.[31] We hypothesize that such TSPY actions could be important for spermatogonial stem cell renewal and sustaining the two consecutive rounds of meiotic divisions in meiosis I and II,[30] which generate four spermatids during spermatogenesis in the testis. Although low levels of TSPY transcripts could be detected in some somatic tissues, such as prostate and brain, the primary normal functions of TSPY are mostly male specific in the testis.

There are numerous normal functions assigned to TSPX through various genetic linkage, DNA sequencing, and transgenic mouse studies. TSPX is ubiquitously expressed in most tissues, with the highest levels in the ovary, brain, and other neural tissues, and presumed to serve certain housekeeping/important functions[14] (**Supplementary Figure 2**). Several studies showed that mutations, duplication, or deletion of *TSPX* are associated with various degrees of intellectual disability.[32–35] TSPX interacts with calcium/calmodulin-dependent serine protein kinase (CASK) and modulates the Tbr-1 regulation of the N-methyl-D-aspartate (NMDA) receptor subunit and other neural genes.[19,35–37] Loss-of-function mutation of *Tspx* in mice results in neurodevelopmental and behavioral abnormalities,[38,39] suggesting that *TSPX* could be an essential gene for neurodevelopment and synaptic functions. TSPX has been demonstrated to be an essential component of the RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) transcriptional repressor complex, which regulates numerous neuronal genes and could also serve as tumor suppressor for various cancers.[40] TSPX is involved in the regulation of cell cycle progression and cell proliferation. Overexpression of TSPX retards cell proliferation and regulates the transition of both the G1 and G2 phases of the cell cycle.[14,31,41–43] It enhances the transforming growth factor-β (TGF-β) signaling in renal and vascular cells and increases the renal expression of TGF-β receptors and TGF-β-mediated fibrogenesis of the kidney in experimental models of diabetes.[41,43] Hence, TSPX could serve important functions in cell cycle regulation and cell proliferation and transcriptional functions and exert various effects on physiology and diseases involving these cellular properties.

Its role in human oncogenesis will be discussed in more detail under a separate section, below.

At present, studies on other members of the TSPY-like intronless genes are limited. Since they are postulated to be derived from the Y-located *TSPY* gene, they could potentially possess similar properties, assigned to *TSPY* and *TSPX*. A frameshift mutation in *TSPYL1* had been linked to high risk for the sudden infant death syndrome (SIDS) among an Amish community in Pennsylvania, USA.[44] Subsequent studies on the European Amish and German populations failed to associate any *TSPYL1* variations with SIDS.[45,46] Other studies suggest that *TSPYL5* and *TSPYL6* could be involved in cell growth and drug sensitivity and breast cancer susceptibility, respectively.[47–49] To shed some lights on the probable functions of these TSPY-like genes, we have performed a data-mining experiment investigating their expression patterns in the Genotype-Tissue Expression (GTEx) Project.[50] Our results show that TSPX and TSPYL5 are expressed in a wide range of the 53 human tissues/cells in the database (**Supplementary Figure 2**). TSPY and TSPYL6 are specifically expressed in the testis, while TSPYL5 shows a preferential testis expression pattern. Both TSPYL1 and TSPYL4 are tandemly integrated within a 30-kb segment on the human chromosome 6q22.1 and show similar preferential expression patterns in the brain and other neural tissues. Since they harbor the conserved SET/NAP domain, they could potentially participate in some of the functions, prescribed to TSPY, TSPX, and other members, for example, *SET* and NAP1-like genes, of the family with similar expression patterns (**Supplementary Figure 2**).

TSPY AS A PROTO-ONCOGENE IN GONADOBLASTOMA AND GERM CELL TUMORS

The gonadoblastoma locus on the Y chromosome (GBY) was initially proposed to explain the high incidence (>60%) of gonadoblastoma in patients with disorders of sex development (DSD), *i.e.*, XY females and XY males with gonadal dysgenesis, harboring residual materials from the Y chromosome.[51,52] Subsequent deletion mapping localized the GBY locus on deletion interval 3 proximal to the centromere on the short arm of the human Y chromosome.[53,54] GBY is the only oncogenic locus on this male-specific chromosome.[55] The *TSPY* transcriptional repeats are the major functional genes located at the critical region of GBY, and hence they constitute the key candidates for the gene(s) responsible for predisposing the dysgenetic gonads of DSD patients to gonadoblastoma development.[56,57] Gonadoblastoma is a benign germ cell tumor and, if untreated, can advance to dysgerminoma or testicular germ cell tumors (TGCTs), depending on the status of the dysfunctional gonads.[58–60] Expression analysis shows that TSPY is abundantly expressed in gonadoblastoma and various types of TGCTs, particularly intense in the carcinoma *in situ* cells (CIS), the precursors for all germ cell tumors.[24,29,61–64] Further, TSPY is also expressed in nongonadal germ cell tumors, such as the intracranial germ cell tumors, of male patients.[65] TSPY expression pattern is correlated with those of various CIS and germ cell markers, such as placental alkaline phosphatase (PLAP), octamer-binding transcription factor 4 (OCT4), proto-oncogene c-Kit (c-Kit),[63] and CD133 (prominin 1), a consensus marker for cancer stem cells[66–69] capable of self-renewal and tumorigenesis (**Figure 3**). These findings suggest the potential role of TSPY in the early tumorigenic initiation and progression of gonadoblastoma and TGCTs and somatic cancers of germ cell origin. Overexpression of TSPY in human HeLa and mouse NIH-3T3 cells accelerates cell proliferation, particularly exacerbating the transition of the G2/M phase of the cell cycle, and upregulates various oncogenes and growth-promoting genes, but represses tumor suppressors and growth
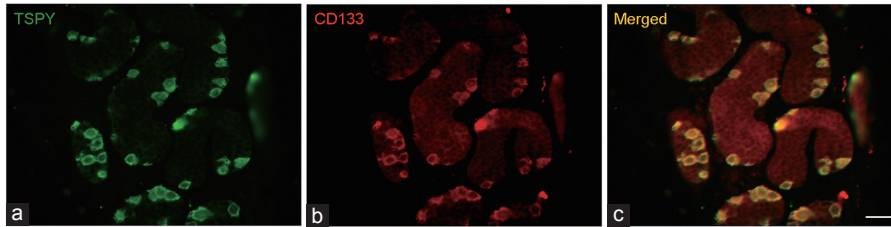
Figure 3: Co-localization of (**a**) TSPY and (**b**) CD133 in the CIS cancer stem cells in a testicular seminoma. Immunofluorescence was performed as previously described,[63] and (**c**) a merged view of **a** and **b**. CIS: carcinoma *in situ* cells; TSPY: testis-specific protein Y-encoded. Scale bar=20 μm.

inhibitors.[70] TSPY is capable of binding to its own exon 1 sequence and amplifying its expression and oncogenic actions.[71] Tumorigenicity assays in athymic mice showed that cells overexpressing TSPY form tumor significantly faster than those without TSPY expression, suggesting that TSPY possesses oncogenic properties when it is ectopically expressed in somatic cells. Transgenic mouse studies showed that *TSPY* promoter is capable of directing the expression of a reporter in germ cells of both sexes,[25] and ectopic expression of *TSPY* gene in germ cells leads to gonadoblastoma-like structures in the ovaries of female mice,[72] thereby supporting its candidacy as the gene for GBY.

TSPY IN SOMATIC CANCERS

As a proto-oncogene on the human Y chromosome, *TSPY* could have significant influence on the oncogenic processes of male-specific cancers, such as prostate cancer,[73,74] and male-biased cancers, such as hepatocellular carcinoma (HCC).[75–78] To establish such possible correlation, TSPY expression was analyzed with immunohistochemistry on archival pathological specimens and RNAs from prostate cancer and HCC.[79] Results from these studies showed that TSPY is abundantly expressed in prostate cancer specimens of various Gleason grades as well as latent cancer of elderly individuals.[80] Interestingly, the levels of TSPY expression could be correlated with the Gleason grades of latent cancer, suggesting that it could act at early stages of prostatic oncogenesis.

HCC is closely associated with hepatic cirrhosis caused by infections of hepatitis viruses (hepatitis B virus [HBV] and hepatitis C virus [HCV]) and other chronic liver diseases or toxicities/injuries.[81–84] The etiologies are complex and might involve contributions of various genetic, cell signaling, and environmental components. There are significant sex differences in the incidence and progression of liver cancer favoring men.[77,85–90] As a proto-oncogene on the male-specific chromosome, ectopic expression of TSPY in HCC could signify a contribution of this male-specific gene in such sexual dimorphism(s) in HCC. To support such postulation, TSPY expression has been analyzed with tissue microarray, individual pathological specimens, and RNAs from tumor and nontumor pairs from HCC patients.[79] The results showed that TSPY is positive in approximately 50% of male HCC specimens, and its expression is closely correlated with those of other markers, such as glypican 3 (GPC3) and forkhead box M1 (FOXM1). To further confirm its involvement in HCC pathogenesis, TSPY expression was analyzed in the Cancer Genome Atlas (TCGA) database, particularly on RNA-Seq transcriptome and DNA methylation datasets on HCC and adjacent nontumor tissues of male patients.[91] The results showed that 33% of HCC were positive for TSPY expression. Significantly TSPY expression is associated with poorer survival of the patients.[91] Additional studies revealed that male specimens of 17% of lung adenocarcinoma, 11% of head-and-neck cancer, and 10% of renal cancer are also positive for TSPY transcripts. TSPY is co-expressed with a network of 53 genes and is associated with DNA hypomethylation and gene expression

in somatic cancers.[91] As a repetitive gene, the *TSPY* transcripts could be underestimated in the common methods used to quantitate gene expression from RNA-Seq data,[92] suggesting that TSPY expression could likely be more common and/or abundant than the data derived from the transcriptome database. Nevertheless, the data-mining study confirmed the TSPY expression in HCC and further demonstrated its expression in other tumor types, thereby substantiating the potential role(s) of this male-specific proto-oncogene in somatic cancers.[91]

In two separate studies, TSPY has also been identified as a highly expressed biomarker in HCC specimens. The first one using cDNA microarray and hybridization approach identified *TSPY* as a highly expressed gene in HCC and demonstrated it as a cancer-testis (CT) antigen detectable as an autoantigen in sera of HCC patients.[93] The second study using proteomic approaches identified TSPY as a highly expressed protein consistently detected in male HCC samples, as compared to those of female specimens.[94] Further studies showed that overexpression of TSPY potentiated HCC cell proliferation and increased colony formation in soft agar and cell invasiveness, corresponding to metastatic properties of these transfected cells,[94] as previously reported.[70] Hence, these studies support the hypothesis that TSPY could exacerbate hepatocarcinogenesis, thereby exerting the male biases observed among the HCC patients.

TSPX AS A TUMOR SUPPRESSOR IN HUMAN CANCERS

TSPX was initially isolated as a gene induced by the TGF-β in lung cancer cells.[14] Early studies demonstrated that overexpression of TSPX arrests cell growth and proliferation,[12,14] thereby affirming its potential role(s) as a tumor suppressor in oncogenesis. Mutations of *TSPX* in selected cancers, such as endometrial tumors and uterine leiomyomas, have been reported.[34,95] Its expression is drastically reduced in lung cancer tissues and cell lines, as compared to normal lung tissues and cell lines.[42] Overexpression of TSPX in lung and breast cancer cells diminishes their proliferation, colony formation, and migratory properties.[12,14,42] As a component of the REST/NRSF transcriptional repressor complex for TGF-β signaling activation,[40] TSPX regulates TGF-β-induced cell cycle arrest in epithelial cells and promotes TGF-β signaling by repressing genes involved in cell growth. Since TSPX expression is inducible with TGF-β,[14] its regulation of TGF-β signaling suggests the likely presence of a feedback mechanism in tumor suppression involving TSPX and TGF-β. Further, TGF-β family serves important functions in numerous developmental and physiological pathways[96] and TSPX is widely expressed in most tissues, such TSPX-TGF-β feedback loop could be critical in modulating these biological processes, particularly on neurodevelopment and neural functions,[97] beyond tumorigenesis.[98]

The HBx protein encoded by HBV is a putative oncoprotein essential for viral replication and oncogenesis.[99] Its expression and/or mutations in infected cells affect cell proliferation, androgen, NF-κB/WNT-β-catenin and ERK/JNK signaling, and other oncogenic events.[100–106] HBx is protected by the proteasome 19S lid

subunit regulatory particle non-ATPase 3 (RPN3) from proteosomal degradation. TSPX abrogates the RPN3-dependent stabilization of HBx by interacting with both HBx and RPN3 and tethering HBx for degradation through the ubiquitin-proteasome pathway.[107] The critical domain has been mapped to the carboxyl acidic domain of TSPX. Hence, TSPX serves as a tumor suppressor and a negative regulator for HBx stability and HBV-associated hepatocarcinogenesis. Interestingly, TSPY lacks such domain and does not interact with RNP3 or HBx and has no effects on the proteosomal degradation of HBx.[107]

THE CONTRASTING PROPERTIES OF TSPY AND TSPX IN CELL CYCLE REGULATION

As discussed above, *TSPY* is a proto-oncogene on the Y chromosome, while its X-homologue *TSPX* behaves as a tumor suppressor. When they are overexpressed, they promote and arrest cell proliferation, respectively.[12,14,70,94] Several large-scale genome-wide association studies have mapped a prostate cancer susceptibility locus to Xp11.22 on the X chromosome,[108,109] where TSPX is located, suggesting that it could be a candidate for such a cancer susceptibility locus. As discussed below, mutations and/or aberrant RNA processing of TSPX could convert it to pro-oncogenic as TSPY, thereby contributing positively to tumorigenesis.

Initial flow cytometry analysis of cells overexpressing TSPY showed a significant decrease in the number of cells at the G2/M stage, suggesting that the cells might transit this cell cycle stage expeditiously.[70] Since the G2/M checkpoints insure that only cells with proper DNA duplication/genome integrity will enter mitosis to yield normal daughter cells, such expedited transition through the G2/M phase could potentially induce genome instability and pass on mutations to the progeny cells.[29] We showed that TSPY binds to type B cyclins and exacerbates the phosphorylation activities of the mitotic cyclin B-CDK1 complex both *in vitro* and *in vivo*.[31] Cyclin B-CDK1 activity is essential for the cell to enter and exit mitosis, and TSPY exacerbation of its kinase function could, at least partially, explain the rapid transition of G2/M phase in cells overexpressing TSPY. A parallel study showed that TSPX also binds to cyclin B, but represses the cyclin B-CDK1 activity. TSPY and TSPX binding and modulation of cyclin B-CDK1 activities are competitive in nature. Domain mapping identified the carboxyl acidic domain of TSPX to be responsible for its inhibitory effect(s) on the cyclin B-CDK1 activities. Importantly, truncation of this acidic domain renders the abbreviated TSPX to be stimulatory in cyclin B-CDK1 activity as TSPY. On the other hand, transposition of the TSPX acidic domain to the carboxyl terminus of TSPY results in an inhibitory molecule on cyclin B-CDK1 activity as intact TSPX protein.[31] Immunofluorescence analysis localized both TSPY and TSPX at the microtubule spindle assembly throughout mitosis, particularly on the metaphase chromosomes.[29] Based on these observations, TSPX is hypothesized to serve normal functions in modulating the cyclin B-CDK1 phosphorylation activities and maintaining integrity of the spindle assembly checkpoints (SACs).[29] TSPY, on the other hand, is specialized in spermatogonial stem cell renewal and male meiotic divisions by exacerbating cyclin B-CDK1 activities[30] and, when expressed in incompatible cells, it competitively disrupts the TSPX-associated SAC integrity and promotes cell proliferation, genome instability, and oncogenesis.[29]

DIFFERENTIAL ACTIONS OF TSPY AND TSPX ON ANDROGEN RECEPTOR SIGNALING

The male sex hormone, androgen, and its receptor, androgen receptor (AR), play key roles in testicular differentiation and spermatogenesis, as well as sexual dimorphisms in development and physiology in somatic organs, such as the brain.[110–116] Any exacerbation of the androgen and/or AR functions by genes on the Y chromosome will greatly amplify such male sex hormone effects in these biological processes. In a yeast two-hybrid study, AR was identified as an interactive protein for TSPY, suggesting the possible involvement of this male-specific proto-oncogene in the male sex hormone/receptor signaling functions.[117] Subsequent studies demonstrated that TSPY and TSPX competitively bind to AR at their SET/NAP domain.[23] The interactive domain for AR has been mapped to the N-terminal and DNA-binding domains. Significantly, recent studies showed that numerous AR variants, such as AR splice variant 7 (AR-V7), lacking the C-terminal ligand-binding domain are consistently expressed in various prostate cancer samples and are constitutively active in transactivation of target genes in a ligand-independent manner(s).[118] Importantly, AR-V7 could be detected in patients with castration-resistant prostate cancer and has been proposed as a diagnostic and prognostic biomarker for prostate cancer.[119,120] Since these AR variants still possess the N-terminal and DNA-binding domains, follow-up experiments showed that TSPY and TSPX are capable of binding to AR variants, including AR-V7. Reporter assays showed that TSPY and TSPX bindings stimulate and repress the transactivation of AR and AR-V7 on their target genes in ligand-dependent and ligand-independent manners, respectively. The inhibitory domain for TSPX has been mapped on to its carboxyl acidic domain, truncation of which renders the abbreviated TSPX molecule to be stimulatory, while its transposition to the carboxyl terminus of TSPY results in an inhibitory hybrid protein in AR/AR-V7 transactivation.[23]

To determine the effects of TSPY and TSPX on the endogenous AR transactivation of target genes, they were individually transfected and overexpressed in androgen-responsive LNCaP prostate cancer cells and analyzed under androgen-induced conditions. Overexpression of TSPY and TSPX stimulates and inhibits cell proliferation, respectively. TSPY and TSPX co-localize with the endogenous AR on the promoters of various target genes and differentially activate and repress their expression, respectively. Transcriptome analysis showed that TSPY upregulates and TSPX represses numerous common canonical pathways associated with cell proliferation, cell growth, and oncogenesis, suggesting the potential contrasting roles of TSPY and TSPX in promoting and suppressing the androgen and AR oncogenic functions in prostate cancer cells, respectively.[23]

TSPY expression is inducible with androgen in LNCaP cells,[121] suggesting that *TSPY* gene could be regulated by AR. To evaluate such possibility, a luciferase reporter directed by a 2.4-kb *TSPY* promoter[25] was used in transfection assays with AR and AR-V7 expression vectors.[23] The results showed that AR and AR-V7 upregulate the TSPY-Luciferase reporter in ligand-dependent and ligand-independent manners, respectively (**Figure 4**). Significantly, inclusion of a TSPY or TSPX expression vector in the assays resulted in stimulation and repression of the luciferase reporter directed by the *TSPY* promoter, respectively. These observations suggest that TSPY and AR/AR-V7 form a positive feedback loop(s) in mediating TSPY expression and the AR/AR-V7 comprehensive gene regulatory programs. TSPX, on the other hand, is a repressor for such positive feedback loop(s) on AR/AR-V7 and TSPY functions, inhibiting the AR/AR-V7 amplification of TSPY expression/tumorigenic actions as well as AR/AR-V7 global transcriptional regulation of responsive genes in cell proliferation and oncogenesis (**Figure 5**).
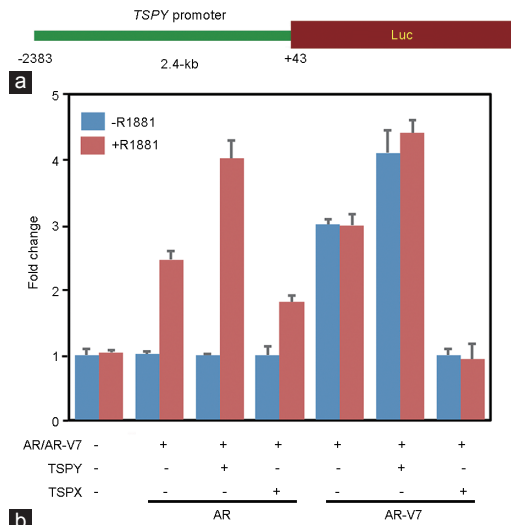
Figure 4: (a) A luciferase reporter directed by a 2.4-kb promoter (−2383 to +43) of the human *TSPY* gene.[25] (b) Reporter assays show AR (full length) or AR-V7 (V7) transactivation of the TSPY-luciferase reporter in the absence (blue bars) and presence (red bars) of synthetic androgen ligand, R1881, in HEK293 cells.[23] Results show that AR and AR-V7 transactivate the *TSPY-luciferase* gene in ligand-dependent and ligand-independent manners, respectively. Inclusion of a TSPY or a TSPX expression vector further exacerbates and inhibits such transactivation activities, respectively. The activity of the TSPY-luciferase of each transfection assay was determined in triplicates and calculated with reference to its activity without AR/AR-V7, TSPY, or TSPX co-transfection and in the absence of ligand (lane 1, from the left). The relative fold changes were then calculated between transfection pairs without and with the ligand R1881 for AR assays. For AR-V7, the relative fold changes were calculated with the reference to TSPY-luciferase activity without ligand (lane 1), since its transactivation on target genes is ligand independent. AR: androgen receptor; AR-V7: AR splice variant 7; TSPX: TSPY homologue on the X chromosome; TSPY: testis-specific protein Y-encoded.

BATTLE OF THE SEXES: TSPY AND TSPX IN HUMAN ONCOGENESIS

The existence of a positive feedback loop(s) between a Y-chromosome gene and AR/AR-V7 signaling is important in various developmental and physiological processes as well as disease pathogeneses with sexual dimorphisms, since they could synergistically amply their respective male-specific functions in these processes.[23,101,103,110–114,118] Although the roles of androgen and AR have been demonstrated to play critical roles in numerous developmental pathways and physiological events,[110–114] TSPY expression is mostly restricted to the testis and to a certain extent the prostate, and hence such positive feedback loop could likely be effective in spermatogenesis and/or prostate development and functions under normal conditions.[8,17,80] Under oncogenic conditions, TSPY is abundantly expressed in numerous cancer types, including gonadoblastoma, testicular germ cell tumors, prostate cancer, hepatocellular carcinoma and cholangiocarcinoma, lung cancer, renal cancer, and head-and-neck carcinoma,[29,56,62–64,80,91,122] some of which are male specific or possess male biases in incidence, progression, and/or treatment outcomes. Such unique male specificities or sexual dimorphisms could be attributed to the oncogenic actions of the male sex hormone androgen and its receptors, the Y-specific oncogene TSPY, or a combination of AR-TSPY positive feedback loop. In particular, prostate differentiation is highly dependent on the male sex hormone and AR-mediated developmental pathways,[123,124] while prostate cancer is highly sensitive to androgen and AR actions in initiation, progression, drug resistance,
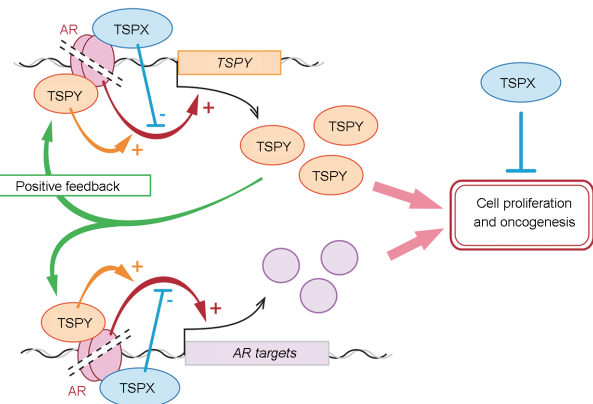

Figure 5: A model of a positive feedback loop between TSPY and AR in gene regulation. *TSPY* expression is regulated by AR actions on its promoter (left, top). The synthesized TSPY protein, in turn, interacts with AR (AR variants) and exacerbates its global transactivation of target genes (left, bottom), including its own expression (left, top), thereby establishing a positive feedback loop in their male-specific functions. TSPX, on the other hand, inhibits AR/AR variant transactivation of *TSPY* and their global gene regulation. The male sex hormone receptors and TSPY synergistically promote cell proliferation and oncogenesis, while TSPX, as a tumor suppressor, counteracts such oncogenic actions (right). AR: androgen receptor; TSPX: TSPY homologue on the X chromosome; TSPY: testis-specific protein Y-encoded.

and metastasis.[125–127] TSPY expression in latent and clinical prostate cancer,[80] therefore, highlights its likely augmentation of androgen and AR functions in these oncogenic processes. AR, in turn, could amply TSPY expression and its effects on cell proliferation, G2/M checkpoints, and genome stability,[29] important for the initiation and progression of prostatic oncogenesis.[125] Significantly, TSPY interacts and intensifies the AR-V7 transactivation of target genes, including its own gene,[23] suggesting that such TSPY-AR-V7 positive feedback loop could play a key role in AR variant-associated advances from localized to metastatic castration-resistant prostate cancer. Accordingly, the Y-located *TSPY* oncogene and AR/AR-V7 synergistically amplify their respective male-specific oncogenic effects in prostate cancer and other male sex hormone-responsive cancers, including hepatocellular carcinoma,[86,101,103,106] thereby greatly magnifying the oncogenic processes of such male-specific/biased human cancers.

As a tumor suppressor, TSPX opposes most of the TSPY oncogenic actions, including cell proliferation, cell cycle regulation, and male sex hormone and receptor transactivation activities.[12,14,23,31,42,70] As discussed above, the C-terminal acidic domain in TSPX, absence in TSPY, is primarily responsible for its tumor suppressor functions, including in proteosomal degradation of the viral oncoprotein HBx, inhibition of cyclin B-CDK1 phosphorylation and cell proliferation, and repression of AR/AR-V7 transactivation of TSPY and global gene regulatory program(s).[23,31,107] Importantly, truncation of the carboxyl acidic domain results in an abbreviated molecule capable of stimulating cyclin B-CDK1 activities and AR/AR-V7 transactivation of responsive genes, thereby converting TSPX to possess oncogenic properties, similar to those of TSPY. Currently, it is uncertain if TSPX could direct the synthesis of truncated version(s) of its protein under diseased conditions, perhaps through either genomic mutation(s) inserting a stop codon ahead of the acidic domain or alternative splicing events deleting the exonic sequence coding for the acidic domain, similar to splicing aberrations resulting in the syntheses of the constitutively active AR variants.[118] Hence, it is conceivable that TSPX could be a

"double-edged sword" capable of functioning as a tumor suppressor and a proto-oncogene in human oncogenesis.

TSPY AND TSPX ACTIONS BEYOND CANCERS

Besides cancers, androgen and AR have been postulated to exert important effects on various normal developmental and physiological processes, contributing to sex differences in brain structures, muscle development, and cardiovascular and neurological physiology, among others.[110–114] Since TSPX is widely expressed in various tissues, particularly in the brain and other neural tissues, it could exert modulatory effects on such male sex hormone actions, thereby modifying the sex hormone-related differences in these developmental and physiological events. On the other hand, the expression of the Y-located TSPY is tightly regulated and is mostly restricted to the testis, prostate gland, and possibly brain.[25,72] Accordingly, its stimulatory functions on androgen and AR could likely be centered on biological processes within these organs in males. However, under diseased conditions, *TSPY* is epigenetically dysregulated and expressed in various somatic cancers, exacerbating the actions on the male sex hormone functions and its own oncogenic functions.[79,80,91] At present, the exact mechanisms in the aberrant activation of the *TSPY* gene are uncertain. The tandem arrangement of its transcriptional units on the Y chromosome has been demonstrated to be a hotspot for length variation and mutation[26] and hence could be a factor for genetic instability, for example, copy number variation[2] and epigenetic dysregulation under diseased environments. In a humanized transgenic mouse model, an 8.5-kb DNA fragment, containing 2.9-kb promoter, 2.8-kb human *TSPY* gene, and 2.8-kb downstream sequence, has been tandemly integrated 50 times onto the Y chromosome of the host genome.[128] The human transgene shows an expression pattern similar, if not identical, to that in humans, *i.e.*, primarily expressed in spermatogonia and spermatocytes in the testis.[128] However, when such Y-located transgene is introduced into the cancerous genetic background of the LADY prostate cancer line, the human *TSPY* transgene is ectopically activated gradually in foci of cancer cells in the early stages and widely expressed in late stages of prostatic oncogenesis, thereby supporting the notion that this Y-located and tandemly repeated human transgene, under its own promoter, could be activated under tumorigenic conditions.[129] On the other hand, when the human *TSPY* gene or a *TSPY* promoter-directed transgene is integrated into the host autosomes,[25,72] the transgenes are expressed in the testis as well as various somatic tissues, particularly neurons of the central and peripheral nervous systems from E12.5 embryonic to adult stages.[25,72] Its neural expression pattern overlaps those of the endogenous mouse Tspx and Cask, an interactive transcription partner for TSPX,[19,35] suggesting that TSPY, if activated, could modulate the functions of TSPX, thereby exerting male-specific effects on neurological processes associated with TSPX functions.

Significantly, other members of the SET/NAP/TSPY protein family serve a wide variety of cellular functions. For example, SET has been demonstrated to serve as a chaperone for histones and inhibit their acetylation, thereby regulating the transcriptional activities as well as chromatin organization during DNA synthesis.[130–133] Previous studies showed that TSPY is capable of interacting with histones and hence could have chaperone functions in histone posttranslational modifications. Further, SET also binds cyclin B, but represses cyclin B-CDK1 kinase activities.[31] Similarly, the inhibitory domain for SET has also been mapped to its C-terminal acidic domain, truncation of which converts it into a stimulatory protein.[31] Interestingly, as an oncoprotein, SET binds to the unacetylated C-terminal domain (CTD)

of p53 and represses the p53 transcriptional activities and tumor suppressor functions.[134–136] The carboxyl acidic domain of SET has been demonstrated to act as a "reader" for the unacetylated CTD of p53 in such acetylation-dependent gene regulation.[137,138] Since TSPX also possesses an acidic domain, abide ~7 times larger, it potentially could possess similar acetylation "reader" function(s) in the regulation of p53 and other acetylation-sensitive transcription factors. However, as a tumor suppressor, it has been demonstrated to stabilize p53 and upregulate p21$^{\text{Waf1/Cip1}}$.[139] These findings suggest that members of this protein family, *i.e.*, TSPY, TSPX, and SET, harbor a conserved SET/NAP domain but could serve differential functions, depending particularly on the absence, presence, size, and/or hydrophilicity of their C-terminal acidic domains.[136] Further, as a Y-located gene, *TSPY* could also compete or exacerbate other autosomal TSPY-like genes, thereby exerting a male-specific effect(s) on their respective functions. Future studies focusing on TSPY synergistic or antagonistic actions on TSPX/TSPYL functions in various biological processes could shed critical insights on its role(s) as a male-specific modifier involved in sex differences in the health and diseases of humans.

AUTHOR CONTRIBUTIONS

YFCL wrote the review; YL and KT performed studies described in the review. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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REFERENCES

1 Hughes JF, Rozen S. Genomics and genetics of human and primate Y chromosomes. *Annu Rev Genomics Hum Genet* 2012; 13: 83–108.
2 Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, *et al*. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003; 423: 825–37.
3 Hughes JF, Page DC. The history of the Y chromosome in man. *Nat Genet* 2016; 48: 588–9.
4 Lyon MF. X-chromosome inactivation. *Curr Biol* 1999; 9: R235–7.
5 Bellott DW, Hughes JF, Skaletsky H, Brown LG, Pyntikova T, *et al*. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature* 2014; 508: 494–9.
6 Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, *et al*. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 1990; 346: 240–4.
7 Chandley AC, Cooke HJ. Human male fertility – Y-linked genes and spermatogenesis. *Hum Mol Genet* 1994; 3: 1449–52.
8 Zhang JS, Yang-Feng TL, Muller U, Mohandas TK, de Jong PJ, *et al*. Molecular isolation and characterization of an expressed gene from the human Y chromosome. *Hum Mol Genet* 1992; 1: 717–26.
9 Stevanović M, Lovell-Badge R, Collignon J, Goodfellow PN. *SOX3* is an X-linked gene related to SRY. *Hum Mol Genet* 1993; 2: 2013–8.
10 Elliott DJ. *RBMY* genes and *AZFb* deletions. *J Endocrinol Invest* 2000; 23: 652–8.
11 Tsend-Ayush E, O'Sullivan LA, Grützner FS, Onnebo SM, Lewis RS, *et al*. *RBMX* gene is essential for brain development in zebrafish. *Dev Dyn* 2005; 234: 682–8.
12 Chai Z, Sarcevic B, Mawson A, Toh BH. SET-related cell division autoantigen-1 (CDA1) arrests cell growth. *J Biol Chem* 2001; 276: 33665–74.
13 Delbridge ML, Longepied G, Depetris D, Mattei MG, Disteche CM, *et al*. *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* 2004; 12: 345–56.
14 Ozbun LL, You L, Kiang S, Angdisen J, Martinez A, *et al*. 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* 2001; 73: 179–93.

15   Lau YF, Kido T, Li Y. The TSPY gene family. In: Lau YF, Chan WY, editors. The Y chromosome and male germ cell biology in health and diseases. Hackensack: World Scientific Publishing Co., Pte., Ltd. 2007. p73–90.

16   Tukiainen T, Villani AC, Yen A, Rivas MA, Marshall JL, *et al*. Landscape of X chromosome inactivation across human tissues. *Nature* 2017; 550: 244–8.

17   Arnemann J, Jakubiczka S, Thüring S, Schmidtke J. Cloning and sequence analysis of a human Y-chromosome-derived, testicular cDNA, TSPY. *Genomics* 1991; 11: 108–14.

18   Vogel T, Dittrich O, Mehraein Y, Dechend F, Schnieders F, *et al*. Murine and human *TSPYL* genes: novel members of the TSPY-SET-NAP1L1 family. *Cytogenet Cell Genet* 1998; 81: 265–70.

19   Wang GS, Hong CJ, Yen TY, Huang HY, Ou Y, *et al*. Transcriptional modification by a CASK-interacting nucleosome assembly protein. *Neuron* 2004; 42: 113–28.

20   Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, *et al*. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res* 2017; 45: D200–3.

21   Lau YF, Lau HW, Kömüves LG. Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. *Cytogenet Genome Res* 2003; 101: 250–60.

22   Muto S, Senda M, Akai Y, Sato L, Suzuki T, *et al*. Relationship between the structure of SET/TAF- Ibeta/INHAT and its histone chaperone activity. *Proc Natl Acad Sci U S A* 2007; 104: 4285–90.

23   Li Y, Zhang DJ, Qiu Y, Kido T, Lau YC. The Y-located proto-oncogene TSPY exacerbates and its X- homologue TSPX inhibits transactivation functions of androgen receptor and its constitutively active variants. *Hum Mol Genet* 2017; 26: 901–12.

24   Honecker F, Stoop H, de Krijger RR, Chris Lau YF, Bokemeyer C, *et al*. Pathobiological implications of the expression of markers of testicular carcinoma *in situ* by fetal germ cells. *J Pathol* 2004; 203 :849–57.

25   Kido T, Lau YF. A *Cre* gene directed by a human *TSPY* promoter is specific for germ cells and neurons. *Genesis* 2005; 42: 263–75.

26   Repping S, van Daalen SK, Brown LG, Korver CM, Lange J, *et al*. High mutation rates have driven extensive structural polymorphism among human Y chromosomes. *Nat Genet* 2006; 38: 463–7.

27   Krausz C, Giachini C, Forti G. TSPY and male fertility. *Genes (Basel)* 2010; 1: 308–16.

28   Svacinova V, Vodicka R, Vrtel R, Godava M, Kvapilova M, *et al*. Sequence recombination in exon 1 of the *TSPY* gene in men with impaired fertility. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2011; 155: 287–98.

29   Lau YF, Li Y, Kido T. Gonadoblastoma locus and the TSPY gene on the human Y chromosome. *Birth Defects Res C Embryo Today* 2009; 87: 114–22.

30   Lau YF, Li Y, Kido T. Role of the Y-located putative gonadoblastoma gene in human spermatogenesis. *Syst Biol Reprod Med* 2011; 57: 27–34.

31   Li Y, Lau YF. TSPY and its X-encoded homologue interact with cyclin B but exert contrasting functions on cyclin-dependent kinase 1 activities. *Oncogene* 2008; 27: 6141–50.

32   Moey C, Hinze SJ, Brueton L, Morton J, McMullan DJ, *et al*. Xp11.2 microduplications including *IQSEC2*, *TSPYL2* and *KDM5C* genes in patients with neurodevelopmental disorders. *Eur J Hum Genet* 2016; 24: 373–80.

33   Vasli N, Ahmed I, Mittal K, Ohadi M, Mikhailov A, *et al*. Identification of a homozygous missense mutation in *LRP2* and a hemizygous missense mutation in *TSPYL2* in a family with mild intellectual disability. *Psychiatr Genet* 2016; 26: 66–73.

34   Le Gallo M, O'Hara AJ, Rudd ML, Urick ME, Hansen NF, *et al*. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. *Nat Genet* 2012; 44: 1310–5.

35   Huang TN, Hsueh YP. Brain-specific transcriptional regulator T-brain-1 controls brain wiring and neuronal activity in autism spectrum disorders. *Front Neurosci* 2015; 9: 406.

36   Tsang KH, Lai SK, Li Q, Yung WH, Liu H, *et al*. The nucleosome assembly protein TSPYL2 regulates the expression of NMDA receptor subunits GluN2A and GluN2B. *Sci Rep* 2014; 4: 3654.

37   Huang TN, Hsueh YP. Calcium/calmodulin-dependent serine protein kinase (CASK), a protein implicated in mental retardation and autism-spectrum disorders, interacts with T-Brain-1 (TBR1) to control extinction of associative memory in male mice. *J Psychiatry Neurosci* 2017; 42: 37–47.

38   Chung WC, Huang TN, Hsueh YP. Targeted deletion of CASK-interacting nucleosome assembly protein causes higher locomotor and exploratory activities. *Neurosignals* 2011; 19: 128–41.

39   Li Q, Chan SY, Wong KK, Wei R, Leung YO, *et al*. *TSPYL2* loss-of-function causes neurodevelopmental brain and behavior abnormalities in mice. *Behav Genet* 2016; 46: 529–37.

40   Epping MT, Lunardi A, Nachmani D, Castillo-Martin M, Thin TH, *et al*. TSPYL2 is an essential component of the REST/NRSF transcriptional complex for TGFbeta signaling activation. *Cell Death Differ* 2015; 22: 1353–62.

41   Chai Z, Dai A, Tu Y, Li J, Wu T, *et al*. Genetic deletion of cell division autoantigen 1

42   retards diabetes- associated renal injury. *J Am Soc Nephrol* 2013; 24: 1782–92.

42   Kandalaft LE, Zudaire E, Portal-Núñez S, Cuttitta F, Jakowlew SB. Differentially expressed nucleolar transforming growth factor-beta1 target (DENTT) exhibits an inhibitory role on tumorigenesis. *Carcinogenesis* 2008; 29: 1282–9.

43   Toh BH, Tu Y, Cao Z, Cooper ME, Chai Z. Role of cell division autoantigen 1 (CDA1) in cell proliferation and fibrosis. *Genes (Basel)* 2010; 1: 335–48.

44   Puffenberger EG, Hu-Lince D, Parod JM, Craig DW, Dobrin SE, *et al*. Mapping of sudden infant death with dysgenesis of the testes syndrome (SIDDT) by a SNP genome scan and identification of TSPYL loss of function. *Proc Natl Acad Sci U S A* 2004; 101: 11689–94.

45   Schubert S, Haas C, Bartsch C, Mirshekarnejad M, Kohrs S, *et al*. Variants in *TSPYL1* are not associated with sudden infant death syndrome in a cohort of deceased infants from Switzerland. *Mol Cell Probes* 2015; 29: 31–4.

46   Hering R, Frade-Martinez R, Bajanowski T, Poets CF, Tschentscher F, *et al*. Genetic investigation of the *TSPYL1* gene in sudden infant death syndrome. *Genet Med* 2006; 8: 55–8.

47   Kim EJ, Lee SY, Kim TR, Choi SI, Cho EW, *et al*. TSPYL5 is involved in cell growth and the resistance to radiation in A549 cells via the regulation of p21(WAF1/Cip1) and PTEN/AKT pathway. *Biochem Biophys Res Commun* 2010; 392: 448–53.

48   Kumar SR, Bryan JN, Esebua M, Amos-Landgraf J, May TJ. Testis specific Y-like 5: gene expression, methylation and implications for drug sensitivity in prostate carcinoma. *BMC Cancer* 2017; 17: 158.

49   Liu M, Li B, Guo W, Zhang X, Chen Z, *et al*. Association between single nucleotide polymorphisms in the *TSPYL6* gene and breast cancer susceptibility in the Han Chinese population. *Oncotarget* 2016; 7: 54771–81.

50   GTEx Consortium. Genetic effects on gene expression across human tissues. *Nature* 2017; 550: 204–13.

51   Page DC. Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads. *Development* 1987; 101 Suppl: 151–5.

52   Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet* 1987; 25: 191–218.

53   Salo P, Kääriäinen H, Petrovic V, Peltomäki P, Page DC, *et al*. Molecular mapping of the putative gonadoblastoma locus on the Y chromosome. *Genes Chromosomes Cancer* 1995; 14: 210–4.

54   Tsuchiya K, Reijo R, Page DC, Disteche CM. Gonadoblastoma: molecular definition of the susceptibility region on the Y chromosome. *Am J Hum Genet* 1995; 57: 1400–7.

55   Affara NA, Lau YF, Briggs H, Davey P, Jones MH, *et al*. Report and abstracts of the first international workshop on Y chromosome mapping 1994. Cambridge, England, April 2-5, 1994. *Cytogenet Cell Genet* 1994; 67: 359–402.

56   Kersemaekers AM, Honecker F, Stoop H, Cools M, Molier M, *et al*. Identification of germ cells at risk for neoplastic transformation in gonadoblastoma: an immunohistochemical study for OCT3/4 and TSPY. *Hum Pathol* 2005; 36: 512–21.

57   Looijenga LH, Hersmus R, Oosterhuis JW, Cools M, Drop SL, *et al*. Tumor risk in disorders of sex development (DSD). B*est Pract Res Clin Endocrinol Metab* 2007; 21: 480–95.

58   Scully RE. Gonadoblastoma. A review of 74 cases. *Cancer* 1970; 25: 1340–56.

59   Ulbright TM. Gonadoblastoma and hepatoid and endometrioid-like yolk sac tumor: an update. *Int J Gynecol Pathol* 2014; 33: 365–73.

60   Ulbright TM, Young RH. Gonadoblastoma and selected other aspects of gonadal pathology in young patients with disorders of sex development. *Semin Diagn Pathol* 2014; 31: 427–40.

61   Lau YF. Gonadoblastoma, testicular and prostate cancers, and the *TSPY* gene. *Am J Hum Genet* 1999; 64: 921–7.

62   Honecker F, Stoop H, Mayer F, Bokemeyer C, Castrillon DH, *et al*. Germ cell lineage differentiation in non- seminomatous germ cell tumours. *J Pathol* 2006; 208: 395–400.

63   Li Y, Tabatabai ZL, Lee TL, Hatakeyama S, Ohyama C, *et al*. The Y-encoded TSPY protein: a significant marker potentially plays a role in the pathogenesis of testicular germ cell tumors. *Hum Pathol* 2007; 38: 1470–81.

64   Li Y, Vilain E, Conte F, Rajpert-De Meyts E, Lau YF. Testis-specific protein Y-encoded gene is expressed in early and late stages of gonadoblastoma and testicular carcinoma *in situ*. *Urol Oncol* 2007; 25: 141–6.

65   Hoei-Hansen CE, Sehested A, Juhler M, Lau YF, Skakkebaek NE, *et al*. New evidence for the origin of intracranial germ cell tumours from primordial germ cells: expression of pluripotency and cell differentiation markers. *J Pathol* 2006; 209: 25–33.

66   Jang JW, Song Y, Kim SH, Kim J, Seo HR. Potential mechanisms of CD133 in cancer stem cells. *Life Sci* 2017; 184: 25–9.

67   Trinh DT, Shibata K, Hirosawa T, Umezu T, Mizuno M, *et al*. Diagnostic utility of CD117, CD133, SALL4, OCT4, TCL1 and glypican-3 in malignant germ cell tumors of the ovary. *J Obstet Gynaecol Res* 2012; 38: 841–8.

68   Hervey-Jumper SL, Altshuler DB, Wang AC, He X, Maher CO, *et al*. The role of CD133+ cells in a recurrent embryonal tumor with abundant neuropil and true rosettes (ETANTR). *Brain Pathol* 2014; 24: 45–51.

69   Gashaw I, Dushaj O, Behr R, Biermann K, Brehm R, *et al*. Novel germ cell markers characterize testicular seminoma and fetal testis. *Mol Hum Reprod* 2007; 13: 721–7.

70   Oram SW, Liu XX, Lee TL, Chan WY, Lau YF. TSPY potentiates cell proliferation

and tumorigenesis by promoting cell cycle progression in HeLa and NIH3T3 cells. *BMC Cancer* 2006; 6: 154.

71 Kido T, Lau YF. The Y-located gonadoblastoma gene *TSPY* amplifies its own expression through a positive feedback loop in prostate cancer cells. *Biochem Biophys Res Commun* 2014; 446: 206–11.

72 Kido T, Schubert S, Schmidtke J, Chris Lau YF. Expression of the human *TSPY* gene in the brains of transgenic mice suggests a potential role of this Y chromosome gene in neural functions. *J Genet Genomics* 2011; 38: 181–92.

73 Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, et al. EAU guidelines on prostate cancer. Part II: treatment of advanced, relapsing, and castration-resistant prostate cancer. *Eur Urol* 2014; 65: 467–79.

74 Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, et al. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. *Eur Urol* 2014; 65: 124–37.

75 Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; 127: S5–16.

76 Yin J, Zhang H, Li C, Gao C, He Y, et al. Role of hepatitis B virus genotype mixture, subgenotypes C2 and B2 on hepatocellular carcinoma: compared with chronic hepatitis B and asymptomatic carrier state in the same area. *Carcinogenesis* 2008; 29: 1685–91.

77 Lee CM, Lu SN, Changchien CS, Yeh CT, Hsu TT, et al. Age, gender, and local geographic variations of viral etiology of hepatocellular carcinoma in a hyperendemic area for hepatitis B virus infection. *Cancer* 1999; 86: 1143–50.

78 Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. *Hepatology* 1995; 22: 1027–33.

79 Kido T, Lo RC, Li Y, Lee J, Tabatabai ZL, et al. The potential contributions of a Y-located protooncogene and its X homologue in sexual dimorphisms in hepatocellular carcinoma. *Hum Pathol* 2014; 45: 1847–58.

80 Kido T, Hatakeyama S, Ohyama C, Lau YF. Expression of the Y-encoded TSPY is associated with progression of prostate cancer. *Genes (Basel)* 2010; 1: 283–93.

81 Shirvani-Dastgerdi E, Schwartz RE, Ploss A. Hepatocarcinogenesis associated with hepatitis B, delta and C viruses. *Curr Opin Virol* 2016; 20: 1–10.

82 Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. *Best Pract Res Clin Gastroenterol* 2014; 28: 753–70.

83 Bravi F, Tavani A, Bosetti C, Boffetta P, La Vecchia C. Coffee and the risk of hepatocellular carcinoma and chronic liver disease: a systematic review and meta-analysis of prospective studies. *Eur J Cancer Prev* 2017; 26: 368–77.

84 Brandon-Warner E, Walling TL, Schrum LW, McKillop IH. Chronic ethanol feeding accelerates hepatocellular carcinoma progression in a sex-dependent manner in a mouse model of hepatocarcinogenesis. *Alcohol Clin Exp Res* 2012; 36: 641–53.

85 Liu WC, Liu QY. Molecular mechanisms of gender disparity in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol* 2014; 20: 6252–61.

86 Ma WL, Lai HC, Yeh S, Cai X, Chang C. Androgen receptor roles in hepatocellular carcinoma, fatty liver, cirrhosis and hepatitis. *Endocr Relat Cancer* 2014; 21: R165–82.

87 Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; 317: 121–4.

88 Wang SH, Chen PJ, Yeh SH. Gender disparity in chronic hepatitis B: mechanisms of sex hormones. *J Gastroenterol Hepatol* 2015; 30: 1237–45.

89 Wang SH, Yeh SH, Chen PJ. The driving circuit of HBx and androgen receptor in HBV-related hepatocarcinogenesis. *Gut* 2014; 63: 1688–9.

90 Yeh SH, Chen PJ. Gender disparity of hepatocellular carcinoma: the roles of sex hormones. *Oncology* 2010; 78 Suppl 1: 172–9.

91 Kido T, Lau YC. Identification of a TSPY co-expression network associated with DNA hypomethylation and tumor gene expression in somatic cancers. *J Genet Genomics* 2016; 43: 577–85.

92 Robert C, Watson M. Errors in RNA-Seq quantification affect genes of relevance to human disease. *Genome Biol* 2015; 16: 177.

93 Yin YH, Li YY, Qiao H, Wang HC, Yang XA, et al. TSPY is a cancer testis antigen expressed in human hepatocellular carcinoma. *Br J Cancer* 2005; 93: 458–63.

94 Li S, Mo C, Huang S, Yang S, Lu Y, et al. Over-expressed testis-specific protein Y-encoded 1 as a novel biomarker for male hepatocellular carcinoma. *PLoS One* 2014; 9: e89219.

95 Sato S, Maekawa R, Yamagata Y, Asada H, Tamura I, et al. Potential mechanisms of aberrant DNA hypomethylation on the X chromosome in uterine leiomyomas. *J Reprod Dev* 2014; 60: 47–54.

96 Derynck R, Miyazono K. The biology of TGF-beta family. *Cold Spring Harb Perspect Biol* 2017; 9: a022095.

97 Meyers EA, Kessler JA. TGF-β family signaling in neural and neuronal differentiation, development, and function. *Cold Spring Harb Perspect Biol* 2017; 9: a022244.

98 Seoane J, Gomis RR. TGF-β family signaling in tumor suppression and cancer progression. *Cold Spring Harb Perspect Biol* 2017; 9: a022277.

99 Koike K. Hepatitis B virus HBx gene and hepatocarcinogenesis. *Intervirology* 1995;

38: 134–42.

100 Ali A, Abdel-Hafiz H, Suhail M, Al-Mars A, Zakaria MK, et al. Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma. *World J Gastroenterol* 2014; 20: 10238–48.

101 Chiu CM, Yeh SH, Chen PJ, Kuo TJ, Chang CJ, et al. Hepatitis B virus X protein enhances androgen receptor-responsive gene expression depending on androgen level. *Proc Natl Acad Sci U S A* 2007; 104: 2571–8.

102 Liu B, Wen X, Huang C, Wei Y. Unraveling the complexity of hepatitis B virus: from molecular understanding to therapeutic strategy in 50 years. *Int J Biochem Cell Biol* 2013; 45: 1987–96.

103 Tian Y, Kuo CF, Chen WL, Ou JH. Enhancement of hepatitis B virus replication by androgen and its receptor in mice. *J Virol* 2012; 86: 1904–10.

104 You X, Liu F, Zhang T, Lv N, Liu Q, et al. Hepatitis B virus X protein upregulates Lin28A/Lin28B through Sp-1/c-Myc to enhance the proliferation of hepatoma cells. *Oncogene* 2014; 33: 449–60.

105 Yu Z, Gao YQ, Feng H, Lee YY, Li MS, et al. Cell cycle-related kinase mediates viral-host signalling to promote hepatitis B virus-associated hepatocarcinogenesis. *Gut* 2014; 63: 1793–804.

106 Zhu R, Zhang JS, Zhu YZ, Fan J, Mao Y, et al. HBx-induced androgen receptor expression in HBV- associated hepatocarcinoma is independent of the methylation status of its promoter. *Histol Histopathol* 2011; 26: 23–35.

107 Kido T, Ou JH, Lau YF. The X-linked tumor suppressor TSPX interacts and promotes degradation of the hepatitis B viral protein HBx via the proteasome pathway. *PLoS One* 2011; 6: e22979.

108 Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet* 2008; 40: 281–3.

109 Hooker S, Hernandez W, Chen H, Robbins C, Torres JB, et al. Replication of prostate cancer risk loci on 8q24, 11q13, 17q12, 19q33, and Xp11 in African Americans. *Prostate* 2010; 70: 270–5.

110 Morford JJ, Wu S, Mauvais-Jarvis F. The impact of androgen actions in neurons on metabolic health and disease. *Mol Cell Endocrinol* 2018: 465: 92–102.

111 Moretti C, Lanzolla G, Moretti M, Gnessi L, Carmina E. Androgens and hypertension in men and women: a unifying view. *Curr Hypertens Rep* 2017; 19: 44.

112 Lucas-Herald AK, Alves-Lopes R, Montezano AC, Ahmed SF, Touyz RM. Genomic and non-genomic effects of androgens in the cardiovascular system: clinical implications. *Clin Sci (Lond)* 2017; 131: 1405–18.

113 Takayama KI. The biological and clinical advances of androgen receptor function in age-related diseases and cancer [Review]. *Endocr J* 2017; 64: 933–46.

114 Takov K, Wu J, Denvir MA, Smith LB, Hadoke PW. The role of androgen receptors in atherosclerosis. *Mol Cell Endocrinol* 2018; 465: 82–91.

115 Dimitriadis F, Tsiampali C, Chaliasos N, Tsounapi P, Takenaka A, et al. The Sertoli cell as the orchestra conductor of spermatogenesis: spermatogenic cells dance to the tune of testosterone. *Hormones (Athens)* 2015; 14: 479–503.

116 O'Hara L, Smith LB. Androgen receptor roles in spermatogenesis and infertility. *Best Pract Res Clin Endocrinol Metab* 2015; 29: 595–605.

117 Kido T, Lau YF. The human Y-encoded testis-specific protein interacts functionally with eukaryotic translation elongation factor eEF1A, a putative oncoprotein. *Int J Cancer* 2008; 123: 1573–85.

118 Lu J, Van der Steen T, Tindall DJ. Are androgen receptor variants a substitute for the full- length receptor? *Nat Rev Urol* 2015; 12: 137–44.

119 Antonarakis ES. Predicting treatment response in castration-resistant prostate cancer: could androgen receptor variant-7 hold the key? *Expert Rev Anticancer Ther* 2015; 15: 143–5.

120 Ciccarese C, Santoni M, Brunelli M, Buti S, Modena A, et al. AR-V7 and prostate cancer: the watershed for treatment selection? *Cancer Treat Rev* 2016; 43: 27–35.

121 Lau YF, Zhang J. Expression analysis of thirty one Y chromosome genes in human prostate cancer. *Mol Carcinog* 2000; 27: 308–21.

122 Lau Y, Chou P, Iezzoni J, Alonzo J, Kömüves L. Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. *Cytogenet Cell Genet* 2000; 91: 160–4.

123 Francis JC, Swain A. Prostate organogenesis. *Cold Spring Harb Perspect Med* 2017. Doi: 10.1101/cshperspect.a030353. [Epub ahead of print].

124 Toivanen R, Shen MM. Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. *Development* 2017; 144: 1382–98.

125 Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. *Genes Dev* 2000; 14: 2410–34.

126 Brand LJ, Dehm SM. Androgen receptor gene rearrangements: new perspectives on prostate cancer progression. *Curr Drug Targets* 2013; 14: 441–9.

127 Bryce AH, Antonarakis ES. Androgen receptor splice variant 7 in castration-resistant prostate cancer: clinical considerations. *Int J Urol* 2016; 23: 646–53.

128 Schubert S, Skawran B, Dechend F, Nayernia K, Meinhardt A, et al. Generation and characterization of a transgenic mouse with a functional human TSPY. *Biol Reprod* 2003; 69: 968–75.

129 Kido T, Schubert S, Hatakeyama S, Ohyama C, Schmidtke J, et al. Expression of a Y-located human proto- oncogene *TSPY* in a transgenic mouse model of prostate cancer. *Cell Biosci* 2014; 4: 9.

130 Gamble MJ, Erdjument-Bromage H, Tempst P, Freedman LP, Fisher RP. The histone chaperone TAF- I/SET/INHAT is required for transcription *in vitro* of chromatin templates. *Mol Cell Biol* 2005; 25: 797–807.

131 Kim DW, Kim KB, Kim JY, Lee KS, Seo SB. Negative regulation of neuronal cell differentiation by INHAT subunit SET/TAF-Iβ. *Biochem Biophys Res Commun* 2010; 400: 419–25.

132 Saavedra F, Rivera C, Rivas E, Merino P, Garrido D, *et al*. PP32 and SET/TAF-Iβ proteins regulate the acetylation of newly synthesized histone H4. *Nucleic Acids Res* 2017; 45: 11700–10.

133 Almeida LO, Neto MP, Sousa LO, Tannous MA, Curti C, *et al*. SET oncoprotein accumulation regulates transcription through DNA demethylation and histone hypoacetylation. *Oncotarget* 2017; 8: 26802–18.

134 Kim JY, Lee KS, Seol JE, Yu K, Chakravarti D, *et al*. Inhibition of p53 acetylation by INHAT subunit SET/TAF-Iβ represses p53 activity. *Nucleic Acids Res* 2012; 40: 75–87.

135 Wang D, Kon N, Lasso G, Jiang L, Leng W, *et al*. Acetylation-regulated interaction between p53 and SET reveals a widespread regulatory mode. *Nature* 2016; 538: 118–22.

136 Wang D, Kon N, Tavana O, Gu W. The "readers" of unacetylated p53 represent a new class of acidic domain proteins. *Nucleus* 2017; 8: 360–9.

137 Laptenko O, Tong DR, Manfredi J, Prives C. The tail that wags the dog: how the disordered C-terminal domain controls the transcriptional activities of the p53 tumor-suppressor protein. *Trends Biochem Sci* 2016; 41: 1022–34.

138 Sullivan KD, Galbraith MD, Andrysik Z, Espinosa JM. Mechanisms of transcriptional regulation by p53. *Cell Death Differ* 2018; 25: 133–43.

139 Tu Y, Wu W, Wu T, Cao Z, Wilkins R, *et al*. Antiproliferative autoantigen CDA1 transcriptionally up-regulates p21(Waf1/Cip1) by activating p53 and MEK/ERK1/2 MAPK pathways. *J Biol Chem* 2007; 282: 11722–31.
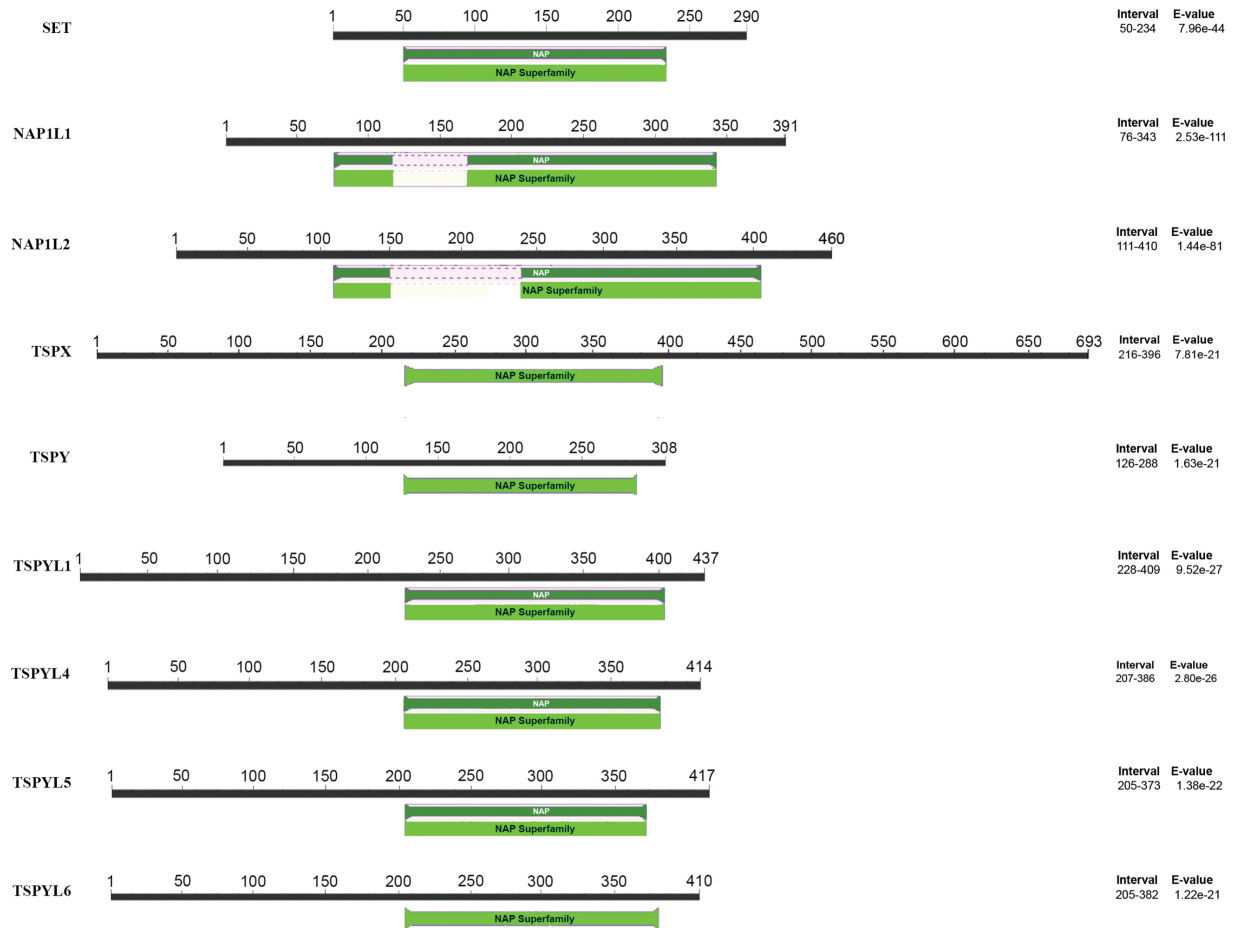
Supplemental Figure 1   Locations of the conserved NAP domains on the members of the SET/NAP/TSPY protein superfamily. The NAP domains were identified by searches of the Conserved Domain database at the National Center for Biotechnology Information (NCBI) portal with the individual protein sequences. The specific intervals harboring the NAP/SET domain (indicated by the dark and light green bars in the middle) and the Expected value (E-value) of respective proteins are listed on the right columns. The E-value is calculated with respect to the overall length of the protein and the probability of matches with the corresponding conserved domain. The lower the E-value, the more significant is the match. Based on the E-values, the TSPY/TSPX and TSPYL proteins could constitute a TSPY-like subfamily of proteins harboring NAP domains somewhat distinct from those of the SET/NAP proteins. Searches were conducted on March 21, 2018.

SET

NAP1L1

NAP1L2

TSPX

TSPY

TSPYL1
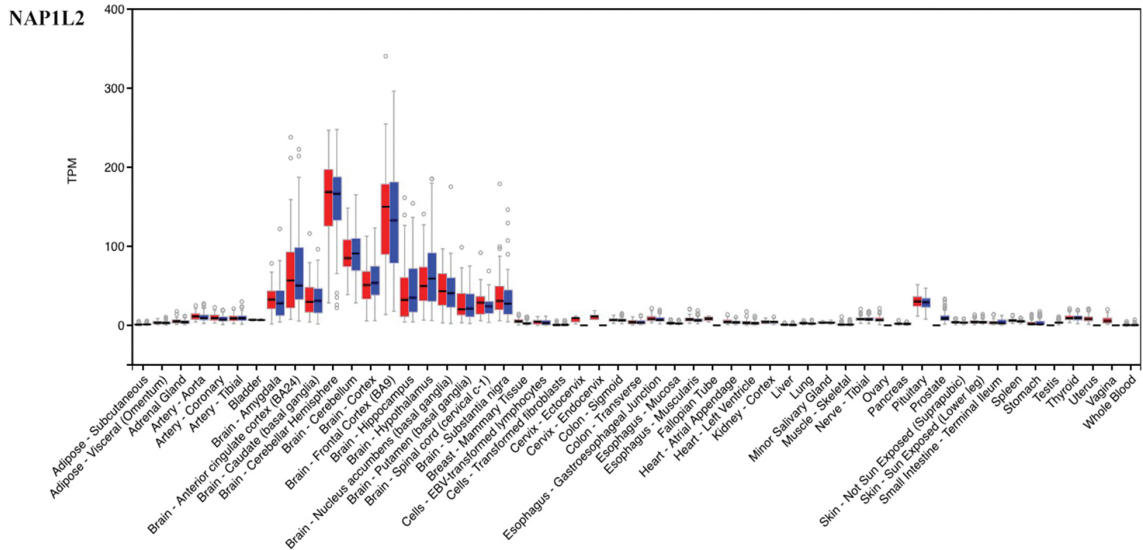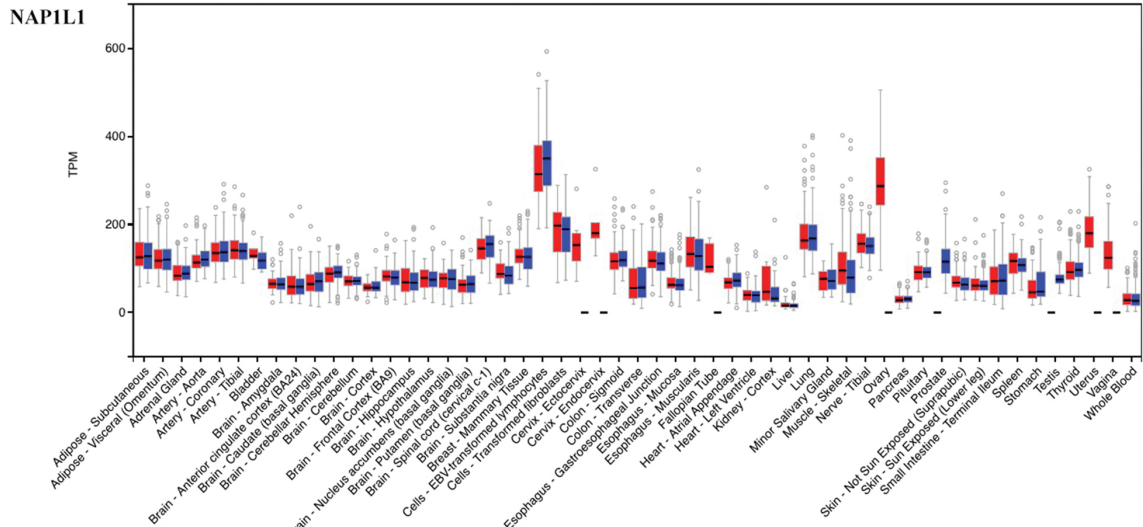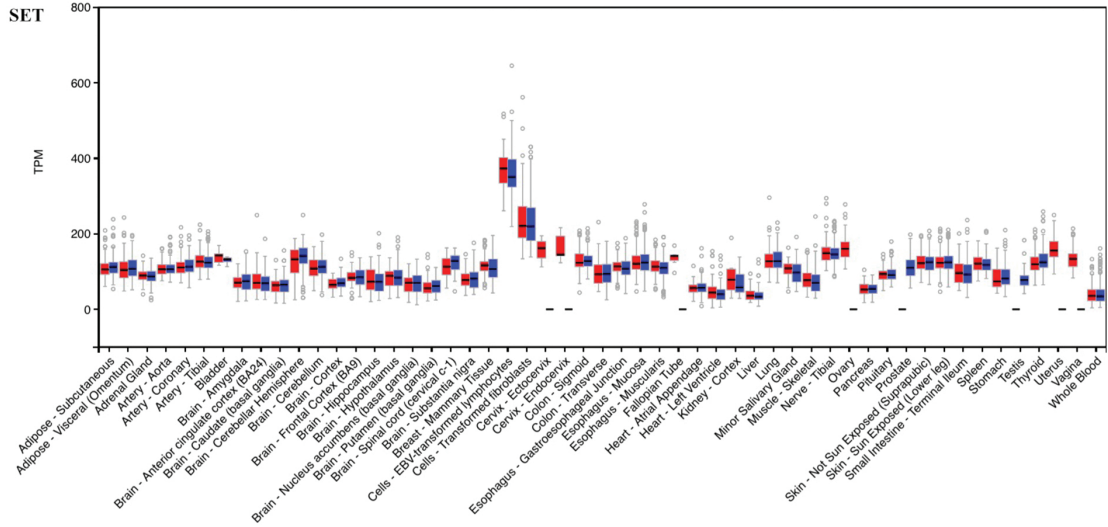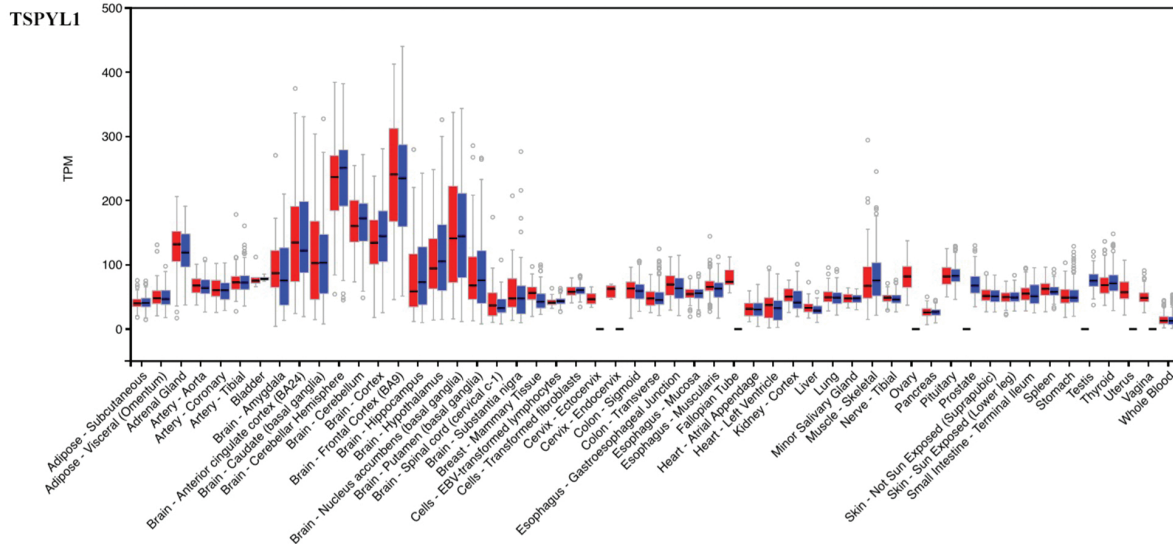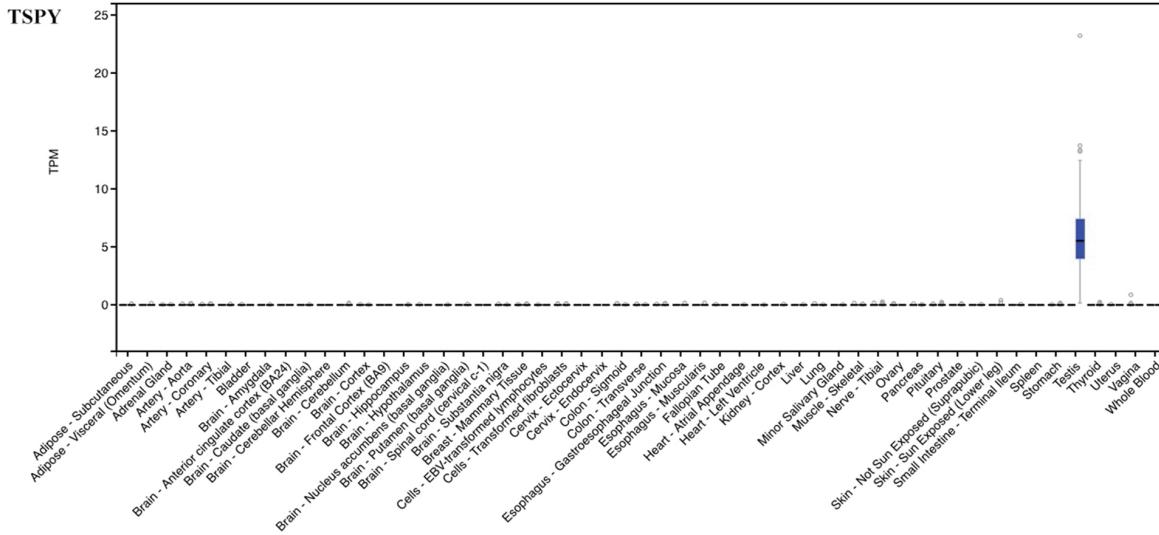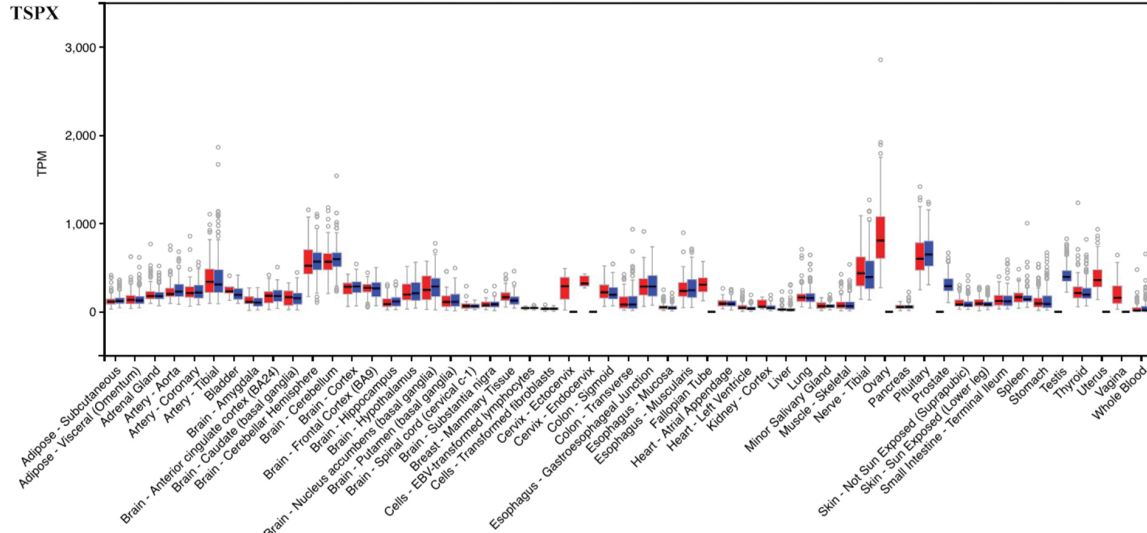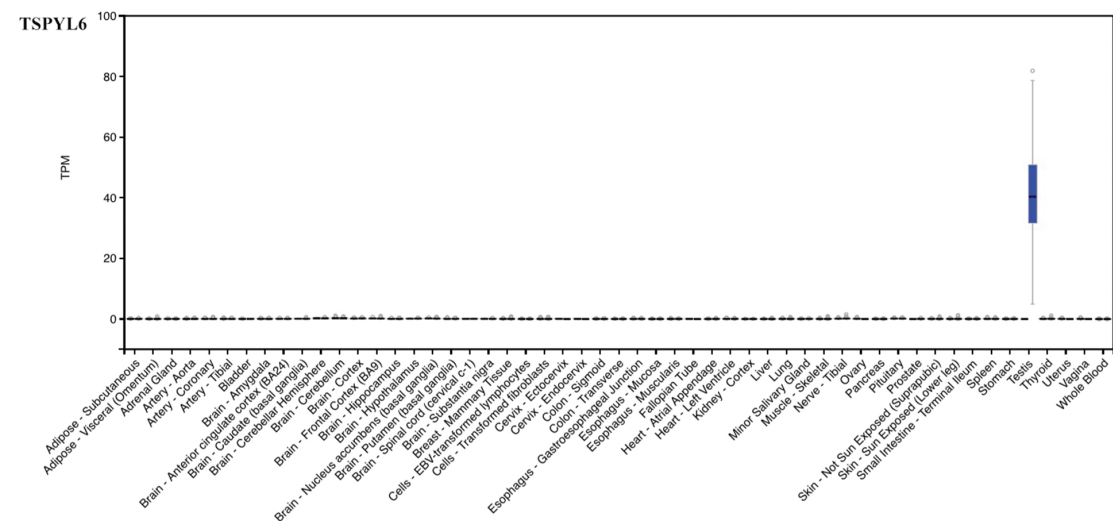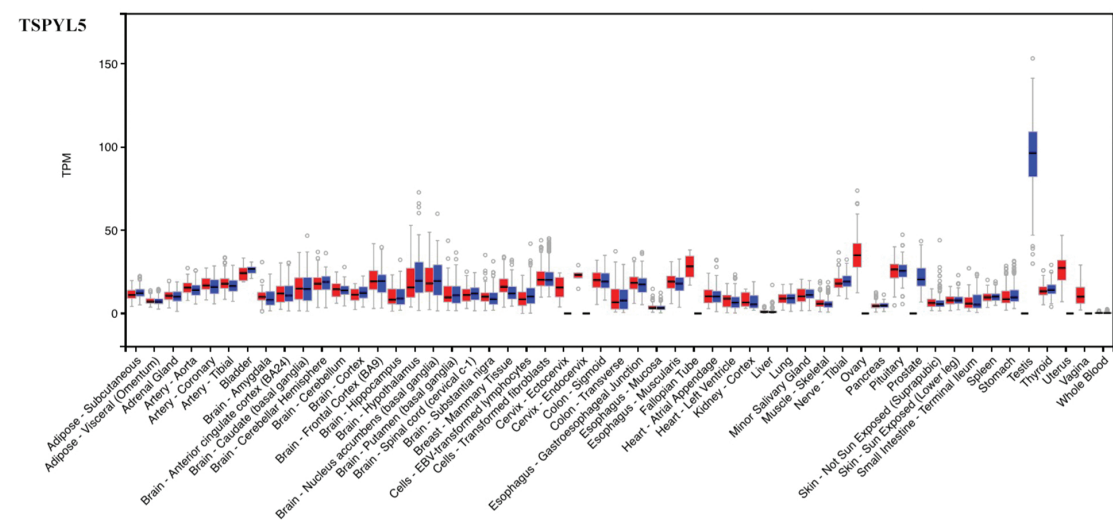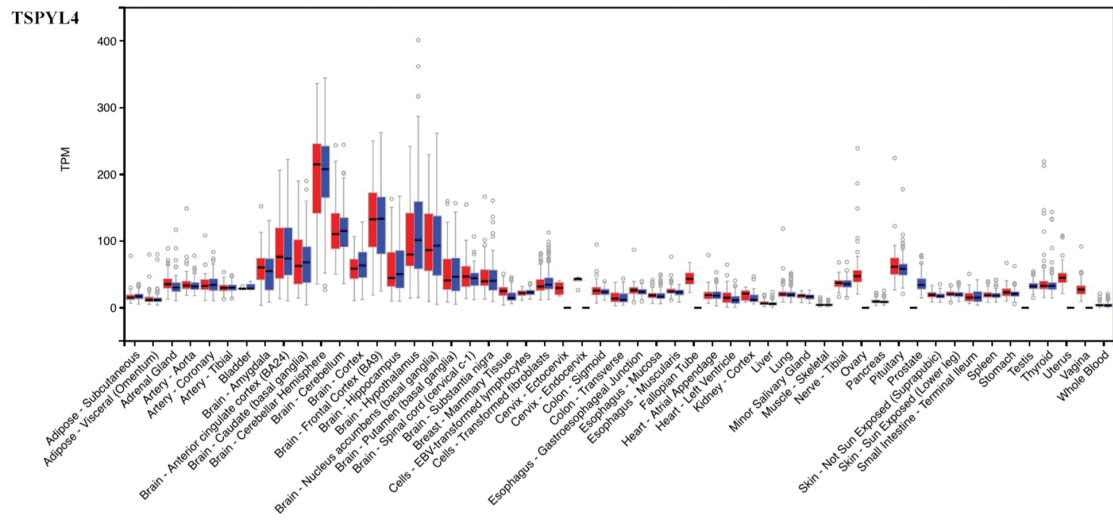
Supplemental Figure 2   Expression patterns of SET/NAP/TSPY family genes in 53 human tissues and cells. Data extracted from the GTEx Project portal. Red and blue represent female and male respectively. TPM = transcripts per million reads.