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INVITED REVIEW

Germ Cell Biology

Roles of the Y chromosome genes in human cancers

Tatsuo Kido, Yun-Fai Chris Lau

Male and female differ genetically by their respective sex chromosome composition, that is, XY as male and XX as female. Although both X and Y chromosomes evolved from the same ancestor pair of autosomes, the Y chromosome harbors male-specific genes, which play pivotal roles in male sex determination, germ cell differentiation, and masculinization of various tissues. Deletions or translocation of the sex-determining gene, *SRY*, from the Y chromosome causes disorders of sex development (previously termed as an intersex condition) with dysgenic gonads. Failure of gonadal development results not only in infertility, but also in increased risks of germ cell tumor (GCT), such as gonadoblastoma and various types of testicular GCT. Recent studies demonstrate that either loss of Y chromosome or ectopic expression of Y chromosome genes is closely associated with various male-biased diseases, including selected somatic cancers. These observations suggest that the Y-linked genes are involved in male health and diseases in more frequently than expected. Although only a small number of protein-coding genes are present in the male-specific region of Y chromosome, the impacts of Y chromosome genes on human diseases are still largely unknown, due to lack of *in vivo* models and differences between the Y chromosomes of human and rodents. In this review, we highlight the involvement of selected Y chromosome genes in cancer development in men.

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INTRODUCTION

Numerous studies have identified various sex differences in the risks, incidence and progression of various human diseases, such as asthma,[1,2] autoimmune diseases,[3,4] schizophrenia,[5,6] autism spectrum disorders,[7,8] cardiovascular disease,[9,10] and non-sex-specific cancers such as liver cancer, bladder cancer, and lung cancer.[11–13] According to the report by Cook and colleagues, 32 out of 36 cancer types showed male preference of cancer mortality in United States for the years between 1977 and 2006.[14] However, the mechanisms responsible for such sex-differences are still largely unknown. The most significant genetic differences between men and women are genes on their sex chromosomes, that is, XY for men and XX for women. Men are prone to X-linked diseases caused by mutations on genes on their X chromosome while ectopic expression of the genes on their Y chromosome could have male-specific effects on normal development, physiology, and diseases. The human Y chromosome can be classified structurally into three regions: (i) male-specific region of the Y chromosome (*MSY*), (ii) pseudoautosomal regions (PAR1 and PAR2), and (iii) heterochromatin region on Yq (**Figure 1**).[15] PARs contain 20 protein-coding genes (16 genes in PAR1 and 4 genes in PAR2) that are also present on the X chromosome.[16] The *MSY* contains 23 protein-coding genes and numerous pseudogenes (**Table 1**).[15,17,18] While genes in PARs are present in both X and Y chromosomes and undergo meiotic recombination similarly with autosomal genes, genes in *MSY* are excluded from meiotic recombination with a homologous chromosome partner. The *MSY* genes evolved during about 300 million years after beginning of X-Y differentiation.[19] The *MSY* genes can be classified into two groups according to their expression patterns. Group-I genes are expressed almost ubiquitously, and Group-II genes are expressed specifically/predominantly in testis (**Table 1**).[20–22] It is postulated that Group-I *MSY* genes function as broadly expressed regulators for gene expression and protein stability as maintaining the ancestral dosage of homologous X-Y gene pairs, e.g., *DDX3Y*, *EIF1AY*, *KDM5D*, *RPS4Y*, *TBL1Y*, *USP9Y*, *UTY*, and *ZFY*.[21] The ubiquitous and/or somatic expressions of *MSY* genes suggest that the balanced expression between *MSY* genes and their X homologs could be crucial to maintain the healthy condition in men. In humans, 12 of 14 functional X-Y paired genes (86%) escape X-inactivation in female,[21] thereby maintaining the dosage balance of X-Y paired genes. On the other hand, Group-II genes, including *HSFY*, *SRY*, RNA-binding motif protein, Y-linked (*RBMY*), and testis-specific protein, Y-encoded (*TSPY*), may play diverse functions from their X homologs.

Transgenic mouse models using knockout strategies are useful tools to determine and infer the functions of respective genes in human health and diseases. However, only 9 of 17 ancestral genes in the human *MSY* are conserved in the mouse Y chromosome.[21] Recent work by Soh *et al.*[23] demonstrated that only 2.2% of mouse *MSY* sequence shares ancestry with the primate *MSYs*. Further, the mouse Y-chromosome long-arm harbors the highly amplified units (85–221 times) containing genes, such as *Sly*, *Ssty1*, *Ssty2*, or *Srsy* that are absent on the primates Y-chromosomes.[23] Accordingly, mouse modeling of human *MSY* genes is difficult, and the impacts of *MSY* genes on human diseases are still largely unknown. Based on genetic mapping studies, three major loci have been assigned to the human *MSY*, that is, testis-determining factor (*TDF*), gonadoblastoma locus

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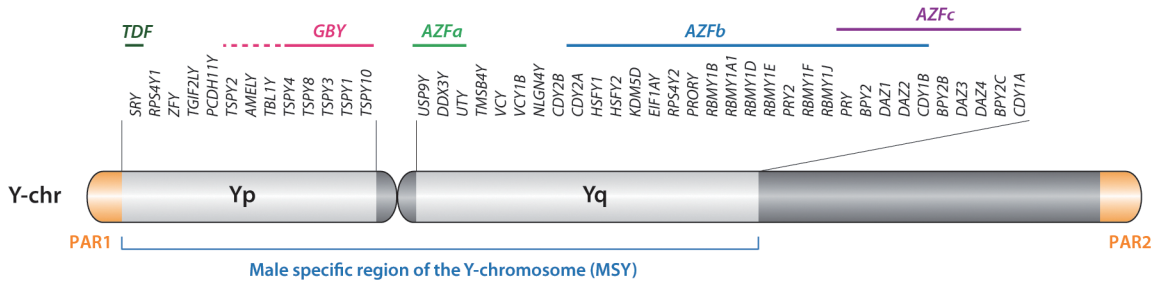

Figure 1: Schematic diagram of human Y chromosome indicating the protein-coding genes within the male-specific region of Y chromosome. *AZFa-c*: the deleted regions identified in azoospermia patients; *GBY*: the gonadoblastoma locus on Y chromosome; PAR1 and PAR2: the pseudoautosomal regions.

Table 1: Protein coding *MSY* genes

Gene/gene family[a]	*Members of multi-copy gene*	*Functional domain in protein product*[17]	*Expression*[20]
AMELY			Testis specific
BPY2	*BPY-2, 2B, 2C*	Winged HTH-like domain	Testis specific
CDY	*CDY-1, 1B, 2A, 2B*	CHROMO domain	Testis specific
DAZ	*DAZ-1, 2, 3, 4*	RRM	Predominantly in testis[c]
DDX3Y (DBY)[b]		DEAD-like helicase	Ubiquitous
EIF1AY		Eukaryotic translation initiation factor 1A	Ubiquitous
HSFY	*HSFY-1, 2*	HSF	Testis specific
KDM5D (SMCY)[b]		PHD zinc finger, jumonji domain	Ubiquitous
NLGN4Y		Carboxylesterase	Ubiquitous
PCDH11Y		Cadherin repeats	Ubiquitous
PRY	*PRY, PRY2*		Testis, heart, lung, white blood cells
RBMY	*RBMY1-A1, 1B, 1C, 1D, 1E, 1F/J*	RRM	Testis specific
RPS4Y	*RPS4Y1, RPS4Y2*	S4 RNA-binding domain	Ubiquitous
SRY		HMG	Predominantly in testis[c]
TBL1Y		WD40 repeats (WD40)	Ubiquitous
TGIF2LY		Homeodomain (HOX)	Testis specific
TMSB4Y (TB4Y)[b]		Thymosin β-actin-binding motif	Ubiquitous
TSPY	>40 copies (copy number varies among cohort)	SET/NAP domain	Predominantly in testis[c]
USP9Y (DFFRY)[b]		Ubiquitin-like domain, ubiquitin C-terminal hydrolase	Ubiquitous
UTY		Jumonji domain, treble-clef zinc finger	Ubiquitous
VCY	*VCY, VCY1B*		Predominantly in testis[c]
XKRY	*XKRY, XKRY2*		Testis specific[22]
ZFY		Zinc fingers (ZnF_C2H2)	Ubiquitous

[a]Gene name and gene ID are listed in Table S1; [b]A popular alias alternatively used; [c]Other tissue(s) also expresses, but testis expresses at the highest level. *MSY*: male-specific region of the Y chromosome; HTH: helix-turn-helix; CHROMO: chromatin organization modifier; RRM: RNA recognition motif; HSF: heat-shock factor; HMG: high-mobility group; NAP: nucleosome assembly protein; SET: SE translocation

on Y chromosome (*GBY*) and azoospermia factor (*AZF*) (**Figure 1**). The *SRY* gene has been demonstrated to be the testis-determining gene,[24,25] while a group of genes, that is, *RBMY* and *DAZ*, have been identified within the *AZF* locus on the long-arm.[15,26,27] The gonadoblastoma (*GBY*) locus was initially mapped to a small region on the short arm of the Y chromosome proximal to the centromere, harboring a gene predisposing dysgenetic gonads of XY sex-reversed patients to develop gonadoblastoma.[28] Subsequent studies showed that *TSPY* is the putative gene for this locus.[29,30] The sex determination and genes for the *AZF* locus have recently been reviewed in details.[27,31,32] The present review focuses on the *MSY* genes and their associations to human tumors, including gonadoblastoma.

EXPRESSION OF Y-LINKED GENES AND HUMAN CANCERS

Various studies have demonstrated that *TSPY* and *RBMY* are ectopically expressed in somatic cells under disease conditions, such as cancer, although they are normally expressed preferentially in testicular germ cells (see below). To explore the changes of *MSY* genes in somatic cancers, we had performed a data-mining study on hepatocellular carcinoma (HCC) using the RNA-Seq gene expression data on 27 pairs of male tumor-nontumor paired samples at the Cancer Genome Atlas (TCGA) project.[33] Our results showed that, in addition to *TSPY* and *RBMY*, other *MSY* genes, that is, *TGIF2LY* and *VCY*, were consistently up-regulated in ~30% cases of liver cancer, while *DDX3Y*, *ZFY*, and *DAZ1* were frequently down-regulated (~70%, **Figure 2**). Since the Y chromosome is unique to men, these observations suggest that the Y chromosome genes could potentially influence on the development, progression and outcomes of liver cancer in a male-specific manner(s).

Testis-specific protein, Y-encoded (TSPY)

The human *TSPY* gene was initially identified as a Y-linked gene specifically expressed in the testis.[34,35] It is tandemly repeated in 20.3-kb highly homologous units, usually in the range of 21–35 copies, on the short arm of the Y chromosome.[15,36–38] The human *TSPY* is expressed in gonocytes in the embryonic testis,[39] spermatogonia, and prophase I spermatocytes at preleptotene to zygotene stages in adult testis.[40] Deletion mapping has localized the *TSPY* repeat units to the critical

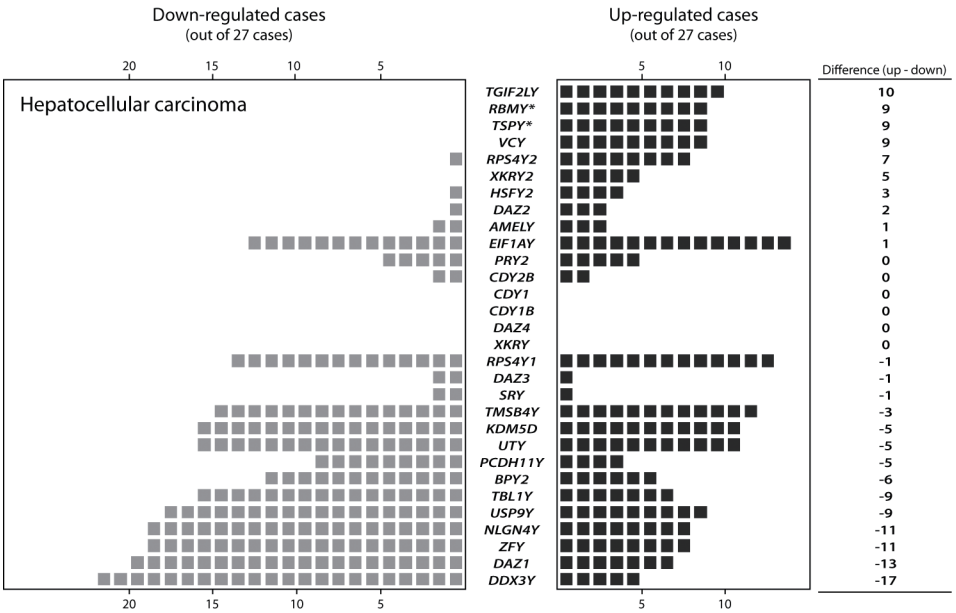

Figure 2: Gene expression profile of MYS protein-coding genes in male liver cancer cases. The case number showing either up-regulation or down-regulation in cancer specimens is indicated for each gene, according to the RNA-Seq gene expression data derived from the database of the Cancer Genome Atlas project. Twenty-seven pairs of tumor and corresponding nontumor tissue were analyzed. Each black or gray square indicates an up or down respectively the expression of the corresponding Y chromosome genes in the tumor to nontumor pairs.

region harboring the *GBY* locus on the short arm.[29,30] It is postulated to serve normal functions in male germ cell differentiation, mitosis, and meiosis,[41,42] but could promote gonadoblastoma development in patients with disorders of sex developments (DSDs) harboring Y chromosome materials including *TSPY*. Indeed, *TSPY* expression has been observed in gonadoblastoma, and various types of germ cell tumors (GCTs), including carcinoma *in situ*/intratubular germ cell neoplasia unclassified (CIS/ITGCNU) (the precursor for all testicular GCTs [TGCTs]), seminoma, and extragonadal intracranial GCT.[43–46] In addition to GCTs, *TSPY* is frequently expressed in some somatic cancers including liver cancer,[47,48] melanoma,[49] and prostate cancer,[50,51] suggesting that *TSPY* can be considered as a cancer-testis antigen (CT-antigen). CT-antigens are group of proteins that are predominantly/specifically in testis under normal conditions, but are ectopically expressed in somatic cancers.[52] Although the biological functions of CT-antigens are currently uncertain, they have been proposed as diagnostic markers and therapeutic targets in cancers.[48,52–54]

Molecular functions of TSPY

TSPY is a member of SE translocation/nucleosome assembly protein 1 (SET/NAP1) superfamily harboring a highly homologous SET/NAP-domain, initially identified in the SET oncoprotein (also called template-activating factor I or TAF-I) and the NAP1.[42,55] X-ray crystallography showed that the SET protein forms a homodimer with a headphone like structure, and the SET/NAP domain occupies the earmuff region, which could be important for protein-protein binding with interactive partners. The N-terminal alpha-helix region contains the binding site for the homodimerization.[56–58] Members of the SET/NAP1 protein family could function as chaperones of histones. In particular, NAP1 plays crucial roles in shuttling and assembling core histones as a histone chaperone.[57,59] SET forms inhibitor of histone acetyltransferase (INHAT) complex with its isoform TAF-Iα and pp32.[60] INHAT complex associates with chromatin to inhibit the histone acetylation mediated by acetyltransferases, thereby suppressing the expression of targeted genes.[60] SET also interacts with transactivators and enhances their respective target gene expression.[54,61] These observations suggest that SET/NAP1 proteins serve a wide range of functions in many biological processes. Recently, we reported that TSPY binds the type B cyclins and enhances the kinase activity of the cyclin-B/CDK1 complex.[62] Correlating with this function, overexpression of *TSPY* leads to shortening of the G2/M phase and acceleration of cell proliferation in *TSPY*-transfected HeLa and 3T3 cells *in vitro* and tumorigenicity in athymic mice *in vivo*.[63] In contrast, its single-copy X-linked homolog TSPX (also termed as TSPY-like 2 [*TSPYL2*], differentially expressed nuclear TGF-β1 target [*DENTT*] or cell division autoantigen 1 [*CDA1*]) inhibits the kinase activity of cyclin-B/CDK1 complex.[62] TSPX protein harbors a 250 amino acids aspartic acid/glutamic acid (D/E)-rich domain at its C-terminus, which is absent in TSPY. The inhibitory function of TSPX has been mapped on the C-terminal D/E-rich domain.[62] Since the SET/NAP-domains of both proteins are well conserved, TSPY and TSPX could play contrasting roles on their common target molecules. Abrogated TSPX expression in lung cancer is associated with accelerated cancer progression.[64] *In vitro* studies also demonstrated that overexpression of TSPX retards cell proliferation.[65] Further, down-regulation of TSPX by nitric-oxide correlates with the glioma stem cell proliferation.[66] These observations suggest that while *TSPY* and *TSPX* originated from the same ancestor gene, they have respectively evolved into two independent genes on the sex chromosomes, and play contrasting roles in human oncogenesis, that is, *TSPY* as a proto-oncogene and *TSPX* as a tumor suppressor gene.[67,68]

Yeast-two hybrid screening using the SET/NAP-domain of TSPY as bait has identified several novel TSPY binding proteins. The first one is the translation elongation factor 1A, eEF1A. The SET/NAP domain of TSPY binds to the domain-III of eEF1A, and enhances protein synthesis.[69] TSPY also binds to the 40S ribosomal component RPS26 (unpublished data), suggesting that TSPY could be associated with the protein synthesis machinery in the cells. Recent studies suggest

that protein synthesis is crucial in the regulation of cell proliferation and cancer progression.[70–73] TSPY may normally support protein synthesis essential for the maintenance of germ cell proliferation, but when ectopically expressed, it could promote cancer growth under diseased conditions. Interestingly, we also showed that TSPY protein binds to the exon-1 of its structural gene and enhances its own expression in prostate cancer cells,[74] suggesting that TSPY could intensify its functions by amplifying its own gene expression through a positive feedback loop. Hence, TSPY could play the role of a transcription regulator, by binding to the DNA/nuclear proteins on the chromatin of target genes. Since *TSPY* is located in the male-only Y chromosome, its functions in the protein synthetic machinery, cell cycle progression, histone chaperone/chromatin modification gene, and regulation could shed new insights on sex disparities associated with the development, progression and treatments responses among numerous somatic cancers, e.g., liver cancer and melanoma, with ectopic *TSPY* expression.

Expression of TSPY in germ cell tumors

Human GCTs can be classified into five types based on various parameters including age at clinical presentation, anatomical sites and histology; e.g., type-I, teratoma/yolk sac tumor; type-II, seminomatous/nonseminomatous GCTs; type-III, spermatocytic seminoma; type-IV, dermoid cyst; type-V, hydatidiform mole.[75] *TSPY* expression is primarily detected in type-II TGCs and type-III spermatocytic seminoma.[44] Type-II TCGs are further subdivided into nonseminomatous GCTs and seminomatous GCTs, including seminoma, dysgerminoma, germinoma, and gonadoblastoma.[75,76] The type-II testicular germ cell tumors (TGCTs) are the most common malignancies among young men aged 15 to 34 years in United States, and its incidence is approximately 1.38–6.31 per 100 000 (years of 1973 to 2001).[77] The incidence of TGCTs is globally increased during the past 70 years, especially among men of European ancestry, and the etiologies of such preference are uncertain.[78,79]

TGCTs, both seminoma and nonseminoma, are derived from CIS/ITGCNU.[75,76,80,81] CIS/ITGCNU cells display a close phenotype to fetal germ cells, suggesting their origin is due to a developmental delay or failure of differentiation of early germ cells (**Figure 3**).[75,82–84] *TSPY* is expressed in gonocytes[85] and most CIS/ITGCNU cells with some minor exceptions (**Figure 3**).[43,44] Upon further oncogenic progression, the seminoma cells maintain *TSPY* expression while, nonseminoma cells do not or rarely express *TSPY* (**Figure 3**).[43,44,86] It has been speculated that the development of nonseminoma TGCT requires reprogramming to embryonic carcinoma state.[75] Indeed, the global gene expression analysis using microarray hybridization strategy indicated that embryonic carcinoma cells showed significant similarities with human embryonic stem cells, while seminoma closely resemble transformed primordial germ cells.[87] Accordingly, *TSPY* expression likely correlates with the germ cell lineage even in maturation-disturbed germ cells, but not with the reprogrammed cells like embryonic carcinoma with acquired pluripotency.

Gonadoblastoma is a subclass of type-II TCGs preferentially developed in the dysgenetic gonads of XY females or individuals with DSD.[75,76,88] The Y chromosome of gonadoblastoma patients frequently lacks sex determination region but retains common region of the short arm, termed *GBY* locus.[28–30] The tandemly repeated units of *TSPY* gene are mapped within *GBY* critical region on the

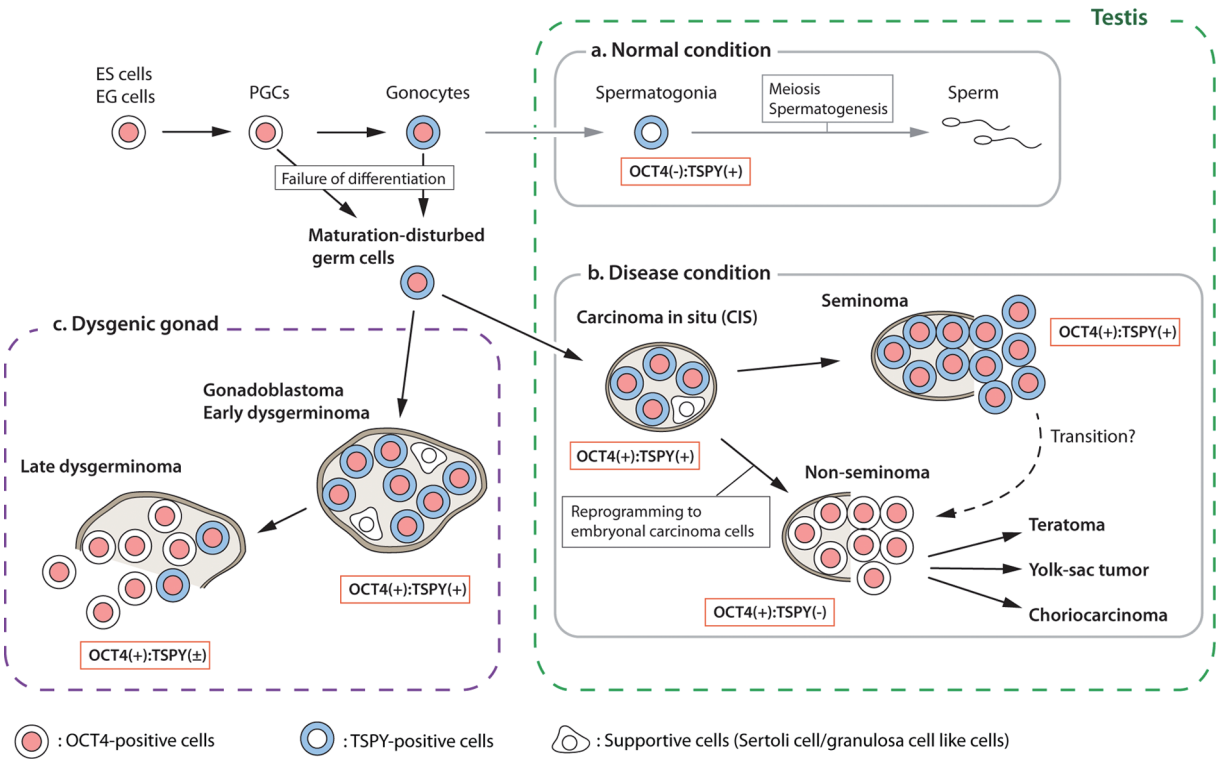

Figure 3: Schematic representation of expressions of testis-specific protein, Y-encoded (TSPY) and OCT3/4 in normal testis and type-II germ cell tumors. Germ cell expresses OCT3/4 until it reaches the maturation status. (Process-a) In normal testis, germ cell mature as spermatogonia, and lost OCT3/4 expression while it expresses TSPY. (Process-b) Failure of germ cell maturation causes development of carcinoma *in situ* (CIS) in the testis. The CIS cells are mostly OCT3/4-positive and TSPY-positive. It further develops into invasive seminoma (OCT3/4-positive and TSPY-positive) or nonseminoma via reprogramming to embryonal carcinoma status (OCT3/4-positive but TSPY-negative). (Process-c) In dysgenic gonad, maturation disturbed germ cells develop gonadoblastoma (OCT3/4-positive and TSPY-positive), and further progress into invasive dysgerminoma (OCT3/4-positive but TSPY-negative).

Y chromosome, hence *TSPY* is considered as a candidate gene for *GBY*, promoting gonadoblastoma development in the dysgenetic gonads of DSD patients.[28–30] Gonadoblastoma morphologically resembles the CIS/ITGCNU in the TGCTs in the testis; gonadoblastoma cells are mixed with granulosa-like cells while TGCT cells are mixed with Sertoli cells in the seminiferous tubules enclosed by myoid cells.[89] Most OCT4-positive gonadoblastoma cells strongly express TSPY as well as the germ cell/placental alkaline phosphatase (PLAP) and the proto-oncogene receptor c-Kit, similar to the testicular CIS.[43,45,86,88] Noticeably, *TSPY* is rarely expressed in the dysgerminoma after progression from gonadoblastoma.[86,88] Dysgerminoma is considered as a counterpart of seminoma based on morphology and expressed biomarkers.[90–92] Loss of *TSPY* expression in dysgerminoma may indicate the dividing characteristics between dysgerminoma and seminoma.

Overall, TSPY is expressed differentially in a subset of GCTs positive for both OCT4 and PLAP biomarkers. Since TSPY is most frequently expressed in the CIS and gonadoblastoma, at early stages of germ cell tumorigenesis, it is postulated to play an important role in early stages of oncogenesis in the immature germ cell lineage. Accordingly, the risk of GCT development/progression is higher in TSPY positive than TSPY negative cases.[93] TSPY could accelerate such progression of GCTs through its functions in cell cycle, protein synthesis, and histone/chromatin modification and gene regulation, as discussed above.

Expression of TSPY in somatic cancers

In addition to type-II and III GCTs, ectopic expression of *TSPY* has been frequently detected in various types of somatic cancers, including HCC,[47,48] melanoma,[49] and prostate cancer.[50,51] Yin *et al*.[48] reported that *TSPY* expression was detected in 50% cases of early stage HCC and 16% cases in undifferentiated stage (later stage of HCC). Our recent studies also demonstrated that TSPY was detected in 19.2% cases in tissue microarray and 46.9% cases in RNA samples isolated from fresh HCC specimens.[47] Further immunohistochemical analysis showed that TSPY is expressed in the glypican 3-positive cells, a biomarker of the HCC.[47,94] In the studies of prostate cancer, TSPY was immunohistochemically detected in the regions positive for alpha-methylacyl-CoA racemase, a biomarker of prostatic intraepithelial neoplasia and prostate cancer cells.[50,95] TSPY expression was more frequently detected in clinical prostate cancer specimens (78%) than latent prostate cancer (47%) and noncancer prostate tissues (50%).[50] These observations clearly indicate that *TSPY* is ectopically activated in somatic cancer cells.

While the correlation between TSPY expression and clinical outcome is still unclear, TSPY has been suggested as a prognostic biomarker and therapeutic target for immunotherapy.[48] Further analysis incorporating clinical outcomes and *TSPY* expression would be important to elucidate the significance of *TSPY* expression on cancer progression and immunotherapy.

Rodent Tspy and human TSPY transgenic mouse models

Although *TSPY* is an evolutionarily conserved gene on the Y chromosomes of mammals including apes and bovines,[96,97] the mouse *Tspy* gene is apparently nonfunctional as it contains multiple in-frame stop codons within the open reading frame.[98] Rat Y chromosome harbors a single functional copy of *Tspy* gene,[99] but its expression pattern is different from human *TSPY*, that is, the rat *Tspy* is expressed only in elongating spermatids while the human *TSPY* is primarily expressed in spermatogonia and spermatocytes,[100] suggesting that the biological functions of the rat *Tspy* could be different from those of human *TSPY*. Accordingly, the gene knockout in rodents might not be a suitable strategy to explore the biological functions of human *TSPY*. To overcome this difficulty, Schubert *et al*.[101] had generated a transgenic mouse line harboring 50 copies of human *TSPY* gene on Y chromosome of the mouse, designated as TgTSPY9. The 8.2-kb transgene contains 2.95-kb the promoter region, 2.8-kb structural gene and 2.45-kb 3' flanking sequence of the human *TSPY* gene. It is predominantly expressed in spermatogonia and spermatocytes at early stages of spermatogenesis, similar to the pattern of *TSPY* in human testis.[101] Expression of human *TSPY* transgene in testicular germ cells of *TgTSPY9* mice does not show any significant effects in fertility or other physiology,[101] consistent with the observation that the copy number of human *TSPY* gene varies among fertile men.[38] By introducing the Y-located *TSPY* transgene of *TgTSPY9* to the LADY mouse model of prostate cancer, we have demonstrated that the Y-located *TSPY* could be aberrantly activated during oncogenesis in the LADY model of prostate cancer.[102] However, while TSPY is expressed in FoxA1-positive epithelial cells and prostate cancer cells in human clinical prostate cancer specimens, *TgTSPY9* transgene was expressed in FoxA1-negative hypercellular stroma areas in the prostate of LADY mice.[102] Such differential expression patterns suggest the potential limitations of current mouse models of prostate cancer in mimicking the ectopic expression of *TSPY* under disease conditions, such as during prostate cancer development.

Azoospermia factor (AZF) genes and cancer

RNA-binding motif protein, Y-linked (*RBMY*) isoforms are encoded by repetitive genes within the *AZF* region, frequently deleted in azoospermia patients (**Figure 1**). RBMY binds to the RNA stem-loops capped by a C[A/U] CAA pentaloop[103] and may participate in the alternative splicing of various testis-specific gene transcripts.[104] Indeed, deletion of *RBMY* resulted in the failure of meiosis.[105] Abnormal expression of *RBMY* was observed in 36% cases of male liver HCC but not in normal liver tissues.[106,107] Over-expression of *RBMY* caused tumorigenicity in mouse fibroblast 3T3 cells,[106] and knock-down of *RBMY* in a liver cancer cell line HepG2 resulted in the reduction of transformation and anti-apoptotic efficiencies.[107] Further, the liver-specific *RBMY* transgenic mice showed accelerated hepatic neoplastic changes in the diethylnitrosamine-induced hepatocarcinogenesis animal model.[107] While the mechanism is still unclear, these observations suggest that the ectopic expression of *RBMY* genes could contribute to HCC development. On the contrary, multiple copies of *BPY2*, *DAZ,* and *CDY1* genes are also mapped onto the microdeletion of *AZFc* region, and deletions of these *AZF* genes are associated with increased risks of seminoma.[108,109] Consistently with these reports, our analysis of TCGA data showed that *DAZ1* and *BPY2* are frequently down-regulated in HCC (**Figure 2**). Although these observations are preliminary in nature, further studies on *AZF* genes could provide new insights into the roles of Y chromosome in cancer development and their usefulness as diagnostic biomarkers.

CONCLUSIONS AND FUTURE ASPECTS

In the past decades, associations between *MSY* genes and diseases have been identified. However, because of differences between human and rodents Y chromosomes[21,110] and difficulties in generating knockout mice of Y chromosome genes,[111] there are still limitations on investigating the roles of human *MSY* genes *in vivo*. As it has been suggested, most *MSY* genes may function as broadly expressed regulators for gene expression, protein stability and maintenance of the dosage of homologous XY gene pairs.[21] Supporting this hypothesis, it was demonstrated that *UTX*

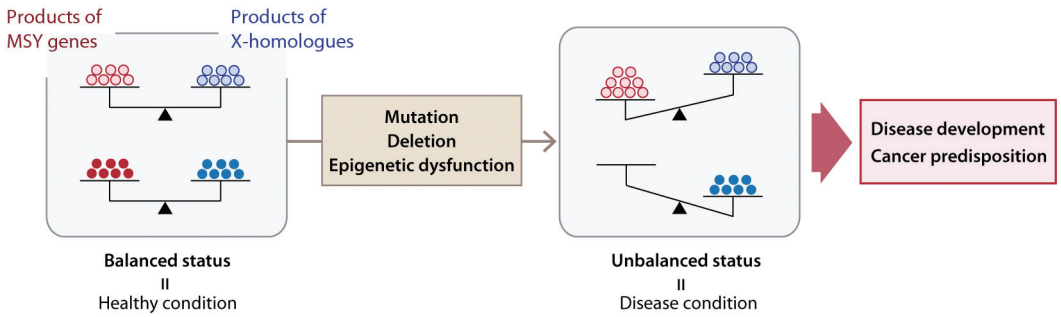

Figure 4: Conceptual illustration of gene balance between male-specific region of the Y chromosome genes and their X homologs in health maintenance and disease development, including cancer, in men.

and *UTY* could play comprehensive, but independent of their demethylase activities, during embryonic development.[112] On the other hand, while *TSPY* displays proto-oncogenic properties,[62,63,69] its X-linked homolog *TSPX* is a tumor suppressor and down-regulated in cancer.[62,65,66] This is the first example for an *MSY* gene and its X-homologue possess distinct and opposing functions. Hence, it is important to establish respective *in vivo* models to elucidate the roles of human *MSY* genes in development and progression of diseases, including cancers.

Recently, an epidemiologic study reported that loss of Y chromosome (LOY) in peripheral blood cells significantly associated with shorter cancer survival and higher risk of cancer incidence in men.[113] LOY in peripheral blood is frequently observed in elder men.[114] According to a clinical study with >40 years of follow-up, Forsberg *et al.* found that LOY in peripheral blood associated with increased risk of both all-caused mortality and cancer mortality, particularly in nonhematological cancers.[113] Further, transcriptome analysis of human peripheral blood samples detected the expression of some Y chromosome genes, e.g., *EIF1AY*, *DDX3Y*, *KDM5D*, *CYorf15B*, *CYorf15A*, and *UTY*.[115] Although the mechanism linking LOY in human peripheral blood and cancer mortality remains to be elucidated, these observations strongly suggest that Y chromosome genes are involved in a wide variety biological processes that have not been fully explored. *MSY* genes play crucial roles in both hormonal regulation and the balance in gene expression and protein stability, as described above. Ectopic expression of one or a few of these Y chromosome genes, such as *TSPY* and *RBMY*, could exacerbate oncogenesis in the absence of proper counter-balance from the other *MSY* genes (**Figure 4**). Further studies of Y chromosome genes from the global aspects, including both coding and noncoding RNA genes, will shed new lights on their roles in health and diseases in men.

AUTHOR CONTRIBUTIONS

TK performed the data-mining experiments. TK and YFCL co-wrote the manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Table S1: Gene name and Entrez gene ID of genes listed in Table 1

Gene/Gene family	*Members of multi-copy gene*	*Gene name*	*Entrez gene ID*
AMELY		amelogenin, Y-linked	266
BPY2	*BPY-2, 2B, 2C*	basic charge, Y-linked, 2	9083, 442867, 442868
CDY	*CDY- 1, 1B, 2A, 2B*	chromodomain protein, Y-linked	9085, 9426
DAZ	*DAZ-1, 2, 3, 4*	deleted in azoospermia	1617, 57055, 57054, 57135
*DDX3Y (DBY)**		DEAD (Asp-Glu-Ala-Asp) box helicase 3, Y-linked	8653
EIF1AY		eukaryotic translation initiation factor 1A, Y-linked	9086
HSFY	*HSFY-1, 2*	heat shock transcription factor, Y linked	86614
*KDM5D (SMCY)**		lysine (K)-specific demethylase 5D	8284
NLGN4Y		neuroligin 4, Y-linked	22829
PCDH11Y		protocadherin 11 Y-linked	83259
PRY	*PRY, PRY2*	PTPN13-like, Y-linked	9081
RBMY1	*RBMY1-A1, 1B, 1C, 1D, 1E, 1F/J*	RNA binding motif protein, Y-linked, family 1	5940, 378948, 5942, 378949, 378950, 159163
RPS4Y	*RPS4Y1, RPS4Y2*	ribosomal protein S4, Y-linked	6192, 140032
SRY		sex determining region Y	6736
TBL1Y		transducin (beta)-like 1, Y-linked	90665
TGIF2LY		TGFB-induced factor homeobox 2-like, Y-linked	90655
*TMSB4Y (TB4Y)**		thymosin beta 4, Y-linked	9087
TSPY	>40 copies (varies among cohort)	testis specific protein, Y-linked	7258, 64591, 728137, 728395, 728403
*USP9Y (DFFRY)**		ubiquitin specific peptidase 9, Y-linked	8287
UTY		ubiquitously transcribed tetratricopeptide repeat containing, Y-linked	7404
VCY	*VCY, VCY1B*	variable charge, Y-linked	9084
XKRY	*XKRY, XKRY2*	XK, Kell blood group complex subunit-related, Y-linked	9082
ZFY		zinc finger protein, Y-linked	7544

*: alternate gene name in parenthesis