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REVIEW ARTICLE

Long noncoding RNA *XIST*: Mechanisms for X chromosome inactivation, roles in sex-biased diseases, and therapeutic opportunities

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Abstract Sexual dimorphism has been reported in various human diseases including autoimmune diseases, neurological diseases, pulmonary arterial hypertension, and some types of cancers, although the underlying mechanisms remain poorly understood. The long noncoding RNA (lncRNA) X-inactive specific transcript (*XIST*) is involved in X chromosome inactivation (XCI) in female placental mammals, a process that ensures the balanced expression dosage of X-linked genes between sexes. *XIST* is abnormally expressed in many sex-biased diseases. In addition, escape from *XIST*-mediated XCI and skewed XCI also contribute to sex-biased diseases. Therefore, its expression or modification can be regarded as a biomarker for the diagnosis and prognosis of many sex-biased diseases. Genetic manipulation of *XIST* expression can inhibit the progression of some of these diseases in animal models, and therefore *XIST* has been proposed as a potential therapeutic target. In this manuscript, we summarize the current knowledge about the mechanisms for *XIST*-mediated XCI and the roles of *XIST* in sex-biased diseases, and discuss potential therapeutic strategies targeting *XIST*.

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Introduction

Noncoding RNAs (ncRNAs) are a class of transcripts that do not encode proteins. In mammalian transcriptomes, only a small fraction of transcripts have the capacity to encode proteins, and the vast majority of RNAs are noncoding, including ribosomal RNAs, transfer RNAs, small RNAs, long noncoding RNAs (lncRNAs), and circular RNAs. The large proportion of noncoding RNAs were once considered to be "transcriptional noise", since their functions were unknown. It was later recognized that ncRNAs are indeed functional, and many of which are crucial for normal cell function. For example, some small RNAs, including small interfering RNAs (siRNAs) and microRNAs (miRNAs), are involved in post-transcriptional gene silencing. A special species of ncRNAs longer than 200 nucleotides known as lncRNAs,[1] are abundant in mammalian transcriptomes and are involved in diverse biological processes ranging from transcriptional regulation, genome organization, genomic imprinting, dosage compensation, and cell differentiation, to tumorigenesis.[2–8] The aberrant expression of many human lncRNAs has been documented in many diseases.[9] For example, X-inactive specific transcript (*XIST* in humans and monkeys, *Xist* in mice) is involved in X chromosome inactivation (XCI) crucial for normal female development in placental mammals,[10,11] and has been implicated in sex-biased diseases in recent years.[12]

LncRNA *XIST* and mechanisms for X chromosome inactivation

XIST is very important for sex determination and dosage compensation in mammals. In most mammals, sex is determined by the X and Y chromosomes, with females being XX and males XY.[13] As a consequence, without dosage compensation, the extra X chromosome in females can lead to unbalanced X-linked gene expression. XCI produces dosage compensation by randomly inactivating one of the X chromosomes in females.[14,15] Regulation of XCI in pre- and post-implantation development occurs differently. In pre-implantation, most mammalian embryos undergo XCI imprinting. Humans lack imprinted XCI and regulate gene expression by X chromosome dampening (XCD) instead.[16,17] In post-implantation development, both human and other placental mammals undergo random XCI.[16,17] The long noncoding transcript *XIST* (19 kb in humans, and 17 kb in mice) has been suggested to play vital roles in random XCI.[17,18] Random XCI has three phases: initiation, establishment, and maintenance,[19] and has been extensively investigated in mice. The *XIST*/*Xist* gene is located in the X inactivation center (XIC),[20–22] and can be transcribed from the X chromosome to be inactivated (Xi), which functions in *cis* to coat Xi and nucleate dynamic protein complexes.[23] *Xist* RNA-containing complexes gradually expand, allowing *Xist* to spread across Xi.[23] Meanwhile, these complexes alter chromatin architecture and thus compact the chromosome, leading to progressive gene silencing along the Xi.[23,24]

Factors involved in transcriptional activation of *XIST*

The initiation stage of random XCI is a stochastic process involving X–X pairing, counting, and *XIST*/*Xist* activation.[19,25–27] In early embryo development, XCI is regulated by *XIST*/*Xist* activators including *Ftx*,[28] *Jpx*[29,30] and RNF12 (encoded by *Rlim*),[31] and inhibitors such as *Tsix*,[32,33] which are located in XIC and are conserved between humans and mice (Fig. 1A). In mice, *Jpx* RNA activates *Xist* transcription in a dose-dependent manner by evicting CTCF[30] (Fig. 1B), a DNA-binding insulator capable of repressing *Xist* expression.[34,35] *Ftx* promotes the transcription of *Xist* through the proximity of their gene loci, which is independent of the *Ftx* RNA products[28] (Fig. 1B). The X-encoded E3 ubiquitin ligase RNF12[31] upregulates mouse *Xist* expression by targeting for degradation the pluripotency factor REX1,[36] which normally activates *Tsix* and represses *Xist* expression through binding to regulatory regions[36,37] (Fig. 1B). The autosomal transcription factor YY1 binds to the 5' region of the *Xist* gene lacking DNA methylation and activates *Xist* expression, in competition with the *Xist* repressor REX1, whereas the methylated copy on the active X (Xa) cannot be bound[38] (Fig. 1B). In addition, the chromatin remodeler SPEN (also known as SHARP) accumulates on the Xi early in mouse XCI to silence *Tsix* and activate *Xist* expression[39] (Fig. 1B). In human pluripotent stem cells, *XIST* expression is silenced by the *de novo* DNA methyltransferases DNMT3A and DNMT3B.[40]

XIST-mediated polycomb recruitment, nuclear scaffolding and XCI

After *Xist* is activated, it establishes XCI through binding and recruiting proteins responsible for coating and spreading, histone modification, DNA methylation and chromatin compaction,[12,41] that together form Barr bodies[19] (Fig. 1D). During this process, active histone marks such as H3K4me1, H3K4me3, H3K9ac, H3K27ac, H4K5ac, H4K8ac, H4K12ac and H4K16ac are gradually lost, while repressive histone marks like H2AK119ub, H3K9me2, H3K9me3 and H3K27me3, and DNA methylation accumulate.[24,42–46] *Xist* RNA contains multiple repeat motifs that can interact with various proteins (Fig. 1C). Upon *Xist* transcription, many *Xist* RNA-binding proteins immediately assemble on the multivalent E-repeat of *Xist* RNA,[47] including PTBP1, MATR3, TDP-43 and CELF1. They form a condensate on the Xi via self-aggregation and protein interactions,[47] restricting *Xist* to the Xi.[23,48–50] *Xist* forms about 50 locally confined loci in open chromatin regions on Xi, each containing 2 *Xist* RNA molecules capable of nucleating supramolecular complexes (SMACs).[23] The complexes gradually expand across the Xi and the dynamics create gradients of local proteins over broad genomic regions along the Xi[23] (Fig. 1D). During this process, the *Xist* RNA's A-repeat binds to the corepressor SPEN/SHARP's C-terminal SPOC domain,[41,51,52] which acts as a molecular integrator to bridge *Xist* RNA to the transcription machinery, nucleosome remodelers and histone deacetylases.[52] The SPOC domain then interacts with the SMRT co-repressor

and further activates pre-loaded histone deacetylase HDAC3 on Xi,[24,53] resulting in the loss of active chromatin marks like H3K27ac.[24] The B-repeat of *Xist* RNA recruits Polycomb repressive complexes PRC1 and PRC2 through directly binding with hnRNPK to establish the repressive chromatin marks H2AK119ub and H3K27me3 on Xi[54–57] and achieving selective X chromosome silencing.[56,58] In humans, the E-repeat may also be required for PRC recruitment and H3K27me3 enrichment.[59] In addition, *XIST*/*Xist* can recruit the m^{6}A machinery to its transcript. In both humans and mice, the A-repeat interacts with the RNA-binding motif (RBM) proteins RBM15 and RBM15B.[60,61] These RBM proteins further recruit the m^{6}A methyltransferase METTL3/14 to specific sites in *XIST*/*Xist* through Wilms tumour-associated protein (WTAP), eventually resulting in m^{6}A formation at adjacent sites.[60,61] In humans, the m^{6}A in *XIST* RNA is responsible for recruiting the m^{6}A reader YTHDC1 to promote gene silencing,[60] although the mechanisms remain elusive. In mice, YTHDC1 protein is recruited to *Xist* RNA through SPEN/SHARP's SPOC domain.[52] The three-dimensional conformation further promotes *Xist* spreading to actively transcribed genes across Xi.[62] During this process, many proteins are involved. The C-repeat of *Xist* RNA is bound by YY1, which tethers *Xist* to inactive X nucleation center on Xi.[63] The A-repeat interacts with the Lamin B receptor (LBR) to recruit Xi to the nuclear lamina, enabling *Xist* to spread across Xi.[64] In addition, the interaction between *Xist* and PRCs is also crucial for *Xist* spreading.[65]

XIST in XCI maintenance

The mechanism of XCI maintenance is less studied. During mouse embryonic stem cell differentiation, *Xist* expression is dispensable for XCI maintenance,[66] whereas in human B cells, *XIST* is required for maintaining the X-inactivation of immune genes.[12] A genome-wide RNAi screen in mouse embryonic fibroblasts identified 32 proteins involved in the maintenance of Xi silencing.[67] One of those proteins, DNMT1, is responsible for CpG dinucleotide methylation maintenance, suggesting that DNA methylation is required for XCI maintenance in mice. A recent study also revealed that the condensate formed by many *Xist* RNA-binding proteins seeded by the *Xist* RNA's E-repeat, is crucial for gene silencing during the *Xist*-independent phase of XCI in differentiating mouse embryonic stem cells.[47] In human B cells, comprehensive identification of RNA-binding proteins by mass spectrometry (ChIRP-MS) uncovered the *XIST*-interacting proteome, which is different from the ones found in embryonic stem cells and myeloid cells.[67] Some cell-specific *XIST*-interacting proteins may also contribute to XCI maintenance. For example, TRIM28, a cofactor of the H3K9me3-specific histone methyltransferase SETDB1,[46,68] only binds *XIST* RNA in B cells.[12] CRISPRi screening further indicates that TRIM28 is indispensable for XCI maintenance in B cells.[12] Therefore, the XCI maintenance mechanisms may be tissue-specific.

Random XCI is crucial for normal female development in placental mammals. Under normal conditions, XCI can balance gene dosage between males and females in placental mammals. However, any mistakes occurring in this process may lead to cell dysfunction and disease. Since *XIST* function is related to sex chromosome gene expression, it has been hypothesized to be related to many sex-biased diseases.

XIST and sex-biased diseases

Sex disparities in disease are common. For example, most cancers show a large sex bias in incidence and mortality.[69,70] The incidence of autoimmune and neurological diseases also differs between sexes.[71] Even for COVID-19-associated illness, the severity and mortality is different for men and women.[72–74] The difference might be attributed to sexual dimorphism[75,76] and gender differences in attitudes and behavior.[77,78] A growing body of evidence has revealed that the lncRNA *XIST*, an important regulator in X chromosome dosage compensation in placental mammals, can play pivotal roles in some sex-biased diseases.

XIST in autoimmune diseases

More than 80% of autoimmune diseases are female dominant, examples being systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). This female bias has been linked to X-linked immune gene dosage.[71,79] The X chromosome is known to contain the largest number of immune response-related genes of the whole human genome.[71,80–82] XCI has evolved to balance gene dosage between males and females. As a result, in female mammals, one of the X chromosomes is inactivated by *XIST* and most genes on the inactive X are silenced. However, a fraction of X-linked genes can still escape from X-inactivation and therefore have biallelic expression in both humans and mice,[83–85] which leads to female-biased gene expression. In humans, about 15% of genes consistently escape from XCI and another 15% of genes vary between individuals or tissues.[86]

Most somatic cells maintain XCI with static enrichment of *XIST* and heterochromatin marks on the Xi, but immune cells exhibit a unique dynamic localization of *XIST* and epigenetic modifications to the Xi following stimulation.[87–90] Although female naïve and activated lymphocytes have similar high levels of *XIST* RNA, naïve lymphocytes lack canonical localization of *XIST* RNA transcripts on the Xi.[90] In murine B cells, it has also been reported that *Xist* RNA disappears from the Xi at the pro-B cell stage with a gradual loss of heterochromatic modifications, while mature B cell activation restores *Xist* RNA localization and the heterochromatic modifications on Xi.[91]

Many studies have shown that altered *XIST* localization on Xi in lymphocytes may promote sex-biased autoimmune diseases. For example, in SLE patients, cellular imaging has shown that both B and T cells exhibit abnormal *XIST* RNA localization patterns without altered expression.[87,88,90] Some recent studies uncovered more mechanistic details regarding autoimmune B cell dysregulation. Pyfrom et al showed that there is a complete lack of *XIST* localization on the Xi in human CD11c$^+$ atypical memory B cells,[87] a unique B cell population expanded in SLE and RA.[92,93] Yu et al further showed that *XIST* is continually required in adult human B cells,[12] finding that some X-linked immune genes in B cells lack promoter methylation, which requires *XIST*

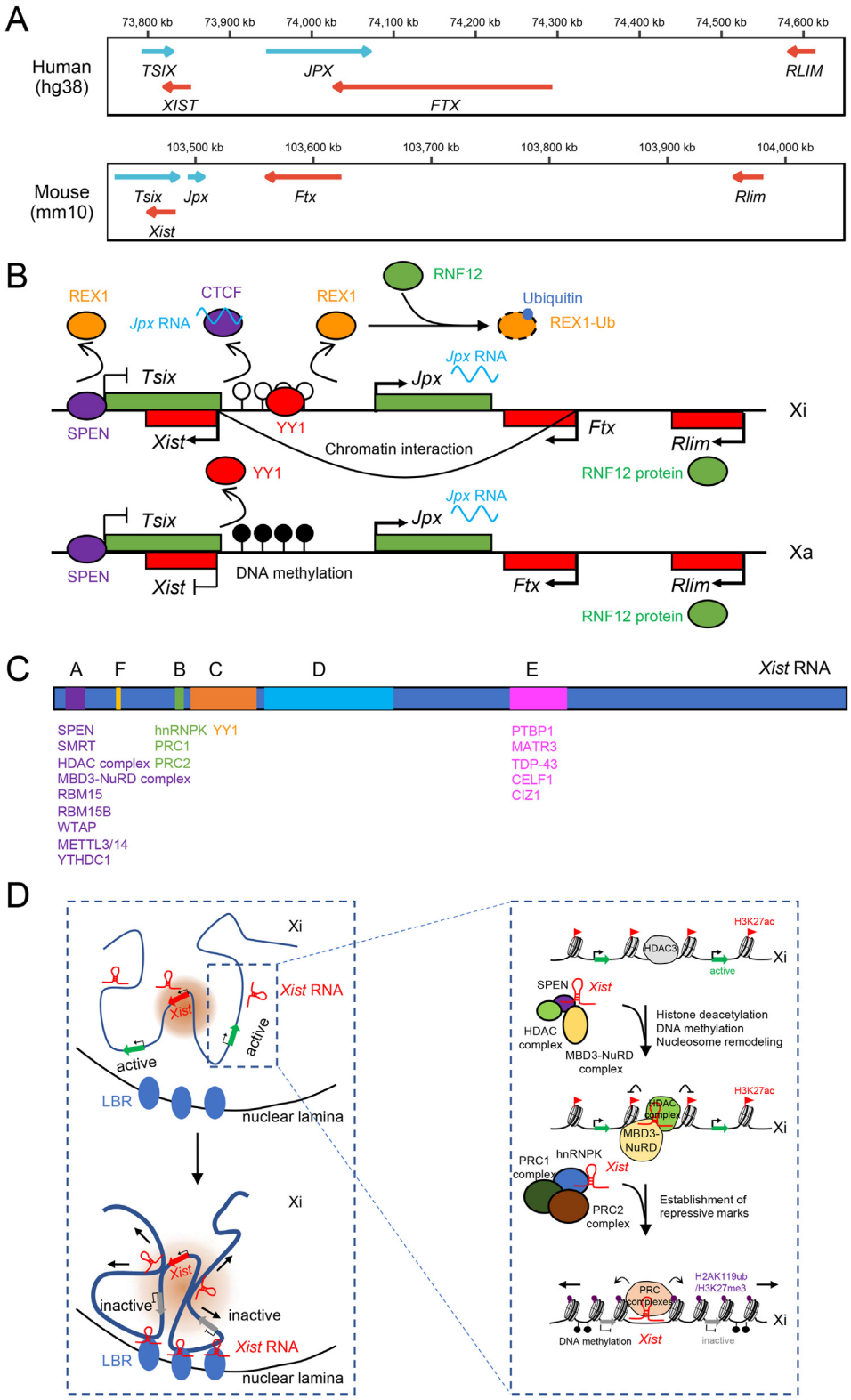

Figure 1 The long noncoding *Xist* RNA and its roles in X chromosome inactivation. **(A)** Genomic arrangement of *XIST*/*Xist* and its regulators in X inactivation center (XIC) in human and mouse. **(B)** Factors involved in the transcriptional activation of mouse *Xist*. *Jpx* RNA transcribed from both X chromosomes interacts with CTCF insulator to release it from the *Xist* regulatory region. YY1 competes with REX1 repressor to bind the *Xist* regulatory region on Xi lacking DNA methylation, while the methylated copy is not bound, achieving selective activation of *Xist*. The repressor REX1 is further recognized by the E3 ubiquitin ligase RNF12, encoded by *Rlim* in XIC, leading to the ubiquitination and degradation of REX1. *Ftx* promotes *Xist* transcription through nuclear proximity of *Xist* and *Ftx* loci, independently of *Ftx* transcripts. However, active *Ftx* transcription is required for *Xist* accumulation. SPEN remodels

for silencing maintenance via enhancer H3K27ac deacetylation. For example, the X-linked Toll-like receptor 7 (*TLR7*), which recognizes single-strand RNA (ssRNA)-containing immune complexes involved in female-biased autoimmunity and ssRNA viral infection,[94] is commonly overexpressed in SLE and RA patients and promotes the formation and activation of $CD11c^+$ atypical memory B cells.[92,93,95] Yu et al also found that in somatic cells, *XIST* complexes are tissue-specific.[12] B cell-specific *XIST* cofactor TRIM28 may inhibit transcription elongation on immune genes such as *TLR7*, possibly through RING domain-mediated sumoylation of the transcription elongation kinase CDK9.[12] SLE patients' T cells also have dispersed *XIST*, altered XCI maintenance and aberrant overexpression of many X-linked genes, but the mechanisms are still unclear.[88]

In summary, in autoimmune disease, *XIST* localization on Xi of some immune cells is lost or changed, leading to altered XCI maintenance. Some *XIST*-dependent immune genes such as *TLR7* can therefore be reactivated, which may be sufficient to promote isotype-switched immune cells and autoimmunity (Fig. 2A).

XIST in sex-biased cancers

For many cancers, the incidence, prevalence, prognosis, and mortality differ greatly between the sexes. For example, males have higher risks of bladder, colorectal, kidney, lung, liver and blood cancers, while females have higher risks of breast and thyroid cancers.[69,70] In this section, we summarize current progress on the roles of *XIST* in some of these sex-biased cancers.

Some genes escaping from X-inactivation involve tumor suppressors

Tumors have significant numbers of genetic mutations. An investigation about the paired tumor-germline exome sequencing data across 21 tumor types identified six genes with higher loss-of-function mutation frequency in male-biased cancers.[96] All six, *ATRX*, *CNKSR2*, *DDX3X*, *KDM5C*, *KDM6A*/*UTX*, and *MAGEC3*, are located in the non-pseudoautosomal region of the X chromosome.[96] These genes can escape from X-inactivation, leading to female-biased expression, and are regarded as tumor suppressor genes as they are frequently mutated in cancer.[97–101] They are therefore referred to as 'escape from X-inactivation tumor-suppressor' genes or EXITS genes.[96] For example, the loss-of-function mutation in the H3K27me3 demethylase gene *KDM6A*/*UTX* mainly occurs in male-biased cancers.[96,102,103] However, female cancers with *KDM6A* mutations usually require homozygous mutations, leading to lower female incidences.[102] Other than disease incidence, gender-biased *KDM6A* expression also leads to different outcomes. The expression of *KDM6A* in female bladder cancer patients is significantly higher than in male patients and is correlated with longer survival and better prognosis of female patients.[104] Conditional knockout of mouse *Kdm6a* increases bladder cancer risk and decreases overall female survival, which, however, does not significantly affect the survival or tumor burden of male mice.[104] These results indicate that female-biased *KDM6A* expression exerts antitumor effects in bladder cancer leading to lower incidence and better prognosis in females.

Oncogenic role of *XIST* in male-biased cancers

XIST is normally not expressed in male somatic tissues. In the tissues involved in some male-biased cancers like bladder, colorectal, and lung tumors, however, *XIST* expression is abnormally elevated,[105–114] and the elevated *XIST* expression correlates with shorter survival and poor prognosis.[105–107,115] In cell lines derived from these cancers, overexpression of *XIST* promotes cell proliferation, migration, invasion and epithelial–mesenchymal transition, and inhibits apoptosis,[114,116,117] while knockdown of *XIST* has the opposite effect,[105,106,109,112–114,116–124] irrespective of the sexual characteristics of cell lines (Table 1). Murine xenograft assays have also shown that for bladder,[118] colorectal,[109] or lung[106,112–114,121–123] cancers, *XIST* silencing inhibits tumor growth in mice, and *XIST* overexpression promotes non-small cell lung cancer (NSCLC) tumor growth in mice.[114] These suggest that *XIST* may play an oncogenic role in male-biased cancers.

Mechanistically, *XIST* serves primarily as a miRNA molecular sponge to regulate the expression of miRNA targets in male-biased cancers (Fig. 2C). For example, upregulated *XIST* can bind miR-124 and promote the expression of Androgen Receptor (*AR*) to facilitate bladder cancer development.[125] *AR* encodes a steroid hormone receptor functioning as a transcription factor to promote the progression of bladder cancer.[126] Although androgen signaling promotes the progression of bladder cancer in both males and females, higher *AR* expression is observed in male patients[127] and associated with higher bladder cancer risk.[126] In mice, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) can induce bladder cancer, with higher male incidence,[128] possibly due to different *AR* expression levels. Knockout of the *AR* gene abolishes BBN-induced bladder carcinogenesis in both male and female mice,[128] indicating that AR-mediated androgen signaling may play a crucial role in the progression of some male-biased cancers, at least in BBN-mediated bladder tumorigenesis. In the presence of androgen, AR can regulate the expression of

the chromatin to silence *Tsix* and activate *Xist*. **(C)** Overview of repeat motifs in mouse *Xist* RNA. The proteins or complexes interacting with these repeats are indicated below. **(D)** The roles of *Xist* in the establishment of random XCI. As shown in the left panel, spatially, *Xist* RNA binds some locally confined loci on the Xi and nucleates local protein gradients to form SMACs (shown as light red circles). *Xist* RNA is tethered to the inactive X nucleation center by YY1. The Xi is recruited to the nuclear lamina through the *Xist*-LBR interaction, and the SMACs gradually expand to silence the whole X chromosome. Arrows indicate the expansion of the complex and the spreading of gene silencing on Xi. In the right panel, enlarged view of *Xist*-mediated chromatin dynamics near gene loci on Xi during XCI is shown. *Xist* RNA interacts with SPEN and further activates HDAC and MBD3-NuRD complexes, enabling removal of active histone marks, remodeling of nucleosomes, and DNA methylation. *Xist* also recruits PRC1 and PRC2 through hnRNPK to establish repressive histone marks, such as H2AK119ub and H3K27me3.

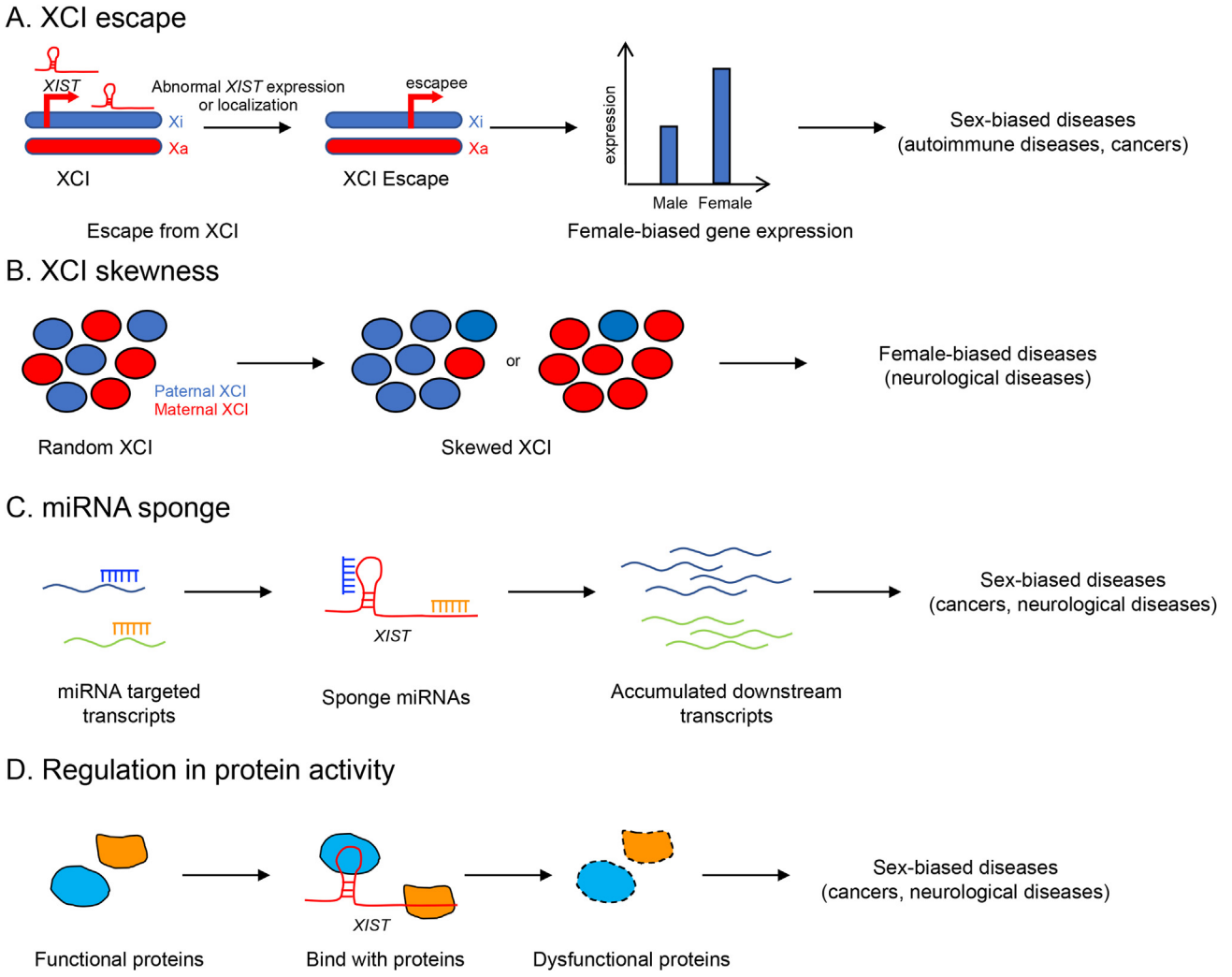

Figure 2 Molecular mechanisms underlying roles of *XIST* in diseases. **(A)** X chromosome inactivation (XCI) escape. In female mammals, some X-linked genes can escape from XCI when *XIST* expression or localization is altered and therefore have biallelic expression, leading to female-biased gene expression, which might contribute to sex-biased diseases. **(B)** XCI skewness. In female mammals, the paternal and maternal X chromosomes generally have a similar opportunity to be silenced in somatic cells. Under specific condition, however, skewed XCI may occur, which may cause disease in females. **(C)** MiRNA sponge. The long noncoding transcript *XIST* can bind various miRNAs, resulting in derepression of miRNA targeted genes and pathology. **(D)** Regulation in protein activity. *XIST* can bind to developmentally critical proteins and affect their activity.

downstream genes, many involved in bladder cancer outgrowth, including β-catenin,[129] CD24,[130] EGFR/ERBB2,[131] and ELK1.[132] AR protein accumulation is also seen in some male breast cancer patients, and high *AR* expression predicts inferior outcomes and poor tamoxifen treatment responses in male breast cancer.[133] In addition, *XIST* can activate the Wnt/β-catenin signaling pathway to accelerate bladder and colon cancer progression by sponging miR-139-5p[105] and miR-34a[109] respectively. *XIST* targets miR-200b-3p to modulate the expression of *ZEB1*,[134] sponges miR-132-3p to activate the MAPK1 signaling pathway,[110] interacts with miR-137 to regulate the EZH2 signaling pathway,[117] binds miR-486-5p to regulate the neuropilin-2 (NRP-2) pathway,[108] inhibits miR-30a-5p to activate ROR1,[135] suppresses miR-93-5p to modulate the HIF-1A/AXL signaling pathway,[136] sponges miR-338-3p to regulate *PAX5* expression,[137] and targets miR-125b-2-3p to regulate the Wee1 signaling pathway,[138] all of which promote colorectal cancer progression. *XIST* can also promote TGF-β-induced epithelial–mesenchymal transition through the miR-367/141-ZEB2[120] and miR-137/Notch-1[124] axes in non-small cell lung cancer. *XIST* promotes *Bcl-2* expression through sponging miR-449a, thus exerting an anti-apoptotic effects in many cancers.[113,139] *XIST* also targets miR-16 to activate *CDK8*,[114] a member of the mediator complex acting as an oncogene,[140] with *XIST*-mediated proliferation and migration of lung cancer cells reversed by miR-16 overexpression.[114]

Beside sponging miRNAs, the lncRNA *XIST* can also directly interact with proteins and affect their functions (Fig. 2D). For example, *XIST* binds to the DNA demethylase TET1 to reduce TET1-mediated demethylation on the tumor suppressor gene *p53*,[141] thereby inhibiting *p53* expression in bladder cancer,[116] with *XIST*-mediated cell proliferation able to be reversed by expression of *p53* in bladder cancer cells.[116] *XIST* also directly binds with the H3K27me3-specific

Table 1 Effects of manipulated *XIST* expression on cell proliferation and tumorigenesis in male-biased cancers.

Cancer types	Cell lines	Gender of cell host	Genetic manipulation in *XIST* expression	Effects on cell proliferation and/or tumorigenesis	References
Bladder cancer	5637	Male	Overexpression	Promotion	116
			Knockdown	Inhibition	105,118
	253J	Male	Knockdown	Inhibition	119
	T24	Female	Knockdown	Inhibition	105,116,118
	RT112	Female	Knockdown	Inhibition	119
Colorectal cancer	HT29	Female	Overexpression	Promotion	117
	LoVo	Male	Knockdown	Inhibition	117
	SW480	Male	Knockdown	Inhibition	109
	HCT116	Male	Knockdown	Inhibition	109
Non-small cell lung cancer	A549	Male	Overexpression	Promotion	114
			Knockdown	Inhibition	106,112–114,120,122–124
	H1299	Male	Overexpression	Promotion	114
			Knockdown	Inhibition	112–114,122–124
	H522	Male	Knockdown	Inhibition	121
	Calu3	Male	Knockdown	Inhibition	121
	H226	Male	Knockdown	Inhibition	120

histone methyltransferase EZH2 to silence the expression of proposed tumor suppressor *KLF2* in NSCLC cells.[106] Methyltransferase-like14 (METTL14) can catalyze the N^6-methyladenosine (m^6A) on *XIST* transcript, which is further recognized by the m^6A reader YTHDF2, leading to *XIST* degradation.[142] *METTL14* is downregulated and *XIST* expression is upregulated in colorectal cancer, and the loss of METTL14 has been shown to be associated with poor prognosis.[142] Knockdown of *METTL14* promotes colorectal cancer proliferation and invasion and substantially abolishes the m^6A level of *XIST*, leading to augmented *XIST* expression,[142] while *METTL14* overexpression results in a remarkable decrease in *XIST* expression level, cell growth and invasion.[142] It has therefore been proposed that METTL14 inhibits colorectal cancer progression by reducing *XIST* expression.[142]

XIST in female-biased and gynecologic cancers

Breast and thyroid cancers are more common in women than in men.[70,143] The role of *XIST* in these female-biased cancers is complex. In normal female breast tissues, *XIST* is highly expressed, whereas in the cancer tissues or cells, *XIST* expression is downregulated relative to adjacent normal tissues or normal cell lines.[144–146] *XIST* expression is also significantly reduced in brain-metastatic breast cancer, and decreased *XIST* expression promotes brain metastasis in breast cancer, while the dCas9-mediated overexpression does not.[147] In breast cancer cells, *XIST* overexpression inhibits their proliferation, migration and invasion, and facilitates their apoptosis, while *XIST* silencing exerts the opposite effect.[145,146] The xenograft tumor assay in BALB/c nude mice confirmed that *XIST* can retard breast tumor growth.[146] *XIST* can activate *CDX1* by sponging miR-155 to depress the growth, migration, and invasiveness of breast cancer.[144] Interestingly, the expression of *Jpx*, an activator of *XIST* expression,[29,30] has also been noted to be downregulated in breast cancer samples.[145] Knockdown of *XIST* or *SPEN*/*SHARP* can promote the recruitment of HDAC3 to the promoter of *PHLPP1*.[145] Since *PHLPP1* encodes a phosphatase able to dephosphorylate AKT, knockdown of *XIST* leads to reduced *PHLPP1* expression and increased AKT phosphorylation.[145] As mentioned above, female-biased expression of *KDM6A*/*UTX* caused by escaping from *XIST*-mediated XCI can act as a tumor suppressor in some male-biased cancers. However, in other female-biased cancers like breast cancer, KDM6A/UTX may play an oncogenic role, since a study reported that KDM6A/UTX can cooperate with H3K4 methyltransferase MLL4 to promote the expression of many oncogenes and prometastatic genes, leading to cell proliferation and invasion in breast cancer cells, both *in vitro* and in a mouse xenograft model.[148]

In thyroid cancer, *XIST* may be oncogenic, contributing to female bias since females have higher expression of *XIST* compared to males. Compared to adjacent normal tissues or normal cell lines, *XIST* expression in thyroid cancer tissues and cell lines is upregulated,[149–151] regardless of gender. In addition, *XIST* expression positively correlates with thyroid cancer progression.[149,150] In both male-derived and female-derived thyroid cancer cells, *XIST* knockout inhibited cell proliferation, migration and invasion[149–151] (Table 2), and its oncogenic role has been confirmed in the xenograft tumor assay in female nude mice.[150] *XIST* regulates thyroid cancer progression by functioning as a competing endogenous RNA (ceRNA) to sponge miRNAs. For example, *XIST* can enhance the expression of the receptor tyrosine kinase *MET* by sponging miR-34a, resulting in increased phosphorylation of PI3K and AKT.[150] *XIST* also upregulates *CLDN1* expression through interaction with miR-101-3p, thereby promoting cell proliferation, migration, and invasion of thyroid cancer.[151] In addition, in papillary thyroid carcinoma, *XIST* targets miR-141 to promote cell proliferation and invasion,[149] although the downstream processes are yet to be elucidated.

Table 2 Effects of manipulated *XIST* expression on cell proliferation and tumorigenesis for female-biased cancers.

Cancer types	Cell lines	Gender of cell host	Genetic manipulation in *XIST* expression	Effects on cell proliferation and/or tumorigenesis	References
Breast cancer	MCF7	Female	Overexpression	Inhibition	145,146
			Knockdown	Promotion	146,147
	MDA-MB-231	Female	Overexpression	Inhibition	146
			Knockdown	Promotion	146
	SKBR3	Female	Knockdown	Promotion	147
	ZR75-1	Female	Knockdown	Promotion	147
	MDA-MB231BrM2a	Female	Overexpression	Inhibition	147
	M10	Female	Knockdown	Promotion	145
Thyroid cancer	TPC-1	Female	Knockdown	Inhibition	149,151
	KAT18	Unspecified	Knockdown	Inhibition	150
	FTC113	Male	Knockdown	Inhibition	150

As an important participant in XCI in female mammals, aberrant expression of *XIST* has also been implicated in ovarian and cervical cancers, two common gynecologic malignant tumors.[70] Similarly to breast cancer, *XIST* expression in ovarian cancer tissues is downregulated compared to adjacent normal tissues.[152] In addition, *XIST* expression correlates with ovarian cancer development, with downregulation in advanced stages, and higher expression is associated with better prognoses.[152] In ovarian cancer cell lines, *XIST* overexpression suppresses cell proliferation,[153–155] while *XIST* knockdown has the opposite effect.[153] *XIST* suppresses cancer progression through sponging hsa-miR-214-3p[154] and miR-106a.[155] In recurrent ovarian tumors, *XIST* expression is also decreased compared to paired primary tumors and is associated with resistance to the anticancer agent Taxol.[156] The loss of *XIST* also induces ovarian cancer stem cells to acquire Taxol resistance through modulation of the miR-93-5p/KMT2C axis.[153] These findings indicate that *XIST* might not only be a biomarker for the diagnosis and prognosis of cancers, but also a potential therapeutic target for ovarian cancer. Notably, two recent studies have claimed that *XIST* promotes the proliferation, invasion, and migration of ovarian cancer cells by modulating the miR-335/BCL2L2 axis[157] and regulating miR-149-3p.[158] These findings require reconciliation with prior observations.

In cervical cancer, *XIST* expression is elevated,[159–161] in contrast to breast or ovarian cancer. *XIST* knockdown in cervical cancer cell lines like SiHa, HeLa, C33A and Me180 cells inhibits cell proliferation, blocks the cell cycle, and promotes apoptosis.[159–161] Reduced tumor growth is also observed in the murine xenograft assay after *XIST* silencing.[161] In cervical cancer, *XIST* accelerates cancer progression by sponging various miRNAs, thereby derepressing the expression of oncogenic genes targeted by these miRNAs. For example, *XIST* interacts with miR-200a to upregulate *Fus* expression,[159] binds miR-140-5p to promote *ORC1* expression,[160] and targets miR-889-3p to derepress *SIX1* expression.[161] It has been suggested that high expression of *XIST* is associated with unfavorable prognosis of cervical cancer patients.[159] Taken together, these studies suggest that the role of *XIST* in tumorigenesis shows an organ-dependent pattern for female-biased and gynecologic cancers.

XIST in other sex-biased diseases

XIST in neurological disorders

Neurological disorders are nervous system-related and include those associated with neurodevelopment (autism spectrum disorders, schizophrenia, Rett syndrome, and Down syndrome) and neurodegeneration (Parkinson's, Alzheimer's and Huntington's diseases). Many neurological diseases show sex-bias. For example, autism spectrum disorder is a male-biased disease with three times more frequent observation in males than in females.[162] Rett syndrome is a female-biased progressive neurodevelopmental disorder. Parkinson's disease (PD) is a male-biased neurodegenerative disorder, and Alzheimer's disease (AD) is a chronic neurodegenerative disease with higher prevalence in females.[163] Some genes related to neuronal plasticity and cognitive process are located on the X chromosome. Nearly 20% of them, including *MECP2*, *FMR1*, and *CDKL5*, are correlated with neurodevelopmental diseases.[164] It has therefore been suggested that *XIST* and XCI status may be responsible for the sex-bias of neurological diseases. In this section, we discuss *XIST* and its function in XCI in neurological diseases as a microRNA sponge, the phenomenon of XCI skewness (XIS) in some female patients and X-linked heterozygous mutation diseases related to XCI.

XIST or XCI with neurodevelopmental diseases

A common feature of neurodevelopmental disease patients is that most female patients show XCI skewness as compared to normal females. XCI skewness is a phenomenon in which one X chromosome is more active than the other.[162] A study reported that a rare C-to-G mutation in the *Xist* promoter may lead to XCI skewness, and cells may prefer to inactivate X chromosome with this mutation.[165] Interestingly, XCI skewness is increased in autistic females compared to normal females.[166] However, this C-to-G mutation in *Xist* promoter was not detected and whether XCI

skewness is responsible for the female-relative decreased susceptibility of autism spectrum disorder is still unclear.

Female-biased Rett syndrome is mainly caused by the heterozygous mutation of X-linked methyl-CpG-binding protein (*MECP2*).[167] MeCP2 is a DNA methylation reader with both repressive and activating function with different cofactors.[168] Rett syndrome is exclusively observed in females because *MECP2* mutation is lethal in males during embryogenesis.[169] Deletions and nonsense mutations of *MECP2* are more severe than missense mutations and probably cause cells to preferentially inactivate X chromosomes.[170] Although there is no known direct connection between *XIST* and Rett syndrome symptoms, downregulation of *Xist* caused by knocking down of bone morphogenetic protein (BMP)/TGF-β signaling pathway members can activate *MECP2* gene expression on Xi allele in mouse embryonic fibroblasts.[167] This study also showed that restoration of the wild-type allele of *MeCP2* could be a promising therapeutic strategy of Rett syndrome in future.[167]

XIST in neurodegenerative diseases

XIST can serve as a miRNA sponge to regulate gene expression in neurodegenerative diseases. For the male-biased Parkinson's disease, *XIST* expression is generally upregulated and can sponge miR-199a-3p to enhance *Sp1* gene expression. Sp1 promotes the transcription and translation of leucine-rich repeat kinase 2 (*LRRK2*), a key PD-related gene.[171–174] Overexpressing miR-199a-3p or knocking down *XIST* by shXIST can inhibit apoptosis and promote cell proliferation, which can rescue neurodegeneration.[175] Furthermore, *in vivo* study in a PD mouse model showed that lentivirus vectors carrying shXIST or overexpression of miR-199a-3p mimics can alleviate Parkinson's disease-associated symptoms.[175]

AD involves the accumulation of β-amyloid (Aβ) peptide, which is a cleavage product of the amyloid precursor protein (APP). *Xist* expression was significantly upregulated in AD mice and cell models,[176,177] where inflammation and injury of nerve cells occurred.[177] *Xist* is a molecular sponge of miR-124 that targets *BACE1*, an enzyme crucial for the cleavage of APP and serving as a biomarker of AD. *Xist* silencing could reduce *BACE1* expression through miR-124.[176] Apart from sponging miRNA, *Xist* might also be involved in the progression of AD through its protein interaction. *Xist* could recruit the histone methyltransferase EZH2 to deposit H3K27me3 mark on the *NEP1* promoter region to repress its expression. NEP1 is an enzyme responsible for Aβ degradation. *Xist* knockdown resulted in increased expression of *NEP1* and alleviated Aβ-induced neuronal inflammation and damage.[177] Therefore, *XIST* may be a potential therapeutic target for AD.

XIST in pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a female-biased disease characterized by the proliferation and overgrowth of dysfunctional pulmonary artery endothelial cells, leading to right heart failure.[178] An epidemiology study showed that the approximate ratio of PAH females to male is 4:1.[179] The higher incidence in female may be explained by the higher expression of *XIST* in females since upregulation of *Xist* can promote PAH related phenotypes in murine model cells of plexiform PAH.[180] EH_{ITSN} (C-terminal protein fragments of intersectin-1) is generated during inflammation associated with PAH and can promote endothelial cell (EC) proliferation via activation of MAPK p38, ELK1 and FOS.[181] Qin et al expressed EH_{ITSN} in pulmonary artery endothelial cells (PAECs) from both male and female donors, and observed that female EH_{ITSN}-transfected PAECs have a higher proliferation rate.[180] Moreover, the *Xist* levels are also upregulated in both male and female EH_{ITSN}-transfected PAECs compared with controls, but female transfected PAECs showed more dramatic *Xist* increases. Treating female EH_{ITSN}-transfected PAECs with PenNPF, an EH_{ITSN} inhibitory peptide, can also reduce *Xist* levels and EC proliferation. Meanwhile, knockdown of *Xist* by siRNA can also impair the cell proliferation in female EH_{ITSN}-transfected PAECs. Increased *Xist* levels have also been detected in PAH patients with increased *ELK1* and decreased *KLF2*, known targets of *Xist* with roles in EC proliferation and anti-angiogenic effects only in female idiopathic PAH patients.[180] In summary, higher *XIST* in females may explain the female-bias feature, and upregulation of *XIST* in PAH patients may operate through upregulation of *ELK2* and downregulation of *KLF2*, both related to PAH.[106,180,182]

Although PAH is female-biased, the five-year survival rate from diagnosis in women is higher than in men.[183] This higher survival rate may stem from either the protective effect of sex hormones or women's better response to current treatment.[184] There is evidence that estrogen, which plays a vital role in the development of secondary sex characteristics, may attenuate the PAH phenotype in both males and females.[185,186] As a result, since females have higher circulating estrogens than males, they may be better protected.[183]

Therapeutic strategies targeting *XIST*

XIST expression and/or its modification appear to be altered during the progression of many sex-biased diseases. It therefore could be used as a potential biomarker for the diagnosis and prognosis of several diseases. In addition, studies in cell and mouse models have shown that genetic manipulation of *XIST* expression can potentially inhibit the progression of many diseases including bladder, colorectal and lung cancers. *XIST* could therefore be regarded as an important therapeutic target for these diseases.

Some commercially available drugs can regulate *XIST* expression although detailed mechanisms remain ambiguous. 5-fluorouracil, cisplatin, mitomycin and adriamycin are effective chemotherapies for colorectal cancer. High level of *XIST* expression in colorectal cancer cells promotes resistance to these chemotherapies through the XIST/miR-30a-5p/ROR1 axis.[135] Atractylenolide II, traditionally prescribed for melanoma treatment by Chinese medicine practitioners, is able to induce G1 cell-cycle arrest and apoptosis in B16 melanoma cells by modulating the expression of cell cycle-related genes or the phosphorylation level of related proteins.[187] When applied to colorectal cells, atractylenolide II downregulates *XIST* expression and reverses the effect of XIST/miR-30a-5p/ROR1 axis in modulating the chemosensitivity of colorectal cancer cells.[135] Platycodin D exerts anti-tumor effects in multiple

cancers, including lung cancer,[188] gastric cancer,[189] hepatocellular carcinoma,[190] and bladder cancer.[191] In bladder cancer cells, platycodin D treatment can inhibit *XIST* expression and regulate the XIST/miR-335 axis to slow bladder cancer progression both *in vitro* and *in vivo*.[191]

Beside these drugs, some molecules specifically targeting the lncRNA *XIST* can also be designed. Multiple approaches targeting lncRNAs have been developed, including small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs) and clustered regularly interspaced short palindromic repeats (CRISPR).[6,192] siRNAs targeting specific lncRNA can trigger RNA-induced silencing complex to degrade the lncRNA, which has been adopted by many groups to knockdown *XIST* expression in various cancer cells such as the colorectal cancer cell line LoVo and NSCLC cell line A549. Clinically, some siRNA drugs have already been used to treat patients, such as Onpattro (patisiran) for the treatment of hereditary transthyretin amyloidosis with polyneuropathy.[193] ASOs function through binding with specific RNA and recruiting RNase H to degrade the RNA of interest. Some ASO drugs have also been approved, including nusinersen to treat spinal muscular atrophy.[194] CRISPR uses single guide RNAs to guide the Cas9 nuclease to cleave specific DNA sequences. However, its utilization for *XIST* needs further investigation and optimization, since *XIST* function is required for normal female mammals and off-target effects need to be considered.

In addition, the mechanisms underlying *XIST*-mediated XCI could be relevant to treatment of diseases like Down syndrome, caused by chromosome 21 trisomy, and associated with intellectual disability, hematopoietic disorders and early-onset Alzheimer's.[195] Jiang et al proposed to use *XIST* to silence the whole extra chromosome 21. They transfected *XIST* on the gene-rich core of one chromosome 21 in stem cells from Down syndrome patients and successfully reduced chromosome 21 transcriptional outputs to near-normal levels.[196] Chiang et al also found that *XIST* could rebalance chromosome 21 dosage in trisomic induced pluripotent stem cells (iPSCs).[197]

Concluding remarks

Sex disparities in disease are common, and traditional therapies without consideration of sex differences sometimes cause disparity of efficacy between sexes. *XIST* plays pivotal roles in modulating the progression of many sex-biased diseases, and it functions in these diseases through at least four different mechanisms: XCI escape, XCI skewness, miRNA sponge, and regulation of the activity of interacting proteins (Fig. 2). *XIST*-mediated XCI is crucial for normal female development in mammals, and any alteration in *XIST* expression or localization may cause escape from XCI, which might be a double-edged sword for females. On the one hand, the escaped gene expression might protect females from some diseases, such as male-biased cancers and even COVID-19. For example, female immune cells can have biallelic *TLR7* expression due to XCI escape, which could in turn stimulate the cells to produce more type 1 interferon early in SARS-CoV-2 infection, therefore protecting females from COVID-19.[198] On the other hand, the elevated expression of these escapees may also be detrimental to the immune response under normal conditions. Hence 80% of autoimmune disease patients are female.[71] XCI skewness seems to be harmful to females, which is often observed in female-biased neurodevelopmental diseases. As a long transcript, *XIST* can bind large numbers of miRNAs and proteins and affect their function, which would promote or inhibit the progression of some diseases.

Our review also indicates that *XIST* expression or modification might be a biomarker for the diagnosis and prognosis of some diseases. Besides, *XIST* may be an excellent therapeutic target for some diseases, and several potential therapeutic strategies targeting *XIST* have been proposed. It should be noted that there remain many unknowns in the *XIST* field and sex-biased diseases. First, despite the fact that *XIST* expression is abnormally expressed in some sex-biased diseases and manipulated *XIST* expression can affect the progression of several diseases, whether and how *XIST* contributes to the sex disparity in the incidence and mortality of diseases need further elucidation. Most research to date has focused merely on the relationship between *XIST* expression and diseases without consideration of sex differences, as is done for most male-biased cancers. This has greatly limited interpretation. LncRNAs can regulate gene expression both in *cis* and in *trans*.[199] The ectopic expression system used by some groups to study the effect of elevated *XIST* expression on disease progression may be useful to elucidate the *trans*-acting effects of *XIST*, which, however, may not always reflect the real effect of increased expression in endogenous *XIST* since its chromosomal localization may differ. For example, mislocalized (but unaltered) expression of *XIST* may contribute to female-biased autoimmunity.[12,87,88,90] Second, conflicting results have been observed for *XIST* function by different research groups. For example, while most studies suggested that *XIST* has an anticancer effect on ovarian cancer,[153–156] some have claimed that *XIST* might exert an oncogenic role in its progression.[157,158] This inconsistency may arise from many causes including different stages or subtypes of disease progression at sampling. Third, for some diseases, the relationship between *XIST* and disease progression was explored only by utilizing quantitative reverse transcription PCR to measure the expression of genes of interest, and therefore a global understanding of *XIST* function on transcriptome variation is lacking. Transcriptome-wide approaches, such as RNA-sequencing (RNA-Seq) or single cell RNA-Seq, could be applied to address the detailed mechanisms underlying the progression of *XIST*-mediated diseases in future.

Author contributions

JL, ZM, and LY studied the literature and drafted the manuscript under the supervision of QM. JL produced the figures. TW and GL assisted in manuscript collation and review. QM conceived the review, obtained funds and provided critical input and is the corresponding author. All authors contributed to the article and approved the submitted version.

Conflict of interests

The authors declare no conflicts of interest.

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