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, 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. The aberrant expression of many human lncRNAs has been documented in many diseases. 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, and has been implicated in sex-biased diseases in recent years.

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. 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. 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 dosage compensation (XCD) instead. In post-implantation development, both human and other placental mammals undergo random XCI. The long noncoding transcript XIST (19 kb in humans, and 17 kb in mice) has been suggested to play vital roles in random XCI. Random XCI has three phases: initiation, establishment, and maintenance, and has been extensively investigated in mice. The XIST/Xist gene is located in the X inactivation center (XIC), and can be transcribed from the X chromosome to be inactivated (Xi), which functions in cis to coat Xi and nucleate dynamic protein complexes. Xist RNA-containing complexes gradually expand, allowing Xist to spread across Xi. Meanwhile, these complexes alter chromatin architecture and thus compact the chromosome, leading to progressive gene silencing along the Xi.

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. In early embryo development, XCI is regulated by XIST/Xist activators including Ftx, Jpx, Rnf12 (encoded by Rlim), and inhibitors such as Tsix, 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 (Fig. 1B), a DNA-binding insulator capable of repressing Xist expression. Ftx promotes the transcription of Xist through the proximity of their gene loci, which is independent of the Ftx RNA products (Fig. 1B). The X-encoded E3 ubiquitin ligase RNF12 upregulates mouse Xist expression by targeting for degradation the pluripotency factor REX1, which normally activates Tsix and represses Xist expression through binding to regulatory regions. 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 (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 in human pluripotent stem cells. Xist expression is silenced by the de novo DNA methyltransferases DNMT3A and DNMT3B.
and further activates pre-loaded histone deacetylase HDAC3 on Xi, resulting in the loss of active chromatin marks like H3K27ac. 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 and achieving selective X chromosome silencing. In humans, the E-repeat may also be required for PRC recruitment and H3K27me3 enrichment. In addition, XIST/Xist can recruit the m6A machinery to its transcript. In both humans and mice, the A-repeat interacts with the RNA-binding motif (RBM) proteins RBM15 and RBM15B. These RBM proteins further recruit the m6A methyltransferase METTL3/14 to specific sites in XIST/Xist through Wilms tumour-associated protein (WTAP), eventually resulting in m6A formation at adjacent sites. In humans, the m6A in XIST RNA is responsible for recruiting the m6A reader YTHDC1 to promote gene silencing, although the mechanisms remain elusive. In mice, YTHDC1 protein is recruited to Xist RNA through SPEN/SHARP’s SPOC domain. The three-dimensional conformation further promotes Xist spreading to actively transcribed genes across Xi. 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. The A-repeat interacts with the Lamin B receptor (LBR) to recruit Xi to the nuclear lamina, enabling Xist to spread across Xi. In addition, the interaction between Xist and PRCs is also crucial for Xist spreading.

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, whereas in human B cells, XIST is required for maintaining the X-inactivation of immune genes. A genome-wide RNAi screen in mouse embryonic fibroblasts identified 32 proteins involved in the maintenance of Xi silencing. 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. 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. Some cell-specific XIST-interacting proteins may also contribute to XCI maintenance. For example, TRIM28, a cofactor of the H3K9me3-specific histone methyltransferase SETDB1, only binds XIST RNA in B cells. CRISPRi screening further indicates that TRIM28 is indispensable for XCI maintenance in B cells. 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. Even for COVID-19-associated illness, the severity and mortality is different for men and women. The difference might be attributed to sexual dimorphism and gender differences in attitudes and behavior. 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. The X chromosome is known to contain the largest number of immune response-related genes of the whole human genome. 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, 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.

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. 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. 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.

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. 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, a unique B cell population expanded in SLE and RA. Yu et al. further showed that XIST is continually required in adult human B cells, finding that some X-linked immune genes in B cells lack promoter methylation, which requires XIST localization on Xi.
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, is commonly overexpressed in SLE and RA patients and promotes the formation and activation of CD11c⁺ atypical memory B cells. Yu et al also found that in somatic cells, XIST complexes are tissue-specific. 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. 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.

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. 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. All six, ATRX, CNKSR2, DDX3X, KDM5C, KDM6A/UTX, and MAGEC3, are located in the non-pseudoautosomal region of the X chromosome. 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. They are therefore referred to as ‘escape from X-inactivation tumor-suppressor’ genes or EXITS genes. For example, the loss-of-function mutation in the H3K27me3 demethylase gene KDM6A/UTX mainly occurs in male-biased cancers. However, female cancers with KDM6A mutations usually require homozygous mutations, leading to lower female incidences. 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. 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. 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, and the elevated XIST expression correlates with shorter survival and poor prognosis. In cell lines derived from these cancers, overexpression of XIST promotes cell proliferation, migration, invasion and epithelial—mesenchymal transition, and inhibits apoptosis, while knockdown of XIST has the opposite effect, irrespective of the sexual characteristics of cell lines (Table 1). Murine xenograft assays have also shown that for bladder, colorectal, or lung cancers, XIST silencing inhibits tumor growth in mice, and XIST overexpression promotes non-small cell lung cancer (NSCLC) tumor growth in mice. 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. AR encodes a steroid hormone receptor functioning as a transcription factor to promote the progression of bladder cancer. Although androgen signaling promotes the progression of bladder cancer in both males and females, higher AR expression is observed in male patients and associated with higher bladder cancer risk. In mice, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) can induce bladder cancer, with higher male incidence, possibly due to different AR expression levels. Knockout of the AR gene abolishes BBN-induced bladder carcinogenesis in both male and female mice, 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.
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-139-5p105 and miR-34a109 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 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-ZEB2120 and miR-137/Notch-1124 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...
histone methyltransferase EZH2 to silence the expression of proposed tumor suppressor KLF2 in NSCLC cells.\textsuperscript{106} Methyltransferase-like14 (METTL14) can catalyze the N\textsubscript{6}-methyladenosine (m\textsubscript{6}A) on XIST transcript, which is further recognized by the m\textsubscript{6}A reader YTHDF2, leading to XIST degradation.\textsuperscript{102} 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.\textsuperscript{102} Knockdown of METTL14 promotes colorectal cancer proliferation and invasion and substantially abolishes the m\textsubscript{6}A level of XIST, leading to augmented XIST expression,\textsuperscript{102} while METTL14 overexpression results in a remarkable decrease in XIST expression level, cell growth and invasion.\textsuperscript{102} It has therefore been proposed that METTL14 inhibits colorectal cancer progression by reducing XIST expression.\textsuperscript{102}

\textit{XIST} in female-biased and gynecologic cancers

Breast and thyroid cancers are more common in women than in men.\textsuperscript{70,143} The role of \textit{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.\textsuperscript{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.\textsuperscript{147} In breast cancer cells, XIST overexpression inhibits their proliferation, migration and invasion, and facilitates their apoptosis, while XIST silencing exerts the opposite effect.\textsuperscript{145,146} The xenograft tumor assay in BALB/c nude mice confirmed that XIST can retard breast tumor growth.\textsuperscript{146} XIST can activate CDX1 by sponging miR-155 to depress the growth, migration, and invasiveness of breast cancer.\textsuperscript{144} Interestingly, the expression of Jpx, an activator of XIST expression,\textsuperscript{29,30} has also been noted to be downregulated in breast cancer samples.\textsuperscript{145} Knockdown of XIST or SPEN/SHARP can promote the recruitment of HDAC3 to the promoter of PHLPP1.\textsuperscript{145} Since PHLPP1 encodes a phosphatase able to dephosphorylate AKT, knockdown of XIST leads to reduced PHLPP1 expression and increased AKT phosphorylation.\textsuperscript{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 \textit{in vitro} and in a mouse xenograft model.\textsuperscript{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,\textsuperscript{149–151} regardless of gender. In addition, XIST expression positively correlates with thyroid cancer progression.\textsuperscript{149,150} In both male-derived and female-derived thyroid cancer cells, XIST knockout inhibited cell proliferation, migration and invasion\textsuperscript{149–151} (Table 2), and its oncogenic role has been confirmed in the xenograft tumor assay in female nude mice.\textsuperscript{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.\textsuperscript{150} XIST also upregulates CLDN1 expression through interaction with miR-101-3p, thereby promoting cell proliferation, migration, and invasion of thyroid cancer.\textsuperscript{151} In addition, in papillary thyroid carcinoma, XIST targets miR-141 to promote cell proliferation and invasion,\textsuperscript{149} although the downstream processes are yet to be elucidated.

| 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 | \textsuperscript{116} |
| | 253J | Male | Knockdown | Inhibition | \textsuperscript{116} |
| | T24 | Male | Knockdown | Inhibition | \textsuperscript{116} |
| | RT112 | Female | Knockdown | Inhibition | \textsuperscript{116} |
| Colorectal cancer | HT29 | Female | Overexpression | Promotion | \textsuperscript{117} |
| | LoVo | Male | Knockdown | Inhibition | \textsuperscript{117} |
| | SW480 | Male | Knockdown | Inhibition | \textsuperscript{117} |
| | HCT116 | Male | Knockdown | Inhibition | \textsuperscript{117} |
| | A549 | Male | Knockdown | Inhibition | \textsuperscript{117} |
| Non-small cell lung cancer | H1299 | Male | Overexpression | Promotion | \textsuperscript{114} |
| | H522 | Male | Knockdown | Inhibition | \textsuperscript{114} |
| | Calu3 | Male | Knockdown | Inhibition | \textsuperscript{114} |
| | H226 | Male | Knockdown | Inhibition | \textsuperscript{114} |

\textsuperscript{114} J. Li et al.
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.151 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 while XIST knockdown has the opposite effect.154 XIST suppresses cancer progression through sponging hsa-miR-214-3p154 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-335/BCL2L2 axis.157 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 axis157 and regulating miR-149-3p.158 These findings require reconciliation with prior observations.

In cervical cancer, XIST expression is elevated159–161 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.151 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

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**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    |
|              | MDA-MB-231 | Female              | Knockdown                               | Promotion                                        | 146,147    |
|              | SKBR3      | Female              | Overexpression                          | Inhibition                                       | 146        |
|              | ZR75-1     | Female              | Knockdown                               | Promotion                                        | 146        |
|              | MDA-MB231BrM2a | Female          | Overexpression                          | Inhibition                                       | 147        |
| Thyroid cancer | M10        | Female              | Knockdown                               | Promotion                                        | 145        |
|              | TPC-1      | Female              | Knockdown                               | Inhibition                                        | 149,151    |
|              | KAT18      | Unspecified         | Knockdown                               | Inhibition                                        | 150        |
|              | FTC113     | Male                | Knockdown                               | Inhibition                                        | 150        |

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As for thyroid cancer, XIST expression on cell proliferation and tumorigenesis for female-biased cancers.
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, down-regulation 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. 175,176 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 EH2TSN (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 EH2TSN in pulmonary artery endothelial cells (PAECs) from both male and female donors, and observed that female EH2TSN-transfected PAECs have a higher proliferation rate. 180 Moreover, the Xist levels are also upregulated in both male and female EH2TSN-transfected PAECs compared with controls, but female transfected PAECs showed more dramatic Xist increases. Treating female EH2TSN-transfected PAECs with PenNPF, an Eh2TSN inhibitory peptide, can also reduce Xist levels and EC proliferation. Meanwhile, knockdown of Xist by siRNA can also impair the cell proliferation in female EH2TSN-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.

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-fluourouracil, 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. 183 Atractylolide 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, atractylolide 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, \(^1\)\(^\_\)\(^8\) gastric cancer, \(^1\)\(^\_\)\(^9\) hepatocellular carcinoma, \(^1\)\(^\_\)\(^9\) \(^1\) and bladder cancer. \(^1\)\(^\_\)\(^1\) 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 \(in\) \(vivo\). \(^1\)\(^\_\)\(^9\)

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). \(^6\), \(^1\)\(^\_\)\(^9\) 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. \(^1\)\(^\_\)\(^9\) 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. \(^1\)\(^\_\)\(^9\) 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. \(^1\)\(^\_\)\(^5\) 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. \(^1\)\(^\_\)\(^6\) Chiang et al also found that \(XIST\) could rebalance chromosome 21 dosage in trisomic induced pluripotent stem cells (iPSCs). \(^1\)\(^\_\)\(^7\)

**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. \(^1\)\(^\_\)\(^8\) 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. \(^7\)\(^1\) 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. \(^1\)\(^\_\)\(^9\) 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. \(^1\)\(^2\), \(^8\), \(^8\) Second, conflicting results have been observed for \(XIST\) function by different research groups. For example, while most studies suggested that \(XIST\) has an oncogenic effect on ovarian cancer, \(^1\)\(^5\)\(^\_\)\(^3\)–\(^1\)\(^5\)\(^\_\)\(^6\) some have claimed that \(XIST\) might exert an oncogenic role in its progression. \(^1\)\(^5\)\(^\_\)\(^7\), \(^1\)\(^5\)\(^\_\)\(^8\) 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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