

Expression and Function of a Large Non-coding RNA Gene XIST in Human Cancer

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Abstract

Background X inactive-specific transcript (XIST) RNA is involved in X chromosome silencing in female cells and allows X chromosome equilibration with males. X inactive-specific transcript expression has been found to be dysregulated in a variety of human cancers when compared to normal cells; meanwhile, the inactivated X chromosome has been noted to be conspicuously absent in human cancer specimens, whereas X chromosome duplications are widely noted. The specific pathways whereby changes in X chromosome status and XIST expression occur in cancer remain incompletely described. Nevertheless, a role for XIST in BRCA1-mediated epigenetic activity has been proposed.

Methods Here we review the data regarding XIST expression and X chromosome status in a variety of female, male, and non-sex-related human cancers.

Conclusions It is not yet known whether X chromosome duplication, XIST dysregulation, and over-expression of X-linked genes represent important factors in tumorigenesis or are simply a consequence of overall epigenetic instability in these cancers.

Introduction

The X inactive-specific transcript (XIST) belongs to a class of RNA molecules known as non-coding transcripts (NCT), which also includes non-coding RNAs (ncRNAs) such as microRNA. These ncRNAs are sometimes transcribed

from DNA genes that have exons and introns, and they can therefore have various splicing isoforms. Non-coding transcripts appear to be ubiquitous, have important roles in human cells, and have been implicated in tumorigenesis; the expression of NCTs has been found to be dysregulated in cancer cells [1]. The XIST gene is located in the X inactivation center (XIC), and its product is transcribed from the inactive X chromosome (Xi) [2]. It then spreads along the X chromosome from which it was transcribed. This ncRNA plays an important role in the initiation of X inactivation in female cells, which achieves dosage equilibration with males.

The exact molecular mechanisms whereby XIST initiates X inactivation are still under investigation; meanwhile, there is considerable evidence that XIST plays other important roles in the differentiation, proliferation, and genome maintenance of human cells. Specifically, it has been suggested that dysfunctional expression of XIST may have a pathologic role in cancer, possibly related to changes in gene expression from alterations in the stability of heterochromatin. Meanwhile, it is possible that cancer cells might have specific factors that allow for X inactivation outside of the embryonic development context. For example, special AT-rich sequence-binding protein-1 (SATB1), a proposed silencing factor of XIST in X chromosome inactivation, is expressed in embryonic stem cells and thymic lymphoma; unlike in normal adult somatic cells, XIST gene silencing can occur in the presence of SATB1 in thymic lymphoma [3]. Meanwhile, XIST silencing function has been reported in transgenic male fibrosarcoma cell lines, again suggesting a special context in cancer cells whereby XIST-induced X chromosome inactivation can occur, even in a post-differentiation state [4]. Thus, the functions of XIST, in addition to developmentally orchestrated X inactivation, represent an important area for further investigations. Our understanding

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of the conditions in which XIST functions continues to evolve, and improvements in genetic technology have allowed a rapid expansion of our knowledge of genomic expression patterns in normal cells and cancer cells. This review discusses the published data that have implicated XIST as having a role in cancer, as well as potential mechanisms.

X chromosome inactivation and cancer

Current theories regarding the process of X inactivation in females have their foundation in the Lyon hypothesis, whereby one copy of the X chromosome in female somatic cells is inactivated [5, 6]. Initially, the heterochromatic Barr body was discovered to be a marker of female sex status as a “nucleolar satellite” seen in the cells of female mammals [7]. The Barr body was subsequently identified to be Xi, and XIST was identified as an important mediator of X inactivation [2]. The exact nature of the Barr body initially was incompletely understood; nevertheless, its histological absence in the nuclei of malignant cells, especially in those of poorly differentiated or undifferentiated tumors, was notable. This phenomenon was more prominent in the cells of breast and cervical cancer specimens, and Barr body duplications were also observed [8].

Loss of the Barr body has since become a significant, consistent observation in female cancers (and is reviewed in Pageau et al. [9]). Gain or loss of chromosomes is a common phenomenon throughout the cancer genome, but the X chromosome in females represents a special case. Expression of more than one X chromosome in female cells generally is considered incompatible with life; for this reason, X inactivation occurs. This is in contrast to autosomes, where two copies are active. As such, the active X chromosome (Xa) seems more prone to transformation; only one “hit” might be required to inactivate a tumor-suppressor gene or activate an oncogene on Xa. Several genes with oncogenic potential (both as tumor suppressors and as oncogenes) have been identified on the X chromosome; these are reviewed by Spatz et al. [10]. The exact mechanisms whereby alterations in X chromosome inactivation, changes in X chromosome copy number, and/or changes in Barr body number occur in female (and male) cancer cells, as well as their compatibility with survival in these cells, remain undetermined.

XIST expression in female cancers

Although XIST typically is expressed by all female somatic cells, XIST expression has been found to be lost in female breast, ovarian, and cervical cancer cell lines

[11, 12]. Meanwhile, in cell lines where XIST expression is lost, analysis of methylation of X-linked genes reveals a loss of Xi as well; and, the majority of these cell lines had multiple copies of Xa. Through analysis of allelic status of the multiple Xas, it has been found that three patterns of Xi loss are present; some cells lost Xi without gain of Xa, some lost Xi and underwent multiplication of Xa, and a minority underwent reactivation of Xi [11].

Benoit et al. investigated X chromosome gene expression and performed Barr body staining in ovarian cancer cell lines. Compared with normal ovarian epithelial cells, half of the cancer cell lines demonstrated an undetectable level of XIST, and in these cell lines, loss of Barr body was observed [12]. Other investigators found that in ovarian cancer cell lines with a reduction of XIST expression (measured as the level of XIST RNA), the sensitivity of the cells to treatment with Taxol was reduced, suggesting that the expression of XIST in human ovarian cancer cells may serve as a prognostic marker with respect to chemotherapeutic response. This may be due to direct modulation by XIST of the toxic effects of Taxol, or by the reactivation of X-linked “resistance-specific genes” should the absence of XIST cause increased X chromosome expression; it also is possible, of course, that the relationship is not causal, and that loss of XIST is a consequence of genetic instability in cancer cells that also have acquired resistance to chemotherapy [13].

Ganesan et al. reported in 2002 that the breast and ovarian tumor suppressor BRCA1 “decorates” the XY body containing the essentially inactivated X chromosome in male mouse testis and co-localizes with XIST and macro-histone H2A1 (MH2A1) in the nucleus of female cells [14]. The XY body refers to the specialized nuclear territory formed by the sex chromosomes of mammalian spermatocytes. Furthermore, by examining XIST staining in a human breast cancer cell line lacking normal BRCA1 expression, they showed that loss of BRCA1 was associated with a lack of focal XIST staining on Xi. When BRCA1 was reintroduced via recombinant retroviral infection, XIST staining was restored. Meanwhile, in both cell lines, XIST RNA levels were not affected. These results suggested that BRCA1 localizes to the Xi, where it interacts with XIST RNA. These authors also determined that loss of BRCA1 led to increased expression of a green fluorescence protein (GFP) transgene located on Xi, indicating BRCA1 may have a role in stabilization of X inactivation [14, 15]. Meanwhile, BRCA1 deficiency and concomitant loss of Xi both have been observed in the highly aggressive, sporadic basal-like cancers of the breast [16].

Conversely, Pageau et al. determined that while BRCA1 may appear to overlap in two dimensions, they actually did not co-localize with XIST RNA when viewed in three dimensions; so, while BRCA1 did indeed appear to have a role in replication and maintenance of the tightly packed

heterochromatin within the nucleus, it did not appear to have a direct role in XIST localization on the Xi [17]. Rather, its relatively broad effects on chromatin and gene regulation are likely the reason for changes in X inactivation status, as opposed to a specific interaction with XIST [18]. Further investigation of this controversial relationship between BRCA1 and XIST led to similarly conflicting results. Xiao et al. found that while BRCA1 does distribute along the XY body in male spermatocytes, it was conspicuously absent on the XIST-rich Xi in female somatic cells; again, co-localization of XIST and BRCA1 was absent. They also found that in breast cancer cell lines lacking both BRCA1 and normal XIST staining, reconstitution of wild-type BRCA1 did not restore normal XIST localization, in contrast to the findings of Ganesan et al., as described above. Likewise, there was no change in XIST staining when BRCA1 was silenced using shRNA or siRNA [19].

The discrepancy in BRCA1/XIST co-localization between these studies may represent a different level of stringency required to define co-localization, differences in technique, or differing sensitivities of the probe used for XIST. It also has been suggested that the BRCA1 mutant cell line used by Xiao et al. expresses BRCA1 with a single exon deleted, a splice alternate that might occur naturally and has been shown to have some wild-type properties. Despite these differences, it has been proposed that sufficient data exist to support the influence of BRCA1 on XIST localization along Xi [20].

Some investigators have suggested that in BRCA1-deficient tumors, there is substantial overall genetic instability that leads to X chromosomal changes including loss of Xi or duplication of Xi or Xa. It has been reported that the frequent duplication of X chromosomes in breast cancer cell lines appeared to be primarily due to Xa duplication and unrelated to BRCA1 status, whereas loss of Barr body in these cell lines appears to be due to Xi loss rather than Xi reactivation [21]. In a recent investigation of human breast cancer tissue from patients with a variety of germline BRCA1 mutations, XIST RNA domains (corresponding to Xi) were detected in a significant number of samples; and, among tumors with identical BRCA1 mutation, a variety of XIST staining patterns were observed. Thus, genetic instability, rather than BRCA1-related epigenetic instability, may lead to the observed perturbations in Xi and the deficiency of XIST staining [22]. Interestingly, it has recently been described that BRCA1 deficiency leads to increased levels of XIST in breast cancer cell lines, which appears to be in part due to expression of XIST from Xa, normally regulated by BRCA1 [23]. Thus, XIST detection does not necessarily signify the presence of Xi in cancer cells, although Xi has historically been considered to be the site of XIST

transcription [23]. Clearly, further studies are needed to determine the specifics of causality between BRCA1 deficiency, heterochromatin and genomic instability, and Xi loss or defective XIST localization. Based on the data described, XIST appears to have a role in female cancer, though the specifics remain elusive.

XIST and male cancer

Normally, X inactivation occurs early in embryonic development in females and in specific epigenetic contexts in female somatic cells [3]. Meanwhile, as males have only one X chromosome, the only period in which XIST is normally expressed in males is in the germline; diploid primary spermatocytes exhibit X inactivation and formation of the aforementioned XY body during spermatogenesis. X inactive-specific transcript has been shown to localize to this XY body in spermatocytes [24]. Meanwhile, strong expression of XIST (which is completely absent in male somatic tissues) has been demonstrated in mature testis; these levels; however, are lower than those found in female somatic tissues [25].

Subsequent studies revealed that male mice lacking the XIST gene still undergo transcriptional silencing of X-linked genes, and furthermore they undergo normal spermatogenesis and develop an XY body; this suggests that X inactivation in these spermatocytes is XIST-independent [26]. Meanwhile, the patterns of X chromosome methylation observed in female cells undergoing X inactivation and those of male spermatocytes with XY body formation differ; these differences, as well as a reduced density of chromatin in the XY body in comparison with a Barr body, may facilitate reactivation of selected X-linked genes during spermatogenesis [26]. Thus, the process of X inactivation in female cells and male germ cells appears to be different.

Testicular germ cell tumors (TGCTs) are derived from germ cells and include seminomas and nonseminomas. X inactive-specific transcript expression patterns in TGCTs have been extensively studied. In normal XY males, TGCTs have long been known to display gain of X chromosomes. Over a decade ago, germ cell tumor tissues were shown to have expression of XIST as determined by polymerase chain reaction (PCR), in contrast with normal male blood, where no XIST expression is detected [27]. Subsequent studies showed that XIST expression is actually more common in seminomatous TGCTs (and in corresponding cell lines) than in nonseminomatous TGCTs, and there were supernumerical X chromosomes present in both types [28]. Methylation patterns and X chromosome linked gene expression were also studied in these TGCTs in order to determine the inactivation status of the supernumerical X chromosomes; it was found that the

expression of XIST in the samples was not associated with the methylation of X-linked genes or even X chromosome inactivation. Ultimately, the authors suggest that the role of XIST in these male tumors may be distinct from its role in normal female X inactivation, and that the acquisition of additional X chromosomes may actually contribute to oncogenesis [28].

X inactive-specific transcript has meanwhile emerged as a potential serum marker for TGCTs in male patients. In somatic cells, the XIST gene on the active X chromosome of males and females is typically methylated on the 5' end, whereas the allele on Xi is typically unmethylated. Thus, only in female cells would an unmethylated XIST gene be expected to be present. However, Kawakami et al. demonstrated that in addition to tumor expression of XIST, some patients with TGCT have detectable unmethylated XIST in plasma [29]. Like the level of XIST expression (as described above), the methylation status of the XIST gene appears to differ between seminomatous and nonseminomatous TGCTs as well [30]. Similarly, the 5' region of XIST has been found to be relatively more unmethylated in the serum of patients with prostate cancer than in normal male serum [31].

Male patients with Klinefelter syndrome (47XXY) have been found to express XIST. In fact, any somatic expression of XIST in men suspected of Klinefelter syndrome has been suggested as a serum marker for the condition [32]. Meanwhile, along with testicular atrophy and hormonal imbalances, these patients have an increased risk of developing malignancies of the lung and breast, non-Hodgkin lymphoma, and testicular tumors; interestingly, their risk of prostate cancer appears to be reduced [33]. The overall increased risk of malignancy may be due to overexpression of oncogenic X-linked genes in the absence of adequate inactivation of their supernumerical X chromosome. Kawakami et al. compared expression levels of XIST in the small-cell cancer cell line PSK-1 established from a patient with Klinefelter syndrome. When compared to the peripheral blood of the same patient, the expression of XIST was lost in the cancer cell line. Meanwhile, the cancer cells actually were found to have an additional X chromosome—three total. However, while the germline XXY cells had the expected chromosomal profile of one Xa and one Xi, the cancer cells had three active X chromosomes. Based on X expression profiling, these were found to represent duplication of the Xa, and complete loss of Xi [11]. In normal XY men with male breast cancer, X chromosomal duplications are also frequently found [34, 35].

XIST and other cancers

X inactive-specific transcript expression has been found to be dysregulated in a variety of non-sex-related cancers in humans and in mice. In cell lines created from the tissue of

both a male patient and a female patient with collecting duct carcinoma of the kidney, the XIST gene, along with several other chromosome X genes, was found to have an increase in copy number [36]. X inactive-specific transcript gene amplification has also been detected in microsatellite-unstable sporadic human colorectal cancer tissue when compared to matched normal colorectal epithelium [37].

In an investigation of the cytochrome P450 genotype on the formation of benzo[a]pyrene (B[a]P)-induced proximal small intestine tumors in mice, it was found that there was “striking” upregulation of the XIST gene after 8 weeks of B[a]P treatment, which was abolished after 12 weeks. Furthermore, based on the expression profiles of several Y chromosome genes in the cells, it was speculated that XIST may actually silence genes on the Y chromosome through its role in histone modification and DNA methylation [38]. Meanwhile, there was a detectable level of XIST transcript in pre-neoplastic cells located in the gastric fundus of a male mouse infected with *Helicobacter felis* (a mouse model for *H. pylori* infection, which can lead to gastric cancer in humans); as expected, there was no expression of XIST detected in the normal male mouse fundus [39].

X inactive-specific transcript expression and X inactivation status have been studied in men and women with non-Hodgkin lymphoma demonstrating supernumerary X chromosomes. In seven men with lymphoma in which additional X chromosomes were present, XIST expression was found to be absent in the tumor tissue. Markers of X chromosome methylation in these samples, meanwhile, were negative, suggesting the supernumerary X chromosomes remained active [40]. Development of multiple Xa in previously normal XY male cells must represent a duplication of the active X chromosome, whereas Xa duplication in female cells may arise in more than one way (duplication of Xa versus reactivation of Xi). In an investigation of female lymphoma with supernumerary X, the two scenarios appeared to occur with equal frequency according to expression and methylation analysis [40]. Meanwhile, XIST gene deletion has been reported in leukemia without evidence of Xi reactivation [41].

Conclusions

The X chromosome is distinctive in both male and female cells. In male cells, it represents a unique unpaired chromosome; in both male and female cells, it undergoes a developmentally orchestrated process of silencing, which is relatively permanent for the lifespan of female cells. Thus, one active X chromosome is normally present in human cells; this rule is violated in cancers in both sexes, where supernumerary X chromosomes are observed along with dysregulation of XIST expression. It remains to be

determined whether the X chromosome duplications/reactivations observed in tumors found in women, men, and men with Klinefelter syndrome are involved in the development of cancer and the mechanisms whereby this occurs. Alternatively, the X chromosome duplications and reactivations observed in cancer cells may be a symptom of epigenetic instability and elevated non-disjunction frequencies, which lead to chromosomal abnormalities in many cancers.

Along with changes in X chromosome nuclear content, cancer cells in women and men have shown downregulation and upregulation of XIST, respectively; cancer cells from patients with Klinefelter syndrome, meanwhile, demonstrate the downregulation of XIST observed in female cancer cells. The functions and role of the XIST gene in the loss of normal X chromosome ploidy in human cells bears further investigation, as does its interaction with BRCA1 in female cancer. One strategy would be to investigate the genetic sequence, as well as the presence of any mutations, of the XIST gene in human cancer tissues; then, functional studies of the effects of wild-type and mutated XIST in cancer cells could be performed. The XIST gene may represent an important modulator of tumor growth and development in men and women.

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