



Effect of Non-Coding Region RNA Gene XIST (X-Inactive Specific Transcript) on Human Breast Cancer

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ABSTRACT

X chromosome is a sex chromosome found in both women and men. The inactivation of the X chromosome is linked to a non-coding region of RNA known as the XIST gene (X-inactive specific transcript). This gene is located in the X inactivation center (XIC X-inactive center). The XIST gene is a region that belongs to the RNA group, non-coding transcripts (NCT), also known as microRNA. Breast cancer (Breast Cancer) is a type of cancer that commonly affects women, but men can develop breast cancer, but the chances are small, about 1 in 1000. Breast cancer is a cancerous neoplasm that is malignant, and occurs in the mammary gland. The presence of a specific XIST gene on the X chromosome and the prevalence of breast cancer, which is mostly in women, raises the idea that there is an influence of this gene on breast cancer (breast cancer) in the epigenetic process. The XIST gene related to microRNA has an opportunity to be looked at because certain microRNAs have a greater or lesser level (concentration) in cancer cells than normal cells. This is a new opportunity to continue to be developed as a consideration for a new treatment method involving gene therapy.

1. Introduction

X chromosome is a sex chromosome found in both women and men. However, a unique mechanism of inactivation of the x chromosome in normal (XX) women is that only one X chromosome is transcribed. This is related to the non-coding region of RNA known as the XIST gene (X-inactive specific transcript). This gene is located in the X inactivation center (XIC X-inactive center). The XIST gene is a region that belongs to the RNA group, non-coding transcripts (NCT), also known as microRNA.¹

This X chromosome inactivation mechanism works in epigenetic regulation. Epigenetics is a change in gene expression but does not change the gene sequence.

Research on inactivation of the X chromosome is mostly done on human embryonic stem cells (hESC). As an initiating factor for the X chromosome inactivation, this Xist gene will be expressed in low levels, especially before the process of differentiation of the X and hESC chromosomes.²

The X chromosome will be inactivated randomly, starting with the embryo in the pre-gastrulation stage. This allows the inactivation pattern of the X chromosome to be passed on to the offspring, resulting in a genetic mosaic pattern of cells both paternally and maternally.³

However, there is a mechanism of XIST gene dysregulation in several variations of cancer cells when compared to normal cells. The absence of this gene in some types of cancer clearly indicates an epigenetic symptom which of course will be closely related to the development of cancer cells itself.

Breast cancer (Breast Cancer) is a type of cancer that commonly affects women, but men can develop breast cancer, but the chances are small, about 1 in 1000. Breast cancer is a cancerous neoplasm that is malignant, and occurs in the mammary gland. Cancer cells are formed from normal cells in a complex process called transformation, which consists of the initiation and promotion stages.

At the initiation stage, a change occurs in the genetic material of the cell which provokes the cell to become malignant. This change in the genetic material of cells is caused by an agent called a carcinogen, which can be a chemical, virus, radiation or sunlight. Meanwhile, at the promotion stage, a cell that has undergone initiation will turn malignant. Cells that have not passed the initiation stage will not be affected by the promotion. Because it requires several factors for malignancy (a combination of sensitive cells and a carcinogen). Breast cancer can be classified in several ways including genetic. The genetic classification includes DNA micro-arrays.

DNA micro-array is a method that begins by comparing normal cells with cancer cells and looking at the differences in genetic expression between the two types of cells. Although these differences in genetic expression do not necessarily indicate the characteristics of cancer cell oncogens, several groups of researchers consider that some groups / clusters of genes have a tendency to leave genetic traces on other cells until the same genetic expression occurs, which is called genetic profile. Thus, the functional dynamics of genes and genomes can be observed such as mRNA

transcription processes, identification of binding domains of nucleic acid proteins, single-nucleotide polymorphism analysis.

Breast cancer can occur because of several genetic factors passed down from parents to children. Genetic factors in question are mutations in several genes that play an important role in the formation of breast cancer. The genes in question are genes that are oncogeneous and genes that suppress tumor. Tumor suppression genes that play an important role in the formation of breast cancer include the BRCA1 gene and the BRCA2 gene.

The magnitude of the influence of genes on this disease of course led to various studies to find all the genes that affect breast cancer. The presence of a specific XIST gene on the X chromosome and the prevalence of breast cancer, which is mostly in women, raises the idea that there is an effect of this gene on breast cancer (breast cancer) in the epigenetic process. The XIST gene related to microRNA has an opportunity to be looked at because certain microRNAs have a greater or lesser level (concentration) in cancer cells than normal cells. In cancer cells the overexpression of certain mRNAs because they are free of microRNAs, or the over-suppression of certain mRNAs leads to overexpression of their alternative mRNAs. Returning the microRNA level to normal levels is one method of cancer therapy. This is a new opportunity to continue to be developed as a consideration for a new treatment method involving gene therapy.

Expression and Mechanism of the XIST gene

Gen X inactive-specific transcript (XIST) belongs to a class of RNAs known as “non-coding transcripts (NCTs)”, in the real sense not without coding, but rather as microRNA. NCTs have an important role in human cells, and also have an association with the tumorigenesis process, this was found by the dysregulation of NCTs expression in cancer cells.

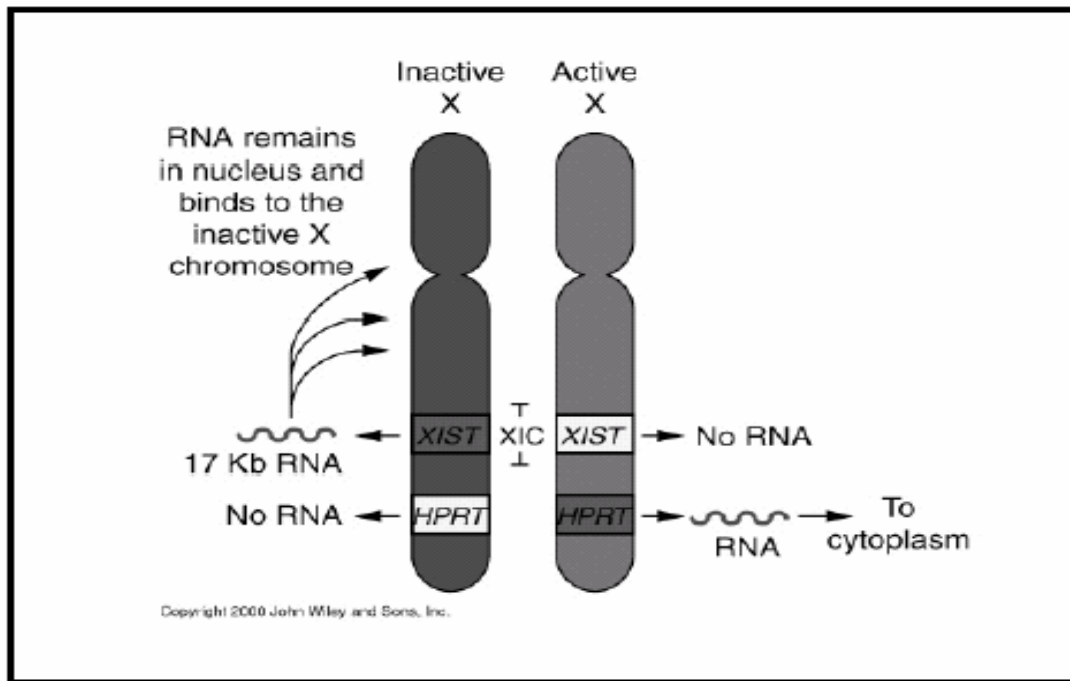


Figure 1. Gen XIST (X- inactive specific transcript)

This XIST gene is in the XIC (X-inactive center) region with a length of about 17 kb. Contains 6 repeating sequences, and only at location 5' repeat A is known to have a function. Repeat A contains 7.5 copies of the sequence which are estimated to fold to form a 2-rod loop structure. However, the relationship between the inactivation mechanism and the loop-shaped structure at repeat A is not certain. This gene can replace the previously active X chromosome to become inactive by expressing a nuclear compartment which acts as a means of transcription. Furthermore, repressive chromatin modification such as Polycomb Group (PCG) related to histone H3K27 trimethylation and H2A histone mono-ubiquitination, and incorporation of macro H2A histone variants can be observed from Xi.³

PcG protein is a transcription repressor and maintains silencing of developmental control genes. Apart from the presence of PcG protein, to maintain

the inactivation of the X chromosome, DNA methylation is required. The presence of Dnmt1 DNA methyltransferase activity shows that the gene is integrated into X inactivation (Xi). Recently, a new protein has been identified that is required for the maintenance of DNA methylation patterns, and gene repression in yang Xi. The SmcHD1 protein contains the ATP binding domain

and hinge domains which are also present in SMC type structural chromosome proteins. Interestingly, Xist localization and recruitment PCG were normal in the absence of SmcHD1, indicating that the initiation of X inactivation was normal. This identifies SmcHD1 as an important factor for X inactivation maintenance and provides

the first clue to a potentially new epigenetic mechanism involved in conveying DNA methylation patterns.³

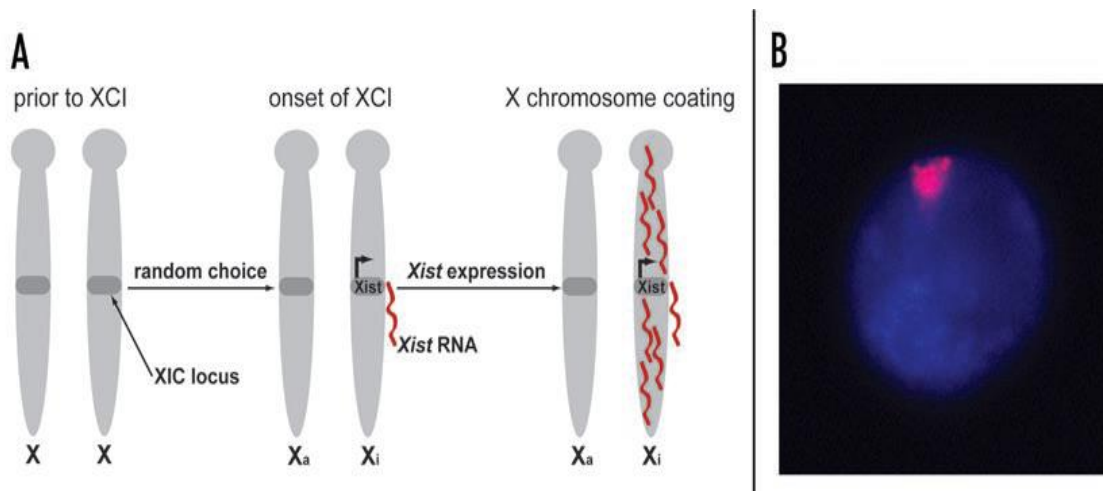


Figure 2. Mechanism of random X chromosome inactivation.³

In general, the X chromosome has the same gene composition. So that the random inactivation mechanism can occur on any chromosome, the presence of the XIST gene at the XIC locus will initiate the chromosome to be inactive. XIST will undergo posttranscript regulation in the form of microRNA formation. MicroRNA is a noncoding RNA, very small

(21 or 22 bases) produced from fragmented precursors. MicroRNA prevents the translation of mRNA transcripts into proteins by complementing to form base pairing with functional mRNAs so that the translation is off, and X chromosome inactivation occurs.

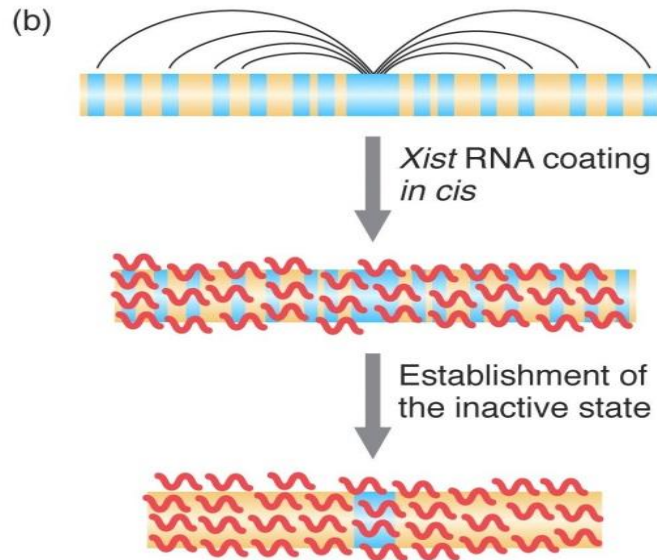


Figure 3. X chromosome inactivation

However, the complete mechanism for this gene is still being developed. With the premise that this X chromosome inactivation involves a complex epigenetic process, many factors are involved. This of

course will not stop being investigated until the specific factors and real mechanisms are found.

Breast Cancer

Breast cancer includes cancer of the neoplasm

(mass of abnormal tissue that grows excessively and does not coordinate with the surrounding normal tissue) which is malignant, and occurs in the mammary gland. Cancer cells are formed from normal cells in a complex process called transformation, which consists of the initiation and promotion stages. At the initiation stage, a change occurs in the genetic material of the cell which provokes the cell to become malignant. This change in the genetic material of cells

is caused by an agent called a carcinogen, which can be a chemical, virus, radiation or sunlight. Meanwhile, at the promotion stage, a cell that has undergone initiation will turn malignant. Cells that have not passed the initiation stage will not be affected by the promotion. Therefore, several factors are needed for the occurrence of malignancy (a combination of sensitive cells and a carcinogen).⁴

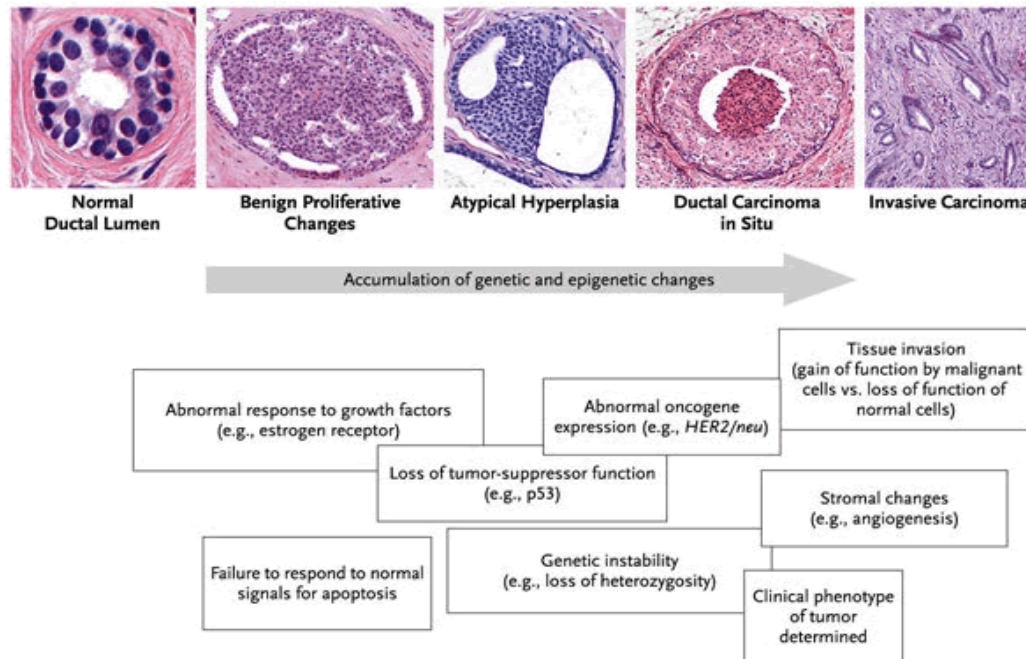


Figure 4. Breast cancer carcinogenesis

Breast cancer can be classified in several ways including genetic. Genetic classification, one of which includes DNA micro-arrays. DNA micro-array is a method that begins by comparing normal cells with cancer cells and looking at the differences in genetic expression between the two types of cells. Although these differences in genetic expression do not necessarily indicate the characteristics of cancer cell oncogenes, several groups of researchers consider that some groups / clusters of genes have a tendency to leave genetic traces on other cells until the same genetic expression occurs, which is called genetic profile. Thus, the functional dynamics of genes and genomes can be observed such as mRNA transcription processes, identification of binding domains of nucleic

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Breast cancer can occur due to several genetic factors passed down from parents to children. Genetic factors in question are mutations in several genes that play an important role in the formation of breast cancer. The genes in question are genes that are oncogeneous and genes that suppress tumor. Tumor suppression genes that play an important role in the formation of breast cancer include the BRCA1 gene and the BRCA2 gene.

2. Discussion

Cancer is a genetic and epigenetic disease. In genetic processes, cancer can occur due to changes in

DNA sequences or what is known as mutations, while epigenetics can be changes in chromatin and methylation in the promoter region. The X chromosome duplication and reactivations observed in cancer cells may be a symptom of epigenetic instability and a high frequency of nondisjunction, which can lead to chromosomal abnormalities in many cancers. As the X chromosome changes, the cancer cells in women and men are shown to have XIST downregulation and upregulation, in this case to

indicate the XIST downregulation observed in female cancer cells. Epigenetic regulation is important for maintaining the main stability of cells, both for continuing the function of organs and tissues. Undue changes in cell identity must be avoided. Failure to avoid it can lead to tumor development and disease. In cancer, the epigenetic impact that occurs due to gene silencing affects the progression of cancer cells, and this occurs in tumor suppressor genes.

Diseases involving imprinted genes			
Prader-Willi syndrome	Hypotonia, short stature, obesity, small hands and feet, mild mental retardation	Paternal deficiency for the HBII-85 C/D box snoRNA cluster	Nicholls et al (1989), Sahoo et al (2008)
Angelman syndrome	Neurogenetic disorder	Maternal deficiency for the UBE3A gene	Kishino et al (1997), Malcolm et al (1991), Matsuura et al (1997)
Beckwith-Wiedemann syndrome	Overgrowth, increase in cancer risk	IGF2 overexpression	Ohlsson et al (1993)
Disease modulated by X inactivation pattern in female patients			
RETT syndrome	Disease severity	X-linked MeCP2 mutation	Archer et al (2007), Huppke et al (2006)
Haemophilia	Severe disease in heterozygous female patients	X-linked factor VIII mutation	Favier et al (2000)
Autism	Potential association with non-random X inactivation	Unknown X-linked gene	Talebizadeh et al (2005)
X-linked hypohidrotic ectodermal dysplasia	Severe disease in heterozygous female patients	X-linked ectodysplasin A (EAD) gene mutation	Lexner et al (2008)
Chronic granulomatous disease	Severe disease in heterozygous female carriers	X-linked gp91-phox mutation	Anderson-Cohen et al (2003)
Epigenetic gene silencing contributing to disease			
Fragile X syndrome	Neurological disease onset and severity	CGG repeat expansion causes silencing of fragile X mental retardation 1 (FMR1)	Bassell and Warren (2008)
Cancer	Cancer progression	Silencing of tumour suppressor genes	Gal-Yam et al (2008), Laird and Jaenisch (1996)

Table 1. Epigenetic related to diseases.⁵

The function and role of the XIST gene in the loss of normal X on the ploidy chromosome of human cells is undergoing further investigation, such as the interaction of this XIST gene with BRCA1 in breast cancer. One of the strategies to investigate the genetic

sequence, as well as a marker for the presence of the XIST gene mutation in human cancer tissue, is to functionally study the different effects of XIST on wild types and mutations in cancer cells.⁶

Table II. Gain and loss of X chromosomes in human tumours			
Type of tumour	X chromosome		References
	Gain	Loss	
Testicular germ cell tumour	Yes		XIST expression leading to X inactivation of extra X chromosomes Kawakami et al (2004a), Looijenga et al (1997)
Lymphoma	Yes		No change McDonald et al (2000)
Recurrent ovarian cancer		Yes	Loss of XIST expression correlates with shorter disease free period in taxol treated patients Huang et al (2002)
Breast cancer	Xa	Xi loss	Can be associated with XIST activation on the active X chromosome Sirchia et al (2009), Vincent-Salomon et al (2007)
Cervical cancer	Xa	Xi loss	Kawakami et al (2004b)

Table 2. Gain and loss of X chromosome in human tumors.⁵

The table shows that in breast cancer there is an increase in the number of active X chromosomes (Xa), and a decrease in the number of inactive X chromosomes (Xi). This can be attributed to the activation of the XIST gene. The XIST gene can represent an important modulator of tumor growth and development in both men and women. The data summarized above suggests that the loss of BRCA1 can cause certain characteristics of Xi's features to be impaired, including the normal localization of XIST RNA. The association between loss of BRCA1 overexpression and failure of proper XIST localization was observed not only in cultured cells, but also in some BRCA1 in human primary tumor cells. These results imply that loss of XIST localization is a natural marker in certain BRCA1 deficient tumor cells. Acute suppression of BRCA1 synthesis led to an increased incidence of reactivation of the GFP transgene located in Xi. However, it is interesting to speculate that perhaps the loss of the BRCA1 gene in certain cells in women will have the additional effect of causing increased expression of some X chromosome genes, which may play a role in the development of breast and ovarian cancer.⁶

A subset of malignant breast cancer and ovarian tumors has been shown to detect reduced Barr body.

The Barr body is an inactive X chromosome that has condensed to form a solid complement that appears to be near the nucleus when observed under a microscope, and can be detected by certain colors.⁷

Likewise, in men with Klinefelter's syndrome (genotype XXY) who have a predisposition to gynecomastia and breast cancer, this again suggests a role for increased dose of gene X in breast growth and carcinogenesis. One of the most common cytogenetic abnormalities observed in male breast cancer is an increase in the number of X chromosomes. The excess X chromosome allows an X chromosome inactivation mechanism, so that only 1 X chromosome is active. This mechanism will certainly be different from that of women, because the initiation of the Y chromosome will be an additional, more complex epigenetic factor.⁸

The presence of a specific XIST gene on the X chromosome and the prevalence of breast cancer, which is mostly in women, raises the idea that there is an effect of this gene on breast cancer (breast cancer).⁹

The XIST gene related to microRNA has an opportunity to be looked at because certain MicroRNAs have a greater or lesser level (concentration) in cancer cells than normal cells. In cancer cells the overexpression of certain mRNAs because they are free of microRNAs, or the over-

suppression of certain mRNAs leads to overexpression of their alternative mRNAs. Returning the microRNA level to normal levels is one method of cancer therapy.

3. Conclusion

Inactivation of the X chromosome is related to the XIST gene. This gene is located in the X inactivation center (XIC X-inactive center). The XIST gene is a region that belongs to the RNA group, non-coding transcripts (NCT), also known as microRNA. The presence of a specific XIST gene on the X chromosome and the prevalence of breast cancer, which is mostly in women, raises the idea that there is an influence of this gene on breast cancer (breast cancer) in the epigenetic process. The XIST gene related to microRNA has an opportunity to be looked at because certain MicroRNAs have a greater or lesser level (concentration) in cancer cells than normal cells. In cancer cells the overexpression of certain mRNAs because they are free of microRNAs, or the over-suppression of certain mRNAs leads to overexpression of their alternative mRNAs. Returning the microRNA level to normal levels is one method of cancer therapy. This is a new opportunity to continue to be developed as a consideration for a new treatment method involving gene therapy.

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