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XIST in Brain Cancer

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Abstract

Long non-coding RNAs (lncRNAs) make up the majority of the human genome. They are a group of small RNA molecules that do not code for any proteins but play a primary role in regulating a variety of physiological and pathological processes. X-inactive specific transcript (XIST), one of the first lncRNAs to be discovered, is chiefly responsible for X chromosome inactivation: an evolutionary process of dosage compensation between the sex chromosomes of males and females. Recent studies show that XIST plays a pathophysiological role in the development and prognosis of brain tumors, a heterogeneous group of neoplasms that cause significant morbidity and mortality. In this review, we explore recent advancements in the role of XIST in migration, proliferation, angiogenesis, chemoresistance, and evasion of apoptosis in different types of brain tumors, with particular emphasis on gliomas.

Introduction

The central dogma of molecular biology states that genetic information is transcribed from DNA to messenger RNA (mRNA), which then gets translated into proteins [1]. However, at least 80% of the DNA is non-protein coding, previously referred to as “junk DNA” [2]. Recently, the regulatory role of the non-coding genetic transcripts, called non-coding RNAs, became prominent in many biological processes. Long non-coding RNAs (lncRNAs), each more than 200 nucleotides in length, constitute more than 50% of the non-coding portion of the DNA [3]. As opposed to mRNAs, lncRNAs are more localized in the nucleus and primarily regulate gene expression epigenetically, transcriptionally, post-transcriptionally, translationally and proteomically [4]. The alteration of their biological function drives them to mediate the pathogenesis of many diseases, including cancer development and progression.

The lncRNA X-inactive specific transcript (XIST) is the main regulator of X chromosome inactivation (XCI), a major biological process that occurs in females during fetal development [5]. Compared to the X chromosome, the Y chromosome present in males carries a small number of genes, thus a compensatory mechanism is required to equalize the genetic dosage between both sexes. Since females have two X chromosomes, one of them undergoes the process of XCI to achieve this genetic balance.

XCI is described as a complex, multi-step process [6]. It is initiated by the binding of XIST to the X inactivation center present on the target X chromosome. XIST then spreads along the X chromosome and coats it with the help of Heterogeneous Nuclear Ribonucleoprotein U (HnrnpU), a family of proteins that have DNA and RNA binding sites. Following that, Polycomb Repressive Complexes (PRC1 and PRC2) are recruited by XIST to mediate epigenetic histone modifications, including H2AK119 mono-ubiquitination (H2AK119ub) and H3K27 trimethylation (H3K27me3) respectively, which repress the transcriptional ability of the X chromosome [7]. Finally, the inactivated X chromosome is tethered to the periphery of the nucleus, forming a Barr body. Figure 1 summarizes this process.

Parallel to its main biological function in XCI, XIST was found to have an evident role in carcinogenesis [8]. Not only does it promote cancer growth and progression, but it also acts as a prognostic factor in various types of cancers, including colorectal, lung, and brain cancers [9,10]. XIST's tumorigenic function is exerted through downstream proteins regulated by microRNAs (miRNAs), which are short non-coding RNAs. Like lncRNAs, miRNAs also affect gene expression post-transcriptionally by either mRNA cleavage or translational repression [11]. To mediate tumor progression, XIST acts as a molecular sponge that binds to and represses the activity of different tumor suppressive miRNAs [12].

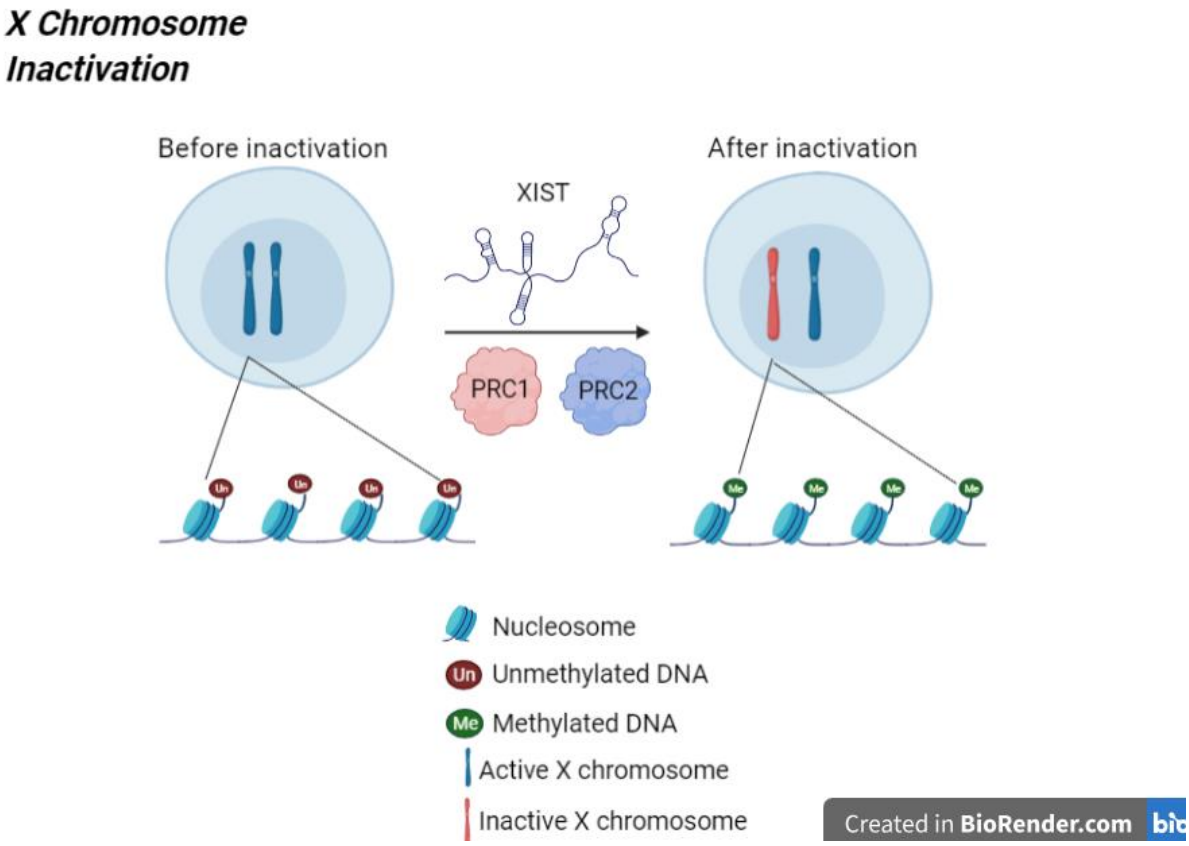

Figure 1. X chromosome Inactivation [13]. This figure depicts the process of XCI, which is mediated by lncRNA XIST. PRC1 and PRC2 are recruited by XIST to facilitate the histone modifications required for X chromosome silencing. Finally, the inactive X chromosome forms a Barr body. Created by BioRender.com

Brain Tumors Overview

Brain tumors constitute 1% of cancers in the United States but 2% of cancer deaths. They are an extremely heterogeneous group of diseases that can be broadly split into gliomas, such as astrocytomas and meningiomas, and non-gliomas, which include pituitary tumors and neuroblastoma. Brain tumors are unique in that they are not staged; however, the 2016 WHO classification system set out the grading for many types including astrocytomas [14].

Glioblastoma (GBM), a grade IV astrocytoma, is the most common aggressive malignant brain tumor. 15% of all brain tumors and nearly 50% of malignant brain cancers are glioblastomas, usually presenting with non-specific symptoms such as headaches or vomiting. Glioblastomas are considered as a disease of old age since more than half of the cases occur in patients 65 years or older. The median survival for glioblastoma is eight months with a 5-year survival rate of just over 7% [15]. Known for its resilience, glioblastoma tumors are incurable with recurrence being rapid, leading some patients to opt out for symptomatic therapy. Current management guidelines dictate maximal surgical resection, coupled with radiotherapy and chemotherapy, specifically temozolomide which was shown to increase survival to 14.6 months [16].

Glioblastoma tumors can be classified into primary and secondary tumors. 90% of all glioblastoma tumors in elderly patients are primary. These show molecular amplification of the epidermal growth factor receptor (EGFR), loss of heterozygosity of 10q, p16INK4A and phosphatase and tensin homolog (PTEN) mutations [17]. Secondary glioblastomas are seen more commonly in younger patients, with TP53 mutations occuring in those tumors. Glioblastoma can also be split into different subtypes based on the histology, neuronal stem cell markers, or isocitrate dehydrogenase (IDH) mutations [18]. Discovery of more biomarkers may also help in early interference, which will hopefully improve patients' life expectancies.

Role of XIST in Brain tumors

Besides its developmental role in X chromosome inactivation, XIST has an evident function in tumorigenesis [12,20]. Since primary malignant brain tumors have poor overall survival rates, their molecular pathogenesis is under study [21]. Like other cancers, XIST is implicated in brain tumor development in terms of proliferation, migration, invasion, angiogenesis, chemoresistance and evasion of apoptosis. XIST accomplishes this through complex interaction with various miRNAs and their downstream targets inside the cell, summarized in the following table and figure (Table 1 & Figure 3).

a. Migration and Proliferation

The knockdown of XIST suppresses cell migration and proliferation in gliomas, pituitary tumors, and neuroblastomas. Due to XIST's sponging function, several microRNAs are often inhibited from carrying out their function. Following XIST silencing, miR-133a is upregulated in gliomas, which reduces tumor migration and proliferation [22]. Cells with overexpressed miR-133a display a significant decrease in the mRNA and protein expression levels of SRY-related HMG box 4 (SOX4), a regulator in the epithelial mesenchymal transition (EMT), which is an important step in tumor metastasis. This is further reinforced by the upregulation of E-cadherin and alpha-catenin and the downregulation of N-cadherin and vimentin upon the silencing of XIST. E-cadherin is a cell-to-cell adhesion molecule whose loss in cancerous cells is one of the hallmarks of EMT [23].

XIST is also linked to the expression of chemokine receptor CXCR7, which promotes invasion and proliferation in glioma cells [24,25]. CXCR7 expression is also correlated to glioma grades, and in vivo experiments from the same study revealed that the pharmacological inhibition of CXCR7 after irradiation results in glioma regression and prolonged survival [26]. MiR-137, a tumor suppressor in endometrial and colorectal cancer, downregulates CXCR7 in gliomas, and thus reduces the proliferative abilities of the tumor [24,27,28]. Since XIST binds to miR-137 and represses it, its silencing allows miR-137 to maintain its function.

MiR-137 is downregulated in glioma cell lines, and its introduction to glioma tumor cells suppresses their proliferation and invasion [29]. Through targeting Chromosome Segregation 1 Like protein (CSE1L) in oligodendroglial tumors, it represses anchorage-independent growth and

inhibits tumor cell migration. MiR-137 also reduces COX-2 expression in gliomas, which has oncogenic properties such as enhancing tumor proliferation and migration. Its expression level is also associated with poor survival and high-grade tumors [30]. Furthermore, miR-137 downregulates Rac1, a protein that regulates cell proliferation. In the presence of XIST, miR-137 is inhibited, leading to the overexpression of Rac1. To suppress glioma cell proliferation induced by XIST, miR-137 should be overexpressed or Rac1 inhibited [31].

In glioma cell lines, a study demonstrates that miR-204-5p can directly bind to XIST and negatively regulate XIST expression [32]. When XIST is silenced and miR-204-5p is upregulated, migration and proliferation are suppressed. A separate study reported that miR-204-5p inhibits tumor migration and invasion in gliomas by regulating ezrin expression, a cell membrane protein that has several oncogenic functions, most notably invasion and migration [33].

XIST's role in migration and proliferation in glioma can also be attributed to the miR-329-3p/CREB1 axis [34]. CREB1 is usually highly expressed in glioma cells and is responsible for cell proliferation and invasion. However, when the tumor cell is transfected with miR-329-3p, CREB1 expression declines and its oncogenic properties are suppressed. To prevent the loss of invasion and proliferation, XIST acts as a tumor promoter by sponging miR-329-3p. MiR-329-3p, similar to other miRNAs, acts as a tumor suppressor in several cancers, including breast [35] and cervical cancers [36].

XIST performs a similar function in glioblastoma cells. In a study investigating the IRS1/PI3K/Akt pathway in GBM cells, XIST is found to be a competing endogenous RNA (ceRNA) of miR-126, a tumor-suppressor miRNA that is a regulator of the IRS1/PI3K/Akt signaling pathway in GBM [37]. This pathway is a signaling pathway activated upon the binding of insulin and insulin-like growth factor (IGF) to their receptors and carries out many of the hormone's metabolic functions inside the cell. The knockdown of XIST in GBM cell lines reduces migration and proliferation attributed to its sponging of miR-126 and the miRNA's regulation of the glucose metabolism pathway. Another study further reinforces the tumor-suppressing role of miR-126 in glioblastoma by highlighting its interaction with the famous proto-oncogene KRAS. By directly targeting KRAS mRNA, it suppresses the migration and proliferation capabilities of the tumor [38].

Knockdown of XIST inhibits cell proliferation, migration, and invasion in human glioblastoma stem cells through the upregulation of miR-152 [39]. When compared with controls, XIST silencing was found to significantly decrease cell proliferation as well as colony formation, with a higher frequency of cells found in the G1 and S phases rather than the G2/M phase. MiR-152 is a tumor-suppressor miRNA that is hypermethylated in gliomas, and when it is downregulated, cell proliferation is promoted [40]. Its presence, however, limits cell invasion in gliomas [41].

In neuroblastoma, XIST promotes migration and proliferation via DNA histone methylation of the Dickkopf WNT Signaling Pathway Inhibitor 1 (DKK1), a glycoprotein that has tumor-suppressive effects in neuroblastoma, including loss of proliferation, mobility, and survivability [42]. Epigenetic modification of the DKK1 gene is achieved through a third party, the EZH2 protein, to which XIST binds. Testing the effect of XIST knockdown in neuroblastoma mouse models achieved the expected results: mice had longer survival times and smaller tumor volumes.

XIST is further implicated in neuroblastoma tumorigenesis by negatively affecting miR-653-5p, downregulating it and preventing it from targeting hexokinase 2 (HK2), a serine protease that is overexpressed in neuroblastoma [43]. Increased expression of HK2 attenuates the effects of miR-653-5p on the suppression of migration and proliferation. Another study detected elevated HK2 levels in neuroblastoma metastatic variants compared to local variants, further highlighting its role in invasion [44]. XIST silencing in neuroblastoma can also lead to reduced cell cycle progression and proliferation through the upregulation of miR-375 and downregulation of L1 cell adhesion molecule (L1CAM), a membrane glycoprotein that is part of the immunoglobulin superfamily cell adhesion molecules and functions in cell-to-cell adhesion [45]. L1CAM is a target of miR-375, which XIST normally silences, and its repression can lead to increased suspension of tumor cells in G0/G1 stages of the cell cycle and reduced cell proliferation.

In pituitary neuroendocrine tumors, miR-424-5p suppresses tumor invasion and proliferation through regulating bFGF, and this effect is reversed when XIST competitively binds to miR-424-5p, leading to increased tumorigenicity of the cancer [46]. Further studies had been conducted investigating the relationship between bFGF and pituitary endocrine tumors, revealing that raised levels of bFGF are correlated with tumor size and negatively correlated with patient survival [47].

The dysregulation of XIST plays a significant role in the proliferative ability and activation of migration and invasion in gliomas, neuroblastomas, and pituitary neuroendocrine tumors. XIST achieves this through its ability to act as a molecular sponge and reduce the expression of a multitude of tumor-suppressive miRNAs, subsequently ameliorating their effect on carcinogenesis.

b. Angiogenesis

During the process of solid tumor growth, angiogenesis is required to support tumor development by providing enough nutrition, oxygen, and energy to the neoplasm [48]. Angiogenesis is the process of formation of new blood vessels and is tightly regulated by a number of stimulating as well as inhibiting factors acting on endothelial cells [49]. It is noteworthy to mention that brain tumors are highly angiogenic [50].

XIST silencing in glioblastoma cells suppresses the migration of human brain microvascular endothelial cells [51]. This is possibly linked to the finding that glioma xenograft models with XIST knockdown had significantly reduced numbers of vessels. XIST enhances glioma angiogenesis by sponging, and thus down-regulating, the oncogenic microRNA termed miR-429. Meanwhile, XIST silencing in pituitary neuroendocrine tumor upregulates miR-424-5p, which in turn decreases the expression of basic fibroblast growth factor (bFGF) [46]. bFGF is implicated in promoting tumor angiogenesis, meaning that XIST silencing possibly has an anti-angiogenic effect [52].

XIST knockdown increases blood-tumor barrier permeability and inhibits glioma angiogenesis by suppressing the transcription factors forkhead box C1 (FOXC1) and zonula occludens 2 (ZO-2) expression, primarily through the upregulation of miR-137 [24]. The above-mentioned transcription factor, FOXC1, promotes glioma angiogenesis through increasing the expression of chemokine receptor 7b (CXCR7). During states of hypoxia within human brain microvascular endothelial cells, XIST sponges miR-485-3p and inactivates its role in downregulating the expression of SOX7 [53]. The result is an activation of VEGF signalling as well as the ERK1/2 and Akt signalling pathways, all of which participate in angiogenesis.

c. Chemoresistance

Temozolomide is an alkylating agent used to treat and prevent the progression of brain tumors. It is spontaneously converted to an active methylating agent without metabolic conversion by the liver, allowing it to retain a high bioavailability [54] Additionally, its lipophilic nature permits it to cross the blood brain barrier, thus making it available to be administered orally [55]. Temozolomide methylates the N^7 and O^6 positions of guanine and the O^3 position of adenine to achieve its antitumor effect. O^6-methylguanine pairs with thymine instead of cytosine on the opposite DNA strand during replication. Since this pairing is highly cytotoxic, the DNA mismatch repair pathway is activated, and the process of apoptosis results [54].

The poor prognosis associated with brain tumors may be attributed to the increasing chemoresistance of the tumor cells. Of the many factors determining tumor response to therapy, expression of O^6-methylguanine-DNA methyltransferase (MGMT) is the most prominent [55-57]. MGMT is a DNA repair enzyme that removes the alkyl group from guanine's O^6 position and transfers it to a cysteine residue found on its own active site. Absence of MGMT, which is achieved by hypermethylation of the gene promoter, renders the tumor cells more sensitive to temozolomide when compared to tumor cells with normal levels of MGMT. Conversely, hypomethylation of the MGMT promoter, or upregulation of MGMT, is associated with resistance to temozolomide.

XIST promotes resistance to temozolomide in glioma cells by indirectly regulating the expression of MGMT [58]. When XIST is silenced, the resistant glioma cells become responsive to the treatment and the half maximal inhibitory concentration (IC50) of the drug reduces. To achieve chemoresistance, XIST acts as a molecular sponge and binds miR-29c to inhibit its function. MiR-29c belongs to the miR-29 microRNA family which suppresses DNA methylation of tumor suppressor genes. Besides its function as a tumor suppressor, miR-29c mediates chemosensitivity of the tumor cells by downregulating MGMT [59]. It does so by binding and thus inhibiting Specificity Protein 1 (SP1), a transcription factor known to bind to MGMT's gene promoter and upregulate its expression. Therefore, XIST knockdown prevents miR-29c binding, which in turn improves the response of glioma cells to temozolomide.

d. Evasion of Apoptosis

Apoptosis, programmed cell death, plays a critical role in development and homeostasis. However, dysregulated apoptosis is a feature of many diseases, including autoimmune conditions and cancers. In fact, evading apoptosis is one of the hallmarks of cancer, making it a valuable target in cancer treatment [60]. LncRNAs have been found to regulate gene expression at the epigenetic, transcriptional and post-transcriptional levels, possibly influencing cellular growth, proliferation, metabolism and even apoptosis. Hence, studies have attempted to explore and map out these regulatory networks.

XIST has been shown to inhibit the apoptosis of glioblastoma tumor cells through a variety of pathways. An early study comparing the apoptosis rates of Glioblastoma Stem Cells (GSCs) with and without XIST reports that GSCs with XIST have lower apoptosis rates. Further experiments revealed that inhibiting miR-152 could balance these rates, hinting that XIST could be exerting its effect through downregulating miR-152 [39] . More recently, a study found that XIST knockdown in U251 and U87MG glioblastoma cells, coupled with hypoxic conditions, promotes cellular apoptosis. Further experiments revealed that this effect is mediated through the miR-126-dependent IRS1/ PI3K/ Akt pathway [37]. Apoptosis was also found to be induced through the regulation of Bcl-2 expression, which occurs through the crosstalk between XIST and miR-204-5p [32]. Similarly, another study found that XIST inhibits cellular apoptosis in glioblastoma through once again sponging the effect of a microRNA, miR-329-3p in this case, leading to the overexpression of CREB1 [34].

These studies show that there could be multiple regulatory axes through which glioblastoma cells can inhibit apoptosis. An analogous result was also documented in neuroblastoma, another tumor of neural tissue, where XIST's role in modulating the miR-375/ L1CAM pathway, yet again through a similar sponging mechanism of the microRNA [45]. All of these axes could present valuable molecular targets to improve glioblastoma management and prognosis.

Table 1. MicroRNAs regulated by XIST.

Cancer Type	XIST Expression Level	XIST Target miRNA	XIST Regulation of Target miRNA	Downstream Target	Functional Impact	Ref.
Gliomas	Upregulated	miR-133a	↓	SRY-related HMG box 4 (SOX4)	↑ Migration ↑ Proliferation	(22)
		miR-137	↓	C-X-C chemokine receptor type 7 (CXCR7)	↑ Proliferation	(24,25)
			↓	CXCR7, Forkhead box C1 (FOXC1) and zonula occludens 2 (ZO-2)	↑ Angiogenesis	(24)
			↓	Rac Family Small GTPase 1 (Rac1)	↑ Proliferation	(31)
		miR-204-5p	↓	?	↑ Proliferation	(32)
			↓	B-cell lymphoma 2 (Bcl-2)	↓ Apoptosis	(32)
		miR-329-3p	↓	CAMP Responsive Element Binding Protein 1 (CREB1)	↑ Proliferation ↑ Migration ↓ Apoptosis	(34)
		miR-126	↓	IRS1/ PI3K/ Akt pathway	↑ Proliferation ↑ Migration ↓ Apoptosis	(37)

		miR-152	↓	?	↑ Proliferation ↑ Migration ↓ Apoptosis	(39)
		miR-429	↓	?	↑ Angiogenesis	(51)
		miR-485-3p	↓	SRY-Box Transcription Factor 7 (SOX7)	↑ Angiogenesis	(53)
		miR-29c	↓	Specificity Protein 1 (SP1)	↑ Chemoresistance	(58)
Neuroblastoma	Upregulated	miR-375	↓	L1 Cell Adhesion Molecule (L1CAM)	↑ Proliferation ↓ Apoptosis	(45)
		miR-653-5p	↓	Hexokinase 2 (HK2)	↑ Migration ↑ Proliferation	(43)
Pituitary Neuroendocrine Tumors	Upregulated	miR-424-5p	↓	Basic fibroblast growth factor (bFGF)	↑ Migration ↑ Proliferation ↑ Angiogenesis	(46)

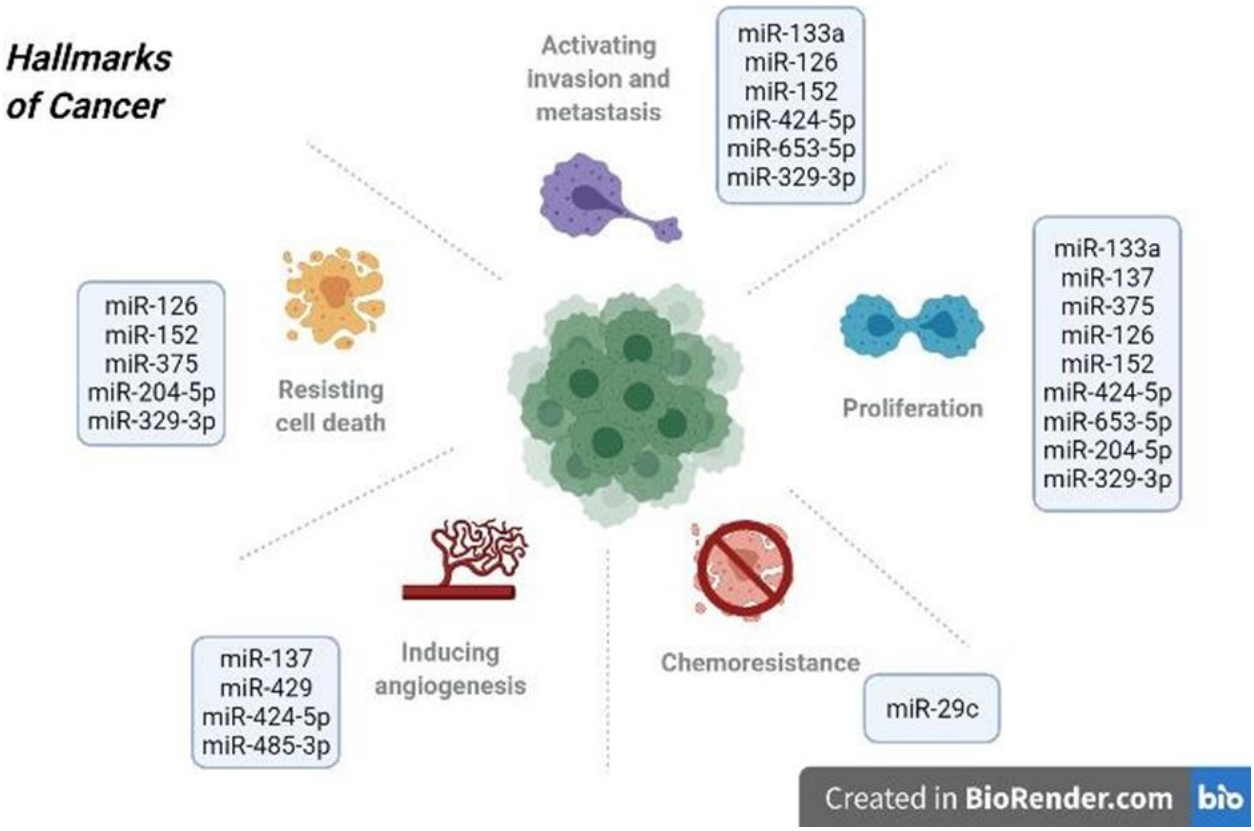

Figure 2. Hallmarks of Cancer. This figure shows the miRNAs that are sponged by XIST to promote tumor development. Created by BioRender.com

Discussion

Despite their role as major biological regulators, lncRNAs are essential contributors to tumor development and progression. They are known to be widely involved in the pathogenesis of brain tumors. XIST, one of the first lncRNAs discovered in mammals, is responsible for X chromosome inactivation, which occurs to ensure equal X-linked genetic expression between males and females. Like other lncRNAs, XIST contributes to brain tumor development in terms of proliferation, migration, angiogenesis, and chemoresistance.

Brain tumors are among the most lethal cancer types: over two thirds of adults diagnosed with glioblastoma - the most aggressive brain tumor - die within 2 years of diagnosis [61]. This is due to multiple challenges faced while managing brain tumors, the first being their location behind the blood-brain barrier (BBB), a tight mechanism made to protect neuronal cells from substances found in the circulation [62]. The downside of this is the inadequacy of many chemotherapeutic agents to cross the barrier and act on the tumor. Furthermore, brain tumors have a distinctive microenvironment, in addition to unique genetic and epigenetic features, often making them resistant to most conventional therapy [63,64]. The tumor microenvironment of glioblastoma is a heterogenous inflammatory environment, inducing mainly drug resistance and immunosuppression, hence providing pathways for tumor evasion [65]. In addition, glioblastoma is characterized by cellular and intratumoral heterogeneity, making it a particularly difficult tumor to study. The combination of all these features makes brain tumors tough to treat, and thus it is crucial to find alternative therapeutic targets.

The potential of lncRNAs as alternative therapeutic targets lies in the fact that they are easily detected in body fluids such as plasma, saliva, urine, etc [66]. In addition, their expression is selective to tumor cells, which provides specific targets in therapeutics, thus reducing the risk of adverse effects in traditional chemotherapeutic drugs. The H19 gene is a prime example of such specificity, as its product is an oncofetal lncRNA not expressed in normal adult cells [67]. Depending on the function of the lncRNA in the tumor, it would be necessary to either upregulate it or downregulate it in order to achieve the therapeutic effect desired. As the knockdown of XIST in gliomas, neuroblastomas, and pituitary neuroendocrine tumors has tumor-suppressive effects, we suggest that the goal of pharmaceutical developers should be to silence its expression.

MEG3 is one lncRNA that has shown promising results in cancer therapeutics [68]. A study done on mouse models showed that overexpression of MEG3 in glioblastoma stem-like cells (GSCs) suppresses its growth. Furthermore, they showed that the use of valproic acid, an epigenetic targeting drug used to treat seizure disorder in glioblastoma patients, increases the levels of MEG3 and results in tumor shrinkage when given *in vivo* in mice injected with GSCs. This suggests that epigenetic modifications, via epigenetic targeting drugs such as valproic acid, are a potential therapeutic target for the inhibition of tumor growth. Thus, it may be possible to develop a drug that epigenetically silences XIST in brain tumors. *In vivo* studies investigating the effect of XIST knockdown in neuroblastoma have yielded promising results, but unfortunately have not advanced to clinical stages yet [42].

Other lncRNAs such as H19 have been well-studied and their use for targeted therapy has proceeded to clinical trials. As mentioned previously, H19 is an oncofetal gene that has been linked to early tumor recurrence [67]. Researchers have taken advantage of this specificity, targeting the H19-expressing cells in the neoplasm through the DNA plasmid BC-819. BC-819 contains diphtheria toxin fragment A (DTA), a toxin secreted by *Corynebacterium diphtheriae* that inhibits protein synthesis in human cells [69]. DTA is under transcriptional control of the H19 promoter, and its expression can be initiated by H19 transcription factors present in tumor cells, thus leading to selective destruction of the tumor. In a phase 2b clinical trial, 47 patients with recurrent, multiple non-muscle invasive bladder tumors that were unresponsive to prior intravesical therapy underwent a 6-week course of BC-819 therapy. 33% of patients achieved full tumor ablation and 64% had no recurrent tumors at 3 months. The treatment was approved for a phase 3 clinical trial, which is underway.

In light of the fact that lncRNAs are linked to the pathogenesis of many tumors, various technologies have been developed to interfere with their expression. Some of the most common approaches to silence lncRNAs include small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), small molecule compounds, and the CRISPR/Cas9 system [70]. ASOs are synthetic single-stranded oligodeoxynucleotides that are capable of lncRNA knockdown through the activation of RNaseH-mediated degradation [71]. ASOs have shown promising results in hepatocellular carcinoma, where chemoresistance of the cancerous cells was reduced by the introduction of Floxuridine-integrated ASO with anti-Bcl-2 [72]. ASOs show numerous

advantages, most notably that the FDA has approved their use in various non-cancerous diseases [73]. Although these technologies have not yet been widely used to target XIST in gliomas, we propose that such a technology can have promising results.

Biomarkers are an essential component of personalized medicine. Each biomarker's effect on survival can informally be visualized as the sum of the biomarker's prognostic and predictive effects [74]. Given the uniqueness of gliomas, biomarkers' roles are also expected to exhibit distinct patterns of effects. For example, there is currently no routine use of serum biomarkers for glioblastoma for follow-up [75]. In fact, single molecule markers may not be able to sufficiently follow dynamics of diseases like cancer, leading some to begin delineating multiple marker profiles.

Only recently have studies begun thoroughly exploring lncRNAs as potential tumor biomarkers. XIST has been shown to have potential multiple roles in a variety of cancers. A study by Salama et al. highlighted the role that XIST can play as a non-invasive immune biomarker for breast cancer [76]. A different study by Lan et al. even looked at the role that serum exosomal XIST can play in breast cancer recurrence [77]. On the other hand, Xu et al. showed how XIST can be a biomarker for cisplatin chemosensitivity in non-small cell lung cancer, helping design treatment regimens [78]. Deng et al. pursued a different approach and delineated a role in tumor staging as XIST levels were significantly associated with lymphatic and distant metastases [79]. These represent but a select few roles that have been discovered; a more detailed discussion can be found in Wang et al. [80].

Given XIST's role that has been elucidated in other tumors [80,81] and its involvement in a plethora of pathways, it could have a role in a multiple marker profile for glioma prognosis. Such multi-marker panels may help patients avoid biopsy and have a role in treatment individualization, prognosis stratification, and response prediction. A recent GBM panel was proposed by Wang et al. involving CD44, ABCC3, TNFRSF1A, and MGMT which can help classify GBM patients into five strata, each with a different survival time and treatment response [82]. Some panels involving XIST have been proposed for other tumors such as a lung cancer multi-panel looking at XIST with TSIX, hnRNPu, Bcl-2, and BRCA1 [83]. Additional lab and clinical studies would need to be done to evaluate the links between XIST and clinical outcomes for gliomas as part of such a panel.

Besides brain tumors, XIST also plays an oncogenic role in other cancers. XIST is highly upregulated in various cell lines of non-small cell lung cancer [84-86], causing proliferation of cancer cells through promoting iASPP, an oncogenic inhibitor of p53 [87]. High levels of XIST are also reported in breast cancer [88], specifically in doxorubicin-resistant breast cancer cells [89]. Furthermore, XIST is shown to exert an oncogenic role in bladder cancer, by binding to different microRNAs such as miR-124, miR-133a, miR139-5p and miR335 [90,91].

In spite of its overwhelming oncogenic role, XIST has been shown to act as a tumor-suppressor in various other tumors. In ovarian cancer, XIST sponges miR-106a, inhibiting cell proliferation and inversely promoting apoptosis in multiple cell lines *in vitro* [92]. The results were further fortified in mice xenografts, where XIST significantly reduced tumor size in ovarian cancer. Similar conclusions were drawn in other cancer cell lines, including prostate cancer, renal cell carcinoma, and oral squamous cell carcinoma, where XIST's interaction with various microRNAs resulted in tumor suppression [93-95]. In a clinical setting, low expression of XIST in prostate cancer patients was correlated with poor clinical features, such as metastasis, advanced clinical stage, higher preoperative PSA levels, and shorter survival times [95]. Thus, the abnormal expression of XIST can lead to complex outcomes that warrant further study.

Apart from cancer, XIST also modulates several other neuropathological processes. In Parkinson's disease, XIST inhibits miR-199a-3p to regulate Sp1 expression as well as promote disease progression by targeting LRRK2 [96]. Furthermore, the downregulation of XIST reduces ischemia/reperfusion-induced neurological impairment via the miR-362/ROCK2 axis, which promotes neuronal proliferation [97]. On the other hand, by mediating miR-92a/Itgα5 or KLF4 axis, XIST lessens cerebral vascular injury following ischemic stroke [98]. Finally, XIST silencing was found to limit neuronal apoptosis following spinal cord injury by blocking the PTEN/PI3K/AKT signaling pathway [99].

As summarized in this discussion, the role of XIST in various pathologies was shown to either lead to disease progression or suppression. The focus of our paper is on XIST's function in the tumorigenesis of brain cancers, especially glioblastoma, which has an unfavorable outcome in terms of management and curability. XIST's ability to act as a potential therapeutic target and

biomarker in brain tumors remains understudied, and we believe that targeting it alongside its associated microRNAs can yield promising results.

Contribution

SE, KS, and SA: manuscript design. SE, KS, SA, RQ, AAO, AHO, OH: literature collection and summary. SE, KS, SA, RQ, AAO, AHO, OH: drafting of manuscript. SE, KS, RQ, AAO: figure drawing. SA, SE, KS: revising of the manuscript. All authors have read and approved the final submitted manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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