INVOLVEMENT OF LONG NON-CODING RNAS IN GLIOBLASTOMA AND RESEARCH APPROACHES AND TECHNIQUES TO STUDY LNCRNAS IN TUMORS: A REVIEW

O. BOUCHRA[1], A. ESSALIHI[1], K. KHADIRI[2], Z. KHADROUF[2], M. KARKOURI[1,3]

• • •

[1]Laboratory of Cellular and Molecular Pathology, Faculty of Medicine and Pharmacy,
Hassan II University of Casablanca, Casablanca, Morocco
[2]Laboratory of Immunology and Biodiversity, Faculty of Sciences, Ain Chock,
Hassan II University of Casablanca, Casablanca, Morocco
[3]Pathology Department, University Hospital Ibn Rochd, Casablanca, Morocco

CORRESPONDING AUTHOR
Oumaima Bouchra, PhD; e-mail: oumaimabouchra@gmail.com

ABSTRACT – Glioblastoma is recognized as the most aggressive tumor of the central nervous system, accounting for the majority of gliomas (57.3%) and approximately 15% of all brain tumors. Additionally, it represents 48.3% of primary malignant intracranial tumors. Overall survival extension among patients with glioblastoma remains challenging despite improvements in post-surgical treatments, including chemotherapy and radiotherapy. Consequently, the development of novel therapeutic approaches specifically targeting glioma is of critical clinical relevance. Long non-coding RNAs, a class of non-coding RNAs longer than 200 nucleotides (nt), once considered as "noise" of transcription, are now acknowledged to play a critical role in a variety of molecular processes at the transcriptional, post-transcriptional, and epigenetic levels, as well as in numerous physiological and pathological processes. A dysregulation of lncRNAs in glioma affects and alters many signaling pathways. A significant number of these lncRNAs have been discovered due to advances in high-throughput sequencing technology, which has also revealed their strong correlation with tumor development and progression, as well as their interaction with microRNAs. Nowadays, lncRNAs are thought to be promising targets for diagnostic, prognostic, and therapeutic purposes. In this review, we will describe some lncRNAs and detail their classification and cellular localization, as well as their mechanisms of action and how they affect and alter many signaling pathways in glioblastoma. We will also discuss several research strategies for identifying and characterizing lncRNAs in tumors.

KEYWORDS: Bioinformatic tools, Glioma, Glioblastoma, lncRNAs, Molecular pathways, Research approaches, Signaling pathways, Therapeutic targets, Transcription regulation.

INTRODUCTION

Glioblastoma (GBM) constitutes the majority of gliomas (57.3%), approximately 15% of brain tumors, and 48.3% of primary malignant intracranial tumors[1]. Classification based solely on histology, patient age, tumor location, and size has failed to predict prognosis in GBM patients[2]. While these variables are recognized as markers of survival and treatment outcomes among patients, none have reliably fore-casted outcomes precisely[3]. Furthermore, overall survival (OS) extension in GBM patients has remained elusive despite advancements in post-surgical treatments such as chemotherapy with temozolomide (TMZ) and radiotherapy[4]. GBM cases with similar clinical and histological characteristics exhibit varying survival rates[5]. During carcinogenesis, normal cells acquire genetic and epigenetic changes, including DNA methylation, histone modification, and regulation by non-coding RNA (ncRNAs), a recently iden-tified mechanism that has become the focus of considerable studies due to its involvement in tumor development and progression. Over the past decade, a significant number of these ncRNAs have been discovered through the application of next-generation sequencing (NGS) technology[6-9].

It has been estimated that approximately 97% of the human genome is composed of ncRNAs [7] which are not translated into proteins and can be classified as housekeeping ncRNAs or regulatory ncRNAs based on their length[8,10]. Short ncRNAs also referred to as sncRNAs include small interfering RNAs (siR-NAs), piwi-interacting RNAs (piRNAs), tRNA-derived fragments (tRFs), small nucleolar RNAs (snoRNAs), and small nuclear ribonucleic acid (snRNAs), all containing 200 nt or fewer (<200 nt)[11,12]. Long non-cod-ing RNAs (lncRNAs) are longer than 200 nt (>200 nt) and represent the largest class with over 60,000 genes in the human genome. Many ncRNAs are tissue-specific and cancer-specific, forming a complex network of reciprocal interconnections[13,14]. Moreover, they can impact the fate and survival of can-cer cells through mechanisms such as chromatin remodeling, signal transduction, transcriptional, and post-transcriptional modifications. Some function as tumor suppressors, while others act as oncogenes. These ncRNAs have been recognized as potential targets for diagnostic, therapeutic, and prognostic applications[7].

Briefly, microRNAs (miRNAs), approximately 20–22 nt in length, are highly conserved across species. They regulate gene expression by binding to the 3'-untranslated region (3'-UTR) of target mRNAs[15]. Con-sidering their structure, miRNAs lack the poly(A) tail typically found in mRNAs[7]. An increasing number of miRNAs have been demonstrated to play crucial roles in glioma, as confirmed by various studies[16-18].

LncRNAs, as described above, are a class of non-protein-coding transcripts whose lengths vary from 200 nt to 100 kilobases (kb)[19]. They are considered regulatory RNA transcripts[20], encoded by 4–9% of the whole genome, and more abundant than mRNAs in mammals[21]. LncRNAs share several structural similarities with mRNAs, including size, promoter structure, RNA polymerase II transcription, and splic-ing. Additionally, many lncRNAs have a poly(A) tail at the 3' end and a 7 mG cap at the 5' end, similar to mRNAs. However, unlike mRNAs, lncRNAs do not encode proteins[22-24]. On average, lncRNAs consist of nearly 2.8 exons, fewer than those found in protein-coding genes. Unlike mRNAs, lncRNAs lack an open reading frame (ORF)[25,26]. While previously claimed as 'transcriptional noise', lncRNAs are now recognized for their involvement in various molecular processes, such as transcriptional regulation, chromatin mod-ification, and post-transcriptional regulation[24,27,28]. Moreover, they play crucial roles in physiological and pathological processes, including apoptosis, metabolism, differentiation, cell growth, and proliferation, and are involved in the tumor microenvironment[24,29]. According to a previous study, approximately 18% of lncRNAs are linked to human tumors, while less than 10% of human protein-coding genes are asso-ciated with human malignancies[29]. Numerous studies have demonstrated that aberrant lncRNA expres-sion is implicated in cancer development and progression, influencing cell survival, growth, invasion, metastasis, and tumor angiogenesis. Another study focused on profiling the expression of lncRNAs in glioma has confirmed their significant role in pathogenesis and tumor development and progression[30]. These lncRNAs influence cell growth and metastasis[31], suggesting their involvement in glioma initia-tion and progression[32]. Accordingly, lncRNAs are considered potential biomarkers for glioma diagnosis, prognostic, and targeted therapy[19]. LNCipedia, one of the largest databases for lncRNAs, currently com-piles 127,802 transcripts from 56,946 lncRNA genes[26]. Furthermore, studies have found that 40% of the thousands of lncRNA genes (around 4000–20,000) identified by GENCODE and ENCODE projects are specifically expressed in the brain[33].

In this review, we will provide an overview of the types of lncRNAs and briefly discuss their biogen-esis. We will also summarize the latest findings regarding the involvement of lncRNAs in GBM, their underlying mechanisms, as well as their potential use as cancer biomarkers, prognostic indicators, and therapeutic targets. Additionally, we will outline the interaction between miRNAs and lncRNAs in GBM, highlighting current research approaches for studying lncRNAs.

LOCALIZATION AND BIOGENESIS OF LNCRNAS

LncRNAs are divided into nuclear and cytoplasmic subgroups and their activity depends on their localization[26,34]. LncRNAs located in the nucleus are primarily implicated in transcriptional and epigenetic regulation by recruiting chromatin remodeling and modification complexes. In contrast, lncRNAs exported to the cytoplasm participate in post-transcriptional regulation, affecting protein translation, mRNA stability, and the competitive endogenous RNA (ceRNA) network[9].

According to several studies, lncRNAs are categorized into five groups depending on their genomic location and chromosomal position: Intronic lncRNAs, which are generated from the antisense strand of protein-coding genes; Intergenic lncRNAs, which originate from intergenic regions; Sense lncRNAs, derived from the coding strand and can embody the whole sequence of a protein-coding gene or overlap with only a subset of it; Antisense lncRNAs, transcribed from the reverse direction of mRNA; and Bidirectional lncRNAs, transcribed from adjacent transcription start sites either in sense or antisense direction[19,35] (Figure 1). Accounting for approximately 98-99% of the human genome, non-coding regions are interposed between coding regions[35]. Many studies have demonstrated a high conservation of long intergenic non-coding RNAs (lincRNAs) within species. These lincRNAs exhibit expression characteristics similar to protein-coding genes, such as the presence of histone H3K36 trimethylation in the gene body and histone H3K4 trimethylation at their 5' end, known as the "K4-K36" domain, indicating active transcription. Studies have shown that more than 70% of lincRNAs containing the K4-K36 domain exhibit signs of active transcription, a percentage comparable to that found in protein-coding genes (approximately 72%)[36]. Furthermore, lincRNAs demonstrate higher conservation compared to antisense and intronic transcripts and are more stable than intronic lncRNAs. It's interesting to highlight that some lncRNAs can act as RNA and protein-coding genes at the same time. An example is SRA (steroid receptor RNA activator), which not only translates into a protein but also serves as a scaffold for forming complexes with several co-activator and repressor proteins, thereby regulating gene transcription. To date, lincRNAs and antisense lncRNAs are considerably more documented and known than intronic and sense lncRNAs[35].
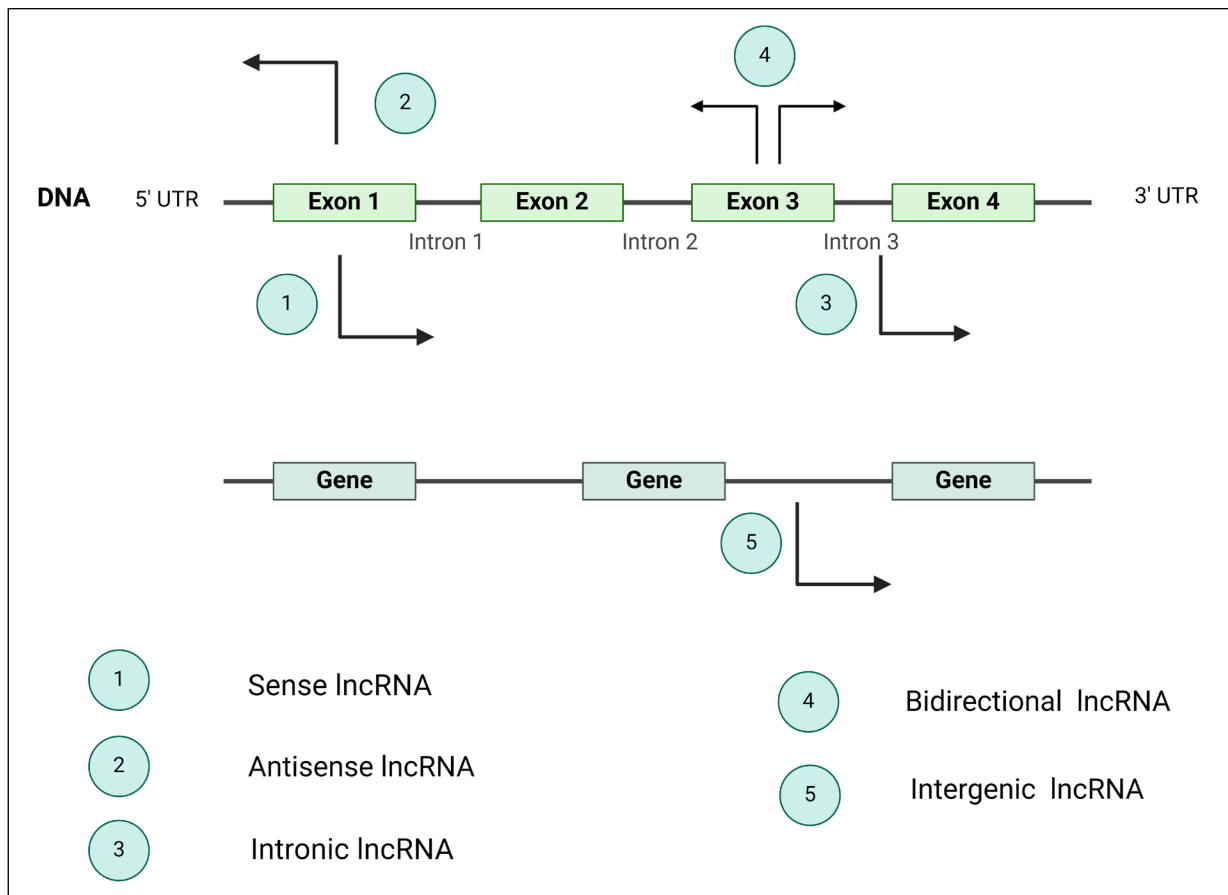

Figure 1. Genomic locations of Long non-coding RNAs (LncRNAs). (Created with BioRender.com)

As previously mentioned, chromatin-localized lncRNAs within the nucleus play significant roles in transcriptional regulation. They act on DNA sequences in a cis manner, thereby regulating gene expression in proximity or in a trans manner, regulating distant gene expression. LncRNAs modulate biological pathways through various mechanisms at the genomic, transcriptional, and post-transcriptional levels [7]. Based on their modes of gene expression regulation, lncRNAs can be categorized into four principal types: signal, decoy, guide, and scaffold[37-39].

LNCRNAS AS SIGNAL MOLECULES

LncRNAs acting as signal molecules are assumed to regulate downstream gene transcription[9]. They impact the transcription of target genes by binding to the promoter and blocking the preinitiation complex (PIC) formation or by interacting with transcription factors. These lncRNAs can function independently or via interacting with proteins such as transcription factors[35]. They can target genes located nearby (cis-regulation) or further away (trans-regulation)[40]. To negatively regulate gene expression, some cis-lncRNAs act via chromatin modification, recruiting chromatin modification complexes, such as the polycomb repressive complex (PRC) or Rpd3 small histone deacetylase complexes (Rpd3S HDAC), as well as other chromatin modifiers. Additionally, some lncRNAs contribute to positive regulation. For example, the HOXA transcript at the distal tip (HOTTIP) recruits Mixed-lineage leukemia (MLL) chromatin-modifying complex to maintain an active domain over the 5' end of the homeobox A gene cluster (HOXA). When acting in trans, lncRNAs may bind to chromatin modification complexes, transcription elongation factors, or RNA polymerases independently of sequence complementarity to modify transcription[35].

LNCRNAS AS DECOY MOLECULES

LncRNA often acts as a decoy molecule by blocking specific molecular pathways. They serve as molecular decoys by binding to specific DNA sequences, thereby inhibiting the interaction of functional protein molecules such as transcription factors and chromatin remodeling proteins with their DNA or mRNA targets. The restriction of transcription factor activity results in the suppression of downstream gene transcription. In some specific tissues and certain tumor cells, lncRNAs also compete with mRNA molecules to block the inhibitory effect of miRNA on mRNA, thereby upregulating the expression of target genes[9].

LNCRNAS AS GUIDE MOLECULES

LncRNAs can act as guide molecules by aiding specific proteins to reach their target locations and exert their biological functions[9]. Alternatively, they can directly bind to proteins and induce their relocation throughout the genome, thereby influencing gene expression. The regulation of gene expression by lncRNAs involves both repressive and activating complexes, often interacting with transcription factors[41].

LNCRNAS AS SCAFFOLD MOLECULES

LncRNA can function as a "central platform" facilitating interactions among various molecules and proteins. Furthermore, their scaffold properties enable the assembly of distinct macromolecular complexes, thereby integrating information across diverse signaling pathways[41]. LncRNAs may also modulate gene expression by base pairing with translation factors or ribosomes to regulate translation or by binding to splicing factors to influence splicing[19].

MECHANISMS OF ACTION OF LNCRNAS IN GLIOBLASTOMA

LncRNAs are classified into oncogenes and tumor suppressors. In this section, we will highlight the most studied lncRNAs. **Table 1** summarizes the lncRNAs discussed in this review, while **Table 2** lists other dysregulated lncRNAs implicated in GBM.

Table 1. A summary of the main dysregulated lncRNAs in glioma.

Dysregulated lncRNAs	
Oncogenes	HOTAIR
	HOTAIRM1
	HOTTIP
	NEAT1
	MALAT1
	H19
	CCAT2
	XIST
	ANRIL
	MEG3
	DANCR
	HULC
	CRNDE
	SNHG5
	PlncRNA-1
	Linc-OIP5
Oncosuppressors	BCYRN1
	MEG3
	MALAT1
	MATN1-AS1
	CASC2

Table 2. Other lncRNAs dysregulated on glioma.

Other lncRNAs dysregulated on glioma			
LncRNAs	**Expression**	**Mechanism**	**Reference**
TUG1	Upregulated	Recruits polycomb to methylate H3K27 histone	126
		Inhibits miR-144, and reverse its effect on occludini, ZO-1, and claudin-5	127
ASLNC22381 ASLNC20819	Upregulated	Target growth factor-related IGF-1 pathway	128
NNT-AS1	Upregulated	miR-494-3p/ PRMT1 axis	129
TUNAR	Downregulated	miR-200a–Rac1 axis	130
ECONEXIN	Upregulated	Regulates TOP2A by sponging miR-411-5p	131
ADAMTS9-AS2	Upregulated	FUS/MDM2 axis	132
AGAP2-AS1	Upregulated	TFPI2/EZH2-LSD1	133
UCA1	Upregulated	miR-182-5p/MGMT axis	134
PVT	Upregulated	miR-128-3p/GREM1	135
TUSC7	Downregulated	Sponging miR-23b	136
RAMP2-AS1	Downregulated	DHC10/NOTCH3/HES1 pathway	137
BDNF-AS	Downregulated	ADAR/p53	138
SOX2OT	Upregulated	upregulates ALKBH5	139
LOC441204	Upregulated	ß -catenin/p21/cdk4 cascade	140
ZEB1-AS1	Upregulated	miR-200c/141-ZEB1 axis	141
Linc-ROR	Downregulated	May modulate Krüppel-like factor 4 (KLF4)/ c-MYC pathway	142
Linc-p21	Downregulated	Scaffold with SETDB1 and DNMT1	143
LINC00657	Downregulated	Target of miR-190a-3p	144
RNCR3	Downregulated	Target KLF16/ miR-185-5p	145

ONCOGENES

HOX Transcript Antisense Intergenic RNA (HOTAIR)

HOTAIR, a ~2.2-kb lncRNA, belongs to the Homeobox superfamily (HOX) and is transcribed from the Homeobox C (HOXC) locus[42,43]. This lncRNA is located on chromosome 12q13, and its higher expression is correlated with tumor progression, invasion, metastasis, and poor prognosis in various cancers, including GBM[26,44]. Studies have demonstrated that HOTAIR interferes with cell cycle progression and is associated with glioma patient survival, serving as a biomarker that defines molecular subtypes of glioma[45]. HOTAIR interacts with the polycomb repressive complex 2 (PRC2), facilitating cell cycle progression[46]. Moreover, HOTAIR is regulated by the glioblastoma proliferative pathway, primarily through interactions with bromodomain proteins, such as bromodomain-containing 4 (BRD4), which binds to HOTAIR and increases its levels[43]. Recent reports suggest that HOTAIR can also affect histone methylation, thereby inducing target gene silencing[26]. In a study, the knockdown of HOTAIR in cell lines inhibited proliferation, invasion, and migration while inducing cell cycle arrest in the G0/G1 phase and apoptosis. These findings suggest that knocking down HOTAIR exerts tumor-suppressive effects in human glioma cells, thereby supporting the evidence for its oncogenic role in glioma[42]. HOTAIR has been reported to mediate proliferation in GBM cells through the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) and Wnt/b-catenin pathways[26]. Another study demonstrated that HOTAIR knockdown inhibits the expression of fibroblast growth factor 1 (FGF1) and suppresses the activity of mitogen-activated protein kinase 1/2 (MEK1/2) and phosphatidylinositol 3-kinase (PI3K) /Akt pathways by upregulating miR326, which targets FGF1. This knockdown induces cell cycle arrest and apoptosis, while suppressing cell proliferation, invasion, and migration in human glioma cells[42,43].

Additionally, HOTAIR expression correlates with angiogenesis through upregulating vascular endothelial growth factor A (VEGFA) expression and transporting VEGFA to endothelial cells via extracellular vesicles released from glioma cells[47]. HOTAIR promotes glioma progression by modulating glutaminase levels through sponging miR-126-5p as a ceRNA[48]. Knockdown of HOTAIR using miR-148b-3p as a target RNA increases the permeability of the blood-tumor barrier (BTB) and reduces levels of tight junction-related proteins, including zonula occludens 1 (ZO-1), occludin and claudin-5, by specifically targeting the stimulatory factor 1 (USF1), thereby improving drug delivery[43]. Higher serum levels of exosomal HOTAIR were found in patients with recurrent GBM, correlating with poor responses to TMZ and worse outcomes[26].

HOTAIR Myeloid-Specific 1 (HOTAIRM1)

HOTAIRM1, positioned between the loci Homeobox A1 (HOXA1) and Homeobox A2 (HOXA2), was initially discovered as a myeloid-specific regulator of the HOXA gene family. Commonly expressed in myeloid lineage cells, HOTAIRM1 modulates the transcription of specific genes through chromosome remodeling during myeloid cell differentiation and maturation. This lncRNA is highly expressed in high-grade astrocytomas and is positively associated with the development and progression of gliomas[26,33]. In GBM, HOTAIRM1 is highly expressed and promotes tumor cell proliferation, migration, and invasion through various mechanisms[33]. *In vivo*, experiments from one study indicated that silencing HOTAIRM1 reduced tumor growth by modulating HOXA1 gene expression. HOTAIRM1 promotes histone H3K9 and H3K27 demethylation and decreases DNA methylation levels via displacing epigenetic modifiers G9a and EZH2 away from the HOXA1 transcription starting point[49]. Capture-C analysis has revealed that HOTAIRM1 allows DNA looping between the HOTAIRM1 locus and the HOXA genes. These findings collectively support HOTAIRM1's role as an enhancer for transcriptional activation[33,50]. In another study, silencing HOTAIRM1 suppressed glioma proliferation, epithelial-to-mesenchymal transition (EMT), cell migration, and invasion, and increased sensitivity to TMZ *in vitro*. Moreover, lncRNA HOTAIRM1 was reported to act as a sponge for miR-129-5p and miR-495-3p to induce invasion and migration in glioma cell lines. Furthermore, enhanced HOTAIRM1 expression was linked to higher inflammation and activation of a T cell-mediated immune response[26].

HOTTIP

HOTTIP, situated at the terminus of the HOXA gene family[26], shows decreased expression in gliomas. However, *in vitro* studies have demonstrated that its overexpression suppresses proliferation and induces apoptosis. A negative correlation has been observed between HOTTIP and Brain and Reproductive Organ Expressed (BRE) which is a BRCA1-A complex subunit[41,51]. BRE is important in preventing both

replicative and DNA damage-induced premature senescence[52]. Furthermore, decreased BRE expression led to a downregulation of CCNA (cyclin A) and CDK2 expression, while simultaneously increasing the expression of p53[41,51]. A further study indicated that knocking down HOTTIP inhibits hypoxia-induced EMT in glioma cells by increasing miR-101 levels, thereby suppressing metastasis through regulating Zinc finger E-box-binding homeobox 1 (ZEB1) expression[26]. Therefore, HOTTIP is involved in EMT and metastasis in glioma by targeting miR-101, thus inducing downregulation of ZEB1 expression. HOTTIP is highly expressed in metastatic glioma tissues compared to non-metastatic glioma tissues[41,53]. According to these findings, HOTTIP might serve as a relevant biomarker and potential therapeutic target in GBM[26,53].

NUCLEAR ENRICHED ABUNDANT TRANSCRIPT 1 (NEAT1)

NEAT1 is a 3.7 kb long nuclear lncRNA located on chromosome 11, transcribed from the multiple endocrine neoplasia gene (MEN1)[54]. During GBM carcinogenesis and TMZ resistance, NEAT1 expression is upregulated, promoting proliferation, migration, invasion, and inhibiting apoptosis. Its expression is modulated by EGFR pathway activity via Signal transducer and activator of transcription 3 (STAT3) and Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling pathways[33,43,55]. Furthermore, NEAT1 promotes GBM cell growth and invasion by enhancing β-catenin nuclear transport and reducing Inhibitor of β-Catenin and TCF (ICAT), Glycogen Synthase Kinase 3 Beta (GSK3B), and Axin levels through binding to EZH2 which mediate the H3K27 trimethylation at their promoters. NEAT1 contributes to therapeutic resistance by affecting β-catenin activity. Conversely, TMZ induces an increase in NEAT1 levels through the expression of the High Mobility Group Box 1 (HMGB1) protein, which subsequently activates the Toll-like Receptor 2 (TLR2) signaling pathway[43,55]. The last evidence showed that the downregulation of miR-370-3p in GBM contributes to increased NEAT1 expression, which suggests that the increased expression of NEAT1 occurred by reducing its inhibition[33,56].

METASTASIS-ASSOCIATED LUNG ADENOCARCINOMA TRANSCRIPT 1 (MALAT1)

MALAT1, also known as nuclear-enriched transcript 2 (NEAT2), is located on chromosome 11q13.1[26] and is transcribed from the MALAT1 gene[23,54]. MALAT1 generates approximately 8000 nucleotide transcripts that predominantly reside within the nucleus[23]. This lncRNA acts as a transcriptional regulator and forms molecular scaffolds for ribonucleoprotein (RNP) complexes[18]. It also binds to other RNAs or proteins (e.g., miRNAs, transcription factors, or RNA-binding proteins) and interferes with their function[23]. It was reported that MALAT1 is involved in cell cycle progression, cell migration, and cancer metastasis[18]. MALAT1 acts as a transcriptional repressor via binding to miR-199a as a ceRNA. It also increases zinc fingers and homeoboxes 1 (ZHX1) levels, known to induce carcinogenesis in several cancers[57]. In addition, MALAT1 upregulates ras-associated protein-1 b (Rap1b), stathmin 1 (STMN1), RAB5A, and autophagy-related protein 4D (ATG4D) levels by binding miR-101, thereby promoting glioma progression[58]. Furthermore, MALAT1 induces chemoresistance to TMZ in glioblastoma cells via inhibiting miR-101[59] and miR-203[60]. Wnt inhibitory factor 1 (WIF1) reduces non-canonical Wnt/β-catenin signaling via downregulating MALAT1, thus inhibiting glioblastoma cell migration[43,61]. Besides that, a repression of miR-155 and a rise in F-box and WD repeat domain-containing 7 (FBXW7) function, leads to a decrease in the expression of several oncoproteins in glioma cells[43,62]. Inversely, in a recent study, they found that MALAT1 expression was lower in GBM tissues than in normal brain tissues. This is consistent with another study where MALAT1 was found to suppress both growth and cell invasion. They also proposed that MALAT1 functions through the ERK/MAPK (extracellular signal-regulated kinase/mitogen-activated protein kinase) signaling pathway, resulting in the upregulation of MMP2 (matrix metalloproteinase 2) and a decrease in Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) expression, thereby inactivating this signaling cascade[63]. However, available evidence highlights the conflicting roles of MALAT1, raising questions about whether it acts as an oncogene or a tumor suppressor[23].

H19 IMPRINTED MATERNALLY EXPRESSED TRANSCRIPT

H19 is a 2.3 kb long lncRNA located on chromosome 11p15.5 downstream of insulin growth factor 2 (IGF2)[64]. H19 has a remarkably high expression in several cancers. In gliomas, H19 is implicated in cell growth, and invasion[65], and correlates with glioma grade[66]. H19 is also involved in the regulation of glioma vascular endothelial cells[67]. High expression of H19 is associated with improved survival in GBM patients[26].

Furthermore, H19 targets miR-342 to induce Wnt5a expression, thereby promoting the Wnt5a/β-catenin signaling pathway in glioma cells. Knockdown of H19 or overexpression of miR-342 inhibits glioma cell proliferation, migration, and angiogenesis[68]. H19 has also been reported to bind to the c-Myc gene inducing tumorigenesis. *In vitro* studies indicate that H19 promotes resistance to TMZ in glioma cell lines[26]. Moreover, H19 stimulates endothelial cell proliferation, migration, and tube formation through the H19-miR29a-Vasohibin 2 (VASH2) axis[18,67]. H19 modulates glioma angiogenesis and progression via inhibiting miR-29a, which normally targets the angiogenic factor VASH2[67,68]. Additionally, H19 targets hypoxia-inducible factor 1 subunit alpha (HIF-1α) and regulates VEGF expression by acting as a ceRNA on miR-138[43,69].

COLON CANCER-ASSOCIATED TRANSCRIPT 2 (CCAT2)

CCAT2 is a 1752-bp lncRNA[70], located in the 8q24 gene, initially identified in colorectal cancer. A study has shown that CCAT2 is highly expressed in glioma tissues and correlates positively with advanced tumor stages. Silencing CCAT2 has been shown to inhibit glioma cell proliferation and tumorigenesis[71]. Knockdown of CCAT2 leads to an increase in miR-424 levels and a decrease in VEGFA expression, thereby suppressing glioma cell proliferation and angiogenesis and inducing apoptosis. CCAT2 functions as a sponge for miR-424, promoting VEGFA expression and stimulating glioma progression through activation of the PI3K /AKT signaling pathway[72].

X-INACTIVE SPECIFIC TRANSCRIPT (XIST)

XIST is located on chromosome Xq13.2, specifically within the X chromosome inactivation center (XIC), and comprises sequences ranging from 15,000 to 20,000 nt[73]. It plays a crucial role in the transcriptional silencing of one X chromosome in female mammals during development[74]. XIST is also implicated in tumorigenicity and angiogenesis in glioma by sponging miR-429[75]. Additionally, XIST targets miR-133a, reducing its expression level in glioma through the XIST/miR-133a/ SRY-box transcription factor 4 (SOX4) axis. MiR-133a, in turn, directly binds to the 3' UTR of SOX4, a known transcription factor related to EMT. SOX4 is upregulated in glioblastoma and is associated with tumor progression and poor prognosis. Moreover, *in vitro*, the knockdown of XIST resulted in reduced expression of SOX4, leading to decreased proliferation, migration, and invasion. This knockdown also increased levels of E-cadherin and α-catenin while reducing levels of N-cadherin and vimentin[29].

Another study revealed that XIST knockout attenuates cell growth in glioblastoma cells and reduces glucose uptake by binding to miR-126 through the insulin receptor substrate 1 (IRSI)/ PI3K/Akt pathway[76].

ANTISENSE NON-CODING RNA IN THE INK4 LOCUS (ANRIL)

ANRIL is a 3.8-kb RNA transcribed from the opposite direction of the Inhibitor of Cyclin-Dependent Kinase 4a - Alternative Reading Frame - Inhibitor of Cyclin-Dependent Kinase 4b cluster (INK4b-ARF-INK4a) in the short arm of chromosome 9p21.3. ANRIL also shares a bidirectional promoter with p14ARF gene[77] and is considerably upregulated in GBM. Studies have revealed that homozygous deletion of the INK4b-ARF-INK4a locus in GBM leads to a co-deletion of ANRIL. Conversely, GBM cases without copy number alterations in the INK4b-ARF-INK4a locus present an upregulation of all genes within the locus, including ANRIL, CDKN2A, and CDKN2B. This evidence indicates that in the absence of ANRIL, the INK4b-ARF-INK4a locus is repressed in GBM[78].

SUPPRESSORS LNCRNAS

Brain Cytoplasmic RNA 1 (BCYRN1)

BCYRN1 also known as BC200, is a sense-transcribed lncRNA located on chromosome 2 between the calmodulin 2 gene (CALM2) and the epithelial cellular adhesion molecule (EPCAM) gene[79]. *In vitro* studies have demonstrated that BCYRN1 affects apoptosis, proliferation, migration, and invasion and suppresses tumor formation *in vivo*. It has been reported that BCYRN1 acts as a competitive sponge for miR-619-5p, thereby modulating glioma progression through inactivation of the CUE domain containing 2

(CUEDC2)/ phosphatase and tensin homolog (PTEN)/AKT/p21 pathway. Both knockdown of BCYRN1 and overexpression of miR-619-5p resulted in the activation of the AKT signaling pathway, increased P-AKT protein expression, and decreased PTEN and P21 expression via CUEDC2 downregulation. Conversely, BCYRN1 knockdown promoted cell growth, colony formation, and migration, while inhibiting apoptosis both *in vitro* and *in vivo*. Furthermore, BCYRN1 expression was reported to be lower in glioma tissue and significantly decreased in more advanced glioma grades. Decreased BCYRN1 expression correlates with poor prognosis[18].

MATERNALLY EXPRESSED GENE 3 (MEG3)

MEG3 is located on chromosome 14q32.3 and spans approximately 1.7 kb of length[80]. MEG3 activates both p53-dependent and independent pathways and is considered a tumor suppressor in glioma[44]. Overexpression of MEG3 increases p53 levels through downregulating murine double minute 2 (MDM2)[81]. MEG3 inhibits GBM cell growth by sequestering miR-19a and miR-93. Additionally, elevated expression of MEG3 along with Wnt/β-catenin in glioma cells may suppress cell growth and metastasis[26]. Other studies have shown that MEG3 overexpression results in cell death, suppresses proliferation, and is downregulated in glioma cell lines[82]. In GBM, hypermethylation with DNA methyltransferase 1 (DNMT1), resulted in a complete gene silencing[83]. Conversely, the interaction of MEG3 with miR-19a, suppresses PTEN expression, promoting glioma cell proliferation, migration, and invasion[41,84].

CANCER SUSCEPTIBILITY CANDIDATE 2 (CASC2)

CASC2 is located on chromosome 10 and is considered a tumor suppressor in various human tumors, including glioma[85]. In glioma tissue and GBM cell lines, CASC2 is underexpressed. However, its high expression suppresses the proliferation, migration, and invasion of GBM cells and inhibits the Wnt/β-catenin pathway by targeting miR-21[41]. Overexpressed miR-21 represses CASC2 through sequence-specific binding. The resistance of GBM cells to TMZ is partly due to increased autophagy. Current evidence showed that TMZ sensitivity was CASC2 dependent in glioma stem cells and that a CASC2 overexpression induces inhibition of autophagy and accumulation of lipid peroxides, leading to cell death. It has been proposed that increased CASC2 expression mediates inhibition of autophagy via binding miR-193a-5p and regulating mTOR expression[41,86].

Molecular Pathways of lncRNAs in Glioblastoma

LncRNAs alter many signaling pathways and cellular processes in glioma[43]. In this section, we will highlight the most affected pathways in glioma.

PI3K/Akt/mTOR pathway

This pathway is the most altered in cancer cells, promoting cell growth, proliferation, survival, apoptosis, and autophagy[87]. The lncRNA CASC2, downregulated in gliomas, is a major regulator of this pathway. In addition to its modulation of the Wnt/β-catenin signaling pathway, CASC2 influences glioma growth and resistance to TMZ by increasing the PTEN expression and suppressing the p-Akt pathway through miR-181a sponging[88].

Differentiation antagonizing non-coding RNA (DANCR) is a lncRNA located on chromosome 4 and overexpressed in several cancers as a tumor-associated lncRNA. Its overexpression correlates with cell proliferation, invasion, and metastasis[89]. DANCR competitively binds to miR-33a-5p, miR-33b-5p, miR-1-3p, miR206, and miR-613, resulting in upregulated AXL expression, a receptor tyrosine kinase that activates the PI3K/Akt/ NF-κB pathway, thereby promoting cisplatin resistance in gliomas[90].

Highly upregulated in liver cancer (HULC) is an oncogenic lncRNA located on chromosome 6, upregulated in glioma[91]. Its overexpression induces proliferation, migration, invasion, and angiogenesis through cell cycle regulation. HULC also reduces cell-matrix interaction to inhibit tumor

cell apoptosis and regulates endothelial cell-specific molecule 1 (ESM-1) via the PI3K/Akt/mTOR pathway[92].

Colorectal neoplasia differentially expressed (CRNDE) is a lncRNA, localized on chromosome 16 and upregulated in gliomas[93]. This lncRNA competitively binds to miR136-5p to suppress Bcl-2 and Wnt2, thereby blocking the PI3K/Akt/mTOR signaling pathway[94]. CRNDE knockdown induces a decreased phosphorylation of P70S6K, a signaling molecule in the mTOR pathway[95].

MAPK pathway

MAPKs (mitogen-activated protein kinases), activated by growth factors, cytokines, and environmental stress, are implicated in cell growth, apoptosis, proliferation, and migration[96]. MALAT1 exerts a tumor-suppressive function over the ERK/MAPK signaling pathway. Additionally, MALAT1 inhibits MMP2, a crucial enzyme in invasion, thereby inhibiting cell proliferation and invasion[43].

MATN1 antisense RNA 1 (MATN1-AS1) upregulation inhibits GBM cell proliferation and invasion via targeting downstream components of the MAPK pathway while promoting GBM cell apoptosis. Blocking the MAPK signaling pathway has been reported to inhibit cell invasion in GBM cells[97].

Small nucleolar RNA host gene 5 (SNHG5) is an oncogene lncRNA in glioblastoma. Activated by the transcription factor Yin Yang 1 (YY1), SNHG5 promotes cell proliferation and inhibits apoptosis. Its oncogenic role is mediated through stimulation of the p38/MAPK axis[98,99].

Wnt/β-catenin pathway

The Wnt/β-catenin signaling pathway regulates cell proliferation and cell fate, playing a crucial role in embryonic development and tissue homeostasis. Activation or mutation of components within this pathway affects various human cancers and other diseases. Additionally, overexpressed CASC2 blocks the Wnt/β-catenin signaling pathway, thereby suppressing migration, invasion, and proliferation in glioma[43]. The oncogenic lncRNA microRNA 22 host gene (MIR22HG) is highly dysregulated in glioblastoma. MIR22HG targets miR-22-3p and miR-22-5p to activate the Wnt/β-catenin pathway. Normally, miR-22-3p and miR-22-5p target secreted frizzled-related protein 2 (SFRP2) and protocadherin-related 15 (PCDH15) to inactivate this pathway. Studies have shown an increased level of both MIR22HG and miR-22 in glioma stem cells and glioblastoma[99].

Notch pathway

Notch signaling pathway is also involved in fundamental functions, including cell survival, apoptosis, differentiation, and development. Dysregulation of this pathway has been implicated in several diseases, including cancer[43]. Long intergenic ncRNA-OIP5 (Linc-OIP5) is identified as an oncogenic lncRNA highly expressed in high-grade glioma.

Knockdown of Linc-OIP5 inhibited cell proliferation *in vitro* and suppressed tumor growth *in vivo*. This lncRNA upregulates Jagged-1 (Jag1), Notch1, and hairy and enhancer of split-1 (Hes1) levels, thereby promoting tumor cell proliferation and metastasis. Linc-OIP5 also modulates Yes1 Associated Transcriptional Regulator (YAP) expression and participates in the activation of Notch signaling cascade[100]. Similarly, a related study on glioma cells showed that the knockdown of prostate cancer-up-regulated long noncoding RNA 1 (PlncRNA-1) significantly reduces apoptosis, cell proliferation, and colony formation. PlncRNA-1 was also found to activate the Notch pathway by expressing Notch-1, Jag-1, and Hes-1[101].

Research approaches and techniques to study lncRNAs in tumor

An emerging number of lncRNAs are being identified as high-throughput sequencing technology advances. However, the function of a huge number of these lncRNAs in tumors is still unknown. Previous research has shown that lncRNA function in tumors can be determined by bioinformatic tools, as well as experimental approaches including characteristic analysis, functional studies, and molecular mechanism analysis[9].

Screening of lncRNAs

Several RNA sequencing techniques may provide useful insight regarding the identity, expression, and stability of RNAs, including lncRNAs as well. Screening of differentially expressed lncRNAs is carried out using high-throughput sequencing technologies, such as NGS[102]. A variety of RNA sequencing technologies are available, including standard or single-cell RNA sequencing (RNA-seq/scRNA-seq), which captures samples of the total transcriptome, and Global or Precision Run-On sequencing (GRO-seq/PRO-seq), which captures nascent transcripts using tagged nucleosides or analogs. Other sequencing technologies are employed as well[102-104].

Depending on the experimental objectives, various biological samples such as tissues, cells, exosomes, plasma, whole blood, and serum, can be used to screen aberrant lncRNAs. High-throughput sequencing technologies provide high sensitivity, capable of detecting rare transcripts with only a few copies, thereby facilitating the identification of novel genes and transcripts across a wide detection range. However, its high cost restricts its utility in diagnostic applications[102]. The lncRNAs can also be screened from databases like the Cancer Genome Atlas (TCGA) database, a tumor-related multi-omics database containing DNA, RNA, protein, and other omics data[105].

On the other hand, direct identification of lncRNAs from sequencing datasets does not involve selective library preparation. Alternatively, identifying a potential non-coding region of the cancer genome may be facilitated by chromatin immunoprecipitation (ChIP) coupled with next-generation sequencing (ChIP-seq)[102,106] or with the assay for transposase-accessible chromatin using sequencing (ATAC-seq)[107]. Additionally, lncRNA candidates can also be determined by cross-referencing the locus coordinates with a variety of comprehensive databases that compile lncRNAs or predict the coding potential of a specific region[102].

Besides, if the coding potential has not yet been assessed, several machine learning tools are available to perform this computation from the very onset. Among a long list, Coding-Potential Assessment Tool (CPAT), FlExible Extraction of LncRNAs (FEELnc), and Predictor of Long non-coding RNAs and Messenger RNAs based on k-mer scheme (PLEK) have been extensively evaluated for their suitability in working with human cancer datasets[102,108]. Aside from well-established projects like Reference Sequence (RefSeq), Ensembl Genome Browser (Ensembl), and Functional annotation of the mammalian genome (FANTOM), a plethora of specialized resources have emerged to assign lncRNA identity and provide valuable annotations. Some of these resources are specifically designed for cancer research, such as Long non-coding RNA to Cancer (Lnc2Cancer) or The Cancer-Specific Circular RNA Database (CSCD). Many of the resources presented valuable insights about transcript localization, gene expression, evolutionary conservation, mutations, and their associations with diseases like cancer. While their primary purpose is determining whether the locus of interest is transcribed as a lncRNA, these resources provide much more information beyond that[102]. Upon screening lncRNAs, the expression of potential lncRNAs is evaluated through both *in vivo* and *in vitro* studies. In addition, the association between the expression of these lncRNAs and clinical parameters is investigated to determine the significance of potential lncRNAs in diagnosis and therapy[9].

Experimental Approaches

Characteristic Analysis of lncRNAs

The most extensively studied properties of lncRNAs include genomic and cell localization, coding potential, secondary structure, and disease association. These characteristics can be evaluated using bioinformatic tools, as mentioned earlier. Additionally, a comprehensive analysis is conducted on their full-length structure. Other software that can be used to provide an in-depth annotation of lncRNAs includes LncBook: A curated knowledgebase of human long non-coding RNAs (LncBook), Long Non-Coding RNA database (LncRNAdb) v2.0, NONCODE: An integrated knowledge database dedicated to non-coding RNAs (NONCODE), and LncRNADisease: a database for long-non-coding RNA-associated diseases (LncRNADisease)[109,110]. The molecular processes of lncRNAs are first determined by knowing their cellular localization. Fluorescence *in situ* hybridization (FISH) is a well-known technique for determining the cellular localization of potential lncRNAs[102,111]. LncATLAS: a comprehensive knowledgebase of long non-coding RNA atlases (LncATLAS) is a software that can also predict lncRNA cellular localization. However, the prediction data must be validated experimentally[9,112].

Before proceeding to more advanced experiments, it is advisable to validate the basic characteristics of the targeted lncRNAs. Therefore, we will outline some of the primary techniques that are utilized for characterizing lncRNAs, as well as their potential limitations. Northern blotting has been widely employed for the analysis of specific RNA expression, enabling quantification, determination of RNA size, and assessment of RNA quality. Recent advancements in protocols have reduced chemical usage and improved specificity, yet this fundamental method remains crucial for the primary characterization of lncRNAs. Moreover, this technique remains one of the most direct methods for studying the circular configuration of circRNAs[102]. The target RNA can also be reverse transcribed with RT-qPCR (Reverse Transcriptase Polymerase Chain Reaction), providing a highly sensitive and convenient assay. However, it is necessary to select appropriate reference genes and to carefully consider confounding factors such as contamination from genomic DNA[102,113].

Particularly, to identify unknown variants of lncRNAs suspected to undergo splicing, the technique RACE (rapid amplification of cDNA ends) is very useful. This technique uses reverse transcription with a 5' or 3' primer of a known sequence of the target RNA to generate cDNAs, followed by PCR amplification. Then, coupled with high-throughput sequencing, a technique also called RACE-Seq is used to characterize the RACE fragments[102,114,115].

RNA-FISH (RNA fluorescence *in situ* hybridization) is the standard technique used to visually detect and localize RNAs within cellular compartments and cells of interest. This technique uses fluorescent probes that target specific RNAs which is crucial for elucidating the mechanisms of various lncRNAs. However, the abundance of repetitive elements within lncRNAs presents a difficulty in detecting an authentic lncRNA signal. This could lead to high-abundance probe binding, off-target RNAs instead of the targeted lncRNA. Moreover, lncRNA signals in the nucleus are visualized as "bright blobs," which makes it more challenging to distinguish them from background signals[102,116,117].

Functional Study of lncRNAs

In vitro and *in vivo* investigations are frequently used to validate the functions of lncRNAs. *In vitro* experiments are performed by overexpressing or knocking down particular lncRNAs to evaluate their effects on phenotype, changes in biological behaviors such as EMT and stem cell markers, and their response to treatment thereby examining their role in tumorigenesis. Experiments *in vivo* involve using a virus as a vector to knock down or overexpress a specific lncRNA which is then transfected into cells. These cells are initially screened for resistance and evaluated using qRT-PCR to quantify the levels of overexpression or knockdown of the target lncRNA. Subsequently, the transfected cell lines are injected into tumorigenic animal models such as nude mice to confirm the tumorigenic potential of the candidate lncRNAs[9,118].

Molecular Mechanism analysis of lncRNAs

LncRNAs are recognized for their significant role in cancer dysregulation at both transcriptional and post-transcriptional levels. At the post-transcriptional level, extensive research has focused on the involvement of lncRNAs in RNP complexes. Numerous techniques are employed to explore interactions between lncRNAs and RBPs, among these techniques we identify[28]: RNA immunoprecipitation (RIP) techniques and lncRNA pull-down.

RIP is one of the earliest techniques to identify RNAs bound to specific RNA-Binding Protein (RBP) complexes. After tissue and cell lysis, the RNA-protein complexes are isolated through immunoprecipitation using antibodies targeting specific proteins. However, RIP faces limitations, particularly in detecting RNAs with low binding affinity to the protein of interest. This limitation arises from the instability observed in RNA-protein complexes when covalent cross-linking is not employed, resulting in the loss of specific RNAs during the washing step designed to eliminate non-specific bindings. Despite these challenges, the RIP techniques have proven effective in elucidating significant RNA-protein interactions related to cancer[102,119]. Chakrabarti et al[120] developed crosslinking and immunoprecipitation (CLIP) techniques to solve the problem of low specificity of RIP. The principle of this technique is to create covalent bonds between RBPs and their binding RNAs using ultraviolet (UV) light. Their method, using UV light is highly efficient because it selectively forms covalent bonds without causing crosslinking between proteins themselves. This property significantly enhances the technique's specificity. Another CLIP variant, CLIPSeq (crosslinking and immunoprecipitation sequencing) has emerged as the gold standard technique for identifying endogenous RNA–protein interactions. This technique involves crosslinking, RNA fragmentation, purification, and subsequent sequencing[120].

Significant progress in CLIP methods has emerged following the success of CLIP-seq to increase the specificity, such as hybrid CLIP (hiCLIP)[102] which identifies RNA duplexes: (mRNA–mRNA and mRNA–lncRNA) bound to RBPs. Another notable method is the iCLIP (individual-nucleotide resolution CLIP), which precisely maps RBP binding sites at the nucleotide level[102,121].

RNA pull-down is another technique specially used to detect proteins bound to specific lncRNAs[122]. Lately, the RNA pull-down protocol has been updated, significantly enhancing its effectiveness by generating antisense DNA oligonucleotide primers designed to selectively and specifically bind to the lncRNA of interest. As with any molecular experiment, the effectiveness of RNA pull-down also depends on the pre-analytical conditions of the tissue or cell fixation, which can impact the molecular integrity. Similar to RIP techniques, RNA pull-down can be pursued by RNA high-throughput sequencing, to get a comprehensive set of RNA targets for a lncRNA of interest[123]. Both RNA-pull down and RIP experiments are valuable for assessing the binding ability of lncRNAs with histone modifiers enzymes at the chromatin level and RNA-binding proteins at the transcriptional level[9]. As previously mentioned, the northern blot analysis is used to determine lncRNA expression and identify distinct splicing variants[122].

Many other Protein Interaction Assays are available to study lncRNA-protein complexes including the dot-blot assay, a well-established method particularly relevant for identifying protein binding sites on lncRNAs. This technique involves *in vitro* biotinylation and transcription of target lncRNAs. These labeled lncRNAs are then incubated with recombinant proteins and subsequently treated with proteinase K to dissociate the complexes. The purified lncRNAs are hybridized into nylon or PVDF membranes dotted with antisense DNA oligonucleotides and visualized using streptavidin-HRP signals[102].

Another technique, mass spectrometry (MS), is widely applied to identify proteins bound to lncRNAs of interest. MS analyzes molecules based on their mass and charge, enabling the identification and quantification of proteins in complex mixtures. Stable-isotope labeling by amino acids in cell culture (SILAC) is often employed to facilitate quantification and avoid false-positive outcomes. This method involves culturing two cell populations in media containing either light or heavy amino acids. Proteins extracted from these cells are then subjected to MS analysis[102,124].

At the chromatin level, the expression levels of target DNA methylases, and histone modifiers are respectively assessed with northern blot and western blot analysis. DNA-FISH (DNA fluorescence *in situ* hybridization) assays are also employed to investigate the impact of lncRNAs on the chromatin state of target genes[9].

At the transcriptional level, qRT-PCR is used to explore alterations in the expression of target mRNAs[9]. *In situ* hybridization (ISH) of lncRNAs is another technique utilized to determine the expression levels of specific lncRNAs and provides insights into their cellular localization. Knocking down target genes has long been regarded as the gold standard assay to determine the endogenous function of lncRNAs[122]. Additional techniques used in the study of lncRNAs include Au-NP assay (Gold nanoparticle-based assays) and HRM analysis (high-resolution melting). HRM analysis, a real-time PCR technique utilizing fluorescent labels to detect melting curve variations, is employed for both mutation detection in nucleic acid sequences and estimation of the methylation status. Previous studies suggested that HRM is a rapid, convenient, and reliable assay with high sensitivity and specificity. Importantly, it is also cost-effective compared to alternative techniques[125].

CONCLUSIONS

LncRNAs are considered potential biomarkers and promising therapeutic candidates for cancer diagnosis and therapy. Many lncRNAs are upregulated in glioma, GBM, and various other tumors and diseases. Inhibiting certain lncRNAs can positively impact the dysregulated pathways involved in pathogenesis, while overexpression of others may positively regulate signaling pathways. Mechanisms of action of lncRNAs are diverse, with many functioning through sponging other macromolecules such as DNA, RNA, and proteins, thereby influencing multiple signaling pathways. Properly classifying lncRNAs as oncogenes or tumor suppressors and assigning them to specific pathways is challenging yet crucial, especially given the rapid increase in identified lncRNAs. The transition from lncRNA-based diagnostics to therapeutics is actively studied, with recent technological advances in oligonucleotide/nanoparticle therapy providing promising tools for cancer treatment based on lncRNAs. Despite the predominant focus on protein-coding genes in cancer research, the significant roles of lncRNAs in cancer development and their clinical relevance are increasingly recognized. However, the mechanisms of action and the identification of lncRNA isoforms remain insufficiently understood, similar to protein isoforms that can exhibit distinct expression patterns and functionalities. Further research is necessary to fully elucidate the roles of lncRNAs and to successfully target them for therapeutic purposes in GBM, other cancers, and various diseases.

AVAILABILITY OF DATA AND MATERIAL:

The data and materials used in this review can be accessed through the original publications cited in the reference list.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

CONSENT FOR PUBLICATION:

All the authors approved the publication of this manuscript.

ETHICS COMMITTEE INFORMED CONSENT:

Ethics approval is not required for this study.

FUNDING:

This research received no external funding.

INFORMED CONSENT:

Informed consent is not required for this study.

REFERENCES

1. Omar Rabab'h, Al-Ramadan A, Shah J, Lopez-Negrete H, Gharaibeh A. Twenty Years After Glioblastoma Multiforme Diagnosis: A Case of Long-Term Survival. Cureus 2021; 13: e16061.
2. Henriksen M, Johnsen KB, Andersen HH, Pilgaard L, Duroux M. MicroRNA Expression Signatures Determine Prognosis and Survival in Glioblastoma Multiforme – a Systematic Overview. Mol Neurobiol 2014; 50: 896-913.
3. Gravendeel LAM, Kouwenhoven MCM, Gevaert O, De Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LBC, Kloosterhof NK, De Moor B, Eilers PHC, Van Der Spek PJ, Kros JM, Sillevis Smitt PAE, Van Den Bent MJ, French PJ. Intrinsic Gene Expression Profiles of Gliomas Are a Better Predictor of Survival than Histology. Cancer Res 2009; 69: 9065-9072.
4. Zhang L, Liu F, Weygant N, Zhang J, Hu P, Qin Z, Yang J, Cheng Q, Fan F, Zeng Y, Tang Y, Li Y, Tang A, He F, Peng J, Liao W, Hu Z, Li M, Liu Z. A novel integrated system using patient-derived glioma cerebral organoids and xenografts for disease modeling and drug screening. Cancer Lett 2021; 500: 87-97.
5. Liu Q, Qi C, Li G, Su W. Prediction of the Outcome for Patients with Glioblastoma with lncRNA Expression Profiles. BioMed Res Int 2019; 2019: 5076467.
6. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell 2011; 144: 646-674.
7. Grillone K, Riillo C, Scionti F, Rocca R, Tradigo G, Guzzi PH, Alcaro S, Di Martino MT, Tagliaferri P, Tassone P. Non-coding RNAs in cancer: platforms and strategies for investigating the genomic "dark matter." J Exp Clin Cancer Res 2020; 39: 117.
8. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics. Oncol Rep 2017; 37: 3-9.
9. Gao N, Li Y, Li J, Gao Z, Yang Z, Li Y, Liu H, Fan T. Long Non-Coding RNAs: The Regulatory Mechanisms, Research Strategies, and Future Directions in Cancers. Front Oncol 2020; 10: 598-817.
10. Ponting CP, Oliver PL, Reik W. Evolution and Functions of Long Noncoding RNAs. Cell 2009; 136: 629-641.
11. Romano G, Veneziano D, Acunzo M, Croce CM. Small non-coding RNA and cancer. Carcinogenesis 2017; 38: 485-491.
12. Diamantopoulos MA, Tsiakanikas P, Scorilas A. Non-coding RNAs: the riddle of the transcriptome and their perspectives in cancer. Ann Transl Med 2018; 6: 241-253.
13. Arun G, Diermeier SD, Spector DL. Therapeutic Targeting of Long Non-Coding RNAs in Cancer. Trends Mol Med 2018; 24: 257-277.
14. Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nat Rev Cancer 2018; 18: 5-18.
15. Bartel DP. Metazoan MicroRNAs. Cell 2018; 173: 20-51.
16. Huang T, Alvarez AA, Pangeni RP, M. Horbinski C, Lu S, Kim SH, James CD, Raizer J, Kessler J, Brenann CW, Sulman EP, Finocchiaro G, Tan M, Nishikawa R, Lu X, Nakano I, Hu B, Cheng SY. A regulatory circuit of miR-125b/miR-20b and Wnt signalling controls glioblastoma phenotypes through FZD6-modulated pathways. Nat Commun 2016; 7: 12885.
17. Ahir BK, Lakka SS. Elucidating the microRNA-203 specific biological processes in glioblastoma cells from comprehensive RNA-sequencing transcriptome profiling. Cell Signal 2019; 53: 22-38.
18. Mu M, Niu W, Zhang X, Hu S, Niu C. LncRNA BCYRN1 inhibits glioma tumorigenesis by competitively binding with miR-619-5p to regulate CUEDC2 expression and the PTEN/AKT/p21 pathway. Oncogene 2020; 39: 6879-6892.
19. Shi J, Dong B, Cao J, Mao Y, Guan W, Peng Y, Wang S. Long non-coding RNA in glioma: signaling pathways. Oncotarget 2017; 8: 27582-27592.
20. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009; 23: 1494-1504.
21. Ding Y, Wang X, Pan J, Ji M, Luo Z, Zhao P, Zhang Y, Wang G. Aberrant expression of long non-coding RNAs (lncRNAs) is involved in brain glioma development. Arch Med Sci 2020; 16: 177-188.
22. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016; 17: 47-62.
23. Fawzy MS, Toraih EA, Abdallah HY. Long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1): A molecular predictor of poor survival in glioblastoma multiforme in Egyptian patients. Egypt J Med Hum Genet 2017; 18: 231-239.

24. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021; 22: 96-118.
25. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigó R. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.
26. Yadav B, Pal S, Rubstov Y, Goel A, Garg M, Pavlyukov M, Pandey AK. LncRNAs associated with glioblastoma: From transcriptional noise to novel regulators with a promising role in therapeutics. Mol Ther Nucleic Acids 2021; 24: 728-742.
27. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-DiNardo D, Kanduri C. Kcnq1ot1 Antisense Noncoding RNA Mediates Lineage-Specific Transcriptional Silencing through Chromatin-Level Regulation. Mol Cell 2008; 32: 232-246.
28. He RZ, Luo DX, Mo YY. Emerging roles of lncRNAs in the post-transcriptional regulation in cancer. Genes Dis 2019; 6: 6-15.
29. Luo C, Quan Z, Zhong B, Zhang M, Zhou B, Wang S, Luo X, Tang C. lncRNA XIST promotes glioma proliferation and metastasis through miR-133a/SOX4. Exp Ther Med 2020; 19: 1641-1648.
30. Zhang XQ, Sun S, Lam KF, Kiang KMY, Pu JKS, Ho ASW, Lui WM, Fung CF, Wong TS, Leung GKK. A long non-coding RNA signature in glioblastoma multiforme predicts survival. Neurobiol Dis 2013; 58: 123-131.
31. Ma K Xiao, Wang H Jie, Li X Rong, Li T, Su G, Yang P, Wu J wen. Long noncoding RNA MALAT1 associates with the malignant status and poor prognosis in glioma. Tumor Biol 2015; 36: 3355-3359.
32. Bian E, Li J, Xie Y, Zong G, Li J, Zhao B. LncRNAs: New Players in Gliomas, With Special Emphasis on the Interaction of lncRNAs With EZH2. J Cell Physiol 2015; 230: 496-503.
33. DeSouza PA, Qu X, Chen H, Patel B, Maher CA, Kim AH. Long, Noncoding RNA Dysregulation in Glioblastoma. Cancers 2021; 13: 1604.
34. Chen LL. Linking Long Noncoding RNA Localization and Function. Trends Biochem Sci 2016; 41: 761-772.
35. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biol 2013; 10: 924-933.
36. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009; 458: 223-227.
37. Balas MM, Johnson AM. Exploring the mechanisms behind long noncoding RNAs and cancer. Non-Coding RNA Res 2018; 3: 108-117.
38. Wang KC, Chang HY. Molecular Mechanisms of Long Noncoding RNAs. Mol Cell 2011; 43: 904-914.
39. Maruyama R, Suzuki H. Long noncoding RNA involvement in cancer. BMB Rep 2012; 45: 604-611.
40. Katsushima K, Kondo Y. Non-coding RNAs as epigenetic regulator of glioma stem-like cell differentiation. Front Genet 2014; 5: 1664-8021.
41. Katsushima K, Jallo G, Eberhart CG, Perera RJ. Long non-coding RNAs in brain tumors. NAR Cancer 2021; 3: 2632-8674.
42. Ke J, Yao Y long, Zheng J, Wang P, Liu Y hui, Ma J, Li Z, Liu X Bai, Li Z Qing, Wang Z hua, Xue Y Xue. Knockdown of long non-coding RNA HOTAIR inhibits malignant biological behaviors of human glioma cells via modulation of miR-326. Oncotarget 2015; 6: 21934-21949.
43. Kim SH, Lim KH, Yang S, Joo JY. Long non-coding RNAs in brain tumors: roles and potential as therapeutic targets. J Hematol Oncol 2021; 14: 77.
44. Zeng T, Li L, Zhou Y, Gao L. Exploring Long Noncoding RNAs in Glioblastoma: Regulatory Mechanisms and Clinical Potentials. Int J Genomics 2018; 2018: 2895958.
45. Zhang JX, Han L, Bao ZS, Wang YY, Chen LY, Yan W, Yu SZ, Pu PY, Liu N, You YP, Jiang T, Kang CS. HOTAIR, a cell cycle–associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. Neuro-Oncol 2013; 15: 1595-1603.
46. Zhang K, Sun X, Zhou X, Han L, Chen L, Shi Z, Zhang A, Ye M, Wang Q, Liu C, Wei J, Ren Y, Yang J, Zhang J, Pu P, Li M, Kang C. Long non-coding RNA HOTAIR promotes glioblastoma cell cycle progression in an EZH2 dependent manner. Oncotarget 2015; 6: 537-546.
47. Ma X, Li Z, Li T, Zhu L, Li Z, Tian N. Long non-coding RNA HOTAIR enhances angiogenesis by induction of VEGFA expression in glioma cells and transmission to endothelial cells via glioma cell derived-extracellular vesicles. Am J Transl Res 2017; 9: 5012-5021.
48. Liu L, Cui S, Wan T, Li X, Tian W, Zhang R, Luo L, Shi Y. Long non-coding RNA HOTAIR acts as a competing endogenous RNA to promote glioma progression by sponging miR-126-5p. J Cell Physiol 2018; 233: 6822-6831.
49. Li Q, Dong C, Cui J, Wang Y, Hong X. Over-expressed lncRNA HOTAIRM1 promotes tumor growth and invasion through up-regulating HOXA1 and sequestering G9a/EZH2/Dnmts away from the HOXA1 gene in glioblastoma multiforme. J Exp Clin Cancer Res 2018; 37: 265.
50. Shi T, Guo D, Xu H, Su G, Chen J, Zhao Z, Shi J, Wedemeyer M, Attenello F, Zhang L, Lu W. HOTAIRM1, an enhancer lncRNA, promotes glioma proliferation by regulating long-range chromatin interactions within HOXA cluster genes. Mol Biol Rep 2020; 47: 2723-2733.
51. Xu LM, Chen L, Li F, Zhang R, Li Z yang, Chen FF, Jiang XD. Over-expression of the long non-coding RNA HOTTIP inhibits glioma cell growth by BRE. J Exp Clin Cancer Res 2016; 35: 162.
52. Shi W, Tang MK, Yao Y, Tang C, Chui YL, Lee KKH. BRE plays an essential role in preventing replicative and DNA damage-induced premature senescence. Sci Rep 2016; 6: 23506.
53. Zhang S, Wang W, Liu G, Xie S, Li Q, Li Y, Lin Z. Long non-coding RNA HOTTIP promotes hypoxia-induced epithelial-mesenchymal transition of malignant glioma by regulating the miR-101/ZEB1 axis. Biomed Pharmacother 2017; 95: 711-720.
54. Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, Chess A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics 2007; 8: 39.
55. Chen Q, Cai J, Wang Q, Wang Y, Liu M, Yang J, Zhou J, Kang C, Li M, Jiang C. Long Noncoding RNA NEAT1, regulated by the EGFR Pathway, Contributes to Glioblastoma Progression Through the WNT/ β -Catenin Pathway by Scaffolding EZH2. Clin Cancer Res 2018; 24: 684-695.

56. Lulli V, Buccarelli M, Ilari R, Castellani G, De Dominicis C, Di Giamberardino A, D'Alessandris QG, Giannetti S, Martini M, Stumpo V, Boe A, De Luca G, Biffoni M, Marziali G, Pallini R, Ricci-Vitiani L. Mir-370-3p Impairs Glioblastoma Stem-Like Cell Malignancy Regulating a Complex Interplay between HMGA2/HIF1A and the Oncogenic Long Non-Coding RNA (lncRNA) NEAT1. Int J Mol Sci 2020; 21: 3610.

57. Liao K, Lin Y, Gao W, Xiao Z, Medina R, Dmitriev P, Cui J, Zhuang Z, Zhao X, Qiu Y, Zhang X, Ge J, Guo L. Blocking lncRNA MALAT1/miR-199a/ZHX1 Axis Inhibits Glioblastoma Proliferation and Progression. Mol Ther - Nucleic Acids 2019; 18: 388-399.

58. Fu Z, Luo W, Wang J, Peng T, Sun G, Shi J, Li Z, Zhang B. Malat1 activates autophagy and promotes cell proliferation by sponging miR-101 and upregulating STMN1, RAB5A and ATG4D expression in glioma. Biochem Biophys Res Commun 2017; 492: 480-486.

59. Cai T, Liu Y, Xiao J. Long noncoding RNA MALAT 1 knockdown reverses chemoresistance to temozolomide via promoting micro-RNA -101 in glioblastoma. Cancer Med 2018; 7: 1404-1415.

60. Chen W, Xu XK, Li JL, Kong KK, Li H, Chen C, He J, Wang F, Li P, Ge XS, Li FC. MALAT1 is a prognostic factor in glioblastoma multiforme and induces chemoresistance to temozolomide through suppressing miR-203 and promoting thymidylate syn-thase expression. Oncotarget 2017; 8: 22783-22799.

61. Vassallo I, Zinn P, Lai M, Rajakannu P, Hamou MF, Hegi ME. WIF1 re-expression in glioblastoma inhibits migration through attenuation of non-canonical WNT signaling by downregulating the lncRNA MALAT1. Oncogene 2016; 35: 12-21.

62. Cao S, Wang Y, Li J, Lv M, Niu H, Tian Y. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by sup-pressing miR-155 expression and activating FBXW7 function. Am J Cancer Res 2016; 6: 2561-2574.

63. Han Y, Wu Z, Wu T, Huang Y, Cheng Z, Li X, Sun T, Xie X, Zhou Y, Du Z. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by downregulation of MMP2 and inactivation of ERK/MAPK signaling. Cell Death Dis 2016; 7: e2123.

64. Li C, Lei B, Huang S, Zheng M, Liu Z, Li Z, Deng Y. H19 derived microRNA-675 regulates cell proliferation and migration through CDK6 in glioma. Am J Transl Res 2015; 7: 1747-1764.

65. Raveh E, Matouk IJ, Gilon M, Hochberg A. The H19 Long non-coding RNA in cancer initiation, progression and metastasis – a proposed unifying theory. Mol Cancer 2015; 14: 184.

66. Shi Y, Wang Y, Luan W, Wang P, Tao T, Zhang J, Qian J, Liu N, You Y. Long Non-Coding RNA H19 Promotes Glioma Cell Invasion by Deriving miR-675. PLoS One 2014; 9: e86295.

67. Jia P, Cai H, Liu X, Chen J, Ma J, Wang P, Liu Y, Zheng J, Xue Y. Long non-coding RNA H19 regulates glioma angiogenesis and the biological behavior of glioma-associated endothelial cells by inhibiting microRNA-29a. Cancer Lett 2016; 381: 359-369.

68. Zhou Q, Liu ZZ, Wu H, Kuang WL. LncRNA H19 Promotes Cell Proliferation, Migration, and Angiogenesis of Glioma by Regu-lating Wnt5a/β-Catenin Pathway via Targeting miR-342. Cell Mol Neurobiol 2022; 42: 1065-1077.

69. Liu ZZ, Tian YF, Wu H, Ouyang SY, Kuang WL. LncRNA H19 promotes glioma angiogenesis through miR-138/HIF-1α/VEGF axis. Neoplasma 2020; 67: 111-118.

70. Xin Y, Li Z, Zheng H, Chan MTV, Ka Kei Wu W. CCAT2: A novel oncogenic long non-coding RNA in human cancers. Cell Prolif 2017; 50: e12342.

71. Guo H, Hu G, Yang Q, Zhang P, Kuang W, Zhu X, Wu L. Knockdown of long non-coding RNA CCAT2 suppressed proliferation and migration of glioma cells. Oncotarget 2016; 7: 81806-81814.

72. Sun SL, Shu YG, Tao MY. LncRNA CCAT2 promotes angiogenesis in glioma through activation of VEGFA signaling by sponging miR-424. Mol Cell Biochem 2020; 468: 69-82.

73. Wang W, Min L, Qiu X, Wu X, Liu C, Ma J, Zhang D, Zhu L. Biological Function of Long Non-coding RNA (LncRNA) Xist. Front Cell Dev Biol 2021; 9: 645647.

74. Wutz A. Gene silencing in X-chromosome inactivation: advances in understanding facultative heterochromatin formation. Nat Rev Genet 2011; 12: 542-553.

75. Cheng Z, Li Z, Ma K, Li X, Tian N, Duan J, Xiao X, Wang Y. Long Non-coding RNA XIST Promotes Glioma Tumorigenicity and Angiogenesis by Acting as a Molecular Sponge of miR-429. J Cancer 2017; 8: 4106-4116.

76. Cheng Z, Luo C, Guo Z. LncRNA-XIST/microRNA-126 sponge mediates cell proliferation and glucose metabolism through the IRS1/PI3K/Akt pathway in glioma. J Cell Biochem 2020; 121: 2170-2183.

77. Aguilo F, Di Cecilia S, Walsh MJ. Long Non-coding RNA ANRIL and Polycomb in Human Cancers and Cardiovascular Disease. Curr Top Microbiol Immunol 2016; 394: 29-39.

78. Paul Y, Thomas S, Patil V, Kumar N, Mondal B, Hegde AS, Arivazhagan A, Santosh V, Mahalingam K, Somasundaram K. Genet-ic landscape of long noncoding RNA (lncRNAs) in glioblastoma: identification of complex lncRNA regulatory networks and clinically relevant lncRNAs in glioblastoma. Oncotarget 2018; 9: 29548-29564.

79. Samson J, Cronin S, Dean K. BC200 (BCYRN1) - The shortest, long, non-coding RNA associated with cancer. Non-Coding RNA Res 2018; 3: 131-143.

80. Choudhari R, Sedano MJ, Harrison AL, Subramani R, Lin KY, Ramos EI, Lakshmanaswamy R, Gadad SS. Long noncoding RNAs in cancer: From discovery to therapeutic targets. Advances in Clinical Chemistry 2020; 95: 105-147.

81. Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, Ansell PJ, Zhao J, Weng C, Klibanski A. Activation of p53 by MEG3 Non-coding RNA. J Biol Chem 2007; 282: 24731-24742.

82. Zhang S, Guo W. Long non-coding RNA MEG3 suppresses the growth of glioma cells by regulating the miR-96-5p/MTSS1 signaling pathway. Mol Med Rep 2019; 20: 4215-4225.

83. Li J, Bian EB, He XJ, Ma CC, Zong G, Wang HL, Zhao B. Epigenetic repression of long non-coding RNA MEG3 mediated by DNMT1 represses the p53 pathway in gliomas. Int J Oncol 2016; 48: 723-733.

84. Qin N, Tong GF, Sun LW, Xu XL. Long Noncoding RNA MEG3 Suppresses Glioma Cell Proliferation, Migration, and Invasion by Acting as a Competing Endogenous RNA of miR-19a. Oncol Res Featur Preclin Clin Cancer Ther 2017; 25: 1471-1478.

85. Wang P, Liu Y hui, Yao Y long, Li Z, Li Z qing, Ma J, Xue Y xue. Long non-coding RNA CASC2 suppresses malignancy in human gliomas by miR-21. Cell Signal 2015; 27: 275-282.

86. Jiang C, Shen F, Du J, Fang X, Li X, Su J, Wang X, Huang X, Liu Z. Upregulation of CASC2 sensitized glioma to temozolomide cytotoxicity through autophagy inhibition by sponging miR-193a-5p and regulating mTOR expression. Biomed Pharmacother 2018; 97: 844-850.

87. Peng Y, Wang Y, Zhou C, Mei W, Zeng C. PI3K/Akt/mTOR Pathway and Its Role in Cancer Therapeutics: Are We Making Headway? Front Oncol 2022; 12: 819128.

88. Liao Y, Shen L, Zhao H, Liu Q, Fu J, Guo Y, Peng R, Cheng L. LncRNA CASC2 Interacts With miR-181a to Modulate Glioma Growth and Resistance to TMZ Through PTEN Pathway. J Cell Biochem 2017; 118: 1889-1899.

89. Thin KZ, Liu X, Feng X, Raveendran S, Tu JC. LncRNA-DANCR: A valuable cancer related long non-coding RNA for human cancers. Pathol Res Pract 2018; 214: 801-805.

90. Ma Y, Zhou G, Li M, Hu D, Zhang L, Liu P, Lin K. Long noncoding RNA DANCR mediates cisplatin resistance in glioma cells via activating AXL/PI3K/Akt/NF-κB signaling pathway. Neurochem Int 2018; 118: 233-241.

91. Yan H, Tian R, Zhang M, Wu J, Ding M, He J. High expression of long noncoding RNA HULC is a poor predictor of prognosis and regulates cell proliferation in glioma. OncoTargets Ther 2016; 10: 113-120.

92. Zhu Y, Zhang X, Qi L, Cai Y, Yang P, Xuan G, Jiang Y. HULC long noncoding RNA silencing suppresses angiogenesis by regulating ESM-1 via the PI3K/Akt/mTOR signaling pathway in human gliomas. Oncotarget 2016; 7: 14429-14440.

93. Ellis BC, Molloy PL, Graham LD. CRNDE: A Long Non-Coding RNA Involved in CanceR, Neurobiology, and DEvelopment. Front Genet 2012; 3: 1664-8021.

94. Li DX, Fei XR, Dong YF, Cheng CD, Yang Y, Deng XF, Huang HL, Niu WX, Zhou CX, Xia CY, Niu CS. The long non-coding RNA CRNDE acts as a ceRNA and promotes glioma malignancy by preventing miR-136-5p-mediated downregulation of Bcl-2 and Wnt2. Oncotarget 2017; 8: 88163-88178.

95. Wang Y, Wang Y, Li J, Zhang Y, Yin H, Han B. CRNDE, a long-noncoding RNA, promotes glioma cell growth and invasion through mTOR signaling. Cancer Lett 2015; 367: 122-128.

96. Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. Oncogene. 2007; 26: 3279-3290.

97. Han N, Yang L, Zhang X, Zhou Y, Chen R, Yu Y, Dong Z, Zhang M. LncRNA MATN1-AS1 prevents glioblastoma cell from proliferation and invasion via RELA regulation and MAPK signaling pathway. Ann Transl Med 2019; 7: 784-784.

98. Chen L, Gong X, Huang M. YY1-Activated Long Noncoding RNA SNHG5 Promotes Glioblastoma Cell Proliferation Through p38/MAPK Signaling Pathway. Cancer Biother Radiopharm 2019; 34: 589-596.

99. Rezaei O, Tamizkar KH, Sharifi G, Taheri M, Ghafouri-Fard S. Emerging Role of Long Non-Coding RNAs in the Pathobiology of Glioblastoma. Front Oncol 2021; 10: 625884.

100. Hu G, Wu L, Kuang W, Chen Y, Zhu X, Guo H, Lang H. Knockdown of linc-OIP5 inhibits proliferation and migration of glioma cells through down-regulation of YAP-NOTCH signaling pathway. Gene 2017; 610: 24-31.

101. Wang X, Yan Y, Zhang C, Wei W, Ai X, Pang Y, Bian Y. Upregulation of lncRNA PlncRNA-1 indicates the poor prognosis and promotes glioma progression by activation of Notch signal pathway. Biomed Pharmacother 2018; 103: 216-221.

102. Carter JM, Ang DA, Sim N, Budiman A, Li Y. Approaches to Identify and Characterise the Post-Transcriptional Roles of lncRNAs in Cancer. Non-Coding RNA 2021; 7: 19.

103. Gardini A. Global Run-On Sequencing (GRO-Seq). Methods Mol Biol 2017; 1468: 111-120.

104. Mahat DB, Kwak H, Booth GT, Jonkers IH, Danko CG, Patel RK, Waters CT, Munson K, Core LJ, Lis JT. Base-pair-resolution genome-wide mapping of active RNA polymerases using precision nuclear run-on (PRO-seq). Nat Protoc 2016; 11: 1455-1476.

105. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015; 19: A68-77.

106. Raha D, Hong M, Snyder M. ChIP-Seq: A Method for Global Identification of Regulatory Elements in the Genome. Curr Protoc Mol Biol 2010; Chapter 21: Unit 21.19.1-14.

107. Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods 2017; 14: 959-962.

108. Antonov IV, Mazurov E, Borodovsky M, Medvedeva YA. Prediction of lncRNAs and their interactions with nucleic acids: benchmarking bioinformatics tools. Brief Bioinform 2019; 20: 551-564.

109. Lina Ma, Jiabao Cao, Lin Liu, Qiang Du, Zhao Li, Dong Zou, Vladimir B Bajic, Zhang Zhang, LncBook: a curated knowledgebase of human long non-coding RNAs. Nucleic Acids Res 2019; 47: D128-D134.

110. Bao Z, Yang Z, Huang Z, Zhou Y, Cui Q, Dong D. LncRNADisease 2.0: an updated database of long non-coding RNA-associated diseases. Nucleic Acids Res 2019; 47: D1034-1037.

111. Rodriguez-Mateos P, Azevedo NF, Almeida C, Pamme N. FISH and chips: a review of microfluidic platforms for FISH analysis. Med Microbiol Immunol 2020; 209: 373-391.

112. Mas-Ponte D, Carlevaro-Fita J, Palumbo E, Hermoso Pulido T, Guigo R, Johnson R. LncATLAS database for subcellular localization of long noncoding RNAs. RNA 2017; 23: 1080-1087.

113. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin Chem 2009; 55: 611-622.

114. Olivarius S, Plessy C, Carninci P. High-throughput verification of transcriptional starting sites by Deep-RACE. BioTechniques 2009; 46: 130-132.

115. Lagarde J, Uszczynska-Ratajczak B, Santoyo-Lopez J, Gonzalez JM, Tapanari E, Mudge JM, Steward CA, Wilming L, Tanzer A, Howald C, Chrast J, Vela-Boza A, Rueda A, Lopez-Domingo FJ, Dopazo J, Reymond A, Guigó R, Harrow J. Extension of human lncRNA transcripts by RACE coupled with long-read high-throughput sequencing (RACE-Seq). Nat Commun 2016; 7: 12339.

116. Orjalo AV, Johansson HE. Stellaris® RNA Fluorescence In Situ Hybridization for the Simultaneous Detection of Immature and Mature Long Noncoding RNAs in Adherent Cells. Methods Mol Biol 2016; 1402: 119-134.

117. Dunagin M, Cabili MN, Rinn J, Raj A. Visualization of lncRNA by Single-Molecule Fluorescence In Situ Hybridization. Methods Mol Biol 2015; 1262: 3-19.

118. Luo ML. Methods to Study Long Noncoding RNA Biology in Cancer. Adv Exp Med Biol 2016; 927: 69-107.

119. Bierhoff H. Analysis of lncRNA-Protein Interactions by RNA-Protein Pull-Down Assays and RNA Immunoprecipitation (RIP). Methods Mol Biol 2018; 1686: 241-250.

120. Chakrabarti AM, Haberman N, Praznik A, Luscombe NM, Ule J. Data Science Issues in Studying Protein–RNA Interactions with CLIP Technologies. Annu Rev Biomed Data Sci 2018; 1: 235-261.
121. Haberman N, Huppertz I, Attig J, König J, Wang Z, Hauer C, Hentze MW, Kulozik AE, Le Hir H, Curk T, Sibley CR, Zarnack K, Ule J. Insights into the design and interpretation of iCLIP experiments. Genome Biol 2017; 18: 7.
122. Feng Y, Hu X, Zhang Y, Zhang D, Li C, Zhang L. Methods for the study of long noncoding RNA in cancer cell signaling. Methods Mol Biol 2014; 1165: 115-43.
123. Torres M, Becquet D, Guillen S, Boyer B, Moreno M, Blanchard MP, Franc JL, François-Bellan AM. RNA Pull-down Procedure to Identify RNA Targets of a Long Non-coding RNA. J Vis Exp 2018; 134: 57379.
124. Mann M. Functional and quantitative proteomics using SILAC. Nat Rev Mol Cell Biol 2006; 7: 952-958.
125. Kolenda T, Ryś M, Guglas K, Teresiak A, Bliźniak R, Mackiewicz J, Lamperska K. Quantification of long non-coding RNAs using qRT-PCR: comparison of different cDNA synthesis methods and RNA stability. Arch Med Sci 2019; 17: 1006-1015.
126. Katsushima K, Natsume A, Ohka F, Shinjo K, Hatanaka A, Ichimura N, Sato S, Takahashi S, Kimura H, Totoki Y, Shibata T, Naito M, Kim HJ, Miyata K, Kataoka K, Kondo Y. Targeting the Notch-regulated non-coding RNA TUG1 for glioma treatment. Nat Commun 2016; 7: 13616.
127. Cai H, Xue Y, Wang P, Wang Z, Li Z, Hu Y, Li Z, Shang X, Liu Y. The long noncoding RNA TUG1 regulates blood-tumor barrier permeability by targeting miR-144. Oncotarget 2015; 6: 19759-19779.
128. Mei Shao, Wenyun Liu, Yu Wang. Differentially expressed LncRNAs as potential prognostic biomarkers for glioblastoma. Cancer Genetics 2018; 226: 23-29.
129. Zheng D, Chen D, Lin F, Wang X, Lu L, Luo S, Chen J, Xu X. LncRNA NNT-AS1 promote glioma cell proliferation and metastases through miR-494-3p/PRMT1 axis. Cell Cycle 2020; 19: 1621-1631.
130. Dai J, Ma J, Yu B, Zhu Z, Hu Y. [ARTICLE WITHDRAWN] Long Noncoding RNA TUNAR Represses Growth, Migration, and Invasion of Human Glioma Cells Through Regulating miR-200a and Rac1. Oncol Res Featur Preclin Clin Cancer Ther 2018; 27: 107-115.
131. Deguchi S, Katsushima K, Hatanaka A, Shinjo K, Ohka F, Wakabayashi T, Zong H, Natsume A, Kondo Y. Oncogenic effects of evolutionarily conserved noncoding RNA ECONEXIN on gliomagenesis. Oncogene 2017; 36: 4629-4640.
132. Yan Y, Xu Z, Chen X, Wang X, Zeng S, Zhao Z, Qian L, Li Z, Wei J, Huo L, Li X, Gong Z, Sun L. Novel Function of lncRNA ADAMTS9-AS2 in Promoting Temozolomide Resistance in Glioblastoma via Upregulating the FUS/MDM2 Ubiquitination Axis. Front Cell Dev Biol 2019; 7: 217.
133. Luo W, Li X, Song Z, Zhu X, Zhao S. Long non-coding RNA AGAP2-AS1 exerts oncogenic properties in glioblastoma by epigenetically silencing TFPI2 through EZH2 and LSD1. Aging 2019; 11: 3811-3823.
134. Cheng M, Wang Q, Chen L, Zhao D, Tang J, Xu J, He Z. LncRNA UCA1/miR-182-5p/MGMT axis modulates glioma cell sensitivity to temozolomide through MGMT-related DNA damage pathways. Hum Pathol 2022; 123: 59-73.
135. Chao Fu, Dongyuan Li, Xiaonan Zhang, Naijie Liu, Guonan Chi, Xingyi Jin. LncRNA PVT1 Facilitates Tumorigenesis and Progression of Glioma via Regulation of MiR-128-3p/GREM1 Axis and BMP Signaling Pathway. Neurotherapeutics 2018; 15: 1139-1157.
136. Shang C, Guo Y, Hong Y, Xue Y xue. Long Non-coding RNA TUSC7, a Target of miR-23b, Plays Tumor-Suppressing Roles in Human Gliomas. Front Cell Neurosci 2016; 10: 235.
137. Liu S, Mitra R, Zhao MM, Fan W, Eischen CM, Yin F, Zhao Z. The Potential Roles of Long Noncoding RNAs (lncRNA) in Glioblastoma Development. Mol Cancer Ther 2016; 15: 2977-2986.
138. Lv X, Gu C, Guo S. Activation of BDNF-AS/ADAR/p53 Positive Feedback Loop Inhibits Glioblastoma Cell Proliferation. Neurochem Res 2020; 45: 508-518.
139. Liu B, Zhou J, Wang C, Chi Y, Wei Q, Fu Z, Lian C, Huang Q, Liao C, Yang Z, Zeng H, Xu N, Guo H. LncRNA SOX2OT promotes temozolomide resistance by elevating SOX2 expression via ALKBH5-mediated epigenetic regulation in glioblastoma. Cell Death Dis 2020; 11: 384.
140. Lin TK, Chang CN, Tsai CS, Huang YC, Lu YJ, Chen WJ, Lin YH, Chung IH, Lin KH. The long non-coding RNA LOC441204 enhances cell growth in human glioma. Sci Rep 2017; 7: 5603.
141. Meng L, Ma P, Cai R, Guan Q, Wang M, Jin B. Long noncoding RNA ZEB1-AS1 promotes the tumorigenesis of glioma cancer cells by modulating the miR-200c/141-ZEB1 axis. Am J Transl Res 2018; 10: 3395-3412.
142. Feng S, Yao J, Chen Y, Geng P, Zhang H, Ma X, Zhao J, Yu X. Expression and Functional Role of Reprogramming-Related Long Noncoding RNA (lincRNA-ROR) in Glioma. J Mol Neurosci 2015; 56: 623-630.
143. Bao X, Wu H, Zhu X, Guo X, Hutchins AP, Luo Z, Song H, Chen Y, Lai K, Yin M, Xu L, Zhou L, Chen J, Wang D, Qin B, Frampton J, Tse HF, Pei D, Wang H, Zhang B, Esteban MA. The p53-induced lincRNA-p21 derails somatic cell reprogramming by sustaining H3K9me3 and CpG methylation at pluripotency gene promoters. Cell Res 2015; 25: 80-92.
144. Chu L, Yu L, Liu J, Song S, Yang H, Han F, Liu F, Hu Y. Long intergenic non-coding LINC00657 regulates tumorigenesis of glioblastoma by acting as a molecular sponge of miR-190a-3p. Aging 2019; 11: 1456-1470.
145. Zhang L, Cao Y, Wei M, Jiang X, Jia D. Long noncoding RNA-RNCR3 overexpression deleteriously affects the growth of glioblastoma cells through miR-185-5p/Krüppel-like factor 16 axis. J Cell Biochem 2018; 119: 9081-9089.