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The roles of long non-coding RNAs in biological properties of human glioma.

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Abstract

Glioma is the most common and aggressive primary adult brain tumor. The median survival time of the glioma patients is less than 15 months under conventional treatments. The etiology of the glioma is still unknown. The emerging evidence suggested that many factors contributed to human glioma formation, metastasis, relapse, and resistant to radiation, and chemotherapy/therapies resistance. Recent reports showed that the long non-coding RNAs (*lncRNAs*) had multifunctional roles in regulating human glioma tumorogenesis processes through both transcriptional and post-transcriptional regulation of gene expression. In this review, the related *lncRNAs* which have been reported were summarized, the functions of the *lncRNAs* which acted as oncogenes or tumor suppressor genes during human glioma development was discussed, and the current mechanisms of *lncRNAs* was elaborated in a variety of biological properties of human glioma.

Keywords: Glioma, Tumorigenesis, *LncRNAs*.

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Introduction

Glioma is the most common and aggressive primary brain tumor in adult. Gliomas make up about 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors. The exact causes of gliomas are still not known. Different oncogenes work in the development of gliomas. Different cancer genes have synergistic in the development of glioma. Treatment for brain gliomas depends on the location, cell type, and grade of malignancy. So far, there is no way to cure gliomas. Surgery, radiation therapy, and chemotherapy are combined together to treat the human gliomas. The median survival time of the glioma patients is less than 15 months under conventional treatments. The etiology of the glioma is unknown till now [1-4]. According to aggressiveness, the World Health Organization (WHO) classified them into Grades 1 and 2 or Low-Grade Gliomas (LGG), and Grades 3 and 4 or High-Grade Gliomas (HGG). Human glioma is characterized by a wide clinical and histological heterogeneity, because 35-40% of them have epigenetic modifications as the underlying mechanism driving malignancy [5]. Clinicians and scientists all over the world can't predict the clinical evolution of each patient who is diagnosed with this human glioma till now.

The global human genome project which is started at 1990's is expected to understand the human Genetic information especially genes information which encodes functional proteins. Finally, only 20000 protein coding genes were discovered [6-8]. More than 98% of eukaryotic transcriptomes compose of non-coding RNAs with no functional protein-coding capacity [9-11]. Investigators categorized the non-coding RNAs (*ncRNAs*) as short *ncRNAs*, mid-size *ncRNAs*, and long non-coding RNAs (*lncRNAs*) by their lengths. *LncRNA* is a large class of ncRNA which have a length of more than 200 nucleotides (nt) and plays important roles in lots of physiological and pathological processes [12]. *LncRNAs* have many roles in regulating embryonic pluripotency, differentiation, development and various diseases, especially in cancers [13-16]. Recently, many evidences demonstrated that *lncRNAs* take part in many signalling pathways related to human glioma progression, invasion, metastasis, and drug-resistance [17-19]. In most reviews, the relationship of the *lncRNAs* and glioma has been analysed. It is known that expression of different types of *lncRNAs* in different pathological grade of gliomas, or even in the same pathological grade of gliomas expression is not the same. The recurrence rate and survival time of the patients with the same pathological grade were also different. Whether this difference is related to the expression of different *lncRNAs* is still unclear. In this review, the updated research data will be focused and the current knowledge of *lncRNAs* contributing to these processes in human glioma occurrence and development will be summarized.

The Function of the I*ncRNAs*

In human genome, no more than 2% of human genome sequence can be transcribed into protein. Most of the human genome sequence can't be transcribed into functional protein. They are transcribed into non-coding RNA (*ncRNAs*), which

include short ncRNA, mid-size ncRNA, and *lncRNA*. *LncRNAs* may be classified according to their mode of action and functions in cells such as, 1) mediators on signalling pathway, 2) serving as molecular decoys, 3) work as molecular guides for the ribonucleoprotein complexes to certain specific chromatin site, and also have 4) scaffold function for the proper complex formation [8,15]. According to GENCODE gene annotation V22, there are 15,900 human *lncRNA* genes that can produce 27,670 long non-coding RNA transcripts. However, there are only 9,894 small non-coding RNAs genes in the human genome [20]. The expression of *lncRNAs* has tissue specificity. It has been suggested through multiple studies that the brain and central nervous system express the greatest amount of *lncRNAs* of any tissue type. They have important roles in regulating transcriptional and non-transcriptional processes [21].

X-inactive specific transcript (Xist) which is the first *lncRNA* was discovered in 1990 by Brown et al. first. They found Xist as a novel protein non-coding RNA only expressed by inactive X-chromosomes only in female mammals in their study. It was revealed that Xist can silence one of X-chromosome activation by coating one X-chromosome leading to its epigenetic function [22]. Later, many *lncRNAs* were described successively as a sort of important regulators.

The nucleus is a highly structured cellular compartment. The chromatin-associated processes such as DNA replication, transcription, RNA processing and RNA export were organized and regulated in nucleus. Recent studies showed that *lncRNAs* had the complex secondary structures and played roles in these processes including mRNA splicing, nuclear localization, cell survival, cell cycle, and migration [19,21,23-26]. Many studies have indicated that *lncRNAs* have roles in various cancers as prognostic markers. For example, *HOTAIR* has a role in colorectal cancer and *MALAT1* has a role in non-small cell lung cancer [27-29]. Many reports showed they also had roles in breast cancer. In high-grade glioma *HOTAIR* is overexpressed whose upregulation is predictive of poor prognosis. In addition, *HOTAIR* has an important role in cell cycle progression, but the mechanism is not clear till now [29,30].

In different human glioma subtypes (Astrocytoma, less dendritic cell tumor, tumor of the ventricular canal, and glioblastoma) *lncRNAs* are differentially expressed and some *lncRNAs* are associated with biological characteristics of human glioma in the same subtype. In human glioma several *lncRNAs* may possibly play a vital role in cancer occurrence, metastasis, drug resistance and recurrence [8,13,16]. Many studies have shown that inhibiting or overexpression of specific *lncRNAs* can have an effect on the process of human glioma progression, showing a potential therapeutic application of *lncRNAs* in human glioma. Some specially *lncRNA* may not only affect the biologic processes of human glioma, but also modulate the function of the vascular endothelial cells which is associated with the Blood-Tumor Barrier (BTB) that contributes to the failure of conventional chemotherapy by restricting sufficient drug molecules delivery to tumor tissues [18]. If we know the mechanism how the *lncRNA* influence the BTB, we can find a new method to improve the prognosis of glioma. The purpose of this review was to summarize the involvement of different *lncRNAs* in the human glioma aggression, metastasis, and chemoradiotherapy resistance processes.

lncRNAs in Human Glioma Bioprocesses

Many studies have shown *lncRNAs* involving in occurrence and development of human glioma as tumorigenic factors or tumor suppressor. These *lncRNAs* have the aberrant expression level in malignant glioma compared to normal tissues. *LncRNAs* can work as molecular signalling mediators which modulate a certain set of gene expression [12]. *LncRNAs* that serve as molecular decoys can take proteins or RNAs away from a specific location [31]. *LncRNAs* can take part in the assembly of protein complexes. *LncRNAs* work as the molecular guides through locating certain ribonucleoprotein complexes to a specific target site on the chromatin [17].

H19, a 2.3 kb carcinogenic *lncRNA* which locates on human chromosome 11p15.5, doesn't contain any known open reading frames [32,33]. *H19* is located on the downstream of Insulin-like Growth Factor 2 (*IGF-2*). They share the same imprinting mechanism. *H19* has been well studied in many different cancers, including bladder, breast, colon, glioma, pancreatic, liver, and ovarian cancers [34-39]. Recent study shows that C-Myc regulates these two genes (*H19* and *IGF-2*) independently and does not affect *H19* imprinting. C-Myc binds to evolutionarily conserved E-boxes near the imprinting control region to facilitate histone acetylation and transcriptional initiation of the *H19* promoter. C-Myc significantly induces the expression of the *H19* noncoding RNA in diverse cell types, including glioblastoma. C-Myc up-regulates *H19*, down-regulates *IGF2* transcripts and does not affect imprinting of the *H19/IGF2* locus [40]. *In vivo* assays overexpression of *H19* promotes tumor development after subcutaneous injection of *H19*-recombined cells into SCID mice [34].

Similar to *H19*, non-coding RNA Hox transcript antisense intergenic RNA (*HOTAIR*) has been characterized as a negative prognostic factor in breast and colon cancer patients. In Zhang's study, it was found that *HOTAIR* expression was closely associated with glioma grade and poor prognosis [41]. A study showed that low expression of *HOTAIR* can inhibit cell invasion, decrease cell proliferation and alter cell cycle progression. Down-regulation of *HOTAIR* can induce cell cycle G_0/G_1 arrest [42]. This suggested that *HOTAIR* played an important role in glioma molecular classification and may serve as a novel therapeutic target for classical and mesenchymal glioma subtypes. Xue et al. found that *HOTAIR* was the target of miR-326 which mediated the tumor-suppressive effects of *HOTAIR* knockdown on glioma cell lines [43]. Overexpressing of *miR-326* reduced the *FGF1* expression which played an oncogenic role in glioma by activating PI3K/AKT and MEK 1/2 pathways. They got the same findings *in vivo* [44-46]. These results provided a new potential therapeutic strategy for glioma treatment. The *HOTAIR*-

miR-326-FGF1 axis might represent a promising therapeutic strategy for the treatment of human glioma. A research by Zhang et al. demonstrated that in GBM cells, *HOTAIR* regulated cell cycle progression predominantly via the *HOTAIR* 5' domain-PRC2 axis, which was *EZH2*-dependent. *HOTAIR* 5'domain-PRC2 is a new regulatory axis that modulates cell cycle progression in GBM cells. Previous studies have indicated that *EZH2* was overexpressed in glioma stem-like cells and adult glioblastoma patient samples [28,47,48].

In order to know whether other *lncRNAs* influence the profiling of human glioma, Kraus finished a research this is to find *lncRNAs* that have roles in human glioma [49]. They found that not only *H19* and but also *HOXA6as*, *Zfhx2as* and *BC200* are suitable as normalisers in glioma and normal brain. These *lncRNAs* are applicable for the accurate normalisation of *lncRNA* expression profiling in various glioma alone and in combination with brain tissue. This enables to perform valid longitudinal studies, e.g. of glioma before and after malignisation to identify changes of *lncRNA* expressions probably driving malignant transformation [21,50,51]. We only know that these *lncRNAs* have important roles in profiling of human glioma, but the true mechanism is not clear.

So many studies show that there is a kind of cell which have self-renewal capacity and differentiation potential in cancer tissue [52,53]. The first solid CSCs were identified in human breast carcinoma by Hajj et al. [54]. They isolated a small cellular subpopulation which has self-renewal capacity from breast tumors patients. Marco used custom microarrays to examine Ultra-Conserved Regions' (UCR) expression across samples from different tissues and different types of cancer. The expression in embryonic stem cells of selected UCRs was validated by real time PCR. In their study, they found the *uc. 283-plus lncRNA* was highly expressed in some solid cancers and associated with pluripotency. It is showed in their research that *uc.283-plus* was over-expressed in glioma samples. The high expression of *uc.283-plus* in glioma is correlated with a 'cancer stem cell phenotype', a well-studied event occurring in glioma [55,56]. Lujambio et al. identified RNA in the *uc.283* genomic region but transcribed from the opposite strand in various types of cancer cell lines [57]. They also showed that *uc.283-minus* could be regulated by epigenetic alteration. Recently, Hudson et al. produced a list of the possible ucRNA-mRNA interactions based on sequence complementarity according to the thermodynamics of the loop-loop RNA interactions [58-60]. The mechanism of the *lncRNA uc.283* working in the human glioma is not clear till now. These studies may be a starting point for the further characterization of *lncRNA uc.283* in human glioma tissues and the role mechanism of the *lncRNA uc.283*.

As everyone knows, there is Blood-Brain Barrier (BBB) in the body. The BBB is a highly selective permeability barrier that separates the circulating blood from the brain Extracellular Fluid (BECF) in the central nerve system. The BBB acts very effectively to protect the brain from many common bacterial infections [61]. It can also affect the absorption and effects of drugs. One of the reasons listed for the failure of brain chemotherapy is the presence of BBB [62]. The BBB is frequently impaired in brain tumor, creating the BTB [63]. BBB and BTB are different. The existence of BTB contributes to the failure of conventional chemotherapy by restricting sufficient drug molecules delivery to tumor tissues [18,64,65]. There are two methods for drugs cross the BTB: paracellular or transcellular. The first method is the main route of absorption for chemotherapy drug molecules [65-68]. If we can know the regulatory roles of *lncRNAs* on BTB permeability, we may investigate novel methods to safely open the BTB. For BTB, when BTB permeability is increased, the expressions of tight junction proteins including ZO-1, occludin, and claudin-5 will be significantly down regulated [69]. Taurine upregulated gene 1 (*TUG1*) is a *lncRNA* located at chromosome 22q12 which was originally identified that plays crucial roles in vision system [18,70]. Beside, *TUG1* is required for regulating carcinogenesis in several of tumors [71,72]. Heng et al. have finished a research whose purpose was to know the *lncRNA TUG1*'s role in BTB. Their research indicated that knockdown of *TUG1* increased BTB permeability via binding to miR-144 and then reducing endothelial cells tight junction protein expression by targeting *HSF2* [18]. In their recently study [73], they found that *TUG1*'s up expression was associational with the tumor-induced endothelial cell proliferation, migration and tube formation. Their study indicated that knockdown of the *TUG1* reduced the expression of vascular endothelial growth factor A (VEGFA). *MiR-299* worked as a bridge in this progress. The mechanism of this progress maybe *TUG1* make the VEGFA up-expression through the *miR-299* was down-expression. In future, *TUG1* may provide a novel therapeutic target for glioma treatment.

As we know, human glioma is the most common and most aggressive malignant primary brain tumor in adult. The prognosis of disease is poor and the reason for this disease is not clear. In recent years, many researches indicated that *lncRNAs* not only have important roles in many normal bio-progresses but also in many diseases especially in many cancers such as human glioma and breast cancer. In this review, we summary the *lncRNAs* which have roles in the human glioma. *H19*, *HOTAIR*, *HOXA6as*, *Zfhx2as* and *BC200*, *uc.283-plus* and *TUG1* have roles in the human glioma, but they work in different progresses of the disease (Table 1). In this review, we summarized the roles of different *lncRNAs* in the glioma. The regulation mechanisms of *lncRNA* in the glioma biological processes are not clear. We think the *lncRNAs* not only have roles in the biological processes of the glioma but also related to the recurrence rate and survival time of the patients. The epigenomic reprogramming by *lncRNAs* can be the real reason for the human glioma. Till now, the human glioma is mainly classified according to the pathological type. Recent studies show that the prognosis of the human glioma is related to the expression of *lncRNAs* type and the expression quantity of the *lncRNAs*. *LncRNAs* may be useful future therapeutic targets for human glioma and can be used to classify the human glioma types. *LncRNAs* could serve as a new target for targeted therapy. Therefore, clarifying the

mechanisms of *lncRNAs* in the various biological processes of the human glioma will be a critical step in exploring new strategies in future cancer therapy. *LncRNAs* may be related to the pathological grading of glioma. Different patients with the same pathological grade have the different disease progress. *LncRNAs* may be better able to respond to the disease process and to the extent of the glioma's nausea. *LncRNAs* may be a better target for clinical treatment of gliomas.

Table 1. *The characterized lncRNAs with potential roles in glioma.*

Gene	Roles
lncRNAs	mRNA splicing, nuclear localization, cell survival, cell cycle, and migration Cancer occurrence, metastasis, drug resistance and recurrence
HOTAIR	A role in colorectal cancer, predictive of poor prognosis, cell cycle progression, glioma molecular classification
MALAT1	A role in non-small cell lung cancer
FGF1	An oncogenic role in glioma by activating PI3K/AKT and MEK 1/2 pathways
H19	Overexpression of *H19* promotes tumor development after subcutaneous injection of *H19*-recombined cells into SCID mice
HOXA6as, Zfhx2as and *BC200*	Profiling of human glioma
uc.283-plus	Correlated with a 'cancer stem cell phenotype'

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