Identification of an EMT-related lncRNA signature in glioma

Chuming Tao
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China.
Scientific Research Center, East China Institute of Digital Medical Engineering, Shangrao, China

Qing Hu
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China.
Scientific Research Center, East China Institute of Digital Medical Engineering, Shangrao, China

Haitao Luo
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China.
Scientific Research Center, East China Institute of Digital Medical Engineering, Shangrao, China

Peng Wang
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China

Zewei Tu
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China

Hua Guo
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China

Kai Huang
Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China

Xingen Zhu (✉ zxg2008vip@163.com)
The Second Affiliated Hospital of Nanchang University https://orcid.org/0000-0002-8556-0941

Research

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Abstract

Background Epithelial-mesenchymal transition (EMT) has been implicated in the invasion and progression of gliomas, the present study investigated EMT-related long noncoding (Inc)RNAs in gliomas.

Methods Candidate lncRNAs screened from a glioma dataset in The Cancer Genome Atlas were used to construct EMT-related lncRNA coexpression networks in order to identify EMT-related lncRNAs; these were validated using the Chinese Glioma Genome Atlas (CGGA). Gene set enrichment analysis and principal component analysis (PCA) were used to annotate lncRNA functions. We established an EMT-related lncRNA signature composed of 9 lncRNAs (HAR1A, LINC00641, LINC00900, MIR210HG, MIR22HG, PVT1, SLC25A21-AS1, SNAI3-AS1, and SNHG18) that could distinguish between low- and high-risk glioma patients. The functions of the identified lncRNAs were evaluated by constructing a competing endogenous RNA network.

Results The low-risk group had longer overall survival (OS) than the high-risk group (P<0.0001). High-risk patients also had no deletion on chromosome arms 1p and/or 19q, expressed wild-type isocitrate dehydrogenase, and had higher World Health Organization (WHO) tumor grade. The signature was an independent factor significantly associated with OS (P=0.041, hazard ratio=1.806). These findings were validated in the CGGA dataset. PCA also revealed differences in EMT status between low- and high-risk patients. Competing endogenous RNA network showed that complex lncRNA–microRNA–mRNA interactions potentially contributing to EMT progression in glioma.

Conclusion Our results demonstrate that the EMT-related 9-lncRNA signature has prognostic value for glioma patients.

Introduction

Gliomas are the most common type of malignant brain tumor in adults[1]. Especially, the glioblastoma (WHO grade IV) is the most invasive and aggressive glioma subtype with the highest mortality rate [2; 3]. It is said that epithelial-mesenchymal transition (EMT) is closely related to glioma malignancies[4; 5], and we have known that lncRNAs orchestrate multiple cellular processes by modulating EMT in a variety of tumors cells[6]. Even though the detailed mechanisms of lncRNAs interact with EMT to promote glioma’s development and progression are not fully understood, their elucidation is critical for developing effective diagnosis and treatments.

The transdifferentiation of epithelial cells into motile mesenchymal cells—a process known as epithelial-mesenchymal transition (EMT)—plays an important role in development, wound healing, and stem cell behavior, and also contributes to the pathology of fibrosis and cancer progression [7]. Tumor cells undergoing EMT acquire resistance to apoptosis and antitumor drugs while disseminating to distant sites [8]. Thus, EMT is a hallmark of carcinogenesis, and strategies targeting EMT pathways have therapeutic potential for cancer treatment [9].
Long noncoding (Inc)RNAs are RNA molecules longer than 200 bases that do not encode proteins [10; 11] and have been shown to function as master regulators of gene expression in normal biological processes and in diseases such as cancer [12]. As an important component of competing endogenous (ce)RNA networks, IncRNAs act as micro (mi)RNA sponges to regulate protein encoding and translation in cancer [13; 14]. For example, Upregulated TMPO antisense transcript 1 is a ceRNA that sponges miR-140-5p in gastric cancer cells, thereby alleviating inhibition of SRY-box transcription factor 4, an EMT regulator [15]. Although IncRNAs related to EMT have been identified [16], little is known about their role in glioma. The IncRNA–microRNA–mRNA ceRNA networks link the function of protein-coding mRNAs with that of non-coding RNAs such as microRNA, long non-coding RNA, pseudogenic RNA, and circular RNA. We learned that LINC01133 inhibits gastric cancer progression and metastasis by acting as a ceRNA for miR-106a-3p to regulate adenomatous polyposis coli (APC) gene expression and the Wnt/β-catenin pathway[17]. And the SMAD5-AS1 target miR-135b-5p to inhibits diffuse large B cell lymphoma proliferation in the same pathway[18]. So, the deregulation of ceRNA networks may an important factor in lead to human diseases including cancer[13].

In this study, we used data from The Cancer Genome Atlas (TCGA) to establish an EMT-related IncRNA signature for glioma that was subsequently validated using data from the Chinese Glioma Genome Atlas (CGGA). We also established a IncRNA-mediated ceRNA network to investigate the downstream targets and mechanisms of EMT-related IncRNAs (Fig. 1).

Material And Methods

Patients and datasets

Clinical information of glioma patients was obtained from TCGA (https://cancergenome.nih.gov/) and CGGA (http://www.cgga.org.cn) websites; the 2 datasets were used for training and validation, respectively. Clinicopathologic information of the study population is shown in Fig. S1.

LncRNA profiling

The LncRNA profile was determined using an established mining method[19]. Briefly, genes were identified as protein-coding or noncoding based on their Refseq IDs, and only long noncoding genes in the NetAffx annotation files were retained. A total of 14,142 LncRNAs were obtained from the TCGA dataset, among which 215 were EMT-related as determined by gene set enrichment analysis (GSEA) (http://www.broadinstitute.org/gsea/index.jsp) [20]. Ultimately, 162 EMT-related LncRNAs from TCGA and 152 EMT-related LncRNAs from CGGA were retained to construct EMT LncRNA coexpression networks (P ≤ 0.001) using Cytoscape v3.7.0 software (The Cytoscape Consortium, San Diego, CA, USA).

Establishment of a LncRNA signature

To establish a LncRNA signature for predicting survival, univariate Cox analysis was carried out with the 2 datasets. We screened LncRNAs with prognostic significance and identified 16 EMT-related LncRNAs that were closely associated with glioma patient survival (P < 0.01). We then assigned a risk score using the
least absolute shrinkage and selection operator (LASSO) Cox regression algorithm [21; 22; 23] and defined the inclusion criteria for the 9 lncRNAs and their constants, selecting the optimal penalty parameter $\beta$ for a minimum 10-fold cross validation in the training set. The risk score was calculated with the following formula:

$$\text{Risk score} = \beta_{\text{gene1}} \times \text{expr}_{\text{gene1}} + \beta_{\text{gene2}} \times \text{expr}_{\text{gene2}} + \ldots + \beta_{\text{genen}} \times \text{expr}_{\text{genen}}$$

where expr_{genen} is the expression level of a lncRNA. Low- and high-risk groups were distinguished based on median risk score.

**Construction of a ceRNA network**

The interaction of lncRNAs, miRNAs, and mRNAs in glioma was predicted based on overlapping miRNA seed sequence binding sites in lncRNAs and mRNAs. The interaction between the 9 identified EMT-related lncRNAs and miRNAs was first predicted using the MiRcode database, which provides whole-transcriptome human miRNA target predictions based on the GENCODE annotation and includes > 10,000 long noncoding RNA genes. miRTarBase, TargetScan, and miRDB databases were then used to identify aberrantly expressed miRNA–mRNA pairs. We analyzed only target mRNAs that matched the databases. Finally, lncRNA–miRNA and miRNA–mRNA pairs were merged based on shared miRNAs into a ceRNA network of EMT-related lncRNAs, which was visualized using Cytoscape v3.7.0 software.

**Statistical analysis**

The expression of EMT-related lncRNAs between the two risk groups and their relationship with WHO grades were analyzed using one-way ANOVA. To compare glioma characteristics with different clinical pathology risk scores, a one-way ANOVA or t-test was conducted to compare risk scores for patients grouped by clinical or molecular pathology characteristics. Uni- and multivariate Cox regression analyses and principal component analysis (PCA) were performed using R v3.6.3 and SPSS v22 software applications (SPSS Inc, Chicago, IL, USA). GSEA was performed to investigate the functions of DEGs between the 2 groups of gliomas. Survival status was determined by univariate Cox regression analysis; the Kaplan–Meier curve was generated using Prism v6 software (GraphPad, La Jolla, CA, USA). Operating characteristic (ROC) curves were used to study the prediction efficiency of the risk signature, age, grade IDH-mutant status, and 1p/19q codeletion status. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the “clusterProfiler” package of R software ($P_{\text{adj}}<0.05$ and $Q<0.05$). GSEA was used for functional annotation of genes. A 2-sided $P$ value $< 0.05$ was considered significant for all statistical tests.

**Results**

**Identification of prognostic EMT-associated lncRNAs in glioma**
We extracted 14,142 and 4304 lncRNAs from the TCGA and CGGA datasets, respectively, and determined their expression profiles. A total of 215 EMT-related genes were extracted by GSEA (HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION M5930, ANASTASSIOU_MULTICANCER_INVASIVENESS_SIGNATURE M2572). We identified 69 EMT-related lncRNAs by constructing EMT-related lncRNA coexpression networks (cor = 0.6, P < 0.0001). Ultimately, 162 and 152 EMT-related lncRNAs were identified from TCGA and CGGA, respectively.

**Establishment of an EMT-related lncRNA signature in glioma**

To identify prognostic lncRNAs, expression data for each lncRNA in TCGA and CGGA were analyzed with a univariate Cox proportional hazards regression model (P < 0.01). We selected 137 and 92 lncRNAs related to glioma patient prognosis from TCGA and CGGA, respectively. We screened lncRNAs with prognostic significance in both datasets and identified 16 that were significantly associated with overall survival (OS) of glioblastoma patients (P < 0.01). LASSO Cox regression was used to identify the optimal prognostic lncRNAs in the TCGA data set and establish the risk signature. Nine of the 16 candidate lncRNAs retained their prognostic significance and were thus selected as independent prognostic markers (Fig. 2a–c). All the 9 EMT-related lncRNAs, LINC00900, MIR210HG, MIR22HG, PVT1, and SNHG18 were found to be risky lncRNAs with HR > 1, and HAR1A, LINC00641, SLC25A21-AS1, and SNAI3-AS1 were protective lncRNAs (HR < 1) (Table 1). Based on the median risk score, we divided glioma patients into low- and high-risk groups (Fig. S2); OS was longer in the former than in the latter (P < 0.0001; Fig. 2d). Then, we use the CGGA(n = 508) dataset as a validation dataset to calculate the risk scores. We found a significant difference in OS between the two risk groups (P < 0.0001; Fig. 2e).

**Table 1**

| Symbol           | HR  | Low95 | High95 | P value     | β Value |
|------------------|-----|-------|--------|-------------|---------|
| HAR1A            | 0.26| 0.20  | 0.36   | 5.49E-18    | -0.015  |
| LINC00641        | 0.37| 0.32  | 0.43   | 7.35E-38    | -0.053  |
| LINC00900        | 6.35| 4.70  | 8.59   | 2.67E-33    | 0.417   |
| MIR210HG         | 2.20| 1.86  | 2.61   | 7.51E-20    | 0.036   |
| MIR22HG          | 2.33| 1.96  | 2.77   | 5.13E-22    | 0.007   |
| PVT1             | 4.87| 3.82  | 6.20   | 9.08E-38    | 0.146   |
| SLC25A21-AS1     | 0.25| 0.20  | 0.31   | 8.65E-39    | -0.410  |
| SNAI3-AS1        | 0.12| 0.07  | 0.21   | 1.54E-15    | -0.366  |
| SNHG18           | 2.66| 2.30  | 3.06   | 1.79E-41    | 0.012   |

**Validation of the 9-lncRNA prognostic risk signature**
We ranked the risk scores of patients in the TCGA training set and analyzed their distribution and plotted survival status (Fig. 3a). The heatmap revealed differences in the expression patterns of the prognostic lncRNAs between low- and high-risk patient groups. The expression of 4 protective lncRNAs was upregulated whereas that of 5 risk-associated lncRNAs was downregulated in patients with low risk scores; the opposite trends were observed in patients with high risk scores. These results were confirmed in the CGGA validation set (Fig. 3b).

**Expression of 9 EMT-related lncRNAs and relationship with patient prognosis**

We evaluated the expression of the 9 EMT-related lncRNAs according to tumor grade in the TCGA training set and CGGA validation dataset. LINC00900, MIR210HG, MIR22HG, PVT1, and SNHG18 increased whereas HAR1A, LINC00641 SLC25A21-AS1, and SNAI3-AS1 decreased with tumor grade (Fig. 4a, c). LINC00900, MIR210HG, MIR22HG, PVT1, and SNHG18 levels were higher whereas those of HAR1A, LINC00641 SLC25A21-AS1, and SNAI3-AS1 were lower in the high-risk group than in the low-risk group (Fig. 4b, d). The results from the two data are consistent. We also examined the significance of these 9 lncRNAs for patient survival using the TCGA dataset. Based on the expression level of each lncRNA, the survival time associated with each gene differed significantly between high- and low-risk groups (Fig. S3). Uni- and multivariate Cox regression analyses showed that the 9-lncRNA signature was an independent factor significantly associated with OS (TCGA: \( P = 0.041 \), hazard ratio [HR] = 1.806; CGGA: \( P < 0.001 \), HR = 1.855) (Fig. 5c-f).

**Prognostic performance of risk scores and relationship with clinical status of glioma patients**

Receiver operating characteristic (ROC) curves of the TCGA dataset showed that risk score (area under the ROC curve [AUC] = 0.771), age (AUC = 0.823), tumor grade (AUC = 0.813), 1p/19q codeletion status (AUC = 0.638), and isocitrate dehydrogenase (\( IDH \)) gene mutation status (AUC = 0.847) could accurately predict survival in glioma patients (Fig. 5a). These results were confirmed using the CGGA dataset (risk score, AUC = 0.730; age, AUC = 0.630; tumor grade, AUC = 0.775; 1p/19q codeletion status, AUC = 0.613; and \( IDH \) mutation status, AUC = 0.726) (Fig. 5b), demonstrating that the prognostic signature was reliable and effective. Uni- and multivariate Cox regression analyses performed using the TCGA dataset showed that risk score, age, WHO grade, and \( IDH \) mutation status were all related to OS (Fig. 5c, d); this was confirmed using the CGGA validation dataset (Fig. 5e, f). These results suggest that risk score derived from EMT-related lncRNAs is an independent factor that predicts the prognosis of glioma patients.

**PCA and functional annotation of EMT-related lncRNAs**

We carried out PCA to investigate differences between low- and high-risk groups based on EMT-related and overall gene expression profiles (Fig. 6a–d). Low- and high-risk glioma patient groups were distinguished based on EMT gene expression levels. We also found that EMT status was associated with a specific lncRNA signature. We performed GO and KEGG pathway analyses of the top 3000 genes that
were differentially expressed between low- and high-risk groups and found that differentially expressed genes (DEGs) were enriched in the following GO terms: M phase of cell cycle, Apoptosis, Tumor necrosis factor-mediated signaling pathway, and Regulation of cell adhesion. Additionally, KEGG pathway analysis revealed that Focal adhesion, Adherens junction, Pathways in cancer, and Cell adhesion molecules were significantly enriched (Fig. 6e, f). Functional annotation by GSEA showed that DEGs between the 2 groups were enriched in Epithelial-mesenchymal transition, E2F targets, P53 Pathway and IL6/JAK/STAT3 signaling (Fig. 6g). These results indicate that the two risk groups strongly correlated with the malignancy of glioma cells.

**LncRNA-mediated ceRNA network and mechanisms of glioma tumorigenesis**

To further investigate the regulatory role of lncRNAs in glioma, a lncRNA–miRNA–mRNA network was established by integrating expression profile data and regulatory relationships. We identified 220 EMT-related lncRNA–miRNA pairs comprising 4 lncRNAs and 28 miRNAs. miRNA–mRNA pairs were predicted using the miRTarBase, TargetScan, and miRDB databases; all target mRNAs with matches in these databases were included in the analysis. In total, 145 target mRNAs (Table 2) were included in the coexpression network that combined lncRNA–miRNA and miRNA–mRNA pairs (Fig. 7a). This ceRNA network revealed complex lncRNA–miRNA–mRNA interactions that could potentially contribute to EMT in glioma. GO and KEGG pathway enrichment analyses showed that target genes of ceRNAs were mainly enriched in the following biological functions and pathways: Vasculogenesis, Adherens junction organization, Ras protein signal transduction, Mitogen-activated protein kinase signaling, miRNAs in cancer, Transcriptional misregulation in cancer, Focal adhesion, and Glioma (Fig. 7b, c). We also identified genes of related functions and pathways and examined the enrichment of each functional pathway (Fig. 7d–g). Thus, the EMT-related ceRNA network plays an important role in glioma progression.
### Table 2
The EMT-related lncRNAs, miRNAs, and mRNAs included in the ceRNA network

| The type of RNAs | Gene symbols |
|-----------------|--------------|
| **LncRNA**      | MIR22HG, HAR1A, MIR210HG, PVT1 |
| **miRNA**       | hsa-miR-23b-3p, hsa-miR-20b-5p, hsa-miR-363-3p, hsa-miR-17-5p |
|                 | hsa-miR-139-5p, hsa-miR-125b-5p, hsa-miR-27a-3p, hsa-miR-125a-5p |
|                 | hsa-miR-24-3p, hsa-miR-206, hsa-miR-140-5p, hsa-miR-129-5p, |
|                 | hsa-miR-107, hsa-miR-33a-3p, hsa-miR-1244, hsa-miR-10a-5p |
|                 | hsa-miR-216b-5p, hsa-miR-876-3p, hsa-miR-490-3p, hsa-miR-217 |
|                 | hsa-miR-425-5p, hsa-miR-135a-5p, hsa-miR-22-3p, hsa-miR-3619-5p |
|                 | hsa-miR-761, hsa-miR-212-3p, hsa-miR-375, hsa-miR-613 |
| **mRNA**        | TNFAIP3, KIF23, SLC12A5, LCOR, E2F2, MET, RHOC, PHLPP2, TRIP10, PIP4K2A, GINS4, |
|                 | RRM2, MMP11, KPNA2, ZNF217, SGMS1, SAMD12, SFRP1, PDGFRA, CDKN1A, RBM47, |
|                 | ELAVL2, RUNX3, PRKCE, BTG3 |
|                 | DLGAP5, SLC16A9, PAX3, SAT1, BCL7A, GOLGA8A, RAP1B, LAMC1, WDR37, KLF10, |
|                 | EIF4EBP1, HOXA3, VEGFA, CMTM4, LHFPL4, RAPGEF4, NACC2, FZD6, RPS6KA5, KLF12, |
|                 | AKAP11, PPIA, AMOT, MAP3K9, E2F1, KIAA0513, AMOTL2, HOXA1, TEF, CNN3, RNF165, |
|                 | SPRY4, SNCG, RIMS3, EZH2, HAS2, PTGFRN, NR3C2, SMOC1, SCAMP5, WEE1, DUSP5, |
|                 | TGFBR3, CADM2, GNG12, DUSP10, TRPM6,IGF1R, NF1, HOXC6, CBX6, ELOVL2, GABBR2, |
|                 | MYT1, RPS6KA1, CPEB3, SPRY2, ERBB3, TRIM29, CALU, ARC, INMT, CCDC65, POLQ, |
|                 | SOD2, EXPH5, WT1, NR2F2, NET1, CA2, CRY2, ITGA2, KANK4, COL4A4, MMP2, FAM129A, |
|                 | PDK1, LRRCL2, MAP3K5, ENDOU, PDPN, CSRNP3, ZNF25, MXI1, FAM84B, TPPP, PPP1R3B, |
|                 | FGFR9, DDN, GATA2, EN2, MYO1B, ARPC5, F3, BCL11B, FBLIM1, BCR, KIAA1147, VDR, |
|                 | NR5A2, BRWD3, DENND5B, GABRB1, TAO1K1, C1S, CNNM2, FGFR1L, RUNX1T1, E2F7, |
|                 | SMAD7, ELAVL4, NR1D2, DACH1, ENPEP, KIAA1109, HMGB2, HOXA10, LAT52, PDE10A, |
|                 | RAP2A |

### Discussion
In this study, we identified a 9-lncRNA signature that could distinguish between low- and high-risk glioma patients based on the median risk score in TCGA. These 9 EMT-related lncRNAs have prognostic significance in glioma: patients in the low-risk group had longer OS than those in the high-risk group. Deletion of chromosomal arms 1p and/or 19q and IDH mutations are considered as useful biomarkers in glioma [2; 24; 25]; we evaluated the risk scores of IDH and 1p/19q codeletion in the TCGA dataset and found that both were related to OS. The results were confirmed using the CGGA dataset, indicating that the 9-lncRNA signature is reliable and effective for predicting prognosis. We also established a lncRNA–miRNA–mRNA ceRNA network to predict the regulatory relationships of the 9 lncRNAs; functional enrichment analysis of downstream target genes revealed that the lncRNAs are involved in tumorigenesis and cancer progression.
As an intricate genetic procedure, EMT can be utilized by both normal and tumor epithelial cells to enable them to separate from neighboring cells and migrate[26], and have influenced invasive, cancer progression and therapy resistance property of cancer cells[27; 28]. Previous reports indicate that Snail[29; 30], ZEB[31; 32], Twist[33] families and some other signaling pathways genes[34; 35; 36] have been identified as master regulators in EMT progress. More and more research shows lncRNAs epigenetically orchestrate EMT, and have promote our understanding of the molecular mechanisms that lncRNA-mediated in cancer initiation and malignancy progression[6]. LncRNAs play a critical role in diverse cellular processes in both normal and disease states [12; 37]. Specifically, lncRNAs promote or act as decoys to repress transcription, or function as epigenetic regulators or scaffolds that interact with various proteins to form ribonucleoprotein complexes [38; 39]. LncRNAs are involved in multiple signaling pathways including p53, nuclear factor κB (NF-κB), phosphatidylinositol 3-kinase/protein kinase B, and Notch, which have been linked to EMT. Among the 9 EMT-related IncRNAs identified in this study, LINC00900, MIR210HG, MIR22HG, PVT1, and SNHG18 were associated with increased risk of glioma whereas HAR1A, LINC00641, SLC25A21-AS1, and SNAI3-AS1 were protective. PVT1 is upregulated in various carcinomas, and its overexpression is associated with poor survival in patients [40]. PVT1 functions act as a ceRNA to protect mRNAs from miRNA-mediated repression and associated with the development of resistance to common chemotherapeutic agents[41; 42]. MiR210HG sponges miR-1226-3p to facilitate breast cancer cell invasion and metastasis and EMT via regulation of mucin-1c; and MIR210HG upregulation was shown to be associated with advanced International Federation of Gynecology and Obstetrics (FIGO) stage, metastasis, and poor prognosis in cervical cancer [43; 44]. SNHG18 promotes resistance of glioma to radiotherapy by repressing semaphorin 5A [45]. LINC00641 expression level was found to be decreased in breast cancer tissue, which was negatively related to tumor size, lymph node metastasis, and clinical stage [46]. SNAI3-AS1 could bond up-frameshift protein 1 (UPF1), regulate Smad7 expression, and activate TGF-β/Smad pathway, to induce EMT in hepatocellular carcinoma[47], and it also acts an important factor in the ceRNA network in Adipose Tissue From Type 2 Diabetes[48]. Therefore, the close relationship between the IncRNA and EMT we screened and the important role of IncRNA in the ceRNA network have been further confirmed in other tumors or diseases.

Although some of the identified IncRNAs have not been previously reported as associated with EMT, our results suggest that they may directly or indirectly be involved in this process. There were some limitations to our study. Firstly, our analyses were based on publically available datasets and require confirmation in other patient populations. Moreover, experiments should be carried out to validate the roles of the identified IncRNAs and their targets and the IncRNA–miRNA–mRNA ceRNA network in glioma.

Conclusions

The 9-EMT–related IncRNA signature established in this study has prognostic value for glioma patients, while the ceRNA network provides insight into the molecular basis of glioma progression as well as potential therapeutic targets for its treatment.
Abbreviations

TCGA
The Cancer Genome Atlas; CGGA: The Chinese Glioma Genome Atlas; EMT: epithelial-mesenchymal transition; OS: overall survival; GO: gene ontology; MF: Molecular function; KEGG: Kyoto Encyclopedia of Genes and Genomes; AUC: Area under the curve; GSEA: Gene set enrichment analysis; ROC: Receiver operating characteristic curve;

Declarations

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Availability of data and materials

The datasets analysed during the current study are available in the TCGA (http://cancergenome.nih.gov/) and CGGA (http://www.cgga.org.cn/) repository.

Author contributions

XZ and KH designed the research. CT, QH, and HL created tables, and figures, and were involved in manuscript writing and proofreading. HG gave analysis advice, and performed the literature search. XZ and KH supervised the research and critically read the draft manuscript. All authors proofread and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 1

Study flowchart.
Figure 2

Risk signature consisting of 9 EMT-related lncRNAs. (a, b) Inclusion criteria for the 9 EMT-related lncRNAs and their constants; the optimal penalty parameter $\beta$ was selected for a minimum 10-fold cross validation of the training set. (c) Seven EMT-related lncRNAs. (d, e) Kaplan–Meier OS curves of patients in the TCGA (d) and CGGA (e) datasets assigned to high- and low-risk glioma groups based on risk score. (f) Heatmap
of the 9 EMT-related IncRNAs in low- and high-risk glioma. The distribution of clinicopathologic features was compared between low- and high-risk groups. **P<0.01, ***P<0.001.

**Figure 3**

Distribution of risk score, OS, and gene expression in the TCGA and CGGA datasets. (a, b) Distribution of risk score and OS. (c) Heat map of the expression of 9 EMT-related IncRNAs in low- and high-risk groups. Rows and columns show IncRNAs and patients, respectively.
Figure 4

Expression of 9 EMT-related IncRNAs according to tumor grade and risk group. ***P<0.001.
Figure 5

Predictive efficiency of the 9 EMT-related lncRNA glioma risk signature. (a, b) Association between WHO grade, age, 1p19q codeletion status, IDH mutation status, and survival rate. (c–f) Uni- and multivariate Cox regression analyses of the association between clinicopathologic factors (including risk score) and OS of patients in the TCGA (c, d) and CGGA (e, f) datasets.
Figure 6

Distinct EMT status in low-risk vs high-risk glioma patients. (a) PCA between low- and high-risk glioma patient groups based on total gene expression profiles. (b) PCA between low- and high-risk groups based on EMT-related genes. (c, d) PCA of low- and high-risk groups based on all candidate EMT-related lncRNAs (c) and the 9 identified EMT-related lncRNAs (d). (e, f) GO and KEGG pathway enrichment analyses of DEGs in low- and high-risk groups. (g) Significant enrichment of EMT-associated functions in the high-risk group, as determined by GSEA.
Figure 7

EMT-related ceRNA network and functional analysis of EMT-related IncRNAs. (a) Global view of the ceRNA network in glioma. Circles, triangles, and diamonds represent IncRNAs, miRNAs, and mRNAs, respectively. (b, c) GO (b) and KEGG pathway enrichment (c) analyses of mRNAs in the network. The horizontal axis shows the number of enriched genes and the color intensity represents the corrected P
value. (d, f) Genes in significant bioprocesses. (e, g) Cluster analysis of genes and pathways with related functions, and enrichment of each functional pathway.

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