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Original Paper

H19 Functions as a Competing Endogenous RNA to Regulate EMT by Sponging miR-130a-3p in Glioma

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Key Words
H19 • Epithelial-to-mesenchymal transition • Glioma

Abstract
Background/Aims: Glioma is one of the most devasting tumors and confers dismal prognosis. Long noncoding RNAs(lncRNAs) have emerged as important regulators in various tumors including glioma. A classic lncRNA-H19, which is found to be highly expressed in human glioma tissues and cell lines, and is associated with tumor progression thus predicating clinical outcomes in glioma patients. However, the overall biological functions and their mechanism of H19 in glioma are not fully understood. ***Methods:*** Firstly, we analyzed H19 alterations in different grades of glioma tissues through an analysis of 5 sequencing datasets and qRT-PCR was performed to confirm the results. Next, we evaluated the effect of H19 on glioma cells migration, invasion and EMT process. Luciferase assays and RIP assays were employed to figure out the correlation of H19 and SOX4. ***Results:*** H19 was overexpressed in glioma tissues. Down-regulation of H19 led to the inhibition of migration, invasion and EMT process with a reduction in N-cadherin and Vimentin. H19 and SOX4 are both direct target of miR-130a-3p. H19 could compete with SOX4 via sponging miR-130a-3p. ***Conclusion:*** Taken together, these results provide a possible function of H19 as an oncogene in glioma tissues and provide a potential new therapeutic strategy for human glioma.

Introduction

Glioma is the most common primary tumor in the brain. The most malignant form, glioblastoma multiforme (GBM), is one of the most aggressive human tumors and is characterized by invariably aggressive biological features [1, 2]. Despite invasive therapeutic strategy, maximal surgical resection followed by radiotherapy with chemotherapy, the median survival of GBM patients is less than 16 months [3, 4]. Therefore, it is essential to Figure out the mechanism contributing to the aggressive phenotype of glioma. Epithelial-mesenchymal

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transition (EMT), a biological process in which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties becoming mesenchymal cells [5]. At present, EMT has also been shown to occur in a variety of epithelial tumors, such as breast cancer [6], colorectal cancer [7] and esophageal squamous cell carcinoma [8] etc. Glioma occurs in neuroectodermal tissue and is classified as neuroepithelial tumors, which is one of the most common malignant tumors in nervous system. Malignant glioma surrounded into the normal tissue in the early stage of tumor formation, which is the main reason for the malignant glioma progression and recurrence [9]. Therefore, our focuse on malignant glioma EMT process play an important role in reversing the malignant progression and reducing the recurrence.

Long non coding RNA (lncRNA) is a class of non-protein coding transcripts with more than 200 base pairs in size [10]. As a large number of novel lncRNAs are found and annotated, the mechanism of lncRNA need to be explored in depth. In 2011, Pandolfi etc. officially launched ceRNA hypothesis: the transcriptions could competitively combine with same microRNAs via its endogeneous microRNA-recognition elements (MREs), reducing the inhibition of miRNAs to target transcripts and improve the expression of target transcriptions, to realize the "communication" between transcriptions [11]. After that, many studies have revealed that this ceRNA activities of lncRNA play important roles in proliferation, apoptosis, invasion and metastasis processes in tumor cells [12-16]. The lncRNA H19 is one of the pivotal players in tumorigenesis [17]. H19 is a maternally expressed and paternally imprinted 2.7 kb gene and it resides close to the telomeric region of chromosome 11p 15.5 [17, 18]. Recent studies have highlighted the oncogenetic effects of H19 in various cancers such as gastric, colorectal and breast cancers [19-21]. Our previous study revealed that H19 was highly expressed in gliomablastomas and could promote glioma cell invasion by inducing miR-675 expression [22]. However, deeper mechanism of H19 as a ceRNA regulating the phenotype of glioma needs to be explored.

In this article, we showed that H19 was significantly upregulated in malignant glioma cells and the clinical malignant glioma tissue samples compared to the low grade samples. Ectopic overexpression of H19 induced EMT process in LN229 glioma cells, while, knockdown of H19 decreased EMT process in U87 and U251 cells. In addition, knockdown of H19 decreased glioma growth *in vivo*. Moreover, we found that H19 functions as a competing endogenous RNA to regulate EMT process by sponging miR-130a-3p in glioma, which provide a theoretical basis for the molecular diagnosis and Precision Medicine of glioma in the future.

Materials and Methods

Human tissue samples

Twenty frozen glioma tissues were obtained from the Department of Neurosurgery at the First Affiliated Hospital of Nanjing Medical University. Tissue samples were obtained at surgery and immediately frozen in liquid nitrogen. The use of human glioma tissues were approved by Research Ethics Committee of Nanjing Medical University (Nanjing, Jiangsu, China) and written informed consent was obtained from all patients.

Cell culture

The human glioma cell lines U87, U251, A172, LN229 and U118 were purchased from the Chinese Academy of Sciences Cell Bank(Shanghai, China) and were mantained in Dulbecco's modified Eagle's medium(DMEM) with high glucose and sodium pyruvate supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 100 mg/mL streptomycin). Normal human astrocytes (NHAs) were obtained from Lonza (Basel, Switzerland) and cultured in the provided astrocyte growth media supplemented with rhEGF, insulin, ascorbic acid, GA-1000, L-glutamine and 5% FBS. All the cells

were incubated at 37°C in a humidified atmosphere with 5% CO2. Neurosphere culture cells were grown in stem cell medium consisting of DMEM/F12 (Gibco) supplemented with 1% N2, 2% B27 (Invitrogen), 20 ng/mL epidermal growth factor, and fbroblast growth factor–2 (Invitrogen) as previously indicated [23].

Lentiviral packaging and stable cell line establishment

The lentiviral packaging kit was purchased from Genechem (Shanghai, China). A lentivirus carrying hsa-miR-130a-3p or hsa-miR-negative control (miR-NC) was packaged in the human embryonic kidney cell line, 293T, and the virus were collected according to the manufacturer's instruction. Stable cell lines were established by infecting lentivirus into U87, U251 and LN229 cells, followed by puromycin selection.

RNA isolation and quantitative real-time PCR(qRT-PCR)

RNA was extracted from harvested cells or human tissue samples with Trizol reagent according to the manufacturer's instructions (Invitrogen). A stem-loop-specifc primer method was used to measure the expression levels of miR-130a-3p, as described previously [24, 25]. Expression of U6 was used as an endogenous control [26]. The cDNAs were amplifed by qRT-PCR using SYBR Premix ExTaq (Takara) on a 7900HT system. Primers were purchased from Ribobio. And fold changes were calculated by relative quantifcation (2-ΔΔCt). The PCR reaction for H19 was performed as previously described [22]. Each experiment was performed in triplicate.

Protein extraction and immunoblotting

Protein extraction and Western blot were performed as described previously [27].

Cell migration and invasion assay

Migration and Invasion assays were performed as previously described [22].

Luciferase assay

U87 and U251 cells were seeded in a 24-well plate and co-transfected with the WT or Mut SOX4 or H19 report plasmid, a Renilla luciferase (pRL) plasmid, and the miR-130a-3p mimic or miR-ctrl. Luciferase activities were analyzed after transfection 24h using the Promega Dual Luciferase Reporter Assay System (WI, USA).

RIP assay

MS2bs-based RNA Binding Protein Immunoprecipitation Assay (RIPA) was adopted to test the combination of lncRNA and miRNA. After the co-transfection of pcDNA3-control-MS2bs, pcDNA3-H19-12X-MS2bs and miR-130a-3p or miR-133-3p mimics, we used the anti-MS2bs Magnetic Beads for RIP experiments and finally used the Real time PCR to detect the H19 and miR-130a-3p (miR-133-3p as a negtative control).

In vivo study

Male BALB/c-A nude mice at 6 weeks of age were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences. We investigated the therapeutic potential of H19 using U87 glioma cells in a xenograft model. The mice were randomly assigned into two groups and intracranially implanted with 5×10^5 U87 cells (pretreated with lentivirus H19 or negative control sequences) using a stereotactic instrument. Bioluminescence imaging was used to detect intracranial tumor growth. The mice were anesthetized, injected intraperitoneally with D-luciferin at 50 mg/mL and imaged with the IVIS Imaging System (Caliper Life Sciences) for 10-120 s. The Living Images software package (Caliper Life Sciences) was used to determine the integrated flux of photons (photons per second) within each region. Additionally, the overall survival of the mice was monitored during the experimental period. All animal experiments were approved by the Animal Management Rule of the Chinese Ministry of Health (document 55, 2001) and were conducted in accordance with the approved guidelines and experimental protocols of Nanjing Medical University.

Statistical analysis

All experiments were performed three times. And all data are presented as mean±standard error (SD). Data were analyzed with SPSS 10.0. Statistical. Evaluation of the data was performed by t test (two-sided) and one-way-ANOVA. P<0.05 was considered statistically significant.

Results

H19 is overexpressed in glioma tissues and cell lines

Firstly, we analyzed H19 expression level in 5 independent glioma gene expression datasets (CGGA, TCGA, REMBRANDT, GSE16011 and GSE4290) to examine the association between H19 and glioma grades (Fig. 1A). One-way ANOVA showed that H19 was significantly overexpressed in high grade glioma (HGG) tissues (P<0.01 for CGGA, TCGA and REMBRANDT and P=0.1857, P=0.0129 for GSE16011 and GSE4290, respectively). Next, we investigate the correlation between H19 expression and overall survival using Kaplan-Meier survival curve analysis with a long-rank comparison. HGG samples expressing higher level of H19 were associated with decreased survival compared with low grade glioma (LGG) samples with lower H19 levels in CGGA, TCGA, REMBRANDT and GSE16011 (P=0.0110, P=0.214, P=0.0011 and P<0.0001 respectively) (Fig. 1B). Further, we validated the expression levels of H19 in 10 LGG and 10 HGG (10 grade II, 4 III and 6 IV) samples by qRT-PCR and the results were consistent with the 5 datasets mentioned above (Fig. 1C). Moreover, the levels of H19 expression in glioma cell lines (U87, U251, A172 and U118) were significantly higher than normal astrocyte cells (Fig. 1D). These results indicated that H19 maybe act as a tumor promoter in glioma tissues.

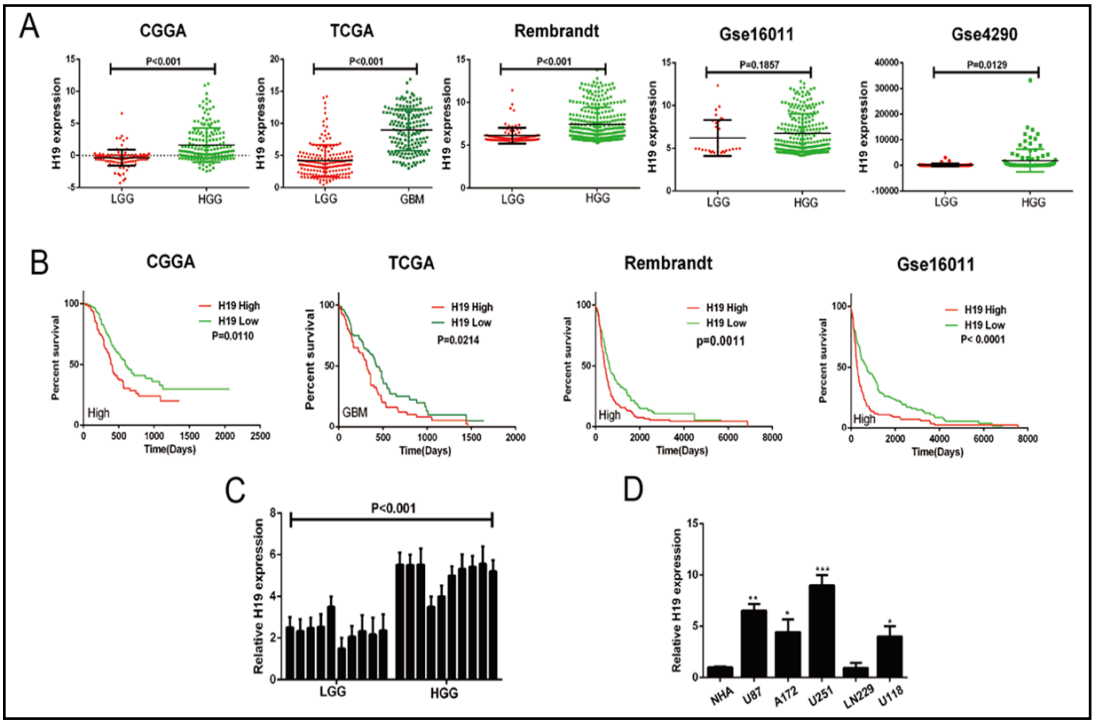

Fig. 1. H19 is overexpressed in glioma tissues and cell lines. A: Relative expression of H19 in glioma tissues was analyzed by using different datasets. Differences in variables between groups were evaluated by one-way ANOVA. B: Kaplan-Meier overall survival curve was depicted according to H19 expression levels. C: H19 expression was analyzed by qRT-PCR in glioma tissues. D: H19 expression was analyzed by qRT-PCR in glioma cell lines and Normal human astrocytes. Error bars indicate mean±standard errors of the mean. *P<0.05, **P<0.01.

H19 overexpression induces EMT process in glioma cells

To further investigate the effect of H19 on glioma progress, we overexpressed H19 in LN229 (LN229-H19), which expresses lower level of H19 than other glioma cells (Fig. 1D), and qRT-PCR showed that LN229-H19 expressed higher level of H19 than LN229-Vector (Fig. 2A). Compared with other epithelial-type tumors, E-cadherin was poorly expressed in gliomas due to the absence of the structure of epithelial cells such as basement membranes. Therefore, researches on EMT of glioma are more concerned with the expression changes of mesenchymal phenotype markers: Vimentin and N-cadherin. As expected, mRNA expression levels of N-cadherin and Vimentin were higher in LN229-H19 (Fig. 2B). The protein levels showed similar results (Fig. 2C). Gain-of-H19 function could result to EMT progress in GBM cells, we asked whether loss of H19 could cause sensitization. We infected U87 and U251, which express high level of H19 as showed above, with H19 shRNA and confirmed by qRT-PCR analysis (Fig. 2D). Similarly, we next examined the mRNA and protein expression level of N-cadherin and Vimentin in U87-shH19, U87-Vector and U251-shH19 and U251-Vector cell lines. As expected, down-regulation of H19 induced decreased expression of N-cadherin and Vimentin (Fig. 2E and F). Together, these results suggest that H19 overexpression contributes to development of EMT progression.

Overexpression of H19 in GBM cells promotes migration, invasion and neurosphere formation

EMT processes are usually accompanied by changes in cell mobility and invasiveness. Therefore, we tend to focus on the effects of H19 on mobility and invasion in GBM cells. As shown in Fig. 3A and D, overexpression of H19 in LN-229 promoted the migration and invasion ability confirmed by wound healing and transwell assays. Meanwhile, knockdown of H19 in U87 and U251 weakened their migration and invasion abilities (Fig. 3B, C and D). These findings suggest that overexpression of H19 could induce glioma cell malignancy.

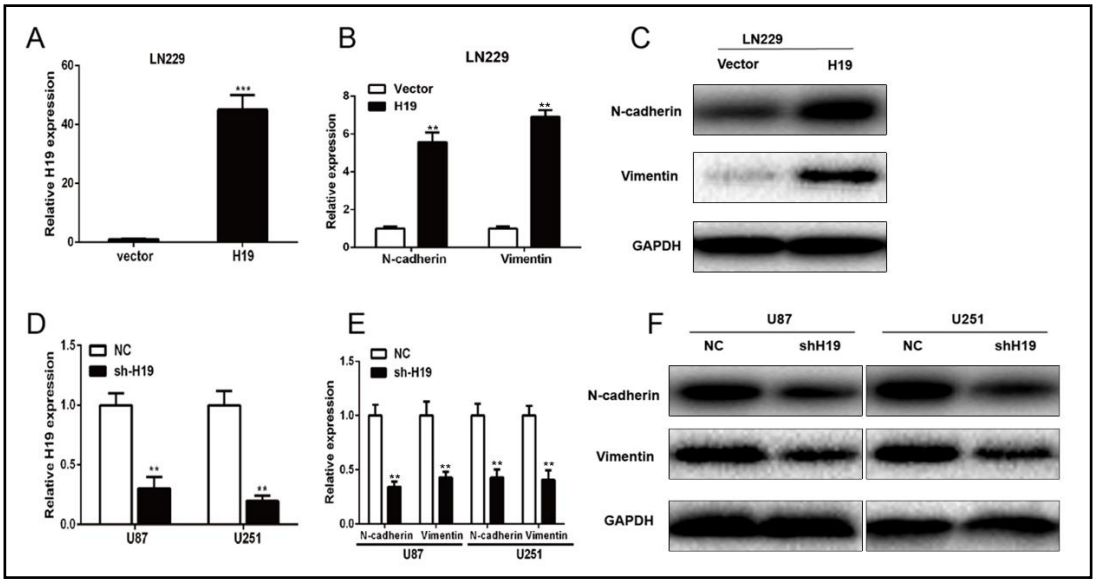

Fig. 2. H19 overexpression induces EMT process in glioma cells. A: H19 transfection in LN229 cells was confirmed by qRT-PCR. B: RT-PCR showed that overexpression of H19 could elevate the mRNA level of N-cadherin and Vimentin. C: Western-blot test found that overexpression of H19 in LN-229 cells could increase the level of N-cadherin and Vimentin. D: H19 knockdown lentivirus transfection in U87 and U251 cells was confirmed by qRT-PCR. E: qRT-PCR showed that knockdown H19 could reduce the mRNA level of N-cadherin and Vimentin. F: Western-blot test found that knockdown H19 could reduce the level of n-cadherin and Vimentin. The data represent the mean±SD from three independent expriments. *P<0.05, **P<0.01.

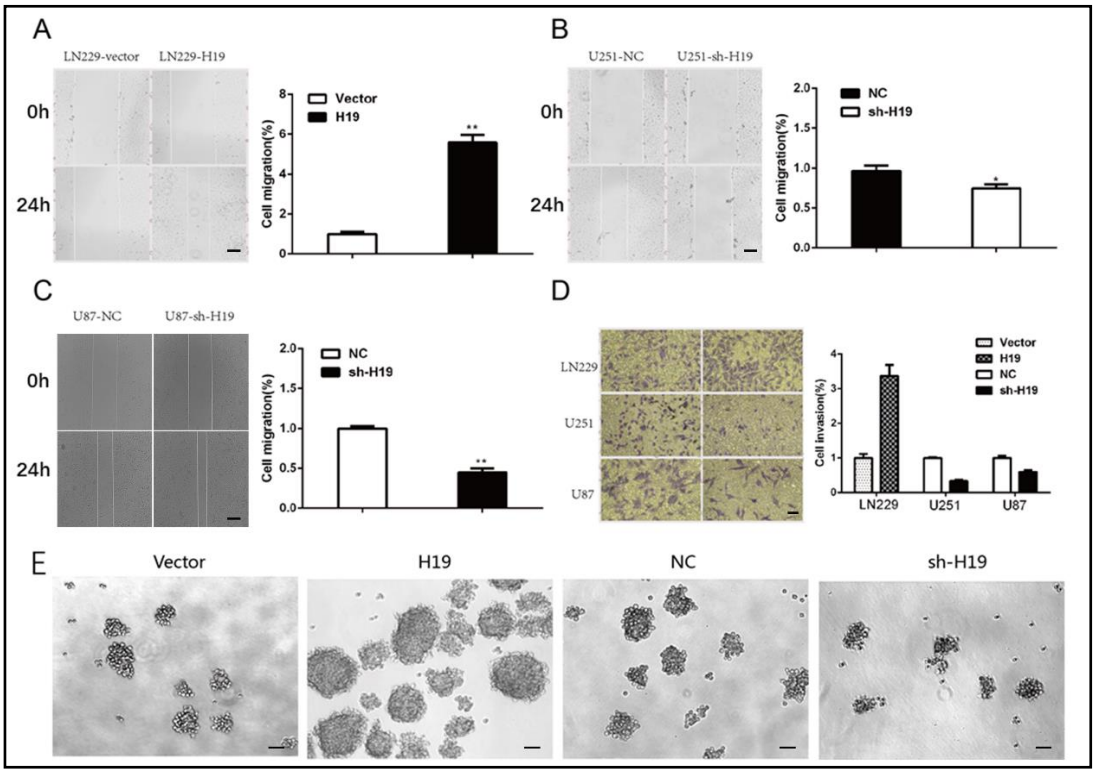

Fig. 3. Overexpression of H19 in GBM cells promotes migration, invasion and neurosphere formation. A, B and C: Wound healing assays were used to determine the migration of glioma cells 24 hours post-transfection. Scale bar=200μm. D: Invasion of glioma cells were measured by Marigel-coated transwell assays 24 hour post-transfection. Scale bar=100μm. E: Neurosphere formation capacity was determined by self-renewal assays. Scale bar=150μm. The data represent the mean±SD from three independent experiments. *P<0.05, **P<0.01.

Previous studies suggested that EMT process correlated with tumor stem cells formation. So, we became interested in exploring the effect of H19 on GBM self-renewal ability. We achieved "stem-like" neurosphere from U87 glioma cell lines by growing them in stem-like conditions. As shown in Fig. 3E, H19 overexpression promoted neurosphere formation while reduced H19 expression induced opposite results.

H19 overexpression promotes GBM xenograft growth in vivo

Considering the remarkable aggressive effects of H19 on GBM cells *in vitro*, we extended our investigation to examine whether H19 could accelerate gliomablastoma growth *in vivo* using nude mice. Before implantation, we knocked down H19 in U87-luciferse cells with lentivirus infection. Bioluminescence imaging showed the inhibition of tumor growth in the H19 knocking-down(U87-shH19-luc) group compared with the control group (Fig. 4A and B). Surival analysis also demonstrated significantly better outcome for the animals injected with U87-shH19-luc cells (Fig. 4C).

H19 and SOX4 are target genes of miR-130a-3p

To further understand the molecular action of H19 in glioma tissues, online bioinformatics databases (DIANA Tools) were employed. We observed that H19 sequence contains the potential binding sites of numerous miRNAs. We then performed dual luciferase reporter assays to determine the most interactive miRNA from top five predictions (mir-454-3p, mir-4617-3p, mir-29b-3p, mir-130a-3p and mir-29c-3p). Briefly, HEK293T cells were co-transfected with plasmid encoding these miRNAs or control sequence and luciferase plasmid harboring the sequence of H19. The results showed that mir-130a-3p could maximumly

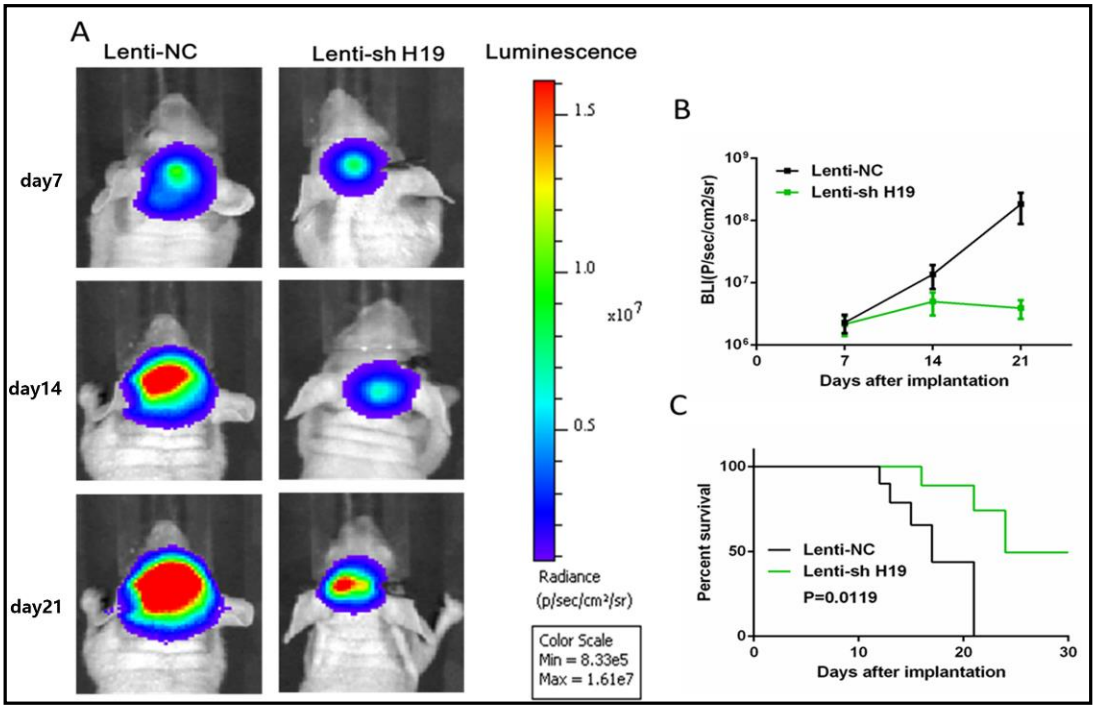

Fig. 4. H19 overexpression promotes GBM xenograft growth in vivo. A: Representative images of mice implanted with intracranial tumors formed by U87 on days 7, 14 and 21. B: Plot of the Fluc activity by bioluminescence imaging for intracranial tumors. C: Kaplan-Meier survival curve of mice injected with U87 transfected with Lenti-NC or Lenti-shH19 lentivirus. *P<0.05, **P<0.01.

suppress H19-driven luciferase activity (Fig. 5A). Thus, we chose miR-130a-3p as object for further study. Then, we performed luciferase reporter assays to determine whether miR-130a-3p could directly bind to H19. U87 and U251 cells were co-transfected with vectors harboring wild-type or mutant H19 binding sites and miR-130a-3p mimics (Fig. 5B). Luciferase activity in U87and U251 cells was markedly reduced after co-transfected with wild-type vector and miR-130a-3p mimics (Fig. 5C). Next, we co-transfected pcDNA3-H19-12X-MS2bs plasmid or vector plasmid (pcDNA3-control-MS2bs) with MS2bs-Flag plasmid. RNA immunoprecipitation (RIP) assays were performed and the results showed that miR-130a-3p was detected in MS2 immunoprecipitates and its levels were significantly increased in MS2 immunoprecipitates purified from cells overexpressing H19 (Fig. 5D). Notably, H19 knockdown significantly increased the expression of miR-130a-3p while overexpression of H19 in LN229 cells decreased the miR-130a-3p level (Fig. 5E).

To identify the underlying role and mechanism of mir-130a-3p, several bioinformatic algorithm (TargetScan, miRDB, microT-CDs and miRWalk) were applied to predict potential targets and SOX4 was chosen as our object (Fig. 5F). Sox4 is an important member of the SoxC (SRY-related high-motility group box) transcription factors family and is the first transcription factor found in the SOX family [28]. Numerous studies have suggested that SOX4 is over expressed in different cancers and correlative with tumor development [29, 30]. The regulation effect of Sox4 on EMT is mainly related to TGF-β/Smad pathway, Wnt signaling pathway and its own transcription factor activity [31, 32]. Similarly, we then performed luciferase reporter assays to determine whether miR-130a-3p could directly bind to the 3'-UTR of SOX4. U87 and U251 cells were co-transfected with vectors harboring wild-type or mutant SOX4 3'-UTRs and miR-130a-3p mimics (Fig. 5G). Luciferase activity in U87and U251 cells was markedly reduced after co-transfected with wild-type vector and miR-130a-3p mimics (Fig. 5H). In addition, after miR-130a-3p mimics infection in U87 and U251, Sox4 protein expression was significantly decreased (Fig. 5I). Taken together, H19 and SOX4 are both direct targets of miR-130a-3p.

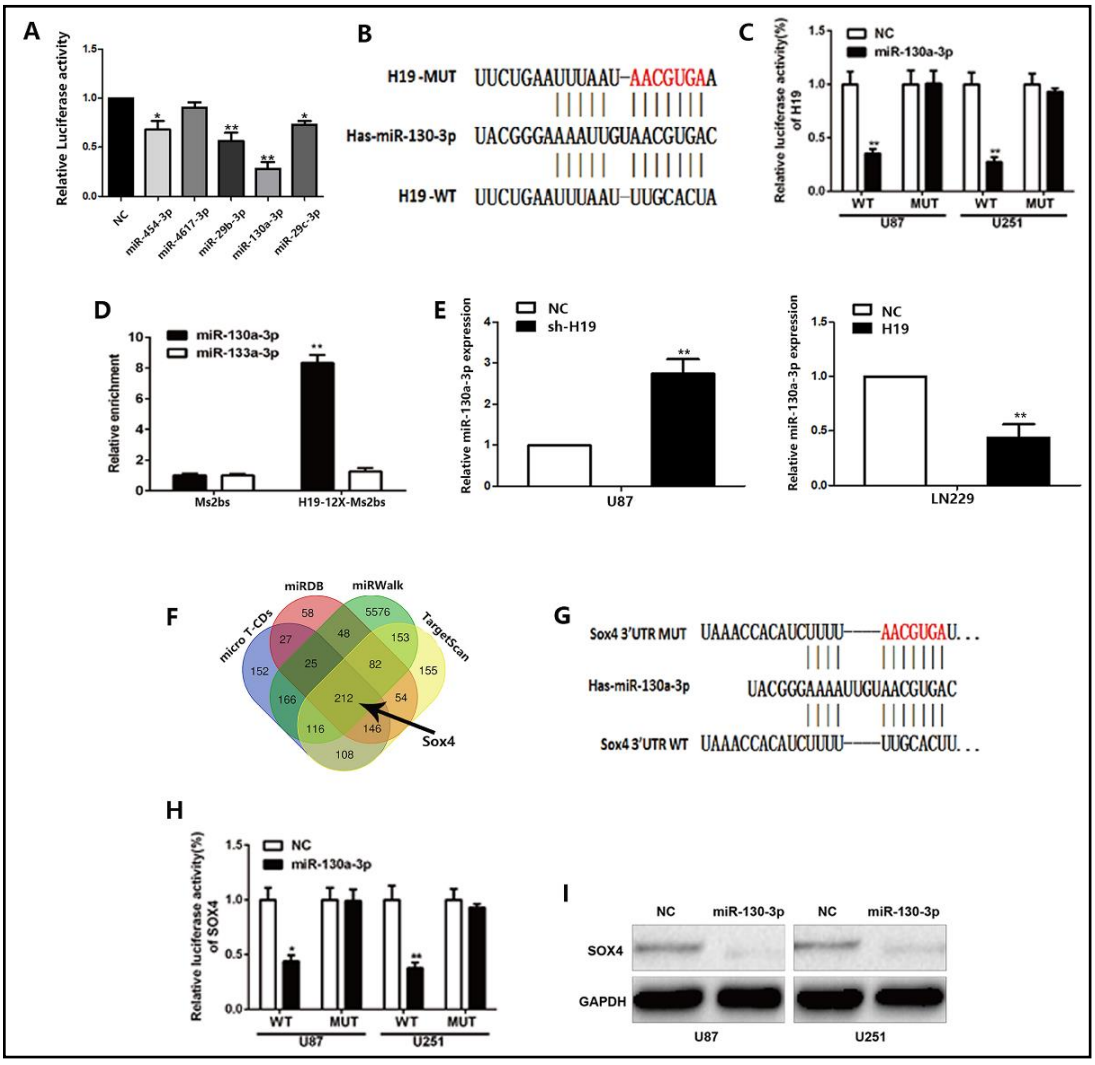

Fig. 5. H19 and SOX4 are target genes of miR-130a-3p. A: H19 luciferase reporter plasmid was co-transfected with 5 various miRNA-coding plasmids into HEK-293T cells. B: Bioinformatics predicts the binding sites of miR-130a-3p with H19. C: The dural luciferase reporter gene experiment confirmed that miR-130a-3p could bind to H19. D: The RIP assays showed that H19 could significantly enrich miR-130a-3p compared to the miR-133a -3p non-binding with H19. E: Left: qRT-PCR analysis of miR-130a-3p expression in U87 transfected with shH19 virus. Right: qRT-PCR analysis of miR-130a-3p expression in LN229 transfected with H19. F: Venn diagram displaying miR-130a-3p predicted to target Sox4 by four prediction algorithms. G: Bioinformatics predicts the binding sites of miR-130a-3p with SOX4. H: The dural luciferase reporter gene experiment confirmed that miR-130a-3p could bind to SOX4. I: Western-blot experiment was performed to inhibit SOX4 protein levels after transfection of miR-130a-3p in U87 and U251 cells. *P<0.05, **P<0.01.

H19/miR-130a-3p/SOX4 pathway facilitates EMT program in glioma cells

Based on the ceRNA hypothesis, ceRNA1 and ceRNA2 are regulated by competitive binding of miRNAs, we want to further identify the interactions between H19, miR-130a-3p and SOX4. Firstly, we analysed SOX4 expression in glioma tissues via four datasets: CGGA, REMBRANDT, GSE4290 and GSE16011. As shown in Fig. 6A, GBM tissues express higher level of Sox4 than normal brain tissues. Next, according to H19 expression, each dataset were divided into two groups: H19 Low and H19 High. Fig. 6B indicated that Sox4 expression level in H19 Low groups is inferior to that in H19 High groups (Fig. 5B). These evidences suggested that H19 expression is positively correlated with the expression of SOX4 in glioma

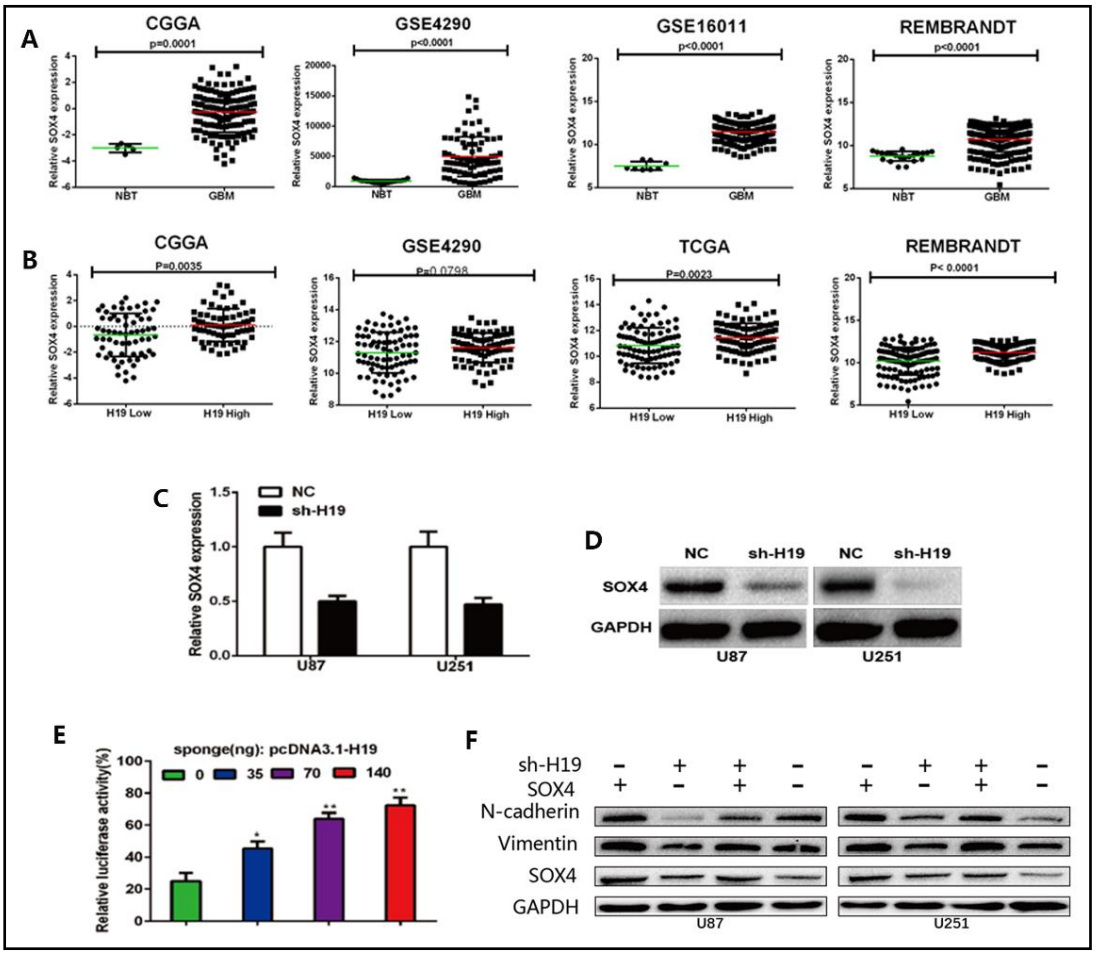

Fig. 6. H19/miR-130a-3p/SOX4 pathway facilitates EMT program in glioma cells. A: The analysis of the clinical glioma database revealed that SOX4 was highly expressed in malignant glioma. B: Compared with H19 low expression group, SOX4 was highly expressed in H19 high expression group. C, D: qRT-PCR and Western blot experiments in U87 and U251 cells. knockdown of H19 inhibited the mRNA and protein expression level of SOX4. E: In 293T cells, the co-transfection of miR-130a-3p, SOX4 wild report plasmid and H19 overexpression plasmid (0, 35,70,140)ng found that the inhibitory effect of miR-130a-3p on the activity of SOX4 wild report plasmid was gradually reduced with the increase of H19. F: H19 effect on SOX4 downstream Sox2-catenin and EMT Markers was evaluated by Western-blot. *P<0.05, **P<0.01.

tissues. we infected U87 and U251 cells with H19 shRNA or control shRNA to knock down H19 expression. Then, SOX4 mRNA and protein expression were verified. The results found out that downregulated H19 could decrease SOX4 expression (Fig. 6 C and D). In order to verify whether the regulation of H19 to SOX4 was mediated by endogenously sponging miR-130a-3p, we co-transfected 293T cells with H19 overexpression virus and SOX4 report plasmid. Compared to the Vector group, overexpression of H19 significantly weakened the inhibitory effect of miR-130a -3p to SOX4 (Fig. 6 E). Collectively, these results demonstrate that H19 acts as an endogenous sponge by sequestering miR-130a-3p and thus abolishing the miR-130a-3p induced repressing effect on the SOX4.

Ikushima et al. reported that SOX4-SOX2 signaling pathway plays an important role in maintaining the characteristics of glioma stem cells [33, 34]. To further explore the causal role of H19 in glioma progression, SOX2 and *E*MT markers N-cadherin, Vimentin were measured. Predictable, all these kinds of proteins were down-regulated when H19 was knockn-down in U87 and U251 cells, suggesting that H19 may affect the biological function of glioma cells by influencing the expression of SOX2. Morever, the down-regulation could reversed by SOX4

overexpression (Fig. 6E). These observations reinforced the contribution of H19/miR-130a-3p/SOX4 molecular axis to EMT processes in glioma cells.

Discussion

Over the past decades, growing evidence has stressed the association of lncRNAs with tumor aggressive potential and the possibility use of lncRNAs as cancer biomarkers [35, 36]. Moreover, different lncRNAs have been repoted to have different effects on glioma [37-39]. H19, one of the earliest discovered lncRNAs, plays a powerful role during tumorigenesis process [17]. Its function can be divided into two parts: a developmental reservoir of miR-675 to suppress its targets [40, 41]; and a modulator of miRNAs or proteins via their binding [20, 42]. Jia P et al. found that knockdown of H19 suppressed glioma induced angiogenesis by inhibiting miR-29a [43]. Similarly, Zhao H et al. found that H19 might regulate the tumor growth and metastasis via miR-140 dependent iASPP regulation [44]. Our group previously stated the oncogenic role of H19 on promoting glioma cell invasion by inducing miR-675 expression [22]. However, thus far, little is known about the clinical and biological function of H19 during glioma invasion processes. Epithelial-mesenchymal transition (EMT) is an important process leading to the enhancement of glioma invasion ability [9]. In this study, we determined that We found that overexpression of H19 can enhance the invasive ability of glioma and can elevate the expression levels of EMT markers: N-cadherin and Vimentin while knockdown of H19 taking the opposite trend, which suggests that H19 may function as an oncogenic LncRNA to promote EMT in glioma cells.

Further bioinformatics and luciferase assays revealed that H19 and SOX4 are both targets of miR-130a-3p. Wei H and colleagues found that miR-130a as an oncogenic miRNA that targets PTEN to drive malignant cell survival and tumor growth [45]. Additionally, miR-130a was reported as an oncogene by directly targeting TGFβR2 in gastric cancer [46]. The expression level and biological functions of miR-130a-3p in glioma remains unclear yet. The clinical importance of SOX4 has been paid more and more attention. Numerous studies have shown that SOX4 may lead to tumor development and promote its development. In the study of human breast cancer, SOX4 was overexpressed and correlates with (ER-/PR-/HER2-) subtype of breast cancer. Further research found SOX4 can promote the progression of breast cancer by regulating epithelial-mesenchymal transformation (EMT) [47]. Other studies indicated that SOX4 was overexpressed in bladder tumor [48] and hepatocellular carcinoma [49] and it can regulate EMT to promote metastasis of cancer. In consistent with the former report concering SOX4 in glioma [50], we found SOX4 was upregulated in high grade glioma tissues compared with low grade ones, and the result showed that a positive correlation exists between H19 and SOX4. Furthermore, after koncking down H19 levels, SOX4 expression was significantly suppressed. Meanwhile, dual luciferase reporter assay showed that H19 overexpression could obviously weaken the inhibitory effect of miR-130a-3p on SOX4. RIP assay indicated that H19 overexpression could sponge more miR-130a-3p. These results suggested that H19 could regulate SOX4 expression via sponging miR-130a-3p.

Conclusion

We demonstrated that lncRNA H19 is an independent prognostic factor for glioma patients and promote the progression of glioma both *in vitro* and *in vivo*. This study provides the first connection between H19/miR-130a-3p/SOX4 axis and EMT progrecesses in glioma cells. A deeper characterization of the function and downstream signaling pathways influenced by H19/miR-130a-3p/SOX4 pathway dysregulation may provide novel insights into the underlying mechanism of progression of glioma. These results will help to open new possibilities for therapeutic intervention.

Abbreviations

GBM (glioblastoma multiforme); EMT (epithelial-to-mesenchymal transition); SOX4 (SRY-box 4); HGG (high grade glioma); LGG (low grade glioma); NHAs (normal human astrocytes).

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Disclosure Statement

No conflict of interest exists.

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