Research Article

Silencing of Long Noncoding RNA HLA Complex P5 (HCP5) Suppresses Glioma Progression through the HCP5-miR-205-Vascular Endothelial Growth Factor A Feedback Loop

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Long noncoding RNA (IncRNA) HLA complex P5 (HCP5) is correlated with multiple diseases, especially cancers. However, it remains to be further studied whether HCP5 is involved in the malignant behaviors of gliomas. This study is aimed at investigating the role and regulation mechanisms of HCP5 in gliomas. HCP5 expression in glioma tumor tissues and its association with glioma patients’ survival were analyzed based on RNA-sequencing data. The expression of HCP5 was also examined in glioma cells. Then, HCP5 was downregulated in U251 cells and/or primary glioblastoma cells to explore its effects on cell proliferation and migration. The influence of HCP5 downregulation on tumor growth was confirmed in xenograft mice. About the mechanism, we investigated whether HCP5 functioned via interacting with microRNA- (miR-) 205 and regulating vascular endothelial growth factor A (VEGF-A) expression in gliomas. Results showed that HCP5 upregulation was found in glioma tissues and cell lines. Patients with high HCP5 expression showed lower survival probability and shorter survival time. HCP5 downregulation inhibited cell proliferation and migration and mitigated tumor growth. miR-205 was downregulated in glioma cells. Knockdown of HCP5 led to miR-205 upregulation and VEGF-A downregulation. miR-205 overexpression exhibited the similar effects as HCP5 downregulation on cell viability and proliferation. And VEGF-A overexpression could reverse the effects of HCP5 downregulation on cell viability and proliferation, as well as tumor growth. In conclusion, HCP5 silencing suppressed glioma progression through the HCP5-miR-205-VEGF-A feedback loop.

1. Introduction

Gliomas are the type of primary neoplasias that occur in the brain, accounting for over eighty percent of primary brain tumors [1, 2]. Glioblastomas (GBMs), the most lethal primary gliomas, are grade IV gliomas classified by the World Health Organization [3, 4]. Currently, multiple therapeutic strategies have been applied in gliomas, such as surgical resection, chemotherapy, and radiotherapy; however, the prognosis is still very poor. The median survival is only 15 months in GBM patients, and 5-year survival rate postdiagnosis is proven to be 5.1% [5, 6]. Hence, the underlying mechanisms of glioma progression need further studies [4].
effects of HCP5, it is uncertain whether HCP5 has a similar role in gliomas. Up to now, only one study reported that HCP5 was upregulated in gliomas and its downregulation mitigated the malignant biological behavior of glioma cells [17].

miRs are endogenous short noncoding RNA strands with ~22 nucleotides that are responsible for 40%-60% of posttranscriptional regulation of gene expression [18]. Current thinking holds that lncRNAs function in cancers through serving as sponges for miRs. For example, HCP5 contributed to the epithelial-mesenchymal transition (EMT) of colorectal cancer via interacting with miR-139-5p [19]. In anaplastic thyroid cancer, HCP5 knockdown showed a tumor-suppressive function, which was proven to be correlated with the upregulation of miR-128-3p [20]. For gliomas, HCP5 has been demonstrated to affect the malignant behavior of glioma cells via interacting with a tumor suppressor, miR-139, along with the alteration of Runt-related transcription factor 1 [17]. miR-205 is a highly conserved miRNA in many species. Studies have demonstrated that miR-205 could act as tumor promotor or suppressor in different cancers [21]. It has been demonstrated to be downregulated and functioned as a tumor suppressor in gliomas [22]. However, whether other miRs including miR-205 lie downstream of HCP5 in gliomas still needs to be further studied.

This study is aimed at evaluating the role HCP5 in gliomas both in vitro and in vivo. Moreover, the molecular mechanism of HCP5 in the progression of gliomas was analyzed mainly focusing on the miR-205/vascular endothelial growth factor A (VEGF-A) axis.

2. Material and Methods

2.1. RNA-Sequencing Data Processing and Analysis. Based on the RNA-sequencing data (n = 9,736 for tumor samples; n = 8,587 for normal samples) from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects, a web-based tool named Gene Expression Profiling Interactive Analysis (GEPIA: http://gepia.cancer-pku.cn/) was used to analyze gene expression [23]. The differential expression analyses of HCP5 were performed via GEPIA in several cancer types, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESE; tumor: n = 306; normal: n = 13), cholangiocarcinoma (CHOL; tumor: n = 36; normal: n = 9), colon adenocarcinoma (COAD; tumor: n = 275; normal: n = 349), esophageal carcinoma (ESCA; tumor: n = 182; normal: n = 286), kidney chromophobe (KICH; tumor: n = 66; normal: n = 53), acute myeloid leukemia (LAML; tumor: n = 173; normal: n = 70), stomach adenocarcinoma (STAD; tumor: n = 408; normal: n = 211), and GBM (tumor: n = 163; normal: n = 207). Survival analysis of 224 glioma patients was also analyzed by GEPIA. These patients were assigned into two groups according to the median value of HCP5 expression.

2.2. Cell Culture. Commercially available human GBM cell lines, including U87 MG (ATCC® HTB-14™) and A172 (ATCC® CRL-1620™), were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). Normal human astrocytes (NHA) and human GBM cell line U251 were both obtained from the China Academy Sinica Cell Repository (Shanghai, China). GBM cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco), while NHA cells were grown in MCDB-131 medium (Sigma; St. Louis, MO, USA) containing 3% FBS and 10× G-5 Supplement (Gibco). Primary glioblastoma cell line was established as previously described [24]. Briefly, tumor tissue was obtained from one GBM patient. After removing the vessels, clotted blood, and charred tissue, sample was dissociated by Collagenase Type IVa (250 U/mL) and Pronase E (2.5 U/mL) for 1 h at 37°C. Then, cells were centrifuged at 300 × g for 5 min at 4°C, resuspended in DMEM containing 10% FBS, and put in a cell culture flask. The cell culture medium was changed every 2 days. Cells were all maintained in a humidified incubator with an atmosphere of 5% CO₂ at 37°C.

2.3. Cell Transfection. Small interfering RNAs (siRNAs) against HCP5 (si-HCP5#1, si-HCP5#2, and si-HCP5#3), their negative control (si-NC), miR-205 mimics, and scrambled miRs (NC mimics) were synthesized by GenePharma Co., Ltd. (Shanghai, China). Short hairpin RNA (shRNA) targeting HCP5 (sh-HCP5) and its negative control (sh-NC) were cloned into pGPU6/GFP/Neo plasmid by GenePharma Co., Ltd. Full-length human VEGF-A gene was ligated into pcDNA3.1 plasmid (Invitrogen, Carlsbad, CA, USA), with empty pcDNA3.1 vector as a control (pcDNA3.1). These vectors were transfected into U251 cells using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions. Transfected cells were collected at 48 h after transfection to do the downstream experiments.

2.4. Cell Viability Assay. U251 or primary glioblastoma cells (2 × 10⁵ cells per well) were seeded in 96-well plates. After transfection, 20 μL 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Promega, Madison, WI, USA) per well was added on transfection, a linear scratch was created by a 200 μL pipette tip, and cellular debris were removed by washing with phosphate buffer saline for three times. After culturing for another 48 h in serum-free medium, images of the scratched region were photographed by a microscope equipped with a camera (Nikon, Tokyo, Japan).

2.5. Scratch Wound-Healing Assay. U251 or primary glioblastoma cells (4 × 10⁵ cells per well) were seeded in 6-well culture plates. Cells were transfected with siRNA against HCP5 (si-HCP5) or control siRNA (si-NC). 48 h after transfection, a linear scratch was created by a 200 μL pipette tip, and cellular debris were removed by washing with phosphate buffer saline for three times. After culturing for another 48 h in serum-free medium, images of the scratched region were photographed by a microscope equipped with a camera (Nikon, Tokyo, Japan).

2.6. Proliferation Assay. Cell proliferative ability was determined by 5-ethynyl-2'-deoxyuridine (EdU) cell proliferation assay kit (RiboBio, Guangzhou, China) as previously described [25]. Firstly, transfected cells were seeded into
2.7. In Vivo Xenograft Experiments. Eight-week-old male C57BL/6 nude mice, obtained from the Guangdong Medical Laboratory Animal Center (Guangzhou, China), were divided into sh-HCP5 and sh-NC groups, or divided into pcDNA3.1-VEGF-A, pcDNA31, pcDNA31+sh-HCP5, and pcDNA3.1-VEGF-A+sh-HCP5 groups (n = 10 per group). After transfection with indicated plasmid, U251 cells (5 × 10⁶ cells per mouse) were subcutaneously injected into mice. Tumor volumes were recorded every 5 days from day 8 after injection according to the following formula: volume (mm³) = length × width²/2. All mice were euthanized by intraperitoneal injection of 100 mg/kg pentobarbital at 4 weeks postinjection, and tumor tissues were excised for further experiments. The animal experiments were carried out according to the Guide for the Care and Use of Experimental Animals of the National Institutes of Health, and the experimental protocol was approved by the Ethics Committee of the Shannxi Provincial People’s Hospital (Shanxi, China).

2.8. Dual-Luciferase Reporter Assay. The binding sequence of miR-205 and HCP5 was predicted by IntraRNA 2.0 online software (http://http://rna.informatik.uni-freiburg.de/). The wild-type or mutant sequence of HCP5 was cloned into pmirGLO dual-luciferase vector to construct luciferase reporter vectors, named HCP5 WT or HCP5 MT, respectively, and dual-luciferase reporter assay was carried out according to a previous study [26]. HEK293T cells were seeded into 96-well plates, followed by cotransfection with luciferase reporter vectors (HCP5 WT or HCP5 MT) and miRs (NC mimics or miR-205 mimics). After transfection for 48 h, the luciferase activity of transfected cells was determined by a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA), as suggested by the manufacturer’s instructions. Renilla luciferase was an internal reference.

2.9. Quantitative Reverse Transcription PCR (qRT-PCR). Total RNA extracted by TRIzol reagent (Invitrogen) was used as a template for the synthesis of first-strand cDNA with the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen), and all steps were performed as the manufacturer’s instructions. HCP5 expression levels were obtained using a One Step SYBR® PrimeScript™ PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China). The expression level of miR-205 was assessed using an All-in-One™ miRNA qRT-PCR reagent kit (GeneCopeia Inc., Rockville, MD, USA). Besides, VEGF-A mRNA expression levels were measured by the PrimeScript™ 1st Strand cDNA Synthesis kit (TaKaRa) and the TB Green™ Premix Ex Taq™ II (TaKaRa) for reverse transcription and qRT-PCR, respectively. Primers used in this study are shown in Table 1, and the relative quantification of genes was calculated by the 2-ΔΔCt method [16]. GAPDH was chosen as the housekeeping gene for HCP5 and VEGF-A, whereas U6 was used as that for miR-205.

2.10. Western Blot Analysis. Proteins in U251 cells and tumor tissues were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) (both from Beyotime Biotechnology, Shanghai, China). After centrifugation, proteins in the supernatant were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Then, extracted proteins were loaded (50 μg/lane) and separated by dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis. Subsequently, proteins were transferred from the gel to the polyvinylidene difluoride membranes. Protein-bound transfer membranes were blocked by 5% nonfat milk for 1 h at room temperature, and then, membranes were successively incubated with the primary antibodies (VEGF-A (ab214424) or GAPDH (ab181602), both were from Abcam (Cambridge, UK)) and HRP-conjugated secondary antibody (goat anti-rabbit, ab205718, Abcam, Cambridge, UK). Specific protein bands were visualized by using an ECL detection reagent (GE Healthcare, Braunschweig, Germany). Band intensity of VEGF-A was quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA), with normalization to GAPDH.

2.11. Statistical Analysis. All experiments were repeated three times. Data are presented as the mean ± standard deviation (SD). Statistical analysis was carried out with GraphPad Prism 6 software (GraphPad, San Diego, CA, USA). The differences between two groups were analyzed by Student’s t test. The differences among groups were compared by one-way analysis of variance (ANOVA) with Bonferroni post hoc test. Survival analysis was performed by the Kaplan-Meier method. P < 0.05 was considered as statistically significant.

| Table 1: The primers used for real-time PCR. |
|---|---|
| Name | Primer sequences (5’ to 3’) |
| miR-205 | Forward: GGTTCCCTTACCTCACCAGG  
Reverse: CAGTGGCTGTGCTGAGGAT |
| U6 | Forward: CGCTTCACGAATTGGGCAGTCAT  
Reverse: GACTTCCTCTGTTGCTTTG |
| HCP5 | Forward: CACTGGCTGTGACCTGTT  
Reverse: TCTTGGGTGATTTGAGGCTT |
| VEGF-A | Forward: AGCTCATCTCCTCATTGTCG  
Reverse: CAATGACCCTTCACTGACC |
| GAPDH | Forward: GACAAGCTTCGTTCTCACAG |
**Figure 1**: Potential correlation between HCP5 overexpression and glioma. (a) The expression of HCP5 in diverse cancer types, which was analyzed by GEPIA. (b) HCP5 expression was assayed in GBM using GEPIA software. The boxes show the median and interquartile range, and the whiskers show the minimum and maximum values. (c) Prognostic value of HCP5 in glioma patients detected by GEPIA. (d) The expression of HCP5 in GMB cell lines and normal NHA cells, analyzed by qRT-PCR. Data are presented as the mean ± SD. *P < 0.05. TPM: transcripts per million.
Figure 2: Continued.
Figure 2: Continued.
Figure 2: HCP5 downregulation inhibited cell viability and migration and mitigated tumor growth. U251 cells were transfected with si-NC, si-HCP5#1, si-HCP5#2, or si-HCP5#3. (a) HCP5 expression was determined by qRT-PCR. (b) Cell viability was assessed by MTS assay. (c) Cell migration was examined by scratch wound-healing assay. (d) Proliferation was determined by EdU incorporation proliferation assay. U251 cells were transfected with sh-NC or sh-HCP5 and then injected into nude mice. (e) Tumors isolated from nude mice. (f) Tumor volume was recorded every 5 days from day 8 after injection. (g) HCP5 expression in primary glioblastoma cells (pGCL) and NHA cells was measured by qRT-PCR. pGCL cells were transfected with si-HCP5#2. (h) HCP5 expression level was analyzed. (i) Cell viability was assessed by MTS assay. (j, k) The migration of U251 cells was examined by scratch wound-healing assay after transfection with si-HCP5#2 and si-NC. And the wound-healing rate was measured. (l) EdU was used to examine the proliferation of pGCL cells. Data are presented as the mean ± SD. *P < 0.05; **P < 0.01.
Figure 3: Continued.
3. Results

3.1. HCP5 Was Aberrantly Upregulated in Glioma Specimens and Commercial Glioma Cells. We firstly compared HCP5 expression in cancer tissue samples and healthy control samples. Results in Figure 1(a) showed that HCP5 levels in CESC, CHOL, COAD, ESCA, KICH, LAML, and STAD were all prominently higher than those in normal samples (all \( P < 0.05 \)). Similarly, HCP5 expression was significantly upregulated in GBM samples when compared to normal samples (all \( P < 0.05 \), Figure 1(b)). Using the RNA-sequencing data from the Chinese Glioma Genome Atlas, the relationship between HCP5 expression and the prognosis of 224 glioma patients was analyzed. Results revealed that patients with high HCP5 expression had lower survival probability and shorter survival times than those with low HCP5 expression (both \( P < 0.0001 \), Figure 1(c)). Moreover, the expression of HCP5 in GBM cell lines (i.e., U87 MG, U251, and A172) was notably higher than that in normal NHA cells (all \( P < 0.05 \), Figure 1(d)). These results implied that HCP5 overexpression might be correlated with GBM progression.

3.2. HCP5 Downregulation Inhibited Cell Proliferation and Migration and Mitigated Tumor Growth. The influences of HCP5 on the malignant behaviors of GBM were analyzed via silencing HCP5 both in vitro and in vivo. In this study, we designed three siRNAs against HCP5. HCP5 levels in cells transfected with the three siRNAs against HCP5 were significantly lower than those in cells with si-NC (all \( P < 0.05 \), Figure 2(a)), and si-HCP5#2 and si-HCP5#3 showed higher knockdown efficiency than si-HCP5#1. Figure 2(b) shows that cell viability was apparently mitigated after HCP5 downregulation. Wound-healing assay in Figure 2(c) showed that knockdown of HCP5 decreased cell migration, as the migration distance was obviously reduced following HCP5 downregulation (both \( P < 0.05 \)). As depicted in Figure 2(d), compared with the si-NC group, the percentage of EdU-positive cells was decreased by HCP5 downregulation (both \( P < 0.01 \)). Furthermore, the effects of HCP5 on GBM were investigated in nude mice. Tumor growth was slower in mice injected with HCP5-silenced U251 cells compared to those injected with U251 cells transfected with sh-NC (all \( P < 0.05 \), Figures 2(e) and 2(f)). Additionally, the effects of HCP5 on GBM were also evaluated in primary glioblastoma cell line. We found that HCP5 expression was higher in primary glioblastoma cells than in NHA cells (all \( P < 0.05 \), Figure 2(g)). Downregulation of HCP5 in primary glioblastoma cells significantly suppressed cell viability, migration, and the percentage of EdU-positive cells (all \( P < 0.05 \), Figures 2(h)–2(l)). These data suggested that HCP5 was involved in tumorigenesis, as silencing of HCP5 suppressed cell proliferation and migration in vitro, and repressed tumor growth in vivo.

3.3. HCP5 Downregulation Repressed Cell Proliferation via Interaction with miR-205. The regulatory mechanism of HCP5 in GBM was further studied. miR-205 expression was found to be apparently downregulated in GBM cell lines including U87 MG, U251, and A172 cells relative to NHA cells (all \( P < 0.05 \), Figure 3(a)). We also found that miR-205 level was obviously higher in HCP5-silenced U251 cells than the si-NC group (all \( P < 0.05 \), Figure 3(b)), which indicated that there might be a negative correlation between miR-205 and HCP5 in GBM. The potential binding sites
1.5
1.0
0.5
0.0
Control si-NC
si-HCP5#2

Relative mRNA expression of VEGF-A

(a)

2.5
2.0
1.5
1.0
0.5
0.0
sh-NC sh-HCP5 sh-NC sh-HCP5 sh-NC sh-HCP5

Relative expression level

(b)

sh-NC sh-HCP5

Relative protein expression

(c)

Figure 4: Continued.
between HCP5 and miR-205 were graphically marked in Figure 3(c). HCP5 expression was prominently downregulated after miR-205 overexpression (P < 0.05, Figure 3(d)). Results in Figure 3(e) illustrated that the luciferase activity was markedly reduced in HEK293T cells that were cotransfected with HCP5 WT and miR-205 (P < 0.05), but a similar phenomenon was not found in cells with HCP5 MT. Moreover, both cell viability and the proportion of EdU-positive cells were dramatically lowered by miR-205 or si-HCP5.

And compared with the si-HCP5 or miR-205 mimic group, cell viability and proliferation were further decreased in the si-HCP5+miR-205 mimic group (all P < 0.05, Figures 3(f) and 3(g)). These results confirmed that HCP5 might act as a sponge of miR-205 in U251 cells.

3.4. HCP5 Downregulation Repressed GBM Proliferation through Downregulating VEGF-A. Finally, we investigated whether HCP5 regulated GBM progression by modulating
VEGF-A through miR-205. qRT-PCR assay showed that compared with the si-NC group, VEGF-A mRNA expression was significantly repressed in HCP5-silenced cells (P < 0.05, Figure 4(a)). Moreover, HCP5 and VEGF-A mRNA levels were strikingly decreased while miR-205 expression was markedly increased in HCP5-silenced tumor tissues, compared to the sh-NC group (all P < 0.05, Figure 4(b)). Unsurprisingly, western blot results in Figure 4(c) indicated that VEGF-A protein expression levels were reduced both in HCP5-silenced U251 cells and tumor tissues. Additionally, a rescue experiment was used to further confirm whether HCP5 functioned via regulating VEGF-A. Figure S1 illustrates that VEGF-A was successfully overexpressed in U251 cells transfected with pcDNA3.1-VEGF-A. In U251 cells, we found that VEGF-A overexpression not only increased cell viability and the proportion of EdU-positive cells but also reversed the influence of HCP5 downregulation on these malignant behaviors (all P < 0.05, Figures 4(d) and 4(e)). Similarly, upregulation of VEGF-A promoted tumor growth and abrogated the effect of sh-HCP5 on tumor growth in vivo (all P < 0.05, Figures 4(f) and 4(g)). These data proved that HCP5 downregulation functioned in GBM through downregulating VEGF-A.

4. Discussion

GBM is a devastating disease that is related to the dysregulation of multiple IncRNAs. In this study, we found that HCP5 was aberrantly upregulated in GBM patients and cell lines. Patients with high HCP5 expression showed shorter survival time. In vitro experiments proved that HCP5 downregulation could suppress cell viability, migration, and proliferation in U251 cells. HCP5 knockdown also repressed tumor growth in xenograft mice. Further experiments demonstrated that HCP5 functioned via sponging miR-205 to positively regulate VEGF-A in gliomas.

Accumulating evidence has proven that HCP5 was involved in the tumorigenesis of many cancers [27, 28]. Recently, researchers come to realize the importance of RNA sequencing in exploration for genetic mechanisms underlying human diseases [29]. RNA-sequencing data from TCGA and GTEx illustrated that HCP5 was upregulated in many types of cancers including gliomas, which implied that HCP5 possessed oncogenic potential in gliomas. In addition, lower survival probability and shorter survival time were shown in patients with high HCP5 expression. In vitro experiments showed the increase of HCP5 in GBM cells. The upregulation of HCP5 in gliomas was consistent with a previous study [17]. Taken together, we hypothesized that HCP5 might be an oncogenic gene in gliomas.

Two main distinguishing features of gliomas are rapid proliferation and angiogenesis [30]. Migration contributes to the high mortality of gliomas, and blocking cancer cell metastasis is considered to be a promising avenue for the treatment of this disease [31]. Studies have confirmed that HCP5 promoted the proliferation and migration in clear cell renal cell carcinoma and gastric cancer [32, 33]. A previous study has pointed out that HCP5 downregulation decreased cell viability and migration in U87 and U251 cells [17]. In line with these studies described above, this study found that HCP5 knockdown inhibited the migration and proliferation of U251 and primary glioblastoma cells and prevented tumor growth in xenograft mice, which suggested that HCP5 exerted a tumor-promoter role in gliomas.

Studying the molecular mechanisms of HCP5 may provide innovative strategies for glioma therapy. The importance of miR-205 in gliomas has been evidenced in recent studies. For example, miR-205 was downregulated not only in glioma tissues but also in glioma cell lines, and it could inhibit EMT and tumor growth of gliomas [22]. miR-205 has been reported to be a target of many IncRNAs in gliomas [34, 35]. Hence, our study explored whether miR-205 was a target of HCP5 in gliomas. We found that there was a negative correlation between miR-205 and HCP5, and HCP5 could directly bind to miR-205 in GBM cells. Furthermore, miR-205 overexpression showed the similar effects as HCP5 downregulation on GBM cell viability and proliferation. These results confirmed that HCP5 functioned via targeting miR-205.

Many features of cancers such as migration, angiogenesis, and permeabilization of blood vessels are commonly correlated with VEGF-A [36]. Vascularization plays an important role in tumor progression, and aberrant angiogenesis is a hallmark of GBM [37]. Many studies indicated that there were binding sequences between miR-205 and VEGF-A, and miR-205 could target VEGF-A to inhibit the progression of multiple cancers [38–40]. Thus, we selected the VEGF-A as the downstream target gene of miR-205 and further analyzed whether VEGF-A was a downstream factor of HCP5. In this study, we found that VEGF-A was downregulated in HCP5-silenced U251 cells and tumor tissues. Moreover, the viability and proliferation of GBM cells as well as tumor growth could be enhanced by VEGF-A overexpression, and the upregulation of VEGF-A reversed the impacts of HCP5 downregulation on these features. Our results, combined with previous studies, suggested that HCP5 might affect glioma progression through the miR-205/VEGF-A axis.

5. Conclusions

Upregulation of HCP5 was found in glioma tissues and cell lines. HCP5 knockdown induced miR-205 upregulation, followed by the downregulation of VEGF-A, resulting in the repression of tumor cell proliferation and migration as well as tumor growth in gliomas. To our knowledge, this is the first time to report the correlation between HCP5 and miR-205 in gliomas. Targeting HCP5 and miR-205 might provide insight into a new strategy for glioma therapy.

Abbreviations

lncRNA: Long noncoding RNA
HCP5: HLA complex P5
VEGF-A: Vascular endothelial growth factor A
GBM: Glioblastoma
miR: MicroRNA
EMT: Epithelial-mesenchymal transition
ANOVA: One-way analysis of variance.  
SD: Standard deviation  
PMSF: Phenylmethanesulfonyl  
RIPA: Radioimmunoprecipitation assay  

VEGF-A pathway. (iv) downregulation repressed cell proliferation via interaction included within the article.  

mined by western blot analysis.  
cells acted as a control. VEGF-A protein expression was deter-
fected with pcDNA3.1-VEGF-A. U251 cells were transfected  
Figure S1: VEGF-A was overexpressed in U251 cells trans-

Conflicts of Interest  
The data used to support the findings of this study are  

Additional Points  
Highlights. (i) HCP5 was aberrantly upregulated in glioma samples and cells. (ii) HCP5 silencing inhibited cell proliferation and migration and reduced tumor growth. (iii) HCP5 downregulation repressed cell proliferation via interaction with miR-205. (iv) HCP5 functioned via the miR-205/VEGF-A pathway.  

Data Availability  
The data used to support the findings of this study are included within the article.  

Supplementary Materials  
Figure S1: VEGF-A was overexpressed in U251 cells trans-

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