Glioma is the most common primary malignancy in the brain, and vasculogenic mimicry (VM) is one of the blood supply methods. Here we investigated the possibility that IncRNAs regulate the stability of transcription factors through the SMD pathway, which affects proliferation, migration, invasion, and the ability to form VMs in glioma. Expression of PABPC5, HCG15, and ZNF331 was detected by real-time qPCR or western blot in glioma. Cell Counting Kit-8, Transwell assays, and in vitro VM tube formation were used to investigate PABPC5, HCG15, and ZNF331 function in cell proliferation, migration, invasion, and VM, respectively. ChIP assays were used to ascertain the interaction between ZNF331 and LAMC2 or PABPC5. PABPC5 and HCG15 were highly expressed in glioma cells. ZNF331 was lowly expressed. PABPC5 bound HCG15 to increase its stability. Knockdown HCG15 reduced the degradation of ZNF331 mRNA by the SMD pathway. ZNF331 inhibited transcription through binding to the promoter region of LAMC2 and PABPC5 and inhibited the ability to form VMs in glioma cells. The PABPC5/HCG15/ZNF331 feedback loop plays an important role in regulating VM formation in glioma and provides new targets for glioma treatment.

INTRODUCTION
Glioma is the most common and malignant central nervous system tumor in humans, accounting for 60% of central tumors, with a mortality rate of more than 98%. Currently, glioma treatment methods mainly include surgery, radiation therapy, and chemotherapy. Although the symptoms can be improved by active treatment, treatment effects are still not satisfactory, the recurrence rate is high, the 2-year survival rate is only 15%–26%, and the average median survival time is only 12–18 months. Gliomas are primary intracranial malignancies with the highest degree of vascularization and characterized by micro-angiogenesis and angiogenesis. Currently, anti-vascular therapies include tyrosine kinase inhibitors and recombinant humanized monoclonal antibodies. Various cancer treatments have been extensively studied, and similarly targeted anti-angiogenic drugs can be effective for glioma treatment. Several related studies have concluded that vasculogenic mimics help to generate anti-angiogenic therapeutic resistance, resulting in poor anti-angiogenic treatment effects. Therefore, research regarding the vasculogenic mimic mechanism of glioma cells can provide new treatment strategies for glioma chemotherapy and improve treatment effects.

Maniotis et al. proposed the concept of vasculogenic mimicry (VM) when they studied melanoma in 1999. Some studies have shown that tumor cells directly generate vascular channels and are independent on endothelial cells. Vascular channels are blood vessel-like pipe structures formed by malignant tumor cells in a state of hypoxia to meet the needs of their own blood supply to develop their own growth; this provided a new perspective regarding tumor cell plasticity. Tumor samples are used for immunohistochemistry (IHC) to identify VM; tumor VM periodic acid Schiff (PAS) staining is positive, and CD31 or CD34 staining is negative, indicating the presence of matrix-associated vascular channels. In recent years, research regarding anti-VM has become a new direction for glioma treatment. Therefore, the study of tumor vascular mimicry-related genes and signal transduction pathways is of great significance. Studies have reported that glioma VM is positively correlated with malignancy and promotes glioma migration, invasion, and metastasis.
binding protein cytoplasmic 5) is a member of the cytosolic poly(A) binding protein family, binds to the protein at the 3′ end of the poly(A) tail of most eukaryotic mRNAs, and is located on chromosome Xq21.3/Yp11.2. Studies have suggested that PABPC5 is involved in metabolism of DNA and RNA in mitochondria. The PABPC5 gene is composed of at least two exons and one intron and an uninterrupted ORF (open reading frame). Studies have found that PABPC5 is located on translocation breakpoint DX214, associated with premature ovarian failure in ovarian disease, and high expression of PABPC5 is closely related to the poor prognosis of ovarian cancer patients. At present, there are no reports of glioma research.

Imbalance of non-coding RNA (ncRNA) is related to the progression of various tumors and plays an important regulatory role in tumorigenesis and development, including long ncRNA (lncRNA; >200 nt) and microRNA (miRNA; ~22 nt). IncRNA is involved in various cellular processes, such as proliferation, migration, invasion, and apoptosis. A large amount of evidence proves that IncRNA plays a key role in the progression of gliomas and also has important significance for the diagnosis and treatment of gliomas. HCG15 (Human leukocyte antigen complex group 15) is located on chromosome 6p21. At present, HCG15 has not been reported in glioma and VM.

STAU (Staufen) is a key mRNA transport and localization factor in Drosophila. Combination of the Staufen paralog Stau1 in mammals with the 3′ UTR region of intermolecular and intramolecular double-stranded structures triggers degradation of target mRNA; this degradation process is called Staufen-mediated mRNA decay (SMD). SMD is a STAU1 mediated mRNA degradation pathway, which STAU1 combines with STAU1 binding site (SBS) formed when the Alu element of IncRNAs recognizes and pairs with the Alu element of target mRNA 3′UTR during translation, and then recruits the ATP-dependent RNA helicase up-frameshift 1 (UPF1) to bind and promote the degradation of target mRNA. UPF1 can detect and degrade mRNA transcripts containing premature stop codons (PTCs), specifically accelerating the target degradation of gene mRNA. Studies have reported that approximately 1% of human mRNA is regulated by STAU1, so SMD is an important post-transcriptional regulatory pathway. The role of SMD in glioma needs further investigation.

In cancer research, LINC00993 regulates the development of breast cancer by inhibiting CDKN2A transcription. ZNF331 (zinc-finger protein 331) is located on chromosome 19q13.42, which encodes a zinc-finger protein containing the KRAB (Kruppel-related box) domain found in transcriptional repressors. Studies have reported that ZNF331 methylates in the promoter region of human gastric cancer cells, which inactivates them and increases the growth and invasion capabilities of gastric cancer cells. Low expression of ZNF331 indicates a poor prognosis in colorectal cancer patients. At present, no research of ZNF331 regulating VM of gliomas has been reported.

LAMC2 (laminin subunit gamma 2) is a family of extracellular matrix glycoproteins. It is the main non-collagen component of the basement membrane and is involved in regulating a variety of biological processes, including cell adhesion, differentiation, migration, signaling, neurite growth, and metastasis. LAMC2 promotes the migration and invasion of lung cancer cells through the Protein kinase B (PKB or AKT) signaling pathway. Studies have reported that LAMC2 is highly expressed in U87 and U251 glioma cells. LAMC2 plays a key role in formation of glioma vascular mimicry through the AKT and ERK (extracellular regulated protein kinases) signaling pathways, and it increase the malignancy degree of glioma. The tumor blood supply channel is formed by deformation of the extracellular matrix, so LAMC2 is a landmark protein for VM. ZNF331 has not been found to regulate the transcription of LAMC2 and, thus, to regulate VM in glioma.

In this study, we investigated the expression and function of PABPC5, HCG15, and ZNF331 in glioma tissue and glioma cell lines and studied the role of PABPC5, HCG15, and ZNF331 in regulating glioma VM. These results will provide new molecular mechanisms for glioma development and provide new insights into glioma treatment.

RESULT

**PABPC5 and HCG15 Were Highly Expressed in Glioma Tissues and Cells, and Knockdown of PABPC5 and HCG15 Inhibited VM Formation**

Western blot was used to detect PABPC5 expression in glioma tissues (12 normal brain tissues, 12 low-grade glioma tissues [World Health Organization [WHO] I–II], and 12 high-grade glioma tissues [WHO III–IV]) and glioma cells (U87 and U251). As shown in Figures 1A and 1B, compared with the normal brain tissue group, expression of PABPC5 was significantly increased in low-grade glioma tissue and high-grade glioma tissue (p < 0.01); the expression of PABPC5 was significantly higher than in low-grade glioma tissues (p < 0.05). In U87 and U251 glioma cells, the expression level of PABPC5 was significantly higher than in the normal human astrocyte (NHA) group (p < 0.05). To further explore the function of PABPC5 in gliomas and construct knockdown PABPC5 cells, we applied two knockdown plasmids to transfect glioma cells and tested the transfection efficiency. We selected the higher knockdown efficiency. The #2 plasmid was subjected to subsequent experiments (p < 0.01) (Figure S1B). The results showed that knockdown of PABPC5 affected proliferation, migration, invasion, and VM in glioma cells. As shown in Figures 1C–1E, compared with the PABPC5 (–)–NC group, the proliferation, migration, invasion, and VM formation ability of cells in the PABPC5 (–) group were significantly reduced (p < 0.01). The expression of LAMC2 in the control group was not significantly different from that of the PABPC5 (–)–NC group; compared with the PABPC5 (–)–NC group, the expression of LAMC2 in the PABPC5 (–) group was significantly reduced (p < 0.01 and p < 0.05) (Figure 1F).

HCG15 expression was detected in glioma tissues (12 normal brain tissues, 12 low-grade glioma tissues [WHO I–II], and 12 high-grade glioma tissues [WHO III–IV]) and in glioma cells (U87 and U251).
by real-time qPCR technology. The results are shown in Figures 1G and 1H. Compared with the normal brain tissue group, the expression of HCG15 in low-grade glioma tissue (p < 0.05) and high-grade glioma tissue (p < 0.01) was significantly higher than in normal brain tissue. The expression of HCG15 in high-grade glioma tissue was significantly higher than in low-grade glioma tissue (p < 0.05). The expression of HCG15 in U87 and U251 glioma cells was significantly higher than in the NHA group (p < 0.01 and p < 0.05). To further analyze the effects of HCG15 knockdown and overexpression on the biological behavior of gliomas, we constructed knockdown and overexpression HCG15 cell lines. In knockdown HCG15 cell lines, we transfected with two knockdown plasmids and tested transfection efficiency. We selected #2 with the higher knockdown efficiency for subsequent experiments (p < 0.01) (Figure S1C). We tested transfection efficiency for glioma transplanted with overexpression of HCG15; its expression increased about 3 times (p < 0.01) (Figure S1D). We investigated the effects on proliferation, migration, invasion, and VM ability in glioma cells. The results are shown in Figures 1I–1L. Compared with the control group, there was no statistical difference between the HCG15 (+)-NC and HCG15 (+)-NC groups; compared with the HCG15 (-)-NC group, the HCG15 (+) group had significantly reduced proliferation, migration, invasion, and VM ability of glioma cells (p < 0.01, p < 0.05) and significantly reduced expression levels of the vascular mimicry-related molecule LAMC2 in glioma cells (p < 0.01). Compared with the HCG15 (+)-NC group, the HCG15 (+) group significantly increased the proliferation, migration, invasion, and VM ability in glioma cells (p < 0.01) and increased expression levels of the vascular mimicry-related molecule LAMC2 in glioma cells (p < 0.01).

**PABPC5 Promoted VM Formation in Glioma Cells by Increasing the Stability of HCG15**

Next, the effect of PABPC5 knockdown on HCG15 was detected. The results are shown in Figure 2A. Compared with the control group, the expression of HCG15 was not significantly different in the PABPC5 (-)-NC group; compared with the PABPC5 (-)-NC group, the expression level of HCG15 was significantly reduced in PABPC5 (-) group (p < 0.01). To study the relationship between PABPC5 and HCG15, RNA immunoprecipitation (IP) experiments were used to detect whether there was a direct binding effect between PABPC5 and HCG15. As shown in Figure 2B, in the anti-PABPC5 group, HCG15 enrichment was significantly higher than in the anti-immunoglobulin G (IgG) group (p < 0.01 and p < 0.05). Furthermore, the effect of PABPC5 knockdown on HCG15 nascent was detected; there was no statistical difference between the control group and the PABPC5 (-)-NC group, and there was no difference between the PABPC5 (-)-NC group and the PABPC5 (-) group (Figure 2C). Half-life experiments (Figure 2D) confirmed that there was no significant statistical difference between the control group and the PABPC5 (-)-NC group; compared with the PABPC5 (-)-NC group, the half-life of HCG15 was significantly reduced in the PABPC5 (-) group (p < 0.01 and p < 0.05). Knockdown of PABPC5 combined with knockdown and overexpression of HCG15 were performed in a glioma cell line. Furthermore, the effects of PABPC5 knockdown cotransfection with overexpression of HCG15 on proliferation, migration, invasion, and VM ability VM was analyzed in glioma cells. The results are shown in Figures 2E–2H. Compared with the control group, the PABPC5 (-) + HCG15 (-)-NC group and the PABPC5 (-) + HCG15 (+)-NC group significantly inhibited proliferation, migration, invasion, and VM ability and reduced expression levels of LAMC2 protein (p < 0.01 and p < 0.05); compared with the PABPC5 (-) group, the PABPC5 (-) + HCG15 (-)-NC group had significantly inhibited glioma cell proliferation, migration, invasion, and VM formation and significantly reduced expression levels of LAMC2 (p < 0.01 and p < 0.05). Compared with the PABPC5 (-) + HCG15 (+)-NC group, HCG15 (+) reversed the inhibitory effect of PABPC5 (-) on proliferation, migration, invasion, VM ability of VM and LAMC2 protein expression in PABPC5 (-) + HCG15 (+) (p < 0.01 and p < 0.05).

**Figure 1. Endogenous Expression of PABPC5 and HCG15 and the Effect of PABPC5 and HCG15 on the Biological Behavior of Glioma Cells**

(A) PABPC5 protein expression levels in normal brain tissue (NBT), low-grade glioma tissue (LGGT), and high-grade glioma tissue (HGGT). The protein expression and corresponding ID values of PABPC5 in NBT, LGGT, and HGGT are shown; the data are expressed as mean ± SD (n = 12). Compared with the NBT group, "p < 0.01; compared with the LGGT group, "p < 0.05. (B) PABPC5 protein levels in normal human astrocytes (NHAs) and U87 and U251 cells. The protein expression and corresponding ID values of PABPC5 in NHAs and U87 and U251 cells are shown; the data are expressed as mean ± SD (n = 3). Compared with the NHA group, "p < 0.05. (C) The effect of knockdown on PABPC5 on the ability of proliferation was measured with CCK-8 in U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (+)-NC group, "p < 0.05. (D) The effect of knockdown of PABPC5 on the migration and invasion ability of U87 and U251 cells by was tested by Transwell test. Representative images and corresponding statistical analysis diagrams are shown. Data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (+)-NC group, "p < 0.01; the scale bar indicates 50 m. (E) Three-dimensional culture was used to determine the effect of knockdown of PABPC5 on the VM formation ability of U87 and U251 cells. Representative images and corresponding statistical analysis plots are shown. Data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (+)-NC group, "p < 0.01. The scale bar indicates 50 μm. (F) Knockdown of PABPC5 regulates LAMC2 protein expression levels in U87 and U251 cells. The data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (+)-NC group, "p < 0.05 and *p < 0.01. (G) HCG15 expression in NBT, LGGT, and HGGT. Data are expressed as mean ± SD (n = 12). Compared with the NBT group, "p < 0.01 and "p < 0.05. Compared with the LGGT group, "p < 0.05. (H) HCG15 expression in NHAs and U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the NHA group, *p < 0.01 and *p < 0.05. (I) Detecting the effects of knockdown and overexpression of HCG15 on proliferation. Data are expressed as mean ± SD (n = 3). Compared with the HCG15 (+)-NC group, *p < 0.01 and "p < 0.05. (J) Detecting the effects of knockdown and overexpression of HCG15 on the migration and invasion ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the HCG15 (+)-NC group, "p < 0.01. Compared with the HCG15 (+)-NC group, the scale bar indicates 50 μm. (K) Effect of knockdown and expression of HCG15 on the VM formation ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the HCG15 (+)-NC group, *p < 0.01. Compared with the HCG15 (+)-NC group, the scale bar indicates 50 μm. (L) Detecting the effects of knockdown and overexpression of HCG15 regulates LAMC2 protein expression levels in U87 and U251 cells, respectively. Data are expressed as mean ± SD (n = 3). Compared with the HCG15 (+)-NC group, "p < 0.01. Compared with the HCG15 (+)-NC group, #p < 0.01.
Molecular Therapy: Oncolytics

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ZNF331 was Lowly Expressed in Glioma Tissues and Cells, and Overexpression of ZNF331 Inhibited VM Formation

Western blot was used to detect ZNF331 expression in glioma tissues (12 normal brain tissues, 12 low-grade glioma tissues [WHO I–II], and 12 high-grade glioma tissues [WHO III–IV]) and in glioma cells (U87 and U251). As shown in Figures 3A and 3B, compared with the normal brain tissue group, the expression of ZNF331 was significantly reduced in low-grade glioma tissue and high-grade glioma tissue (p < 0.01), and the expression of ZNF331 was significantly lower than in low-grade glioma tissues (p < 0.01). In U87 and U251 glioma cells, the expression levels of ZNF331 were significantly lower than in the NHA group (p < 0.01). The effects of ZNF331 knockdown and overexpression on the biological behavior of gliomas were examined. Knockdown and overexpression of ZNF331 were constructed in glioma cells. The tumor cells were tested for transfection efficiency. We selected #1 with the higher knockdown efficiency for subsequent experiments (p < 0.01) (Figure S1E). We tested the transfection efficiency of overexpressing ZNF331 glioma cells. Its expression increased about three times (p < 0.01) (Figure S1F). The results are shown in Figures 3C–3E. Compared with the control group, no significant statistical difference was found between the ZNF331 (-)–NC group and the ZNF331 (+)–NC group. Compared with the ZNF331 (-)–NC group, the ZNF331 (+) group had significantly enhanced cell proliferation, migration, invasion, and VM formation ability and enhanced LAMC2 protein expression levels (p < 0.01 and p < 0.05). Compared with the ZNF331 (+)–NC group, in the ZNF331 (+) group, proliferation, migration, invasion, and VM formation ability were significantly reduced, as were LAMC2 protein expression levels (p < 0.01). After PABPC5 knockdown in glioma cells, compared with the control group, there was no significant statistical difference between the PABPC5 (–)–NC group. Compared with the PABPC5 (–)–NC group, the expression of ZNF331 mRNA and protein was significantly increased in the PABPC5 (–) group (p < 0.01 and p < 0.05) (Figure 3G). After knockdown and overexpression of HCG15 in glioma cells, compared with the control group, there was no significant statistical difference between the HCG15 (–)–NC group and the HCG15 (+)–NC group. Compared with the HCG15 (–)–NC group, the expression of ZNF331 mRNA and protein was significantly increased in the HCG15 (–) group (p < 0.01). Compared with the HCG15 (+)–NC group, the expression of ZNF331 mRNA and protein was significantly reduced in the HCG15 (+) group (p < 0.01) (Figure 3H). The stable PABPC5 knockdown cotransfection with HCG15 knockdown or overexpression glioma cells were constructed. The effect on ZNF331 mRNA and protein was examined. The results are shown in Figure 3I. Compared with the control group, the PABPC5 (–) + HCG15 (–)–NC group and PABPC5 (–) + HCG15 (+)–NC group had significantly increased mRNA and protein levels of ZNF331 (p < 0.01 and p < 0.05). The PABPC5 (–) + HCG15 (–)–NC group compared with the PABPC5 (–) + HCG15 (–)–NC group had significantly higher mRNA and protein expression levels of ZNF331 (p < 0.01 and p < 0.05). Compared with the PABPC5 (–) + HCG15 (+)–NC group, HCG15 (+) reversed the inhibitory effect of PABPC5 (–) on the mRNA and protein expression of ZNF331 in PABPC5 (–) + HCG15 (+) group (p < 0.01 and p < 0.05).

HCG15 Promoted the Degradation of ZNF331 and Enhanced the VM Formation of Glioma Cells through the SMD Pathway

By applying a bioinformatics database (IntaRNA), it was predicted that HCG15 may bind to the 3’ UTR of ZNF331 mRNA through a specific sequence Alu element. We applied a dual-luciferase gene reporting analysis system to verify this. Compared with the ZNF331-3’ UTR-Wt + HCG15 (+)–NC group, the luciferase activity of the ZNF331-3’ UTR-Wt + HCG15(+) group significantly decreased (p < 0.05), but there was no statistical difference between the ZNF331-3’ UTR-Mut + HCG15 (+) group and the ZNF331-3’ UTR-Mut + HCG15 (+)–NC group (Figure 4A). We applied RNA IP experiments to verify the relationship between HCG15 and STAUI, STAUI, and ZNF331. We found that HCG15 (ZNF331 mRNA) was significantly higher in the anti-STAUI group than in the anti-IgG group (p < 0.01) (Figure 4B). To determine whether STAUI and UPF1 participate in the interaction between HCG15 and ZNF331 mRNA, U87 and U251 cells were transfected with STAUI and UPF1 knockdown plasmids. Nascent RNA experiments were performed; we found that the HCG15 (–)–NC group, HCG15 (+)–NC group, STAUI (–)–NC group, and UPF1 (–)–NC group compared with the HCG15 (–)–NC group, STAUI (–)–NC group, and UPF1 (–)–NC group showed no significant statistical difference, meaning that there were no nascent ZNF331 mRNA changes. In the HCG15 (–)–NC group, STAUI (–)–NC group, and UPF1 (–)–NC group, the half-life of ZNF331 mRNA was significantly longer than in the HCG15 (–)–NC group, STAUI (–)–NC group, and UPF1 (–)–NC group (p < 0.01 and p < 0.05). Compared

Figure 2. PABPC5 Enhanced the Biological Behavior of Glioma Cells by Increasing the Stability of HCG15

(A) Knockdown of PABPC5 affects HCG15. Data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (–)–NC group, “p < 0.01. (B) Validating the binding interaction between PABPC5 and HCG15. Data are expressed as mean ± SD (n = 3). Compared with the respective anti-normal IgG groups, “p < 0.01. (C) Knockdown of PABPC5 to detect nascent HCG15 in U87 and U251 cells; data are expressed as mean ± SD (n = 3). (D) Effect of knockdown of PABPC5 on HCG15 half-life. Data are expressed as mean ± SD (n = 3). Compared with the control group, “p < 0.01. Compared with the PABPC5 (–)–NC group, “p < 0.01 and “p < 0.05. (E) Detecting the effects of knockdown of PABPC5, knockdown of HCG15, and overexpression of HCG15 on the proliferation ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the control group, “p < 0.01. Compared with the PABPC5 (–) + HCG15 (–)–NC group, #p < 0.05 and ##p < 0.01. Compared with the PABPC5 (–) + HCG15 (+)–NC group, &p < 0.05 and **p < 0.01. (F) Detecting the effects of knockdown of PABPC5, knockdown of HCG15, and overexpression of HCG15 on the migration and invasion ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the control group, “p < 0.01. Compared with the PABPC5 (–) + HCG15 (–)–NC group, #p < 0.05 and **p < 0.01. Compared with the PABPC5 (–) + HCG15 (+)–NC group, &p < 0.05. The scale bar indicates 50 μm. (G) Detecting the effects of knockdown of PABPC5, knockdown of HCG15, and overexpression of HCG15 on the VM ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the control group, “p < 0.01. Compared with the PABPC5 (–) + HCG15 (–)–NC group, #p < 0.05. Compared with the PABPC5 (–) + HCG15 (+)–NC group, &p < 0.05. The scale bar indicates 50 μm. (H) Detecting the effects of knockdown of PABPC5, knockdown of HCG15, and overexpression of HCG15 on LAMC2 protein expression in U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the control group, “p < 0.01.”
with the HCG15 (+)-NC group, the half-life of ZNF331 mRNA was significantly reduced in the HCG15 (+) group (p < 0.01) (Figures 4C–4E). In addition, compared with the STAUI (-)-NC and UPF1 (-)-NC groups, the protein expression of ZNF331 was significantly increased in the STAUI (-) and UPF1 (-) groups (p < 0.01 and p < 0.05) (Figures 4F and 4G). We further knocked down or overexpressed STAUI and knocked down HCG15 in glioma cells and detected changes in ZNF331 protein expression. The results are shown in Figure 4H. Compared with the control group, the expression levels of the HCG15 (-) + STAUI (-)-NC and HCG15 (-) + STAUI (+)-NC group were significantly increased (p < 0.01 and p < 0.05). Compared with the HCG15 (-) + STAUI (-)-NC group, the HCG15 (-) + STAUI (-) group had significantly increased expression levels of ZNF331 protein (p < 0.01 and p < 0.05). Compared with the HCG15 (+) + STAUI (+)-NC group, STAUI (+) significantly reversed the effect of HCG15 (-) on increasing the expression level of ZNF331 in the HCG15 (+) + STAUI (+) group (p < 0.01 and p < 0.05).

Furthermore, ZNF331 was knocked down or overexpressed in glioma cells, which were transfected with HCG15 (-) at the same time. The results are shown in Figures 4F–4K. Compared with the HCG15 (-)-NC + ZNF331 (+)-NC group, the HCG15 (-)+ZNF331 (+) group had a stronger ability to inhibit glioma cell proliferation, migration, invasion, and VM formation (p < 0.01). Compared with the HCG15 (-)-NC + ZNF331 (-)-NC group, HCG15 (-)+ZNF331 (-) reversed the inhibitory effect of HCG15 (-) on the VM formation ability of U87 and U251 cells. The changes in expression of PABPC5 mRNA and protein are detected in U87 and U251 cells after knockdown and overexpression of ZNF331. Data are expressed as mean ± SD (n = 3). Compared with the control group, PABPC5 mRNA expression was significantly decreased in the HCG15 (-) group (p < 0.01 and p < 0.05). Compared with the control group, the protein expression of PABPC5 was significantly decreased in the ZNF331 (+) group (p < 0.01 and p < 0.05). In previous experiments, we detected that ZNF331 regulated the expression of LAMC2 protein and affected the ability of VM formation. This time we also used knockdown and overexpression of ZNF331 glioma cells to detect the expression of PABPC5 protein. The results are shown in Figure 5E. Compared with the control group, the ZNF331 (-)-NC group and ZNF331 (+)-NC group had no significant statistical difference. Compared with the ZNF331 (-)-NC group, the protein expression of PABPC5 was significantly increased in the ZNF331 (-) group (p < 0.01 and p < 0.05). Compared with the ZNF331 (+)-NC group, the protein expression of PABPC5 was significantly decreased in the ZNF331 (+) group (p < 0.01 and p < 0.05).

Knockdown of PABPC5 and HCG15 and Overexpression of ZNF331 Alone or in Combination Inhibited Glioma Growth and Prolonged the Survival Time of Nude Mice

The above hypothesis was confirmed by in vivo tumor model experiments. First, subcutaneous transplantation tumor technology was used to clarify the effects of PABPC5 knockdown, HCG15 knockdown, and overexpression of ZNF331 on glioma growth volume alone or in combination (Figures 6A and 6B). There was no significant difference between the control group and the negative control (NC) group. Compared with the NC group, PABPC5 (-) group, HCG15

Figure 3. ZNF331 Was Lowly Expressed in Gliomas and Inhibited the Formation of Vascular Mimicry (A) ZNF331 protein expression levels in NBST, LGST, and HOGT. Data are expressed as mean ± SD (n = 12). Compared with the NBST group, **p < 0.01. Compared with the LGST group, #p < 0.01. (B) ZNF331 protein expression levels in NHA and U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the NH4 group **p < 0.01. (C) Detecting the effects of knockdown and overexpression of ZNF331 on the proliferation ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the ZNF331 (-)-NC group, **p < 0.01. Compared with the ZNF331 (+)-NC group, **p < 0.01 (D) Detecting the effect of ZNF331 on the migration and invasion ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the ZNF331 (-)-NC group, *p < 0.05 and **p < 0.01. Compared with the ZNF331 (+)-NC group, #p < 0.01. The scale bar indicates 50 μm. (E) Detecting the effects of ZNF331 on the VM formation ability of U87 and U251 cells. Data are expressed as mean ± SD (n = 3). Compared with the ZNF331 (-)-NC group, **p < 0.01. Compared with the ZNF331 (+)-NC group, #p < 0.05 and #p < 0.01. The scale bar indicates 50 μm. (F) Detecting the effects of knockdown and overexpression of ZNF331 regulates LAMC2 protein expression levels in U87 and U251 cells, respectively. Data are expressed as mean ± SD (n = 3). Compared with the ZNF331 (-)-NC group, **p < 0.01 and *p < 0.05. Compared with the ZNF331 (+)-NC group, #p < 0.01 and #p < 0.01. (G) Expression levels of ZNF331 mRNA and protein are detected in U87 and U251 cells after knockdown of PABPC5. Data are expressed as mean ± SD (n = 3). Compared with the PABPC5 (-)-NC group, *p < 0.05 and **p < 0.01. (H) The expression levels of ZNF331 mRNA and protein are detected in U87 and U251 cells after knockdown and overexpression of HCG15. Data are expressed as mean ± SD (n = 3). Compared with the control group, *p < 0.05 and **p < 0.01. Compared with the PABPC5 (-) + HCG15 (-)-NC group, #p < 0.05 and #p < 0.01. Compared with the PABPC5 (-) + HCG15 (+)-NC group, &p < 0.05 and &p < 0.01.

ZNF331 Bound Directly to the Promoter Regions of LAMC2 and PABPC5

We predicted from the bioinformatics database (JASPAR) that ZNF331 may have potential binding sites with the promoter regions of PABPC5 and LAMC2. The above hypothesis was confirmed by ChIP experiments verified that ZNF331 combined with LAMC2 and PABPC5. The results are shown in Figures 5A and 5B. ZNF331 could be seen at the binding sites of LAMC2 and PABPC5, which proved that there was binding in the promoter region. Interestingly, we found that ZNF331 not only altered LAMC2 but also affected PABPC5. ZNF331 was knocked down or overexpressed in glioma cells. The changes in LAMC2 mRNA and PABPC5 mRNA were analyzed by real-time qPCR (Figures 5C and 5D). Compared with the control group, the ZNF331 (-)-NC group and ZNF331 (+)-NC group showed no significant statistical difference. Compared with the ZNF331 (-)-NC group, the expression of LAMC2 and PABPC5 mRNA was significantly increased in the ZNF331 (-) group (p < 0.01). Compared with the ZNF331 (+)-NC group, LAMC2 and PABPC5 mRNA expression was significantly reduced in the ZNF331 (+) group (p < 0.01 and p < 0.05). In previous experiments, we detected that ZNF331 regulated the expression of LAMC2 protein and affected the ability of VM formation. This time we also used knockdown and overexpression of ZNF331 glioma cells to detect the expression of PABPC5 protein. The results are shown in Figure 5E. Compared with the control group, the ZNF331 (-)-NC group and ZNF331 (+)-NC group had no significant statistical difference. Compared with the ZNF331 (-)-NC group, the protein expression of PABPC5 was significantly increased in the ZNF331 (-) group (p < 0.01 and p < 0.05). Compared with the ZNF331 (+)-NC group, the protein expression of PABPC5 was significantly decreased in the ZNF331 (+) group (p < 0.01 and p < 0.05).
(-) group, ZNF331 (+) group, and PABPC5 (-) + HCG15 (-) + ZNF331 (+) group tumor volumes were significantly lower (p < 0.01). No significant difference was found between the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group. Compared with the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group, the PABPC5 (-) + HCG15 (-) + ZNF331 (+) group had the most obvious effect of lower tumor volume (p < 0.01). The effect of PABPC5 knockdown, HCG15 knockdown, and ZNF331 overexpression on the survival time of nude mice was studied using orthotopic transplantation tumor technology (Figure 6C). There was no significant statistical difference between the control group and the NC group. Compared with the NC group, the PABPC5 (-) group, HCG15 (-) group, ZNF331 (+) group, and PABPC5 (-) + HCG15 (-) + ZNF331 (+) group had significantly longer survival times (p < 0.01 and p < 0.05). No significant difference was found between the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group. Compared with the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group, the PABPC5 (-) + HCG15 (-) + ZNF331 (+) group had the longest survival time. Finally, we wanted to clearly show whether there was a statistical difference in the number of VMs among the group. Subcutaneously transplanted tumors in nude mice were surgically excised, and the tumor tissues were sectioned with CD34-PAS dual staining. IHC was used for VM detection. No significant difference was found between the control group and the NC group. Compared with the NC group, the number of VMs in the PABPC5 (-) group, HCG15 (-) group, ZNF331 (+) group, and PABPC5 (-) + HCG15 (-) + ZNF331 (+) group were reduced significantly (p < 0.01 and p < 0.05). No significant difference was found between the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group. Compared with the PABPC5 (-) group, HCG15 (-) group, and ZNF331 (+) group, the number of VMs was significantly reduced in the PABPC5 (-) + HCG15 (-) + ZNF331 (+) (p < 0.05) (Figure 6D).

**DISCUSSION**

This is the first study to verify that PABPC5 and HCG15 are highly expressed in glioma tissues and cells. In glioma cell knockdown, PABPC5 inhibits proliferation, migration, invasion, and VM formation by reducing the stability of HCG15. ZNF331 is lowly expressed in glioma tissues and cells. Knockdown of HCG15 inhibits degradation of ZNF331 mRNA through the SMD pathway and increases the expression of ZNF331 in glioma cells to inhibit the malignant biological behavior of glioma cells and formation of VM. ZNF331 not only directly binds to the LAMC2 promoter region of VM-related proteins but also binds to the promoter region of PABPC5. At the same time, ZNF331 plays the role of transcription repressor; it inhibits transcription of PABPC5 and LAMC2. PABPC5, HCG15, and ZNF331 construct a feedback loop to regulate VM formation (Figure 6E).

RBP plays an important role in regulating the formation of VM of tumor cells by binding to single- or double-stranded RNA. RBPs-BNIP3 is highly expressed in melanoma cells, and RBP-BNIP3 binds to CD47; it induces VM formation in the cell’s hypoxic state and mediates cell adhesion and migration of extracellular matrix. Fus is highly expressed in glioma tissues and cells, and knockdown of Fus inhibits formation of glioma cell VM through the feedback regulation loop formed by circ_002136/miR-138-5p/STAU1. This study found that PABPC5 was highly expressed in glioma tissues and cells and that knockdown of PABPC5 inhibited proliferation, migration, invasion, and VM formation of glioma cell. At the same time, PABPC5 inhibited expression of the VM marker protein LAMC2 protein level. That indicates that PABPC5 plays an oncogenic role in glioma cells.

Recent studies have demonstrated that lncRNAs are abnormally expressed in a variety of tumors; they are involved in regulation of tumorigenesis and development. The IncRNA HOXA-AS2 is highly expressed in glioma tissues and cells, and its expression is inhibited by binding to mir-373. It increases the expression of EGFR and promotes VM of glioma cells. At the same time, the IncRNA MIR155HG promotes the growth of glioma cells through the mir-185/ANXA2 axis. Our previous work found that HCG15 is highly expressed in glioma tissues and cells. Knockdown of HCG15 reduced the expression level of LAMC2 protein and inhibited the proliferation, migration, invasion, and VM formation of glioma cells. Overexpression of HCG15 increased the protein expression level of LAMC2 and promoted malignant biological behavior of glioma cells. It has been suggested that HCG15 plays a tumor-promoting role in glioma cells.
We previously reported the mechanisms of various lncRNAs regulating the formation of glioma cell VM. For example, SNHG16 and USF1 are highly expressed in glioma tissues and cells and play a role in promoting VM formation by combining miR-212-3p and miR-429.43 TUG1 plays the role of an oncogenic gene through negatively regulating the effect of miR-299 and enhancing angiogenesis in tumors.44 Numerous studies have confirmed that RBPs regulate their stability by binding to lncRNAs and participate in the development of tumors.45 HuR directly combines with HOTAIR to enhance its stability, which negatively regulates the expression of miR-7 and promotes migration and invasion in head and neck squamous cell carcinoma cells.46 In this study, a bioinformatics database RBP map search revealed that PABPC5 and HCG15 had potential binding sites. RNA IP experiments were used to verify this specific binding effect. Furthermore, we found that the expression level of HCG15 was decreased after PABPC5 knockdown in glioma cells, whereas the nascent HCG15 was not transcribed but the half-life of HCG15 was shortened. In other words, HCG15 stability was decreased after PABPC5 knockdown in glioma cells.

![Figure 5. ZNF331 Bound to the Promoter Regions of LAMC2 and PABPC5](image-url)
The stability of HCG15 was changed by knockdown of PABPC5, which was not caused by HCG15 nascent RNA. When knockdown of PABPC5 was combined with knockdown of HCG15, the effect was strengthened further regarding proliferation, migration, invasion, and VM formation ability in glioma cells. Overexpression of HCG15 reversed the inhibitory effect of PABPC5 on the proliferation, migration, invasion, and VM formation ability of glioma cells. Therefore, PABPC5 bound HCG15 to regulate the ability of VM formation by changing stability of HCG15 in glioma cells.

ZNF331 plays a transcriptional inhibitory role in tumorigenesis and development. ZNF331 was originally identified in thyroid tumors; it is a member of the KRAB zinc-finger protein family and has a transcriptional regulatory function as a transcription repressor. ZNF331 inhibits the growth of esophageal and gastric cancers, and other studies have found that ZNF331 inhibits colony formation and cell proliferation in colorectal cancer cells and induces G1/S arrest during development of colorectal cancer. Because the ZNF331 promoter region is often methylated, it loses its role as a tumor suppressor gene, so ZNF331 plays a tumor-suppressive role in colon cancer.
found that ZNF331 was lowly expressed in glioma tissues and cells and that its expression level decreased as the glioma tissue pathological grade increased. Overexpression of ZNF331 reduced the expression level of LAMC2 protein and inhibited proliferation, migration, invasion, and VM formation ability in glioma cells.

The SMD pathway is ubiquitous in mammalian cell development. It has been reported that IncRNAs bind to the 3' UTR of transcription factors via Alu elements to form an SBS. After binding to STAU1, it directly recruits the ATP-dependent RNA helicase UPF1 to trigger STAU1-mediated mRNA degradation. The SMD pathway participates in a variety of cellular life processes, including differentiation of mouse myoblasts into myotubes and differentiation of mouse adipocytes, and can regulate the migration of human HaCaT keratinocytes. STAU1, as a post-transcriptional regulator, function with IncRNA, and then they are involved in epidermal end differentiation in gastric cancer cell lines and regulate cyclin-dependent kinase gene transcription and expression, thereby affecting the proliferation and apoptosis of gastric cancer cells.

In this study, the bioinformatics software Repeat Masker and IntaRNA were used to predict that there were Alu elements in ZNF331 and that there was a potential binding site between the Alu element of HCG15 and the 3' UTR of ZNF331. Dual-luciferase reporter gene and RNA IP experiments were used to verify this targeted binding effect and the binding site. We found that knockdown of HCG15 significantly increased the expression level of ZNF331 mRNA and extended its half-life. Overexpression of HCG15 significantly reduced the expression level of ZNF331 mRNA and shortened its half-life. Whereas the transcription of nascent ZNF331 mRNA was not affected by HCG15, so HCG15 combined with ZNF331 via Alu to form SMD to change its stability. In the SMD pathway, STAU1 binds to SBS formed in combination of IncRNA and target mRNA through Alu elements, and then recruits UPF1 to play a role in post-translational modification. Therefore, we knocked down STAU1 and UPF1 in glioma cells. We observed that each increased the expression level of ZNF331 mRNA and extend its half-life to increase its stability and inhibit LAMC2 expression, inhibiting formation of VM in glioma cells. Simultaneous knockdown of HCG15 and STAU1 further reduced the protein expression of ZNF331. Overexpression of STAU1 reversed knockdown of HCG15, which increased ZNF331 protein expression. This proved the interaction between HCG15 and STAU1. Therefore, it was suggested that HCG15 regulates the expression level of ZNF331 by participating in the STAU1-mediated mRNA decay mechanism and then regulates VM formation. IncRNAs participate in molecular regulatory networks through the SMD pathway, which has attracted increasing attention from researchers. The IncRNA SNHG5 is highly expressed in colorectal cancer cells, and knockdown of SNHG5 blocks STAU1-mediated decay of SPATA2 mRNA and inhibits growth of colorectal cancer cells through the SMD pathway.

It is well known that LAMC2 is a VM formation-related protein, and involved in the biological process of tumor vascular mimic formation. The transcription factor EphA2 activates the AKT pathway in high-serum-treated prostate cancer cells to increase the expression of MMP-2 and LAMC2, increasing VM formation ability. ZEB1 binds directly to the LAMC2 promoter region to exert transcriptional repression, so ZEB1 inhibits migration and invasion of prostate cancer cells. Studies have reported that low expression of EphA2 or LAMC2 inhibits VM formation in glioma cells. In this study, ChIP experiments were performed to confirm the specific binding and binding sites of ZNF331 and LAMC2 promoter region. Knockdown of ZNF331 increased the expression levels of LAMC2 mRNA and protein, and the proliferation, migration, invasion, and VM formation ability of glioma cells was significantly enhanced, suggesting that ZNF331 acts as a transcriptional repressor in gliomas to regulate VM formation. Further, the ChIP experiment was used to confirm that ZNF331 had a binding effect and binding site with the promoter region of the upstream RBP PABPC5, and knockdown of ZNF331 also increased PABPC5 mRNA and protein expression levels. ZNF331/ PABPC5 formed a feedback loop to play an important role in regulating the formation of glioma VM.

Finally, in this study, knockdown of PABPC5, knockdown of HCG15, and overexpression of ZNF331 were performed alone or in combination in nude mice. It was confirmed that knockdown of PABPC5, knockdown of HCG15, and overexpression of ZNF331 alone could effectively inhibit the growth of transplanted tumors and prolong the survival of nude mice. Combined knockdown of PABPC5, knockdown of HCG15, and overexpression of ZNF331 had the best antitumor effect and the longest survival time in nude mice. The potential value of PABPC5 knockdown, HCG15 knockdown, and ZNF331 overexpression alone or association was suggested.

In summary, this study demonstrates, for the first time, that PABPC5 and HCG15 are highly expressed in glioma tissues and cells, that PABPC5 and HCG15 are targeted for binding, that HCG15 acts on ZNF331 through SMD, and that ZNF331 is lowly expressed in glioma tissues and cells. Knockdown of PABPC5 reduced the stability of HCG15, and knockdown of HCG15 is through the SMD pathway to prevent its degradation of ZNF331 mRNA. ZNF331 binds to the LAMC2 promoter region, inhibiting downstream LAMC2 transcription and, thus, inhibiting glioma cell VM formation ability. ZNF331 combines with the promoter region of PABPC5 and inhibits upstream PABPC5 transcription to form a feedback regulatory loop. Therefore, they inhibit the effects of proliferation, migration, invasion, and VM formation in glioma cell. The purpose of this study is to demonstrate that the PABPC5/HCG15/ZNF331 feedback loop plays an important role in regulating glioma VM formation. The feedback loop provides new strategies for glioma treatment and new directions for glioma intervention.

MATERIALS AND METHODS

Cell Lines and Cell Culture

The glioma cell lines U87 and U251 and the human embryonic kidney 293T (HEK293T) cells were purchased from the Cell Resource Center of the Shanghai Institute of Biological Sciences. Primary NHAs were purchased from ScienCell Research Laboratory. The cells were preserved in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The 293T cell line was cultivated in DMEM/F-12 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.
cultured in DMEM or RPMI 1640 medium (Gibco, Carlsbad, CA, USA) in 5% CO₂ and 37°C incubators.

Human Tissue Specimens
Normal brain tissue (NBT; n = 12) was selected as an NC for patients with traumatic craniocerebral trauma and craniotomy; gliomas and surgical patients were selected according to pathologists to determine the pathological grade according to WHO classification. All glioma tissues were classified into low-grade glioma (WHO I–II, n = 12) and high-grade glioma (WHO III–IV, n = 12). The above tissues were obtained from patients at Shengjing Hospital affiliated with China Medical University. All patients voluntarily provided signed informed consent. Human tissue specimens were approved by the hospital ethics committee.

CD34-Labeled Endothelium PAS Dual Staining
VM was detected by CD34-PAS in glioma tissues. The assay was performed as reported previously.63

Real-Time qPCR
The real-time qPCR assay was performed as reported previously.63 The expression levels were normalized to endogenous controls, and the relative quantification of the RNA was expressed by the 2primer method. The PCR primer sequences are shown in Table S1.

Cell Proliferation Assay
Cell viability was detected using CCK-8 (Beyotime Biotechnology, China). The process of preformation and application reagents were consistent with a previous report.63

Plasmid Construction and Cell Transfection
PABPC5 short hairpin RNA (PABPC5(-)), HCG15 short hairpin RNA (HCG15(-)), the HCG15 full-length plasmid (HCG15(+)), ZNF331 short hairpin RNA (ZNF331(-)), the ZNF331 full-length plasmid (ZNF331(+)), STAU1 short hairpin RNA (STAU1(-)), the STAU1 full-length plasmid (STAU1(+)), UPF1 short hairpin RNA (UPF1(-)), and their respective non-targeting sequences (NC) were synthesized (GeneChem, Shanghai, China). Reagents for transfection systems and drugs for screening cells were identical to a previous report.63 The plasmid sequences are shown in Table S2.

Western Blot
This procedure and the reagents used were consistent with a previous report.63 The primary antibodies were as follows: PABPC5 (1:500, ab-clonal, Wuhan, China), ZNF331 (1:2,000, Proteintech, Rosemont, IL, USA), LAMC2 (1:500, Santa Cruz Biotechnology, USA), and GAPDH (1:10,000, Proteintech, Rosemont, IL, USA). The secondary antibodies were diluted (1:10,000, Proteintech, Rosemont, IL, USA); PABPC5, ZNF331, and LAMC2 were rabbit resistance, and GAPDH was mouse resistance. The ratio of the IDV(Integrated Density Value) integral density of the target band to the GAPDH band was used as the relative expression level of the target protein, and statistical analysis was performed.

Cell Migration and Invasion Assay
Cell migration and invasion were measured using a polycarbonate membrane with a pore size of 8 μm (Corning Life Sciences, NY, USA) in a 24-well chamber. Specific operational details have been described previously.63

In Vitro VM Tube Formation Assay
Each well was covered with 300 μl Matrigel (Becton Dickinson, Franklin Lakes, NJ, USA) in a 24-well plate, which was placed on a flat surface for coagulation. Specific operational details have been described previously.63 Independent observers calculated the total number of tubular structures for each image for statistical analysis.

ChIP Assays
ChIP assays were performed using the Simple ChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology, Danvers, MA, USA) according to the manufacturer’s instructions. Specific operational details have been described previously.63 The primer sequence is shown in Table S3.

Reporter Assay Kit (Promega) was used according to a previous report.63

In Vivo Xenograft Mouse Model
For in vivo xenograft experiments, 4-week-old athymic nude mice (BALB/c) were purchased from the Cancer Institute of the China Academy of Medical Science, and the mice were randomly divided into 5 groups: a control group and PABPC5(-)-NC, HCG15(-)-NC+ZNF331(+)-NC, PABPC5(-), HCG15(-), ZNF331(+), and PABPC5(-)+HCG15(-)+ZNF331(+) groups. When subcutaneous
xenografts were administered to each nude mouse, \(3 \times 10^5\) glioma cells were injected into the right axillary area. According to the estimation formula,\(^{63}\) tumor volume was measured every 7 days after implantation until 42 days after implantation. Finally, the presence of VM was detected by CD34-PAS dual stain. Survival analysis experiments were performed using a Kaplan-Meier survival curve. \(3 \times 10^5\) glioma cells were injected into the right striatum by stereotactic technique, and the survival time was recorded. All experiments with mice were conducted strictly in accordance with the China Medical University Application for Laboratory Animal Welfare and Ethical Review (201702 Edition), approved by the Ethics Committee of China Medical University.

**Statistical Analysis**

All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA) and SPSS 19.0 software (IBM, New York, NY, USA). All values were derived from the mean ± standard deviation (SD) of more than three independent experiments. The above results were analyzed by Student’s t test or one-way analysis of variance (ANOVA). There was a statistically significant difference (at least \(p < 0.05\)).

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at [https://doi.org/10.1016/j.omto.2020.03.017](https://doi.org/10.1016/j.omto.2020.03.017).

**AUTHOR CONTRIBUTIONS**

Y.L. contributed to experiment design, manuscript draft, and data analysis. F.J. contributed to experiment implementation, manuscript draft, and data analysis. Y.X. designed the experiments. X.R., C.Y., J.M. contributed to experiment design, manuscript draft, and data analysis. J.Z., P.W., X.L., and J.M. conceived or designed the experiments. D.W., and S.S. performed the experiments. Y.Y. and L.S. analyzed the data. J.Z., P.W., X.L., and J.M. conceived or designed the experiments, and wrote the manuscript. All authors read and approved the final manuscript.

**CONFLICTS OF INTEREST**

The authors declare no competing interests.

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