INTRODUCTION

As the most frequent malignant tumor, gastric cancer (GC) is a major cause of tumor-related death.\(^1,2\) Because of the unconspicuous early symptoms, many GC patients were diagnosed at advanced stages.\(^3\) Although GC can be treated by surgical resection in the clinic, its invasive metastasis greatly increased the difficulty of operation.\(^4\) Thus, it is imperative to investigate the underlying molecular mechanism of GC genesis and progression and develop a new therapeutic regimen.

Circular RNA (circRNAs) are characterized by the closed-loop structure and the absence of 5′heads or 3′polyadenylated tail.\(^5\) which made them more stable and resistant to the degradation of exonuclease.\(^6\) Due to the superior characteristics of circRNA, it was found to exert vital effects in the research of new tumor biomarkers.\(^7\) Currently, multiple circRNAs have been found to play an important role in regulating the progress of cell biology in various human diseases.\(^8,9\) Consequently, circRNAs may be promising biomarkers for the management of diverse human diseases.
For instance, the high expression of circGSK3B in hepatocellular carcinoma (HCC) was verified and upregulated circGSK3B enhanced HCC cell malignant phenotype. The high expression of circ_0006220 in non-small-cell lung carcinoma (NSCLC) was closely involved in promoting cell growth, migration, and invasion. In addition, vast data have shown that circRNA regulates the function of drug resistance in human tumors. For example, circ_0014235 could mediate biological progress and gefitinib-resistance in NSCLC.

Bai et al. disclosed that hsa_circ_0004674 reinforced the doxorubicin (DXR) resistance in osteosarcoma (OS) via Wnt/β-catenin signal pathway. Hsa_circ_0043691 is originated from 5′-nucleotidase, cytosolic IIIB (NT5C3L), located in chr17: 39983677–39983878 and contains 201 nucleotides. Hsa_circ_0043691 was observably overexpressed in GC, indicating that hsa_circ_0043691 may have a vital function in GC progression. Therefore, it was essential to further exploit the possible molecular mechanisms of hsa_circ_0043691 in GC.

2 MATERIALS AND METHODS

2.1 Clinical human specimens

Thirty-nine pairs of fresh GC samples and paired non-cancerous tissue samples were removed from GC patients at Fuyong People’s Hospital. No donors in this paper had received preoperative treatment and signed written informed consent prior to surgery. All acquired tissue samples were quickly transferred to a liquid nitrogen container. Our work was supported by the Research Ethics Committee of Fuyong People’s Hospital.

2.2 Cell transfections

Several GC cell lines, including N87, HGC27, and AGS, were bought from ATCC. Human normal gastric cell (GES-1) and GC cell line (MKN-45) were commercially gained from Chinese Academy of Sciences (Shanghai, China). The RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) was used to culture cells under a moist environment with 5% CO2 at 37°C.

Small interfering RNAs (siRNAs) targeting hsa_circ_0043691 (si-hsa_circ_0043691), miR-1294 mimic (miR-1294), inhibitor (in-miR-1294) and their relative control (si-NC, miR-NC and in-miR-NC) were constituted from GenePharma. Plasmid of pcDNA3.1-PBX3 (PBX3), and pcDNA3.1 (pcDNA) were composed and obtained from Origene. Transient transfection of the above-mentioned oligonucleotides or vectors were executed by Lipofectamine 3000 (Thermo Fisher).

2.3 qRT-PCR

Total RNA was isolated through trizol reagents (TaKaRa) in line with the guidebook. The cDNA was obtained by using the PrimeScriptVR RT kit (Promega). SYBR Green PCR Master Mix (BioTNT Biotechnologies) was employed to measure the expression levels of mRNAs and genes, and all primer sequences were presented in Table 1. The relative expression level of hsa_circ_0043691, miR-1294 and PBX3 was determined by the 2−ΔΔCt quantification method and normalized to β-actin and U6.

2.4 CircRNA stability analysis and subcellular localization analysis

To analyze hsa_circ_0043691 stability, total RNA from GC cells were co-cultured with 3 U/mg RNase R at 37°C for 30 min. The cells that were untreated with RNase R (RNase R-) were regarded as the internal control. Moreover, 2 mg/ml Actinomycin D (Sigma-Aldrich) was employed to challenge GC cell lines (AGS and MKN-45) for 0, 4, 8, 12, and 24 h. Finally, levels of hsa_circ_0043691 and linear NT5C3L were ascertained by qRT-PCR.

Hsa_circ_0043691 localization in cytoplasm or nucleus was evaluated by PARIS Kit (Life Technologies) in AGS and MKN-45 cells. GC cells were cultured with 200 μl Lysis Buffer. After centrifugation, the supernatant (cytoplasmic RNA) and the remaining fraction (nuclear RNA) were separated and the expression level of hsa_circ_0043691 was measured by qRT-PCR.

| Name                   | Primers for qRT-PCR (5′-3′)                      |
|------------------------|--------------------------------------------------|
| hsa_circ_0043691       | Forward TGGGAGACTCTATCGGGGAC                      |
|                        | Reverse CCTGGAGAAAAACCTTGTCA                     |
| NT5C3L                 | Forward ACAGCATGTCAGTGCTCTAGG                    |
|                        | Reverse TAGAAGCATCCCCATGTGCC                    |
| PBX3                   | Forward GACATCGGGCGACATCCTCC                     |
|                        | Reverse TGTTTCTTGTGTCGGCT                       |
| miR-1294               | Forward GCGGAGTGTGAGTGGGTGGC                     |
|                        | Reverse CTCAACTGTGTCGGAG                      |
| β-actin                | Forward TGGATCAGCAAGCAGAGGAG                    |
|                        | Reverse TCGGCCACATTGTA                           |
| U6                     | Forward CTCGCTTGGCAGACGAGA                      |
|                        | Reverse CGAATTGTGCGCTTGTG                      |
2.5 | Colony formation analysis

GC cells (2 × 10³ cells in each well) were implanted into 6-well plates for 2 weeks at 37°C. Next, 4% paraformaldehyde (Beyotime) and 0.1% crystal violet (Beyotime) were applied to fix and stain the forming colonies cells. Ultimately, the colonies were photographed and counted under a microscope (Leica).

2.6 | Wound healing and transwell assays

In 6-well plates, a single-line wound was made by 200μl sterile pipette tips on a beeline of the wells. The wound healing was observed, presented, and calculated in 0 and 24h by the inverted microscope (Leica).

GC cells were added to the upper chamber of the 24-well Transwell inserters (8 μm; Costar; Corning) with 200μl serum-free medium, in which the upper chamber was coated without (for migration analysis) or with (for invasion analysis) the diluted Matrigel (BD Bioscience). The lower chamber was appended with RPMI-1640 medium including 10% FBS; 24h later, cells across the filters were counted and imaged by using a light microscope (magnification, x100; Leica) after dyeing with crystal violet.

2.7 | Cell apoptosis assay

After 48h transfection, the GC cells were simultaneously stained with Annexin V-FITC and PI (Yeasen) under dark condition for 20 min. The apoptosis rate of cells was determined and counted by a flow cytometer (Beckman).

2.8 | Western blot assay

Total protein was separated using RIP (Beyotime). First of all, 30μg protein was segregated by SDS-PAGE, and then transferred onto the PVDF membranes (Sigma-Aldrich). The membranes were co-cultured with the primary antibodies, including anti-Bcl-2 (1:500, ab182858, Abcam), anti-Bax (1:500, ab32503, Abcam), anti-ASCT2 (1:500, ab187692, Abcam), anti-GLS1 (1:500, ab156876, Abcam), anti-PBX3 (1:500, ab109173, Abcam), and anti-β-actin (1:500, ab8226, Abcam) overnight. Then, the membranes were challenged with secondary antibodies (Goat Anti-Rabbit IgG H&L, 1:5000, ab205718, Abcam) for 60 min. Ultimately, the protein signals were visualized by the eýoECL Plus Kit (Beyotime) and counted via the Image Lab software (NIH).

2.9 | Tube formation assay

Human umbilical vein endothelial cells (HUVECs) were employed to assess the angiogenic ability. The 96-well plates were smeared with 80μl/well Matrigel (BD Bioscience). Then, HUVEC cells and GC cells of AGS and MKN-45 were co-cultured for 24h. The capillary-like branches were observed, imaged, and counted using a microscope.

2.10 | Glutamine, glutamate, and α-KG level assay

The cultured GC cells were suspended in assay Buffer for 24h, and then the glutamine, glutamate determination kit or α-KG colorimetric assay kit (BioVision) were used to detect the concentrations of glutamine, glutamate or α-KG base on the manufacturer’s project.

2.11 | Dual-luciferase reporter assay

The online websites of circinteractome and targetscan were applied to measure the relationship between miR-1294 and hsa_circ_0043691 or PBX3. The wild-type (WT) and mutant (MUT) binding sequences of miR-1294 on hsa_circ_0043691 or PBX3 were designed, amplified and assembled into the pmirGLO vectors (Promega) to establish luciferase reporter vectors (hsa_circ_0043691 WT, PBX3 3’UTR WT, hsa_circ_0043691 MUT, or PBX3 3’UTR MUT). Subsequently, the above vectors were co-transfected with miRNA mimics or miR-1294 mimic into AGS and MKN-45 cells. Finally, luciferase activity was investigated using the dual-luciferase reporter assay kit (Promega).

2.12 | RIP and pull-down assays

RIP assay was implemented using the Magna RIP kit (Millipore). In short, the experimental cells were lysed using RIP lysis buffer (Millipore) for 48h, followed by cultivating with AGO2 or IgG antibody overnight at 4°C to prepare co-precipitated RNAs. Ultimately, the enrichment of miR-1294 and hsa_circ_0043691 was assayed by qRT-PCR.

GC cells were lysed and co-cultured with biotinylated miR-1294 WT probe (Bio-miR-1294 WT), miR-1294 MUT probe (Bio-miR-12194 MUT) and control probe (Bio-NC; Sangon) at 4°C overnight using the pull-down kit from Thermo Fisher. Finally, hsa_circ_0043691 levels were examined through qRT-PCR.

2.13 | Animal experiments

All animal tests were authorized by the Animal Ethics Committee of Fuyong People’s Hospital. Twelve 6-week-old BALB/c mice from Vital River (Beijing, China) were randomly divided into two groups (six mice/group). 6 × 10⁶ MKN-45 cells transfected with sh-hsa_circ_0043691 or its control (sh-NC) were hypodermically implanted into the nude mice. The xenograft volume was monitored every seven days. The tumors were harvested after 28 days, then weighed, and further used for additional assays.
2.14 Statistical analysis

With three independent experiments, the data in this work were presented as the mean ± standard deviation (SD). Student's t test, or ANOVA, was employed to assess the difference between two groups or among multiple groups. The survival outcomes of GC patients were analyzed by Kaplan–Meier method, and the relationships among hsa_circ_0043691, miR-1294 and PBX3 in GC tissues were measured using the Pearson correlation coefficient. p < 0.05 was deemed as statistically different.

3 RESULTS

3.1 Hsa_circ_0043691 expression was heightened in GC

The qRT-PCR assay exhibited that hsa_circ_0043691 expression was prominently higher in GC tissues (tumor) compared to matched non-tumor tissues (normal) (Figure 1A). The expression of hsa_circ_0043691 was higher in stage III (n = 20) than that in stage I+II (n = 19) (Figure 1B) and higher in the lymph node metastasis (n = 18) than that in non-metastasis tissues (n = 21) (Figure 1C). Meanwhile, hsa_circ_0043691 high expression was closely linked to larger tumor size (>3 cm) (n = 17) in comparison to smaller tumor size (≤3 cm) (n = 22) (Figure 1D). Furthermore, GC patients with high hsa_circ_0043691 expression level showed shorter survival rates (Figure 1E). The expression level of hsa_circ_0043691 was observably higher in four GC cell lines (N87, HGC27, AGS, and MKN-45) than that in normal GES-1 cells (Figure 1F). Hsa_circ_0043691 was looped and comprised exons 8 of its parental gene NT5C3L (Figure S1A). Then the stability of hsa_circ_0043691 was confirmed. The results showed that hsa_circ_0043691 was resistant to RNase R digestion compared with linear NT5C3L (Figure 1G,H). In comparison to the linear NT5C3L, the half-life of hsa_circ_0043691 was distinctly longer in GC cells (Figure 1I,J). Besides that, convergent primers for NT5C3L mRNA and special divergent primers to amplify hsa_circ_0043691 were designed. The results indicated

FIGURE 1 Hsa_circ_0043691 expression profile. (A) Hsa_circ_0043691 expression level in 39 pairs of GC tissues and corresponding normal tissues. (B) Hsa_circ_0043691 level in GC tissues at stage I+II (n = 19) and stage III (n = 20). (C) CircNT53CL content in GC tissues with (n = 18) or without lymph node metastasis (n = 21). (D) CircNT53CL expression was detected in GC tissues with smaller (≤3 cm) tumor size (n = 17) or larger (>3 cm) tumor size (n = 22). (E) The Kaplan–Meier curve analysis of the overall survival of GC patients. (F) The abundance of hsa_circ_0043691 was determined in GC cell lines and normal GES-1 cells. (G, H) Hsa_circ_0043691 expression level was examined after RNase R digestion. (I, J) The half-life of hsa_circ_0043691 and linear NT5C3L after treatment with Actinomycin D for indicated times. (K, L) Subcellular distribution of hsa_circ_0043691. *p < 0.05.
that hsa_circ_0043691 could be detected only in cDNA, as no products were detected in the extracted gDNA (Figure S1B). In addition, hsa_circ_0043691 was located in GC cells' cytoplasm by qRT-PCR assay (Figure 1K,L). Therefore, these findings indicated that circN-T5C3L, with a stable structure in cytoplasmic, might play an oncogenic role in GC patients.

### 3.2 | Hsa_circ_0043691 knockdown curbed GC cell growth, metastasis and angiogenesis but promoted apoptosis

To research the effect of hsa_circ_0043691 on GC cells, the loss-of-function analysis was implemented. In relation to the control
**FIGURE 3** Deficiency of hsa_circ_0043691 suppressed glutaminolysis in GC cells. (A–E) AGS and MKN-45 cells were transfected with control, si-NC and si-hsa_circ_0043691. The levels of glutamine (A), glutamate (B), and α-KG (C) were analyzed in GC cells. (D, E) Western blot for ASCT2 and GLS1 protein levels. *p < 0.05.

**FIGURE 4** Hsa_circ_0043691 targeted miR-1294. (A) The complementary binding sites between hsa_circ_0043691 and miR-1294 predicted by circinteractome. (B) Analysis of the upregulation or downregulation efficiencies of miR-1294 mimic and inhibitor. (C, D) Dual-luciferase reporter assay, (E, F) RIP assay, (G) RNA pull-down assay were employed to analyze the interaction between miR-1294 and hsa_circ_0043691. (H) MiR-1294 expression level in 39 pairs of GC tissues and corresponding normal tissues. (I) The correlation between hsa_circ_0043691 and miR-1294 expression in GC tissues. (J) MiR-1294 level in GC cells. (K) miR-1294 expression level was evaluated in AGS and MKN-45 cells transfected with si-hsa_circ_0043691 alone or si-hsa_circ_0043691 and in-miR-1294. *p < 0.05.
and si-NC groups, hsa_circ_0043691 was observably decreased in si-hsa_circ_0043691 group (Figure 2A). Thereafter, the colony-forming ability was overtly reduced by the transfection of si-hsa_circ_0043691 (Figure 2B), suggesting that GC cell proliferation in si-hsa_circ_0043691 group was distinctly inhibited. Meanwhile, the findings presented that the migratory and invasive abilities of GC cells were clearly inhibited in si-hsa_circ_0043691 group (Figure 2C–E). In addition, absence of hsa_circ_0043691 facilitated GC cell apoptosis (Figure 2F). Moreover, hsa_circ_0043691 silencing reduced the protein level of Bcl-2 and increased Bax level in AGS and MKN-45 cells (Figure 2G,H). Simultaneously, downregulation of hsa_circ_0043691 distinctly repressed angiogenesis in AGS and MKN-45 cells (Figure 2I).

3.3 Knockdown of hsa_circ_0043691 decreased glutaminolysis in GC cells

The effect of hsa_circ_0043691 on energy metabolism was analyzed in this work. Down-regulation of hsa_circ_0043691 markedly inhibited glutaminolysis metabolites, including glutamine, glutamate, and α-KG levels (Figure 3A–C). Meanwhile, glutaminolysis-related proteins (ASCT2 and GLS1) were quantified by Western blot assay. The outcomes disclosed that hsa_circ_0043691 absence predominantly suppressed the protein levels of ASCT2 and GLS1 in AGS and MKN-45 cells (Figure 3D,E). The results disclosed that knockdown of hsa_circ_0043691 regulated glutaminolysis in GC.

FIGURE 5 Hsa_circ_0043691 knockdown repressed GC cell malignant phenotypes by sponging miR-1294. (A–M) AGS and MKN-45 cells were transfected with si-NC, si-hsa_circ_0043691, si-hsa_circ_0043691+in-miR-NC, or si-hsa_circ_0043691+in-miR-1294. (A) Cell proliferation analysis. (B–D) cell migration and invasion abilities. (E) Cell apoptosis analysis. (F, G) Western blot for apoptosis-related protein levels. (H) Tube formation analysis for angiogenesis. (I) The glutamine level, (J) glutamate level, (K) and α-KG level were assessed. (L, M) Western blot for ASCT2 and GLS1 protein levels. *p < 0.05.
3.4 | Hsa_circ_0043691 targeted miR-1294 in GC cells

To evaluate the potential mechanism of hsa_circ_0043691, the downstream miRNAs of hsa_circ_0043691 were analyzed. As shown in Figure 4A, hsa_circ_0043691 and miR-1294 had complementary binding sites. MiR-1294 abundance was increased by miR-1294 mimic and reduced by miR-1294 inhibitor transfection in AGS and MKN-45 (Figure 4B). Enrichment of miR-1294 restricted the luciferase reporter activity of hsa_circ_0043691 WT group, but not the hsa_circ_0043691 MUT group (Figure 4C,D). Additionally, in RIP assay, miR-1294 and hsa_circ_0043691 had a higher enrichment in Anti-Ago2 group compared with Anti-IgG group (Figure 4E,F). Simultaneously, high abundance of hsa_circ_0043691 was enriched by Bio-miR-1294 WT, but not Bio-miR-1294 MUT or Bio-NC (Figure 4G). The abundance of miR-1294 was particularly reduced in GC tissue samples (Figure 4H), and miR-1294 expression was inversely associated with hsa_circ_0043691 level in GC tissues (Figure 4I). Similarly, a declined miR-1294 level was observed in GC cells (Figure 4I). Furthermore, deletion of hsa_circ_0043691 remarkably increased miR-1294 expression, while this effect was partly overturned by co-transfection of miR-1294 inhibitor in GC cells (Figure 4K).

3.5 | MiR-1294 inhibitor abrogated the effects of hsa_circ_0043691 silencing in GC cells

To additionally realize the regulatory mechanism of hsa_circ_0043691 and miR-1294, rescue assays were conducted in GC cells with the transfections of si-NC, si-circNT5C3L, si-circNT5C3L+in-miR-NC and si-circNT5C3L+in-miR-1294. The results exposed that miR-1294 partially overturned the effects of hsa_circ_0043691 deficiency on cell proliferation (Figure 5A), migration and invasion (Figure 5B-D). The changes in cell apoptosis and apoptosis-related protein levels (Bcl-2 and Bax) that were induced by si-hsa_circ_0043691 were largely weakened by miR-1294 inhibitor (Figure 5E-G). The reduction in angiogenesis (Figure 5H) and glutaminolysis metabolites including glutamine (Figure 5I), glutamate (Figure 5J), and KG level (Figure 5K), as well as the decline in expressions of ASCT2 and GLS1 (Figure 5L,M) in GC cells with hsa_circ_0043691 deletion were rescued by miR-1296 inhibitor.

3.6 | PBX3 was targeted by miR-1294 in GC cells

Next, the target genes of miR-1294 were predicted. The results presented that miR-1294 and PBX3 3′UTR had complementary binding sites (Figure 6A). The results disclosed that miR-1294 overexpression and miR-1294 mimics significantly reduced the expression of PBX3 in AGS and MKN-45 cells. The mRNA and protein expression of PBX3 in AGS and MKN-45 cells transfected with miR-1294 alone or miR-1294 and PBX3 are presented in Figure 6B-I. The results showed that PBX3 was a potential target gene of miR-1294.
clearly diminished the luciferase activities of PBX3 3’UTR WT group, but not PBX3 3’UTR MUT group in AGS and MKN-45 cells (Figure 6B,C). Moreover, PBX mRNA and protein levels were overtly upregulated in GC tissues (Figure 6D,E). PBX3 was inversely related to miR-1294 expression (Figure 6F) and positively linked to hsa_circ_0043691 expression in GC tissues (Figure 6G). Also, an elevation of PBX3 protein level was showed in GC cells (Figure 6H). In addition, PBX3 protein level was reduced by miR-1294 mimic, while this suppressive effect was abrogated by PBX3 overexpression (Figure 6I). si-circNT5C3L transfection abated the level of PBX3, while the effect was counteracted by co-transfection of miR-1294 inhibitor (Figure 6J).

3.7 | MiR-1294 hampered GC cell malignant phenotypes by targeting PBX3

The rescue experiments were conducted in GC cells to further clarify the interplay between miR-1294 and PBX3 in GC cells. Enrichment of miR-1294 repressed cell proliferation (Figure 7A), migratory and invasive abilities (Figure 7B–D), and promoted cell apoptosis (Figure 7E), which were alleviated by the co-transfection of PBX3. Moreover, miR-1294 inhibited Bcl-2 protein level and increased Bax level, while these changes were blocked by PBX3 overexpression (Figure 7F,G). Besides, miR-1294 impeded angiogenesis (Figure 7H), and reduced glutamine (Figure 7I), glutamate (Figure 7J), α-KG contents (Figure 7K), and the protein levels of ASCT2 and GLS1 (Figure 7L,M), whereas these inhibitory effects were partially counteracted by PBX3 overexpression in AGS and MKN-45 cells. To sum up, this data manifested that miR-1294 inhibited GC cell malignant progression by regulating PBX3.

3.8 | Knockdown of circNT5C3L blocked GC tumor growth in vivo

Finally, the in vivo effect of hsa_circ_0043691 was evaluated by a xenograft GC mouse model. Deficiency of hsa_circ_0043691 strikingly repressed tumor volume and weight (Figure 8A,B). Meanwhile,
hsa_circ_0043691 knockdown suppressed the expressions of hsa_circ_0043691 and PBX3, but increased miR-1294 level in xenograft tumor tissues (Figure 8C–E). All data suggested that silencing of hsa_circ_0043691 hindered tumor growth in vivo.

4 | DISCUSSION

As one of the digestive system malignancies, GC is a serious threat to human health because of its difficult diagnosis and poor prognosis. Currently, the treatment of GC in the clinic mainly includes radiotherapy and chemotherapy. However, the 5-year survival results remain unsatisfactory. In this work, hsa_circ_0043691 was verified to act as an oncogene in GC by regulating miR-1294/PBX3 axis, showing that hsa_circ_0043691 might be a new indicator for the diagnosis and therapy in GC patients.

It has been previously found that circRNAs play vital effects in various cancer progression, including GC. For example, overexpression of circHECTD1 advanced GC cell malignant phenotype by energizing glutaminolysis metabolites. Circ_0002570 knockdown could suppress cell proliferation and metastasis in vitro, and restrain tumor growth in vivo. Circ-PTPDC1 was overexpressed in GC, and deficiency of circ-PTPDC1 inhibited GC progression. In our study, hsa_circ_0043691 abundance was higher in GC tissue samples and cells, which was similar to previous research. Nevertheless, lack of large sample sizes is a certain limitation in the study, which will be supplemented in future experiments. Thereafter, silencing of hsa_circ_0043691 distinctly repressed cell proliferation, migration, invasion, angiogenesis, and glutaminolysis metabolites, but boosted GC cell apoptosis in vitro. Consistently, hsa_circ_0043691 silencing impeded tumor growth in vivo. It has been revealed that the WNT/β-catenin pathway target gene c-Myc drives glutaminolysis. Cancer cells have been shown to highly express PI3K-AKT-mTOR and improve glutaminolysis, and PI3K/AKT/mTOR function as modulators for controlling glutaminolysis. Previous studies have demonstrated that circRNAs can activate the WNT/β-catenin and c-Myc pathway, as well as PI3K-Akt pathway to promote glutaminolysis. Therefore, we speculated that hsa_circ_0043691 may also regulate glutaminolysis via these pathways, which needs to be further investigated in future.

In this work, hsa_circ_0043691 and miR-1294 were predicted to have binding sites by using circinteractome bioinformatics software, and this relationship was testified by multiple assays. Previous studies have found that miR-1294 plays an antitumor effect in the malignant phenotype of many of cancer, including esophageal cancer, osteosarcoma, and laryngeal squamous cell carcinoma cell (LSCC). In GC tissues, downregulated miR-1294 was uncovered in GC tissue samples.

FIGURE 8 | Silencing of hsa_circ_0043691 hampered GC tumor growth in vivo. (A) Detection of tumor volumes every 1 week. (B) Tumor size measurement after 28 days. (C–E) Levels of hsa_circ_0043691, miR-1294 and PBX3 in xenograft tumor tissues. *p < 0.05.
and cells. In our paper, miR-1294 expression was also decreased in GC tissue sample, and overexpression of miR-1294 significantly hindered GC cell progression. Furthermore, hsa_circ_0043691 mediated the suppressive role in GC cells by sponging miR-1294.

PBX3 and miR-1294 3′ UTR had complementary sites. Recent evidence has shown that PBX acted as an oncogene and was involved in regulating human malignant tumors, such as nasopharyngeal carcinoma (NPC), ovarian cancer (OC), and GC. PBX3 content was prominently upregulated in GC tissues and cells, which was consistent with previous research. Upregulation of PBX3 largely eliminated the antitumor action of miR-1294 on cell progression.

In conclusion, our study indicated that hsa_circ_0043691 was markedly upregulated in GC, and downregulation of hsa_circ_0043691 repressed GC cell malignant phenotype and glutaminolysis by regulating PBX3 via targeting miR-1294. Hence, hsa_circ_0043691 may serve as a potential target for GC management.

CONFLICT OF INTEREST
The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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