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Original Article

LncRNA HCP5 promotes the progression of gastric cancer through the miR-526b/PBX3 axis

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Abstract: Objectives: To investigate whether LncRNA HLA complex P5 (HCP5) promotes gastric cancer (GC) via the miR-526b/Pre-B Cell Leukemia Homeobox 3 (PBX3) pathway. Methods: Thirteen paired GC and adjacent non-tumorous tissues, along with NCI-N87 GC cells, were analyzed. HCP5 expression levels were measured, and its impact on cell viability, proliferation, and migration were evaluated. Dual-luciferase reporter assays were performed to confirm the direct interactions among HCP5, miR-526b, and PBX3. The effects of HCP5 overexpression or silencing on miR-526b and PBX3 expression were analyzed. A miR-526b mimic was transfected for functional rescue. Results: HCP5 was significantly upregulated, while miR-526b was downregulated in GC tissues. Dual-luciferase assays confirmed the direct binding of HCP5 to miR-526b and of miR-526b to PBX3. In NCI-N87 cells, HCP5 overexpression downregulated miR-526b and upregulated PBX3 expression, whereas silencing HCP5 showed the opposite effects. Moreover, HCP5 overexpression decreased Bax and increased Bcl-2 levels, which was reversed by miR-526b mimic transfection. Functionally, HCP5 enhanced GC cell viability and migration, both of which were suppressed by miR-526b. HCP5 promoted cell proliferation, as evidenced by a reduced proportion of cells in the G0/G1 phase, which was reversed by miR-526b. Conclusions: HCP5 acts as an oncogenic lncRNA in GC by promoting cell viability, migration, and proliferation via the miR-526b/PBX3 axis. Targeting the HCP5/miR-526b/PBX3 axis may represent a promising therapeutic strategy for GC.

Keywords: Competing endogenous RNA, gastric cancer, HCP5, miR-526b, PBX3

Introduction

Gastric cancer (GC) is one of the most common malignancies of the digestive system. In China, the annual incidence of GC exceeds 396,000 cases, with over 288,000 GC-related deaths [1]. Globally, over 1 million new cases of GC are diagnosed annually, with over 769,000 deaths [2]. This highlights the significant global economic burden and public health challenge imposed by GC. Currently, the primary therapeutic approaches for GC include surgery, chemotherapy, and radiotherapy, all of which have provided clinical benefits [3, 4]. However, these therapies offer limited efficacy in advanced GC patients, with the 5-year survival rate below 40% [5, 6]. Thus, identifying novel biomarkers for early GC diagnosis and treatment, and gaining a deeper understanding of the molecular pathological mechanisms of GC, are urgently needed.

Long non-coding RNAs (lncRNAs) are RNA transcripts exceeding 200 nt in length that lack protein-coding capacity [7, 8]. An increasing number of lncRNAs have been identified as tumor markers [9-11]. Among these, lncRNA HLA complex P5 (HCP5) has garnered particular attention for its oncogenic role [12, 13]. HCP5 is upregulated in colorectal cancer (CRC) tissues and cell lines, and its inhibition suppresses CRC cell growth and migration [14]. Similarly, elevated HCP5 expression has been observed in GC patients compared to healthy controls [15]. These findings suggest that HCP5 may serve as a promising biomarker and therapeutic target in various cancers.

Similar to HCP5, miR-526b has been identified in several studies as a key regulator of tumor cell progression and a potential prognostic biomarker. Reduced or absent expression of miR-526b has been observed in breast cancer and

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other malignancies, where it exerts tumor-suppressive effects by targeting downstream genes such as Twist1 [16]. In CRC, upregulation of miR-526b inhibits tumor progression [17]. Furthermore, previous research has demonstrated that miR-526b inhibits the epithelial-mesenchymal transition (EMT) in cervical cancer cells by directly targeting PBX homeobox 3 (PBX3) [18], supporting a functional link between miR-526b and PBX3. Interestingly, PBX3 is also overexpressed in GC cells and plays a pivotal role in promoting tumor cell proliferation [19, 20]. However, studies investigating whether the miR-526b/PBX3 axis contributes to GC pathogenesis are scarce, warranting further investigation to clarify the potential biological significance of this regulatory pathway in GC.

This study aims to investigate the roles of HCP5 and miR-526b in GC cell biology through a series of in vitro experiments and to explore their potential interactions with the downstream target gene PBX3. Specifically, by assessing how HCP5 or miR-526 modulation affects PBX3 expression and evaluating the subsequent impact on GC cell activity, migration, and proliferation, we seek to uncover the functional significance and regulatory mechanism of the HCP5-miR-526b-PBX3 axis in GC.

Methods and materials

Bioinformatics analysis

The stomach adenocarcinoma (STAD) dataset from the Cancer Genome Atlas Project (TCGA) comprises 375 GC and 32 normal stomach (NC) tissue samples. Differentially expressed lncRNAs were identified using the limma package in R (v4.2.1), with thresholds set at $|\log_2 FoldChange| > 1$ and adjusted $P < 0.05$. Additionally, 60 GC and 8 NC tissue samples from the Gene Expression Synthesis (GEO) dataset (GSE26595) were analyzed. To correct for batch effects between the TCGA and GEO datasets, the Combat function from the SVA package in R was utilized. Lasso regression analysis was implemented using the glmnet package in R. The Benjamini-Hochberg (BH) method was used to adjust *P* values for multiple comparisons and control the false discovery rate (FDR), implemented via the p.adjust function in R. The interaction between HCP5 and miR-526b was predicted using the LncTar online tool, while the interaction between miR-526b and PBX3 was predicted using the DIANA-microT-CDS database.

Tissue sample collection

Between March and May 2021, paired GC and adjacent non-tumorous tissues were collected from GC patients undergoing surgical resection at the First Affiliated Hospital of Guangxi Medical University. Inclusion criteria: (1) histopathologically confirmed gastric adenocarcinoma; (2) age ≥ 18 years. Exclusion criteria: (1) concurrent metastatic GC or synchronous malignancy of other organs; (2) receipt of preoperative chemotherapy, radiotherapy, or targeted therapy. This study was approved the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (NO.2022-KY-E-(251)) and conducted in accordance with the Declaration of Helsinki.

GC tissue cDNA microarray

The GC tissue cDNA microarray (TMA) was obtained from Shanghai Autte Biotechnology (China), comprising 13 GC tissues and 13 matched adjacent normal tissues. All samples were collected from patients pathologically diagnosed with gastric adenocarcinoma following surgical resection. Among the 13 patients, 7 were classified as TNM stage III and 6 as stage IV. According to Lauren's classification, 10 cases were of the intestinal type and 3 were of diffuse type. The expression level of HCP5 was analyzed by quantitative real-time PCR (qRT-PCR).

Cell culture

Human GC cell lines (NCI-N87 and HGC-27) and normal human gastric epithelial cell line GES-1 were purchased from the Chinese Academy of Medical Sciences Cell Resource Center. The HEK-293 cell line, derived from human embryonic kidney cells, was sourced from the American Type Culture Collection (ATCC). HGC-27 and HEK-293 cell lines were cultured in Dulbecco's Modified Eagle Medium (ProCell, China), while NCI-N87 and GES-1 cells were maintained in PMI-1640 medium (ProCell).

Lentiviral oligonucleotides transfection

Two shRNAs targeting HCP5 (shHCP5) and a negative control shRNA (shNC) were designed

and inserted into the pLVX-shRNA2-puro vector. The coding sequence (CDS) of human PBX3 was synthesized and cloned into the pLVX-IRES-ZsGreen1 vector. All recombinant constructs were verified by DNA sequencing. Lentiviral particles were transfected into HEK-293 cells. hsa-miR-526b mimics and chemically modified antisense oligonucleotides (hsa-miR-526 inhibitor) were transfected into target cells using Lipofectamine® 2000 reagent (Thermo Fisher Scientific, USA), in combination with the respective lentiviral vectors or oligonucleotides.

Nuclear and cytoplasmic RNA isolation

Total RNA was extracted from HGC-27 cells using the Cytoplasmic and Nuclear RNA Purification Kit (Norgen Biotek, Canada), following the manufacturer's instructions. The extracted RNA samples were stored at -80°C for later analysis.

RNA extraction and qRT-PCR

The expression levels of HCP5, miR-526b, PBX3, U6, and GAPDH were measured using either the resultant cDNA or commercially available tissue cDNA microarrays. qRT-PCR was performed on a qTower 3.2G real-time PCR system (Analytik Jena, Germany) using BeyoFast™ SYBR Green qPCR mix (Bio-Rad, USA). The qRT-PCR protocol included 40 cycles of initial denaturation at 95°C for 2 minutes, followed by denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 30 seconds GAPDH and U6 were used as internal controls for mRNA and miRNA, respectively. Relative gene expression was calculated using the 2^-ΔΔCT method.

Western blot

Total protein was extracted from cells, and concentrations were determined using the BCA Protein Assay kit. Equal amounts of protein (30 μg) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with skimmed milk, membranes were incubated with the following rabbit polyclonal primary antibodies: PBX3 (1:2000), BCL-2 (1:2000), Bax (1:2000), and GAPDH (1:6000). Following TBST buffer washes, the membranes were incubated with a secondary goat anti-rabbit antibody. Protein bands were visualized, and densitometric analysis was conducted using ImageJ software.

Double luciferase reporter gene assay (Dual-LUC)

Predicted miR-526b binding sites in HCP5 or PBX3 sequences were mutated to generate mutant (MUT) constructs. Wild-type (WT) and MUT sequences of HCP5 and PBX3 synthesized by Sangon Biotech (Shanghai, China) were cloned into the psiCHECKTM-2 vector (Promega, USA). Subsequently, HEK-293 cells were co-transfected with the constructed luciferase reporter gene plasmids and either miR-526 mimics or miR-526 inhibitor using Lipofectamine® 2000 reagent.

Cell viability assay (CCK-8)

Following cell attachment, 10 μL of CCK-8 solution was introduced to each well at four time points (0 h, 24 h, 48 h, and 72 h). The cells were incubated for 1 hour at 37°C, and absorbance was subsequently measured at 450 nm using a microplate reader.

Flow cytometry for cell cycle and apoptosis analysis

Cell cycle distribution and apoptosis were analyzed using the Cell Cycle and Apoptosis Detection Kit (C1052; Beyotime Biotechnology, China). Briefly, cells were collected into a single-cell suspension, fixed in 70% ethanol at 4°C overnight, and stained with 0.5 mL propidium iodide solution. Red fluorescence was detected at an excitation wavelength of 488 nm using a flow cytometer. Cell cycle phase distribution was analyzed with ModFit LT 5.0 software.

Transwell migration assay

Cell migration ability was assessed using a Transwell assay. Briefly, 1,000 μL of cell suspension (containing 100,000 cells) was added to the upper chamber of a Transwell insert (BL539A, Corning), and 1,500 μL of complete medium with 10% FBS (10099-141, GIBCO) was added to the lower chamber. After 24 hours of incubation, the Transwell inserts were removed, and the non-migrated cells were washed away with PBS (BL302A, Biosharp). The migrated cells were fixed with 4% paraformaldehyde (P1110, Solarbio) for 30 minutes,

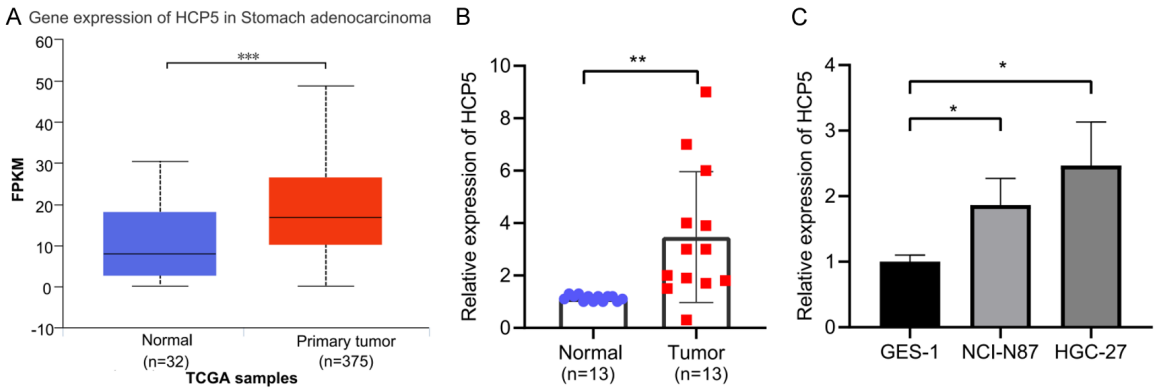

Figure 1. Relationship between LncRNA HLA complex P5 (HCP5) overexpression and the occurrence of gastric cancer (GC). A. Based on the samples from the Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) database, HCP5 expression in GC tissues was compared with that in normal stomach tissues. B. Expression of HCP5 in GC tissue microarray (TMA) examined by qRT-PCR in GC and normal gastric tissues. C. Relative expression of HCP5 in GC and normal gastric epithelial cell lines. *P < 0.05, **P < 0.01, ***P < 0.001.

air-dried, and stained with 0.1% crystal violet (G1063, Solarbio) for 30 minutes. The inserts were washed with PBS, and the number of migrated cells was counted under a microscope in three randomly selected fields of view at 10× magnification, with ImageJ software used for quantification.

Statistical analysis

Potential downstream targets of HCP5 and miR-526b were predicted using StarBase (version 3.0) (http://starbase.sysu.edu.cn/), and a competing endogenous RNA (ceRNA) regulatory network was constructed. GraphPad Prism (version 8.0.1) and SPSS (version 22.0) were utilized for data visualization and statistical analysis. Data were expressed as the mean ± standard deviation (SD). Differences between two groups were assessed using t-tests, while one-way ANOVA followed by LSD post hoc tests was used for multiple-group comparisons. A *p*-value less than 0.05 was considered statistically significant.

Results

HCP5 is upregulated in GC

Bioinformatics analysis of lncRNA-seq data from the TCGA-STAD database revealed that HCP5 expression was significantly increased in GC tissues compared to normal gastric tissues (**Figure 1A**). To verify this finding, qRT-PCR was conducted to assess HCP5 expression in 13 pairs of GC and adjacent normal tissues, which confirmed elevated HCP5 expression in GC tissues (**Figure 1B**). Similarly, HCP5 expression was markedly higher in two GC cell lines (NCI-N87, HGC-27) compared with GES-1 cells, as shown in **Figure 1C**.

HCP5 knockdown inhibited proliferation and promoted apoptosis in GC cells

HGC-27 cells were transfected with HCP5-targeting shRNA (shHCP5). qRT-PCR confirmed effective knockdown of HCP5 (**Figure 2A**). CCK-8 results showed that HCP5 knockdown obviously reduced the proliferative capacity of GC cells (**Figure 2B**). Flow cytometry analysis revealed that HCP5 knockdown led to a remarkable increase in the proportion of GC cells in the G0/G1 phase, accompanied by a decrease in the proportion of cells in the S and G2/M phases (**Figure 2C**, **2D**), suggesting cell cycle arrest at G0/G1. Transwell assay further indicated that HCP5 knockdown significantly reduced GC cell migration compared to the control group (**Figure 2E**, **2F**). Western blot analysis revealed increased Bax and decreased Bcl-2 expression following HCP5 knockdown, indicating enhanced apoptosis (**Figure 2G**). Collectively, these findings suggest that HCP5 silencing inhibits GC cell proliferation and migration, while promoting apoptosis.

HCP5 suppressed the expression of miR-526b

LncRNAs are known to bind miRNAs in the cytoplasm and modulate their function. Studies have reported downregulation of miR-526b in

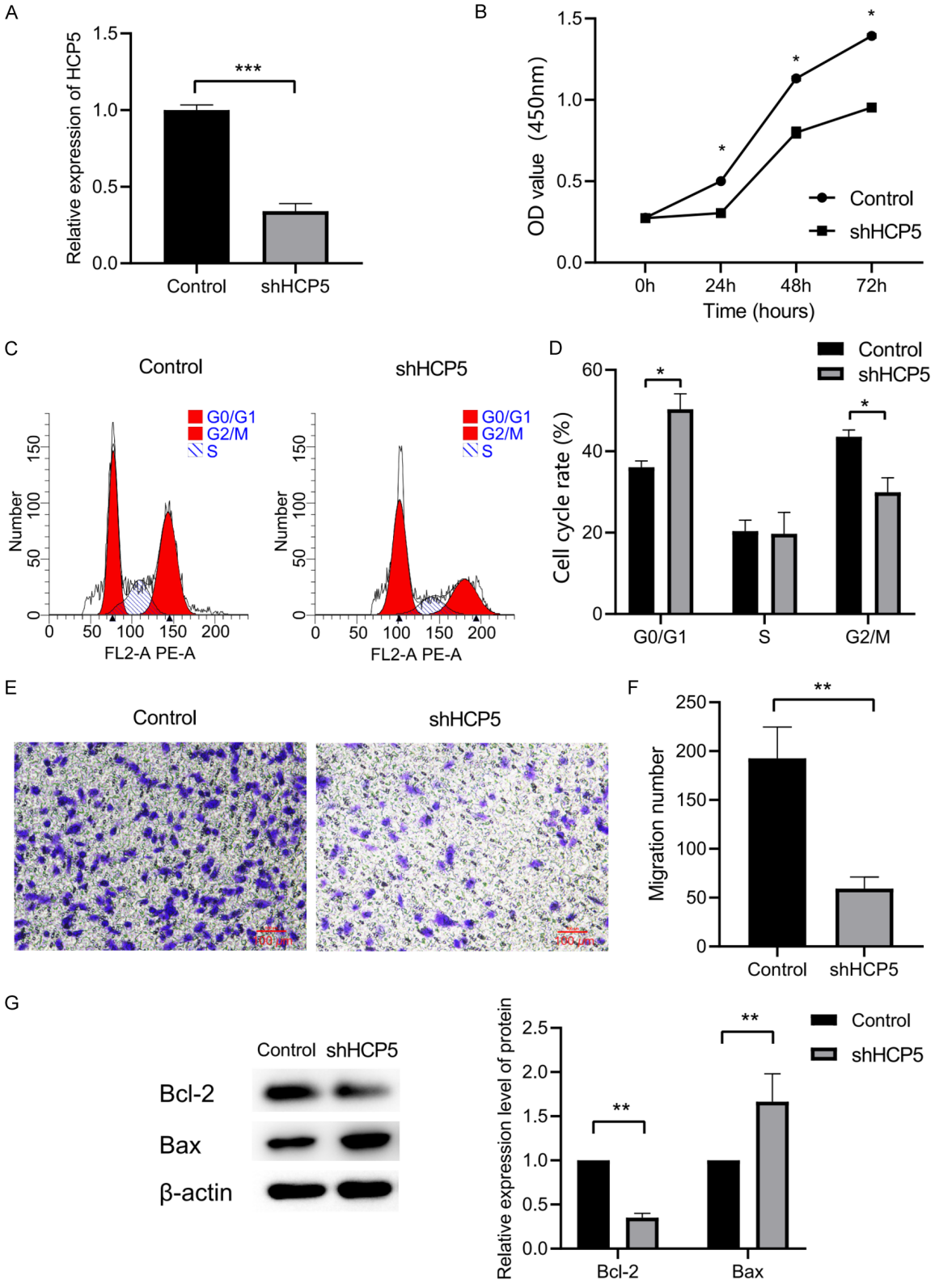

Figure 2. Knockdown of LncRNA HLA complex P5 (HCP5) inhibited proliferation of HGC-27 cells. A. The knockdown efficiency of HCP5 in HGC-27 cells was detected using qRT-PCR. B. The proliferation of HGC-27 cells after HCP5 knockdown was evaluated using CCK-8 assay. C, D. Cell cycle of HGC-27 cells after HCP5 knockdown was evaluated using Flow cytometry. E, F. The migration of HGC-27 cells after HCP5 knockdown was detected using Transwell assay. G. Western blot was used to detect the expression levels of apoptosis-related proteins. Magnification: 100×; *P < 0.05, **P < 0.01, ***P < 0.001.

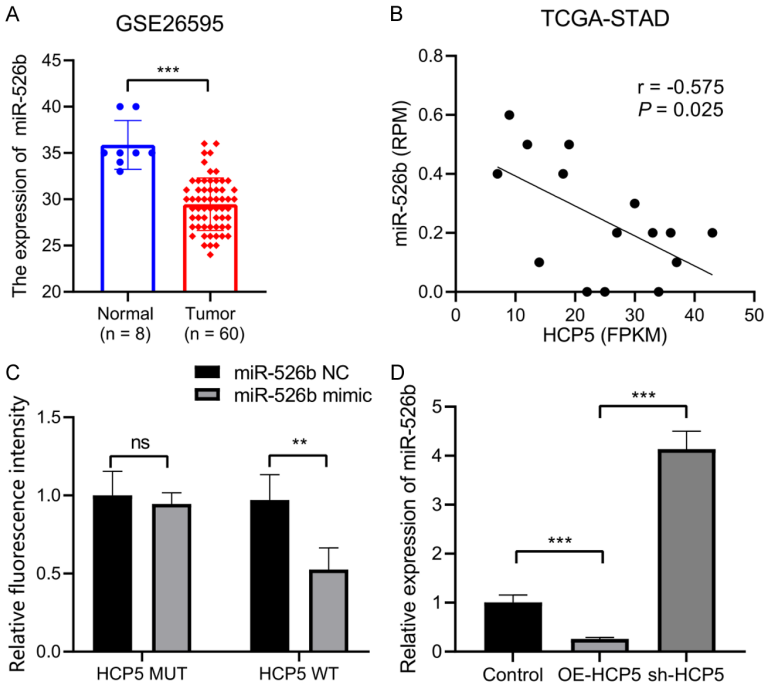

Figure 3. HLA complex P5 (HCP5) mimics suppressed miR-526b expression. A. The GSE26595 dataset showed that the expression of miR-526b in GC tissues was significantly lower than that in normal gastric tissue. B. Correlation analysis of expression of HCP5 and miR-526b in GC tissues based on the Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD). C. Luciferase reporter gene assay showed that miR-526b negatively regulated luciferase activity in the HCP5-WT group, but not in the HCP5-MUT group. D. miR-526b levels in HGC-27 cells with HCP5 knockdown were detected using qRT-qPCR. Ns, no significance; **P < 0.01, ***P < 0.001.

various tumor tissues [16]. Analysis of the GSE26595 database showed that miR-526b expression in GC tissue was much lower than that in normal gastric tissues (**Figure 3A**). Moreover, an inverse correlation between HCP5 and miR-526b expression levels was noted in the STAD database (**Figure 3B**). To investigate whether HCP5 directly interacts with miR-526b, we utilized StarBase v3.0 to predict regulatory targets of HCP5 and miR-526b and constructed a ceRNA network. The analysis revealed possible interactions among HCP5, miR-526b, and PBX3 (Supplementary Figure 1A). Further computational analysis predicted conserved miR-526b-binding motifs (Supplementary Figure 1B) in the HCP5 sequence, suggesting that HCP5 may act as a molecular sponge for miR-526b, thereby modulating PBX3 expression and contributing to GC progression. To validate this hypothesis, a dual-luciferase reporter assay was performed. Co-transfection of HEK-293 cells with miR-526b mimics and wild-type HCP5 (HCP5-WT) significantly reduced luciferase activity compared to the control, while no significant change was observed in the HCP5-MUT group (**Figure 3C**). In addition, HCP5 in HGC-27 cells led to a significant upregulation of miR-526b levels (**Figure 3D**). These findings imply that HCP5 serves as a ceRNA and effectively downregulates miR-526b expression.

Inhibition of miR-526b reversed the effect of HCP5 knockdown on GC cell proliferation

To further elucidate the functional relationship between HCP5 and miR-526b, rescue experiments were conducted in HGC-27 cells. Cells were co-transfected with shHCP5 and a miR-526b inhibitor. CCK-8 and flow cytometry showed that miR-526b inhibition partly reversed the inhibitory effects of HCP5 silencing on cell proliferation in HGC-27 cells (**Figure 4A-C**). Conversely, the miR-526b inhibitor diminished the migratory enhancement induced by HCP5 silencing in HGC-27 cells (**Figure 4D**). Similarly, the WB results showed that the miR-526b inhibitor partially reversed the promoting effect of HCP5 silencing on cell apoptosis in HGC-27 cells. (The expression of Bax, an apoptosis-promoting protein, decreased), as shown in **Figure 4E**. These outcomes demonstrate that HCP5 regulates GC cell growth and apoptosis via miR-526b.

MiR-526b inhibited GC cell proliferation and enhanced its death by directly targeting PBX3

Earlier studies have highlighted PBX3 as a crucial oncogene in GC [19]. STAD data analysis demonstrated a substantial rise in PBX3 expression in GC tissues (**Figure 5A**). In addition, based on the STAD database, miR-526b was negatively correlated with PBX3 (**Figure 5B**). To determine whether miR-526b directly targets PBX3, the miRanda database was used to predict a potential binding region between

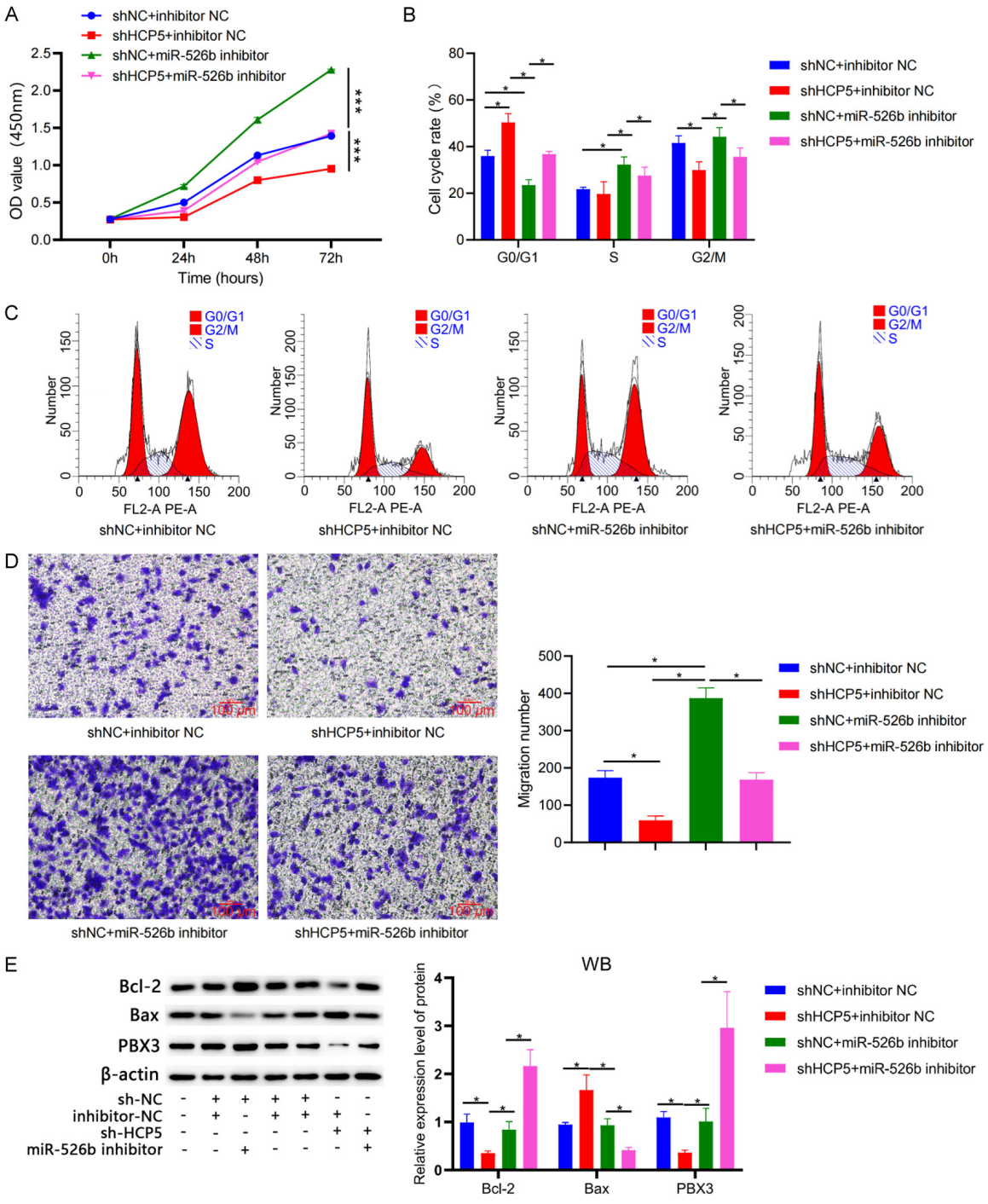

Figure 4. miR-526b inhibitor partially reversed the antitumor effect of HLA complex P5 (HCP5) knockdown in GC cells. miR-526b inhibitor partially reversed the effects of HCP5 knockdown on HGC-27 cell proliferation (A), cell cycle distribution (B, C), and cell migration (D). (E) Effects of HCP5 knockdown on apoptosis-related protein expression in HGC-27 cells. Magnification: 100×; *P < 0.05, ***P < 0.001.

PBX3 and miR-526b (**Figure 5C**). Dual-luciferase reporter assays showed that, relative to the control group, miR-526b mimics decreased luciferase activity in the PBX3-WT group but had no effect on the PBX3-MUT group (**Figure 5D**). Additionally, miR-526b mimics markedly decreased the levels of PBX3 mRNA and protein in HGC-27 cells (**Figure 5E**, **5F**).

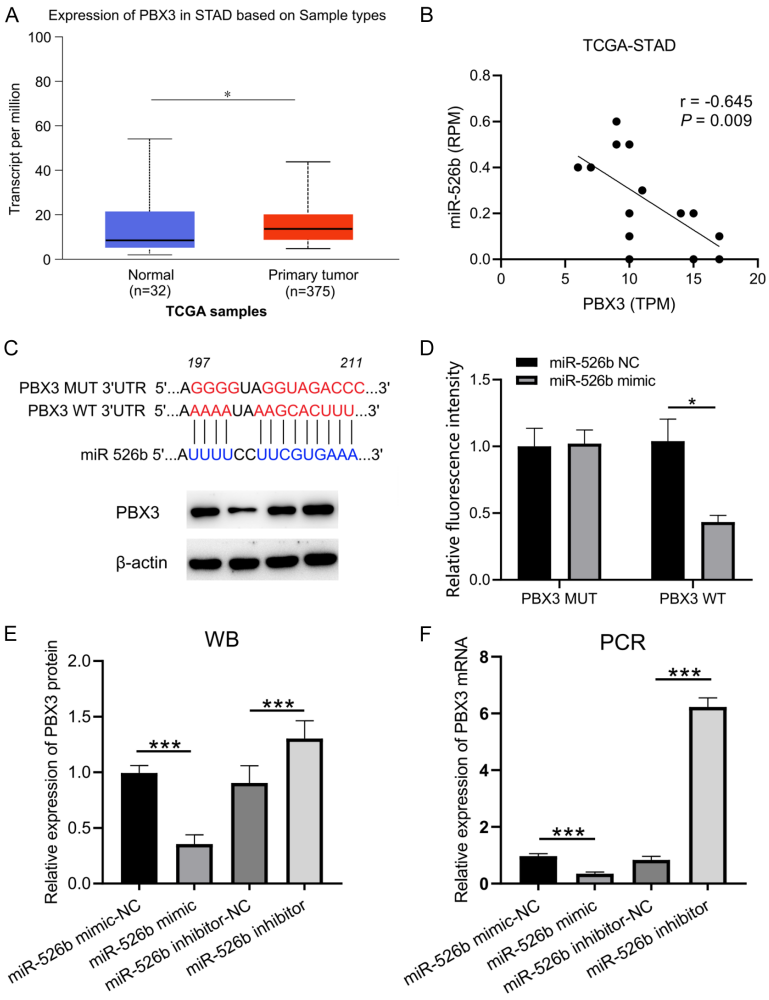

Figure 5. MiR-526b suppressed the malignant behaviors of GC cells by inhibiting the expression of Pre-B Cell Leukemia Homeobox 3 (PBX3). A. The data from the Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) showed that PBX3 expression was significantly upregulated in GC tissues. B. Correlation analysis of miR-526b and PBX3 expression in GC tissues based on TCGA-STAD. C. Putative binding sites between miR-526b and PBX3 were predicted using Bioinformatics analysis. D. Luciferase reporter gene assay showed that miR-526b mimic significantly decreased luciferase activity in PBX3-WT group, but not in the PBX3-MUT group. E, F. Protein and mRNA expression of PBX3 in HGC-27 cells after transfection with miR-526b mimics/inhibitors. *P < 0.05, ***P < 0.001. RPM: Reads per million mapped reads; TPM: Transcript per Kilobase per Million mapped reads.

To further investigate the functional relevance of the miR-526b-PBX3 axis, rescue experiments were performed. CCK-8 and cell cycle assays revealed that PBX3 overexpression significantly mitigated the growth-inhibitory effects of miR-526b mimics in HGC-27 cells (**Figure 6A-C**). Similarly, upregulation of PBX3 reduced the suppressive effect of miR-526b on cell migration (**Figure 6D**). Conversely, elevated PBX3 expression lessened the apoptosis triggered by miR-526b mimics in HGC-27 cells (**Figure 6E**). These results indicate that miR-526b exerts tumor-suppressive effects in GC by directly targeting PBX3.

Discussion

HCP5 is an important oncogenic factor in multiple cancers [21]. Prior research has demonstrated its tumor-promoting roles in lung adenocarcinoma, CRC, triple-negative breast cancer, cervical cancer, prostate cancer, and oral squamous cell carcinoma [15, 17, 22-25]. Yang et al. [26] revealed that HCP5 contributed to CRC progression by aiding EMT. Xu et al. [27] illustrated that HCP5 enhanced the activity of esophageal squamous cell carcinoma via the PI3K/AKT/mTOR signaling pathway. Collectively, these findings propose that HCP5 may serve as a potential therapeutic biomarker for various tumors.

HCP5 modulates cancer progression by regulating a range of miRNAs. In CRC, HCP5 promotes disease progression through the miR-299-3p/PFN1/AKT axis [28]. In ovarian cancer, suppression of HCP5 expression inhibits cell growth, invasion, and migration via the miRNA-525-5p/PRC1 axis [29]. In cervical cancer, HCP5 enhances cellular proliferation and migration via the miR-216a-5p/CDC42 axis [30]. Recent studies have also highlighted the role of HCP5 in GC. Liang et al. [31] reported that HCP5 is overexpressed in GC and promotes cisplatin resistance via the miR-128/HMGA2 axis. Yin et al. [28] found that HCP5 regulates apoptosis in GC cells via the miR-299-3p/SMAD5 axis. Our study found that HCP5 promoted GC cell viability, migration, and proliferation, consistent with previous reports [28, 31, 32]. Importantly, we identified a novel regulatory mechanism whereby HCP5 downreg-

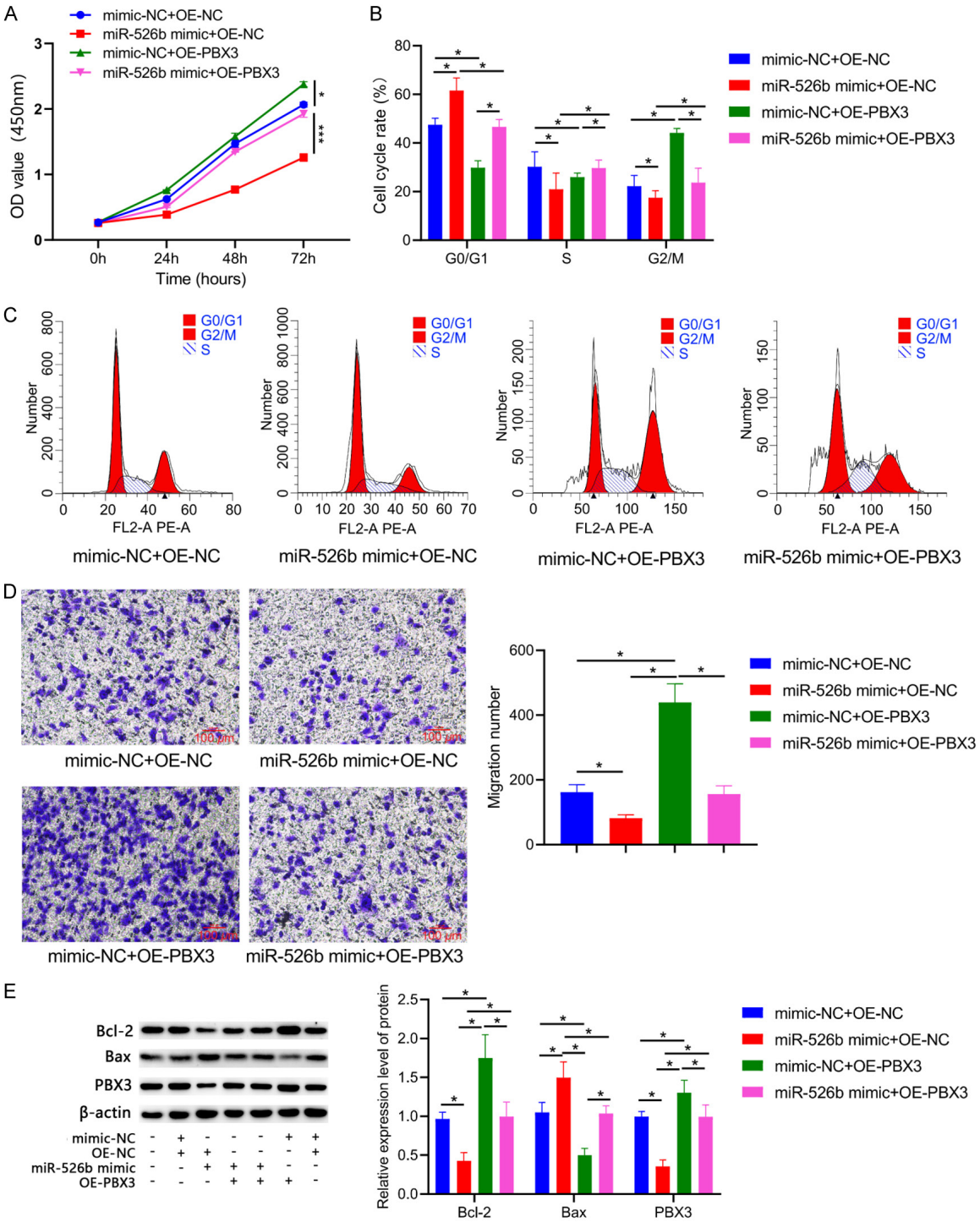

Figure 6. Overexpression of PBX4 partially offset the malignant behavior of miR-526b mimics on HGC-27 cells. A. Cell proliferation; B, C. Cell cycle progression; D. Cell migration; Magnification: 100×; E. Cell apoptosis. a represents the mimic-NC+OE-NC group, b represents the miR-526b mimic+OE-NC group, c represents the miR-526b mimic+OE-PBX3 group, and d represents the mimic-NC+OE-PBX3 group. NC is negative control, OE is Overexpression, *P < 0.05, ***P < 0.001.

ulates miR-526b, leading to upregulation of PBX3, a known oncogene. This axis contributes to reduced apoptosis and enhanced cell viability, migration, and proliferation in GC cells. To

our knowledge, the relationship between HCP5 and miR-526b has not been previously reported, underscoring the novelty of this discovery.

Similar to HCP5, miR-526b also exerts significant regulatory effects in tumors by modulating various downstream target genes. Chen et al. reported that miR-526b inhibited GC cell proliferation and invasion and promoted apoptosis by targeting the KDM4A/YAP1 signaling pathway [33]. Our research validated the tumor-inhibitory function of miR-526b in GC. However, unlike the findings of Chen et al. [33]. We identified PBX3 as a novel downstream target of miR-526b in GC. Specifically, decreased miR-526b expression in GC cells led to GC progression by increasing PBX3 expression. Prior research has highlighted the critical roles of miR-526b and PBX3 in tumor progression. PBX3 is overexpressed in GC cells and is involved in GC cell proliferation. Additionally, in cervical cancer cells, miR-526b inhibits EMT by directly targeting PBX3, thus reducing cancer cell metastasis [18-20]. Our study demonstrated that the regulatory effect of miR-526b on PBX3 is also evident in GC. Specifically, downregulation of miR-526b promotes PBX3 upregulation, subsequently increasing GC cell viability and inhibiting apoptosis, thereby contributing to tumor progression.

PBX3 is a transcription factor belonging to the pre-B-cell leukemia (PBX) family and is closely associated with early human development. Increasing evidence has highlighted its oncogenic potential, particularly in sustaining cancer progression. In terms of GC, PBX3 has been reported to promote EMT [34-36], a process that reduces cell adhesion and enhances cell migration, both of which favor GC cell invasion and metastasis [34, 37, 38]. The findings of our research underscore the critical role of the HCP5/miR-526b/PBX3 axis in GC progression. Combined with the findings of previous studies, promoting EMT may represent one of the mechanisms by which this axis enhances GC development by inhibiting cancer cell apoptosis and promoting proliferation and migration. In addition to promoting EMT, PBX3 may also affect other biological processes influencing tumor progression. For instance, elevated PBX3 expression can increase the activity of MMP9, a key protease involved in metastasis [36, 39], thereby increasing pro-angiogenic signaling and favoring tumor cell growth [36]. Although our findings demonstrate that lncRNA HCP5 may exert oncogenic effects in GC by sponging miR-526b to upregulate PBX3 expression, whether the HCP5/miR-526b/PBX3 ceRNA network promotes GC progression by affecting other biological processes needs further exploration.

Conclusion

HCP5 serves as a potential therapeutic biomarker for GC. This lncRNA promotes GC cell viability, migration, and proliferation by modulating the miR-526b/PBX3 axis. The findings shed light on the molecular mechanisms underlying HCP5-mediated tumor progression and offer a potential therapeutic target for lncRNA-based interventions in GC.

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Disclosure of conflict of interest

None.

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References

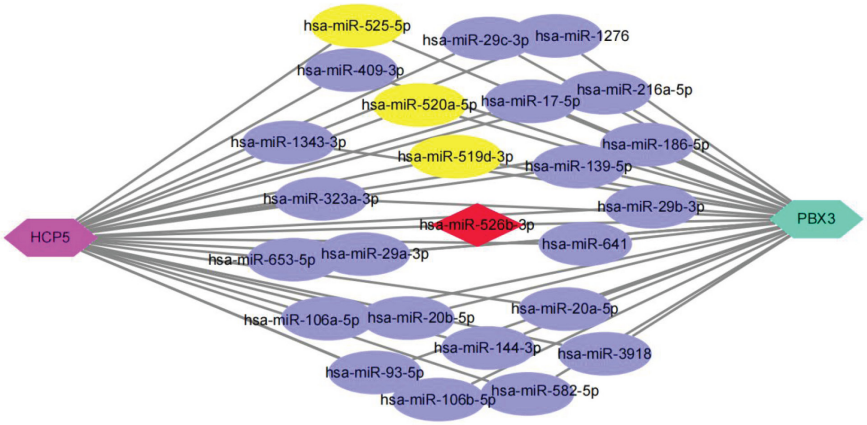
[1] Zheng R, Zhang S, Zeng H, Wang S, Sun K, Chen R, Li L, Wei W and He J. Cancer incidence and mortality in China, 2016. J Natl Cancer Cent 2022; 2: 1-9.

[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[3] Yang L, Bhat AM, Qazi S and Raza K. DLC1 as druggable target for specific subsets of gastric cancer: an RNA-seq-based study. Medicina (Kaunas) 2023; 59: 514.
[4] Deng L, Groman A, Jiang C, Perimbeti S, Gabriel E, Kukar M and Mukherjee S. Association of preoperative chemosensitivity with postoperative survival in patients with resected gastric adenocarcinoma. JAMA Netw Open 2021; 4: e2135340.
[5] Park KB, Jun KH, Song KY, Chin H and Lee HH. Development of a staging system and survival prediction model for advanced gastric cancer patients without adjuvant treatment after curative gastrectomy: a retrospective multicenter cohort study. Int J Surg 2022; 101: 106629.
[6] Ito Y, Miyashiro I, Ishikawa T, Akazawa K, Fukui K, Katai H, Nunobe S, Oda I, Isobe Y, Tsujitani S, Ono H, Tanabe S, Fukagawa T, Suzuki S, Kakeji Y, Sasako M, Bilchik A and Fujita M. Determinant factors on differences in survival for gastric cancer between the United States and Japan using nationwide databases. J Epidemiol 2021; 31: 241-248.
[7] Garcia-Padilla C, Duenas A, Garcia-Lopez V, Aranega A, Franco D, Garcia-Martinez V and Lopez-Sanchez C. Molecular mechanisms of lncRNAs in the dependent regulation of cancer and their potential therapeutic use. Int J Mol Sci 2022; 23: 764.
[8] Oo JA, Brandes RP and Leisegang MS. Long non-coding RNAs: novel regulators of cellular physiology and function. Pflugers Arch 2022; 474: 191-204.
[9] Unfried JP, Sangro P, Prats-Mari L, Sangro B and Fortes P. The landscape of lncRNAs in hepatocellular carcinoma: a translational perspective. Cancers (Basel) 2021; 13: 2651.
[10] Baldini F, Calderoni M, Vergani L, Modesto P, Florio T and Pagano A. An overview of long non-coding (lnc)RNAs in neuroblastoma. Int J Mol Sci 2021; 22: 4234.
[11] Bao G, Xu R, Wang X, Ji J, Wang L, Li W, Zhang Q, Huang B, Chen A, Zhang D, Kong B, Yang Q, Yuan C, Wang X, Wang J and Li X. Identification of lncRNA signature associated with pan-cancer prognosis. IEEE J Biomed Health Inform 2021; 25: 2317-2328.
[12] Li J, Gao C, Liu C, Zhou C, Ma X, Li H, Li J, Wang X, Qi L, Yao Y, Zhang X, Zhuang J, Liu L, Wang K and Sun C. Four lncRNAs associated with breast cancer prognosis identified by coexpression network analysis. J Cell Physiol 2019; 234: 14019-14030.
[13] Zhu TG, Xiao X, Wei Q, Yue M and Zhang LX. Revealing potential long non-coding RNA biomarkers in lung adenocarcinoma using long non-coding RNA-mediated competitive endogenous RNA network. Braz J Med Biol Res 2017; 50: e6297.
[14] Yun WK, Hu YM, Zhao CB, Yu DY and Tang JB. HCP5 promotes colon cancer development by activating AP1G1 via PI3K/AKT pathway. Eur Rev Med Pharmacol Sci 2019; 23: 2786-2793.
[15] Qin S, Yang L, Kong S, Xu Y, Liang B and Ju S. LncRNA HCP5: a potential biomarker for diagnosing gastric cancer. Front Oncol 2021; 11: 684531.
[16] Liu YQ, Cong YZ, Jiang J, Sheng JZ, Li XH, Zhao M and Peng MY. MiR-526b suppresses cell proliferation, cell invasion and epithelial-mesenchymal transition in breast cancer by targeting Twist1. Eur Rev Med Pharmacol Sci 2020; 24: 3113-3121.
[17] Yan F, Ma Y, Liu L, Li L, Deng J and Sun J. Long noncoding RNA HOXD-AS1 promotes the proliferation, migration, and invasion of colorectal cancer via the miR-526b-3p/CCND1 axis. J Surg Res 2020; 255: 525-535.
[18] Li H, Wang J, Xu F, Wang L, Sun G, Wang J and Yang Y. By downregulating PBX3, miR-526b suppresses the epithelial-mesenchymal transition process in cervical cancer cells. Future Oncol 2019; 15: 1577-1591.
[19] Wu J, Gong P and Jiang Z. Knockdown of hsa_circ_0043691 restrains the progression of gastric cancer by decoying miR-1294 to target pre-leukemia transcription factor 3. J Clin Lab Anal 2022; 36: e24733.
[20] Li Y, Sun Z, Zhu Z, Zhang J, Sun X and Xu H. PBX3 is overexpressed in gastric cancer and regulates cell proliferation. Tumour Biol 2014; 35: 4363-4368.
[21] Kulski JK. Long noncoding RNA HCP5, a hybrid HLA class i endogenous retroviral gene: structure, expression, and disease associations. Cells 2019; 8: 480.
[22] Jiang L, Wang R, Fang L, Ge X, Chen L, Zhou M, Zhou Y, Xiong W, Hu Y, Tang X, Li G and Li Z. HCP5 is a SMAD3-responsive long non-coding RNA that promotes lung adenocarcinoma metastasis via miR-203/SNAI axis. Theranostics 2019; 9: 2460-2474.
[23] Zhao J, Bai X, Feng C, Shang X and Xi Y. Long non-coding RNA HCP5 facilitates cell invasion and epithelial-mesenchymal transition in oral squamous cell carcinoma by miR-140-5p/SOX4 axis. Cancer Manag Res 2019; 11: 10455-10462.
[24] Hu R and Lu Z. Long non-coding RNA HCP5 promotes prostate cancer cell proliferation by acting as the sponge of miR-4656 to modulate CEMIP expression. Oncol Rep 2020; 43: 328-336.
[25] Wang L, Luan T, Zhou S, Lin J, Yang Y, Liu W, Tong X and Jiang W. LncRNA HCP5 promotes triple negative breast cancer progression as a

ceRNA to regulate BIRC3 by sponging miR-219a-5p. Cancer Med 2019; 8: 4389-4403.
[26] Yang C, Sun J, Liu W, Yang Y, Chu Z, Yang T, Gui Y and Wang D. Long noncoding RNA HCP5 contributes to epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation and interacting with miR-139-5p. Am J Transl Res 2019; 11: 953-963.
[27] Xu J, Ma J, Guan B, Li J, Wang Y and Hu S. LncRNA HCP5 promotes malignant cell behaviors in esophageal squamous cell carcinoma via the PI3K/AKT/mTOR signaling. Cell Cycle 2021; 20: 1374-1388.
[28] Yin D and Lu X. Silencing of long non-coding RNA HCP5 inhibits proliferation, invasion, migration, and promotes apoptosis via regulation of miR-299-3p/SMAD5 axis in gastric cancer cells. Bioengineered 2021; 12: 225-239.
[29] Wang L, He M, Fu L and Jin Y. Role of lncRNAHCP5/microRNA-525-5p/PRC1 crosstalk in the malignant behaviors of ovarian cancer cells. Exp Cell Res 2020; 394: 112129.
[30] Li X, Chen B, Huang A, Ren C, Wang L, Zhu T, Xiong J, Ding W and Wang H. LncRNA HCP5 enhances the proliferation and migration of cervical cancer via miR-216a-5p/CDC42 axis. J Cancer 2022; 13: 1882-1894.
[31] Liang L, Kang H and Jia J. HCP5 contributes to cisplatin resistance in gastric cancer through miR-128/HMGA2 axis. Cell Cycle 2021; 20: 1080-1090.
[32] Chen S, Ren C, Zheng H, Sun X and Dai J. The effect of long non-coding RNA (lncRNA) HCP5 on regulating epithelial-mesenchymal transition (EMT)-related markers in gastric carcinoma is partially reversed by miR-27b-3p. Med Sci Monit 2020; 26: e921383.
[33] Chen LH, Wang LP and Ma XQ. Circ_SPECC1 enhances the inhibition of miR-526b on downstream KDM4A/YAP1 pathway to regulate the growth and invasion of gastric cancer cells. Biochem Biophys Res Commun 2019; 517: 253-259.
[34] Morgan R and Pandha HS. PBX3 in cancer. Cancers (Basel) 2020; 12: 431.
[35] Li B, Zhang S, Shen H and Li C. MicroRNA-144-3p suppresses gastric cancer progression by inhibiting epithelial-to-mesenchymal transition through targeting PBX3. Biochem Biophys Res Commun 2017; 484: 241-247.
[36] Wang S, Li C, Wang W and Xing C. PBX3 promotes gastric cancer invasion and metastasis by inducing epithelial-mesenchymal transition. Oncol Lett 2016; 12: 3485-3491.
[37] Debnath P, Huirem RS, Dutta P and Palchaudhuri S. Epithelial-mesenchymal transition and its transcription factors. Biosci Rep 2022; 42: BSR20211754.
[38] Jonckheere S, Adams J, De Groote D, Campbell K, Berx G and Goossens S. Epithelial-mesenchymal transition (EMT) as a therapeutic target. Cells Tissues Organs 2022; 211: 157-182.
[39] Jacob A and Prekeris R. The regulation of MMP targeting to invadopodia during cancer metastasis. Front Cell Dev Biol 2015; 3: 4.

A



B

miRNA	GeneID	GeneName	GeneType	TargetSite	Alignment	Class
hsa-miR-526b-3p	ENSG00000206337	HCP5	sense_overlapping	chr6:31433020-31433041[+]	Target: 5' uuaccccaccacuGGCACUUUc 3' :||||||| miRNA : 3' cggagauuuuccuUCGUGAAAg 5'	7mer-m8

Supplementary Figure 1. A. Using StarBase v3.0 to predict regulatory targets of HCP5 and miR-526b and constructed a ceRNA network. B. Computational analysis predicted conserved miR-526b-binding motifs.