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Original Article

MiR-124-3p inhibits proliferation, migration, and epithelial-mesenchymal transformation in gastric cancer by targeting ITGB1

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Abstract: Objective: To investigate the functional interaction between miR-124-3p and integrin β1 (ITGB1) in gastric cancer. Methods: A comprehensive approach integrating bioinformatic prediction with experimental validation was employed. The study used dual luciferase reporter assay, CCK-8 assay, RT-qPCR, western blotting, wound healing assay, and transwell assays to systematically investigate the role of the miR-124-3p/ITGB1 regulatory axis in gastric cancer cell models. Results: This study identified a novel regulatory axis involving miR-124-3p and ITGB1. Mechanistic investigations demonstrated that miR-124-3p directly targets ITGB1, as confirmed by dual-luciferase reporter assays. Aberrant expression of miR-124-3p significantly suppressed ITGB1 mRNA and protein levels, leading to impaired oncogenic properties, including reduced proliferation, migration, and invasion of gastric cancer cells. Furthermore, ITGB1 downregulation inhibited gastric cancer cell growth and invasion while suppressing epithelial-mesenchymal transformation (EMT). Notably, key EMT regulators, such as E-cadherin, were up-regulated. These findings suggest that miR-124-3p-mediated ITGB1 downregulation effectively suppresses gastric cancer progression by inhibiting cell proliferation, invasion, and metastasis. Conclusions: miR-124-3p functions as a tumor-suppressive miRNA that inhibits gastric cancer development through targeting ITGB1. The miR-124-3p/ITGB1 axis provides novel insight and may serve as a promising biomarker for gastric cancer diagnosis and treatment.

Keywords: Gastric cancer, miR-124-3p, ITGB1, proliferation, migration, EMT

Introduction

According to the latest global cancer data, both the incidence and mortality of cancer continue to rise annually, with Asia exhibiting the highest rates. Gastric cancer ranks fifth in global cancer incidence and fourth in cancer-related mortality [1]. Unlike the global trends, China shows a gradual decline in both gastric cancer incidence and mortality [2, 3]. However, the absolute number of gastric cancer cases is expected to increase due to population aging. Early-stage gastric cancer symptoms, including dyspepsia, loss of appetite, reflux, abdominal distension, abdominal discomfort, and melena, are often subtle and easily overlooked. Consequently, most cases are diagnosed at an advanced stage, presenting with complications such as obstruction, anemia, weight loss, and gastrointestinal bleeding, often accompanied by distant metastasis and poor prognosis. Extensive research has been conducted on the mechanisms, progression, diagnosis, and treatment of advanced gastric cancer [4, 5].

MicroRNAs (miRNAs) are a class of evolutionarily conserved, short (about 22 nucleotides), single-stranded RNAs that function as post-transcriptional regulators of gene expression. miRNAs bind to their target mRNAs by complete

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or partial sequence complementarity. Notably, the incomplete pairing mechanism between miRNA and mRNA results in a complex regulatory network: individual miRNAs exert pleiotropic effects through modulating multiple target transcripts simultaneously, while single mRNA 3'UTRs frequently harbor binding sites for diverse miRNA families, enabling combinatorial regulation. miRNAs mediate gene silencing by promoting mRNA degradation, inhibiting translation, or sequestering mRNAs from the translational machinery, ultimately influencing protein expression. These regulatory molecules play essential roles in almost every biologic process, including disease pathogenesis and progression [6]. miRNAs are implicated in tumorigenesis and cancer progression, acting as either tumor suppressors or oncogenic promoters. For example, miR-206 and miR-381 exhibit potent anticancer effects by significantly inhibiting the proliferation of ovarian, breast, prostate, liver, and lung cancer cells [7]. Conversely, oncogenic miRNAs, such as miR-887-3p, promote tumor progression; elevated levels of miR-887-3p suppress apoptosis in pancreatic cancer cells while enhancing their migratory and invasive capabilities [8]. Moreover, miRNAs hold promise as potential drug targets. Hyaluronic acid and milk-derived exosomes, which are inexpensive and readily available, have been utilized as delivery vehicles for miRNA-based therapies. The antitumor efficacy of miR-204 encapsulated in hyaluronic acid and milk exosomes has been demonstrated both *in vitro* and *in vivo*. Histopathologic analysis using hematoxylin-eosin (H&E) staining showed that this approach did not cause tissue or cell damage in major organs, highlighting the potential of miRNA-based therapy as tumor-specific treatment [9]. Additionally, studies have confirmed that miRNA levels can be modulated *in vitro* through overexpression or inhibition, thereby influencing genes and protein expression, as well as cell migration and proliferation [10].

Gastric cancer cells and tissues demonstrate decreased miR-124-3p expression, which correlates with advanced tumor stage, larger tumor size, and metastasis in gastric cancer patients. Functional genomic analysis revealed that miR-124-3p-mediated regulation of EZH2 influences the malignancy of gastric cancer MKN28 cells, affecting their growth and migration rates. Transwell and CCK-8 assays showed that lentiviral transfection targeting MKN28 cells to reduce EZH2 expression significantly suppressed their proliferative and invasive capabilities. However, these effects were reversed following miR-124-3p inhibitor transfection [11]. Additionally, miR-124-3p has been shown to modulate the malignant biologic behavior of various gastric cancer cell lines, including BGC823, SGC7901, and MKN45 [12, 13]. These findings underscore the critical role of miR-124-3p in gastric cancer pathogenesis, highlighting its potential as a target for further research.

Integrins are transmembrane heterodimers composed of alpha and beta subunits, serving as key mediators of cell-cell and cell-extracellular matrix (ECM) interactions. Among them, integrin β1 (ITGB1) plays a crucial role in facilitating cellular adhesion to ECM components and is involved in various biologic processes, including embryogenesis, tissue repair, immune response, tumor cell proliferation, apoptosis, metastasis, and invasion. *ITGB1* is frequently dysregulated in several cancers, including prostate cancer, cholangiocarcinoma, colorectal cancer, and lung cancer, where its aberrant expression is closely associated with malignant biological behaviors such as differentiation, apoptosis, proliferation, invasion, and metastasis [14-17]. In gastric cancer, *ITGB1* is overexpressed, with significantly higher expression levels in advanced gastric cancer compared to early stage disease. High *ITGB1* expression correlates with poor prognosis. Additionally, evidence suggests that *ITGB1* contributes to epithelial-to-mesenchymal transition (EMT) in gastric cancer cells [18]. To investigate the oncogenic role of ITGB1 in gastric cancer, several studies employed transfection techniques to suppress ITGB1 expression. Their findings indicated that *ITGB1* knockdown inhibits gastric cancer cell proliferation, migration, adhesion, and invasion. Moreover, *ITGB1* rescue experiments revealed that upstream regulators exert opposing effects on these oncogenic properties, further supporting ITGB1's role in tumor progression [19, 20]. While substantial research has validated the role of ITGB1 in gastric cancer development, its precise mechanistic network remains to be fully elucidated.

Currently, there is limited research on the miR-124-3p/ITGB1 interaction, and no prior studies

Table 1. Data set information

ID	Data type	Tissue	Platform	Tumor	Normal
GSE19826	mRNA	gastric cancer	GPL570	12	15
GSE33651	mRNA	gastric cancer	GPL2895	40	12
GSE54129	mRNA	gastric cancer	GPL570	111	21
GSE79973	mRNA	gastric cancer	GPL570	10	10
GSE103236	mRNA	gastric cancer	GPL4133	10	9
GSE118916	mRNA	gastric cancer	GPL15207	15	15

have examined this axis specifically in gastric cancer. In this study, we identified ITGB1 as a downstream target of miR-124-3p and investigated how miR-124-3p modulates gastric cancer cell oncogenic properties through ITGB1. Our investigation demonstrated that miR-124-3p functions as a tumor-suppressive microRNA by inhibiting gastric cancer cell growth and migration through direct targeting of *ITGB1*. This study offers a theoretical foundation for elucidating the regulatory network of this microRNA in gastric cancer, offering novel insight into its role in tumor suppression.

Materials and methods

Comparison of gene expression patterns between gastric cancer and normal tissues

Gastric cancer tissue datasets were retrieved from the Gene Expression Omnibus (GEO) database. The selected datasets met the following criteria: (1) all samples were derived from *Homo sapiens*; (2) GEO2R was applicable for data analysis; (3) each dataset contained more than six samples; and (4) both normal and gastric cancer groups were included. A total of six eligible datasets were downloaded (**Table 1**). Differentially expressed genes (DEGs) between normal gastric mucosa samples and gastric cancer samples were identified using the GEO2R online tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/). DEGs were divided into up-regulated and down-regulated groups based on gene expression scores. Intersection analysis of the DEGs from these six data sets was performed to identify the commonly upregulated and downregulated genes.

Screening of key DEGs and KEGG enrichment analysis

Up-regulated DEGs were subjected to protein-protein interaction (PPI) analysis using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://cn.string-db.org/). The resulting *tsv* file was exported for further analysis. CytoHubba plugin within Cytoscape was used to identify hub genes and analyze the interaction network, selecting the top 20 genes with highest degree scores as key DEGs. These 20 pivotal DEGs were then analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david-d.ncifcrf.gov) for functional annotation via KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis. A threshold of $P < 0.05$ was considered significant.

Assessment of ITGB1 expression and its effect on gastric cancer patient survival

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a web-based platform for analyzing TCGA gene expression data and patient survival outcomes. It enables the investigation of differential gene expression, correlation analysis, and prognosis based on TCGA datasets. We used GEPIA to validate ITGB1 expression in gastric cancer and normal gastric mucosa samples, as well as to assess the effect of ITGB1 expression on overall patient survival. Additionally, the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/), an integrative resource combining transcriptomics, proteomics, and systems biology data, was used to map protein expression and distribution across various human cells, tissues, and organs. The HPA database was used to analyze immunohistochemical staining patterns of ITGB1 in both gastric cancer and normal tissues.

Prediction of miRNA upstream of ITGB1

To identify potential upstream regulators of ITGB1, we used MiRTarBase (https://maayanlab.cloud/Harmonizome/resource/MiRTarBase), which predicted ITGB1 as a putative downstream effector of miR-124-3p. Additionally, we employed Encyclopedia of RNA Interactomes (ENCORI) (https://rnasysu.com/encori/panMirDiffExp.php) to validate miR-124-3p expression levels in gastric cancer specimens.

Cell culture and transfection

Human gastric cancer AGS cells and human embryonic kidney 293T cells were obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Both cell lines underwent verification through STR profiling and were confirmed to be free of mycoplasma contamination. Cells were cultured in standard medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin, following the manufacturer's guidelines. They were maintained at 37°C in a humidified incubator with 5% CO_2 to ensure optimal growth conditions and experimental reproducibility.

Hsa-miR-124-3p mimics, a negative control (NC, Hsa-miR-124-3p NC), si-ITGB1, and si-NC were purchased from Genepharma Co., Ltd. (Shanghai, China). Before transfection, cells were inoculated in 6-well plates as per standard procedures. When cell confluence reached 70%-90%, the complete medium was replaced with Opti-MEM Reduced Serum Medium (Gibco, USA) following the Lipofectamine 3000 transfection reagent protocol (Invitrogen, USA). The cells were subsequently transfected using the Lipofectamine 3000 transfection reagent. After 6 hours, Opti-MEM was replaced with a complete growth medium to support cell proliferation. RNA was extracted 24 hours post-transfection, followed by protein extraction 48 hours after the medium change.

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted using the AxyPrep Multisource Total RNA Miniprep Kit (Axygen, USA) following the protocols to ensure RNA purity and integrity. Complementary DNA (cDNA) was synthesized from total RNA using the RevertAid Master Mix (Thermo Fisher Scientific, USA) and the miRNA First Strand cDNA Synthesis Kit (Stem-Loop Method) (Sangon Biotech, China). miR-124-3p and ITGB1 expression levels were analyzed by RT-qPCR using the qPCR SYBR Green Master Mix (Applied Biosystems, USA) and respective primers (Sangon Biotech China). All procedures were performed on ice. U6 and GAPDH were used as reference genes for normalization, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

The specific primer sequences included: hsa-miR-124-3p-F: 5'-AACAAGTAAGGCACGCGGTGA-3'; hsa-miR-124-3p-R: 5'-ATCCAGTGCAGGGTCCAGGG-3'; hsa-miR-124-3p-RT: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATCCGCACTGGATACGACTTGGCA-3'; U6-F: 5'-GCTTCGGCAGCACATATACTAAAAT-3'; U6-R: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; ITGB1-F: 5'-TGGGCTTTACGGAGGAAGTAGAGG-3'; ITGB1-R: 5'-GACACTTGGGACTTTCAGGGATGC-3'; GAPDH-F: 5'-CAGGAGGCATTGCTGATGAT-3'; GAPDH-R: 5'-GAAGGCTGGGGCTCATTT-3'.

Western blotting assay

Total cellular protein was extracted using RIPA buffer supplemented with PMSF (Solarbio, China) to ensure protease inhibition throughout the entire extraction process and protein concentration was determined using BCA Protein Assay Kit (Epizyme, China) by referencing a standard protein curve. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). Membranes were blocked with blocking buffer (Epizyme, China) under shaking conditions, followed by sequential incubation with primary and secondary antibodies.

Primary and secondary antibodies were obtained from Proteintech Group, Inc., including ITGB1 (12594-1-AP, 1:2000), E-cadherin (20874-1-AP, 1:2000), GAPDH (10494-1-AP, 1:2000), HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (SA00001-2, 1:20000).

Dual-luciferase reporter assay

Using bioinformatic analysis, we predicted the miR-124-3p binding site within the ITGB1 gene and we synthesized its 3'UTR fragment. The fragment was cloned into the pYr-MirTarget vector to construct recombinant plasmids: pYr-MirTarget-ITGB1-3'UTR-WT (wild-type) and pYr-MirTarget-ITGB1-3'UTR-MUT (mutant). Both plasmids (WT and MUT) were co-transfected into 293T cells along with miR-124-3p mimics or their negative control (miR-124-3p mimics NC) using Lipofectamine 3000. After 48 hours, luciferase activity was measured using the Dual-Luciferase Reporter Assay Kit (Beyotime, China) to quantify luciferase signals.

Cell counting kit-8 (CCK-8) assay

Cell viability was assessed using the CCK-8 assay (Dojindo, Japan) adhering to manufacturer's protocols. Transfected cells were tryp-

sinized, centrifuged, resuspended, and seeded into 96-well plates at a density of 3000 cells/well in 100 μL of complete growth medium. These plates were then incubated at 37°C in a humidified incubator with 5% CO_2. Once cells adhered to the 96-well plate (about 6 h), 10 ul of CCK-8 reagent was added, followed by incubation for 2 hours. Optical density (OD) at 450 nm was measured using a microplate reader. Additionally, OD readings were recorded at 24, 48, and 72 hours post-incubation.

Wound healing assay

Cell migratory potential was analyzed using a wound healing assay. Prior to seeding, several horizontal reference marks were made on the bottom of a 6-well plate using a ruler. Subsequently, AGS cells were inoculated onto the 6-well plate before transfection procedures. Once a confluent monolayer was formed, perpendicular scratches were introduced using a sterile 200 μL pipette tip, intersecting the pre-drawn horizontal lines. Images were taken at 0 hours and 24 hours, respectively, to assess wound closure. The migration rate (%) was calculated using the formula: Migration rate = (Scratch width at 0 h - Scratch width at 24 h)/Scratch width at 0 h × 100%

Transwell assay

Transwell chambers were sterilized under ultraviolet light for 2 hours before use. Subsequently, the transfected cells were then trypsinized, centrifuged, and resuspended in serum-free medium to obtain a uniform single-cell suspension. The lower chamber was filled with 600 μL of medium containing 20% serum, serving as a chemoattractant. A total of 5×10^4 cells were seeded into the upper chamber, ensuring the absence of bubbles between compartments. After 48 hours of incubation, cells were fixed with 4% paraformaldehyde at room temperature for 5 minutes following phosphate-buffered saline (PBS) washing. After an additional PBS wash, cells were stained with 0.1% crystal violet for 20 minutes, washed again with PBS to remove excess stain, and observed under a microscope.

Statistical analysis

All experimental data were analyzed using SPSS version 25.0 and GraphPad Prism version 9.0 for statistical processing and visualization. Between-group difference was assessed using t-tests or nonparametric tests, while differences among multiple groups were analyzed using one-way ANOVA. Statistical significance was set at: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

Results

Identification of DEGs in gastric cancer

Totals of 98 upregulated DEGs and 117 downregulated DEGs were obtained. Among the 98 upregulated DEGs, 20 key genes were further screened using the STRING database and Cytoscape software. These key DEGs included COL1A1, COL1A2, COL3A1, COL6A3, COL5A1, COL5A2, FN1, BGN, FBN1, LUM, COL4A1, THBS2, COL12A1, SERPINH1, ITGB1, SPARC, BMP1, THBS1, VCAN, COL18A1 (**Figure 1A**, **1B**). Functional enrichment analysis revealed that these 20 key genes were predominantly involved in muscle cell cytoskeleton organization, human papillomavirus infection, protein digestion and absorption, ECM-receptor interactions, focal adhesion regulation, platelet activation, AGE-RAGE signaling in diabetic complications, amoebiasis, cancer-associated proteoglycans, PI3K-Akt signaling, relaxin signaling, and small cell lung cancer (**Figure 1C**).

Elevated ITGB1 expression in gastric cancer and its correlation with unfavorable prognostic outcome

To assess ITGB1 expression pattern in gastric cancer and its prognostic significance, we validated the data using the TCGA database. ITGB1 expression was found to be significantly upregulated in ten cancer types (**Figure 2A**). Specifically, ITGB1 expression was significantly upregulated in gastric cancer ($P < 0.05$, **Figure 2B**). Similarly, immunohistochemical analysis from the HPA database showed strong ITGB1 positivity in gastric cancer samples, whereas normal gastric tissues exhibited negative staining (**Figure 2C**). Subsequent analysis demonstrated a significantly shorter survival duration in patients exhibiting high ITGB1 expression compared to those with low ITGB1 levels ($P < 0.05$, **Figure 2D**).

miR-124-3p directly targets ITGB1 in gastric cancer

Bioinformatic analysis predicted ITGB1 as a downstream target of miR-124-3p. To further

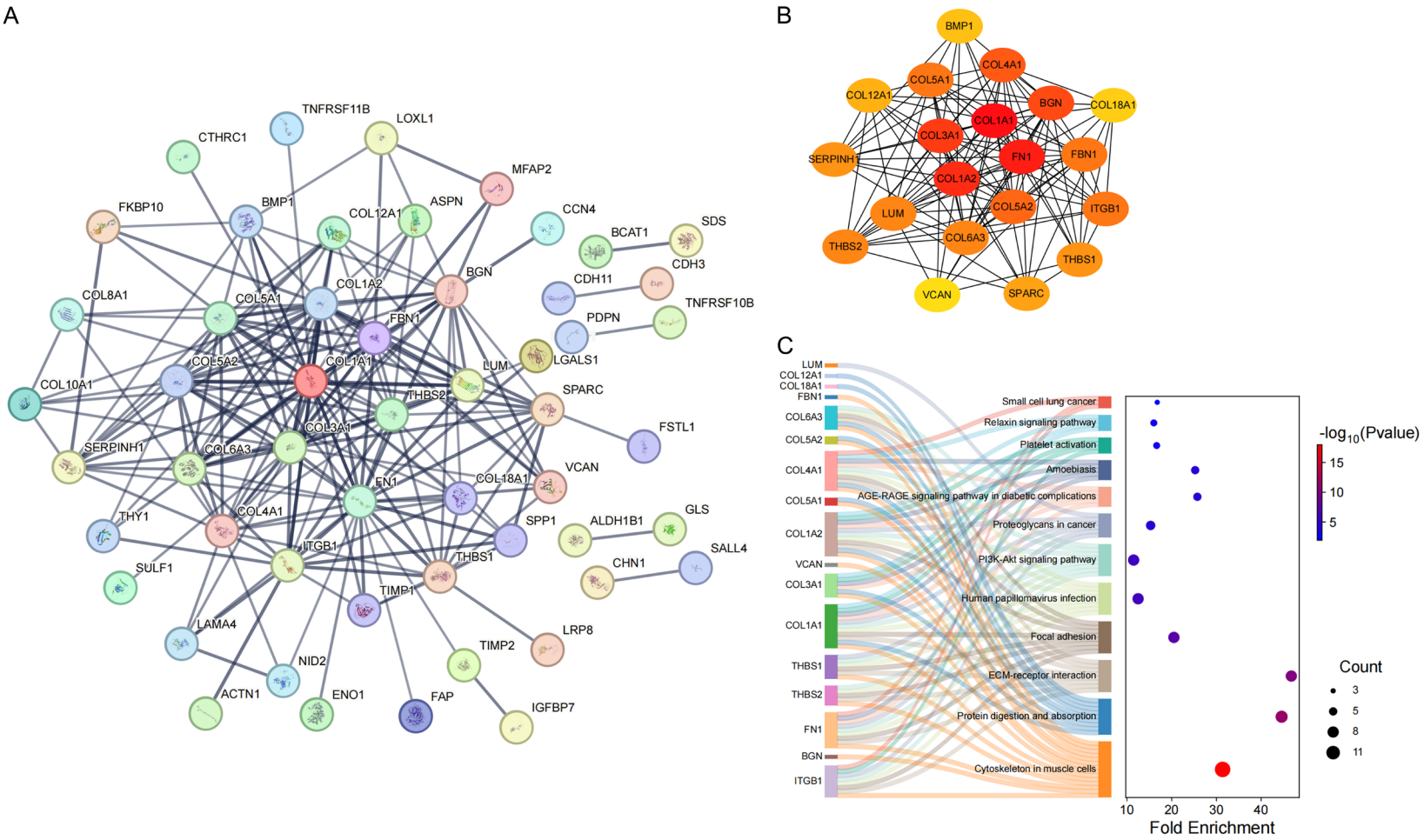

Figure 1. Identification of key differentially expressed genes (DEGs). A. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to draw protein-protein interaction network. B. Cytoscape software was used to draw the degree of interaction among the top 20 key target genes. C. The Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis of 20 key targets using DAVID database.

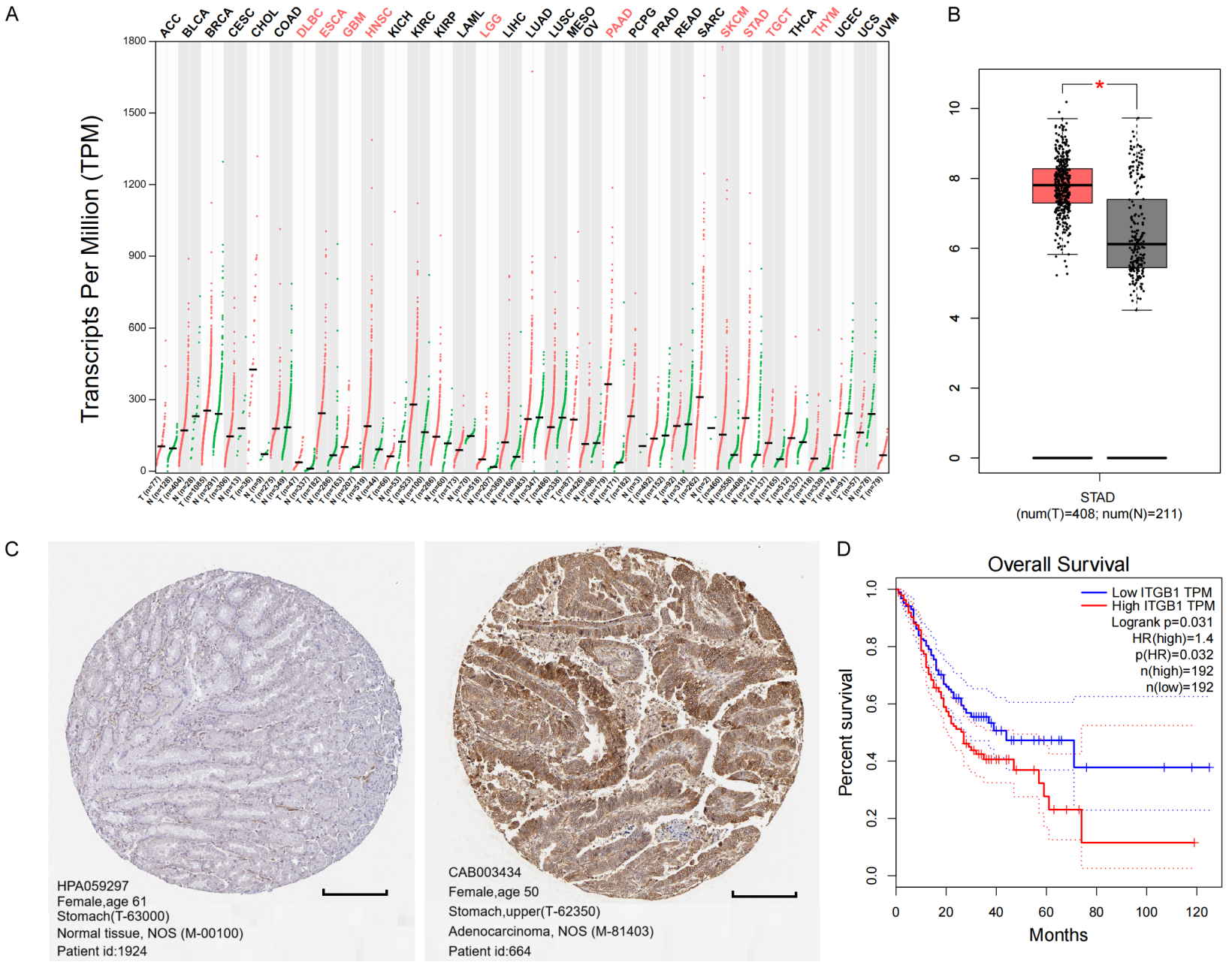
A
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS UVM
Transcripts Per Million (TPM)
1800
1500
1200
900
600
300
0
B
STAD
(num(T)=408; num(N)=211)
C
HPA059297
Female,age 61
Stomach(T-63000)
Normal tissue, NOS (M-00100)
Patient id:1924
CAB003434
Female,age 50
Stomach,upper(T-62350)
Adenocarcinoma, NOS (M-81403)
Patient id:664
D
Overall Survival
Low ITGB1 TPM
High ITGB1 TPM
Logrank p=0.031
HR(high)=1.4
p(HR)=0.032
n(high)=192
n(low)=192
Percent survival
Months

Figure 2. The expression of integrin β1 (ITGB1) in gastric cancer tissues. A. Expression of ITGB1 in 33 cancer types from The Cancer Genome Atlas (TCGA) database; red indicates significantly high expression in cancer tissues. B. Expression of ITGB1 in gastric cancer tissues from TCGA database. C. Expression of ITGB1 in gastric cancer from The Human Protein Atlas (HPA) database. D. The overall survival of gastric cancer patients with high or low ITGB1 expression. Scale bar = 200 μm; magnification is 40×; P < 0.05 (*).

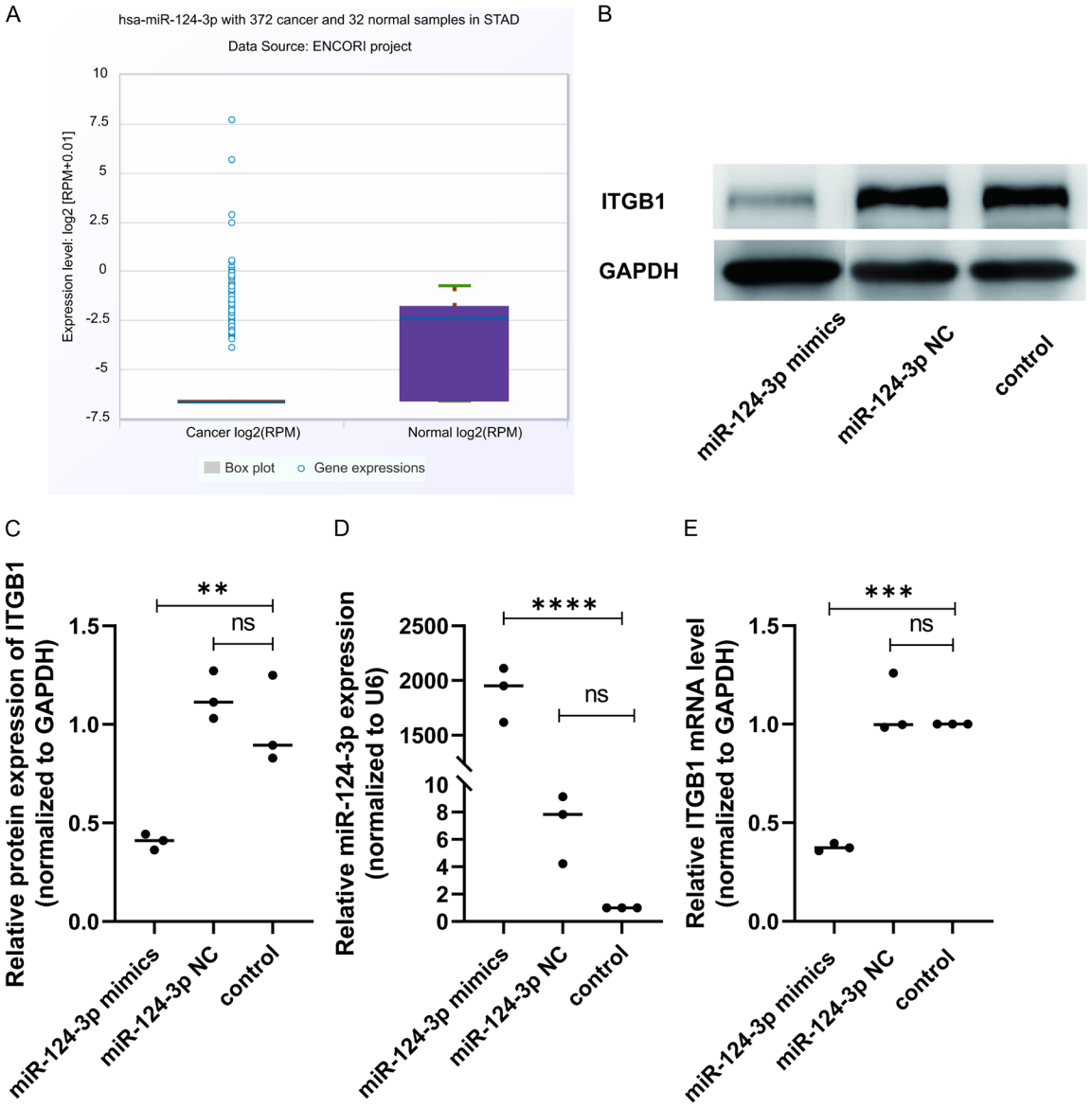

Figure 3. Modulation effects of MiR-124-3p on ITGB1 expression in gastric cancer cells. A. Differential expression of miR-124-3p in gastric cancer was analyzed using The Encyclopedia of RNA Interactomes (ENCORI). B, C. The protein expression of miR-124-3p and ITGB1 after miR-124-3p mimic transfection. D, E. mRNA expression of miR-124-3p and ITGB1 after miR-124-3p mimic transfection. *P* < 0.01 (**); *P* < 0.001 (***); *P* < 0.0001 (****). ns, no significance.

validate this interaction, we used ENCORI database, which confirmed that miR-124-3p expression was significantly downregulated in gastric cancer tissues (**Figure 3A**). To experimentally verify this relationship, ACS cells were transfected with miR-124-3p mimics and the corresponding NC. Western blotting analysis showed a clear downregulation of *ITGB1* pro-

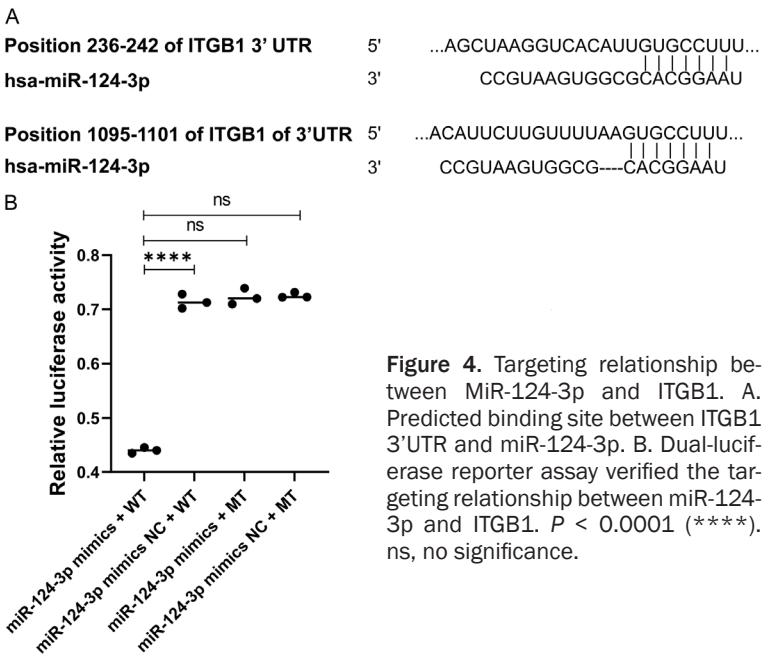

Figure 4. Targeting relationship between MiR-124-3p and ITGB1. A. Predicted binding site between ITGB1 3'UTR and miR-124-3p. B. Dual-luciferase reporter assay verified the targeting relationship between miR-124-3p and ITGB1. $P < 0.0001$ (****). ns, no significance.

tein expression following miR-124-3p mimic transfection (**Figure 3B**, **3C**). Similarly, RT-qPCR results confirmed a significant reduction in *ITGB1* mRNA levels upon miR-124-3p mimic transfection (**Figure 3D**, **3E**).

Further investigation of miR-124-3p targeting ITGB1 was conducted using bioinformatic analysis (**Figure 4A**) and a dual-luciferase reporter assay. The results demonstrated that miR-124-3p mimic transfection remarkably reduced luciferase activity in the WT-ITGB1 vector. However, mutation of the miR-124-3p binding site abolished this effect, confirming that miR-124-3p directly targets ITGB1 (**Figure 4B**).

miR-124-3p suppressed gastric cancer cell growth and invasion

To investigate the effects of miR-124-3p on gastric cancer, gastric cancer cells were transfected with miR-124-3p mimics or corresponding NC. Wound healing assay revealed that miR-124-3p mimic transfection significantly reduced gastric cancer cell migration (**Figure 5A**, **5B**). Transwell assay demonstrated that gastric cancer cell invasion was notably decreased after miR-124-3p mimic transfection (**Figure 5C**). CCK-8 assay showed that gastric cancer cell proliferation was markedly inhibited following miR-124-3p mimic transfection (**Figure 5D**).

ITGB1 knockdown impeded migration, proliferation, and EMT in gastric cancer cells

To determine whether ITGB1 is involved in miR-124-3p-mediated inhibition of gastric cancer cell growth and invasion, cells were transfected with si-ITGB1 or its corresponding NC. RT-qPCR and western blot analyses confirmed successful knockdown of ITGB1 (**Figure 6A**, **6B**). Additionally, ITGB1 knockdown led to a significant increase in E-cadherin expression, a key EMT marker, suggesting a reduction in metastatic potential (**Figure 6C**, **6D**). Further Functional assays demonstrated that the migratory, invasive, and proliferative capacities of si-ITGB1-transfected cells were significantly reduced, mirroring the effects observed following miR-124-3p overexpression (**Figure 7**). In conclusion, miR-124-3p inhibits gastric cancer cell growth and invasion through down-regulating ITGB1, which plays a critical role in the EMT process.

Discussion

ITGB1 (Integrin β1), also known as CD29, is a widely expressed subunit of the integrin family and plays a crucial role in a various intracellular signaling pathways. Comprehensive bioinformatic analysis has identified a panel of genes with elevated expression in gastric cancer tissues, among which ITGB1 emerges as a pivotal gene strongly correlation with patient prognosis. This finding highlights its potential significance in gastric cancer progression, although its underlying mechanisms remain to be fully elucidated.

miRNAs do not regulate single genes in isolation; rather, each miRNA simultaneously targets multiple genes, forming complex and diverse regulatory networks that require further exploration. MiRNA mimics and inhibitors are chemically synthesized duplexes to enforce or inhibit endogenous miRNA activity. They can be delivered by various methods, such as lentivi-

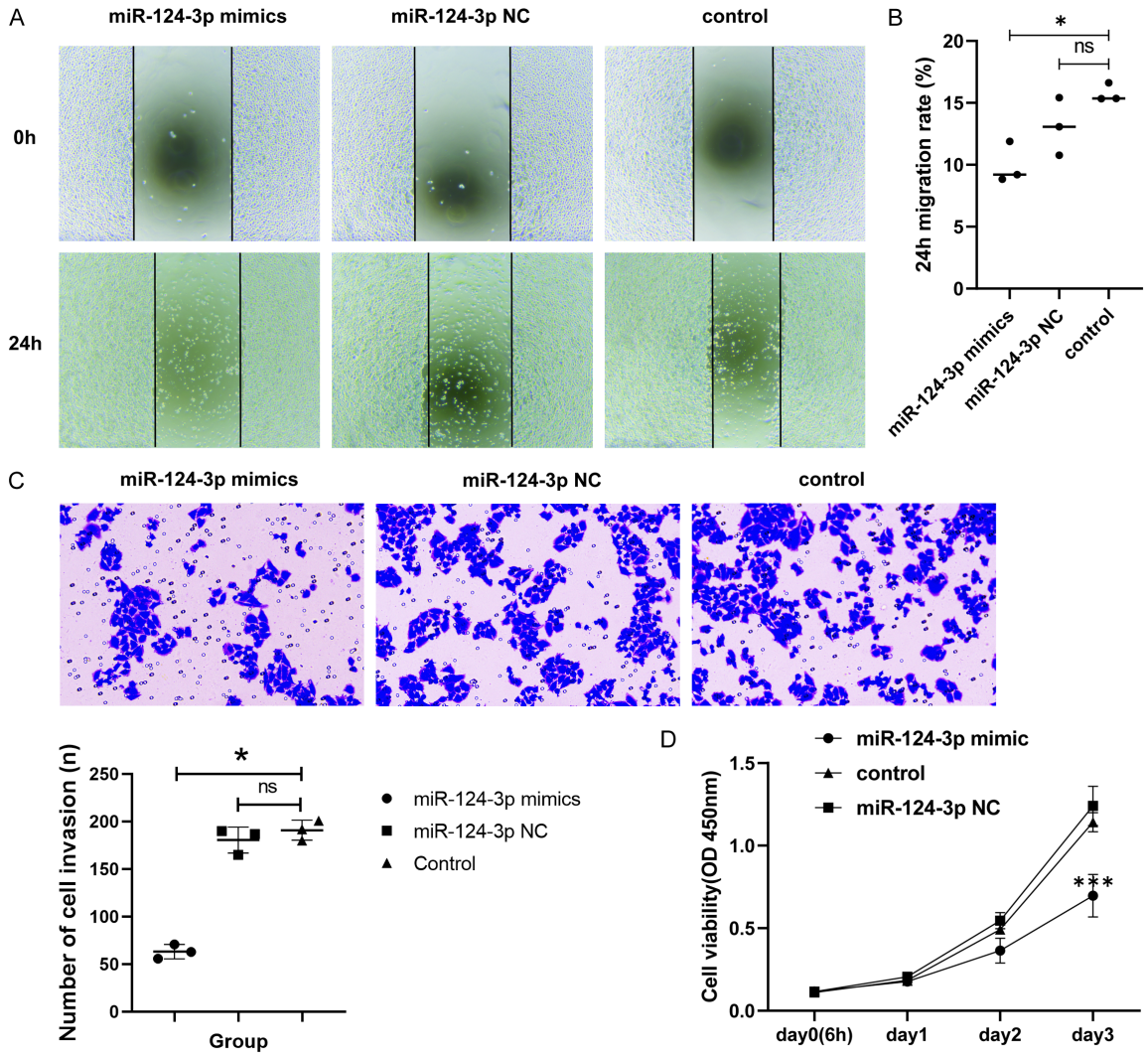

Figure 5. Effects of MiR-124-3p on gastric cancer cell proliferation, migration, and invasion. A, B. AGS cell migration at 0, 24 h after miR-124-3p mimic transfection was detected using Wound-healing assay. C. AGS cell invasion after miR-124-3p mimic transfection was detected using Transwell invasion assay. D. AGS cell proliferation at 6 h, 1, 2, and 3 days after miR-124-3p mimic transfection was detected using CCK-8 assay. $P < 0.05$ (*); $P < 0.001$ (***). ns, no significance.

ruses, lipid-based, or plasmid-mediated transfection. Numerous studies have demonstrated that miRNAs exert profound regulatory effects on tumor cells and animal models, influencing key aspects of tumorigenesis such as proliferation, migration, invasion, and apoptosis [21, 22]. In addition, in terms of drug therapy, miRNAs can modulate drug efficacy by enhancing sensitivity or reducing resistance [23, 24]. Given their crucial role in tumor regulation, miRNAs are regarded as promising diagnostic and therapeutic tools with prognostic value.

This study focuses on miR-124-3p, which has been reported to inhibit bladder cancer cell invasion and metastasis by ITGA3 and plays a critical role in the EMT process, suppressing tumor metastasis through the FAK/Src and FAK/PI3K/Akt pathways [25]. Additionally, miR-124-3p inhibits tumor cell proliferation, invasion and migration while promoting apoptosis. Beyond cancer, it is also involved in the pathogenesis of other diseases, including rheumatoid arthritis, acute myocardial infarction, ulcerative colitis, and hypertension [26].

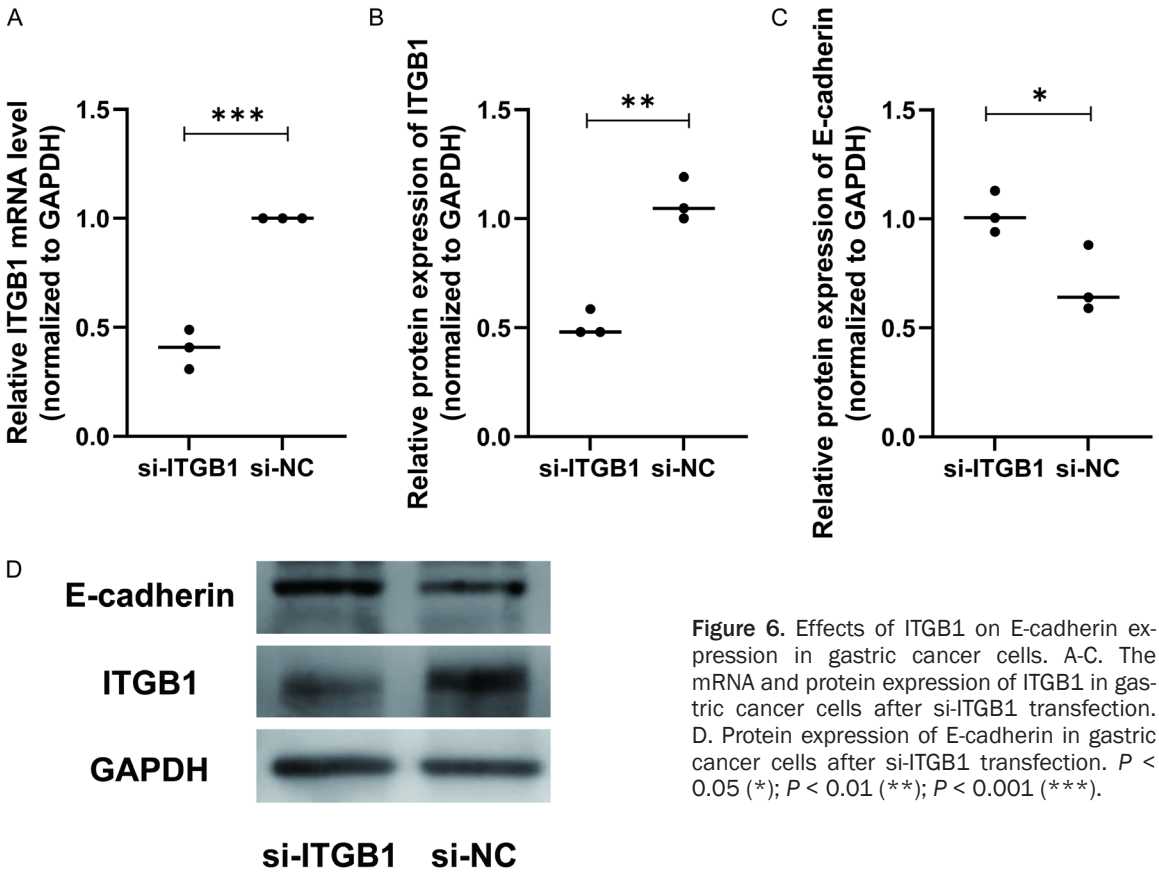

Figure 6. Effects of ITGB1 on E-cadherin expression in gastric cancer cells. A-C. The mRNA and protein expression of ITGB1 in gastric cancer cells after si-ITGB1 transfection. D. Protein expression of E-cadherin in gastric cancer cells after si-ITGB1 transfection. $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***).

However, the precise role of miR-124-3p in gastric cancer progression remains incompletely understood. In this study, we proved that ITGB1 is a direct downstream target of miR-124-3p and that miR-124-3p suppresses gastric cancer progression by downregulating ITGB1. miR-124-3p overexpression led to a significant reduction in ITGB1 mRNA and protein levels, resulting in impaired gastric cancer cell proliferation and motility. To determine whether miR-124-3p exerts its tumor-suppressive effects primarily through ITGB1, we further performed ITGB1 knockdown. The results confirmed our hypothesis: silencing ITGB1 alone inhibited gastric cancer cell growth and invasion, reinforcing its critical role in mediating miR-124-3p's effects. Collectively, miR-124-3p partially represses gastric cancer cell growth and migration through targeting ITGB1, which is implicated in the regulation of EMT.

Despite the insights gained from this study, several limitations remain. Notably, the expression levels of ITGB1 in clinical gastric cancer specimens and its correlation with overall survival require further investigation.

Conclusions

We identified ITGB1 as a novel target gene that interacts with miR-124-3p to promote gastric cancer cell proliferation, metastasis, and EMT. Our findings highlight the critical role of the miR-124-3p/ITGB1 axis in gastric cancer progression, providing a therapeutic target for future intervention.

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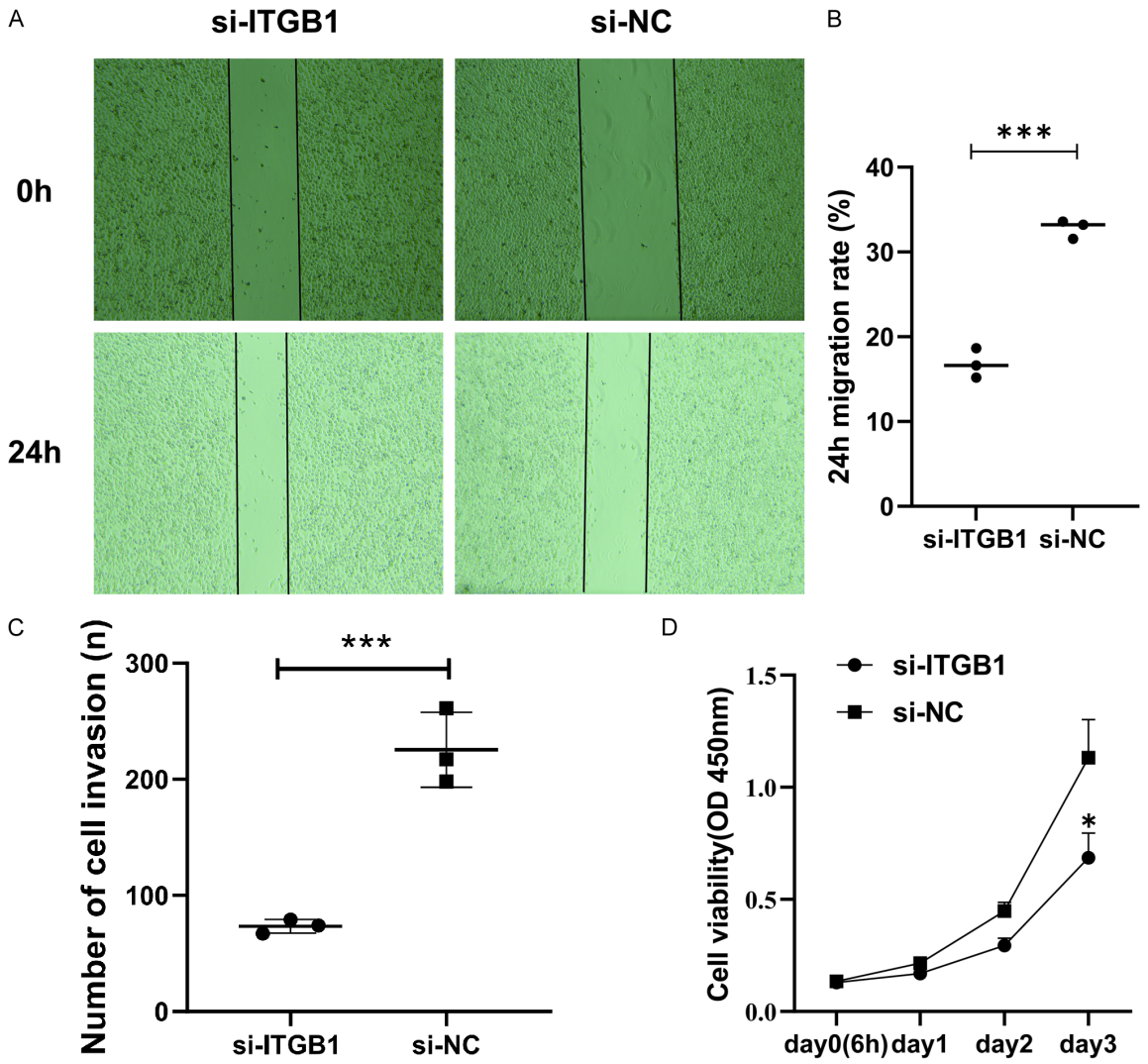

Figure 7. Effects of ITGB1 on gastric cancer cell proliferation, invasion, and migration. A, B. AGS cell migration at 0, 24 h after si-ITGB1 transfection was detected using wound-healing assay. C. AGS cell invasion after si-ITGB1 transfection. D. AGS cell proliferation at 6 h, 1, 2, and 3 days after si-ITGB1 transfection was detected using CCK-8 assay. $P < 0.05$ (*); $P < 0.001$ (***).

Disclosure of conflict of interest

None.

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References

[1] 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.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[3] Gao K and Wu J. National trend of gastric cancer mortality in China (2003-2015): a population-based study. Cancer Commun (Lond) 2019; 39: 24.

[4] Joshi SS and Badgwell BD. Current treatment and recent progress in gastric cancer. CA Cancer J Clin 2021; 71: 264-279.

[5] Smyth EC, Nilsson M, Grabsch HI, van Grieken NC and Lordick F. Gastric cancer. Lancet 2020; 396: 635-648.

[6] Dong H, Lei J, Ding L, Wen Y, Ju H and Zhang X. MicroRNA: function, detection, and bioanalysis. Chem Rev 2013; 113: 6207-6233.
[7] Moradi S, Kamal A, Aboulkheyr Es H, Farhadi F, Ebrahimi M, Chitsaz H, Sharifi-Zarchi A and Baharvand H. Pan-cancer analysis of microRNA expression profiles highlights microRNAs enriched in normal body cells as effective suppressors of multiple tumor types: a study based on TCGA database. PLoS One 2022; 17: e0267291.
[8] Xu X and Zheng S. MiR-887-3p negatively regulates STARD13 and promotes pancreatic cancer progression. Cancer Manag Res 2020; 12: 6137-6147.
[9] Li D, Gong L, Lin H, Yao S, Yin Y, Zhou Z, Shi J, Wu Z and Huang Z. Hyaluronic acid-coated bovine milk exosomes for achieving tumor-specific intracellular delivery of miRNA-204. Cells 2022; 11: 3065.
[10] Lu TX and Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018; 141: 1202-1207.
[11] Huang HG, Tang XL, Huang XS, Zhou L, Hao YG and Zheng YF. Long noncoding RNA LINC00511 promoted cell proliferation and invasion via regulating miR-124-3p/EZH2 pathway in gastric cancer. Eur Rev Med Pharmacol Sci 2020; 24: 4232-4245.
[12] Li Y, Yan J, Wang Y, Wang C, Zhang C and Li G. LINC00240 promotes gastric cancer cell proliferation, migration and EMT via the miR-124-3p/DNMT3B axis. Cell Biochem Funct 2020; 38: 1079-1088.
[13] You L, Wu Q, Xin Z, Zhong H, Zhou J, Jiao L, Song X and Ying B. The long non-coding RNA HOXA11-AS activates ITGB3 expression to promote the migration and invasion of gastric cancer by sponging miR-124-3p. Cancer Cell Int 2021; 21: 576.
[14] Oliveira-Rizzo C, Ottati MC, Fort RS, Chavez S, Trinidad JM, DiPaolo A, Garat B, Sotelo-Silveira JR and Duhagon MA. Hsa-miR-183-5p modulates cell adhesion by repression of ITGB1 expression in prostate cancer. Noncoding RNA 2022; 8: 11.
[15] Lee J, Lee J, Sim W and Kim JH. Soluble TGFBI aggravates the malignancy of cholangiocarcinoma through activation of the ITGB1 dependent PPARγ signalling pathway. Cell Oncol (Dordr) 2022; 45: 275-291.
[16] Wang X and Li T. Ropivacaine inhibits the proliferation and migration of colorectal cancer cells through ITGB1. Bioengineered 2021; 12: 44-53.
[17] Wu B, Xue X, Lin S, Tan X and Shen G. LncRNA LINC00115 facilitates lung cancer progression through miR-607/ITGB1 pathway. Environ Toxicol 2022; 37: 7-16.
[18] Gu W, Sun H, Zhang M, Mo S, Tan C, Ni S, Yang Z, Wang Y, Sheng W and Wang L. ITGB1 as a prognostic biomarker correlated with immune suppression in gastric cancer. Cancer Med 2023; 12: 1520-1531.
[19] Cao C, Han S, Yuan Y, Wu Y, Lian W, Zhang X, Pan L and Li M. Downregulated circular RNA hsa_circ_0000291 suppresses migration and proliferation of gastric cancer via targeting the miR-183/ITGB1 axis. Cancer Manag Res 2019; 11: 9675-9683.
[20] Hu C, Ni Z, Li BS, Yong X, Yang X, Zhang JW, Zhang D, Qin Y, Jie MM, Dong H, Li S, He F and Yang SM. hTERT promotes the invasion of gastric cancer cells by enhancing FOXO3a ubiquitination and subsequent ITGB1 upregulation. Gut 2017; 66: 31-42.
[21] Kim Y, Kim H, Bang S, Jee S and Jang K. MicroRNA-130b functions as an oncogene and is a predictive marker of poor prognosis in lung adenocarcinoma. Lab Invest 2021; 101: 155-164.
[22] Xing S, Tian Z, Zheng W, Yang W, Du N, Gu Y, Yin J, Liu H, Jia X, Huang D, Liu W and Deng M. Hypoxia downregulated miR-4521 suppresses gastric carcinoma progression through regulation of IGF2 and FOXM1. Mol Cancer 2021; 20: 9.
[23] Wei L, Sun J, Zhang N, Zheng Y, Wang X, Lv L, Liu J, Xu Y, Shen Y and Yang M. Noncoding RNAs in gastric cancer: implications for drug resistance. Mol Cancer 2020; 19: 62.
[24] Wang S, Li MY, Liu Y, Vlantis AC, Chan JY, Xue L, Hu BG, Yang S, Chen MX, Zhou S, Guo W, Zeng X, Qiu S, van Hasselt CA, Tong MC and Chen GG. The role of microRNA in cisplatin resistance or sensitivity. Expert Opin Ther Targets 2020; 24: 885-897.
[25] Wang JR, Liu B, Zhou L and Huang YX. MicroRNA-124-3p suppresses cell migration and invasion by targeting ITGA3 signaling in bladder cancer. Cancer Biomark 2019; 24: 159-172.
[26] Li Q, Liu S, Yan J, Sun MZ and Greenaway FT. The potential role of miR-124-3p in tumorigenesis and other related diseases. Mol Biol Rep 2021; 48: 3579-3591.