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Original Article

ITGA5 promotes cervical cancer progression by regulating IMP3 recruitment of HK2 mRNA

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Abstract: Cervical cancer is a prevalent gynecologic malignancy characterized by high rates of invasion and metastasis. Integrin alpha 5 (ITGA5), a member of the integrin family, has been implicated in tumor progression; however, its regulatory role in cervical cancer remains poorly defined. Bioinformatic analyses revealed elevated ITGA5 expression in cervical cancer, which was further validated in patient tissues by immunohistochemistry (IHC). ITGA5 was either silenced or overexpressed in cervical cancer cell lines, and its effects on proliferation, invasion, and migration were assessed using CCK-8, Transwell, and wound healing assays. The *in vivo* effects of ITGA5 knockdown were evaluated through subcutaneous tumor xenografts in nude mice. Mass spectrometry identified insulin-like growth factor II mRNA binding protein 3 (IMP3) as a potential ITGA5-interacting protein. Their interaction was confirmed using co-immunoprecipitation (CO-IP), western blotting, and RNA immunoprecipitation (RIP). ITGA5 was found to be significantly upregulated in cervical cancer and negatively correlated with patient survival. Functionally, ITGA5 promoted proliferation, invasion, and migration of cervical cancer cells *in vitro* and enhanced tumor growth in vivo. Mechanistically, ITGA5 interacted with IMP3, regulating the recruitment of hexokinase 2 (HK2) mRNA by IMP3. Overexpression of HK2 rescued the inhibitory effects of ITGA5 knockdown on cervical cancer progression. This study presents new findings on the pathogenesis of cervical cancer and identifies a possible therapeutic target.

Keywords: Cervical cancer, integrin, ITGA5, IMP3, HK2

Introduction

Cervical cancer (CC) is the fourth most common malignancy among women globally. Although the incidence has declined in some regions due to widespread CC screening and human papillomavirus (HPV) vaccination, the overall global burden remains substantial [1]. In response, the World Health Organization (WHO) launched a global strategy in 2020 to promote the elimination of CC [2]. In line with this effort, the Chinese government has introduced a seven-year action plan (2023-2030) to intensify national elimination efforts. CC remains the most prevalent gynecologic malignancy in China. According to the National Cancer Center's latest data (2022), the incidence and mortality rates of CC in China were 17.69 and 5.52 per 100,000 women, respectively. The prevalence has been rising for over three decades [3], posing a significant threat to women's health in China [4]. Hysterectomy combined with chemotherapy and radiotherapy is the standard treatment for early-stage CC; however, treatment options for locally advanced or metastatic CC often yield poor prognoses. Due to the aggressive nature and high metastatic potential of CC, the 5-year survival rate ranges from 30% to 60% [5]. Thus, there remains an urgent need to explore more effective adjuvant therapies following radical hysterectomy.

Integrins are a family of transmembrane receptor proteins that play critical roles in cell adhesion, signal transduction, tissue development and maintenance, and the pathogenesis of various diseases. They regulate key cellular processes such as proliferation, differentiation, migration, and survival [6]. Integrin function is modulated through mechanisms including con-

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Table 1. Clinicopathological characteristics of patients with CC in our hospital (N=46)

Variable	Number of patients (%)	Patients with LNM (%)
Age (years)		
Median (range)	50 (33-69)	
<50	21 (45.7)	3 (30)
≥50	25 (54.3)	7 (70)
FIGO stage		
IA-IIA	34 (73.9)	0 (0)
IIB-IIIC	12 (26.1)	10 (100)
Histological type		
Squamous cell carcinoma	37 (80.4)	10 (100)
Adenocarcinoma	9 (19.6)	0 (0)
LVSI		
Negative	20 (43.5)	0 (0)
Positive	26 (56.5)	10 (100)
Histologic grade		
G1	10 (21.7)	1 (10)
G2-3	23 (50.0)	9 (90)
Unknown	13 (28.3)	0 (0)
Tumor size		
<2 cm	31 (67)	1 (10)
≥2 cm	15 (33)	9 (90)

CC, cervical cancer; LNM, lymph node metastasis; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space involvement.

formational changes, protein-protein interactions, and intracellular trafficking. They are involved in nearly every phase of cancer development, from tumor initiation to metastatic dissemination and the establishment of pre-metastatic niches [7, 8].

Integrin alpha 5 (ITGA5), a key member of the integrin family, is overexpressed in multiple tumor types and contributes to cancer progression. In non-small cell lung cancer, ITGA5 promotes tumor growth by altering cell adhesion, proliferation, and migration [9]. It is also associated with poor prognosis and tumor immune microenvironment modulation in gastrointestinal cancers [10, 11]. Moreover, ITGA5 has been identified as a gene linked to bone metastasis in breast cancer, promoting tumor progression by activation of the FAK/PI3K/AKT signaling pathway [12, 13]. In CC, ITGA5 enhances angiogenesis and serves as a prognostic risk factor [14].

Insulin-like growth factor II mRNA binding protein 3 (IMP3), a member of the insulin-like growth factor RNA binding protein family, is highly expressed in a variety of malignancies. It regulates numerous genes at the post-transcriptional level [15], and is frequently associated with aggressive tumor phenotypes [16]. IMP3 enhances tumor cell proliferation, invasion, migration, and angiogenesis, and contributes to drug resistance. Its dysregulation affects cancer cell growth, motility, adhesion, and energy metabolism [17, 18].

In this study, we confirmed the carcinogenic role of ITGA5 in CC through both *in vitro* and *in vivo* experiments. Further investigation revealed that ITGA5 promotes CC progression by regulating IMP3-mediated recruitment of hexokinase 2 (HK2) mRNA.

Material and methods

Public database data analysis

Gene expression data from the CC-related dataset GSE9750 were obtained from the GEO database, including 9 CC cell lines, 24 normal cervical tissue samples, and 33 CC tissues. Using R language, we analyzed the expression levels of integrin family proteins, focusing on ITGA5 expression across different groups. In addition, data from 307 CC patients were in The Cancer Genome Atlas (TCGA) were analyzed using Kaplan-Meier (K-M) survival curves to examine the association between gene expression and overall survival (OS).

Human tissue samples and clinical data

Immunohistochemistry was performed on 46 human CC surgical specimens obtained from Suzhou Hospital Affiliated to Nanjing Medical University. All patients had a pathologically confirmed diagnosis of primary CC, including those who had received prior chemotherapy or radiotherapy. These patients underwent surgery in 2021, and the clinicopathological characteristics of these patients were collected from the medical records (**Table 1**), including

age, International Federation of Gynecology and Obstetrics (FIGO) stage in 2008, lymph node metastasis, lymphovascular space invasion, histological grade, and tumor size. All procedures related to clinical samples were approved by the Ethics Committee of Suzhou Hospital Affiliated to Nanjing Medical University (KL901461), and informed consent was obtained from all patients.

Immunohistochemistry (IHC)

CC tissues were fixed in formalin and embedded in paraffin as previously described [19]. Sections approximately 5 μm thick were prepared and incubated overnight at 4°C with a rabbit anti-ITGA5 antibody (1:200 dilution, Abcam, ab150361). Target proteins were visualized using freshly prepared 3, 3-diaminobenzidine (DAB, ASPEN), followed by hematoxylin counterstaining (Solarbio) and dehydration. The sections were subsequently fixed, and images were captured under a microscope. Positive staining appeared brown under microscopic observation. Immunohistochemical results were evaluated based on staining intensity (no =0, weak =1, moderate =2, strong =3) and staining area (<5%=0, <25%=1, 25%-50%=2, 51%-75%=3, >75%=4).

Cell culture and transfection

HeLa (cervical adenocarcinoma) and SiHa (cervical squamous cell carcinoma) cells were purchased from American type culture Collection and cultured in DMEM medium (MeilunBio, China) supplemented with 10% fetal bovine serum (FBS, TransGen Biotech, China) and 1% penicillin-streptomycin (NCM, China). TC-1 cells (mouse lung epithelial cells expressing HPV16 E6 and E7) were purchased from Sebikon Biotechnology and cultured in RPMI 1640 medium (MeilunBio, China) supplemented with 10% FBS and 1% penicillin-streptomycin, at 37°C with 5% CO_2. The plasmids pcDNA3.1-Flag-ITGA5, pcDNA3.1-Flag-IMP3 and pcDNA-3.1-Flag-HK2 overexpressing human ITGA5, IMP3 and HK2 were constructed and cloned into pcDNA3.0 vector (Invitrogen) after PCR amplification.

CC cells were transfected using the X-treme GENE HP DNA infection reagent (Mannheim, Germany) with a plasmid to reagent ratio of 1:3. All siRNA used in this study was obtained from Beijing Tsingke Biotechnology and transfected into cells using Lipofectamine 2000 (Invitrogen, USA), as previously described [20-22].

The siRNA used in this study included: NC siRNA (5'-UUCUCCGAACGUGUCACGU-3'); ITGA5 siRNA 1# (5'-GCAGGGAGUAGUGUUUGUATT-3'); ITGA5 siRNA 2# (5'-CACCCGAAUUCUGGAGUAUTT-3'); IMP3 siRNA 1# (5'-CUAGCGGAUCUCCCACUUU-3'); IMP3 siRNA 2# (5'-GACUAGUGUUCAGGAUCUC-3'); MUS ITGA5 siRNA (5'-GACCUUCUUGCAGCGGGAAUA-3').

Cell proliferation assay

Transfected cells were transferred to 96-well plates at a density of 2500 cells per well. At 0, 24, 48, 72, 96 h after cell adhesion, 20 μL CCK-8 solution (APExBIO, USA) was added to each well. After 4 h of incubation, the absorbance at 450 nm was measured for each well using a microplate reader (Bio-Rad Model 680, Richmond, CA, USA) according to the established protocol [23-25].

Wound healing and transwell assay

In the wound healing assay, cells were plated in six-well plates at a density of 3×10^5 cells per well. After 48 h post-transfection with siRNA or plasmid, the cells were scratched with a 200 μL pipette tip. Images of the wound were captured at 0 h and 48 h using an inverted optical microscope (Olympus, Japan). The proportion of wound healing area was measured using ImageJ software (version 1.54), representing the migration characteristics of cancer cells. For the Transwell assay, 300 μL serum-free medium containing 5×10^4 cells was added to the upper chamber, either uncoated (migration method) or coated (invasion method) with Matrigel, and 700 μL complete medium was added to the lower chamber. After incubation at 37°C for 48 h, the cells that migrated through the 8 μm pore membrane were fixed with methanol and stained with 0.1% crystal violet. Cells in random fields were imaged and counted, as previously described [26, 27].

In vivo tumor growth assay

Fourteen 5-week-old female BALB/c nude mice were maintained under specific pathogen-free conditions. TC-1 cells transfected with si-NC and si-ITGA5 were collected by trypsinization. A total of 1×10^7 cells resuspended in 100 μL PBS

were subcutaneously injected into the axillary side to establish TC-1 xenografts [28]. The fourteen mice were divided into two groups, one group was injected with si-NC transfected cells, and the other group was injected with si-ITGA5 transfected cells. Tumor size (V=0.5× length × width^2) and weight were measured every 3 days and the mice were euthanized by cervical dislocation after 12 days. The tumor was then excised, weighed, measured and photographed. This study was approved by the Animal Ethics and Welfare Committee of Nanjing Medical University.

Immunofluorescence

Excised xenograft tumors were fixed in MDF solution for 24 hours, dehydrated in a graded ethanol series, cleared with xylene, and embedded in paraffin. The tissue sections were cut into 5 μm slices, dewaxed with xylene, and rehydrated in a graded series of ethanol [29-31]. Antigenic repair was performed to expose epitopes, and the sections were blocked with 1% w/v bovine serum albumin (BSA) solution and incubated overnight at 4°C with Abcam anti-KI67 antibodies. The sections were then washed and incubated with fluorescent secondary antibodies (Thermo Scientific). Nuclei were stained with 4', 6-diamino-2-phenylindole (DAPI), and the slides were mounted and imaged using a Zeiss laser fluorescence microscope (Zeiss LSM710).

RNA extraction and reverse-transcription quantitative PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Vazyme) and reverse transcribed into cDNA with the HiScript III RT Super Mix and qPCR kit (R323-01, Vazyme). Real-time PCR was performed using 7500 system (Applied Biosystems, Foster City, CA, USA), SYBR Green Master Mix (Novoprotein Scientific Inc., Shanghai, China) and following gene-specific primers: Human ITGA5 (F, 5'-TTACGGGACTCAACTGCACC-3', R, 5'-AGCCTGAAACACTCAGCCTC-3'); Human IMP3 (F, 5'-actCGTCCaAGatcaagcGGGG-3', R, 5'-AGCCATGCAAAGTGGGAGAT-3'); Human HK2 (F, 5'-ACTCGTCCAAGATCAAGCGG-3', R, 5'-GGATCAGagccACAacGCTT-3'); Human β-Actin (F, 5'-TACATGGCTGGGGTGTTGAA-3', R, 5'AAGAGAGGCATCCTCACCCT-3'); MUS ITGA5 (F, 5'-CCAGCCTGAGCTGTGACTAC-3', R, 5'-AGGAACAGTGAACCGAAGGC-3'); MUS β-Actin (F, 5'-GGAGATCACAGCTCTGGCT-3', R, 5'-GTCGATTGTCGTCCTGAGG-3').

Liquid chromatography/mass spectrometry (LC/MS) analysis

HeLa cells were transfected with Flag-tagged ITGA5 plasmid. After 72 hours, cells were lysed with RIPA buffer, and Flag-ITGA5 was immunoprecipitated using anti-Flag magnetic beads. The immunoprecipitates were analyzed by LC/MS to identify ITGA5-interacting proteins [32, 33].

Western blotting

For protein analysis, cells were harvested 48-72 hours post-transfection, and total protein was extracted using RIPA buffer (Beyotime). Following centrifugation, the supernatant was collected, and protein concentration was quantified using a BCA assay (Beyotime). Proteins were denatured at 100°C for 10 min, and 20 μg of protein per lane was resolved via SDS-PAGE. After electrophoresis, proteins were transferred onto PVDF membranes, which were then blocked in 5% skim milk at room temperature for 1 hour. Membranes were incubated overnight at 4°C with primary antibodies, followed by incubation with HRP-conjugated secondary antibodies at room temperature for 1 hour. After three washes with TBST between each incubation, protein bands were visualized using the ECL Prime detection system [29]. Antibodies used included anti-ITGA5 (1:5000; cat.no.AB150361; Abcam), anti-IMP3 (1:1500; cat.no.12750-1-AP; ProteinTech), anti-β-actin (1:3000; cat.no.66009-1-Ig; ProteinTech) and anti-Flag (1:1000; F9291; Sigma).

Co-immunoprecipitation (CO-IP)

Cells harvested 48-72 hours post-transfection were lysed on ice for 30 minutes using RIPA buffer (Beyotime). Following centrifugation, the supernatants were collected, and 20 μL of Dynabeads Protein A (Vazyme) was added to each sample. The mixtures were incubated with gentle rotation for 3 hours to deplete non-specific proteins. Subsequently, lysates were incubated overnight (12-16 h) at 4°C with either an anti-ITGA5 antibody (Abcam) or anti-Flag antibody-conjugated magnetic beads (AlpalifeBio).

The next day, samples incubated with the anti-ITGA5 antibody were further treated with Dynabeads Protein A for 3 hours. Immunoprecipitates were washed three times with RIPA buffer and eluted using SDS loading buffer. Eluted proteins were denatured at 100°C for 10 minutes and separated by SDS-PAGE.

RNA immunoprecipitation (RIP)

For RNA immunoprecipitation, cell lysates prepared with RIP buffer were incubated with anti-IMP3 and anti-igG antibodies at 4°C. Protein-RNA complexes were purified using 0.5 mg/mL protease K and 0.1% SDS [34]. The interaction between IMP3 and HK2 mRNA was analyzed by RT-qPCR.

Statistical analysis

The experiments were independently repeated three times, and the results were expressed as mean ± standard deviation (SD). Comparisons between two groups were analyzed using the Student's t-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). Statistical analysis of the data was conducted using GraphPad Prism software, with a significance level set at $P<0.05$.

Results

ITGA5 is highly expressed in CC, which is associated with progression and poor prognosis in CC patients

ITGA5 was identified through integrative bioinformatic analysis. The CC-related gene expression dataset GSE9750 was obtained from the GEO database, and expression levels of integrin family members were compared across CC cell lines, patient samples, and normal cervical tissues (**Figure 1A**). Among these, ITGA5 exhibited significantly elevated expression in CC tissues compared to normal controls. High ITGA5 expression was also observed in CC cell lines and patient samples (**Figure 1B**). To evaluate its prognostic relevance, Kaplan-Meier survival analysis using TCGA data revealed that elevated ITGA5 high expression was significantly associated with poor prognosis in CC patients (P=0.0002), establishing it as a risk factor (**Figure 1C**). Based on these findings, ITGA5 was selected for further investigation.

To explore ITGA5 protein expression in clinical samples, we performed immunohistochemistry (IHC) on 46 cervical cancer tissues with associated clinical data (**Figure 1D**). No significant difference in ITGA5 expression was observed between squamous cell carcinoma and adenocarcinoma. However, higher ITGA5 levels were significantly associated with moderately to poorly differentiated tumors, advanced FIGO stages, positive lymph node metastasis, lymphovascular space invasion, and tumor size ≥2 cm (**Figure 1E**), indicating a strong correlation between ITGA5 expression and CC progression.

ITGA5 promotes the proliferation, invasion, and migration of CC cells

To evaluate the role of ITGA5 in the proliferation, invasion, and migration of CC cells in vitro, Hela and SiHa cells were transfected with NC or ITGA5 siRNA, respectively. Knockdown efficiency was confirmed by RT-qPCR (**Figure 2A**, **2B**). CCK-8 assay results showed that ITGA5 silencing significantly reduced CC cell proliferation (**Figure 2C**, **2D**). Wound healing assays demonstrated a marked decrease in migratory capacity following ITGA5 knockdown compared to NC-transfected cells (**Figure 2E**, **2F**). Transwell assays indicated that downregulation of ITGA5 impaired both invasion and migration abilities of CC cells (**Figure 2G**, **2H**). Subsequently, Hela and SiHa cells were transfected with empty vectors or ITGA5 overexpressing plasmids. CCK-8 assays revealed that ITGA5 overexpression significantly enhanced cell proliferation (**Figure 3A**, **3B**). Wound healing assays confirmed increased migratory capacity (**Figure 3C**, **3D**), while Transwell assays demonstrated elevated invasive and migratory abilities in ITGA5-overexpressing cells (**Figure 3E**, **3F**). In conclusion, these findings suggest that ITGA5 promotes CC cell proliferation, invasion, and migration *in vitro*.

The TC-1 cell line, characterized by HPV E6/E7 expression, exhibits robust proliferation and a high tumorigenic potential. Therefore, TC-1 cells were selected for subcutaneous tumor formation experiments. To investigate the effect of ITGA5 on CC cell proliferation *in vivo*, TC-1 cells transfected with NC or ITGA5 siRNA, were evaluated for transfection efficiency by RT-qPCR (**Figure 3G**) and then injected subcutaneously into the armpits of nude mice. Tumor

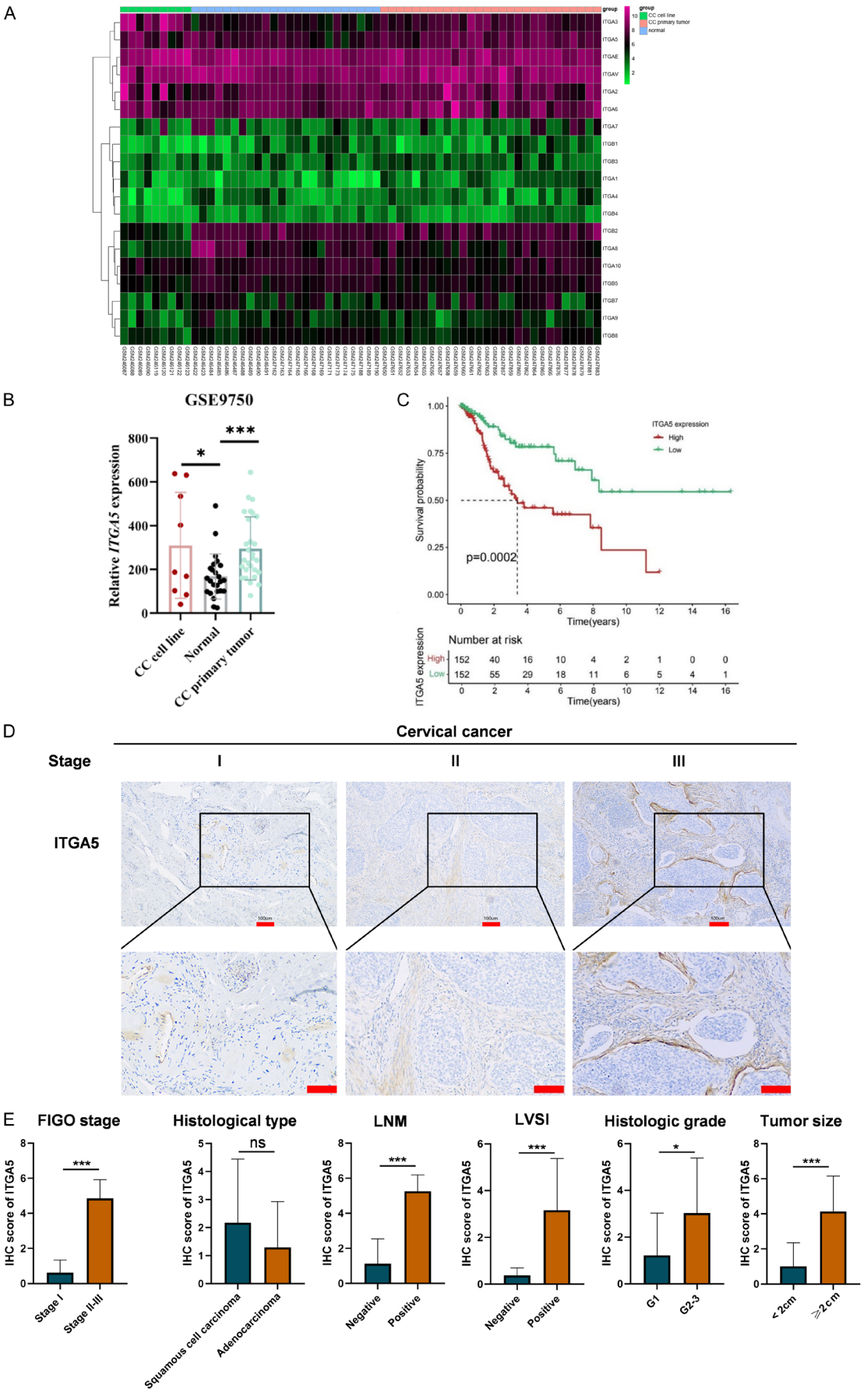
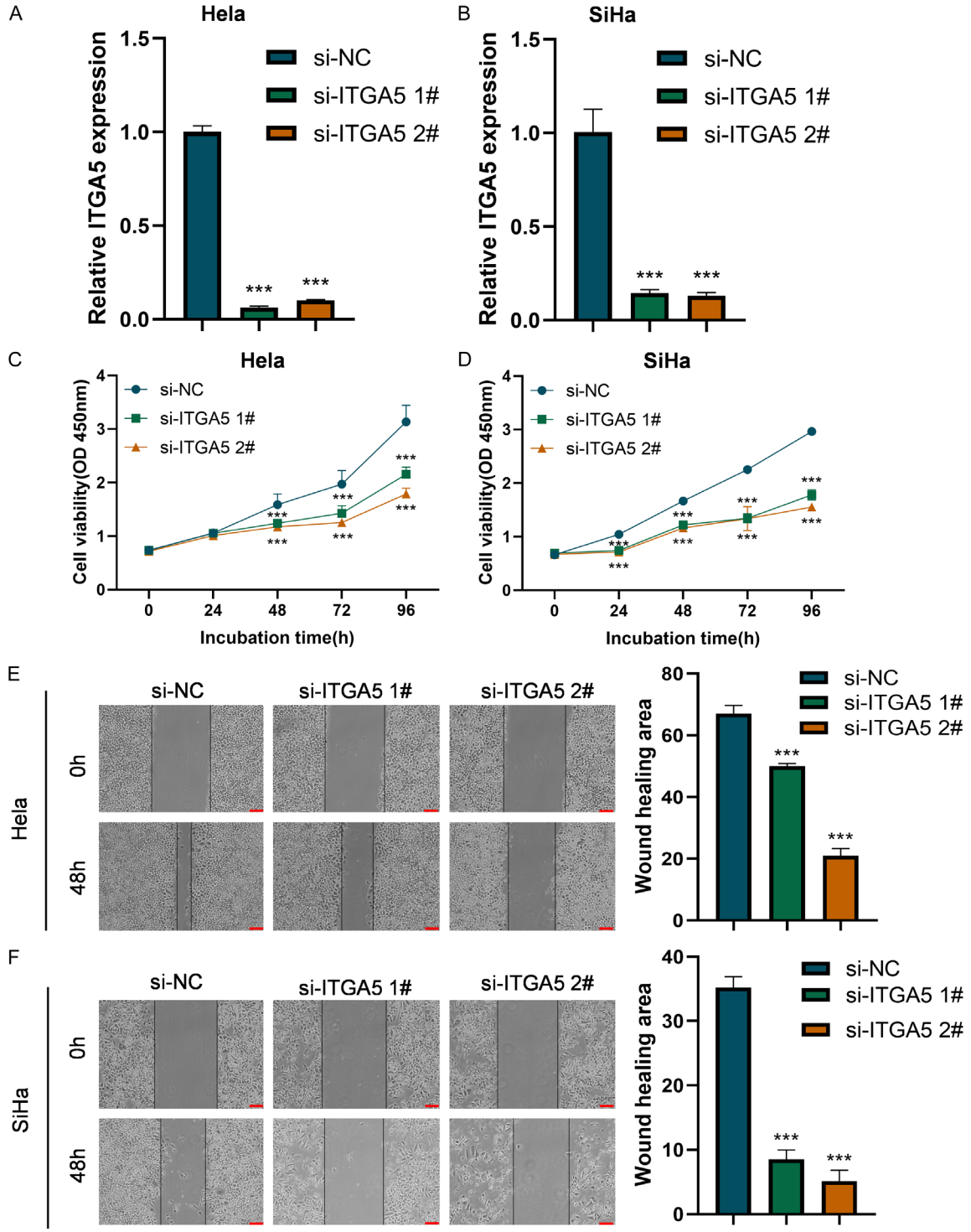

A
group
CC cell line
CC primary tumor
normal
ITGA3
ITGA5
ITGAE
ITGAV
ITGA2
ITGA6
ITGA7
ITGB1
ITGB3
ITGA1
ITGA4
ITGB4
ITGB2
ITGA8
ITGA10
ITGB5
ITGB7
ITGA9
ITGB6
B
GSE9750
Relative ITGA5 expression
CC cell line
Normal
CC primary tumor
C
ITGA5 expression
High
Low
Survival probability
p=0.0002
Time(years)
Number at risk
D
Cervical cancer
Stage
I
II
III
ITGA5
E
FIGO stage
Histological type
LNM
LVSI
Histologic grade
Tumor size
IHC score of ITGA5
Stage I
Stage II-III
Squamous cell carcinoma
Adenocarcinoma
Negative
Positive
G1
G2-3
<2cm
≥2cm

Figure 1. ITGA5 is highly expressed in CC, which is associated with the progression and poor prognosis of CC patients. A. Expression of integrins in cervical cancer cell lines, cervical cancer patients, and normal controls in GSE9750. B. Expression of ITGA5 in different groups in GSE9750. C. Kaplan-Meier analysis of the relationship between ITGA5 expression and survival in patients with CC. D. Representative images of ITGA5 immunohistochemical staining in CC tissues. Scale =100 μm. Enlarged scale =50 μm. E. Distribution of ITGA5 IHC score in tumors grouped by FIGO stage, histologic type, lymph node metastasis (LNM), lymphovascular space invasion (LVSI), histologic grade (G1, well differentiated tumor; G2-3, moderate and poorly differentiated tumor). *P<0.05, **P<0.01, ***P<0.001; ns, not significant.



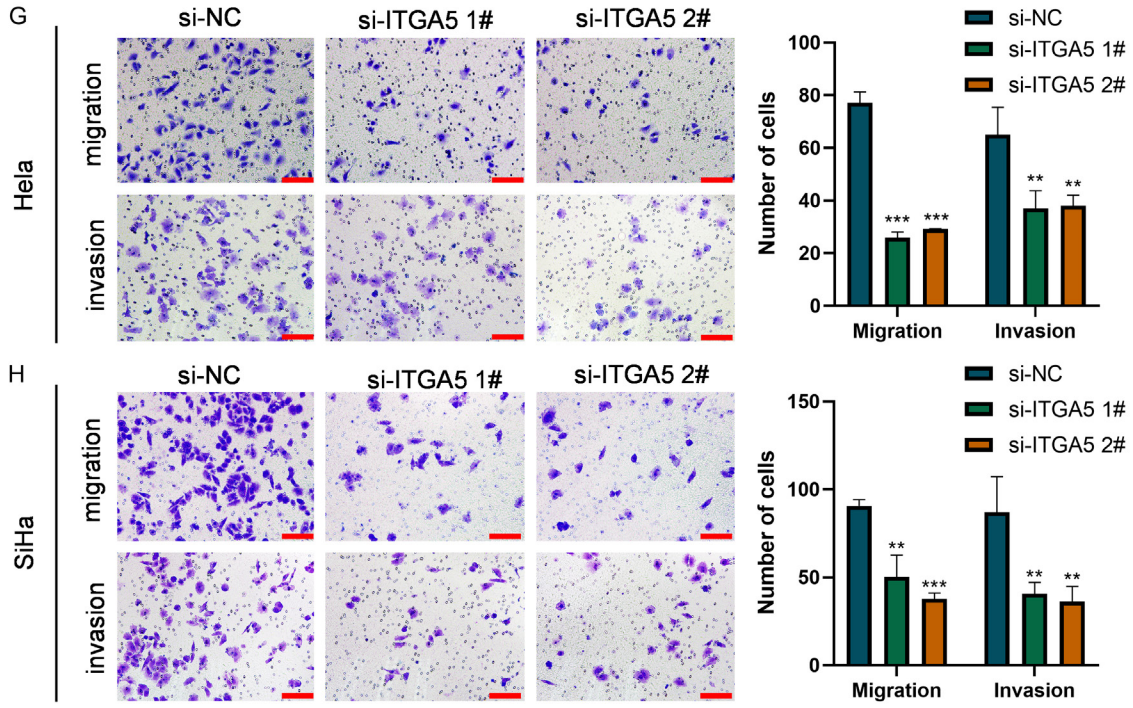


Figure 2. ITGA5 promotes the proliferation, invasion, and migration of CC cells. A, B. ITGA5 mRNA levels after siRNA transfection. C, D. CCK-8 assays showing the proliferation of CC cells after ITGA5 knockdown. E, F. Wound healing assay, showing that the migration of CC cells was reduced after ITGA5 knockdown. G, H. Transwell assays showing that the invasion and migration of CC cells were reduced after ITGA5 knockdown. Scale bar =100 μm. Each experiment was independently repeated three times. *P<0.05, **P<0.01, ***P<0.001 compared with NC.

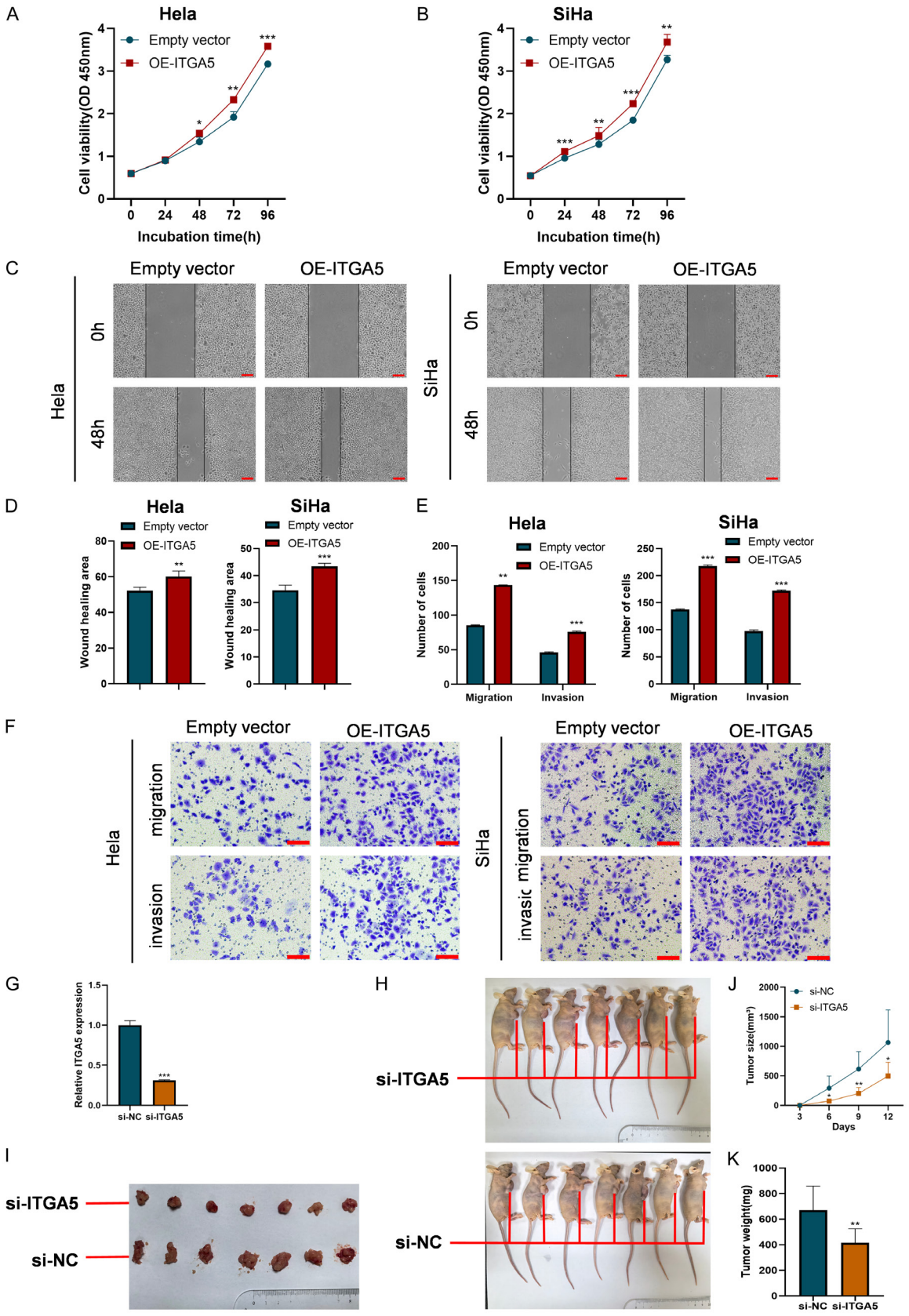
volume and weight were monitored every three days. After 12 days, tumors in the si-ITGA5 group were significantly smaller and lighter than those in the control group (**Figure 3H-K**). Immunofluorescence staining further revealed a marked reduction in Ki-67 positive cells in the si-ITGA5 group (**Figure 3L**, **3M**), supporting the conclusion that ITGA5 promotes CC cell proliferation *in vivo*.

ITGA5 interacts with IMP3

To investigate the molecular mechanism underlying ITGA5-mediated CC progression, CO-IP was performed to extract ITGA5-enriched products, followed by mass spectrometry analysis. Among the molecules interacting with ITGA5, IMP3 showed high abundance (**Figure 4A**). Given that IMP3 is elevated in various cancers and has been previously studied in the context of CC [35], we selected IMP3 for further validation. The interaction between ITGA5 and IMP3 was confirmed by immunoprecipitation assay in Hela and SiHa cells overexpressing IMP3 (**Figure 4B**).

IMP3 promotes the proliferation, invasion, and migration of CC cells

To assess the functional effect of IMP3, its expression was silenced in Hela and SiHa cells (**Figure 5A**, **5B**). CCK-8 assays revealed that IMP3 knockdown significantly impaired CC cell proliferation (**Figure 5C**, **5D**). Wound healing assays showed reduced migratory capacity, and Transwell assays demonstrated that IMP3 inhibition attenuated both invasion and migration (**Figure 5E-H**). Next, we transfected CC cells with IMP3-overexpressing plasmids. The CCK-8 assay revealed that overexpression of IMP3 enhanced the proliferation ability of CC cells (**Figure 6A**, **6B**). The wound healing assay showed that overexpression of IMP3 increased the migration ability of CC cells (**Figure 6C**, **6D**). The Transwell assay results indicated that overexpression of IMP3 enhanced the invasion and migration ability of CC cells (**Figure 6E**, **6F**). In conclusion, these results indicate that IMP3 promotes CC cell proliferation, invasion, and migration *in vitro*.


A
Hela
Empty vector
OE-ITGA5
Cell viability(OD 450nm)
Incubation time(h)
B
SiHa
C
Empty vector
OE-ITGA5
Hela
SiHa
0h
48h
D
Hela
SiHa
Wound healing area
E
Number of cells
Migration
Invasion
F
migration
invasion
G
Relative ITGA5 expression
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Tumor size(mm³)
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I
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Tumor weight(mg)

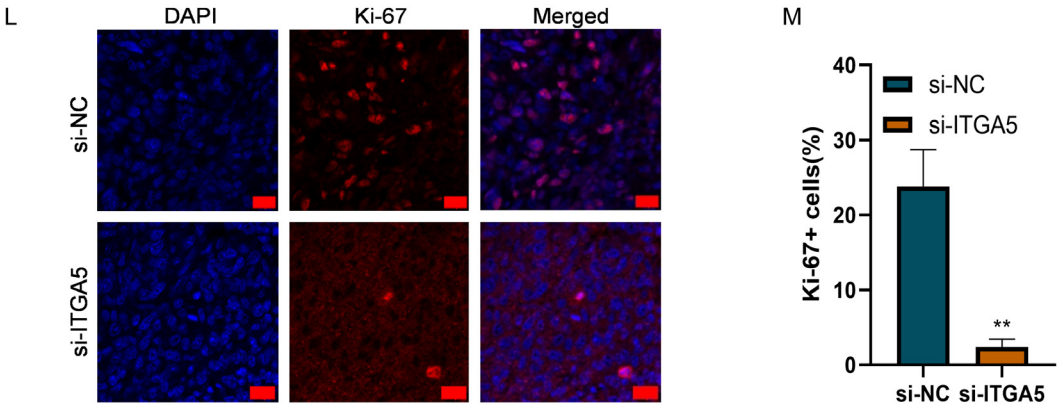


Figure 3. ITGA5 levels are strongly correlated with the progression of CC *in vitro* and *in vivo*. A, B. CCK-8 assays showing the proliferation of CC cells after ITGA5 overexpression. C, D. Wound healing assay, showing that ITGA5 enhances the migration of CC cells. E, F. Transwell assays showed that overexpression of ITGA5 enhanced the invasion and migration of CC cells. Scale bar =100 μm. Each assay was independently repeated three times. *P<0.05, **P<0.01, ***P<0.001 compared with empty vector. G. ITGA5 mRNA levels after mouse siRNA transfection. H. ITGA5-deficient or control TC-1 cells were subcutaneously injected into nude mice. I. Photographs of the collected tumors. J. Measurements of tumor volumes every 3days. K. Measurements of tumor weight. L, M. Immunofluorescence staining of Ki67. Scale bar =20 μm. *P<0.05, **P<0.01, ***P<0.001 compared with NC.

A

Gene names	LFQ intensity ITGA5	LFQ intensity EV
ITGB6	9942000000	35819000
ITGA5	64305000000	166990000
ITGB1	39866000000	60309000
ARFGAP1	145830000	0
RPL36	101800000	0
MYH10	53823000	0
...	...	...
IMP3	7010700	0
BCKDK	6918600	0
...	...	...

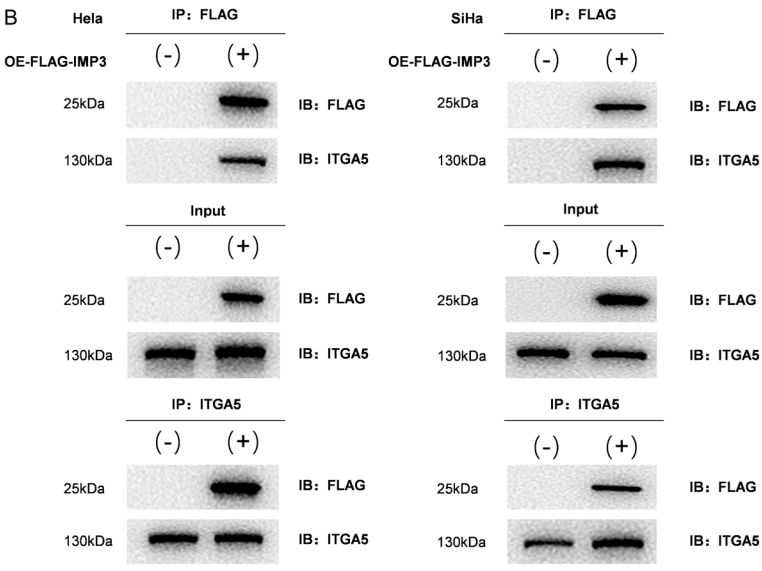
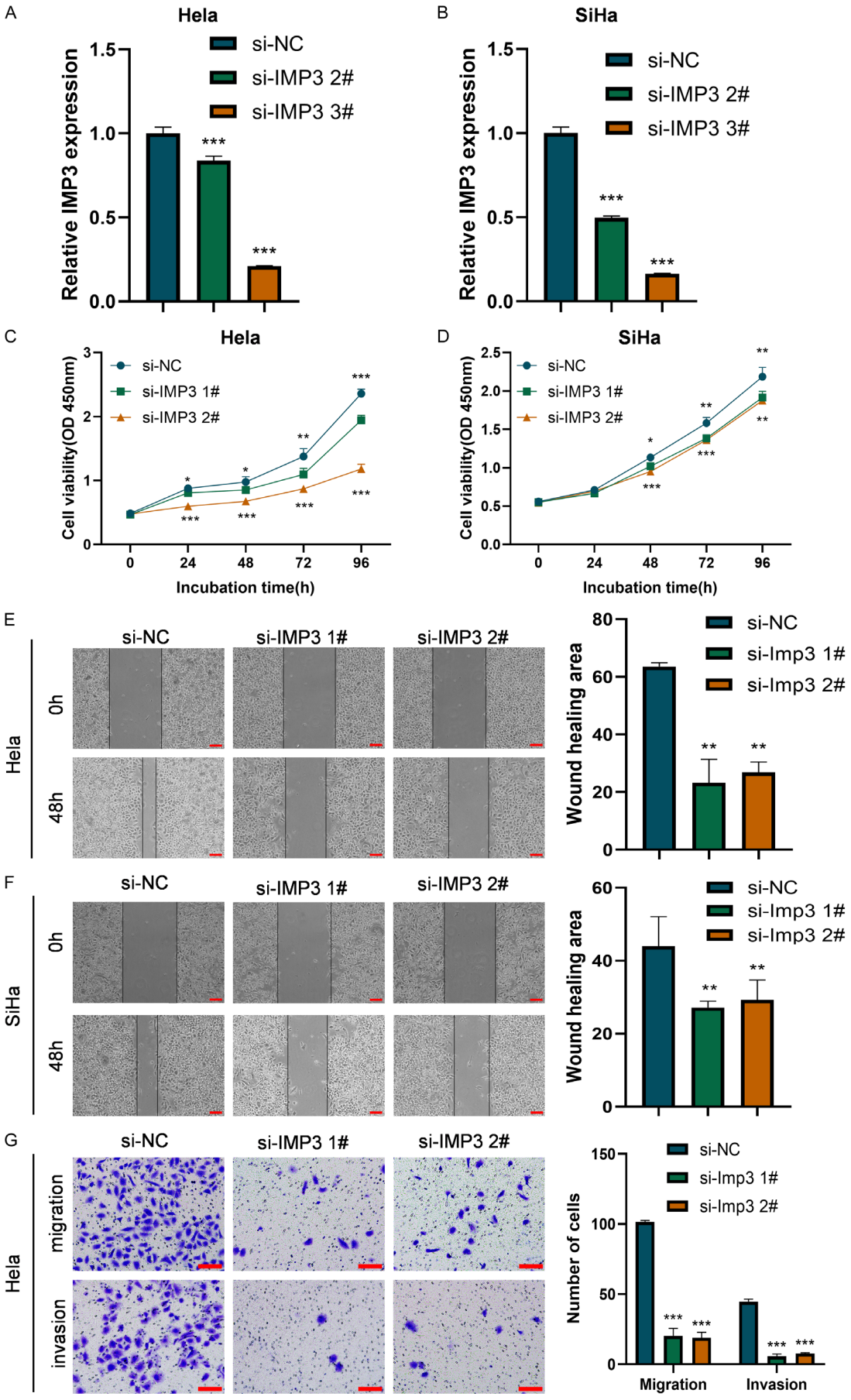


Figure 4. ITGA5 interacts with IMP3. A. Protein expression levels were estimated by LFQ intensity ITGA5/LFQ intensity EV >10. LC-MC/MS identified 100+ proteins in HeLa cells. B. Reciprocal immunoprecipitation (IP) assays of Hela and SiHa cell lysates with anti-Flag beads and anti-ITGA5 antibody.

ITGA5 regulates HK2 mRNA by recruiting IMP3

To explore how the interaction between ITGA5 and IMP3 influences CC progression, we reviewed the literature and found that IMP3 has been reported to stabilize HK2 mRNA [36]. Based on this, *HK2* was selected as a downstream target of ITGA5 and IMP3. We first observed that IMP3 knockdown reduced HK2 mRNA expression in Hela and SiHa cells, whereas IMP3 overexpression increased HK2 mRNA levels (**Figure 7A**, **7B**). Similarly, ITGA5 knockdown significantly downregulated HK2 mRNA expression in both cell lines, while ITGA5 overexpression upregulated its expression (**Figure 7C**, **7D**). To investigate the mechanism further, RIP assays were performed. RT-qPCR analysis of RIP products revealed that IMP3-mediated recruitment of HK2 mRNA was diminished in ITGA5-knockdown Hela and SiHa cells. Conversely, ITGA5 overexpression enhanced IMP3-mediat-


A
Hela
si-NC
si-IMP3 2#
si-IMP3 3#
Relative IMP3 expression
1.5
1.0
0.5
0.0
***
***
B
SiHa
si-NC
si-IMP3 2#
si-IMP3 3#
Relative IMP3 expression
1.5
1.0
0.5
0.0
***
***
C
Hela
si-NC
si-IMP3 1#
si-IMP3 2#
Cell viability(OD 450nm)
Incubation time(h)
0
24
48
72
96
D
SiHa
si-NC
si-IMP3 1#
si-IMP3 2#
Cell viability(OD 450nm)
Incubation time(h)
0
24
48
72
96
E
si-NC
si-IMP3 1#
si-IMP3 2#
Hela
0h
48h
si-NC
si-Imp3 1#
si-Imp3 2#
Wound healing area
80
60
40
20
0
F
si-NC
si-IMP3 1#
si-IMP3 2#
SiHa
0h
48h
si-NC
si-Imp3 1#
si-Imp3 2#
Wound healing area
60
40
20
0
G
si-NC
si-IMP3 1#
si-IMP3 2#
Hela
migration
invasion
si-NC
si-Imp3 1#
si-Imp3 2#
Number of cells
150
100
50
0
Migration
Invasion

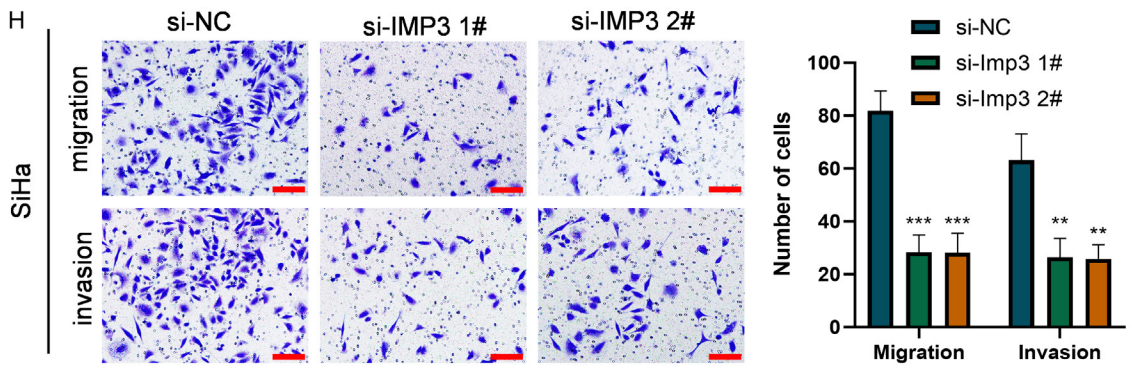


Figure 5. IMP3 promotes the proliferation, invasion, and migration of CC cells. A, B. IMP3 mRNA levels after siRNA transfection. C, D. CCK-8 assays showing the proliferation of CC cells after IMP3 knockdown. E, F. Wound healing assay, showing that the migration of CC cells was reduced after IMP3 knockdown. G, H. Transwell assays showing that the invasion and migration of CC cells were reduced after IMP3 knockdown. Scale bar =100 μm. Each assay was independently repeated three times. *P<0.05, **P<0.01, ***P<0.001 compared with NC.

ed recruitment of HK2 mRNA (**Figure 7E-H**). These findings suggest that ITGA5 promotes HK2 mRNA recruitment by regulating IMP3.

Overexpression of HK2 can reverse the effects of ITGA5 knockdown on CC phenotypes

To further validate whether ITGA5 contributes to CC progression through HK2, we co-transfected HeLa and SiHa cells with ITGA5-targeting siRNA and an HK2 overexpression plasmid. Cell proliferation, invasion, and migration were assessed using CCK-8, Transwell, and wound healing assays (**Figure 8A-F**). Knockdown of ITGA5 alone suppressed these cellular functions, while concurrent HK2 overexpression partially rescued the inhibitory effects. These results indicate that HK2 can partially reverse the effects of ITGA5 knockdown on CC phenotypes.

Discussion

Integrins are heterodimeric transmembrane receptors composed of α and β subunits. As a large family of adhesion molecules, they are involved in key cellular processes such as proliferation, cytoskeletal organization, adhesion, migration, and differentiation. Crucially, integrins mediate both cell-extracellular matrix and intercellular signaling [37]. Due to their ability to enhance malignant phenotypes, integrins have been widely studied as therapeutic targets in cancer [38], and several integrin antagonists are currently in development [39]. Among them, ITGA5 has emerged as a tumor promoting factor in multiple cancer types. It has been recognized as an oncogenic marker, and is implicated in the tumor immune microenvironment of glioma [40]. In gastrointestinal cancers, ITGA5 is a prognostic marker associated with immune infiltration [41], and its expression level correlates with drug resistance and treatment response [42].

In our study, bioinformatic analyses revealed that ITGA5 is significantly overexpressed in CC tissues, a finding corroborated by IHC. Functional assays *in vitro* and *in vivo* further demonstrated that ITGA5 promotes CC cell progression.

Recent studies have shown the role of ITGA5 in various cancers, such as, promoting the malignant phenotype of liver cancer and being a key gene in its proliferation and metastasis [43]. However, the downstream effectors of ITGA5 signaling remain largely undefined. Elucidating the mechanisms by which ITGA5 contributes to tumorigenesis could provide new insight for improving therapeutic strategies in CC. Previous studies have shown that ITGA5 primarily functions as a receptor for fibronectin, forming a heterodimer with integrin β1 [44]. We hypothesized that ITGA5 may also engage with other protein partners through additional pathways. To explore this, we performed mass spectrometry on HeLa cells enriched for ITGA5 and identified IMP3 as a potential binding partner with high binding kurtosis. CO-IP assays confirmed a physical interaction between IMP3 and ITGA5, and functional assays revealed that IMP3 promotes CC progression.

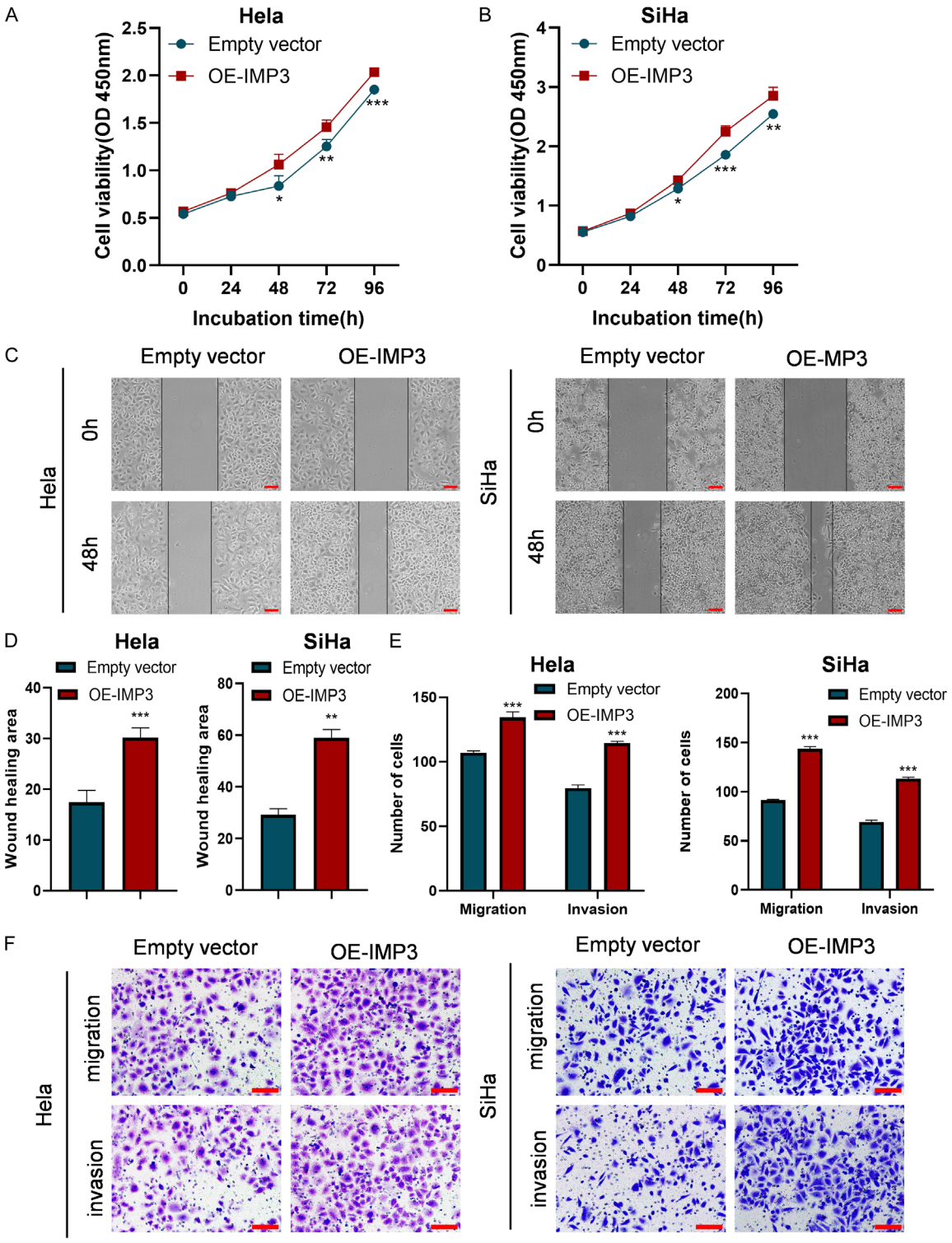


Figure 6. IMP3 promotes the proliferation, invasion, and migration of CC cells. A, B. CCK-8 assays showing the proliferation of CC cells after IMP3 overexpression. C, D. Wound healing assay, showing that IMP3 enhances the migration of CC cells. E, F. Transwell assays showing that overexpression of IMP3 enhanced the invasion and migration of CC cells. Scale bar =100 μm. Each assay was independently repeated three times. *P<0.05, **P<0.01, ***P<0.001 compared with empty vector.

IMP3 is an oncofetal RNA-binding protein known to drive tumor cell proliferation, adhesion, and invasion [45]. Elevated IMP3 expression has been consistently associated with

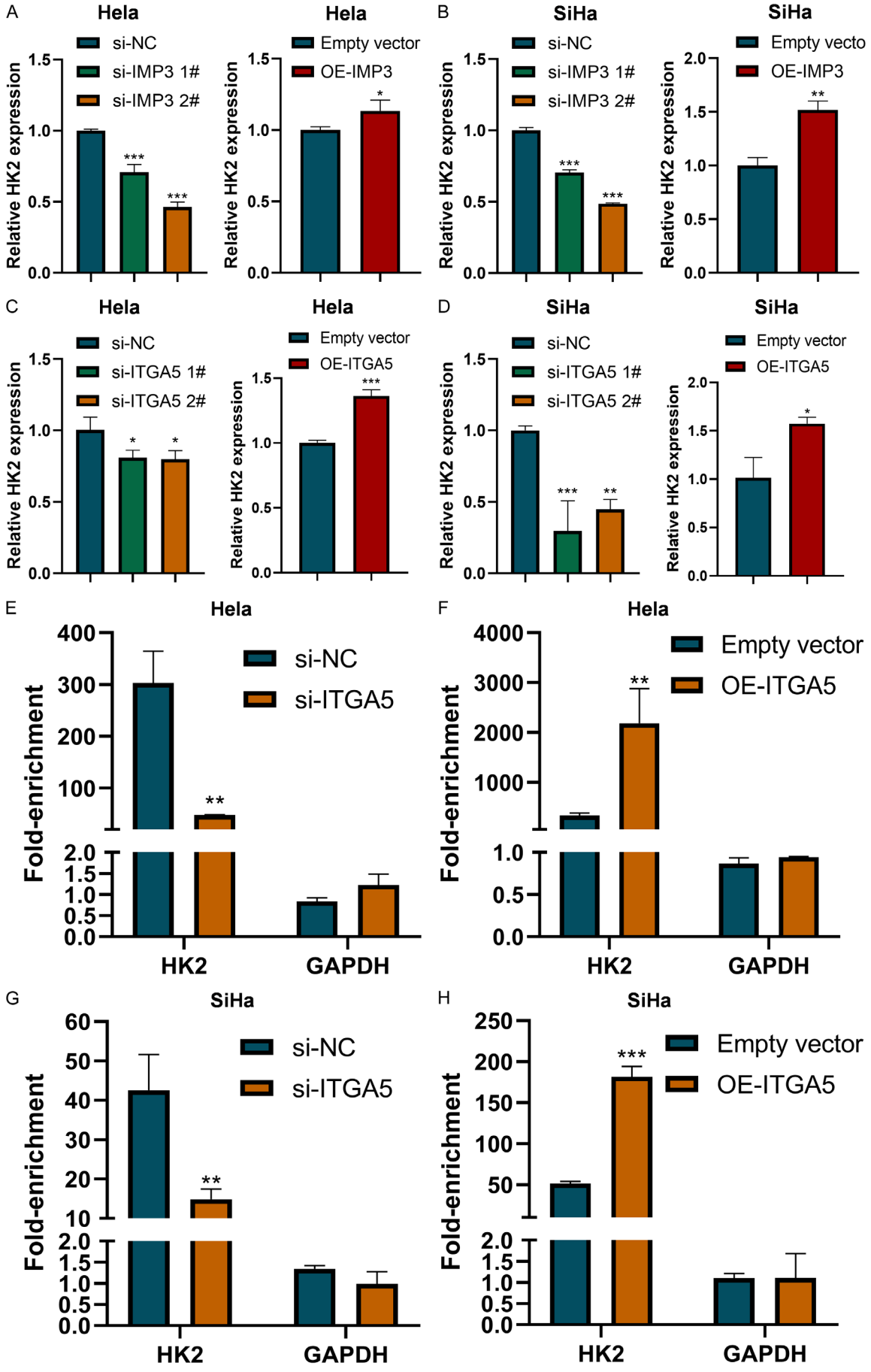

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Figure 7. ITGA5 regulates HK2 mRNA by recruiting IMP3. A, B. Expression levels of HK2 mRNA in Hela and SiHa cells after IMP3 knockdown or overexpression. C, D. Expression levels of HK2 mRNA in Hela and SiHa cells after ITGA5 knockdown or overexpression. E-H. RT-qPCR analysis after RIP revealed HK2 mRNA recruited by the IMP3 protein in the lysates of Hela and SiHa cells after ITGA5 knockdown or overexpression. *P<0.05, **P<0.01, ***P<0.001 compared with NC or empty vector.

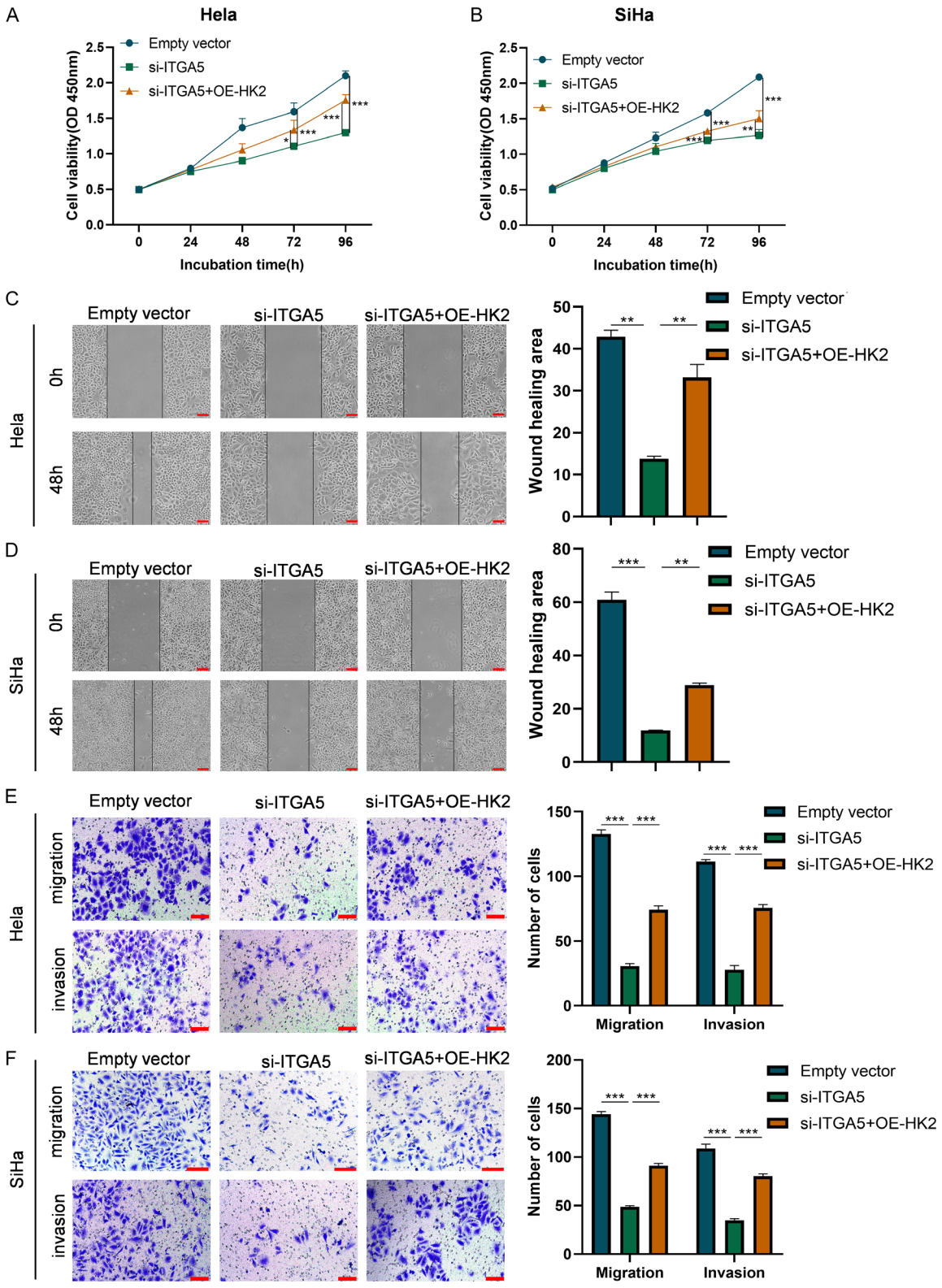

Figure 8. Overexpression of HK2 can reverse the effects of ITGA5 silencing on cervical cancer phenotypes. A, B. CCK-8 assays of cell viability. C, D. Wound healing assays of cell migration. E, F. Transwell assays of cell migration. Each assay was independently repeated three times. Scale bar =100 μm. *P<0.05, **P<0.01, ***P<0.001 compared with si-ITGA5.

poor disease prognosis across various tumor types [46], including rectal cancer, where it functions as an independent prognostic marker [47]. In CC, high IMP3 expression is linked to reduced survival [48]. Previous studies have shown that IMP3 stabilizes HK2 mRNA, promotes aerobic glycolysis, and enhances malignant phenotypes in CC cells [35]. Based on this, we selected HK2 as the downstream effector in our mechanistic model.

HK2, a key rate-limiting enzyme in the glycolytic pathway, is overexpressed in many cancers [49] and contributes to tumor aggressiveness by regulating apoptosis resistance, migration, metastasis and metabolic reprogramming [50, 51]. It has also been implicated in chemotherapy resistance [52]. In our study, RIP assays showed that ITGA5 knockdown diminished IMP3-HK2 mRNA binding, whereas ITGA5 overexpression enhanced this interaction. Rescue experiments further demonstrated that HK2 partially reversed the effects of ITGA5 knockdown on CC phenotypes. In conclusion, our findings reveal that ITGA5 promoted CC progression by facilitating IMP3-mediated recruitment of HK2 mRNA, thereby providing new mechanistic insight into the ITGA5-IMP3-HK2 axis as a possible therapeutic target.

Targeted therapy against ITGA5 continues to be an area of active investigation. MINT1526A, a monoclonal antibody that blocks ITGA5 and exhibits anti-angiogenic properties, has shown promising results. When combined with vascular endothelial growth factor inhibition, MINT-1526A was well-tolerated in a Phase I clinical trial and demonstrated preliminary efficacy [53]. Additionally, integrin α5β1 inhibitors have shown potential in treating breast and prostate cancers [54]. Our findings may contribute to identifying new therapeutic targets for cancer treatment.

Several limitations of this study should be acknowledged. First, the sample size, particularly for subgroups such as adenocarcinoma, was relatively small, limiting the generalizability of the results. Second, while animal experiments confirmed that ITGA5 promotes CC cell proliferation, they did not assess tumor invasion or metastasis, and rescue experiments were not conducted *in vivo*. Lastly, although we demonstrated a relationship between ITGA5, IMP3 and HK2, the underlying molecular mechanisms were not fully elucidated. Future studies are warranted to address these gaps and provide a more comprehensive understanding of ITGA5's role in cervical cancer.

In conclusion, this study demonstrated that ITGA5 promotes CC progression through both *in vivo* and *in vitro* experiments. Mechanistically, ITGA5 facilitates CC development by regulating IMP3-mediated recruitment of HK2 mRNA. These findings may offer new therapeutic targets for CC treatment.

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Disclosure of conflict of interest

None.

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