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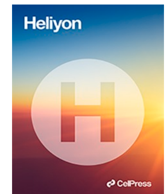

Research article

SIAH1 facilitates the migration and invasion of gastric cancer cells through promoting the ubiquitination and degradation of RECK

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ABSTRACT

Siah E3 ubiquitin protein ligase 1 (SIAH1) has been reported to participate in the development of several human cancers, including gastric cancer. However, the effect and mechanism of SIAH1 on the migration and invasion of gastric cancer cells need be further explored. Here, we first analyzed the clinical value of SIAH1 in gastric cancer, and found that SIAH1 was up-regulated in gastric cancer and associated with a poor prognosis. In addition, silencing of SIAH1 significantly inhibited the migration and invasion of gastric cancer cells through inhibiting the expression of matrix metalloproteinase-9 (MMP9), while overexpression of SIAH1 had the opposite effect. Molecularly, we provided the evidence that reversion-inducing cysteine-rich protein with Kazal motifs (RECK) was a potential substrate of SIAH1. We determined that SIAH1 could destabilize RECK through promoting its ubiquitination and degradation via proteasome pathway. We also found RECK was involved in SIAH1-regulated gastric cancer cell migration and invasion. In conclusion, SIAH1 is up-regulated in gastric cancer, which promotes the migration and invasion of gastric cancer cells through regulating RECK-MMP9 pathway.

1. Introduction

Gastric cancer is one of the common malignant tumors of the digestive system with high prevalence and mortality. It has been reported that more than one million people were diagnosed with gastric cancer every year, and one third of them belonged to China [1]. Despite the rapid development of medical technology in recent years, the 5-year survival rate of gastric cancer patients are still less than 20 % [2]. Therefore, it is an urgent task to further explore the molecular mechanism of gastric cancer and find new therapeutic targets.

Abbreviations: SIAH1, siah E3 ubiquitin protein ligase 1; RECK, reversion-inducing cysteine-rich protein with Kazal motifs; MMPs, matrix metalloproteinases; ASAP3, ArfGAP with SH3 domain, ankyrin repeat and PH domain 3; MET, MET proto-oncogene, receptor tyrosine kinase; EPHB6, EPH receptor B6; CHL, chloroquine; CHX, cycloheximide.

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Siah E3 ubiquitin protein ligase 1 (SIAH1) is a E3 ubiquitin ligase with RING-finger type which is involved in several cellular activities, such as proliferation, apoptosis, DNA damage repair and hypoxia stress [3–6]. It has been reported that SIAH1 is associated with the progression of multiple human cancers, including hepatocellular carcinoma [7], lung cancer [8], breast cancer [9] and colorectal cancer [3]. It was showed that SIAH1 is abnormally expressed in these cancers and is therefore considered a potential marker for them. However, the role and mechanism of SIAH1 acts as a ubiquitin ligase in the migration and invasion of gastric cancer cells remain unclear.

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a glycoprotein localized in cell membrane, which can negatively regulate matrix metalloproteinases (MMPs), thereby inhibiting the role of MMPs in the degradation of extracellular matrix and tumor angiogenesis [10]. It has been reported that RECK is widely expressed in normal human tissues, but is low or no expressed in tumor tissues [11]. Therefore, it is considered to be a tumor suppressor gene. Recent study determined the effect of RECK on the metastasis of gastric cancer. RECK can inhibit stemness gene expression and tumorigenicity of gastric cancer cells by suppressing ADAM-mediated Notch1 activation [12]; RECK is a suppressor of malignancy, and constitutes a good prognostic indicator in gastric cancer [13].

In this study, we determined the effect and mechanism of SIAH1 on the migration and invasion of gastric cancer cells. We provided evidence that SIAH1 could promote the expression of MMP9 through degrading RECK, thus accelerating the migration and invasion of gastric cancer cells. We also confirmed that SIAH1 acted as a ubiquitin ligase for RECK polyubiquitination.

2. Materials and methods

2.1. Tissue specimen

Human non-tumor stomach and gastric cancer tissue samples were obtained from the Nanjing Gaochun People's Hospital (Nanjing, China), immediately transported in liquid nitrogen after excision and eventually stored at −80 °C. All samples were confirmed through the pathological diagnosis. This study was reviewed and approved by the Ethics Committee of the Nanjing Gaochun People's Hospital, and the patients and control subjects provided informed consent (approval no. 2023-116-01).

2.2. Antibodies and plasmids

Antibodies against SIAH1 (cat. A2494), RECK (cat. A6718), MMP9 (cat. A0289), FLAG (cat. AE005), MYC (cat. AE010), MET (cat. A17366), and EPHB6 (cat. A14728) were bought from ABclonal (Wuhan, China). Antibody against ASAP3 (cat. bs-5832R) was purchased from Bioss (Beijing, China). Antibodies against β-actin (cat. 66009-1-Ig) was brought from Proteintech (Wuhan, China). The plasmids of Scramble, shSIAH1, FALG-SIAH1 (WT and C44S), FLAG-VECTOR, MYC-RECK, MYC-VECTOR and HA-Ubiquitin were purchased from Genechem (Shanghai, China).

2.3. Cell culture

The 293T cell and gastric cancer cells SGC-7901 and HGC-27 were bought from FuHeng Biology (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10 % of FBS (TransGen, Beijing, China) in a 5 % CO_2 incubator at 37 °C.

2.4. Transfection and establishment of the stable cell lines

293T cells were co-transfected with packaging plasmids and core plasmids at 1:1:2 using the HighGene transfection reagent (Abclonal) according to the protocol. Seventy-two hours later, the supernatant was collected and then used to infect SGC-7901 and HGC-27 cells. Forty-eight hours after infection, the cells were continuously cultured in the medium containing 2.5 μg/mL puromycin (Sigma, St. Louis, MO, USA). The surviving cells were cultured into cell lines stably expressing Scramble and shSIAH1.

The shRNA double strands were designed to silence SIAH1 as follow:

shSIAH1-F:

GGATCCGGAAAGGCTACTCCACCTTCTTTCAAGAGAAGAAGGTGGAGTAGCCTTTCCTTTTTTG;

shSIAH1-R: AATTCAAAAAAGGAAAGGCTACTCCACCTTCTTCTCTTGAAAGAAGGTGGAGTAGCCTTTCCG.

2.5. Transwell

Transwell invasion assay was carried out using transwell inserts with an 8-μm aperture size (Corning, NY, USA). Briefly, 1×10^4 cells/200 μL suspended in serum-free medium to the upper compartment pre-covered with 1 × Matrigel (BD, Shanghai, China) and medium containing 10 % fetal bovine serum was added to the lower compartment. After incubated at 37 °C for 24 h, cells that had not traversed the upper compartment were removed using cotton-tipped swabs, and cells that invaded to the lower side of the insert were fixed with 4 % paraformaldehyde for 30 min, dyed with 0.3 % Crystal Violet for 30 min, and counted using a microscope. Cell migration was assessed using a similar method without Matrigel.

2.6. Western blotting

The cells were lysed using RIPA buffer and centrifuged at 12000×*g*, 4 °C for 10 min. Equal amounts of proteins were subjected to 6–10 % SDS-PAGE and transferred to PVDF membrane with a pore size of 0.45 μm (Millipore, Billerica, MA, USA). After the membranes had been blocked with 5 % non-fat milk, they were incubated with primary antibodies at 4 °C overnight, followed by incubation with secondary antibodies at 25 °C for 1 h. Bound antibodies were detected using ECL Plus western blotting substrate (Thermo Fisher, Waltham, MA, USA) and visualized using a chemiluminescence detection system (BioRad, CA, USA). Band density was quantified using Image J software. Relative protein levels were determined by standardizing the optical density values of the target proteins and the loading control (β-actin).

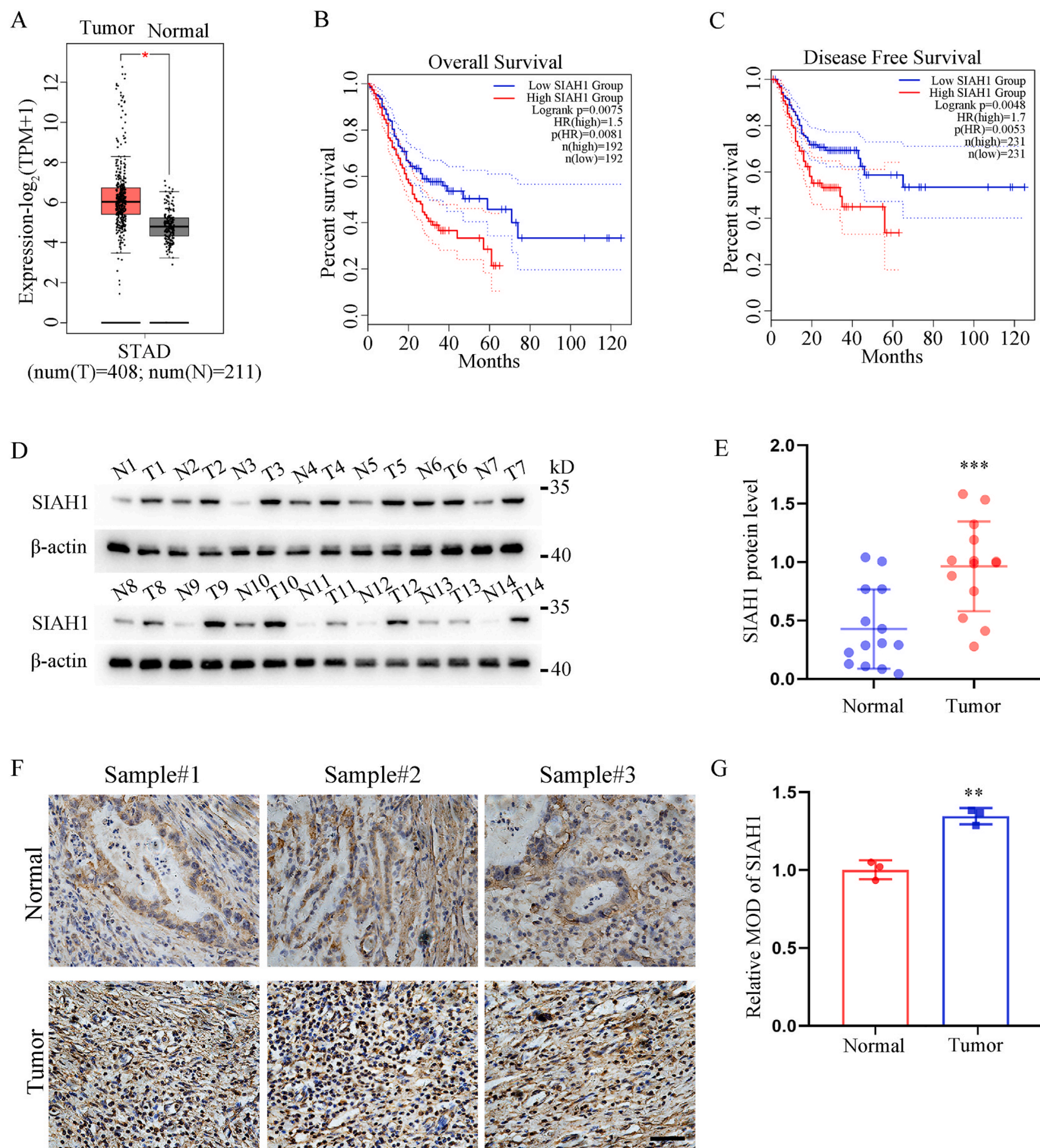

Fig. 1. SIAH1 is up-regulated in gastric cancer and associated with a poor prognosis. A, GEPIA database showed the mRNA level of SIAH1 in gastric cancer tissues (n = 408) and normal stomach tissues (n = 211); **B**, GEPIA database showed the relationship between SIAH1 expression and overall survival; **C**, GEPIA database showed the relationship between SIAH1 expression and disease-free survival; **D**, Representative blots and **E**, quantification showed the protein level of SIAH1 in gastric cancer tissues (n = 14) and normal stomach tissues (n = 14); **F**, Representative images and **G**, quantification of SIAH1 from gastric cancer tissues and normal stomach tissues determined by immunohistochemistry, bar = 50 μm. All data are expressed as the means ± SEM. For D, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in E and G. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

2.7. Immunohistochemistry

The S-P immunohistochemistry kit (Servicebio, Wuhan, China) was used to perform immunohistochemical staining according to manufacturer instructions. Briefly, the sections were dewaxed with dewaxing solution, and then incubated with SIAH1 antibody followed by Biotin-conjugated goat anti-rabbit IgG and HRP-conjugated streptavidin. The reaction was developed by 3, 3′-diaminobenzidine (DAB) chromogenic reagent (Servicebio). Next, hematoxylin was used to stain the nucleus and sections were then dehydrated. Finally, the cover slips were mounted onto the slides with neutral gum. The photos were collected under an Olympus IX-53 microscope (Olympus). The images were processed by Image Pro Plus 6.0 software, and the mean optical density (MOD) represented the expression level of SIAH1.

2.8. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

TRIzol reagent (Thermo Fisher Scientific) was used to extract total RNA from SGC-7901 and HGC-27 cells according to manufacturer instructions. Next, The RNA was reverse transcribed using a FastQuant RT kit (Yeasen, Shanghai, China) to obtain a single stranded cDNA template. MMP9 expression in gastric cancer cells was examined using SYBR Green qPCR master mix (Yeasen) with 40 cycles at 95 °C for 10 s and 60 °C for 30 s. The following primers were used for amplification:

MMP9-F: 5' CCCTGGTCCTGGTGCTCCTG 3';

MMP9-R: 5' CTGCCTGTCGGTGAGATTGGTTC 3'.

2.9. Degradation pathway analysis

SGC-7901 and HGC-27 cells were transfected with FLAG-SIAH1. Twenty-four hours later, the cells were treated with chloroquine (CHL, lysosome inhibitor, 50 μM) or MG132 (proteasome inhibitor, 10 μM) for 6 h. The protein levels of RECK and FLAG-SIAH1 were analyzed using western blotting.

2.10. Cycloheximide (CHX) tracking assay

CHX (50 μM) was using to treated cells silencing or overexpression of SIAH1 for a time gradient (0, 8, 16, 24 h; 0, 4, 8, 12 h). The protein levels of RECK and SIAH1 or FLAG-SIAH1 were analyzed using western blotting. The decrease of RECK protein level with time represents the decrease of its protein stability.

2.11. Ubiquitination assay

FLAG-SIAH1 (WT/C44S), MYC-RECK and HA-Ub were co-transfected into 293T or gastric cancer cells, the proteasomal inhibitor MG132 was used to solidify the ubiquitination process. The cells were lysed using Lysis Buffer (Beyotime, Shanghai, China). Protein levels of FLAG-SIAH1 and MYC-RECK were detected by western blotting. The supernatants were incubated with the MYC antibodies overnight at 4 °C followed by a 4 h incubation with protein A/G-agarose (MedChemExpress, Shanghai, China). After washed with 1 ×

Table 1
Clinicopathological correlation of SIAH1 in human GCs.

Characteristics	Number of cases	SIAH1 expression		p value
		Low (n = 38)	High (n = 38)	
Gender				0.8185
Male	40	21	19	
Female	36	17	19	
Age (years)				0.4686
<60	50	27	23	
≥60	26	11	15	
Tumor size, cm				0.0158
<5	49	30	19	
≥5	27	8	19	
Tumor invasion				0.0093
T1-T2	30	21	9	
T3-T4	46	17	29	
Lymphatic metastasis				0.02
Negative	33	22	11	
Positive	43	16	27	
Distant metastasls				0.0284
M0	67	37	30	
M1	9	1	8	
Clinical stage				0.0189
I-II	31	21	10	
III-IV	45	17	28	

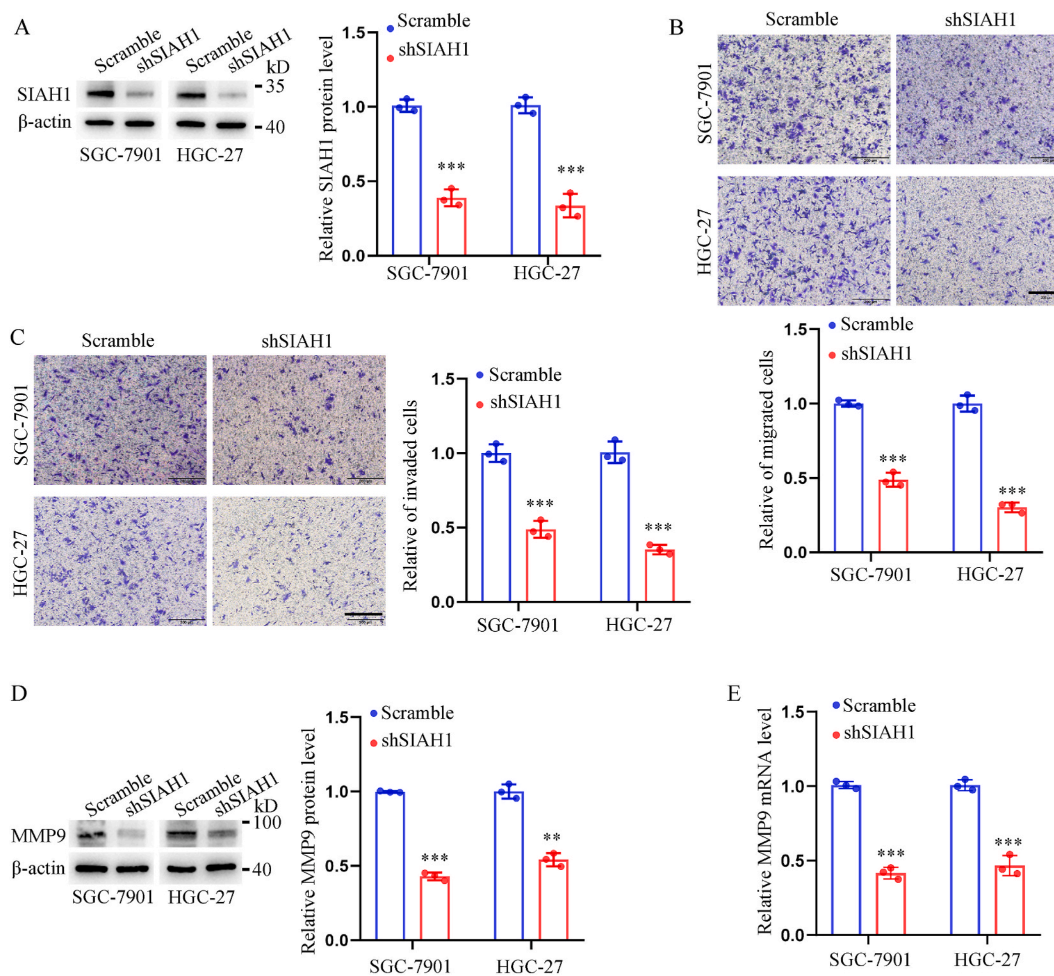

Fig. 2. Silencing of SIAH1 inhibits the migration and invasion of gastric cancer cells. A, Representative blots and quantification showed the protein levels of SIAH1; **B**, Representative images and quantification showed the migrated cells upon SIAH1 was silenced, bar = 200 μm; **C**, Representative images and quantification showed the invaded cells upon SIAH1 was silenced, bar = 200 μm; **D**, Representative blots showed the protein level of MMP9 upon SIAH1 was silenced; **E**, Quantification of the mRNA level of MMP9 upon SIAH1 was silenced. All data are expressed as the means ± SEM. For A and D, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in A-E. $^{**}P < 0.01$, $^{***}P < 0.001$.

PBST, the magnetic beads were boiled in 1 × sample loading buffer for 10 min. The immunoprecipitates were then analyzed by western blotting, and ubiquitination level of RECK was detected by HA antibody.

2.12. Bioinformatics analysis

The survival analysis was directly retrieved from the GEPIA database (http://gepia.cancer-pku.cn/). By entering SIAH1 in the search box, then selecting "survival plots" and determining the tumor type. The relationship between SIAH1 expression level and the survival of gastric cancer patients can be shown.

The method of bioinformatics analysis is referred to the study of Zhang [8]. The UbiBrowser and The Human Protein Atlas databases were used to analyze potential targets of SIAH1 and migration/invasion-related markers. All these data were screened with a Venn diagram (omicstudio).

2.13. Statistical analysis

All experiments were repeated a minimum of three times independently, and representative results are shown. Results are shown as the mean ± structural Equation Modeling (SEM). GraphPad Prism software (GraphPad software Inc., San Diego, CA, USA) was used for statistical analysis and graphs generation. Differences between two groups were analyzed using the Student's *t* -test and between multiple groups were analyzed using one-way analysis of variance and post-hoc tests. Statistical significance was set at $P < 0.05$.

3. Results

3.1. SIAH1 is up-regulated in gastric cancer and associated with a poor prognosis

To explore the role of SIAH1 in the progression of gastric cancer, we first analyzed the mRNA level of SIAH1 in gastric cancer and its association with patient survival using the GEPIA database. It was found that the mRNA level of SIAH1 was higher in gastric cancer tissues than normal stomach tissues (Fig. 1A). Furthermore, taking the median as the baseline, high levels of SIAH1 were associated with poor prognosis in gastric cancer patients (Fig. 1B and C). Subsequently, we collected the human gastric cancer tissue samples from clinic, and then detected the protein level of SIAH1. Obviously, the protein level of SIAH1 in gastric cancer samples was up-regulated (Fig. 1D and E). In addition, we assessed the expression and distribution of SIAH1 in normal stomach tissues and gastric cancer tissues by immunohistochemistry. Correspondingly, the expression of SIAH1 in gastric cancer tissues were increased compared with the normal stomach tissues (Fig. 1F and G). Further analysis of clinical data revealed a correlation between the expression of SIAH1 and the clinicopathological characteristics of gastric cancer patients. It was showed that high levels of SIAH1 were associated with Tumor size ($P < 0.05$), Tumor invasion ($P < 0.01$), Lymphatic metastasis ($P < 0.05$), Distant metastasis ($P < 0.05$) and Clinical stage ($P < 0.05$) (Table 1), the median was used as the benchmark again. These results indicate that SIAH1 is up-regulated in gastric cancer and associated with a poor prognosis.

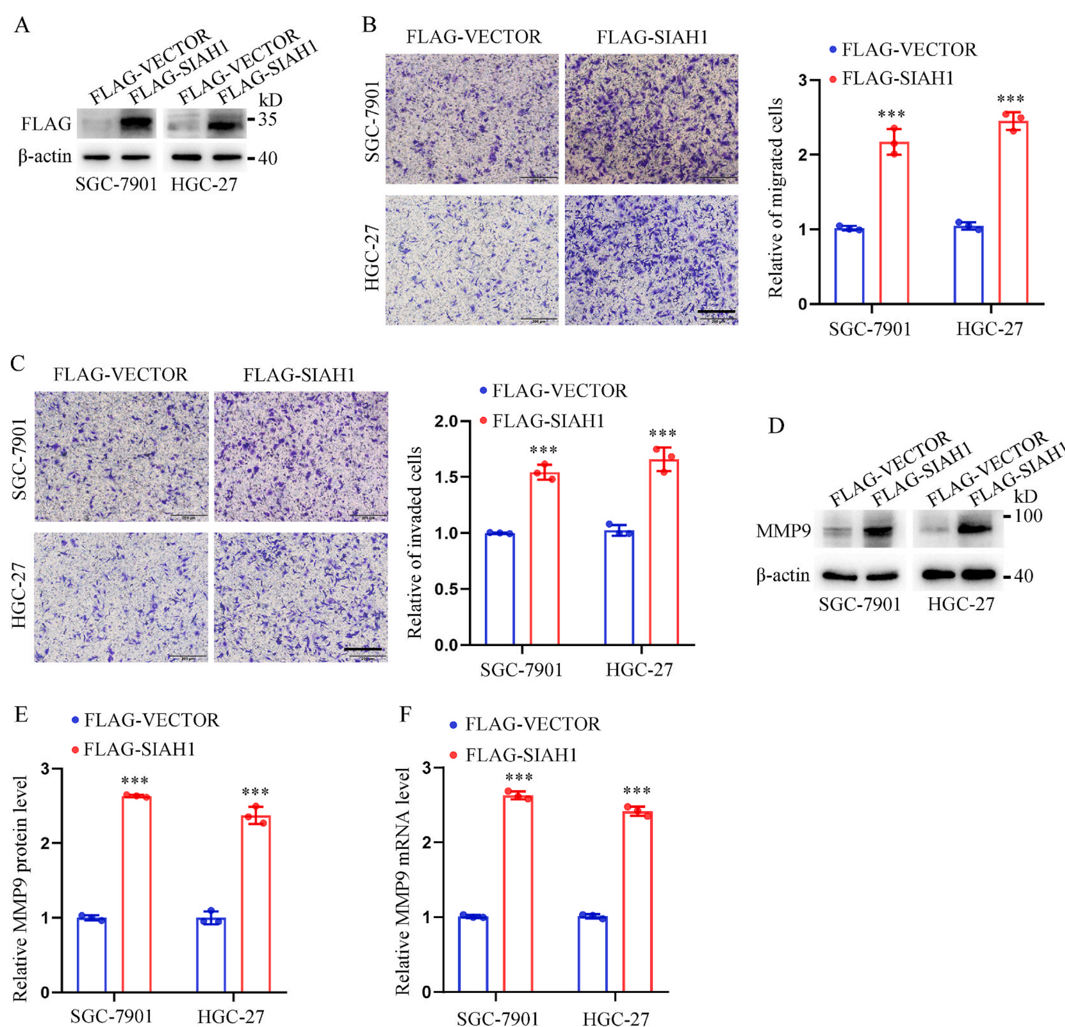

Fig. 3. Overexpression of SIAH1 promotes the migration and invasion of gastric cancer cells. A, Representative blots showed the protein levels of FLAG-SIAH1, IB: FLAG; **B**, Representative images and quantification showed the migrated cells upon SIAH1 was up-regulated, bar = 200 μm; **C**, Representative images and quantification showed the invaded cells upon SIAH1 was up-regulated, bar = 200 μm; **D and E**, Representative blots showed the protein level of MMP9 upon SIAH1 was up-regulated; **F**, Quantification of the mRNA level of MMP9 upon SIAH1 was up-regulated. All data are expressed as the means ± SEM. For A and D, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in B-F. ***$P < 0.001$.

3.2. Silencing of SIAH1 inhibits the migration and invasion of gastric cancer cells

To determine the function of SIAH1 on the migration and invasion of gastric cancer cells, we established a stable cell line silencing SIAH1 in gastric cancer cells using its specific shRNA. The protein level was analyzed by western blotting (Fig. 2A). Next, transwell assay was used to detect the motility behavior of gastric cancer cells. It was showed that, comparing with Scramble group, the migratory cells in the SIAH1 down-regulated group reduced by 55 % and 70 % in SGC-7901 and HGC-27 cells, respectively (Fig. 2B). Uniformly, knock down of SIAH1 also significantly inhibited the invasion of gastric cancer cells (Fig. 2C). Correspondingly, as a biomarker of cell migration and invasion, the protein level of MMP9 was significantly decreased after knock down of SIAH1 (Fig. 2D). Importantly, silencing of SIAH1 also reduced the mRNA level of MMP9 (Fig. 2E). These results indicate that knock down of SIAH1 inhibits the migration and invasion of gastric cancer cells, which was associated with down-regulation of MMP9.

3.3. Overexpression of SIAH1 promotes the migration and invasion of gastric cancer cells

To further verify the effect of SIAH1 on the athletic ability of gastric cancer cells, we transfected FLAG tagged SIAH1 into SGC-7901 and HGC-27. The expression level of FLAG-SIAH1 was determined using western blotting (Fig. 3A). Then, we speculated whether the cell migration and invasion were aggravated upon SIAH1 overexpression. Therefore, 24 h after transfection, the cells were used to evaluate the cell motility. It was found that, the migratory cells in the SIAH1 up-regulated group increased by 120 % and 140 % in SGC-7901 and HGC-27 cells, respectively, comparing with Scramble group (Fig. 3B). Consistently, overexpression of SIAH1 also obviously promoted the invasion of gastric cancer cells (Fig. 3C). Correspondingly, both the protein and mRNA level of MMP9 was significantly increased after overexpression of SIAH1 (Fig. 3D–F). These results indicate that overexpression of SIAH1 promotes the migration and invasion of gastric cancer cells, which was associated with up-regulation of MMP9.

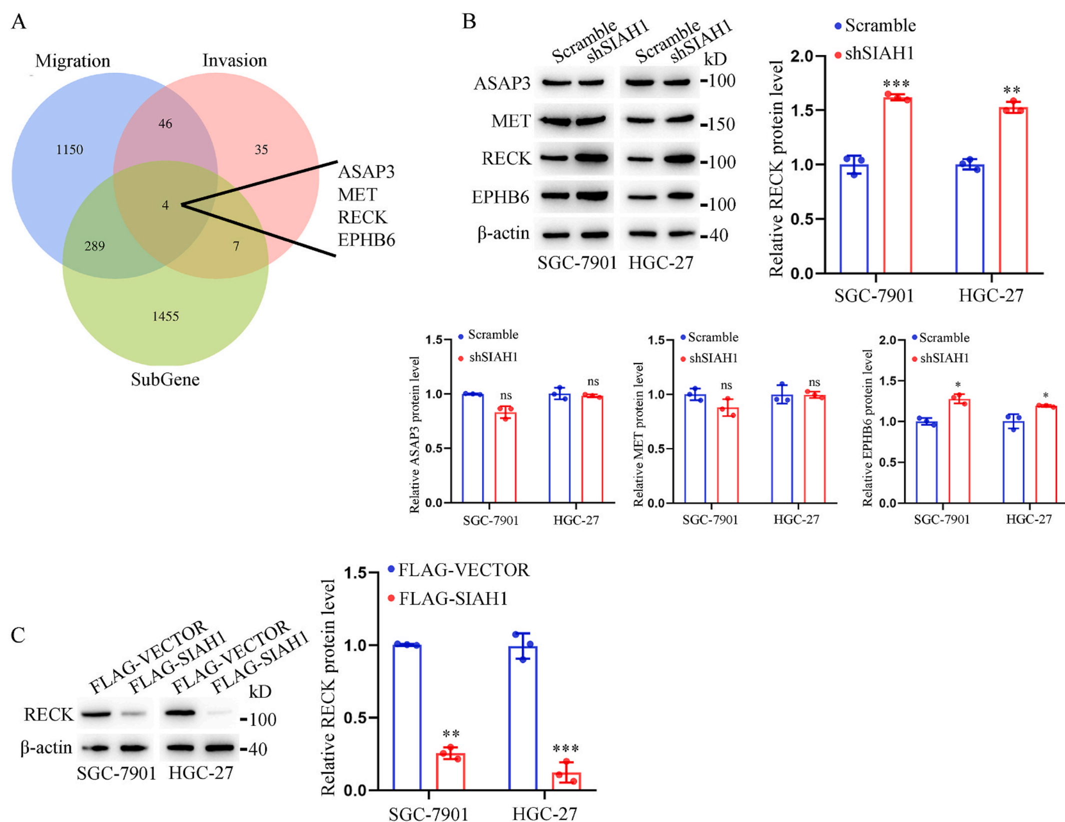

Fig. 4. RECK is a potential substrate of SIAH1. A, The UbiBrowser and The Human Protein Atlas databases were used to analyze potential substrate of SIAH1 and migration/invasion-related markers. All these data were screen with a Venn diagram. **B**, Representative images and quantification showed the protein levels of ASAP3, MET, RECK and EPHB6 upon SIAH1 was silenced; **C**, Representative images and quantification showed the protein levels of RECK upon SIAH1 was up-regulated. All data are expressed as the means ± SEM. For B and C, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in B and C. ns: no significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

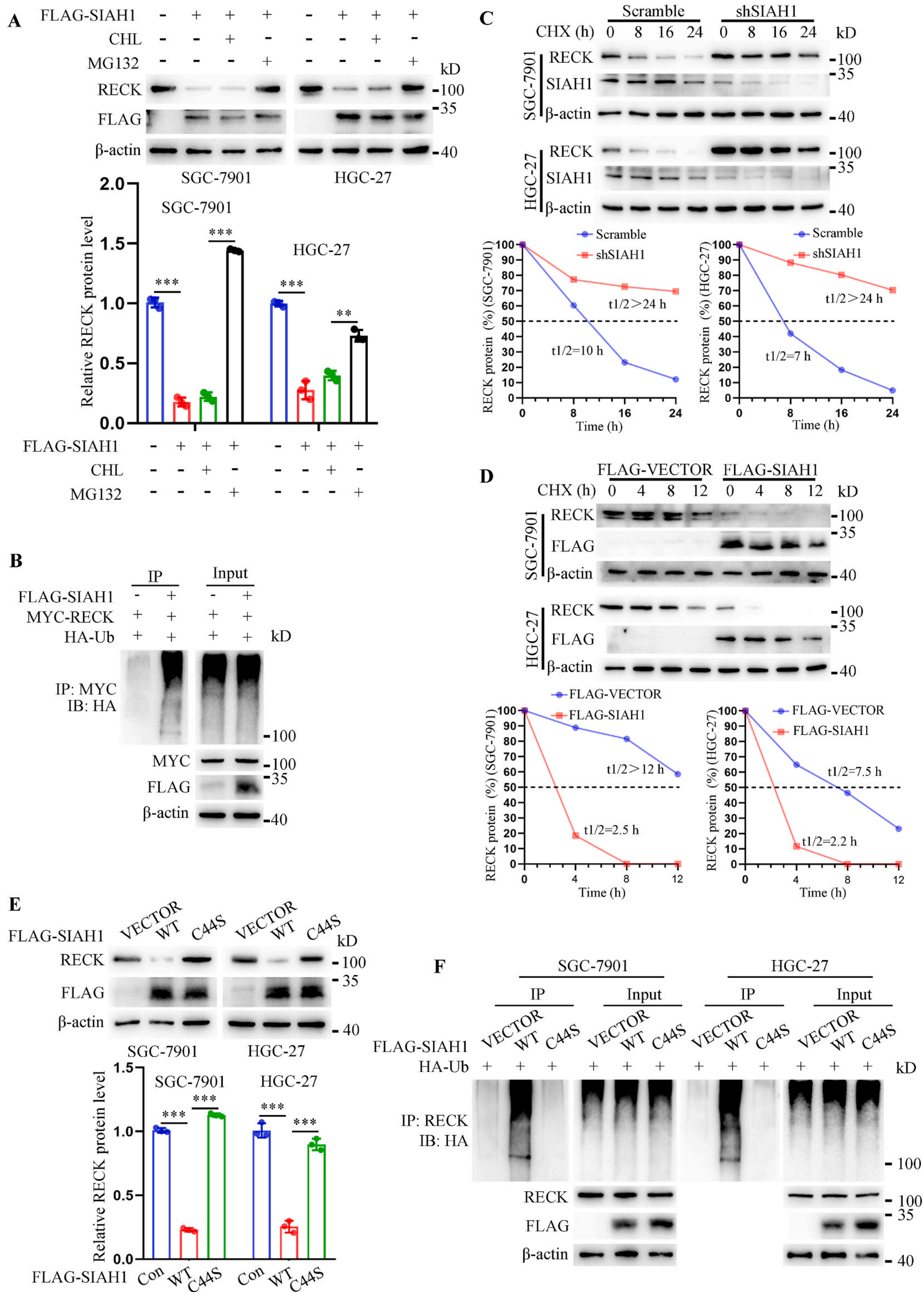

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Fig. 5. SIAH1 destabilizes RECK through promoting its ubiquitination and degradation via proteasome. A, Representative blots and quantification showed proteasome inhibitor MG132 but not lysosome inhibitor chloroquine (CHL) could rescue the degradation of RECK in SIAH1 down-regulated cells. **B**, The effects of the expression of FLAG-SIAH1 on the ubiquitination of MYC-RECK in 293T cells; **C**, Representative blots and quantification showed knock down of SIAH1 increased the stability of RECK in gastric cancer cells; **D**, Representative blots and quantification showed overexpression of SIAH1 decreased the stability of RECK in gastric cancer cells; **E**, Representative blots and quantification showed SIAH1-RING mutant (C44S) significantly attenuated SIAH1-induced down-regulation of RECK in gastric cancer cells; **F**, SIAH1-C44S significantly attenuated SIAH1-induced polyubiquitination of endogenous RECK in gastric cancer cells. All data are expressed as the means ± SEM. For A-F, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in A and E. $^{**}P < 0.01$, $^{***}P < 0.001$.

3.4. RECK is a potential substrate of SIAH1

It has been reported that SIAH1 could regulate the development of multiple human cancers through promoting the ubiquitination and degradation of some substrates, such as β-catenin [14], AKT3 [15], TRB3 [16], and YBX-1 [17]. Thus, we guessed whether SIAH1 promoted the migration and invasion of gastric cancer cells because of its role as a E3 ligase. To detect this guess, we downloaded the potential substrates of SIAH1 from Ubibrowser database, as well as the migration and invasion biomarkers from The Human Protein Atlas database. All these data were screened with a Venn diagram. It was found that there were four potential targets of SIAH1, including ArfGAP with SH3 domain, ankyrin repeat and PH domain 3 (ASAP3), MET proto-oncogene, receptor tyrosine kinase (MET), RECK and EPH receptor B6 (EPHB6) (Fig. 4A). Next, we determined the regulatory role of SIAH1 on these substrates using western blotting. The results showed that the protein level of RECK was the most significantly altered upon silencing of SIAH1 (Fig. 4B). It has been reported that RECK can inhibit the role of MMPs, including MMP9 [10]. Our results above suggest that SIAH1 negatively regulates MMP9. Therefore, although SIAH1 also affects the protein level of EPHB6, it may be more meaningful to analyze the molecular mechanism by which SIAH1 regulates RECK. In addition, SIAH1 had much less effect on EPHB6 than on RECK in our findings. Thus, we will detect the regulation and mechanism of SIAH1 on RECK. Oppositely, it was found that the expression level of RECK was obviously decreased while SIAH1 was up-regulated (Fig. 4C). These results indicate that RECK might be a potential substrate of SIAH1.

3.5. SIAH1 destabilizes RECK through promoting its ubiquitination and degradation via proteasome pathway

It has been reported that SIAH1 plays its role as ubiquitin ligase mainly via the proteasome pathway [14–17]. To determine how SIAH1 degrades RECK, we treated the gastric cancer cells up-regulated SIAH1 with the lysosome inhibitor chloroquine (CHL) and proteasome inhibitor MG132. It was found that MG132 but not CHL obviously rescued the degradation of RECK protein level induced by overexpression of SIAH1 (Fig. 5A). Furthermore, we found that overexpression of SIAH1 significantly increased the ubiquitination of MYC-RECK in 293T cells (Fig. 5B). In addition, we treated the gastric cancer cells with the protein synthesis inhibitor cycloheximide (CHX) upon SIAH1 was silenced or overexpressed to measure the stability of RECK. It was showed that knock down of SIAH1 stabilized RECK while overexpression of SIAH1 destabilized it (Fig. 5C and D).

To further confirm that whether the ubiquitination of RECK by SIAH1 is dependent on its E3 ligase activity, Cys 44 in the RING domain of SIAH1 was converted to serine (C44S). Previous studies verified that this mutant has no E3 ligase activity [8]. The results showed that SIAH1-C44S largely lose the ability of SIAH1 to degrade RECK, compared with wildtype SIAH1 (SIAH-WT) (Fig. 5E). Furthermore, gastric cancer cells transfected with SIAH1-WT significantly increased polyubiquitination of endogenous RECK, while the SIAH1-C44S markedly lose the ability (Fig. 5F). These results indicate that SIAH1 destabilizes RECK through promoting its ubiquitination and degradation via proteasome pathway.

3.6. SIAH1 promotes the migration and invasion of gastric cancer cells through regulating RECK-MMP9 pathway

The results above showed that SIAH1 could promote the migration and invasion of gastric cancer cells through regulating MMP9. RECK has been reported to negatively regulate MMPs, especially MMP9, thus inhibiting the metastasis of human cancers [10,18–20]. To explore whether RECK was involved in the regulation of SIAH1 on MMP9 and the migration and invasion of gastric cancer cells, we transfected MYC-RECK into gastric cancer cells overexpressed SIAH1. The results showed that overexpression of RECK obviously reduced the protein level of MMP9 (Fig. 6A), and decreased the number of migratory and invasive cells induced by SIAH1 up-regulation (Fig. 6B and C). These results indicate that SIAH1 promotes the migration and invasion of gastric cancer cells through regulating RECK-MMP9 pathway.

4. Discussion

SIAH1 is a highly conserved E3 ligase and can regulate transcription factors, nerve conduction factors, hypoxia regulators and other substrates [6,21,22], thereby participating in several cellular ability, such as cell cycle, apoptosis, DNA damage repair and hypoxia stress [4,6,23]. It has been reported that SIAH1 was associated with the growth and metastasis of multiple human cancers, for example, lung cancer [8], hepatocellular carcinoma [24], colorectal cancer [3], breast cancer [25] and glioma [26]. Therefore, SIAH1 has been considered as a potential biomarker of these cancers. In addition, recent study showed that SIAH1 protein level was also noticeably higher in gastric cancer [27]. However, the role and mechanism of SIAH1 on the migration and invasion of gastric cancer cells remains unclear. In this study, we determined that SIAH1 was up-regulated in gastric cancer and associated with a poor prognosis. We also

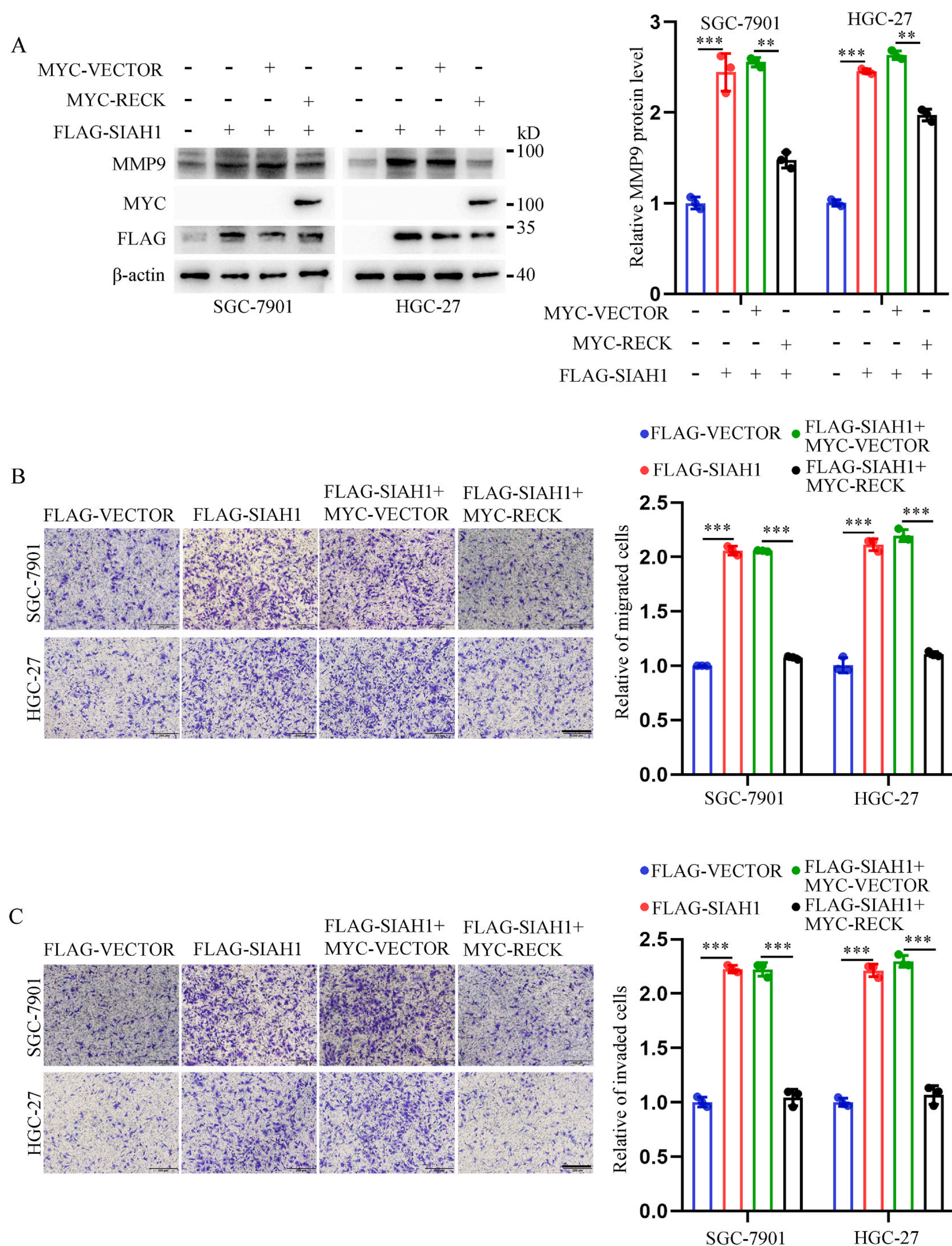

Fig. 6. SIAH1 promotes the migration and invasion of gastric cancer cells through regulating RECK-MMP9 pathway. A, Representative blots showed overexpression of RECK could reduce the protein level of MMP9 induced by SIAH1 overexpression; **B**, Representative images showed overexpression of RECK could decrease the migrated cells induced by SIAH1 overexpression, bar = 200 μm; **C**, Representative images showed overexpression of RECK could decrease the invaded cells induced by SIAH1 overexpression, bar = 200 μm. All data are expressed as the means ± SEM. For A, the protein expression levels of target genes were normalized to those of β-actin (loading control). For statistical analysis, paired *t*-test was used in A-C. $^{**}P < 0.01$, $^{***}P < 0.001$.

confirmed the positive role of SIAH1 on the mobility of gastric cancer cells. We found that knock down of SIAH1 obviously inhibited the migration and invasion of gastric cancer cells, while overexpression of SIAH1 had the opposite effect. However, the mechanism by which SIAH1 regulates gastric cancer cells metastasis and whether it is related to the ligase activity of SIAH1 are still unclear.

RECK encodes a cysteine-rich, extracellular protein with protease inhibitor-like domains whose expression is suppressed strongly in many tumors and cells transformed by various kinds of oncogenes [28]. It has been reported that RECK acts as an cancer-suppressor to inhibit the progression of many human cancers, such as colorectal cancer [29], glioma [30] and breast cancer [31], including gastric cancer [12]. Here, we got four potential substrates of SIAH1 and then verified the regulatory role of SIAH1 on them. We found that SIAH1 significantly negatively regulated the protein level of RECK. Studies have shown that SIAH1 could be involved in cancer progression by promoting the ubiquitination of substrates, including C-MYC, β-catenin, CBP et al. [14,32–35]. We then explored whether SIAH1 was associated with the ubiquitination of RECK. It was found that SIAH1 could promote the ubiquitination and degradation of RECK via proteasome pathway, thereby destabilizing it, which was depended on the ligase ability of SIAH1. In this case, we confirmed that abnormal overexpression of SIAH1 will cause the down-regulation of RECK, which will facilitate the migration and invasion of gastric cancer cells through regulating MMP9. Therefore, up-regulation of SIAH1 may be an important event in the development and malignant progression of gastric cancer.

5. Conclusions

In conclusion, we determine that RECK is a novel substrate of SIAH1. We provide evidence that SIAH1 promotes the migration and invasion of gastric cancer cells through regulating RECK-MMP9 pathway. Our study offers new insights for further study of the role and mechanism of SIAH1 on gastric cancer. However, it has been reported that SIAH1 played different role in different human tumors. For example, SIAH1 could inhibit the invasion and migration of HCC cells by promoting the polyubiquitination of CTR9 [36]; SIAH1 could stabilize Notch1 protein thereby promoting the proliferation of NSCLC cells [8]. In brief, the specific function of SIAH1 is not unified at present, but this instead highlights its research value. In addition, the role of SIAH1 as ubiquitin ligase in gastric cancer progression still needs further exploration, which will be a long process.

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Institutional review board statement

The study was performed according to the principles contained in the Declaration of Helsinki (2013). The study was permitted by the Research Ethics Committee of Nanjing Gaochun People's Hospital (approval no. 2023-116-01).

Informed consent statement

The gastric cancer patients signed an internal regulatory document stating that the remaining samples could be used for academic studies without other informed consent.

Data availability statement

The manuscript includes all datasets which support the conclusions of this paper.

CRediT authorship contribution statement

Xiaohua Zhou: Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fuping Gao:** Visualization, Validation, Supervision, Resources, Formal analysis, Conceptualization. **Guangqi Xu:** Validation, Software, Methodology, Investigation, Data curation. **Yongqiang Puyang:** Validation, Software, Methodology, Formal analysis. **Hongqing Rui:** Supervision, Project administration, Investigation. **Junsheng Li:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32676.

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