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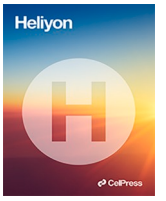

Research article

SIPA1 promotes epithelial-mesenchymal transition in colorectal cancer through STAT3 activation

Youjian Li [a,1], Mengjie Wang [b,1], Lu Jiang [a], Jiehong Jia [a], Fei Pan [a], Wen Li [a], Bochu Wang, PhD, Professor [d], Ke Huang [c], Jie Luo [a,d,*]

[a] College of Pharmacy, National & Local Joint Engineering Research Center of Targeted and Innovative Therapeutics, IATTI, Chongqing University of Arts and Sciences, Chongqing, China
[b] Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[c] College of Landscape Architecture and Life Science/Institute of Special Plants, Chongqing University of Arts and Sciences, Yongchuan, Chongqing, China
[d] Biomedical and Health Engineering Laboratory, Chongqing University, Chongqing, China

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ABSTRACT

Colorectal cancer (CRC) is the third leading cancer type worldwide and accounts for the second highest rate of cancer-related mortality. Liver metastasis significantly contributes to the mortality associated with CRC, but the fundamental mechanisms behind it remain unclear. Signal-induced proliferation-associated protein 1 (SIPA1), a GTPase activating protein, has been shown to promote metastasis in breast cancer. In this study, our objective was to explore the role of SIPA1 in regulating epithelial-mesenchymal transition (EMT) in CRC. The analysis of The Cancer Genome Atlas (TCGA) database revealed that the expression level of SIPA1 mRNA was notably upregulated and exhibited a positively correlated with EMT and STAT3 signaling pathways in CRC. Knockdown of SIPA1 impairs CRC cell proliferation and migration. Further studies on the reliance of SIPA1 on STAT3 signaling for EMT regulation have shown that SIPA1 stimulates the activation of STAT3, resulting in its nuclear translocation. The co-treatment of overexpressed SIPA1 with the STAT3 inhibitor STTITA has shown that SIPA1 regulates the expression of EMT-related markers through STAT3. Our study indicate that SIPA1 promotes CRC metastasis by activating the STAT3 signaling pathway, underscoring the potential of SIPA1 as a therapeutic target for metastatic CRC patients.

1. Introduction

Colorectal cancer (CRC), frequently diagnosed and a prominent contributor to cancer mortality, poses a considerable threat to global health [1].Despite numerous studies enhancing diagnostic techniques and treatment options, the 5-year survival rate is still disappointingly low, mainly due to the issues of tumor metastasis and recurrence [2]. Consequently, it is essential to conduct further explorations into the biomarkers associated with metastasis. These research endeavors could enhance our ability to foresee the

* Corresponding author. College of Pharmacy, National & Local Joint Engineering Research Center of Targeted and Innovative Therapeutics, IATTI, Chongqing University of Arts and Sciences, Chongqing, China.
E-mail address: luojie@cqwu.edu.cn (J. Luo).
[1] Contributed equally to this work.

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likelihood of metastasis and contribute to the formulation of more potent treatment plans designed to curb recurrence.

Signal-induced proliferation-associated protein 1 (SIPA1), a member of the RapGAPs family, and acts as an inhibitor of Rap1 activity [3,4]. The abnormal expression of it that is observed in breast, colorectal, melanoma, and prostate cancer cells, which could contributes to the modulation of tumor cell proliferation, attachment, penetration, and metastasis [5–7]. Previous studies have indicated that a reduction of SIPA1 expression in breast cancer cells can lead to a decrease in tumor cell adhesion, migration, and invasion, which is mediated by the integrin-associated FAK/AKT-MMP9 signaling pathway [8]. Recent research has also identified that SIPA1 targets the EPAS1 gene to augment aerobic glycolysis, thus facilitating cancer cell metastasiss [9]. Furthermore, it has been found that SIPA1 regulates the LINC01615 gene to promote the spread of triple-negative breast cancer [10] and activates the integrin β1 promoter to enhance the invasiveness of breast cancer cells [7]. In a recent study, a proteomic analysis of extracellular vesicles from human CRC cells revealed six differentially upregulated proteins, including SIPA1, that are linked to the increased capabilities of proliferation, migration, and invasion in CRC cells [11]. The exact regulatory mechanism of SIPA1, however, remains to be elucidated, indicating an urgent requirement for additional research.

Signal Transducer and Activator of Transcription 3 (STAT3), functioning as a transcription factor, is integral to the regulation of essential cellular activities including cell proliferation, invasion, immune reactions, and inflammation [12]. The initiation of STAT3's activity is a critical driver of CRC metastasis [13]. The abnormal activation of STAT3 is associated with processes such as epithelial-mesenchymal transition (EMT), matrix metalloproteinase (MMP) production, angiogenesis, and immune system modulation [12,14,15]. Thereby, the inhibition of STAT3 signaling is a potential therapeutic tactic for controlling CRC metastasis. Interleukin-6 (IL-6) is a well-characterized activator of STAT3, alongside other cytokines and growth factors, and Receptor Tyrosine Kinases (RTKs) including EGFR or c-Mett [16], with dysregulated JAK signaling also contributing to the sustained activation of STAT3 in CRC [17]. Consequently, the targeting of STAT3 signaling has become a potential therapeutic strategy to prevent CRC metastasis. Although the involvement of SIPA1 in cancer biology has been noted, its specific impact on EMT in CRC and its relationship with the STAT3 signaling pathway are areas that require further clarification.

Here, our investigation revealed a pronounced association between SIPA1 and the propensity for colorectal cancer to metastasize. Meanwhile, we found the possible molecular mechanism between SIPA1 and CRC metastasis by interfering the expression of SIPA1 in CRC cells. Our study revels a novel mechanism by which SIPA1 modulates epithelial-mesenchymal transition (EMT) in CRC cells and fosters metastasis via the activation of STAT3. This discovery provides new avenues for a better understanding of the metastatic mechanisms within CRC.

2. Methods and materials

2.1. Data collection and processing

The Cancer Genome Atlas (TCGA) provided us with the expression profile data and clinical data for 33 tumor categories, and The Human Protein Atlas (HPA) was consulted for immunohistochemical images of normal and tumor human tissues. It is important to record that this study strictly adhered to the TCGA and UCSC protocols, which allowed for an exemption from ethical review and the requirement for patient consent.

2.2. Expression analysis of SIPA1

The mRNA levels of SIPA1 were examined and contrasted between tissues that are normal and those that are tumorous, utilizing data from The Cancer Genome Atlas (TCGA). The Human Protein Atlas (HPA) was then referenced to compare the protein expression of SIPA1 across normal and tumor tissues.

2.3. Gene set enrichment analysis

The Gene Set Enrichment Analysis (GSEA) was applied in conjunction with the "clusterProfiler" R package [18] to perform the enrichment analysis. The analysis relied on Hallmark MSigDB gene sets as the reference collection for the gene sets involved in the enrichment process.

2.4. Cell lines and antibodies

HEK293 cells, bought from Procell Life Science & Technology Co., Ltd. (Catalog number CL-0001), and SW-480 cells from Nanjing Cobioer Biosciences Co., Ltd., were grown in DMEM medium with 10 % FBS at 37 °C and 5 % CO_2. HCT-116 cells, likewise obtained from Nanjing Cobioer Biosciences Co., Ltd., were nurtured in McCoy's-5A medium with 10 % FBS under the same environmental conditions.

2.5. Western blot

For protein analysis preparation, cells were lysed using a lysis kit (P0013B, Beyotime) that included a protease inhibitor cocktail (HY–K0013, MCE). The protein content was determined by the BCA assay (P0009, Beyotime). Subsequently, proteins were resolved via SDS-PAGE and transferred to PVDF membranes (ISEQ00010, EMD Millipore). These membranes were blocked with 5 % non-fat milk in

TBS buffer at room temperature for a duration of 30 min. The membranes were then exposed to primary antibodies overnight at 4 °C, which included anti-SIPA1 (A19867, ABclonal, 1:1000), anti-phospho-STAT3 (p-STAT3, AP0070, ABclonal, 1:1000), anti-STAT3 (60199-1-Ig, Proteintech, 1:1000), anti-E-cadherin (20874-1-AP, Proteintech, 1:5000), anti-N-cadherin (A19083, ABclonal, 1:1000), anti-Vimentin, and anti-GAPDH (GB11002, Servicebio, 1:2000). After three washes with TBST, the membranes were incubated with the secondary antibodies, HRP-conjugated Goat Anti-Rabbit IgG (AS014, ABclonal, 1:5000) and Anti-Mouse IgG (AS003, ABclonal, 1:5000), at room temperature for 1.5 h. The immunoblots were then visualized using the Tanon 5200 Multi imager.

2.6. CCK8 assay and EdU assay

Cells were plated in 96-well plates at a concentration of 5 × 10^3 cells per well, with some wells receiving treatment to inhibit SIPA1. After a 48-h incubation period, 10 μL of the CCK8 reagent was introduced to each well. Post a subsequent 2-h incubation, the optical density was recorded at 450 nm utilizing a microplate reader.

For the assessment of cell proliferation, an EdU assay was performed following the protocol provided by the manufacturer. Fluorescence microscopy with an Olympus IX73 was utilized to capture the resultant images.

2.7. Colony formation

CRC cells, either subjected to SIPA1 inhibition or not, were seeded in a 6-well plate with 500 cells per well and cultured over a two-week period. Crystal violet staining was utilized to visualize and measure the colonies. The stained colonies were analyzed using the ImageJ software.

2.8. Immunofluorescence assay

CRC cells, either inhibited for SIPA1 or not, were seeded onto coverslips and fixed with a 4 % solution of PFA. Post-fixation, cells were blocked with 1 % FBS for 60 min before being incubated with primary antibodies (anti-SIPA1 and anti-STAT3, both at a dilution of 1:200) at 4 °C throughout the night. Following this, the cells were exposed to secondary antibodies for an hour. The nuclei were subsequently stained with DAPI for a quarter of an hour at room temperature and imaged using an Olympus BX53 confocal microscope.

2.9. Quantitative real-time PCR (RT-qPCR)

Total RNA was harvested utilizing the RNA Easy Fast Tissue/Cell kit (DP451, TIANGEN). Subsequently, cDNA was synthesized with the aid of the ReverAid First Strand cDNA Synthesis Kit (K1622, Thermo scientific). This cDNA was blended with the Genious 2X SYBR Green Fast qPCR Mix (RK21206, Abclonal Technology, Wuhan, China) along with the relevant primers detailed in Supplementary Table S1. The ABI 7300 QuantStudio3 PCR (RT-PCR) System was employed to measure the mRNA expression levels of the genes of interest.

2.10. Wound healing assay

The wound healing assay was employed to measure cell migration capabilities associated with SIPA1 overexpression. HCT116 cells, at 6000 cells per well, were seeded into 6-well plates and organized into equivalent groups. A sterile 10 μl pipette tip was used to create a uniform scratch, forming a cell-free zone after the cells became adherent. The cells were washed twice with PBS and then incubated for 24 h. The migration into the wound was captured using an inverted microscope, and the ImageJ software was applied to quantify the cell migration by assessing the remaining unoccupied area.

2.11. Prognosis analysis

Kaplan-Meier survival analysis with the log-rank test was conducted to explore the link between SIPA1 expression levels and outcomes such as overall survival (OS), progression-free interval (PFI), and disease-specific survival (DSS) in the TCGA dataset. The survival curves were considered significant at a threshold of $*p < 0.05$. Moreover, ROC curves were utilized in the analysis of tumors to evaluate the predictive value of SIPA1 for prognosis.

2.12. Statistical analyses

Statistical processing was carried out with Prism V.9.0 (GraphPad Software, Inc.), and results are presented as mean ± SD, except where indicated otherwise. Data analysis involved the use of a *t*-test, one-way ANOVA, or two-way ANOVA, as applicable, and statistical significance was identified at the levels of $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

3. Results

3.1. SIPA1 is overexpressed in altered carcinomas

Our investigation of SIPA1 mRNA levels in diverse cancers was performed using data sourced from the TCGA database (Fig. 1A). Among the paired 23 tumor types examined, SIPA1 was found to be significantly overexpressed in 15 of them (Fig. 1B). Additionally, when comparing unmatched groups (Fig. 1C) and paired samples from the same patients (Fig. 1D), we observed that the expression of SIPA1 was considerably higher in CRC tumors compared to pericancerous tissues.Significantly, it should be noted that in the cancers studied, there was no significant reduction in SIPA1 expression observed. Further scrutiny of the Human Protein Atlas (HPA) database disclosed that SIPA1 protein expression was observably heightened in pathological samples of CRC (Fig. 1E). These findings reinforce the hypothesis that SIPA1 could be significantly involved in the onset and advancement of CRC.

3.2. Inhibition of SIPA1 moderates tumorigenesis in vitro

To investigate the impact of SIPA1 on tumor cell function, we utilized SIPA1-targeted shRNA to silence SIPA1 in the CRC cell lines. Western blot analysis confirmed the effectiveness of shSIPA1-1 and shSIPA1-2 in significantly inhibiting SIPA1 protein expression (Fig. 2A). Meanwhile, CCK-8 assay showed that the reduction of SIPA1 expression resulted in a notable decrease in the cell proliferation index (Fig. 2B and C). Consistently, EdU assays and colony formation assays were performed to evaluate the effects of SIPA1 knockdown on the proliferative capacity of the CRC cell line. Data showed a drastic reduction in cell growth and cellular DNA synthesis rate following the knockdown of SIPA1 (Fig. 2D–G). Moreover, these results are consistent within the knockdown of SIPA1 in SW480

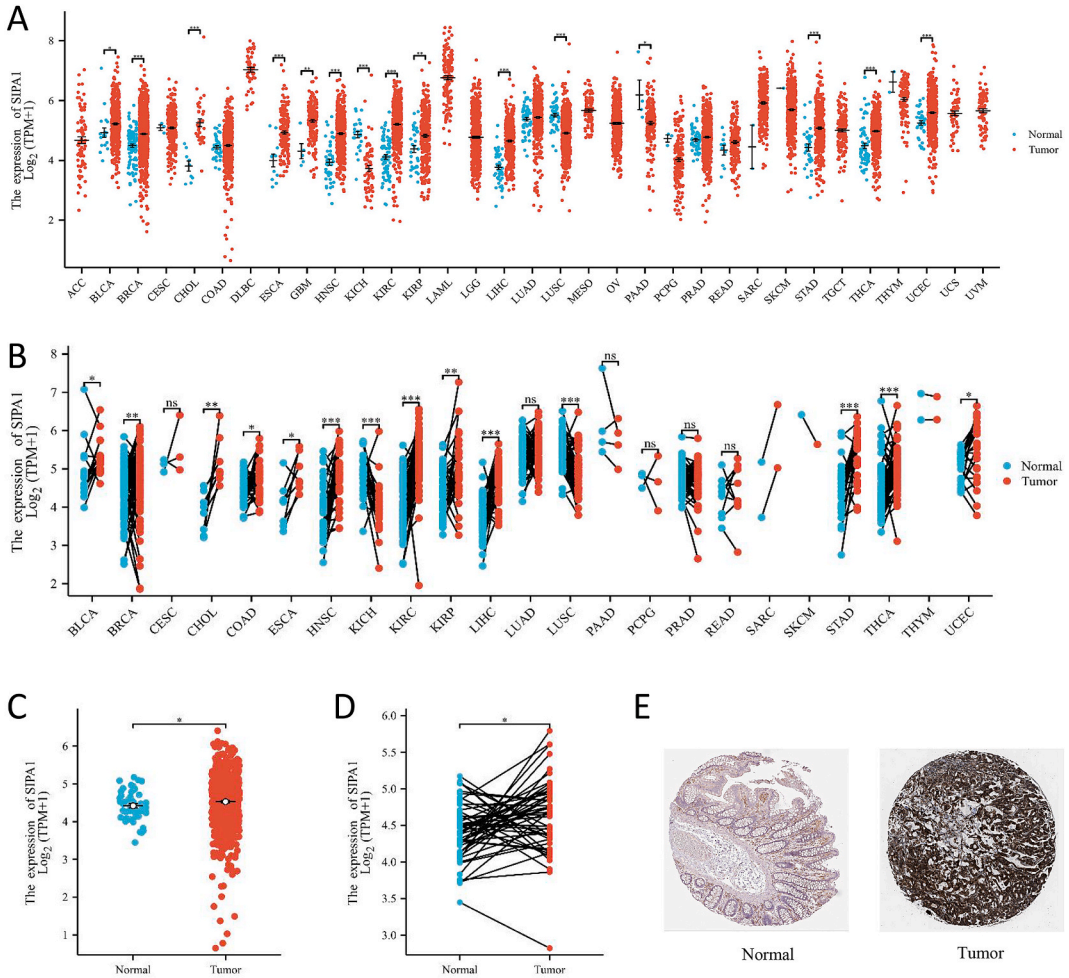

Fig. 1. SIPA1 shows high expression in colorectal cancer. (A–B) In the TCGA database, mRNA expression of SIPA1 was analyzed in 33 tumors and in paired samples of 18 tumors.(C–D) SIPA1 expression in colorectal cancer tumors compared to peri-carcinous tissue in both the paired and unpaired experimental groups. (E) IHC images obtained from the HPA depicted SIPA1 expression in normal and tumor tissues. ns, $P \geq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

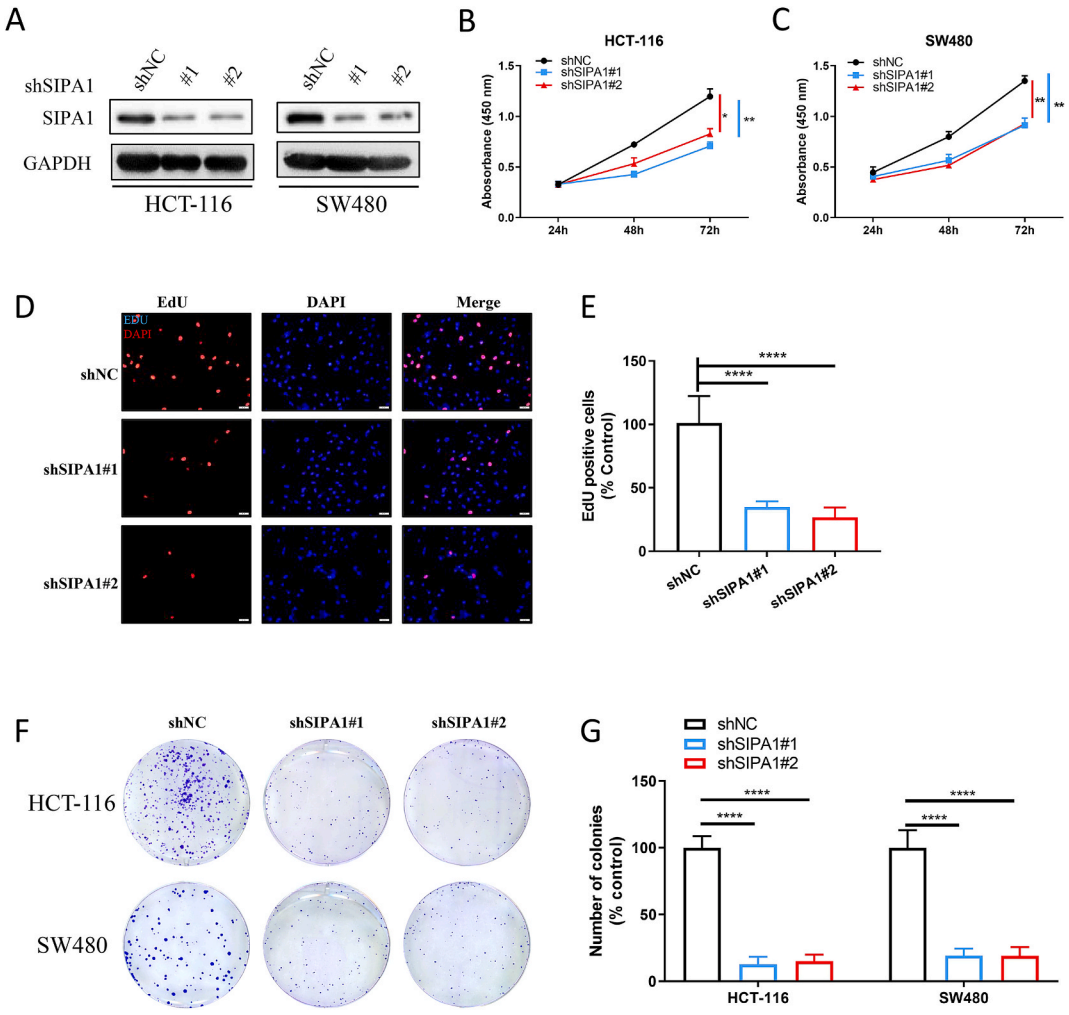

Fig. 2. SIPA1 knockdown inhibits HCT-116 and SW480 cell proliferation and clone formation. (A) Cells were transfected with specific shRNA, and protein expression was analyzed using antibodies. (B–C) CCK8 assay assessed cell proliferation in shCON, shSIPA1#1, and shSIPA1#2 groups of HCT-116 and SW480 cells. (D) Edu assay evaluated DNA synthesis and (E) Percentage of EdU-positive cells in shCON, shSIPA1#1, and shSIPA1#2 groups. (F) Colony formation assay showed clone formation capacity in different group and (G) Colony numbers were counted. Scale bar: 50 μm $^{**}p < 0.01$; $^{****}p < 0.0001$, statistical analysis was performed with one-way ANOVA with Dunn's test for multiple comparisons.

cell line (Supplementary Fig. 1).Based on these findings, we assumed that SIPA1 attribute to the proliferation of CRC cells *in vitro*.

3.3. SIPA1 regulates EMT pathway in CRC

According to the expression value of SIPA1 (to simulate the effect similar to overexpression or knockdown of this gene), the RNAseq samples of TCGA colorectal cancer were categorized into groups based on high and low expression levels, the two groups of which were analyzed seperatively. Molecular Signatures Database was used to select hallmark gene sets to infer the signal pathways that might be associated with the expression of SIPA1 in the gene cluster.By conducting additional GSEA analysis, our findings revealed a notable correlation between elevated SIPA1 levels and the progression of the epithelial-mesenchymal transition (EMT) pathway (Fig. 3A). Moreover, our analysis of the single gene co-expression heat map from the TCGA database revealed a consistent negative correlation between SIPA1 and the EMT marker E-cadherin (CDH1), while displaying a positive correlation with the EMT markers N-cadherin (CDH2) and Vimentin (Fig. 3B). Furthermore, Western blot assay showed that in CRC cells, SIPA1 inhibition enhanced E-cadherin expression and impaired both N-cadherin and Vimentin expression (Fig. 3C and D). Next, we performed *trans*-well assays to investigate the influence of SIPA1 on the migration capability of CRC cells. Data showed that SIPA1 silencing resulted in a significant inhibition of the migration ability in CRC cells (Fig. 3E and F). Collectively, our findings indicate that SIPA1 promotes the EMT process in CRC cells.

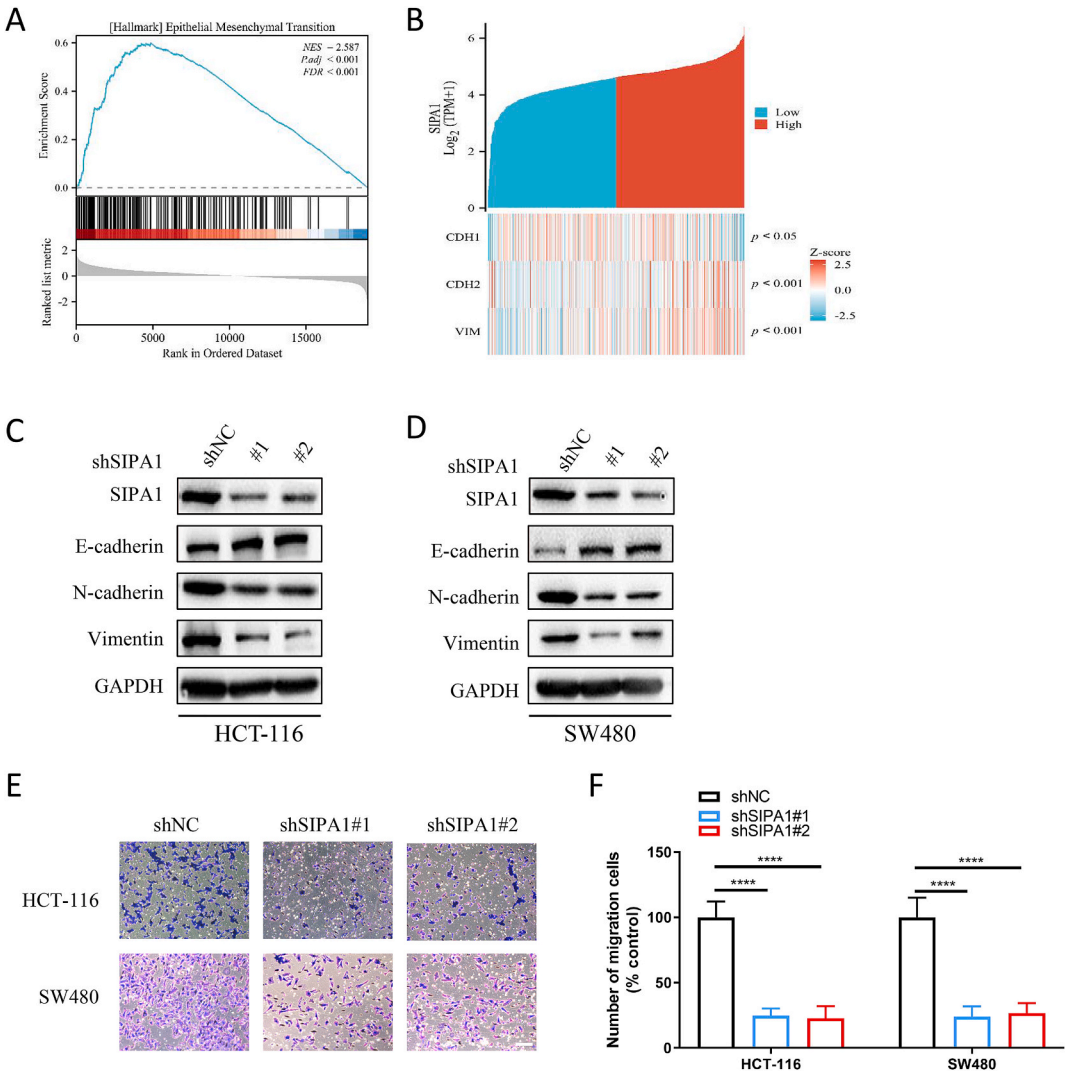

Fig. 3. SIPA1 Regulates EMT Pathway in CRC. (A)GSEA indicated enrichment of SIPA1-associated DEGs in the EMT signaling pathway. (B)The single gene co-expression heat map revealed significant relationships between SIPA1 and EMT markers, such as E-cadherin, N-cadherin, and Vimentin. (C–D) Western blotting demonstrated upregulation of E-cadherin protein expression, while N-cadherin and Vimentin protein expression levels were downregulated in HCT-116 and SW480 cells transfected with SIPA1 shRNA. (E–F) The *trans*-wellrevealed that suppressing the SIPA1 gene expression led to a noteworthy reduction in the migratory capacity of both HCT-116 and SW480 cell lines. Scale bar: 200 μm ****$p < 0.0001$, statistical analysis was performed with one-way ANOVA with Dunn's test for multiple comparisons.

3.4. SIPA1 influences the activation of the STAT3 signaling pathway

To further explore the molecular mechanism of SIPA1 in the EMT regulation, GSEA enrichment analysis was performed. Data showed that SIPA1 may lead activate IL-6/STAT3 signaling pathway (Fig. 4A). Additionally, the single gene co-expression heat map (from the TCGA database) revealed an affirmative relationship between SIPA1 and STAT3 (Fig. 4B). Therefore, we hypothesized that SIPA1 may activate EMT process through STAT3 activation. To confirm this hypothesis, Western blot assays were executed. Data showed that with SIPA1, the phosphorylation of STAT3 was impaired (Fig. 4C and D). The extent of nuclear translocation of STAT3 reflects its functional activity and its transcriptional efficacy. Next, by conducting an assay for the separation of nuclear and cytosolic proteins, we figured out that SIPA1 facilitated the movement of STAT3 into the nuclear compartment (Fig. 4E–H). Furthermore, an immunofluorescence assay demonstrated that SIPA1 manipulation led to a decrease in the concentration of STAT3 within the nucleus. (Fig. 4I). Altogether, these combined results imply that increased SIPA1 expression sets off the activation of the STAT3 signaling pathway.

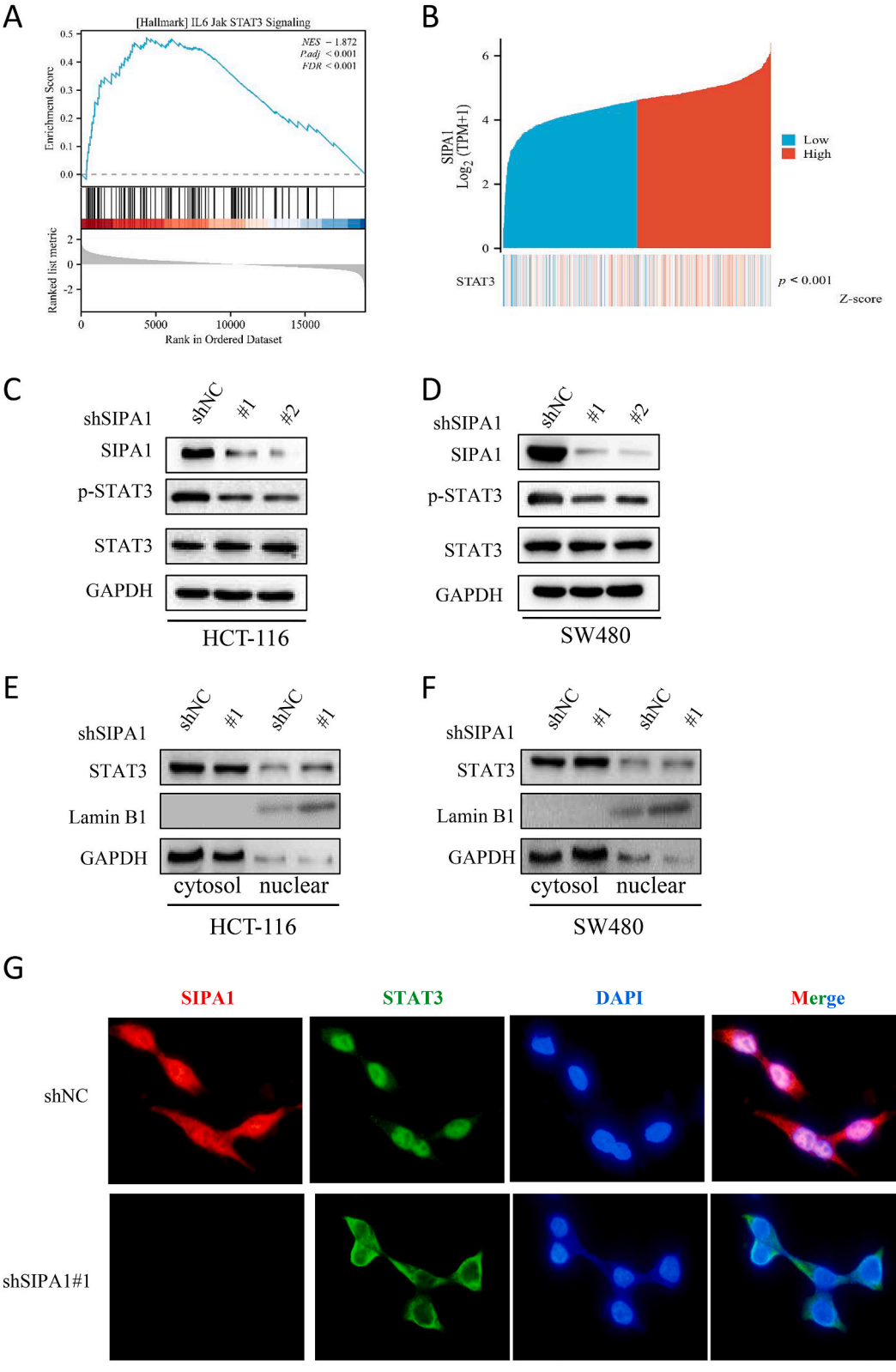

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Fig. 4. SIPA1 influences the activation of the STAT3 signaling pathway. (A) GSEA analysis indicated an association between SIPA1 expression and activation of the IL-6/JAK/STAT3 pathway. (B) The single gene co-expression heat map revealed significant relationships between SIPA1 and STAT3. (C–D) Western blotting was used to detect changes in STAT3 phosphorylation upon SIPA1 intervention in HT-116 and SW480 cell lines. (E–H) Nuclear and cytoplasmic proteins were separated and analyzed by western blotting after SIPA1 intervention in HT-116 and SW480 cell lines. (I) An immunofluorescence assay was conducted to assess changes in the nuclear localization of STAT3 in response to SIPA1 intervention. Scale bar: 50 μm.

3.5. The pro-EMT function of SIPA1 is facilitated through the STAT3 signaling

The phosphorylation of STAT3, which is indicative of its activation, is crucial for the regulation of EMT. Next, we overexpressed SIPA1 in both HCT-116 and SW480 cell lines. And then,we utilized STATTIC, a catalyticactivity inhibitor for STAT3, to abolish its activation. Western blot assay to determine whether the function of SIPA1 is dependenting on STAT3 activation. Results showed that SIPA1 promoted STAT3 activation, reflected by the elevated phosphorylation of STAT3. Additionally, STATTIC abolished the effects of SIPA1 on EMT activation (Fig. 5A and B). Furthermore, given that STAT3 is a critical transcriptional factor, we pursued to examine the potential effects of SIPA1 on the transcriptional levels of E-cadherin, N-cadherin, and Vimentin. The qPCR results demonstrated that SIPA1 upregulates the mRNA expression levels of E-cadherin. With STATTIC addition, we observed that SIPA1-mediated gene regulation was partially abolished (Fig. 5C–H). Moreover, we overexpressed SIPA1 in HCT116 cell line and performed migration assay. The SIPA1 overexpression groups have been shown to enhance migration, while this ability is reversed bythe specific inhibitor STATTTIC (Fig. 5I and J). These results indicate that SIPA1 regulates the EMT capability of CRC cells through the STAT3 signaling pathway.

3.6. SIPA1 as a predictive biomarker for CRC diagnosis and prognosis

To evaluate the diagnostic and prognostic significance of SIPA1 in CRC, we conducted several analyses on temporal diagram by Kapplan-Meier, after the pre-processing of the RNAseq data from TPM format and clinical data that extracting by R language.Firstly, utilizing ROC curves, we assessed the discriminatory power of SIPA1 for the diagnosis of colorectal cancer. The results showed that SIPA1 had a sensitivity and specificity for diagnosing CRC, as indicated by the area under the curve (AUC) of 0.626 (Fig. 6A). Additionally, we performed Kaplan-Meier analysis to determine the predictive value of SIPA1 for clinical outcomes. The results indicated that elevated levels of SIPA1 expression correlated with poorer overall survival (hazard ratio [HR]: 2.03, $P < 0.001$), diminished disease-specific survival (HR: 2.91, $P < 0.001$), and reduced progression-free interval (HR: 1.68, $P < 0.001$) when contrasted with the group exhibiting low SIPA1 expression.(Fig. 6B–D). These findings suggest that the elevated expression of SIPA1 appears to be indicative of an unfavorable prognosis in CRC, suggesting its potential as a biomarker for both the identification and the prognostic assessment of colorectal cancer.

4. Discussion

Our study demonstrates that SIPA1 promotes CRC cells migration and epithelial-mesenchymal transition through STAT3 activation. Additionally, our data further elucidated the upstream regulatory mechanism of STAT3 activation, which plays a crucial part in EMT process. Analyzing and revealing the mechanisms underlying SIPA1-mediated EMT regulation could provide insights into novel therapeutic strategies for CRC.

SIPA1 has been extensively investigated for nearly three decades since its discovery in 1995, particularly in various tumor types [6,7,19]. Our review of TCGA data supports the conclusions of a different research group, indicating an upregulation of SIPA1 across various cancer types, including CRC [11,20,21]. Notably, our analysis did not find any tumors with a reduction in SIPA1 expression. This suggests a potential role for SIPA1 in the processes of tumorigenesis and tumor progression.

Our study demonstrated that knockdown of SIPA1 inhibited cell proliferation in CRC cells, which is consistent to previous study of quantitative real-time PCR and immunohistochemicall staining, refering to SIPA1 plays key roles during the growth and motility of colorectal cancer [22]. Studies by Zhang et al. and Ying Ma et al. both reported that decreasing SIPA1 expression resulted in a marked increase in the growth rate of MDA-MB-231 and MCF7 cells [8,23]. Discordantly, Takahara et al. indicated that knocking out SIPA1 in HSC-3 or HSC-4 cells did not significantly impact cell proliferation, indicating that the functional role of SIPA1 may vary in different cell types and states [5]. However, Ji K et al.'s investigation revealed that beyond mRNA expression in colorectal tumors, no significant links was identified between SIPA1 expression and disease attributes, encompassing TNM classification, recurrence, metastasis, and survival, through an analysis of clinical samples. In addition, the introduction of anti-human SIPA1 hammerhead ribozymes into two colorectal cancer cell lines (HT115 and Caco-2) resulted in an increased invasiveness of the cells after the downregulation of SIPA1. Correspondingly, a smaller but noticeable enhancement in cell migration was also observed after SIPA1 knockdown.

[22]. Such differences may stem from different CRC lines. Meanwhile, the biological role of SIPA1 can be clarified through more clinical sample validation in the future.

EMT is a fundamental cellular process that plays a essential role in cancer metastasis [24,25]. EMT initiates a series of phenotypic changes in cancer cells, transitioning them from an epithelial to a mesenchymal phenotype. This is evidenced by the downregulation of epithelial markers such as E-cadherin and the upregulation of mesenchymal markers including N-cadherin and vimentin [26]. Our research revealed that SIPA1 triggers a decrease on E-cadherin and an increase on both N-cadherin and Vimentin, suggesting that

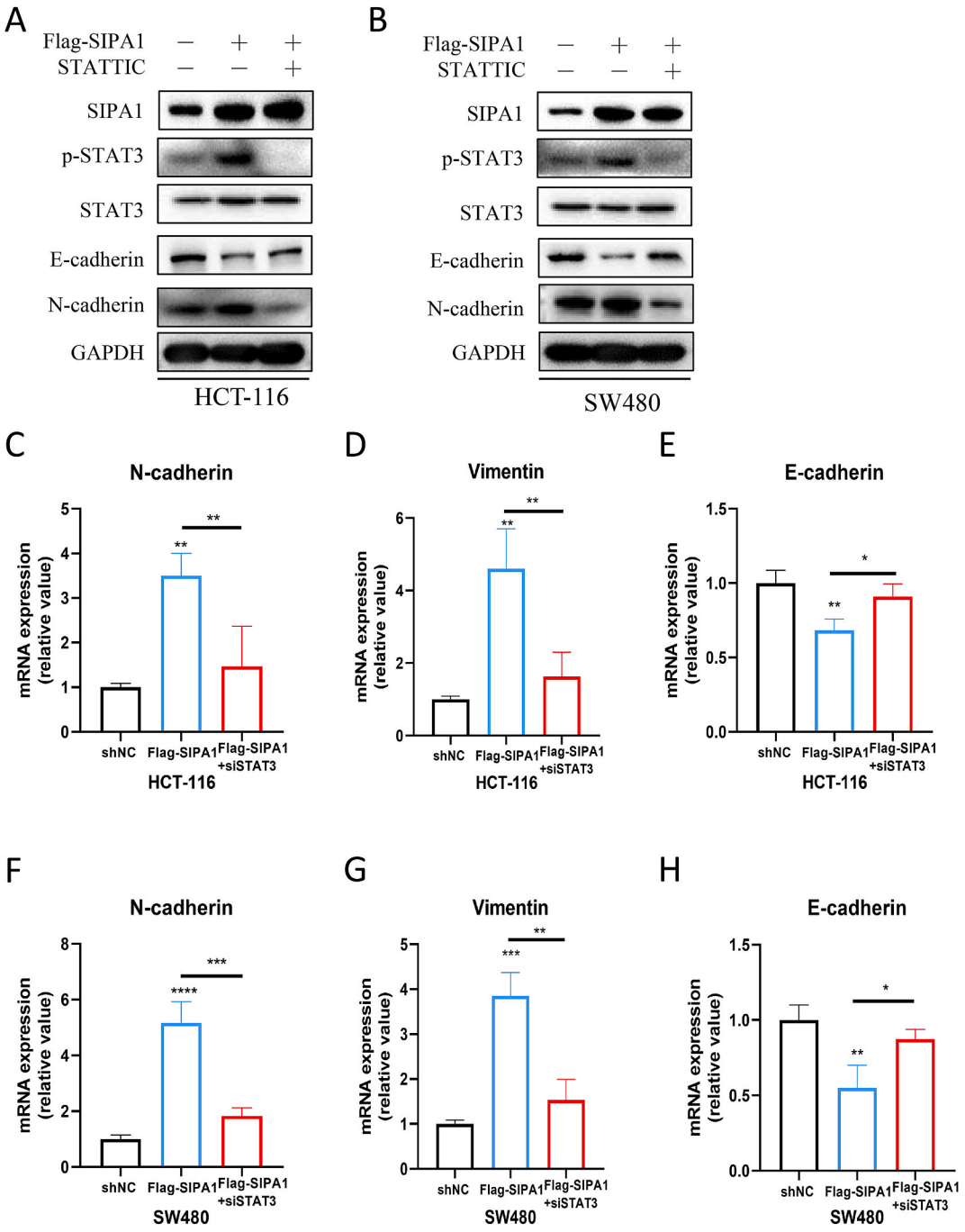

Fig. 5. The pro-EMT function of SIPA1 is mediated by STAT3 signaling pathway. (A–B) SIPA1 promotes EMT activation through the activation of STAT3, and is abolished by the specific STAT3 inhibitor, STATTIC. (C–H) The qPCR results further demonstrated that SIPA1 influenced the mRNA expression levels of E-cadherin, N-cadherin, and Vimentin, which were reversed upon treatment with STATTIC. (I–J) Overexpression of SIPA1 in HCT116 cells promotes migration and is eliminated by a specific STAT3 inhibitor, STATTIC. *$p < 0.05$; **$p < 0.01$, ***$p < 0.0005$; ****$p < 0.001$, statistical analysis was performed with one-way ANOVA with Dunn's test for multiple comparisons.

SIPA1 is involved in promoting the acquisition of mesenchymal features in CRC cells, which facilitates their invasive and migratory behavior.

Additionally, we found that SIPA1-mediated EMT augment was highly associated with STAT3 activation, which was regulated by STAT3 phosphorylation. As a vital transcriptional factor [27], STAT3 is essential for mediating the effects of SIPA1 on the transcriptional levels of E-cadherin, N-cadherin, and Vimentin. Our qPCR results demonstrated that SIPA1 upregulates the mRNA

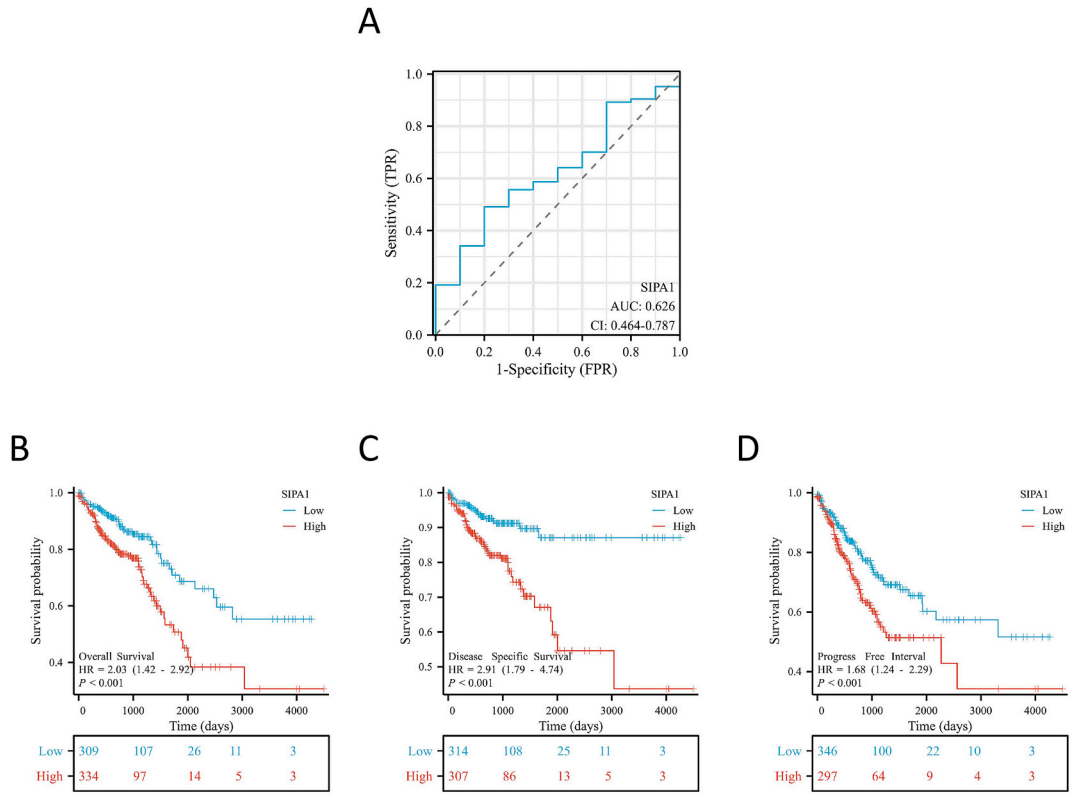

Fig. 6. The predictive value of SIPA1 expression in CRC patients was assessed for diagnosis and clinical outcomes. (A) ROC curve analysis evaluated the diagnostic performance of SIPA1 in CRC. (B–D) Kaplan-Meier analyses compared overall survival, disease-specific survival, and progression-free interval between CRC patients with high and low SIPA1 expression.

expression levels of N-cadherin and Vimentin, downregulates E-cadherin mRNA expression. Notably, when we inhibited STAT3 activity by pharmacological inhibition, the upregulation of these genes by SIPA1 was attenuated. These results indicate that SIPA1 modulates the transcription of these EMT-associated genes via a STAT3-dependent regulatory mechanism.

Importantly, we evaluated the clinical significance of our findings by examining the potential of SIPA1 as both a diagnostic and prognostic indicator in CRC. Our ROC curve analysis indicated that SIPA1 has a sensitivity and specificity for CRC diagnosis. Furthermore, consistent with the study conducted by Liu et al. [11], our Kaplan-Meier analysis demonstrated that high SIPA1 expression is associated with worse OS, DFS, and PFI in CRC patients. These results suggest that SIPA1 is potentially serve as a prognostic biomarker for CRC.

In conclusion, our study provides key insights into SIPA1's role in CRC, showing its regulation of EMT via the STAT3 signaling pathway as instrumental in CRC progression. SIPA1's role as a major regulator of CRC cell invasion and migration is highlighted, indicating its potential as a therapeutic target. Further exploration of the molecular underpinnings and SIPA1-targeted therapeutics may lead to advancements in more effective CRC treatment strategies.

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Data availability statement

The data used to support findings of the study are available from the corresponding author upon request.

CRediT authorship contribution statement

Youjian Li: Visualization, Supervision, Software, Methodology, Investigation. **Mengjie Wang:** Resources, Data curation. **Lu Jiang:** Data curation. **Jiehong Jia:** Formal analysis. **Fei Pan:** Software. **Wen Li:** Formal analysis. **Bochu Wang:** Formal analysis. **Ke Huang:**

Funding acquisition. **Jie Luo:** Writing – review & editing, Writing – original draft, Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34527.

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