BBA - Molecular Cell Research 1871 (2024) 119785

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Promoter hypermethylation-induced downregulation of ITGA7 promotes colorectal cancer proliferation and migration by activating the PI3K/AKT/NF-κB pathway

Jianjun Wang [b,1], Yu Wang [a,1], Jijun Zhu [a,1], Lili Wang [a], Yanlin Huang [e], Huiru Zhang [f], Xiaoyan Wang [a,*], Xiaomin Li [a,c,d,*]

[a] Department of Gastroenterology, Clinical Medical Research Center, Suqian First People's Hospital, The Suqian Clinical College of Xuzhou Medical University, Suqian, China
[b] Department of Histology and Embryology, Wannan Medical College, Wuhu, China
[c] Laboratory of Clinical and Experimental Pathology, National Demonstration Center for Experimental Basic Medical Science Education, Department of Pathology, School of Basic Medical Sciences, Xuzhou Medical University, Xuzhou, China
[d] Department of Pathology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China
[e] School of Medical Imaging, Wannan Medical College, Wuhu, China
[f] School of Clinical Medicine, Wannan Medical College, Wuhu, China

ARTICLE INFO

Keywords:
Colorectal cancer
ITGA7
Methylation
CKAP4
NF-κB

ABSTRACT

We previously reported that integrin alpha 7 (ITGA7) was downregulated in colorectal cancer (CRC) tissues and CRC cell lines and that the lower expression of ITGA7 in CRC tissues was correlated with distant metastasis, suggesting that ITGA7 may function as a suppressor in CRC. The present research was conducted to further investigate the role and mechanisms of ITGA7 in CRC progression. First, bisulfite modification and genomic sequencing (BSP) results showed that the methylation rate of ITGA7 promoter was higher in 10 CRC tissues than in the matched normal tissues. Additionally, 5-Aza-CdR treatment increased ITGA7 expression in CRC cells. Gain-of-function assays revealed the inhibitory role of ITGA7 in CRC cell proliferation and migration. Mechanistically, RNA sequencing, RT-qPCR, and cytoplasm and nuclear separation and rescue assays indicated that knockdown of ITGA7 activated the transcription of MMP9, SETD7, and ADAM15 by enhancing the nuclear translocation of NF-κB. Moreover, CoIP and Western blot suggested a mechanistic model in which ITGA7 binds to CKAP4 to block the interaction of CKAP4 and PI3K p85α and thereby suppress the PI3K/AKT/NF-κB pathway. Accordingly, the current study suggests that ITGA7 functions as a suppressor in CRC progression and that its expression is controlled by promoter methylation.

1. Introduction

Colorectal cancer (CRC) is a common malignant digestive tract cancer with a high risk to human health. Due to increasing prevalence, CRC is now the third most common malignancy and the second most common cause of cancer-related death worldwide [1]. Although more and more studies have discovered several targets driving CRC initiation and development, the underlying mechanisms of CRC occurrence and progression remain unclear. Therefore, in-depth studies are needed to explore the concrete molecular mechanisms of CRC progression and identify effective molecular targets for CRC treatment.

Integrins are a large family of adhesion and signaling proteins composed of an alpha subunit (ITGA) and beta subunit (ITGB) that participate in cell–matrix interactions and cell–cell adhesion to mediate biological functions [2]. In recent years, numerous studies have reported that multiple types of integrins are differentially expressed in various cancers and that the aberrant expression of integrins is closely related to tumorigenesis, angiogenesis, cell proliferation, motility, invasion, and metastasis [3]. Integrins have emerged as promising targets for the treatment of cancer because of their major role in tumor development

* Corresponding authors at: Department of Gastroenterology, Clinical Medical Research Center, Suqian First People's Hospital, The Suqian Clinical College of Xuzhou Medical University, Suqian, China.
E-mail addresses: 726343819@qq.com (X. Wang), lxm1980326@sina.com (X. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbamcr.2024.119785
Received 15 February 2024; Received in revised form 10 June 2024; Accepted 12 June 2024
Available online 15 June 2024
0167-4889/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

[4]. However, while some integrins are highly expressed in several cancer types and function as oncogenic drivers of tumor progression, others appear to suppress malignant tumor behavior. Additionally, in some cancer types, certain integrins exhibited opposing effects.

Integrin alpha 7 (ITGA7), a member of the integrin family, has emerged as a potential therapeutic target for certain cancers. It is upregulated in tongue squamous cell carcinoma, hepatocellular carcinoma, non-small cell lung cancer, endometrial cancer, and glioblastoma [5–9], and its higher expression is positively correlated with poor prognosis and reduced survival. ITGA7 targeting in these types of cancer could suppress cancer cell proliferation, invasion, metastasis, and stemness. In contrast, downregulation of ITGA7 expression has been found in breast cancer, papillary thyroid carcinoma, prostate cancer, and malignant pleural mesothelioma [10–13]. It functions as a tumor suppressor to inhibit the malignant progression of these cancers partially by regulating epithelial-to-mesenchymal transition or binding to tissue inhibitor of metalloproteinase 3 (TIMP3). Moreover, the reduced expression of ITGA7 in breast cancer stem cells (BCSCs) significantly correlates with chemoresistance, which suggests that ITGA7 could act as a predictive marker for chemotherapy responses [14]. However, its role in CRC remains unclear.

We previously reported the downregulation of ITGA7 in CRC tissues and CRC cell lines and the positive correlation of lower expression of ITGA7 with distant metastasis [15]. However, the factors causing the ITGA7 downregulation in CRC remained unknown. In the current study, we reveal the relationship between the hypermethylation of ITGA7 promoter and reduced expression of ITGA7 in CRC. In addition, the inhibitory role of ITGA7 in CRC proliferation and migration was further confirmed using functional assays. We also proposed a novel mechanism in which ITGA7 binds to cytoskeleton-associated protein 4 (CKAP4) to interfere with the interaction of CKAP4 and PI3K p85α, thereby suppressing the PI3K/AKT/NF-κB pathway. Our work is the first to reveal the crucial function of ITGA7 in CRC progression.

2. Materials and methods

2.1. Cell culture

CRC cell lines and the normal colonic epithelial cell line FHC were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in the matched medium with 10 % or 20 % FBS at 37 °C with 5 % CO2.

2.2. Clinical specimens

Human CRC tissues and the matched noncancerous mucosal tissues were collected from patients with general surgery in Yijishan hospital, Wannan Medical College (Wuhu, China). The consents from patients were obtained. The use of clinical materials for research purpose were approved by the Ethics Committee of Wannan Medical College (Wuhu, China). The fresh CRC tissues were immediately frozen in liquid nitrogen and were stored at −80 °C. All cases were diagnosed as colorectal adenocarcinoma.

2.3. RNA extraction, reverse transcription (RT) and real-time quantitative PCR (RT-qPCR)

The total RNAs was extracted using RNAiso Plus reagent (#9109, Takara, Japan) according to the instruction. RT was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (#RR047A, Takara, Japan). RT-qPCR was done using SybrGreen qPCR Mastermix (#DBI-2043, DBI Bioscience, Germany) according to the manufacturer's instructions. GAPDH was used as an internal reference. All primers used in the study were synthesized by Ruibiotech (Guangzhou, China) (Table S1).

2.4. Construction of ITGA7 overexpression plasmid, small interfering RNAs (siRNAs) and transfection

siRNAs used in the study were designed and synthesized by GenePharma (Suzhou, China) and RiboBio (Guangzhou, China) (Table S2). ITGA7 overexpression plasmid was constructed by Ruibiotech (Guangzhou, China). lipofectamine3000 (#L3000015, Invitrogen, Life Technologies, USA) was used for transfection of siRNAs and plasmid as described in the manufacturer's protocol.

2.5. Bisulfite modification and genomic sequencing (BSP)

The methylation status of the CpG island in the ITGA7 promoter was analyzed using the bisulfite modification and genomic sequencing (BSP) method. Bisulfite modification was performed using the EpiTect Bisulfite Kit (#59104, QIAGEN, German) according to the manufacturer's instructions. The fragments of 552 bp containing 50 CpG islands of ITGA7 promoter were amplified using the following specific primers: ITGA7-BSP-Forward, 5′- GGTGATTTATTAGTTTTGGGGT -3′; ITGA7-BSP-Reverse, 5′- CAACCTAAACATCCCCTTAAAAAC -3′. The PCR products were purified and cloned into the pMD19-T vectors. Individual bacterial colonies were picked and sequenced to analyze DNA methylation.

2.6. 5-Aza-CdR treatment

5-Aza-CdR was purchased from Macklin (#A801497, Macklin, China). CRC cells were added 5-Aza-CdR for 48 h. Then, RNA was extracted for RT-qPCR detection.

2.7. Separation of cytoplasm and nucleus fraction

Separation of cytoplasm and nucleus fraction was performed using the Minute nuclear plasma separation kit (#SC-003, Invent Biotechnologies, USA) according to the instruction. Then, the extracts were suffered Western blotting detection. GAPDH was used as cytoplasmic internal reference and H3 was used as nuclear internal reference.

2.8. CCK8, colony formation, EdU, transwell migration assay and wound healing assay

These assays were performed as previous described [15–17].

2.9. Co-immunoprecipitation (CoIP), coomassie blue staining and mass spectrometry (MS)

Protein was extracted from CRC cells using lysis buffer. The cell lysates were shaken slowly for 2 h at 4 °C with Flag, PI3K p85α antibodies or Control IgG (#AC005, ABclonal, USA). Then, ~20 μl of agarose beads (#sc-2003, Santa Cruz, USA) were added into the mixture, which were incubated at 4 °C overnight. The beads were washed, collected, and the proteins were eluted. The eluted proteins were separated by SDS-PAGE followed by coomassie blue staining (#PS111, EpiZyme, China). Then, the differential protein band was subjected to MS analysis in Wininnovate Bio (Shenzhen, China).

2.10. Western blotting

Western blotting was carried out as previous described [15]. These following antibodies were used in Western blotting: anti-ITGA7 (1:500, #bs-1816R, Bioss, China), anti-p65 (1:1000, #YM3111, immunoway, USA), anti-p-p65 (phospho Ser536) (1:1000, #YP0191, immunoway, USA), anti-Flag (1:1000, #20543-1-AP, Proteintech, USA), anti-CKAP4 (1:500, #A7777, ABclonal, USA), anti-PI3K p85α (1:1000, #R22768, ZENBIO, China), anti-Akt (1:1000, #60203-2-Ig, Proteintech, USA), anti-p-Akt (phospho Ser473) (1:1000, #YP0006, immunoway, USA),

anti-Histone H3 (1:1000, #YT2163, immunoway, USA), anti-GAPDH (1:1000, #FD0063, FDbio, China), anti-β-Tubulin (1:1000, #FD0064-50, FDbio, China), HRP labeled goat anti-mouse (1:5000, #FDM007, FDbio, China) and goat anti-rabbit IgG (1:5000, #FDR007, FDbio, China). The protein markers used in the research were purchased from ThermoFisher (#26616, ThermoFisher, USA) and EpiZyme (#WJ103, EpiZyme, China). The protein bands were detected by using FDBio-Dura ECL Kit (#FD8020, FDbio, China).

2.11. Immunofuorescence (IF)

IF was performed according to our previous study [18]. Antibodies of anti-p65 (1:200, #YM3111, immunoway, USA), anti-Flag (1:100, #66008-4-Ig, Proteintech, USA), anti-CKAP4 (1:100, #A7777, ABclonal, USA), anti-PI3K p85α (1:100, #222656, ZENBIO, China), dyLight 488 goat anti mouse IgG (1:200, #RS23210, immunoway, USA) and dylight 594 goat anti rabbit IgG were used in the assay.

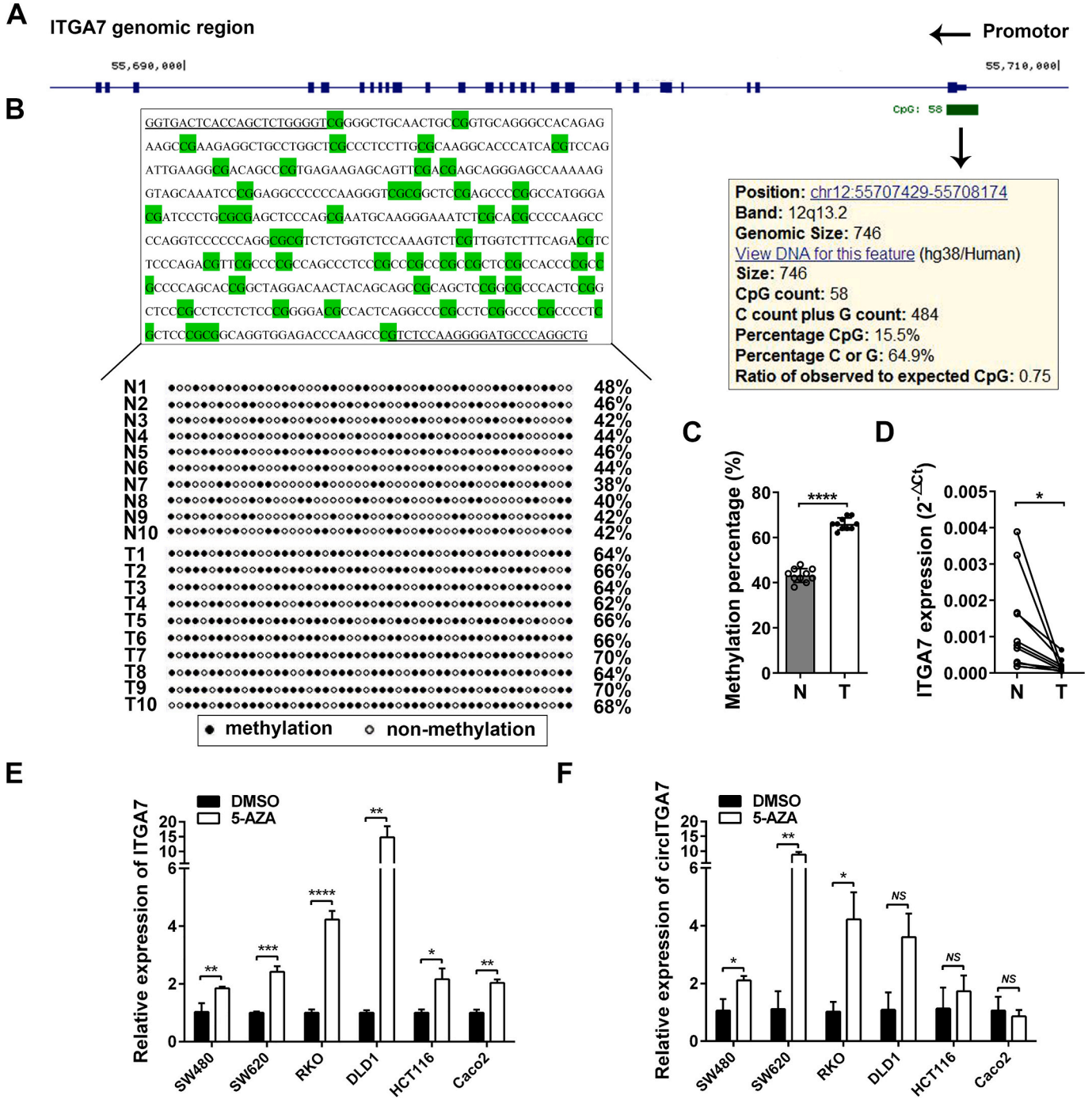

Fig. 1. Hypermethylation of ITGA7 promoter downregulates ITGA7 expression in CRC tissues. (A) The UCSC database showed the CpG island in the promoter region of ITGA7. (B, C) Determination of the degree of ITGA7 promoter methylation in 10 CRC tissues and matched normal tissues using the bisulfite modification and genomic sequencing (BSP) method. (D) The expression of ITGA7 in the same 10 CRC tissues and matched normal tissues was determined using RT-qPCR. N: matched normal tissues; T: CRC tissues. (E, F) The expression of ITGA7 and circITGA7 in CRC cells treated with 5-Aza-CdR for 48 h by RT-PCR analysis.

2.12. Immunohistochemistry

Immunohistochemical staining and the H-score calculation of p-Akt and p-p65 were carried out as in our previous study [17]. Phospho-AKT (Ser473) (1:100; #66444-1-Ig; Proteintech) and phospho-NF-KB p65 (Ser536) (1:100; #310013; ZENBIO) antibodies were used in the assay.

2.13. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, La Jolla, CA, USA). The measurement data were presented as mean ± standard deviation. The comparison between two groups was analyzed using the two-tailed Student's *t*-test. The paired t-test was used to check the difference of ITGA7 expression in

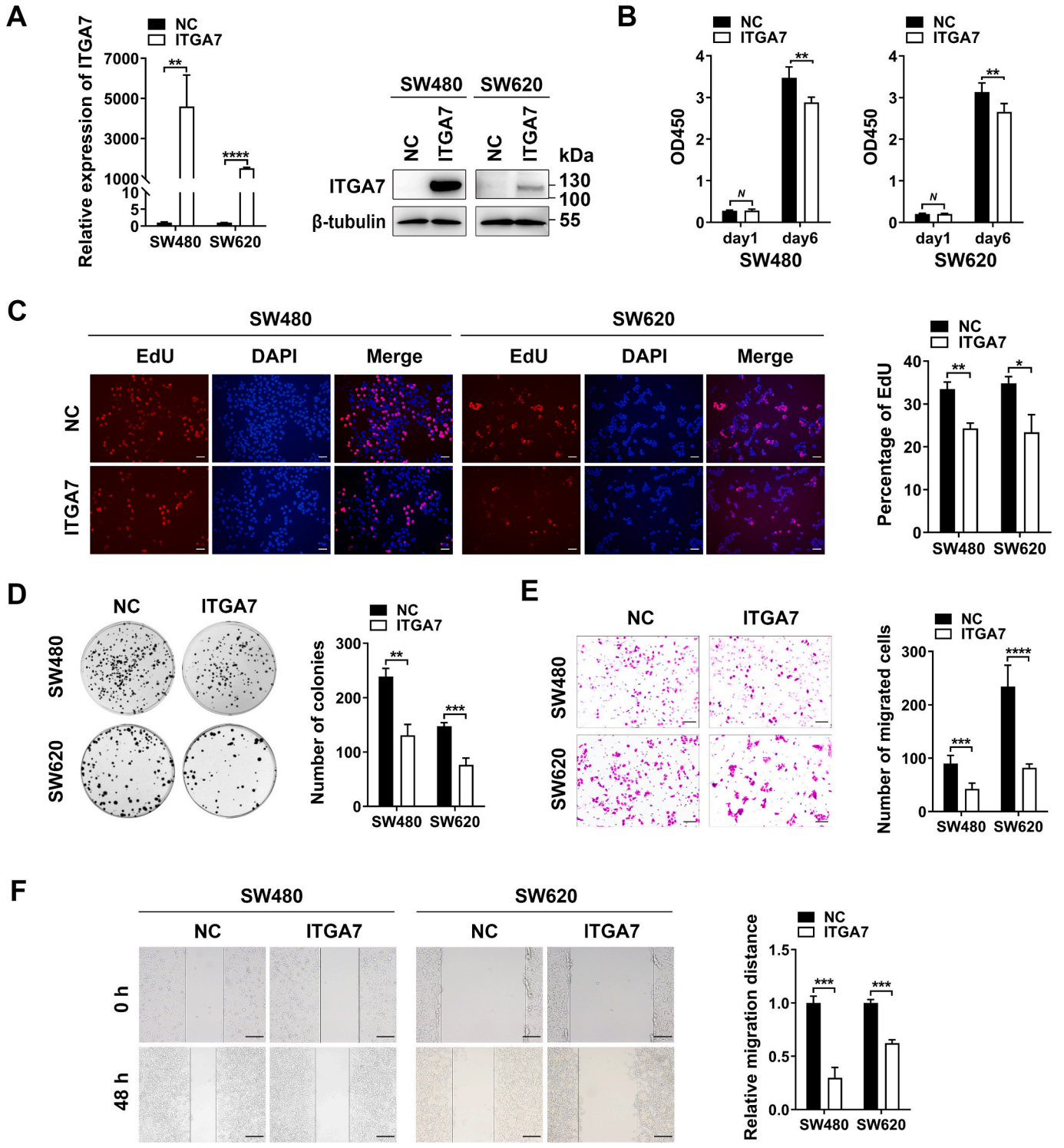

Fig. 2. ITGA7 inhibits the proliferation and migration of CRC cells. (A) The expression of ITGA7 mRNA and protein was upregulated in SW480 and SW620 cells by an ITGA7 overexpression vector. (B) CCK8 and (C) EdU assays showed that the forced ITGA7 expression promoted CRC cell proliferation. Scale bar: 100 μm. (D) A colony formation assay indicated that the elevated ITGA7 increased the number of colonies of SW480 and SW620 cells. (E) Transwell migration (Scale bar: 100 μm) and (F) wound healing (Scale bar: 200 μm) assays demonstrated that ITGA7 overexpression accelerated CRC cell migration.

CRC tissues and the matched normal tissues. Differences were considered to be significant when $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. *N*: not significant.

3. Results

3.1. Hypermethylation of ITGA7 promoter reduces ITGA7 expression in CRC

We previously found that ITGA7 was downregulated in CRC tissues and CRC cell lines and that the reduced ITGA7 expression promoted CRC cell proliferation and migration [15]. However, the factors underlying the ITGA7 decrease in CRC remained unknown. Because mutations and epigenetic alterations are the most common causes of tumor suppressor inactivation, we analyzed the mutation status of ITGA7 in CRC using the cBioPortal for Cancer Genomics database (http://www.cbioportal.org/). The results showed a mutation rate of ITGA7 of just 1.4 % (29/2081) in CRC (Supplementary Fig. S1), which suggested that mutation was not a main participant in the downregulation of ITGA7.

Therefore, we further examined the methylation status of the ITGA7 promoter. First, we analyzed CpG islands in the ITGA7 promoter using the UCSC database (http://genome.ucsc.edu/). The prediction revealed a single CpG island in the promoter region of ITGA7 (Fig. 1A). The GC content of the CpG island was 64.9 %, its size was 484 bp, the ratio of observed to expected CpG was 0.75, and the CpG count was 58 (Fig. 1A). In addition, the methylation degree of the CpG island in the ITGA7 promoter in 10 paired CRC tissues and matched normal tissues was determined using the bisulfite modification and genomic sequencing (BSP) method. The results showed that the CpG island methylation degree of ITGA7 promoter was higher in CRC tissues than in normal tissues (Fig. 1B and C). Correspondingly, the expression of ITGA7 was downregulated in these 10 CRC tissues compared to the matched normal tissues (Fig. 1D). Next, 5-Aza-CdR was used to inhibit DNA methylation in CRC cells, and an increase in ITGA7 and circITGA7 (the circular transcript form of ITGA7) was observed (Fig. 1E and F). These results demonstrated that hypermethylation of the ITGA7 promoter resulted in ITGA7 silencing in CRC.

3.2. ITGA7 inhibits CRC cell proliferation and migration

We previously found that reduced ITGA7 expression in CRC cells could increase proliferation and migration [15]. In the present study, we further performed a gain-of-function assay to confirm the inhibitory role of ITGA7 in CRC progression (Fig. 2A). CCK8 and EdU assays indicated that elevated ITGA7 expression inhibited CRC cell proliferation (Fig. 2B and C). A colony formation assay showed that increased ITGA7 expression suppressed the clonogenicity of CRC cells (Fig. 2D). Additionally, Transwell migration and wound healing assays demonstrated that the upregulation of ITGA7 attenuated the migratory capacity of SW480 and SW620 cells (Fig. 2E and F). These findings revealed the inhibitory role of ITGA7 in CRC cell proliferation and migration.

3.3. Knockdown of ITGA7 upregulates MMP9, SETD7, and ADAM15 transcription

In our previous research, we used RNA sequencing to screen 125 differentially expressed genes in SW480 cells after knockdown of ITGA7 expression [15]. There were 48 upregulated genes, and 26 genes had an expression level higher than 5 FPKM (Fig. 3A and Table S3). Based on the literature, we selected 6 oncogenes among the 26 genes—MMP9, FOSL1, ADAM15, PRMT6, SETD7, and TM4SF1—for further verification of their expression in CRC cells with increased or decreased ITGA7 expression (Fig. 3A). RT-qPCR results showed that ITGA7 knockdown upregulated the expression of MMP9, SETD7, and ADAM15 in SW480, SW620, and HCT116 cells (Fig. 3B). In contrast, overexpression of ITGA7 reduced the expression of these three genes (Fig. 3C). These findings indicated that MMP9, ADAM15, and SETD7 expression is regulated by ITGA7.

3.4. MMP9, SETD7, and ADAM15 participate in the inhibitory role of ITGA7 in CRC cell proliferation and migration

To further validate whether MMP9, SETD7, and ADAM15 were involved in the effect of ITGA7 silencing on CRC progression, we performed rescue experiments. An EdU assay revealed that knockdown of MMP9 or SETD7 restored the enhanced proliferative ability of CRC cells induced by ITGA7 knockdown (Fig. 4A). However, downregulation of ADAM15 was unable to attenuate the effect of ITGA7 on CRC cell proliferation (Fig. 4A). Similar to the EdU results, Transwell migration and wound healing assays demonstrated that the accelerated migratory capacity caused by ITGA7 silencing could be reverted by reduced MMP9, SETD7, or ADAM15 expression (Fig. 4B and C). These results showed that MMP9, SETD7, and ADAM15 participated in the regulatory effect of ITGA7 on CRC malignant behavior.

3.5. Downregulation of ITGA7 expression upregulates MMP9, SETD7, and ADAM15 transcription by increasing NF-κB nuclear transport

To explore the mechanisms by which ITGA7 regulates the transcription of MMP9, SETD7, and ADAM15, we predicted the transcription factors of these genes using the KEGG database (https://www.kegg.jp/kegg/pathway.html) and the Cistrome Data Browser (http://cistrome.org/db/#/). In the KEGG database, we found that NF-κB is the specific transcription factor for MMP9. In the Cistrome Data Browser, we observed the presence of NF-κB binding peaks in the promoters of MMP9, SETD7, and ADAM15 in CRC cell lines. Moreover, in our previous study, the differentially expressed genes induced by decreased ITGA7 expression were enriched in the NF-κB pathway [15]. Therefore, NF-κB is the most likely transcription factor to mediate the regulatory role of ITGA7 in MMP9, SETD7, and ADAM15 transcription.

The NF-κB family contains five members and p65 is the most important subunit for their transcriptional activity. When NF-κB is activated, p65 is phosphorylated and translocates to the nucleus to regulate gene transcription. We found that overexpression of ITGA7 resulted in a decrease in p-p65 while ITGA7 knockdown had the opposite effect (Fig. 5A). In addition, IF indicated that upregulation of ITGA7 inhibited p65 nuclear translocation (Fig. 5B). Furthermore, we isolated the cytoplasmic and nuclear fractions of CRC cells after ITGA7 knockdown or overexpression to further verify the effect of ITGA7 on the subcellular location of p65. As shown in Fig. 5C, ITGA7 downregulation increased the level of nuclear p65. As shown in Fig. 5C, downregulated ITGA7 expression increased the level of nuclear p65. Conversely, ITGA7 upregulation inhibited p65 translocation to the nucleus. Moreover, BAY 11-7082, an inhibitor of the NF-κB pathway, was used to investigate the role of NF-κB in the transcription of MMP9, SETD7, and ADAM15. The results showed that BAY 11-7082 reduced the elevated expression of MMP9, SETD7, and ADAM15 caused by ITGA7 silencing (Fig. 5D).

3.6. ITGA7 suppresses the PI3K/AKT/NF-κB pathway by interfering with the interaction of PI3K p85α and CKAP4 through binding to CKAP4

To further investigate the molecular mechanisms by which ITGA7 regulates NF-κB nuclear transport, we performed a CoIP assay to screen the interacting targets of ITGA7. After Coomassie blue staining, two differential bands in the ITGA7 group were observed compared to the control (Fig. 6A). One band was ITGA7 protein according to the band mass while the second candidate band was subjected to MS identification (Fig. 6A). Among 77 proteins identified by MS, there were 16 proteins (except 2 keratin proteins) with masses ranging from 65 kDa to 70 kDa (Table S4). CKAP4 was the most likely target because it had the highest −10lgP score (Fig. 6B). Furthermore, CoIP and IF confirmed the interaction and colocalization of ITGA7 and CKAP4 (Fig. 6C and D).

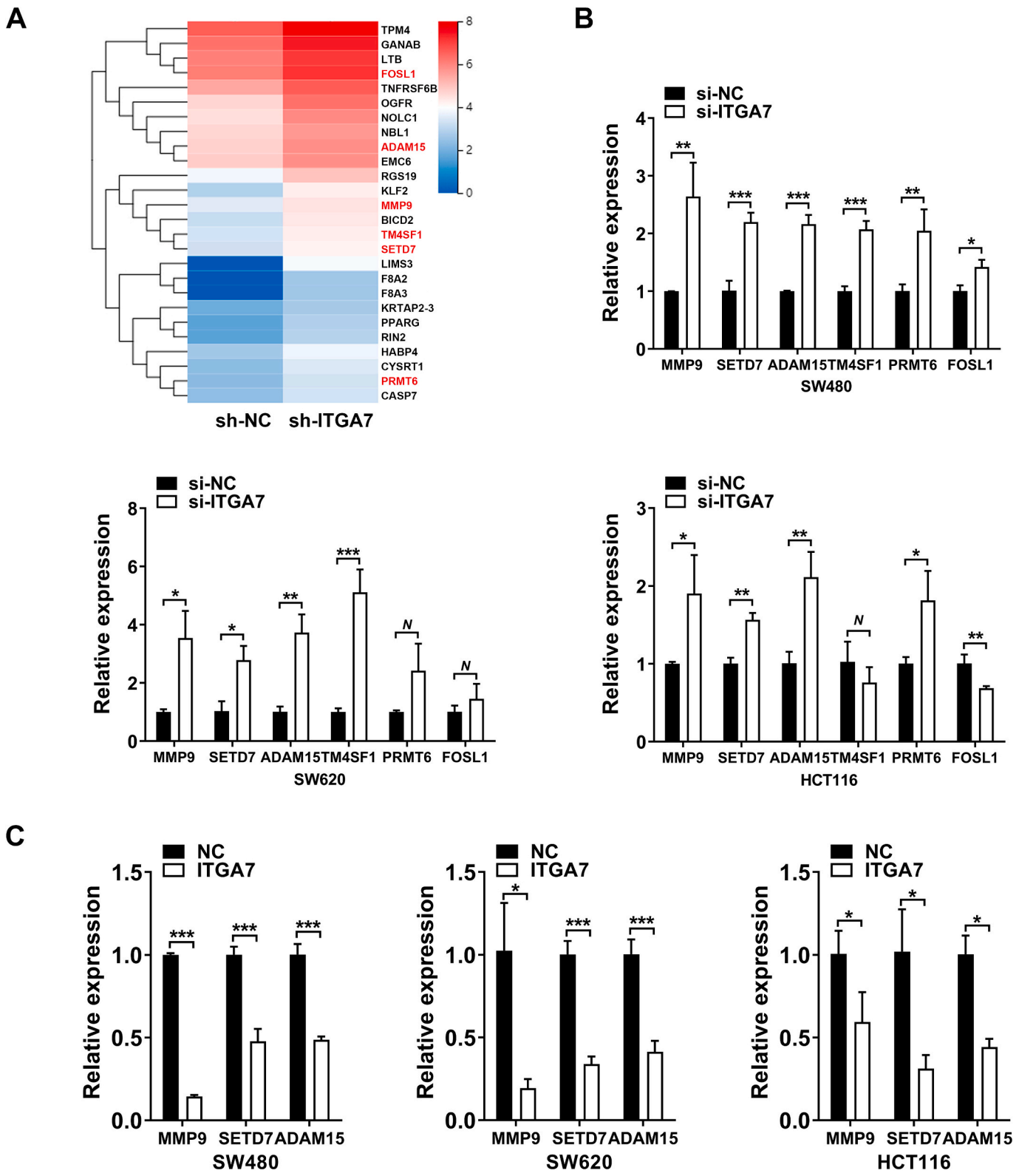

Fig. 3. Knockdown of ITGA7 increases the expression of MMP9, SETD7, and ADAM15 in CRC cells. (A) Heatmap showing the top 26 upregulated genes in SW480 cells with knockdown of ITGA7 expression compared with control cells. Genes in red frames were selected for further verification. (B) The expression of the indicated six genes in SW480, SW620, and HCT116 cells after knockdown of ITGA7 expression was determined by RT-qPCR. (C) MMP9, SETD7, and ADAM15 expression was downregulated in SW480, SW620, and HCT116 cells with overexpression of ITGA7.

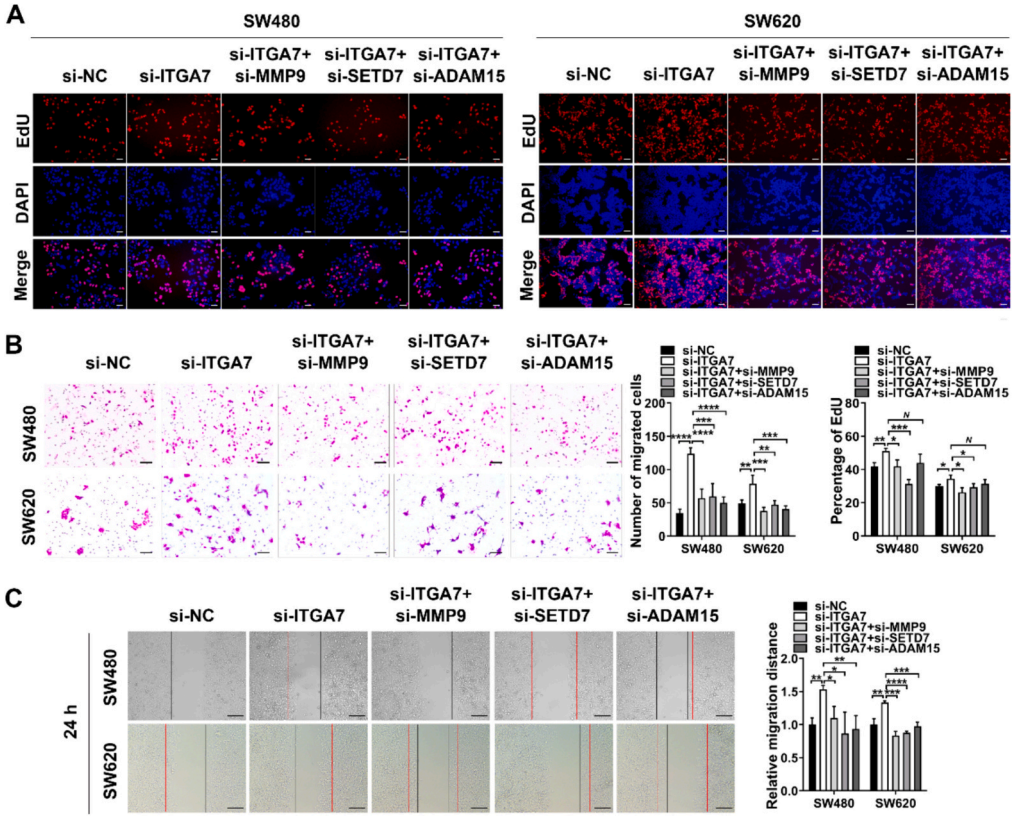

Fig. 4. MMP9, SETD7, and ADAM15 participate in the inhibitory role of ITGA7 in CRC cell proliferation and migration. (A) The proliferation of SW480 and SW620 cells was determined using an EdU assay after their transfection with siRNAs targeting the indicated genes. Scale bar: 100 μm. (B) Transwell migration (Scale bar: 100 μm) and (C) wound healing (Scale bar: 200 μm) assays showed that MMP9, SETD7, or ADAM15 silencing rescued the enhanced migratory ability caused by ITGA7 knockdown. Red line: migration initiation site; black line: migration termination site after 24 h.

CKAP4 has been reported to be upregulated in cancers and to activate the PI3K/AKT pathway by binding to PI3K p85α [19]. We found that neither knockdown nor overexpression of ITGA7 affected the CKAP4 expression level (Fig. 6E). However, CoIP and IF indicated that upregulation of ITGA7 interfered with the binding and colocalization of PI3K p85α and CKAP4 (Fig. 6F and G). Consistent with these findings, ITGA7 upregulation decreased the p-Akt level while ITGA7 knockdown had the opposite effect (Fig. 6H). Because NF-κB is downstream of PI3K/AKT, these data are in accordance with the result that ITGA7 inactivated the NF-κB pathway. To further verify the regulatory role of ITGA7 in the PI3K/AKT/NF-κB pathway, we used immunohistochemistry to assess p-Akt and p-p65 levels in the subcutaneous tumors of nude mice with ITGA7 silencing, which were created in our previous study [15]. The results showed that ITGA7 knockdown increased p-Akt and p-p65 levels in vivo (Fig. 7A).

Taken together, we propose that hypermethylation of ITGA7 promoter suppresses ITGA7 expression in CRC. ITGA7 interferes with the interaction of PI3K p85α and CKAP4 by binding to CKAP4 and thereby suppresses the PI3K/AKT/NF-κB pathway to inhibit CRC cell proliferation and migration (Fig. 7B).

4. Discussion

We previously reported the downregulation of ITGA7 in CRC tissues and CRC cell lines [15]. In the current research, we propose that hypermethylation of ITGA7 promoter reduces the expression of ITGA7 in CRC. Aberrant DNA methylation is one of the common causes of the loss of suppressor genes. In line with our findings, the hypermethylation of ITGA7 promoter in pleural mesothelioma also resulted in reduced ITGA7 expression in cancer cells [13].

In terms of the function of ITGA7 silencing in CRC, we previously revealed that ITGA7 downregulation promoted CRC cell proliferation and migration [15]. In the current study, a gain-of-function assay showed that ITGA7 possessed an inhibitory effect on CRC cell proliferation and migration. Similar to our findings, previous studies found that ITGA7 functions as a suppressor to suppress cancer cell progression in breast cancer, papillary thyroid carcinoma, prostate cancer, and malignant pleural mesothelioma [10–13]. However, in other cancer types, such as tongue squamous cell carcinoma, hepatocellular carcinoma, non-small cell lung cancer, endometrial cancer, and glioblastoma [5–9], ITGA7 served as an oncogene to promote cancer development. The diverse downstream targets and interacting proteins of ITGA7 may explain the opposing functions of ITGA7 in different malignancies. For example, ITGA7 upregulates cancer stem cell markers, such as CD44 and CD133, to promote cancer cell proliferation and stemness in tongue squamous cell carcinoma and hepatocellular carcinoma [5,6]. However, based on RNA sequencing and verification, we proposed MMP9, SETD7, and ADAM15 to be the targets of ITGA7 in CRC. ITGA7 knockdown promoted the transcription of MMP9, SETD7, and ADAM15. MMP9, a well-known metastasis-associated protein, has been reported to promote the proliferation and metastasis of multiple tumors [20,21]. For the other two proteins, more and more studies have demonstrated that SETD7 and ADAM15 play crucial roles in malignant tumor behavior. For example, SETD7 is highly expressed in bladder cancer [22] and triple-negative breast cancer [23], and overexpression of SETD7 promotes the proliferation and migration of cancer cells. In addition, SETD7 is a potential diagnostic biomarker for CRC and knockdown of SETD7 inhibits CRC cell proliferation [24]. Moreover, ADAM15 is upregulated in several types of cancer, such as bladder cancer and non-small cell lung cancer, and the elevated expression of ADAM15 has been associated with the metastatic progression of cancer [25,26].

Consistent with these reports, functional rescue experiments

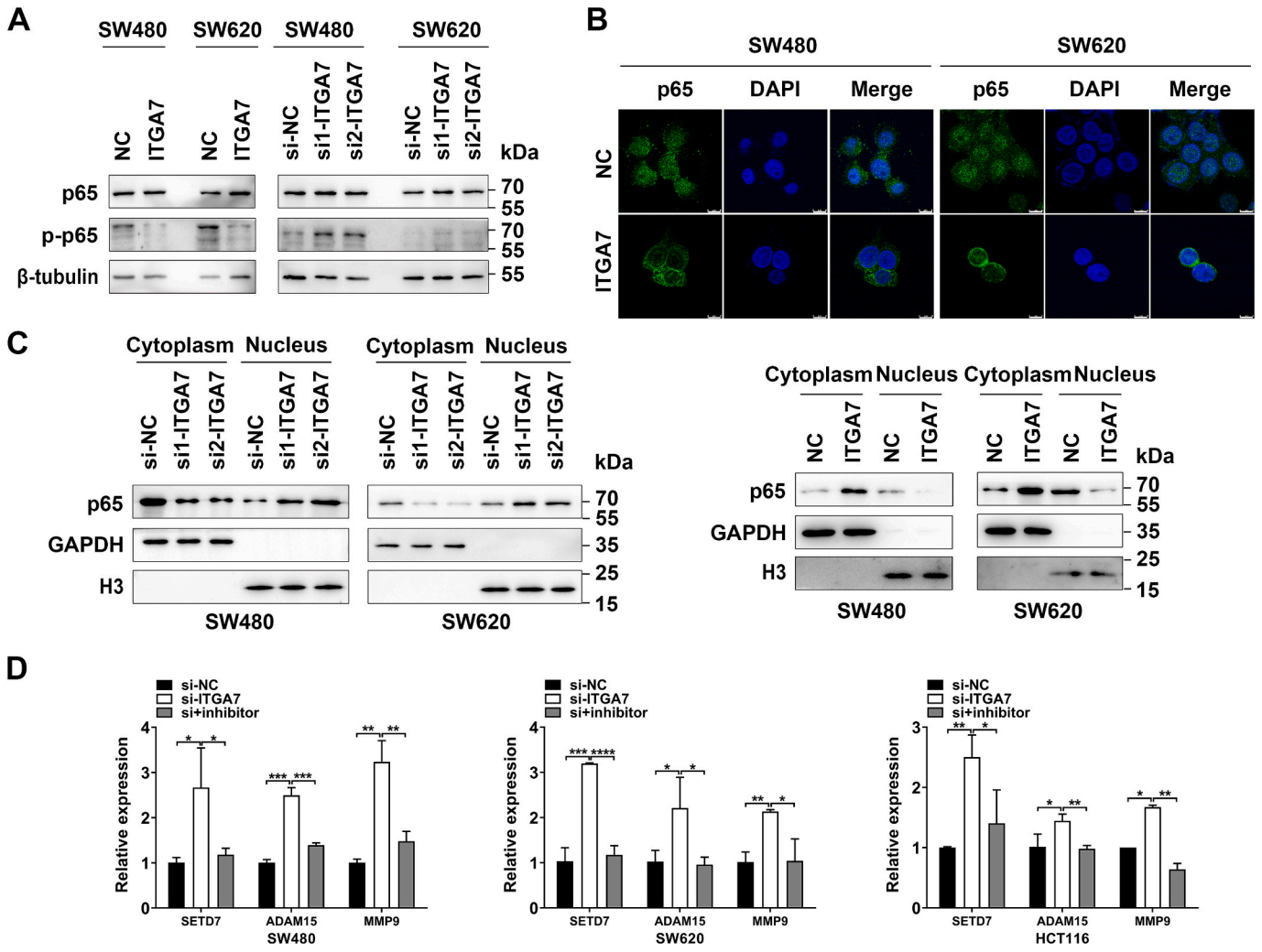

Fig. 5. Downregulation of ITGA7 expression upregulates the transcription of MMP9, SETD7, and ADAM15 by increasing NF-κB nuclear transport. (A) Western blot revealed that ITGA7 upregulation suppressed p65 phosphorylation while ITGA7 knockdown increased the p-p65 level. (B) IF revealed that upregulation of ITGA7 inhibited the nuclear transport of p65. Scale bar: 8 μm. (C) Downregulation of ITGA7 expression increased the nuclear p65 level, whereas overexpression of ITGA7 decreased p65 nuclear transport in SW480 and SW620 cells. (D) The elevated MMP9, SETD7, and ADAM15 transcription induced by reduced ITGA7 expression in SW480 and SW620 cells was blocked by BAY 11-7082 (an inhibitor of the NF-κB pathway).

indicated that knockdown of MMP9 or SETD7 antagonized the cancer-promoting effect of ITGA7 silencing. Nevertheless, knockdown of ADAM15 only attenuated the enhanced migratory ability of CRC cells induced by ITGA7 silencing but did not restore the increased proliferative capacity. These findings demonstrated that ADAM15 merely participated in the regulatory role of ITGA7 in CRC migration. Although we have confirmed that MMP9, SETD7, and ADAM15 mediated the activity of ITGA7, other genes may also contribute to the function of ITGA7 in CRC, and these require further study.

In addition, we presented the mechanisms by which ITGA7 knockdown promoted the transcription of MMP9, SETD7, and ADAM15 by increasing NF-κB nuclear transport. Based on the bioinformatics analysis, NF-κB was the transcription factor for these three genes in CRC cell lines. p65 (RelA) is a subunit of NF-κB with transcriptional activity [27,28]. As the end-point molecule in the NF-κB pathway, the phosphorylation and nuclear translocation of p65 represents NF-κB activation [29]. We observed that ITGA7 knockdown increased the levels of p-p65 and nuclear p65, thereby upregulating MMP9, SETD7, and ADAM15 transcription. Furthermore, the upregulated transcription could be reverted by NF-κB inhibitor. These findings indicated the involvement of NF-κB in the ability of ITGA7 to regulate MMP9, SETD7, and ADAM15 expression.

How ITGA7 regulated NF-κB nuclear translocation is unclear. Tan et al. demonstrated that ITGA7 led to the cytoplasmic translocation of NF-κB by binding to tissue inhibitor of metalloproteinase 3 (TIMP3) in prostate cancer [12]. In our research, we identified CKAP4 as the interacting protein of ITGA7 in CRC. Recent studies have recognized CKAP4 as an oncogene in several types of cancer [30–34], and it is expected to be a therapeutic target in cancer. CKAP4 has been reported to activate the PI3K/AKT pathway by forming a complex with PI3K p85α [19]. In the present work, we found that ITGA7 bound to CKAP4 to interfere with the interaction of CKAP4 and PI3K p85α, thereby suppressing the PI3K/AKT/NF-κB pathway. However, the hypermethylation of ITGA7 promoter in CRC downregulated ITGA7 expression, which resulted in CRC progression. Our results suggest that modulating aberrant epigenetic modifications or administering exogenous ITGA7 to CRC patients with abnormal PI3K/AKT/NF-κB activation may be a promising therapeutic strategy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbamcr.2024.119785.

CRediT authorship contribution statement

Jianjun Wang: Writing – review & editing, Validation, Supervision,

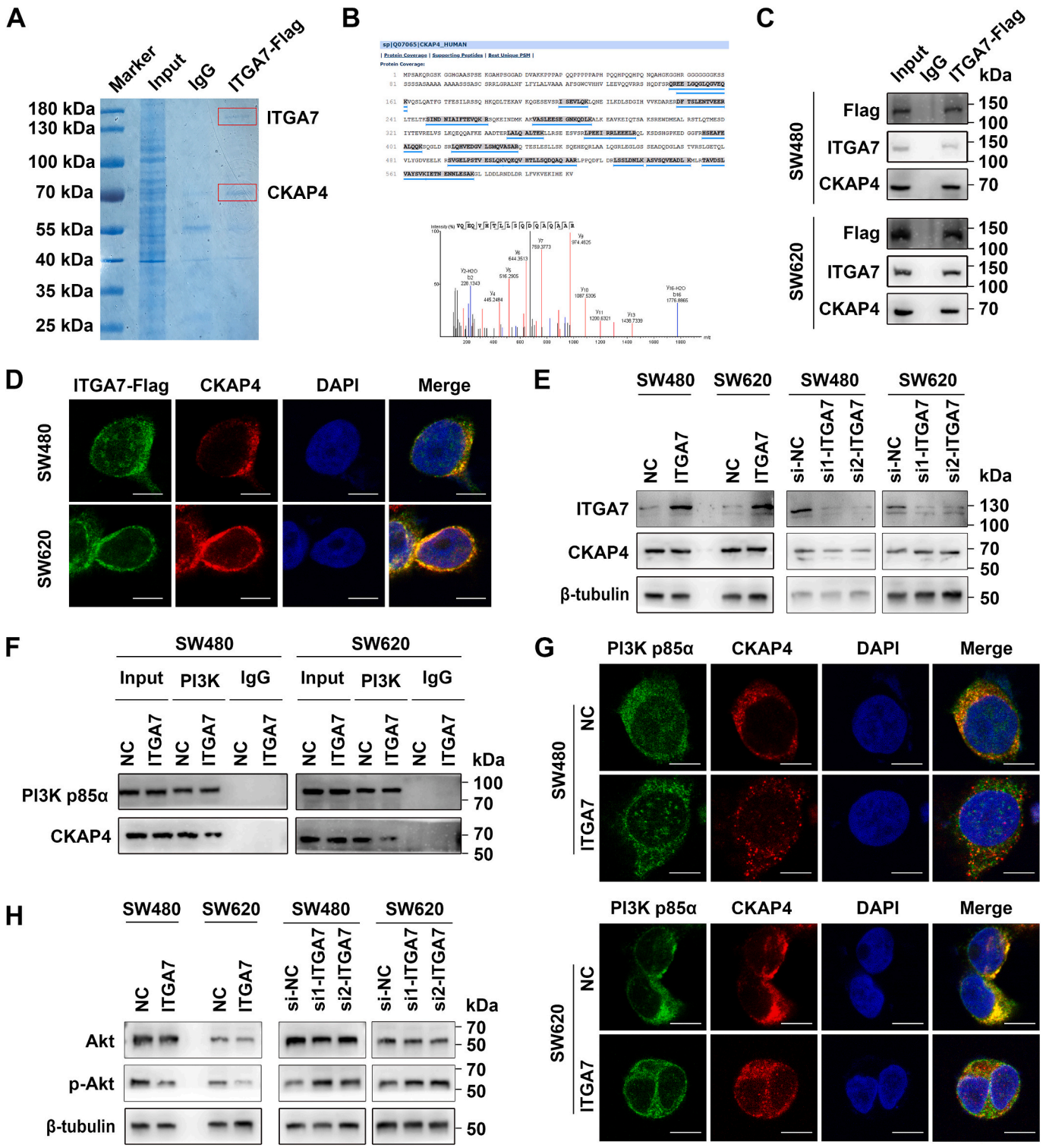

Fig. 6. ITGA7 inhibits the interaction of PI3K p85α and CKAP4 to suppress the PI3K/AKT/NF-κB pathway by binding to CKAP4. (A) A CoIP assay was used to identify the possible interacting targets of ITGA7. Coomassie blue staining showed the ITGA7 band and a differentially expressed band marked by red frames in the ITGA7 overexpression group compared to the control group, and CKAP4 was identified to be the most likely candidate target of ITGA7 by MS. (B) The amino acid sequences of CKAP4 and 17 peptides identified by MS. Below is the peak diagram of one unique peptide of CKAP4. (C) A CoIP assay verified the interaction of ITGA7 and CKAP4 in SW480 and SW620 cells. (D) IF demonstrated the colocalization of ITGA7 and CKAP4 in SW480 and SW620 cells. Scale bar: 8 μm. (E) The expression of CKAP4 in SW480 and SW620 cells after ITGA7 downregulation or upregulation, as determined by Western blot. (F) A CoIP assay indicated that ITGA7 overexpression interfered with the interaction of CKAP4 and PI3K p85α in SW480 and SW620 cells. (G) IF showed that upregulation of ITGA7 inhibited the colocalization of CKAP4 and PI3K p85α in CRC cells. Scale bar: 8 μm. (H) Western blot indicated that ITGA7 upregulation decreased the p-Akt level while ITGA7 knockdown had the opposite effect.

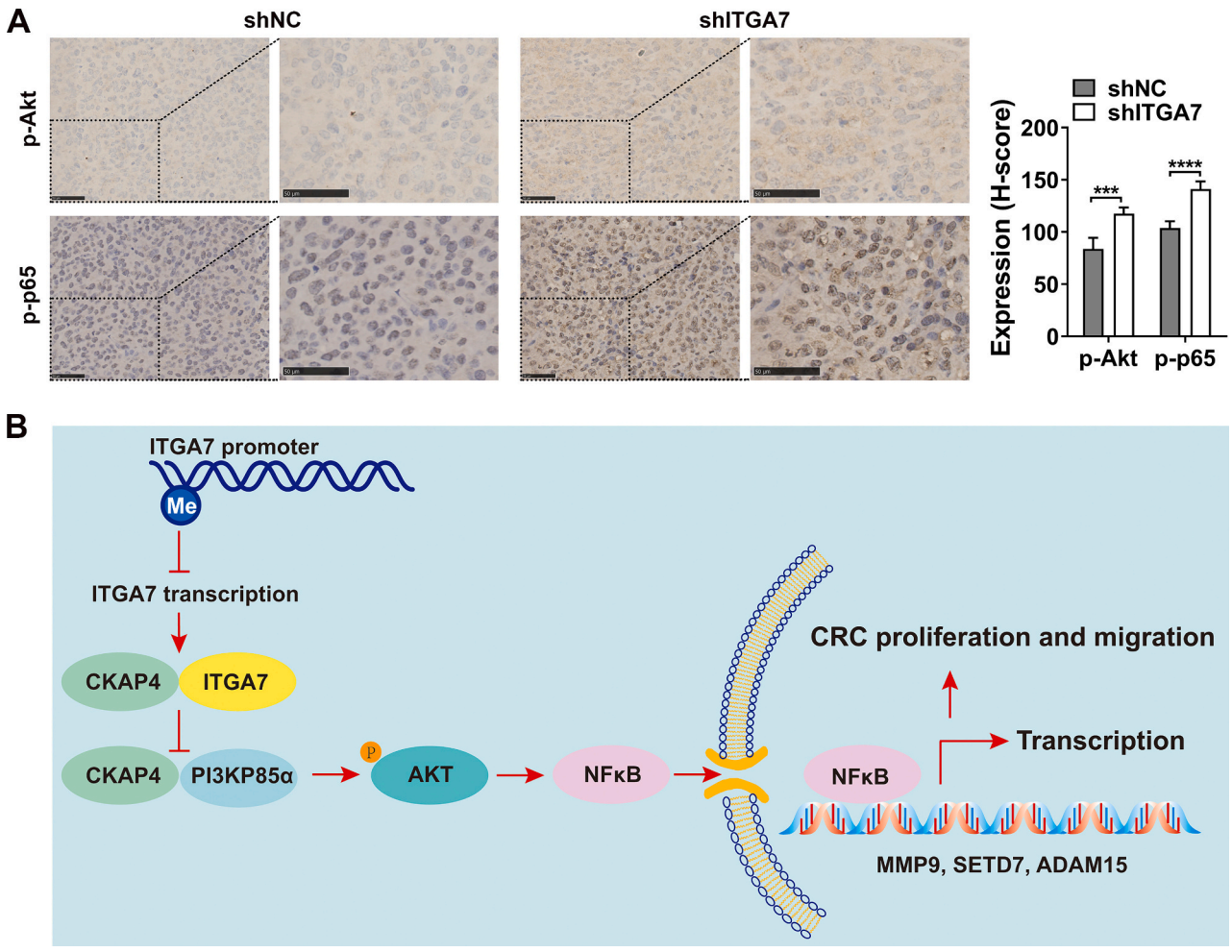

Fig. 7. Knockdown of ITGA7 increases p-Akt and p-p65 levels in vivo. (A) The expression levels of p-Akt and p-p65 were upregulated in the subcutaneous tumors of nude mice with ITGA7 silencing compared to the control group. $n = 5$. Scale bar: 50 μm. (B) Proposed model: hypermethylation of the ITGA7 promoter suppresses ITGA7 expression in CRC. ITGA7 interferes with the interaction of PI3K and CKAP4 by binding to CKAP4, thereby suppressing the PI3K/AKT/NF-κB pathway to inhibit CRC cell proliferation and migration.

Investigation, Funding acquisition, Formal analysis. **Yu Wang:** Writing – review & editing, Formal analysis. **Jijun Zhu:** Writing – review & editing, Supervision. **Lili Wang:** Writing – review & editing, Formal analysis. **Yanlin Huang:** Writing – review & editing, Funding acquisition. **Huiru Zhang:** Writing – review & editing, Funding acquisition. **Xiaoyan Wang:** Writing – review & editing, Supervision, Project administration. **Xiaomin Li:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Funding acquisition, 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.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by Suqian Sci&Tech Program (Grant Number: KY202304), The Social Development Projects of Key R&D Programs in Xuzhou city (Grant Number: KC23247), Startup Fund for Excellent Researchers at Xuzhou Medical University (Grant Number: RC20552212), National Natural Science Foundation of China (Grant Number: 81902479), Guangdong Basic and Applied Basic Research Foundation (Grant Number: 2021A1515010665), Natural Science Foundation of the Anhui provincial High-Education Institutions of China (Grant Number: KJ2021A0828), National College Students Innovation and Entrepreneurship Training Program (Grant Number: 202210368047), College Students Innovation and Entrepreneurship Training Program of Anhui provincial (Grant Number: S202310368012).

References

[1] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. (2021), https://doi.org/10.3322/caac.21660.

[2] S.E. LaFlamme, K.L. Auer, Integrin signaling, Semin. Cancer Biol. 7 (3) (1996) 111–118, https://doi.org/10.1006/scbi.1996.0016.

[3] F. Liu, Q. Wu, Z. Dong, K. Liu, Integrins in cancer: emerging mechanisms and therapeutic opportunities, Pharmacol. Ther. 247 (2023) 108458, https://doi.org/10.1016/j.pharmthera.2023.108458.

[4] I. Łasińska, J. Mackiewicz, Integrins as a new target for cancer treatment, Anticancer Agents Med Chem. 19 (5) (2019) 580–586, https://doi.org/10.2174/1871520618666181119103413.

[5] Z. Lv, Y. Yang, C. Yang, Integrin α7 correlates with worse clinical features and prognosis, and its knockdown inhibits cell proliferation and stemness in tongue

squamous cell carcinoma, Int. J. Oncol. 56 (1) (2020) 69–84, https://doi.org/10.3892/ijo.2019.4927.
[6] J.C. Ge, Y.X. Wang, Z.B. Chen, D.F. Chen, Integrin alpha 7 correlates with poor clinical outcomes, and it regulates cell proliferation, apoptosis and stemness via PTK2-PI3K-Akt signaling pathway in hepatocellular carcinoma, Cell. Signal. 66 (2020) 109465, https://doi.org/10.1016/j.cellsig.2019.109465.
[7] X. Shi, F. Wen, G. Xu, Y. Hu, Integrin-α7 expression positively correlates with CD44 and CD133 expressions, and its high expression associates with advanced tumor features as well as poor survivals in non-small cell lung cancer patients, Transl. Cancer Res. 8 (5) (2019) 1806–1816, https://doi.org/10.21037/tcr.2019.08.31.
[8] M. Liang, C. Liu, T. Lei, S. Guo, J. Min, Effect of integrin α7 on cell proliferation, invasion, apoptosis and the PI3K/AKT pathway, and its association with clinicopathological features in endometrial cancer, Oncol. Lett. 25 (1) (2023) 26, https://doi.org/10.3892/ol.2022.13612.
[9] T.L. Haas, M.R. Sciuto, L. Brunetto, C. Valvo, M. Signore, M.E. Fiori, et al., Integrin α7 is a functional marker and potential therapeutic target in glioblastoma, Cell Stem Cell 21 (1) (2017) 35–50.e9, https://doi.org/10.1016/j.stem.2017.04.009.
[10] A. Bhandari, E. Xia, Y. Zhou, Y. Guan, J. Xiang, L. Kong, et al., ITGA7 functions as a tumor suppressor and regulates migration and invasion in breast cancer, Cancer Manag. Res. 10 (2018) 969–976, https://doi.org/10.2147/cmar.s160379.
[11] Y. Guan, A. Bhandari, E. Xia, L. Kong, X. Zhang, O. Wang, Downregulating integrin subunit alpha 7 (ITGA7) promotes proliferation, invasion, and migration of papillary thyroid carcinoma cells through regulating epithelial-to-mesenchymal transition, Acta Biochim. Biophys. Sin. Shanghai 52 (2) (2020) 116–124, https://doi.org/10.1093/abbs/gmz144.
[12] L.Z. Tan, Y. Song, J. Nelson, Y.P. Yu, J.H. Luo, Integrin α7 binds tissue inhibitor of metalloproteinase 3 to suppress growth of prostate cancer cells, Am. J. Pathol. 183 (3) (2013) 831–840, https://doi.org/10.1016/j.ajpath.2013.05.010.
[13] V. Laszlo, M.A. Hoda, T. Garay, C. Pirker, B. Ghanim, T. Klikovits, et al., Epigenetic down-regulation of integrin α7 increases migratory potential and confers poor prognosis in malignant pleural mesothelioma, J. Pathol. 237 (2) (2015) 203–214, https://doi.org/10.1002/path.4567.
[14] N. Gwili, S.J. Jones, W.A. Amri, I.M. Carr, S. Harris, B.V. Hogan, et al., Transcriptome profiles of stem-like cells from primary breast cancers allow identification of ITGA7 as a predictive marker of chemotherapy response, Br. J. Cancer 125 (7) (2021) 983–993, https://doi.org/10.1038/s41416-021-01484-w.
[15] X. Li, J. Wang, C. Zhang, C. Lin, J. Zhang, W. Zhang, et al., Circular RNA circ ITGA7 inhibits colorectal cancer growth and metastasis by modulating the Ras pathway and upregulating transcription of its host gene ITGA7, J. Pathol. 246 (2) (2018) 166–179, https://doi.org/10.1002/path.5125.
[16] W. Zhang, Y. Lu, X. Li, J. Zhang, L. Zheng, W. Zhang, et al., CDCA3 promotes cell proliferation by activating the NF-κB/cyclin D1 signaling pathway in colorectal cancer, Biochem. Biophys. Res. Commun. 500 (2) (2018) 196–203, https://doi.org/10.1016/j.bbrc.2018.04.034.
[17] X. Li, J. Wang, W. Lin, Q. Yuan, Y. Lu, H. Wang, et al., Circ EXOC6B interacting with RRAGB, an mTORC1 activator, inhibits the progression of colorectal cancer by antagonizing the HIF1A-RRAGB-mTORC1 positive feedback loop, Mol. Cancer 21 (1) (2022) 135, https://doi.org/10.1186/s12943-022-01600-1.
[18] W. Zhang, Y. Lu, X. Li, J. Zhang, W. Lin, W. Zhang, et al., IPO5 promotes the proliferation and tumourigenicity of colorectal cancer cells by mediating RASAL2 nuclear transportation, J. Exp. Clin. Cancer Res. 38 (1) (2019) 296, https://doi.org/10.1186/s13046-019-1290-0.
[19] H. Kimura, K. Fumoto, K. Shojima, S. Nojima, Y. Osugi, H. Tomihara, et al., CKAP4 is a Dickkopf 1 receptor and is involved in tumor progression, J. Clin. Invest. 126 (7) (2016) 2689–2705, https://doi.org/10.1172/jci84658.
[20] Q. Xue, L. Cao, X.Y. Chen, J. Zhao, L. Gao, S.Z. Li, et al., High expression of MMP9 in glioma affects cell proliferation and is associated with patient survival rates, Oncol. Lett. 13 (3) (2017) 1325–1330, https://doi.org/10.3892/ol.2017.5567.
[21] J. Mo, T. Zheng, L. Lei, P. Dai, J. Liu, H. He, et al., MicroRNA-1253 suppresses cell proliferation migration and invasion of osteosarcoma by targeting MMP9, Technol. Cancer Res. Treat. 20 (2021) 1533033821995278, https://doi.org/10.1177/1533033821995278.
[22] J. Lv, Q. Wu, K. Li, K. Bai, H. Yu, J. Zhuang, et al., Lysine N-methyltransferase SETD7 promotes bladder cancer progression and immune escape via STAT3/PD-L1 cascade, Int. J. Biol. Sci. 19 (12) (2023) 3744–3761, https://doi.org/10.7150/ijbs.87182.
[23] L. Gao, J. Zhang, Q. Long, Y. Yang, Y. Li, G. Li, et al., SETD7 promotes metastasis of triple-negative breast cancer by YY1 lysine methylation, Biochim. Biophys. Acta Mol. basis Dis. 1869 (7) (2023) 166780, https://doi.org/10.1016/j.bbadis.2023.166780.
[24] B. Duan, J. Bai, J. Qiu, J. Wang, C. Tong, X. Wang, et al., Histone-lysine N-methyltransferase SETD7 is a potential serum biomarker for colorectal cancer patients, EBioMedicine 37 (2018) 134–143, https://doi.org/10.1016/j.ebiom.2018.10.036.
[25] D.D. Dong, H. Zhou, G. Li, ADAM15 targets MMP9 activity to promote lung cancer cell invasion, Oncol. Rep. 34 (5) (2015) 2451–2460, https://doi.org/10.3892/or.2015.4203.
[26] G. Lorenzatti Hiles, A. Bucheit, J.R. Rubin, A. Hayward, A.L. Cates, K.C. Day, et al., ADAM15 is functionally associated with the metastatic progression of human bladder cancer, PloS One 11 (3) (2016) e0150138, https://doi.org/10.1371/journal.pone.0150138.
[27] S. Mirzaei, S. Saghari, F. Bassiri, R. Raesi, A. Zarrabi, K. Hushmandi, et al., NF-κB as a regulator of cancer metastasis and therapy response: a focus on epithelial-mesenchymal transition, J. Cell. Physiol. 237 (7) (2022) 2770–2795, https://doi.org/10.1002/jcp.30759.
[28] A. Panwalkar, S. Verstovsek, F. Giles, Nuclear factor-kappaB modulation as a therapeutic approach in hematologic malignancies, Cancer 100 (8) (2004) 1578–1589, https://doi.org/10.1002/cncr.20182.
[29] M.Y. Park, H.D. Jang, S.Y. Lee, K.J. Lee, E. Kim, Fas-associated factor-1 inhibits nuclear factor-kappaB (NF-kappaB) activity by interfering with nuclear translocation of the RelA (p65) subunit of NF-kappaB, J. Biol. Chem. 279 (4) (2004) 2544–2549, https://doi.org/10.1074/jbc.M304565200.
[30] Z.Y. Chen, T. Wang, X. Gan, S.H. Chen, Y.T. He, Y.Q. Wang, et al., Cytoskeleton-associated membrane protein 4 is upregulated in tumor tissues and is associated with clinicopathological characteristics and prognosis in hepatocellular carcinoma, Oncol. Lett. 19 (6) (2020) 3889–3898, https://doi.org/10.3892/ol.2020.11499.
[31] M. Cai, W. Wu, S. Deng, Q. Yang, H. Wu, H. Wang, et al., Expression of cytoskeleton-associated protein 4 is associated with poor prognosis and metastasis in nasopharyngeal carcinoma, Exp. Biol. Med. (Maywood) 248 (12) (2023) 1085–1094, https://doi.org/10.1177/15353702231167940.
[32] C.M. Sun, J. Geng, Y. Yan, X. Yao, M. Liu, Overexpression of CKAP4 is associated with poor prognosis in clear cell renal cell carcinoma and functions via cyclin B signaling, J. Cancer 8 (19) (2017) 4018–4026, https://doi.org/10.7150/jca.21226.
[33] T. Luo, K. Ding, J. Ji, X. Zhang, X. Yang, A. Chen, et al., Cytoskeleton-associated protein 4 (CKAP4) promotes malignant progression of human gliomas through inhibition of the hippo signaling pathway, J. Neurooncol 154 (3) (2021) 275–283, https://doi.org/10.1007/s11060-021-03831-6.
[34] K. Iguchi, R. Sada, S. Matsumoto, H. Kimura, Y. Zen, M. Akita, et al., DKK1-CKAP4 signal axis promotes hepatocellular carcinoma aggressiveness, Cancer Sci. 114 (5) (2023) 2063–2077, https://doi.org/10.1111/cas.15743.