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Collagen facilitates the colorectal cancer stemness and metastasis through an integrin/PI3K/AKT/Snail signaling pathway

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ABSTRACT

Purpose: Dynamic remodeling of the extracellular matrix (ECM) around tumor cells is crucial for the tumor progressions. However, the mechanism is not well defined. Here, we aimed to reveal the underlying mechanism of ECM induced metastasis and provide innovative strategy to suppress the distant metastasis induced by ECM.
Materials and Methods: IHC was used to detect the expression of target proteins. H&E staining was used to evaluate the growth of tumor in vivo. Using wound healing and transwell assay, we examined the ability of cell to metastasis. We employed IF and Western blot to detect the expression of target proteins. And qRT-PCR was used to examine the target genes in mRNA level. We also applied flow cytometry to examine the percent of $CD133^+$ cell population.
Results: Herein, we observed elevated expression of type I collagen in colorectal cancer tissues from patients with high metastasis. Additionally, colorectal cancer cells cultured on 2D collagen reveal obviously enhanced capability of metastasis and tumorigenesis both in vitro and in vivo. We demonstrated that the activation of PI3K/AKT signal induced by integrin α2β1 resulted in the enhanced metastatic capability and stemness of colorectal cancer cells. Moreover, we found that Snail worked as the downstream of PI3K/AKT signaling, resulting in the intensive invasion and metastasis of colorectal cancer. Blocking the pathway by applying E7820 successfully reversed the type I collagen induced distant metastasis in colorectal cancer.
Conclusion: Combining E7820 and chemotherapeutic agents to block the integrin α2β1/PI3K/AKT/Snail signaling pathway revealed dramatic enhanced tumor suppression and provided an innovative approach for clinical colorectal cancer treatment.

1. Introduction

Colorectal cancer is a malignancy with the second cause of cancer-associated death worldwide [1]. About half colorectal cancer patients shows positive initial response to the traditional therapies [2]. However, the long-term survival of colorectal cancer patients is still generally unsatisfactory due to the high risk of metastasis after multimodal treatment [3]. Therefore, understanding the mechanism of distant metastasis is urgently needed to facilitate the development of therapeutic strategies or prevent the distant metastasis in colorectal cancer treatment.

Previous evidence indicated that epithelial-mesenchymal transition (EMT) is one of the key molecular steps of cancer cells metastasis [4]. The process of EMT permits the invasion and migration of cells in various cancers, which is also reported to be negatively correlated with the prognosis of colorectal cancer patients [5]. The EMT process is complex, including the loss of cell to cell junctions and apicobasolateral polarity, leading to the generation of mesenchymal cancer cells with invasive property [6]. Compared to those epithelial cancer cells, mesenchymal cancer cells reveal lower expression of cellular adhesion proteins, such as E-cadherin and G-catenin, along with enhanced expression of mesenchymal markers such as N-cadherin and vimentin [7,8]. The alteration of cellular adhesion proteins has been considered as the hallmark of EMT process [9]. Previous reports have demonstrated that several transcription factors, such as Snail, ZEB1 and ZEB2, participate in the regulation of those cellular adhesion proteins expression [10]. However, the cause and specific underlying mechanism of EMT and metastasis in colorectal cancer is still unclear.

The tumor microenvironment is an evolutionarily concept, which is important in a variety of tumor progressions [11], such as sustained growth [12], drug resistance development [13,14], and is implicated in the EMT process of tumor cells as well [15]. It has been suggested that

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the tumor cells and some immune cells from tumor microenvironment could secret TGF-β to induce the EMT process, leading to the generation of disseminated tumor cells and cancer metastasis [16]. Additionally, the role of mechanical force in tumor progression has attracted increasing attention and it has been reported that the extracellular matrix in tumor microenvironment is also involved in the process of EMT. Previous studies have demonstrated that the collagen in extracellular matrix facilitate the EMT of tumor cells in bladder cancer and breast cancer [17]. Moreover, increasing evidence reveals the hypoxia condition could influence the EMT of tumor cells, resulting in the invasion and migration of cancer cells [18]. Given the central involvement of EMT process and tumor microenvironment in tumor metastasis, a better understanding of the underlying mechanism is crucial for targeting microenvironment and EMT process in colorectal cancer which could lay an important basis for treatment in clinic.

In our studies, we observed enriched collagen expression in colorectal tumor tissues from patients with distant metastasis. The collagen in tumor microenvironment activates the pro-survival PI3K/AKT signaling pathway through the membrane surface receptor integrin α2β1, resulting in the tumor promotion in colorectal cancer cells. We also found that the downstream transcription factor Snail down-regulates the expression of E-cadherin, resulting in the EMT of colorectal cancer and distant metastasis. We expounded the role of collagen and underlying mechanism in colorectal cancer cells EMT process and distant metastasis. Additionally, we combined the integrin α2β1 inhibitor E7820 with traditional chemotherapeutic agents, and observed dramatic tumor growth suppression and metastasis inhibition, which provided an innovative approach for colorectal cancer treatment.

2. Materials and methods

2.1. Cell lines culture and reagents

HCT-116 (human colorectal cancer cell line) and CT-26 (mouse colorectal cancer cell line) cells were purchased from the American Type Culture Collection. All cell lines were cultured in RPMI-1640 (Thermo, CA, USA) supplemented with 10% fetal bovine serum (Gibco, CA, USA) in 37 ℃ incubator with 5% CO2. In collagen group, cells were cultured by culture medium with 50 μg/ml dissolved type I collagen. In 2D cultured system, 300 μl mixture (50 μl type I collagen in water, 60 μl 10 × PBS, 15 μl 0.1 N NaOH solution, 175 μl PBS) were added into the 24-well plate. Then the plate was incubated in 37 ℃ for 2 h and the mixture turned into solid collagen. Then the tumor cells were seeded into the 2D collagen plates for a 2D collagen culture system. The type I collagen, type I collagenase, oxaliplatin (clinical chemotherapeutic agent for colorectal cancer) and 5-Fu (clinical chemotherapeutic agent for colorectal cancer) were purchased from Sigma (CA, USA). AZD-8186 (PI3K inhibitor), MK-2206 (AKT inhibitor) and E7820 (integrin α2β1 inhibitor) were purchased from Selleck (CA, USA).

2.2. Patient samples

Colorectal cancer tumor samples were sterilely obtained after the surgery at the First Affiliated Hospital of Wenzhou Medical University and were sent to the laboratory within 2 h. According to the clinical diagnose, samples were divided into metastasis and non-metastasis groups. All experimental procedures were conducted in accordance with the Medical Ethics Standard. All experiments were approved by the First Affiliated Hospital of Wenzhou Medical University. All participating patients received and signed the written informed consent documents. All animal assays were approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University.

2.3. Colony formation analysis

500 HC-T116 or CT26 cells cultured in flask or 2D collagen system were digested and seeded into 6-well plates. Then AZD-8186 (10 nM), MK-2206 (10 nM) or E7820 (5 μM) were added into the culture medium. After 2 weeks, the colony were stained with crystal violet solution (Solarbio, Beijing, China) and counted under a microscope. Each experiment was performed for at least three independent times.

2.4. Flow cytometry

To examine the percentage of CD133 positive cells, HCT116 or CT-26 cells were cultured in flask or 2D collagen system with AZD-8186 (10 nM), MK-2206 (10 nM), E7820 (5 μM) or not for 48 h. Then the cells were digested and collected. Next, cells were incubated with the primary antibody CD133 (eBioscience, CA, USA) for 30 min at room temperature, after washed twice and then re-suspended in PBS. The BD Canto II flow cytometry was (BD Biosciences, USA) was used for the CD133 positive cells analysis. To gate, we used IGG as negative control. We collected 50,000 events for analysis. Each experiment was performed for at least three independent times.

2.5. Wound healing assay

HCT116 or CT-26 cells were cultured in flask or 2D collagen system. Then the cells were digested and seeded into 6-well plates. After 24 h, 10 μl tips were used for wound and AZD-8186 (10 nM), MK-2206 (10 nM), E7820 (5 μM) were added into culture medium. After 12 h, pictures were taken as 10× for analysis by microscope. Each experiment was performed for at least three independent times.

2.6. Cells viability analysis

Cell viability was determined by an MTT assay kit (Solarbio, Beijing, China). Briefly, 3000 HCT-116 or CT-26 cells were seeded into 96-well culture plates. After 12 h, cells were treated with 5-Fu (20 μM) or oxaliplatin (20 μM) in the presence or absence of E7820 (5 μM). After 48 h, 10 μl 0.5 mg/ml MTT solution was added into the culture medium. After 4 h incubation at 37 ℃, the medium was replaced with 100 μl dimethyl sulfoxide and vortexed for 10 min. Absorbance was measured at 570 nm by a microplate reader (Thermo, CA, USA). Each experiment was performed for at least three times.

2.7. siRNA interfering

For Snail knockdown in tumor cells, 2 × 105 HCT116 cells were seeded in a six-well plate and starved in an antibiotic-free growth medium for 24 h before transfection. Snail siRNA (0.125 mg/ml) or a mock siRNA solution was performed for 48 h according to the manufacturer's protocol. The primers were designed and constructed by Gene Pharma Company. The siRNA-Snail sense: 5′-GAT CCG CCT AAC TAC AGC GAG CTG TTC AAG AGA CAG CTC GCT GTA GTT AGG CTT TTT TGG AAA-3′ and: 5′-AGC TTT TCC AAA AAA GCC TAA CTA CAG CGA GCT GTC TCT TGA ACA GCT CGC TGT AGT TAG GCG-3′.

2.8. Real-time PCR

The quantification of gene in mRNA level was detected by real-time PCR using SYBR green dye (Solarbio, Beijing, China). GAPDH was used for normalization. The primers used are listed as follows: human GAPDH forward primer 5′-GGAGCGAGATCCCTCCAAAAT-3′, reverse primer 5′-GGCTGTTGTCATACTTCTCATGG-3′; human integrin β1 forward primer 5′−CCTACTTCTGCACGATGTGATG-3′, reverse primer 5′−CCTTTGCTACGGTTGGTTACATT-3′; human integrin α2 forward primer 5′−CCTACAATGTTGGTCTCCCAGA-3′, reverse primer 5′-AGT AACCAGTTGCCTTTTGGATT-3′; human E-cadherin forward primer 5′-CGAGAGCTACACGTTCACGG-3′, reverse primer 5′-GGGTGTCGAGG GAAAAATAGG-3′; human vimentin forward primer 5′-GACGCCATCA ACACCGAGTT-3′, reverse primer 5′-CTTTGTCGTTGGTTAGCTGGT-3′.

2.9. Western blotting

The HCT-116 cells were cultured in flask or 2D collagen system for 48 h. Then the cells were collected for protein samples with NP40 solution. 20 μg protein samples were separated by SDS-PAGE, followed by transfering to PVDF membranes and detected by immunoblotting with primary antibodies against the primary antibody integrin α2β1 (1:600, Abcam, Cambridge, UK) or actin (1:1000, Abcam, Cambridge, UK) respectively at 4 °C overnight. Then HRP-conjugated secondary antibody (1:1000, Abcam, Cambridge, UK) was incubated for 1 h at room temperature, and visualized by using ECL detection kit (Thermo, CA, USA). Image *J* was used to quantify.

2.10. Immunohistochemistry and H&E staining

Colon tumor samples and mice lungs tissues were fixed in 10% formalin solution. Then the samples were processed, embedded in paraffin, and sectioned at 5 μm for immunohistochemistry. Citric acid and sodium citrate were used for antigen retrieval in a Microwave oven (Media, Beijng, China). Then the sections were incubated with type I collagen (1:200, Abcam, Cambridge, UK) and integrin α2β1 (1:400, Abcam, Cambridge, UK) at 4 °C overnight, followed by signal amplification using an ABC HRP Kit (Thermo, CA, USA) and counter-staining with hematoxylin. Here, we applied IPP 6.0 to quantify the relative expression of collagen. On the other hand, the sections of lung tissues were stained with hematoxylin and eosin (Solarbio, Beijing, China) for cancer metastasis analysis. Image *J* 6.0 software was used to analysis the relative intensity of sections.

2.11. Immunofluorescence staining

HCT-116 cells or CT-26 were cultured in flask or on 2D collagen, then MK-2206 (10 nM) or AZD-8186 (10 nM) or E7820 (5 μM) were added to the 2D culture system. After 48 h, those cells were digested and seeded into the confocal dishes (Solarbio, Beijing, China). After 4 h, the cells were fixed with 4% paraformaldehyde (Solarbio, Beijing, China) and 0.5% Triton X-100 (Solarbio, Beijing, China). Then cells were blocked in 5% bovine serum albumin in PBS for 1 h, and the primary antibody phospho-PI3K (1:500, Abcam, Cambridge, UK), phosphor-AKT (1:500, Abcam, Cambridge, UK), E-cadherin (1:600, Abcam, Cambridge, UK), vimentin (1:500, Abcam, Cambridge, UK) and Snail (1:400, Abcam, Cambridge, UK) were incubated at 4 °C overnight, followed by 594 s antibody (1:800, Abcam, Cambridge, UK) incubation for 2 h. An Olympus confocal microscope (Tokyo, Japan) was used to visualize and intensity analysis was performed by using Image *J*.

2.12. Transwell assay

Migration assay was conducted by using 8-μm transwell chambers (Corning Company, NY, USA). For invasion assay, 100 ul Matrigel mixed with non-serum medium was added to the upper chambers (Matrigel, 1:20; BD Biosciences), and incubated in the 37 °C for 4 h. HCT-116 or CT-26 cells were cultured in flask, medium with dissolved collagen or on 2D collagen for 48 h. Then, 3×10^4 cells suspension in 150 ul non-serum medium were seeded into the upper chambers. 800 ul of 10% FBS medium was added into the lower chambers. After 24 h, transwells were gently washed with PBS, fixed with formalin, removed upper chamber cells with swabs and stained with 0.1% crystal violet. Invaded cells were counted in 3 random fields per well under a 100x microscope. Each experiment was performed in triplicate. For other cases, cells were pre-cultured in different culture systems and then performed migration assay with or without E7820 (5 u M), AZD-8186 (10 nM), or MK-2206 (10 nM).

2.13. ELISA assay

Elisa assay was used to detect the level of type I collagen in different samples. We cultured tumor tissues from colon cancer patients diagnosed with or without distant metastasis for 48 h then the supernatant was obtained for further detection.

First, we coated the microplate with protein from the human or mouse in buffer coating for 2 h at room temperature. After washing the plate with washing buffer, 50 μl BSA (1%) added and incubated for 1.5 h, then washed the plate again. Then 100 μL biotin-antibody collagen I was added and incubated for 1.5 h at 37 °C. After washed with washing buffer for 5 min, 100 μL HRP-Avidin was added and incubated for 1 h at 37 °C. TMB substrate was added and incubated for 20 min (room temperature). The sample was viewed with an ELISA reader at 450 nm wavelength.

2.14. Integrin β1 knockdown by CRISPR–Cas9

pSpCas9(BB)-2A-GFP (P X 458) (a gift from F. Zhang, Addgene plasmid #48,138) was used to generate integrin β1 (human) HCT116 cells in vitro genome editing. Two sgRNA (single guide RNAs) sequences, sg#1 5′-GTGGAGAATGTATACAAGCA-3′ and sg#2 5′- AAGC AGGGCCAAATTGTGGG-3′, for integrin β1 were predicted by Zhang Lab' s website(http://crispr.mit.edu/). To construct the p × 458- integrin β1-KO plasmid, P X 458 was digested and ligated with annealed sgRNAs according to the previous methods. To generate the knockout cell lines, 4 μg sg1 and sg2 plasmid was transfected into 10^5 HCT116 with Lipofectamine® 3000 transfection reagent (Thermo, USA) according to the methods provided by the manufacturer's instructions. After 48 h, high GFP expression cells were sorted by into single clones into the 96-well plate flow cytometry (BD Biosciences FACS Aria II, USA). The single clones were cultured in 96-well plates for another 14 days or a longer time dependent upon the cell growth rate. T7-endonuclease assay or anti- integrin β1 or integrin β1 immunoblotting was used to screen for the integrin β1 deficient clones, respectively. Genome type of the knockout cells was determined by DNA sequencing.

2.15. Animal protocol and treatment

The female nude mice (6¯8 weeks) or BALB/C mice (6¯8 weeks) were purchased from Huafukang (Beijing, China). All mice were fed in SPF room. For tumorigenesis analysis, HCT-116 or CT-26 cells were cultured in flask or on 2D collagen for 48 h, then cells were obtained and injected into mice (1×10^5 HCT-116 cells to nude mice and 5×10^4 CT-26 cells to BALB/C mice). The mice were treated with PBS, AZD-8186 (5 mg/kg, every three days, 2 weeks) or MK-2206 (5 mg/kg, every three days, 2 weeks) or E7820 was (5 mg/kg, every three days, 2 weeks). After 30 days (HCT116) and 15 days (CT-26), the tumor-forming rate were counted (n = 20 in each group).

For lung metastasis analysis, HCT-116 or CT-26 cells were cultured on 2D collagen for 48 h, then cells (2×10^6 HCT116 or 5×10^5 CT-26) were obtained to construct nude mice or BALB/C models. The mice were treated with PBS, AZD-8186 (5 mg/kg, every three days tail vein injection, 2 weeks), MK-2206 (5 mg/kg, every three days tail vein injection, 2 weeks) or E7820 was (5 mg/kg, every three days tail vein injection, 2 weeks). After 30 days (HCT-116) or 15 days (CT-26), the mice were sacrificed and lungs were obtained to count the number of tumor nodules in lungs (n = 10).

For tumor growth suppression analysis, 2×10^6 HCT116 cells were subcutaneously injected into nude mice. When the tumors size reached 7 × 7 mm, oxaliplatin (6 mg/kg) or oxaliplatin (6 mg/kg) combined with E7820 (10 mg/kg) (n = 6) were used to treated the mice by tail vein injection every three days for 2 weeks. The tumor sizes and mice survival were recorded every day (6 mice in each group).

3. Statistical analysis

Each experiment was performed for at least three independent times. Results were presented as mean ± SEM and statistical significance was performed by an unpaired Student's *t*-test by the Graphpad 6.0 software. Statistical analysis between groups was performed by Student's *t*-test for two groups or using one-way ANOVA for multiple groups. The survival rates were determined by Kaplan-Meier survival analysis. *p < 0.05; **p < 0.01; ***p < 0.001; ns, no significant difference.

4. Results

4.1. Type I collagen promoted colorectal cancer cell metastasis and stemness in vitro and in vivo

Type I collagen is the major structural protein of extracellular matrix (ECM) in tumor microenvironment and plays critical roles in cancer metastasis [19]. To determine whether type I collagen is involved in colorectal cancer metastasis, we examined the expression of type I collagen in tumor samples obtained from colorectal cancer patients diagnosed with or without metastasis. Intriguingly, type I collagen exhibited higher expression in tissues from colorectal cancer patients with metastasis compared to the samples from non-metastasis patients (Fig. 1A). This pattern was observed in multiple patients as evidenced by increased accumulation of type I collagen in colorectal cancer patients with metastasis (Fig. 1B, n = 10, p < 0.01). The higher expression of type I collagen was also validated by using ELISA (Fig. S1A, n = 10, p < 0.01). The data suggested that the over-expression of type I collagen may be associated with the cancer metastasis in colon tumors. Thereby, we kept on to investigate whether type I collagen is capable of promoting the distant metastasis of colorectal cancer in vivo. Here, we constructed nude mice models bearing HCT-116 cells and injected type I collagen for 2 weeks when the tumor volume reaching 3 × 3 mm. After 30 days, mice were sacrificed and lungs were obtained to count nude numbers and to perform H&E staining. Compared with mice treated with PBS, we observed robust lung metastasis (Fig. 1C) and increased tumor nodes in lungs from mice treated with type I collagen (Fig. 1 D, n = 10, p < 0.01). Similar results were observed in the mice models bearing CT-26 cells treated with type I collagen (Fig. S1B and C, n = 10, p < 0.01), reminding that type I collagen could efficiently promote the lung metastasis in colorectal cancer. Next, we further validate the role of type I collagen in vitro. Here, we employed three different culture systems to examine the role of type I collagen on migratory behavior. First, we cultured HCT-116 cells in flask with PBS, medium with dissolved collagen or on 2D collagen for 48 h. Then the cells were obtained to perform the migration and invasion assay. According to the data, HCT-116 cells cultured on the 2D collagen extensively increased cell migration and invasion abilities compared with those cells cultured in flask with PBS (p < 0.01), while cells pre-cultured in medium with dissolved collagen had no obvious difference compared to the flask group (Fig. 1E, F), indicating that the 2D collagen culture could facilitate the migration of colorectal cancer cells, which is in line with our previous results in vivo. Previous studies have demonstrated that the successful cancer metastasis is always accompanied by the acquired stemness in metastatic cancer cells. In our study, the

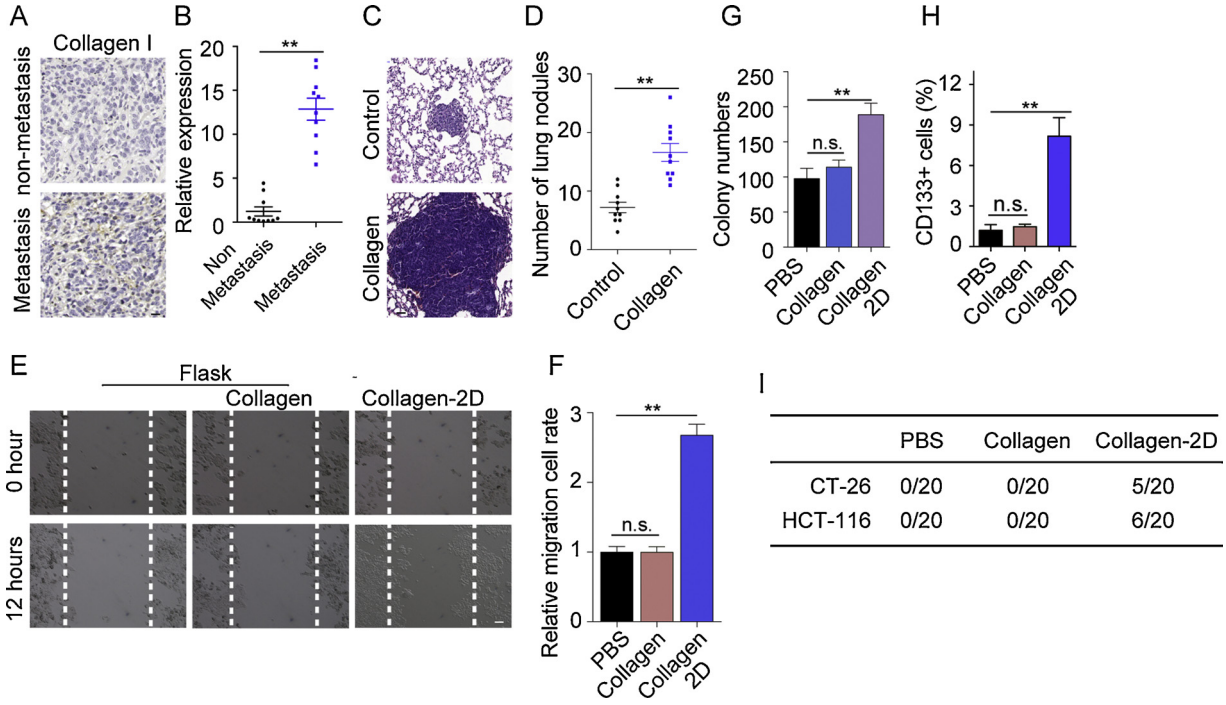

Fig. 1. Type I collagen promoted colorectal cancer cells metastasis in vitro and in vivo.
(A). IHC assay indicated type I collagen had higher expression in the colorectal cancer tissues from patients diagnosed with distant metastasis than that in sample from patient diagnosed without metastasis (scale bar, 40 um). (B). The quantification of type I collagen in colorectal cancer patients with or without distant metastasis (n = 10, p < 0.01). (C). Type I collagen treatment promoted lung metastasis in nude mice models bearing HCT-116 cells (scale bar, 40 um) and resulted in more tumor nodes in mice lung (D, n = 10, p < 0.01). (E) HCT-116 cells pre-cultured on 2D type I collagen had higher ability to invasion compared with those cells cultured in flask, while cells cultured in medium with dissolved type I collagen had no obvious difference. The photos were taken at 0 h and 12 h (scale bar, 40 um). (F) HCT-116 cells pre-cultured on 2D type I collagen had higher ability to migrate compared with those cells cultured in flask, while cells cultured in medium with dissolved type I collagen had no obvious difference (n = 3, p < 0.01). HCT-116 cells pre-cultured on 2D type I collagen had higher ability to form colony (G) and higher percent of CD133 positive cells (H) compared with those cells cultured in flask, while cells cultured in medium with dissolved type I collagen had no obvious difference (n = 3, p < 0.01). (I) HCT-116 or CT-26 cells pre-cultured on 2D collagen had higher tumor-forming rate in mice models compared with those cells cultured in flask, while cells cultured in medium with dissolved type I collagen had no difference (n = 20). * indicated p < 0.05. ** indicated p < 0.01. n.s. indicated not statistically significant.

enhanced colony forming ability of HCT-116 and CT-26 cells was observed in 2D collagen culture group (Fig. 1G and Fig. S1C, n = 3, $p < 0.01$). Consistently, the percentage of $CD133^+$ HCT116 and CT-26 cells were elevated in the type I collagen 2D culture system (Fig. 1H and Fig. S1D, n = 3, $p < 0.01$). To further investigate the stemness induced by collagen, we subcutaneously injected HCT-116 or CT-26 cells (flask, medium dissolved collagen system or on 2D collagen system) into nude or C57 mice and analyzed the tumor formation. We found that HCT116 cells cultured with 2D collagen system revealed dramatic enhanced tumor formation capability compared to PBS or dissolved collagen group. The same result was observed in the CT-26 mice models (Fig. 1I, $p < 0.01$), indicating that collagen could also participate in the stemness regulation of cancer cells. Together, these data suggested that type I collagen could promote colorectal cancer metastasis and stemness in vitro and in vivo.

4.2. Type I collagen regulates colorectal cancer metastasis and stemness through up-regulating integrin α2β1

Our previous results indicated that cancer cells cultured on 2D collagen system revealed enhanced capability of migration and tumorigenesis while tumor cells cultured with dissolved collagen showed no difference to the PBS group (Fig. 1, n = 3, $p < 0.01$). This result reminded us that colorectal cancer cells might transduce the collagen signals through biomechanical force associated receptors. Integrins, serving as transmembrane receptors to facilitate cell-extracellular matrix (ECM) adhesion, have attracted increasing attention due to the function of biomechanical force signals transduction in recent years [20]. In addition, several integrins have been reported to be involved in various tumor progressions, such as cancer initiation, metastasis, drug resistance and so on. Among these, integrin α2β1 was reported to work as a major player in the cancer metastasis process [21]. So next, we kept on to explore whether integrin α2β1 is involved in the type I collagen induced metastasis. First, we cultured HCT-116 cells in three culture systems (flask, medium with dissolved type I collagen, or on 2D collagen) and then examined the expression of integrin α2β1. According to the data, we observed increased mRNA level of both integrin α2 (*ITGA2*) and integrin β1(*ITGB1*) in the 2D culture system (Fig. 2A, $p < 0.01$). Consistently, we detected elevated expression of integrin α2β1 in 2D cultured HCT-116 cells in the protein level (Fig. 2B), reminding that integrin α2β1 in colorectal cancer may participate in the type I collagen induced metastasis. Moreover, we further examined the expression of integrin α2β1 in the samples from colorectal cancer patients diagnosed with or without metastasis. Interestingly, the elevated expression of integrin α2β1 was observed in patients with metastasis (Fig. 2C). This pattern was observed in multiple patients as evidenced by increased accumulation of integrin α2β1 in colorectal cancer patients with metastasis (Fig. 2D, $p < 0.01$). To further investigate the role of integrin α2β1 in metastasis, we employed E7820 (inhibitor of integrin α2β1) to treat mice models bearing HCT-116 cells. After 30 days, we sacrificed the mice and obtained the lungs for H&E staining and counting tumor nodules numbers in lungs. As shown in the Fig. 2E and F, we found that blocking integrin α2β1 by E7820 relieved the lung metastasis induced by type I collagen, indicating that the metastasis induced by type I collagen is integrin α2β1 dependent ($p < 0.01$). Consistently, blockade of integrin α2β1 also reversed the type I collagen induced enhanced ability to invasion and migration in vitro (Fig. 2G, H). Further, we knocked down the *integrin β1* in HCT 116 cells and examined their ability of migration and invasion after cultured on collagen 2D system in vitro and in vivo. According to the data, compared to the wild type HCT 116 cells, knocking down *integrin β1* reversed the enhanced ability to migration and invasion both in vitro and in vivo (Fig. 2 I–K, $p < 0.01$), reminding that integrin α2β1works as an important player in collagen induced metastasis. Additionally, we also found that E7820 treatment could reverse the enhanced colony formation ability induced by type I collagen (Fig. 2L, $p < 0.01$) and reduce the percent of $CD133^+$ cells in both CT-26 and HCT 116 cells induced by type I collagen culture (Fig. 2M, $p < 0.01$). Furthermore, blocking integrin α2β1 remarkably reversed the enhanced tumor-forming ability of both CT-26 and HCT-116 cells induced by type I collagen in vivo (Fig. 2N), suggesting the integrin α2β1 also participate in the stemness regulation induced by type I collagen in colorectal cancer. Taken together, these data suggested that integrin α2β1 worked as the downstream of type I collagen induced metastasis or stemness in colorectal cancer.

4.3. Identification of PI3K/AKT as the downstream mediator of collagen/integrin α2β1 in regulating metastasis

Several studies have confirmed a link between integrin over-expression and activated PI3K/AKT pathway to promote distant metastasis or sustained growth in various tumors [22]. To investigate whether PI3K/AKT is involved in the type I collagen-integrin α2β1 induced metastasis or stemness in colorectal cancer, we detected the expression of p-PI3K and p-AKT in HCT-116 cells cultured in flask or on 2D collagen. Intriguingly, we found that type I collagen 2D culture system could significantly induce the up-regulation of PI3K/AKT signal in HCT-116 cells ($p < 0.01$), while blockade of integrin α2β1 by adding E7820 reversed the phenomenon (Fig. 3A, B, $p < 0.01$). Similar results were obtained in CT-26 cells (Fig. S2A and B, $p < 0.01$), suggesting that PI3K/AKT pathway might work as the downstream of type I collagen-integrin α2β1 in colorectal cancer. Consistently, the enhanced expression of p-PI3K and p-AKT were observed in tumor tissues form colorectal cancer patients with metastasis or mice tumor tissues treated with type I collagen (Fig. S3A, $p < 0.01$). To further validate the role of PI3K/AKT in type I collagen induced metastasis, we cultured HCT-116 cells on 2D collagen, then we employed AZD-8186 (a PI3K inhibitor) or MK-2206 (an AKT inhibitor) to block the PI3K/AKT signals. Then we evaluated the ability of migration of HCT-116 cells by wound healing and migration assays. As excepted, both AZD-8186 and MK-2206 could suppress the enhanced migration and invasion capability of HCT-116 cells induced by type I collagen (Fig. 3C and D, $p < 0.01$). Increasing evidence has demonstrated that PI3K/AKT signal serves as a pro-survival signaling pathways to induced tumor stemness, resulting in the sustained growth in several tumors. In our study, we found that adding AZD-8186 or MK-2206 significantly reversed the enhanced colony-forming ability (Fig. 3E and Fig. S2C, n = 3, $p < 0.01$) as well as reducing the percent of $CD133^+$ cells (Fig. 3F and Fig. S2D, n = 3, $p < 0.01$) of HCT-116 and CT-26 cells treated with type I collagen in vitro. Those results reminded us that PI3K/AKT signal serves as the downstream of type I collagen and integrin α2β1 to induce the regulation of metastasis and stemness in colorectal cancer. To further investigate our conclusion in vivo, we constructed mice models bearing HCT-116 or CT-26 cells and examined the cancer metastasis and tumorigenesis capability. According to the data, we observed that AZD-8186 and MK-2206 efficiently reduced the number of lung nodes in the mice models baring HCT-116 cells cultured on 2D collagen (Fig. 3G, n = 3, $p < 0.01$). Similar results were obtained when tested with CT-26 mice model (Fig. S2E, n = 3, $p < 0.01$). Also, Mice were subcutaneously implanted with HCT-116 (1×10^5) or CT-26 (5×10^4) cells cultured in flask or on 2D collagen into the mice. We found that MK-2206 and AZD 8186 treatment obviously reduced the tumor forming rate (Fig. 3H), which is in line with our previous conclusion. Together, the above data reminded that PI3K/AKT pathway served as the downstream of collagen-integrin α2β1 to participate in the process of distant metastasis and stemness maintain in colorectal cancer.

4.4. Type I collagen promoted colorectal cancer metastasis through inducing EMT transition

PI3K/AKT signal has been demonstrated as a pro-survival signaling pathway to facilitate the cancer stemness maintain and sustained tumor

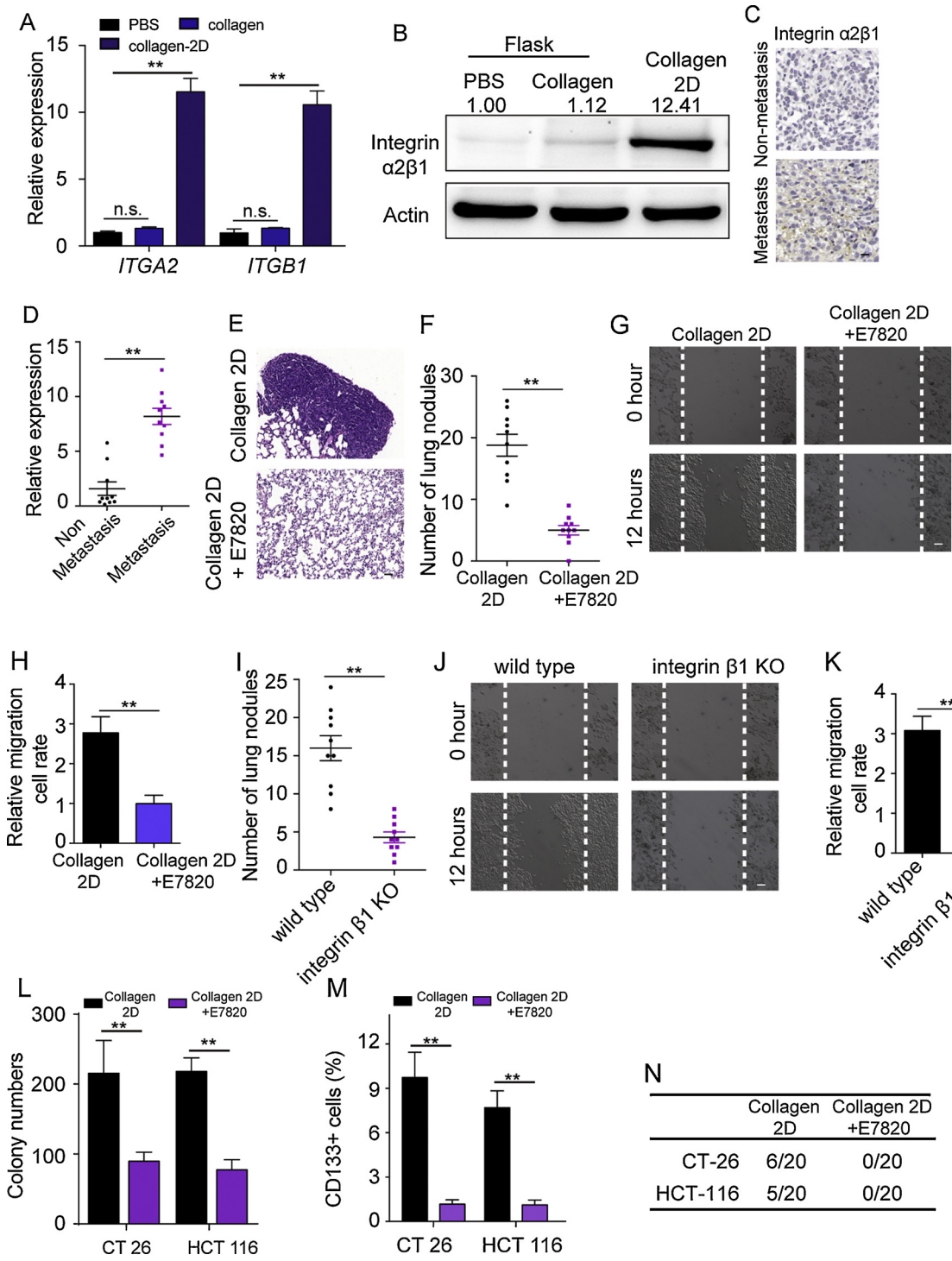

Fig. 2. Type I collagen regulates colorectal cancer metastasis through up-regulating integrin α2β1.

(A). HCT-116 cells cultured on 2D collagen significantly elevated the expression of *ITGA2* (integrin α2) and *ITGB1* (integrinβ1), while cells cultured in medium with dissolved type I collagen had no difference compared with the flask group (n = 3, $p < 0.01$). (B). HCT-116 cells cultured on 2D collagen significantly elevated the expression of integrin α2β1, while cells cultured in medium with dissolved type I collagen had no difference compared with the flask group. The quantification was analyzed by Image *J* software (n = 10, $p < 0.01$). (C). IHC assay indicated that integrin α2β1 had higher expression in colorectal cancer patients diagnosed with distant metastasis compared with that in samples from patient diagnosed without metastasis (scale bar, 40 um). (D). The quantification of integrin α2β1 in colorectal cancer patients with or without distant metastasis (n = 10, $p < 0.01$). (E). HCT-116 cells pre-cultured on 2D collagen promoted lung metastasis in mice models while applying E7820 (5 mg/kg) reversed the phenomenon (scale bar, 40 um). (F). HCT-116 cells pre-cultured on 2D collagen significantly elevated the lung node numbers in mice models while applying E7820 (5 mg/kg) reversed the phenomenon (n = 10, $p < 0.01$). (G–H). HCT-116 cells pre-cultured on 2D collagen promoted cell invasion (G, scale bar, 40 um) and migration (H, n = 3, $p < 0.01$) while applying E7820 (5 u M) reversed the phenomenon. (I–K). Knocking down integrin β1 of HCT-116 cells reversed the enhanced migration and invasion induced by 2D collagen culture both in vivo and in vitro. (L). Applying E7820 (5 u M) reversed the colony-forming ability (I, n = 3, $p < 0.01$) and reduced the CD133 positive cells in (M, n = 3, $p < 0.01$) HCT-116 and CT-26 cells pre-cultured on 2D collagen in vitro. (N). Applying E7820 (5 mg/kg, every three days, 2 weeks) reversed the ability of HCT-116 or CT-26 cells to form tumor in vivo (n = 20). * indicated $p < 0.05$. ** indicated $p < 0.01$. n.s. indicated not statistically significant.

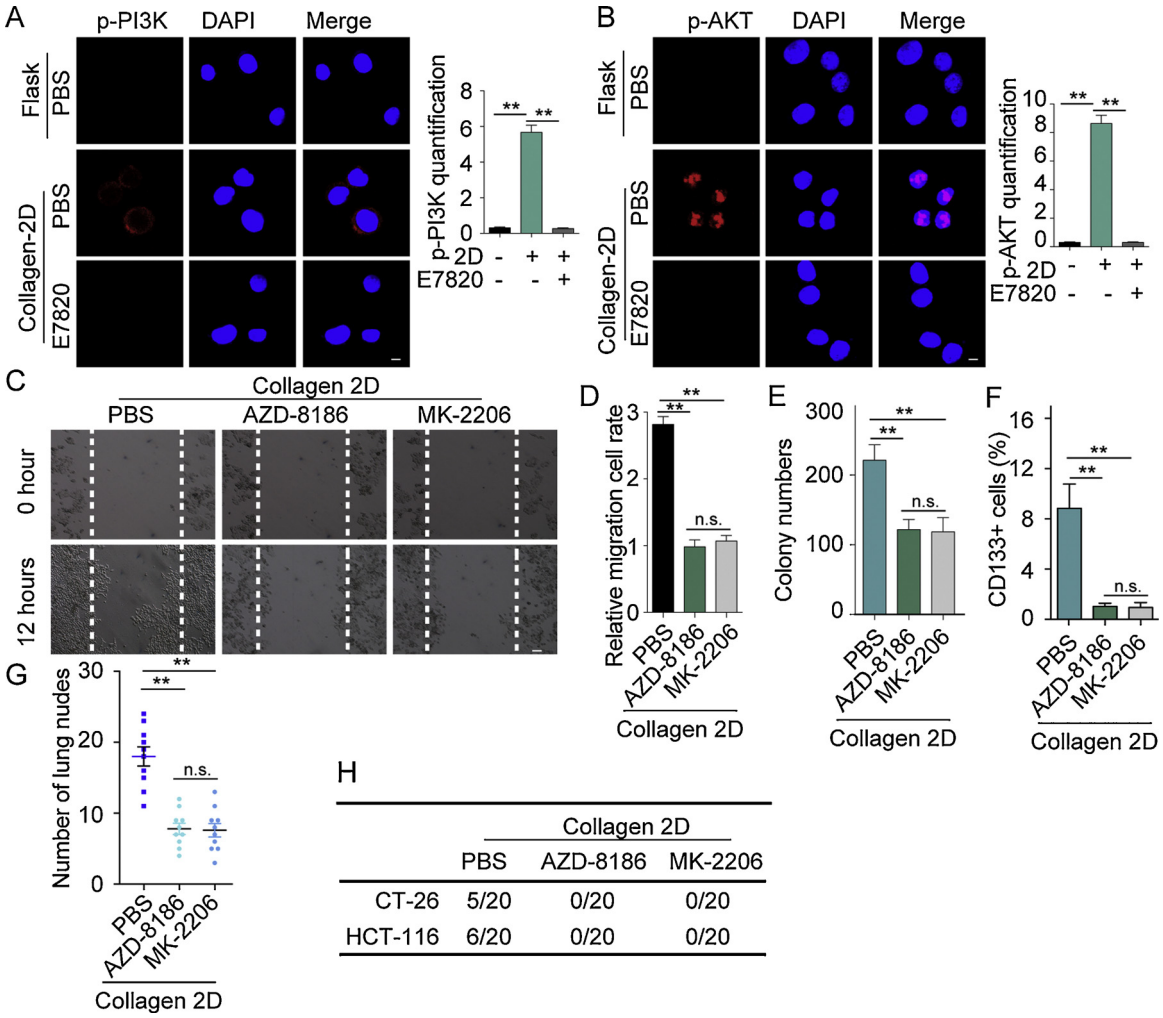

Fig. 3. Identification of PI3K/AKT as the downstream mediator of integrin α2β1 in regulating metastasis.
(A). IF assay indicated that HCT-116 cells cultured on 2D collagen had higher expression of p-PI3K compared with those cells cultured in flask, while adding E7820 (5 u M) reversed the phenomenon (Scale bar, 10 um). The quantification of p-PI3K expression (n = 10, $p < 0.01$). (B). IF assay indicated that HCT-116 cells cultured on 2D collagen had higher expression of p-AKT compared with those cells cultured in flask, while adding E7820 (5 u M) reversed the phenomenon (Scale bar, 10 um). The quantification of p-AKT expression (n = 10, $p < 0.01$). (C) HCT-116 cells pre-cultured on 2D collagen had higher enhanced ability to migrate compared with those cells pre-cultured in flask, while adding AZD-8186 (PI3K inhibitor, 10 nM) or MK-2206 (AKT inhibitor, 10 nM) reversed the phenomenon. The photos were taken at the 0 h and 12 h (Scale bar, 40 um). (D) HCT-116 cells pre-cultured on 2D collagen had higher enhanced ability to invasion compared with those cells pre-cultured in flask, while adding AZD-8186 (PI3K inhibitor, 10 nM) or MK-2206 (AKT inhibitor, 10 nM) reversed the phenomenon. The photos were taken at the 0 h and 12 h (Scale bar, 40 um). Applying AZD-8186 (PI3K inhibitor, 10 nM) or MK-2206 (AKT inhibitor, 10 nM) reversed the colony-forming ability (E, n = 3, $p < 0.01$) and reduced the CD133 positive cells in (F, n = 3, $p < 0.01$) HCT-116 and CT-26 cells pre-cultured on 2D collagen in vitro. (G). Applying AZD-8186 (5 mg/kg, every three days, 2 weeks) or MK-2206 (5 mg/kg, every three days, 2 weeks) reversed the elevated number of tumor nodules in lungs in mice models (n = 10, $p < 0.01$). (H) Applying AZD-8186 (5 mg/kg, every three days, 2 weeks) or MK-2206 (5 mg/kg, every three days, 2 weeks) reversed the elevated tumor forming rate in mice models (n = 20). * indicated $p < 0.05$. ** indicated $p < 0.01$. n.s. indicated not statistically significant.

growth. However, PI3K/AKT signal could not regulate the tumor cells migration or metastasis directly. Intriguingly, previous reports reveal that integrins could regulate the epithelial-mesenchymal transition (EMT) process of tumor cells through the activation of PI3K/AKT signal [23]. Moreover, EMT has been reported as the major process for distant metastasis in many cancers [8]. Previous reports have demonstrated that the down-regulation of E-cadherin and the up-regulation of vimentin has been considered as markers of EMT in tumor cells. E-cadherin works as a major player in cell-cell adhesion and its down-regulation is essential for EMT process and cells migration, while vimentin is upregulated in cells undergoing EMT [16,4]. Here, we examined the expression of E-cadherin and vimentin in HCT-116 cells cultured in flask or on 2D collagen. And we observed that the expression of E-cadherin was significantly reduced in cells cultured on 2D collagen in both mRNA (Fig. 4A, n = 3, $p < 0.01$) and protein levels (Fig. 4C), while vimentin was upregulated in both mRNA (Fig. 4B, n = 3, $p < 0.01$) and protein levels (Fig. 4D), suggesting that type I collagen could induce EMT process in colorectal cancer cells.

Increasing evidence has shown that the transcription factor Snail participates in the transition from epithelia-like cells into mesenchymal-like cells through suppressing E-cadherin expression in many cancer cells [24]. To address whether Snail is involved with the EMT process induced by type I collagen, we detected the expression of Snail in HCT-116 cells cultured in flask or on 2D collagen. We found that 2D collagen culture system resulted in the accumulation of Snail in the nuclear of HCT-116 cells, with statistical significance (Fig. 4E, $p < 0.01$). As the transcriptional factor, Snail has been reported to be activated by PI3K/AKT signal. Additionally, blockade of PI3K or AKT could obviously reverse the accumulation of Snail induced by type I collagen (Fig. 4E), suggesting that Snail worked as the downstream of PI3K/AKT pathway to induce the EMT process in colorectal cancer. This conclusion was further validated by using Snail knockout assays.

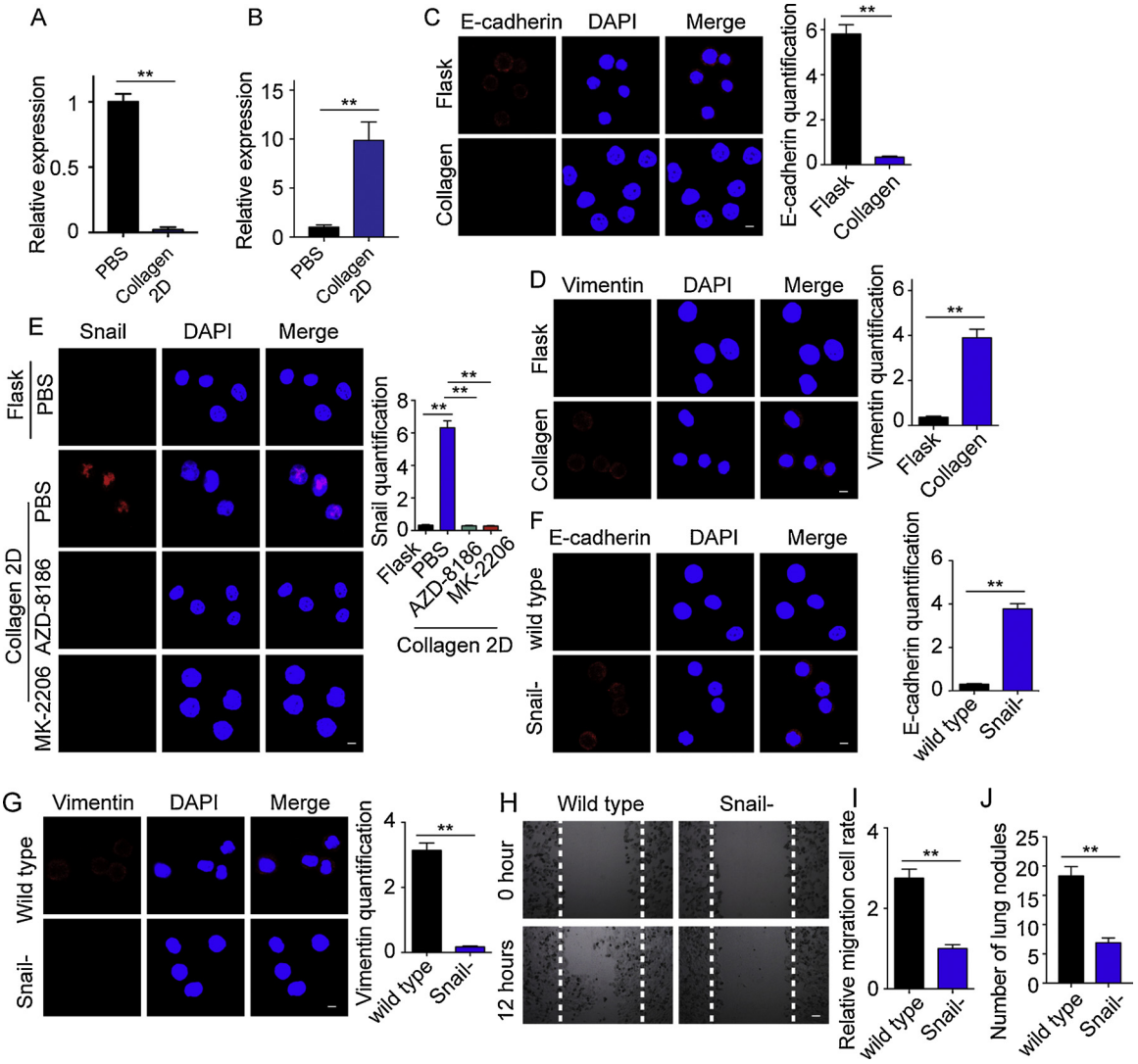

Fig. 4. Type I collagen promoted colorectal cancer metastasis through inducing EMT transition.
(A). HCT-116 cells cultured on 2D collagen had lower expression of E-cadherin compared with those cells cultured in flask in the mRNA level ($n = 3$, $p < 0.01$). (B). HCT-116 cells cultured on 2D collagen had higher expression of Vimentin compared with those cells cultured in flask in the mRNA level ($n = 3$, $p < 0.01$). (C). HCT-116 cells cultured on 2D collagen had lower expression of E-cadherin compared with those cells cultured in flask in the protein level (Scale bar, 10 um). The quantification of the E-cadherin expression was analyzed ($n = 10$, $p < 0.01$). (D). HCT-116 cells cultured on 2D collagen had higher expression of Vimentin compared with those cells cultured in flask in the protein level (Scale bar, 10 um). The quantification of the Vimentin expression was analyzed ($n = 10$, $p < 0.01$). (E). HCT-116 cells cultured on 2D collagen had higher expression of Snail compared with those cells cultured in flask in the protein level, while applying MK-2206 (10 nM) or AZD-8186 (10 nM) reversed the phenomenon (Scale bar, 10 um). The quantification of the Vimentin expression was analyzed ($n = 10$, $p < 0.01$). (F). Compared with the wild type HCT 116 cells, knocking down Snail elevated the reduced E-cadherin induced by collagen (Scale bar, 10 um). The quantification of the E-cadherin expression was analyzed ($n = 10$, $p < 0.01$). (G). Compared with the wild type HCT 116 cells, knocking down Snail reduced the elevated Vimentin induced by collagen (Scale bar, 10 um). The quantification of the Vimentin expression was analyzed ($n = 10$, $p < 0.01$). (H). Compared with wild type HCT 116 cells, knocking down Snail reversed the enhanced invasion ability induced by type I collagen. The photos were taken at the 0 h and 12 h (Scale bar, 10 um). (I). Compared with wild type HCT 116 cells, knocking down Snail reversed the enhanced migration ability induced by type I collagen ($n = 3$, $p < 0.01$). (*J*). Knocking down Snail in HCT-116 reduced the lung nodules numbers induced by type I collagen ($n = 10$, $p < 0.01$). * indicated $p < 0.05$. ** indicated $p < 0.01$. n.s. indicated not statistically significant.

Compared with the wild type cells, 2D collagen culture system failed to induce the down-regulation of E-cadherin (Fig. 4F, $p < 0.01$) and up-regulation of vimentin (Fig. 4G, $p < 0.01$), suggesting that deleting Snail reversed the EMT induced by type I collagen. Consistently, we observed that deleting Snail reversed the enhanced cell migration and invasion ability of HCT-116 cells induced by type I collagen (Fig. 4H, I, $p < 0.01$). The reduced metastatic capability was observed in Snail knockout HCT116 in vivo (Fig. 4J, $p < 0.01$). As excepted, higher expression of Snail was observed in tumor tissues form colorectal cancer patients with metastasis or mice tumor tissues treated with type I collagen (Fig. S3B). Taken together, these data suggested that Snail works as the downstream of type I collagen/integrin α2β1/PI3K/AKT signal to induce the EMT process, resulting in the elevated metastasis in colorectal cancer.

4.5. Blocking integrin α2β1 by E7820 suppressed the cancer metastasis and enhanced the tumor growth inhibition of chemotherapeutic drugs in colorectal cancer

Various studies have evidenced that EMT transition reduce the efficiency of chemotherapeutic drugs for cancer treatment [25]. To enhance the anticancer effects of chemotherapeutic drugs, inhibitors to

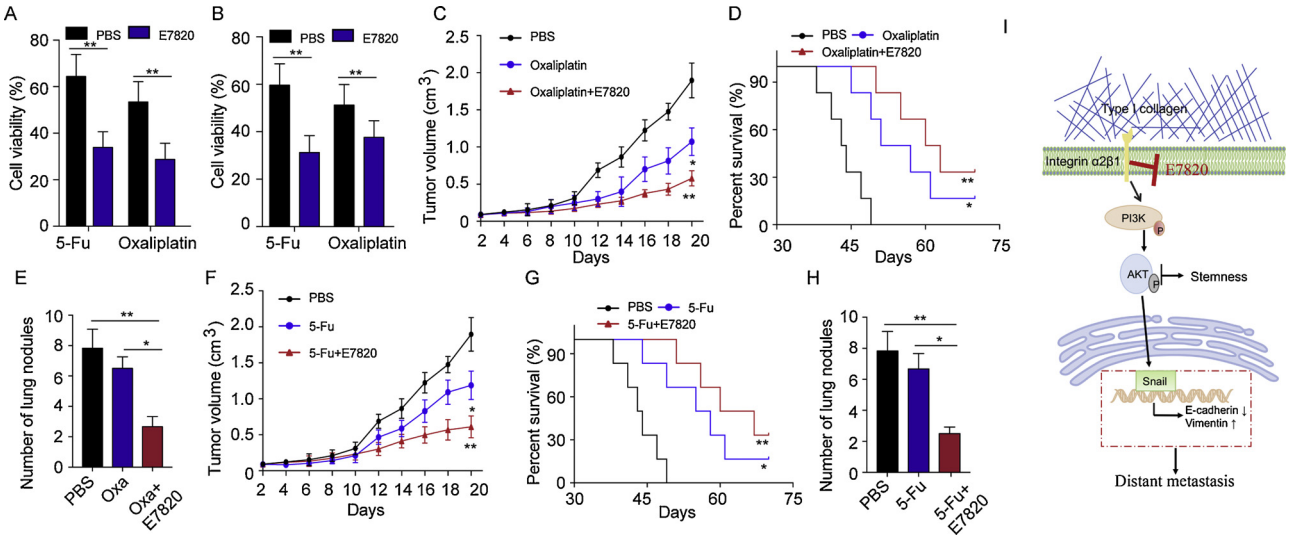

Fig. 5. Blocking integrin α2β1 enhanced the killing ability of chemotherapeutic drugs to colorectal cancer in vitro and in vivo.
(A) HCT-116 cells were treated with 5-Fu (20 u M) or oxaliplatin (20 u M) in the presence or absence of E7820 (5 u M) for 48 h. Then the cell viability was examined by using MTT (n = 3, $p < 0.01$). (B) CT-26 cells were treated with 5-Fu (0.1 u M) or oxaliplatin (30 u M) in the presence or absence of E7820 (5 u M) for 48 h. Then the cell viability was examined by using MTT (n = 3, $p < 0.01$). (C) Tumor volume of mice models bearing HCT-116 cells treated with PBS, oxaliplatin (6 mg/kg) or oxaliplatin (6 mg/kg) combined with E7820 (10 mg/kg) (n = 6) were measured. (D) Survival time of mice models bearing HCT-116 cells treated with PBS, oxaliplatin (6 mg/kg) or oxaliplatin (6 mg/kg) combined with E7820 (10 mg/kg) (n = 6) were recorded. (E) The nude mice models bearing HCT-116 cells were constructed. And the mice were treated with oxaliplatin (6 mg/kg) or oxaliplatin (6 mg/kg) combined with E7820 (10 mg/kg). After 30 days, the mice were sacrificed and lungs were obtained to count the number of lung nodules (n = 6). (F) Tumor volume of mice models bearing HCT-116 cells treated with PBS, 5-Fu (10 mg/kg) or 5-Fu (10 mg/kg) combined with E7820 (10 mg/kg) (n = 6) were measured. (G) Survival time of mice models bearing HCT-116 cells treated with PBS, 5-Fu (10 mg/kg) or 5-Fu (10 mg/kg) combined with E7820 (10 mg/kg) (n = 6) were recorded. (H) The nude mice models bearing HCT-116 cells were constructed. And the mice were treated with 5-Fu (10 mg/kg) or 5-Fu (10 mg/kg) combined with E7820 (10 mg/kg). After 30 days, the mice were sacrificed and lungs were obtained to count the number of lung nodules (n = 6). (I) Schematic diagram showing the signaling pathway involved in type I collagen/integrin α2β1/PI3K/AKT/Snail–induced EMT in colorectal cancer. * indicated $p < 0.05$. ** indicated $p < 0.01$. n.s. indicated not statistically significant.

block the EMT process are developed. Since our previous study have revealed that integrin α2β1 worked as a major player in the type I collagen induced EMT process, so we employed E7820 (an inhibitor of integrin α2β1) to examine whether blocking EMT could elevate the killing ability of chemotherapy for colorectal cancer treatment. Here, we treated HCT-116 cells with 5-Fu in the presence or absence of E7820 for 48 h. Then the cell viability was detected by using MTT. According to the data, we found that combining E7820 significantly enhanced the killing ability of 5-Fu to HCT-116 cells ($p < 0.01$). Also, combining E7820 with oxaliplatin to treat HCT-116 cells achieved similar results (Fig. 5A, $p < 0.01$). Further, we tested the efficacy of chemotherapeutic drugs with or without E7820 on CT-26 cells and obtained similar results (Fig. 5B, $p < 0.01$), suggesting that blocking integrin α2β1 could enhance the killing ability of chemotherapy to colorectal cancer cells in vitro. We kept on to investigate the killing efficiency of chemotherapy by combining with E7820 in vivo. Herein, we constructed nude mice models bearing HCT-116 cells, when the tumor volume reaching 5 × 5 mm, the mice were grouped randomly and received PBS, oxaliplatin monotherapy or combining with E7820. We recorded the tumor volume every 2 days and found that oxaliplatin monotherapy could slow down the tumor growth in some extend, while combining with E7820 significantly inhibit the tumor volume (Fig. 5C, $p < 0.01$). Consistently, combining oxaliplatin with E7820 greatly prolonged the survival time of mice compared with those treated with oxaliplatin monotherapy or control groups (Fig. 5D, $p < 0.01$). Since the type I collagen induced the distant metastasis depending on integrin α2β1-PI3K-AKT-Snail-EMT pathway, the lung metastasis is another important indicator to evaluate the drug efficacy. Here, we also constructed nude mice models bearing HCT-116 cells, when the tumor volume reaching 5 × 5 mm, the mice were grouped randomly and received PBS, oxaliplatin or oxaliplatin combined with E7820. After 30 days, we sacrificed the mice and counted the lung nodules under different treatment systems. According to the data, we observed that control group revealed severe lung metastasis (~8), and oxaliplatin monotherapy slightly reduced the metastasis (~6), while oxaliplatin combining with E7820 significantly reduced the number of lung nodules (~3) (Fig. 5 E, $p < 0.05$). Also, we evaluated the efficacy of 5-Fu combined with or without E7820 on nude mice models bearing HCT-116 cells. In short, we observed that 5-Fu combining with E7820 achieved the dramatic treatment effect. We found that combination 5-Fu with E7820 could slow down the tumor growth (Fig. 5F, $p < 0.05$), prolong the survival time (Fig. 5G, $p < 0.05$) and reduce the lung nodule numbers (Fig. 5H, $p < 0.05$). Together, these results reminded that the application of integrin α2β1 inhibitor could enhance the efficiency of chemotherapeutic drugs.

5. Discussion

Colorectal cancer is an aggressive cancer with large amount of stem-like distinct metastatic cells [26]. During the process of metastasis, those cancer cells could undergo the process of EMT, resulting in the long-distance dissemination and stem-like property [27]. However, the underlying mechanism remains unclear. In our studies, we described the role of collagen from microenvironment to facilitate the tumor growth and metastasis in colorectal cancer. The enriched collagen could activate the PI3K/AKT signaling pathway through the integrin α2β1 in tumor cells, leading to the sustained tumor growth. Furthermore, the PI3K/AKT signal induces the EMT process through activation of transcription factor snail, resulting in the down-regulation of E-cadherin expression. The loss of E-cadherin permits those epithelioid cancer cells to transform into mesenchymal cancer cells with stem-like property, which causes the colorectal cancer metastasis (Fig. 5I). Notably, blockade of integrin α2β1 efficiently suppressed the metastasis and combination of integrin α2β1 inhibitor and chemotherapeutic agents revealed improved curative effects.

Recently, the correlation between the tumor microenvironment and

tumor metastasis has attracted much attention. Previous studies have demonstrated that the tumor associated macrophages and cancer associated fibroblasts could facilitate the tumor metastasis through secretion of pro-tumor cytokines [28]. The extracellular matrix, such as fibronectin, might promote migration or stemness of cancer cells, resulting in the poor prognosis [29]. We found the type I collagen in tumor tissues facilitates the colorectal cancer cells EMT process, as well as enhanced proliferation, which is in line with previous reports. However, we found that the liquid collagen is not capable of inducing the enhanced cancer metastasis and the 2D solid collagen could facilitate the tumor cells migration, reminding that the collagen might participate in the cancer cells metastasis through biomechanical force associated signaling pathways. Indeed, the biomechanical force associated receptor integrin α1β2 was activated in tumor cells cultured with 2D collagen. Moreover, we further expounded that those EMT process was induced by the activation of PI3K/AKT signaling pathway, which was conducted by the membrane surface receptor integrin α2β1. Previous reports have indicated that type I collagen could induced the tumor sustained growth through the activation of PI3K/AKT signals in renal cancer cells. However, the relationship between tumor progression and other type collagen still remained unclear. Except for the type I collagen, some cytokines, such as Interleukin-8, could also promote cancer cells invasion by activating the PI3K/AKT associated pathway through the integrin receptors [22], which remind us that the potential therapeutic target of integrin. In previous report, Yin and his colleagues demonstrated that Interleukin-8 in tumor microenvironment could regulate the expression of integrin β3 in tumor cells, indicating the potential role of integrin in tumor pro-survival signals induced by ECM. Moreover, integrin β3 could efficiently promote the breast cancer cells metastasis through the PI3K/AKT signaling pathway, reminding the indirect connection between PI3K/AKT signal and cancer metastasis. Compared to previous reports, we also firstly proved that the PI3K/AKT signal induced by collagen contributes to Snail excitation and EMT process. Previous studies revealed that the PI3K/AKT signaling pathway is associated with the tumor sustained growth or the development of drugs resistance [30], and this finding reminds us that the participation of PI3K/AKT in cancer metastasis.

To efficiently suppress the sustained growth and high risk of metastasis, it is necessary to block the collagen/integrin α2β1/PI3K/AKT/Snail signaling pathway in tumor progression. In our study, we used E7820, an integrin α2β1 inhibitor, to block the pro-survival signal induced by collagen. To further suppress the tumor growth, chemotherapeutic agents 5-Fu and xeloda were combined with E7820 to evaluate the anticancer effects in mice bearing subcutaneous tumors. This combination significant enhanced the tumor suppression compared to traditional chemotherapy. Furthermore, the application of E7820 efficiently retarded the EMT process and metastasis induced by collagen, leading to prolonged survival time. Combined with previous reports, our research further expounded the role of collagen in tumor development. We also correlated collagen, PI3K/AKT signal and EMT process, which provide feasible targets for clinical colorectal cancer treatment. More importantly, the combination of integrin α2β1 inhibitor and chemotherapeutic agents revealed significantly improved anticancer effects, which might serve as potential strategy for colorectal cancer.

In conclusion, we demonstrated that collagen in tumor microenvironment is capable of facilitate the tumor growth and distant metastasis in colorectal cancer patients through the activation of integrin α2β1/PI3K/AKT/snail signal. Hence, we designed that integrin α2β1 could serve as a new therapeutic target combined with chemotherapy, which might be an innovative approach for clinic colorectal cancer therapy.

Conflict of interest

All authors report no conflicts of interest in this work.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2019.108708.

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