

Decreased collagen type III synthesis in skin fibroblasts is associated with parastomal hernia following colostomy

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Abstract. Parastomal hernia (PH) is a common complication following stoma formation. Abnormal collagen synthesis has been suggested to be involved in PH. The aim of the present study is to explore the effect and mechanism of the collagen synthesis on PH. Data from 157 patients with rectal cancer who received permanent colostomy were retrospectively collected and analyzed to identify the risk factors for PH. Primary culture of skin fibroblasts from patients with or without PH were performed. Cell viability, migration and invasion levels were detected by Cell Counting Kit-8, and wound healing and Transwell assays, respectively. Reverse transcription quantitative polymerase chain reaction and western blot analysis assays were performed to measure the gene and protein expression levels, respectively. The risk factors of sex, body mass index, aperture size and collagen expression were closely associated with the occurrence of PH. $\alpha 1$ (III) procollagen expression levels were significantly increased in patients with PH, while no marked difference in $\alpha 1$ (I) procollagen mRNA expression levels were observed in patients with or without PH. The viability and motility of fibroblasts from the patients with hernia were suppressed. The expression levels of matrix metalloproteinase (MMP)-2 and MMP-9 were decreased while the levels of collagen III and metalloproteinase inhibitor 1 (TIMP-1) were increased in the fibroblasts from the patients with PH. Silencing TIMP-1 expression promoted fibroblast migration and invasion and reversed the patterns of MMP-2, MMP-9 and collagen III expression in fibroblasts from the patients with PH. Decreased collagen III may inhibit the development of PH, potentially

through decreases in TIMP-1 expression. Therefore, the results from the present study may provide a novel target for PH therapy.

Introduction

Parastomal hernia (PH) is a common complication following an ileostomy or colostomy (1). PH rarely appears in the early stages after surgery (0-3%); however, it normally occurs in the first 2 years post-surgery (30-50%), and the risk may persist for >2 decades (2). In addition, PHs may have an adverse effect on the body and quality of life of patients (3). The treatment of PH is difficult as it has a high recurrence rate (4); to the best of our knowledge, the best method for overcoming this is to control initial PH development (5).

PH development may be attributed to multiple risk factors, including age, sex, aperture size, body mass index (BMI) and hypertension (6,7). However, the exact pathogenesis of PH formation remains unclear. It has been demonstrated that a decreased ratio of collagen I/collagen III is involved in PH formation (8). Collagen, as a primary component of the extracellular matrix (ECM), is relevant to the tensile strength in abdominal wall fascial layers. Fibroblasts are a common type of cell involved in the synthesis and metabolism of collagen (9). Therefore, we hypothesized that the synthesis of collagen was associated with the development of parastomal hernia and that the potential regulation mechanism involved fibroblast activity.

To the best of our knowledge, alterations to the ECM are regulated by the matrix metalloproteinases (MMPs), which may lead to collagen degradation (10). The MMPs, which belong to a zinc-dependent endopeptidases family, are essential for ECM remodeling and modification of almost all ECM components, including collagens, fibronectins and proteoglycans (11) that are also involved in normal physiological and pathological processes including angiogenesis and neoplasm metastasis (12,13). MMPs also serve a role in wound healing, in addition to growth factors, cytokines and adhesion molecules (14). In addition, MMPs have been revealed to be associated with each phase of skin wound healing (15,16). Chronic wounds have been demonstrated to be associated with aberrant ECM, and elevated MMPs levels including MMP-2 and MMP-9 (14,17,18). Tissue inhibitor of metalloproteinases (TIMPs) and intrinsic inhibitors of MMPs may regulate the ECM. Metalloproteinase inhibitor 1 (TIMP-1) has also been suggested to be able to affect fibrosis. For example, TIMP-1

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was demonstrated to be involved in organ fibrosis, including the liver and the heart (19,20). A recent study also indicated that TIMP-1 regulated pulmonary fibrosis by activating fibroblasts and promoting proliferation (21). In addition, TIMP-1 contributes to types I and III collagen degradation (22).

However, the association between TIMP-1 and collagen in parastomal hernia remains unclear. Therefore, the aim of the present study was to investigate the potential effect of collagen synthesis in PH.

Materials and methods

Clinical data and specimens. Data from 157 patients with rectal cancer who received permanent colostomy between March 2008 and September 2013 at Beijing Chao-Yang Hospital (Beijing, China) were reviewed retrospectively. All patients underwent abdominal perineal resections. Abdominal CT scans were performed every 6 to 12 months to evaluate the development of PH during the follow-up stage. PH was identified in a total of 55 cases. Information concerning age, sex, BMI (kg/m²), diabetes, hypertension, radiation history, length of hospital stay (days), aperture size and expression of types I and III procollagen mRNA from each patient were collected during outpatient visits in the hospital. Informed consent was provided by each patient and all experiments were authorized by the Ethics Committee of Beijing Chao-Yang Hospital. The dermal tissues and matched normal tissues were obtained from 55 patients with PH for the reverse transcription quantitative polymerase chain reaction (RT-qPCR) and western blot analysis.

Skin fibroblast culture. Briefly, fibroblasts were taken from tissues 1-mm distance away from the center of the skin samples. The tissues were placed on the upper wall of the culture bottle, to which was added Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin in a 90% humidified incubator with 5% CO₂ at 37°C. After 4 h, the culture bottle was turned over carefully and the tissues were immersed into the medium. Primary fibroblast cells were formed and subjected to cell identification following tissue culture for 3-14 days. The cells were amplified, passaged weekly at 80-90% confluence and used at early (between 2nd and 4th) passages (P) to avoid *in vitro* replicative-induced ageing. At P2 or P3, the cells were seeded at 5,000 cells/cm² for all the experiments, unless otherwise stated.

Immunofluorescence microscopy. The fibroblasts were fixed in 4% paraformaldehyde for 20 min at room temperature and extracted in 0.5% Triton X-100 for 10 min. Following washing in PBS, the samples were incubated with rabbit anti-human vimentin monoclonal antibody (cat. no. AX10005; 1:200; Abgent, Inc.) for 1 h at room temperature and then with fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG (cat. no. R37119; 1:100; Molecular Probes; Thermo Fisher Scientific, Inc.). Nuclei were stained with DAPI (5 µg/ml; Abgent, Inc.) for 15 min in the dark and images were observed with a fluorescence DM5000 B microscope (magnification, x200; Leica Microsystems, Inc.).

RNA extraction, cDNA synthesis and RT-qPCR. Total RNA from PH tissues or tissues without PH (WPH) and skin fibroblasts was extracted by TRIzol® (Thermo Fisher Scientific, Inc.). TRIzol® reagent and chloroform were added to the samples and mixed for 5 min. The samples were then centrifuged at 2,000 x g for 10 min at room temperature to recover the supernatant. Next, the supernatant was incubated with an equal volume of isopropyl alcohol at 0°C for 5 min, followed by centrifugation at 12,000 x g at 4°C for 10 min. Following removal of the supernatant, the 75% ethanol was added to wash the precipitate and the RNA was eluted with nuclease-free water. The purity and content for the reverse transcription were determined using NanoDrop 2000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). The cDNA was obtained by RNA with mixture in PrimeScript™ 1st Strand cDNA Synthesis kit (Takara Biotechnology, Co., Ltd.). The reactions were conducted using the following primers: α1 (I) procollagen forward, 5'-GTT CGTCCTTCTCAGGGTAG-3'; α1 (I) procollagen reverse, 5'-TTGTCGTAGCAGGGTTCTTT-3'; α1 (III) procollagen forward, 5'-CGAGGTAACAGAGGTGAAAGA-3'; α1 (III) procollagen reverse, 5'-AACCCAGTATTCTCCACTCTT-3'; β-actin forward, 5'-GGTTACCTCCCATCAGCT-3'; and β-actin reverse, 5'-CAG TGT CCG GAA ATC TCC-3', using a LightCycler system (Roche Diagnostics) using the under the following thermocycler conditions: 94°C for 4 min, then 40 cycles at 94°C for 45 sec, 56°C for 45 sec and 72°C for 2 min. The results were calculated using the 2^{-ΔΔC_q} method (23).

Cell proliferation assay. Cells (~5x10³) were maintained on 96-well plates for the measurement of proliferation using a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.). Following culture for 12, 24 and 48 h, the cells were incubated with CCK-8 solution (10 µl) at 37°C for 2 h. Absorbance was read at 450 nm using an iMark plate reader (Bio-Rad Laboratories, Inc.).

Cell transfection. A total of 2 µg small interfering RNA (siRNA; 5'-UCAACCAGACCACCUUAUAdTdT-3'; Shanghai GenePharma Co., Ltd.) was used to silence TIMP-1 expression. A negative control (NC; cat. no. A06001; Shanghai GenePharma Co., Ltd.) was also included. The cells (4x10⁵) were seeded in 6-well plates. After culture for 24 h, the medium was replaced by Opti-MEM (Invitrogen; Thermo Fisher Scientific, Inc.). The fibroblasts were transfected with TIMP-1 siRNA using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). According to the manufacturer's protocol. The transfection efficiency was assessed using western blot analysis. The subsequent experiments were performed 24 h after transfection.

Wound healing assay. Cell migratory ability was measured using specific wound assay chambers (ibidi GmbH). Fibroblasts (1x10⁶) were seeded onto 24-well plates. Following attachment of the cells, a wound between the fibroblasts was created using a sterile pipette tip (10 µl). Then, the fibroblasts were washed and incubated for 24 h. The cell migration from 0 to 24 h was observed under a phase-contrast inverted microscope (magnification, x200; Olympus Corporation) with a TUCSEN camera. The cell migration was measured with Wimasis Image Analysis (Onimagin Technologies SCA) at 0 and 24 h.

Table I. Patients characteristics.

Variable	Patients with PH (n=55) (%)	Patients without PH (n=102) (%)	P-value
Age, years	66.9±10.4	66.3±9.1	0.708 ^a
Sex			<0.001 ^b
Male	19 (22.6)	65 (77.4)	
Female	36 (49.3)	37 (50.7)	
Body mass index (kg/m ²)	24.7±3.3	23.0±3.4	0.003 ^a
Diabetes			0.671 ^b
Yes	6 (40.0)	9 (60.0)	
No	49 (34.5)	93 (65.5)	
Hypertension			0.306 ^b
Yes	20 (40.8)	29 (59.2)	
No	35 (32.4)	73 (67.6)	
Radiation history			0.884 ^b
Yes	5 (35.7)	9 (64.3)	
No	43 (37.7)	71 (62.3)	
Length of hospital, days	15.8±8.2	15.5±7.6	0.819 ^a
Aperture size, cm	3.3±0.8	2.9±0.7	0.001 ^a
α1 (I) procollagen mRNA (ΔCq)	1.91±0.58	1.99±0.62	0.431 ^a
α1 (III) procollagen mRNA (ΔCq)	2.10±0.54	2.69±0.55	<0.001 ^a

PH, parastomal hernia. ^aStudent's t-test. ^bχ² test.

Transwell assay. The fibroblast invasion capabilities were determined using a Matrigel-coated Transwell assay (Corning Incorporated). Following serum starvation overnight, the fibroblasts were added into the upper chambers in DMEM containing 1% FBS. Concomitantly, the lower chambers were filled with 500 ml DMEM containing 20% FBS. The fibroblasts were incubated in an incubator for 24 h at 37°C. The non-invading fibroblasts blocked by the Matrigel were removed from the upper surface using a wet cotton swab. Following rinsing with PBS, the bottom surface of the filter was fixed with 95% ethanol at room temperature for 10 min and stained with 0.1% crystal violet at room temperature for 5 min. Invasion was detected by counting the stained cells under a light microscope (magnification, x200; Olympus Corporation).

Western blot analysis. The fibroblasts and PH tissues or tissues WPH and were lysed in lysis buffer (RIPA; Beyotime Institute of Biotechnology) and centrifuged at 2,000 x g for 10 min at room temperature for supernatant recovery. The protein concentrations were determined using a BCA kit (Beyotime Institute of Biotechnology). Following separation on 10% SDS-PAGE, the proteins (20 μg/lane) were transferred onto nitrocellulose membranes (EMD Millipore) and blocked with 5% non-fat milk for 1 h at room temperature. Then the membranes were incubated at 4°C overnight with anti-MMP-2 (cat. no. 40994; 1:1,000; Cell Signaling Technology, Inc.), anti-MMP-9 (cat. no. sc-21733; 1:500; Santa Cruz Biotechnology, Inc.), anti-TIMP-1 (cat. no. sc-365905; 1:1,000; Santa Cruz Biotechnology, Inc.), anti-collagen I (cat. no. sc-293182; 1:1,000; Santa Cruz Biotechnology, Inc.), anti-collagen III (cat. no. sc-514601; 1:1,000; Santa Cruz

Biotechnology, Inc.) and anti-β-actin (cat. no. 4970; 1:1,000; Cell Signaling Technology, Inc.) followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (cat. no. 10285-1-AP; 1:2,000, ProteinTech Group, Inc.) and HRP-goat anti-mouse IgG H&L (cat. no. ab205719; 1:2,000; Abcam) secondary antibodies at room temperature for 1 h. The proteins were visualized using an ECL kit (Amersham; GE Healthcare). The density of the blots was measured using the Quantity One software version 2.4 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Differences between groups for categorical data were analyzed by the χ² test and Fisher's exact test and continuous data were analyzed using a Student's t-test. The cumulative incidence rate of PH was calculated by the Kaplan-Meier estimate analysis with log-rank test. Correlations among the risk factors were assessed using logistic regression analysis with a 95% confidence interval (CI) for the inclusion of additional prediction into the model. The clinical features were used as independent variables, while the presence or absence of PH was treated as a dependent variable. The differences between multiple groups were assessed with one-way analysis of variance and Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference. SPSS 16.0 software (SPSS Inc.) was used for the analysis and the results were represented as mean ± standard deviation.

Results

Clinicopathological parameters. A total of 157 patients, including 55 patients with PH and 102 patients without PH, were reviewed. Among them, several parameters (age, history

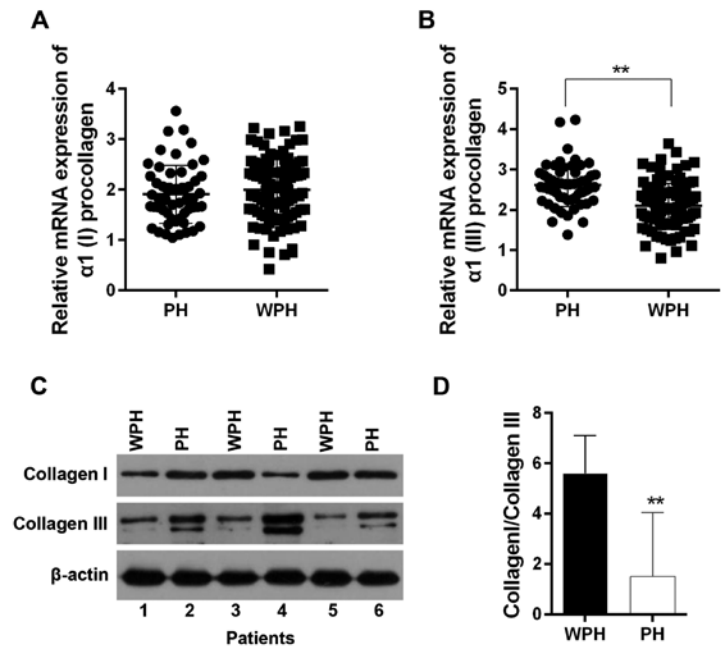


Figure 1. Expressions of (pro) collagen I and III in tissues from patients with PH. The mRNA expression levels of (A) $\alpha 1$ (I) procollagen and (B) $\alpha 1$ (III) procollagen by qPCR. (C) The protein levels of collagen I and III were examined by western blot analysis. (D) The ratio of collagen I to the collagen III protein. ** $P<0.01$ vs. WPH group. PH, parastomal hernia; WPH, tissues without PH.

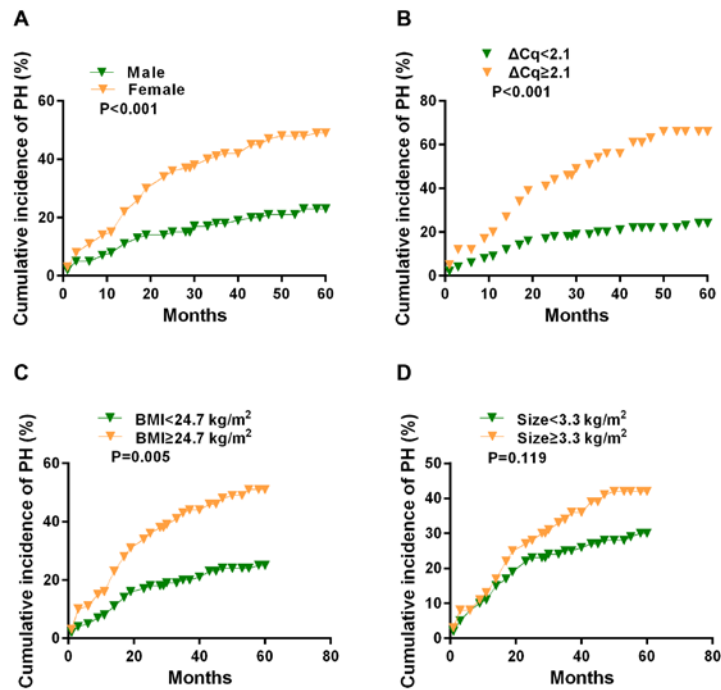


Figure 2. Cumulative incidence rate of PH. The cumulative incidence rate based on the (A) sex, (B) collagen III expression, (C) BMI and (D) aperture size. The cumulative incidence rate of PH was calculated by the Kaplan-Meier estimate analysis with log-rank test. PH, parastomal hernia; BMI, body mass index.

of diabetes, hypertension, radiation history, length of hospital stay and $\alpha 1$ (I) procollagen expression) exhibited no marked association with PH occurrence. However, the sex, BMI, aperture size and $\alpha 1$ (III) procollagen expression levels in the patients with PH were closely associated with the PH (Table I). The hernias occurred at between 7-57 months post-surgery, with an average of 23 months.

Among the 55 pairs of tissues, no marked difference in the $\alpha 1$ (I) procollagen expression was observed between the

dermal tissues of patients with PH and that in tissues from patients without PH (Fig. 1A). By contrast, the $\alpha 1$ (III) procollagen level in dermal tissues of patients with PH was markedly increased compared with those in the tissues from patients without PH ($P<0.05$; Fig. 1B). The protein levels of collagen I were irregular while the collagen III levels were notably increased in the dermal tissues of patients with PH (Fig. 1C). Furthermore, the ratio of collagen I/III was clearly decreased in the dermal tissues of patients with PH (Fig. 1D).

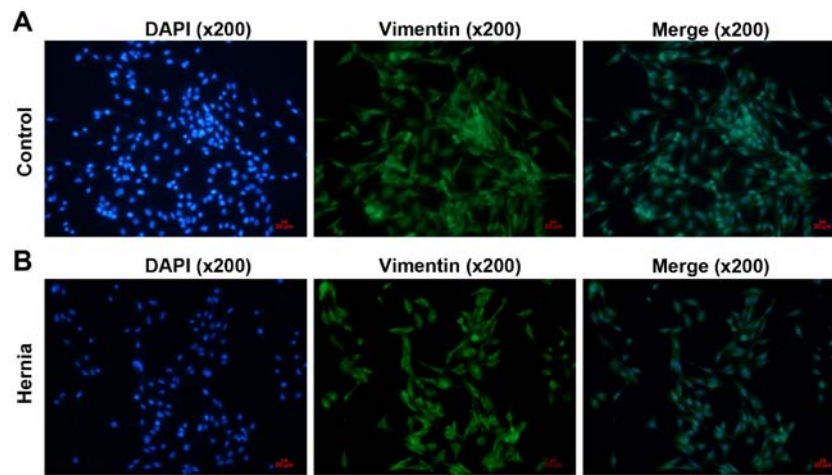


Figure 3. Immunofluorescence for the vimentin. (A and B) The vimentin (green) and nuclei stained with DAPI (blue) in the fibroblasts derived from (A) patients without PH (control) and (B) patients with PH. Magnification, x200. PH, parastomal hernia.

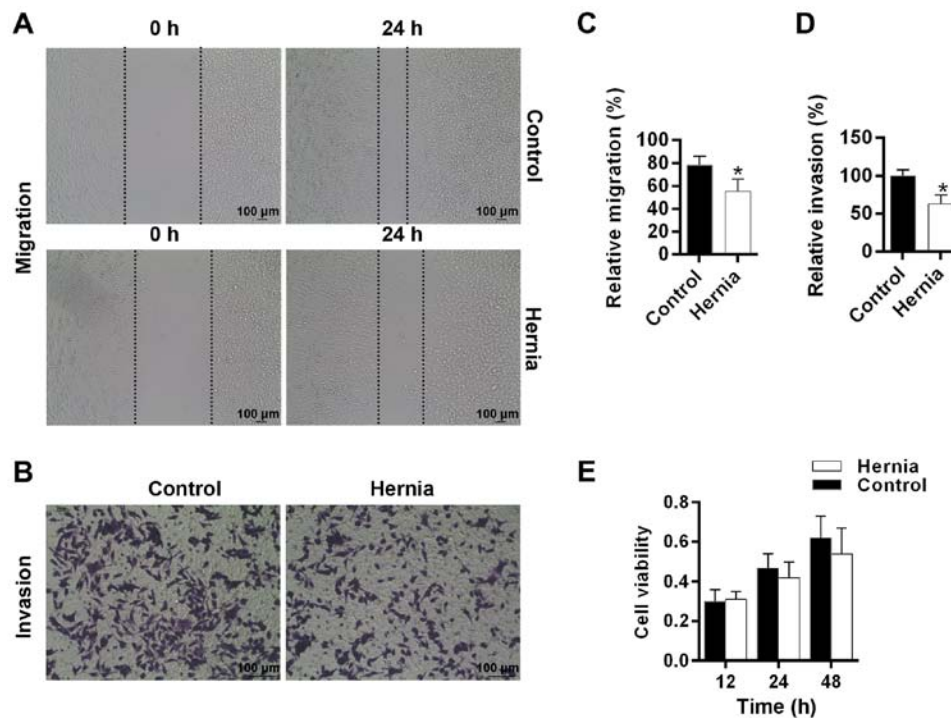


Figure 4. Fibroblast viability, migration and invasion. (A) The fibroblasts derived from patients with PH and those without PH (control) were allowed to migrate into the wound region, and the images were captured at different time points. Scale bar =100 μ m. (B) Fibroblast invasion was determined by cell staining. Scale bar =100 μ m. (C) migration and (D) invasion rate decreased in the hernia group. (E) The fibroblast viability was measured at 12, 24 and 48 h. *P<0.05 vs. control group. PH, parastomal hernia.

According to the Kaplan-Meier analysis, the 5-year cumulative incidence of PH was calculated based on the clinical parameters. The incidence rate in female patients (50.5%) was significantly increased compared with that of male patients (25.4%; P<0.001; Fig. 2A). Patients with increased α 1 (III) procollagen expression (68.9%) exhibited a higher incidence rate compared with those with decreased α 1 (III) procollagen levels (26.7%) (P<0.001; Fig. 2B). The 5-year cumulative incidence rate of patients with a BMI >24.7 kg/m² was 53.1%, which was remarkably increased compared with that of patients with a BMI <24.7 kg/m² (25.4%) (P=0.005; Fig. 2C). In addition, PH incidence rate of patients with an aperture

size >3.3 cm (41.3%) had no significant difference compared with that of patients with an aperture size <3.3 cm (31.5%) (P=0.119; Fig. 2D).

In the logistic analysis, female sex [odds ratio, 2.59; 95% confidence interval (CI), 1.107-6.059; P=0.028], aperture size (Odds ratio, 2.247; 95% CI, 1.280-3.946; P=0.005) and α 1 (III) procollagen (Odds ratio, 0.109; 95% CI, 0.045-0.265; P<0.001) were independent risk factors for PH formation, however, BMI was not associated with PH incidence (Table II).

Effect of fibroblasts on PH. Positive vimentin expression was observed in the fibroblasts derived from tissues from patients

Table II. Factors associated with the occurrence of parastomal hernia.

Variable	Odds ratio	95% confidence interval	P-value
Sex		1.107-6.059	0.028
Male	1		
Female	2.590		
Body mass index (kg/m ²)	1.116	0.983-1.266	0.089
Aperture size	2.247	1.280-3.946	0.005
$\alpha 1$ (III) procollagen mRNA (Δ Cq)	0.109	0.045-0.265	<0.001

Logistic regression was used to perform the statistical analysis.

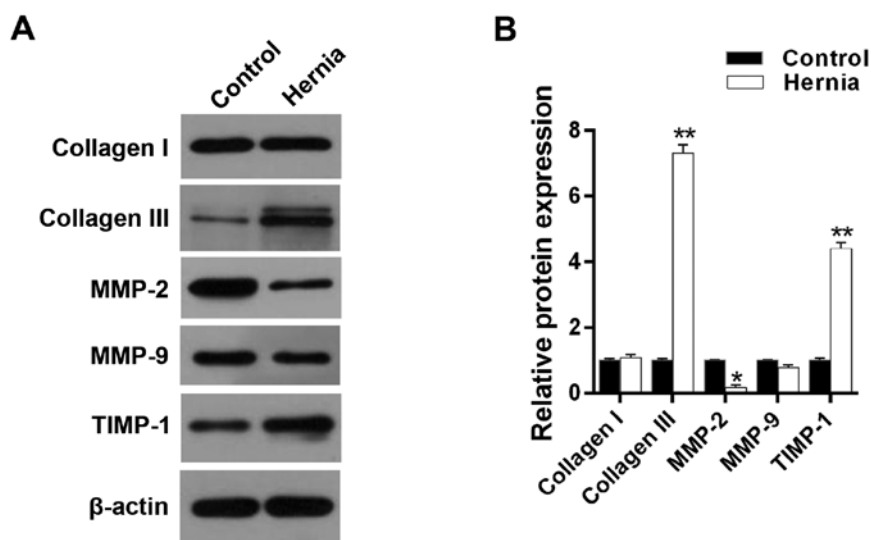


Figure 5. Expression of proteins associated with collagen regulation and metabolism. (A) The protein levels of collagen I, collagen III, MMP-2, MMP-9 and TIMP-1 were detected by western blot analysis. (B) The histogram presents the changes in the levels of these proteins in the fibroblasts derived from patients with PH and those without PH (control). * $P < 0.05$ and ** $P < 0.01$ vs. control group. MMP, matrix metalloproteinase; TIMP-1, metalloproteinase inhibitor 1; PH, parastomal hernia.

with PH and without PH. The cells exhibited long shuttle or flat star shapes (Fig. 3A and B).

The wound closure of the fibroblasts from tissues from patients without PH (control) reached ~80% by 24 h, while only ~50% of the area was denuded by the fibroblasts from PH dermal tissues (Fig. 4A). The invasion rate in the normal fibroblast group was evidently enhanced, while it was notably inhibited in the hernia fibroblast group (Fig. 4B). The data demonstrated that the migration and invasion rates of fibroblasts were significantly suppressed in patients with PH ($P < 0.05$; Fig. 4C and D). In addition, the fibroblast viability was also decreased in patients with PH, compared with patients without PH (Fig. 4E).

To explore the potential effects of collagen synthesis and the ECM on the PH, the protein levels of collagen I, collagen III, MMP-2, MMP-9 and TIMP-1 were detected (Fig. 5A). The collagen I expression levels in the fibroblasts from tissues from patients without PH were not significantly different from those in the fibroblasts from PH dermal tissues. However, among the fibroblasts from PH dermal tissues, the levels of collagen III and TIMP-1 were markedly increased while those of MMP-2 and MMP-9 were evidently inhibited in comparison with the non-PH fibroblasts ($P < 0.05$; Fig. 5B).

Silencing TIMP-1 improves the fibroblast activity. For the fibroblasts from non-PH tissues, the cell-free area in the control-NC group at 24 h was larger compared with that in control-siTIMP-1 group. Similarly, among the fibroblasts from PH dermal tissues, the cell-free area in the hernia-NC group at 24 h was larger compared with that in the hernia-siTIMP-1 group (Fig. 6A). Silencing TIMP-1 notably increased the mean number of normal skin fibroblasts migrating into the bottom chamber. In addition, the number of fibroblasts from PH dermal tissues was significantly increased by silencing TIMP-1, compared with that in hernia-NC group (Fig. 6B). It was also identified that silencing TIMP-1 markedly enhanced the migratory and invasive abilities of fibroblasts ($P < 0.05$; Fig. 6C and D).

Furthermore, the protein levels of the ECM-associated molecules were detected (Fig. 7A). The TIMP-1 expression was significantly suppressed in the groups transfected with siTIMP-1, indicating a successful transfection. It was identified that TIMP-1 silencing markedly inhibited the collagen III expression levels. In addition, the results also suggested that the levels of MMP-2 and MMP-9 were evidently increased by silencing TIMP-1, which indirectly indicated the negative correlation between MMP-2 or MMP-9 and collagen III ($P < 0.05$; Fig. 7B).

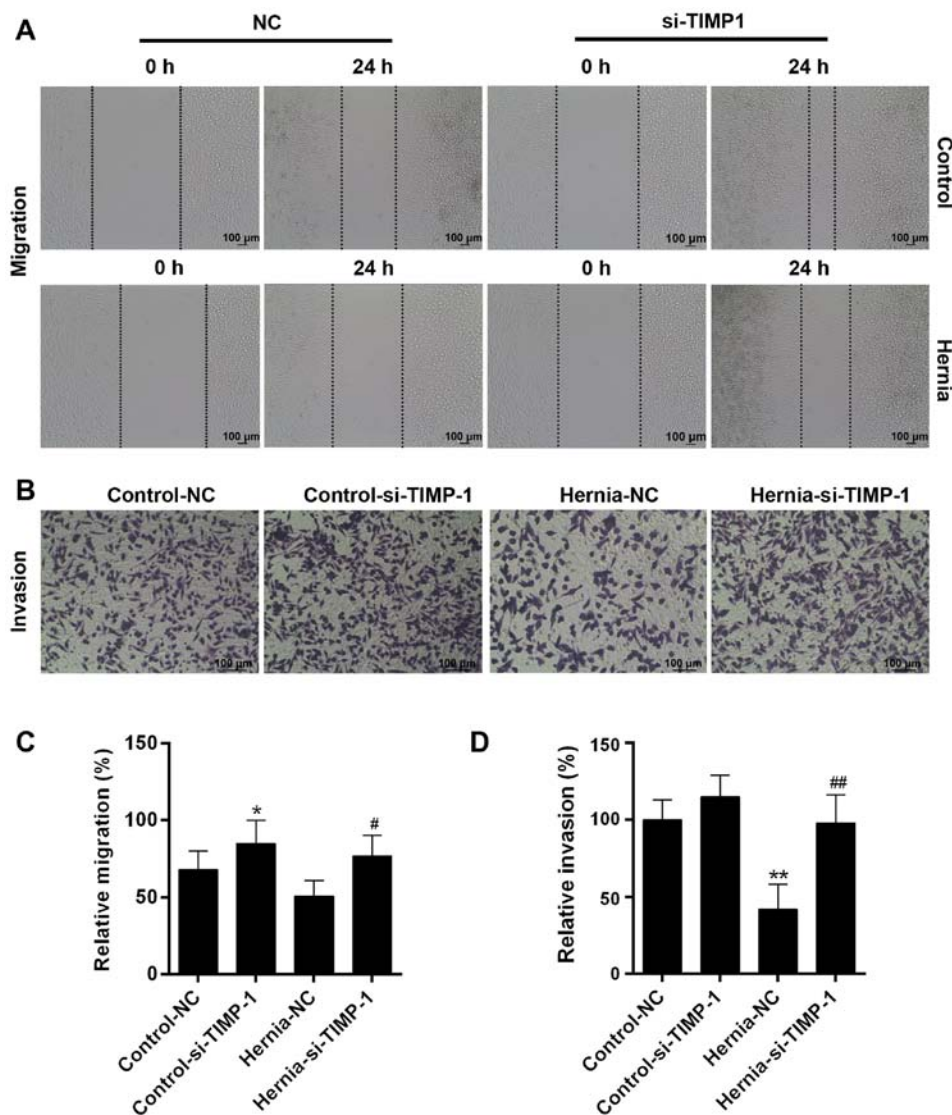


Figure 6. Effects of TIMP-1 silencing on fibroblast migration and invasion. (A) The fibroblasts, which were derived from patients with PH and those without PH (control), were allowed to migrate into the wound region. Scale bar =100 μ m. (B) The invaded fibroblasts were stained. Scale bar =100 μ m. (C) The number of migrated fibroblasts was counted. (D) The number of stained invasive cells was calculated. * $P<0.05$ and ** $P<0.01$ vs. control-NC group, # $P<0.05$ and ## $P<0.01$ vs. hernia-NC group. TIMP-1, metalloproteinase inhibitor 1; PH, parastomal hernia; si, small interfering; NC, negative control.

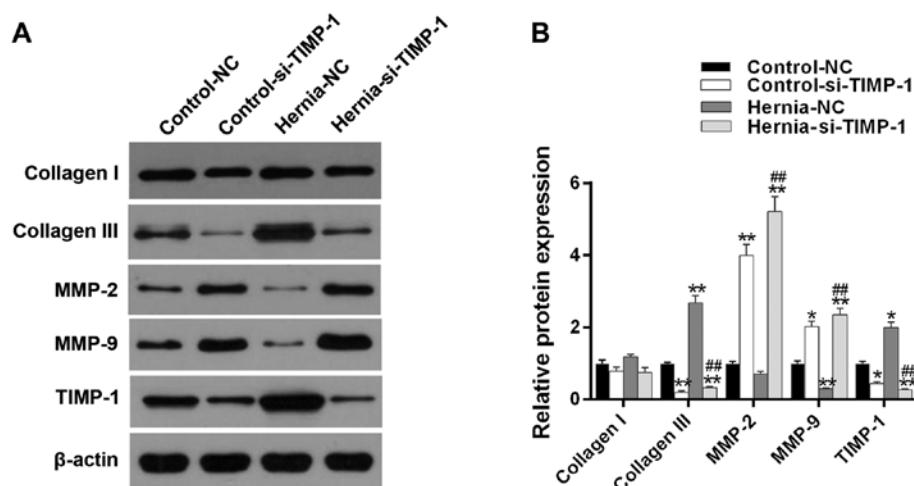


Figure 7. Effects of TIMP-1 silencing on the expression of collagen I, collagen III, MMP-2 and MMP-9. (A) The protein expressions of collagen I, collagen III, MMP-2, MMP-9 and TIMP-1 were determined by western blot analysis in the fibroblasts derived from patients with PH and those without PH (control). (B) Quantitative data of protein expression were analyzed. * $P<0.05$ and ** $P<0.01$ vs. control-NC group, ## $P<0.01$ vs. hernia-NC group. TIMP-1, metalloproteinase inhibitor 1; si, small interfering; MMP, matrix metalloproteinase; PH, parastomal hernia; NC, negative control.

Discussion

PHs are commonly presented as asymptomatic, however, they may result in morbidities due to leakage, dermatitis, perforation, intermittent obstruction and strangulation (24). The exact incidence rate of PH has been very difficult to assess due to lack of a consistent definitions (6). Several risk factors have been identified to be crucial, in spite of a paucity of reliable data. Age, sex, BMI, laparoscopic surgical approach, trans-peritoneal route of stoma creation and aperture size have been suggested as independent risk elements for PH development (7,25). In the present study, it was identified that sex, aperture size and collagen III expression were independent risk factors for PH incidence. In addition, it was also demonstrated that the decreased viability, migration and invasion of the skin-derived fibroblasts from PH patients were enhanced by silencing TIMP-1 and that TIMP-1 may regulate the genes associated with collagen regulation and metabolism.

The occurrence of PH may be attributed to a delayed or weakened healing process, but all the potential risk factors have not been comprehensively identified. In addition, multiple risk factors may be divided into three subcategories, including: Disease (ulcerative colitis, constipation, obesity and cancer); patient (age, sex, malnutrition, etc.); and surgery (emergency operation, postoperative infection) (26,27). The follow-up time following stoma has been demonstrated to be a vital factor in estimating an exact incidence rate of PH; the PH incidence rate was increased with longer follow-up time (28). In the present study, it was demonstrated that risk factors, including diabetes, hypertension, radiation history and length of hospital stay were not markedly different between patients with PH and those without PH. The average BMI of patients with PH was evidently increased compared with those without PH. However, in the logistic regression analysis, BMI was not identified as a significant independent factor for PH development; this observation was consistent with data from a previous study (29). Aperture size was revealed to be a potential independent factor of herniation; however, whether limiting the size of the aperture truly attenuates PH formation remains unknown (7,30). In addition, age was a potential risk factor for PH development. A retrospective review over a >10-year follow-up period of 782 patients demonstrated that PH incidence was increased in the elderly population (31). The present study identified that the PH incidence was increased in female patients; this may be explained by the fact that females usually have thinner abdominal muscle and thicker fat layers (32).

Furthermore, the present study also demonstrated that the levels of procollagen III expression were associated with the PH development and that collagen III expression was increased in patients with PH, indicating that collagen may be involved in PH incidence. Disordered collagen levels and decreased type I collagen and type III collagen expression levels have been observed previously in tissue biopsies from patients with hernias (33). The collagen I/III ratio of patients was revealed to be associated with hiatal hernia (34). Types I and III are the dominant components of collagen in the derma. Type I collagen is mature, mechanically stable and is demonstrated to be associated with tissue strength (35). The present study revealed that the collagen III expression levels were elevated in patients with PH and that the ratio of collagen I/III was

markedly decreased, suggesting that collagen III might serve a crucial role in PH occurrence.

Hernias have been suggested to be associated with a poor quality ECM, which systemically alters collagen turnover (36). ECM stability is directly associated with the levels of collagen synthesis and degradation (37). Due to the constituents of the ECM, collagen degradation may exert a crucial effect on the morphogenesis, development, tissue remodeling and repair processes (38). Furthermore, ECM degradation may be induced by MMPs (10). TIMPs have been demonstrated to exert an effect on cell proliferation, differentiation, migration, apoptosis and anti-angiogenesis processes (39). TIMP-1 may also regulate the levels of MMP-2 and MMP-9. In the present study, it was observed that the levels of MMP-2 expression were evidently decreased, while the TIMP-1 levels were notably enhanced in the hernia fibroblasts, suggesting that MMP-2 may be suppressed by TIMP-1. MMP-9 levels were slightly decreased in the hernia fibroblasts, and this may be due to an association between enhanced MMP-9 expression and poor healing (40). To additionally explore the effects of MMP-2 and MMP-9 on the levels of collagen III, TIMP-1 expression was silenced. The results indicated that silencing TIMP-1 reversed the decrease in viability, migration and invasion of fibroblasts from skin tissues of patients with PH. It has been indicated that MMP-2 is able to degrade interstitial collagen I, II and III, while MMP-9 cleaves collagen I and III (41,42). The results from the present study are consistent with previous studies that silencing TIMP-1 increased the expression levels of MMP-2 and MMP-9 and inhibited the expression of collagen III in the hernia fibroblasts. The inhibited expression of collagen III through TIMP-1 silencing was similar to the results from the clinicopathological analysis.

This study had a number of limitations; for example, the direct correlation between collagen III and MMP-2, the effect of over-expressed TIMP-1 on MMP-2 and MMP-9 were not analyzed. A more comprehensive and direct method of validating the results of the present study will be conducted in the future.

In conclusion, the present study demonstrated that collagen III levels were markedly and independently associated with the occurrence of PH. In addition, the potential regulatory mechanisms of collagen III were closely associated with TIMP-1, MMP-2 and MMP-9 expression. These results may provide novel therapeutic targets for PH.

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Availability of data and materials

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

FZ and FC made substantial contributions to the conception and design of the study. XY, YL and JC were responsible

for data acquisition, analysis and interpretation. XY and YL were involved in drafting the article and critically revising it for important intellectual content. All authors provided final approval of the version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All experiments were authorized by the Ethics Committee of Beijing Chao-Yang Hospital. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was provided by each patient.

Patient consent for publication

Informed consent was provided by each patient.

Competing interests

The authors declare that they have no competing interests.

References

- Carne PW, Frye JN, Robertson GM and Frizelle FA: Parastomal hernia following minimally invasive stoma formation. *ANZ J Surg* 73: 843-845, 2003.
- Celik S, Firat Kocaay A and Akyol C: Parastomal Hernia, pp185-199, 2017. <http://dx.doi.org/10.5772/intechopen.68876>. Accessed August 30, 2017.
- van Dijk SM, Timmermans L, Deerenberg EB, Lamme B, Kleinrensink GJ, Jeekel J and Lange JF: Parastomal hernia: Impact on quality of life? *World J Surg* 39: 2595-2601, 2015.
- Kwiat M and Kawata M: Avoidance and management of stomal complications. *Clin Colon Rectal Surg* 26: 112-121, 2013.
- Shah NR, Craft RO and Harold KL: Parastomal hernia repair. *Surg Clin North Am* 93: 1185-1198, 2013.
- Sohn YJ, Moon SM, Shin US and Jee SH: Incidence and risk factors of parastomal hernia. *J Korean Soc Coloproctol* 28: 241-246, 2012.
- Hong SY, Oh SY, Lee JH, Kim DY and Suh KW: Risk factors for parastomal hernia: Based on radiological definition. *J Korean Surg Soc* 84: 43-47, 2013.
- Skibiński R, Pasternak A, Szura M, Solecki R, Matyja M and Matyja A: Parastomal hernia-contemporary methods of treatment. *Pol Przegl Chir* 87: 531-537, 2015.
- Ma X, Liu Y, Wang Q, Chen Y, Liu M, Li X, Xiang R, Wei Y, Duan Y and Han J: Tamoxifen induces the development of hernia in mice by activating MMP-2 and MMP-13 expression. *Biochim Biophys Acta* 1852: 1038-1048, 2015.
- Nagase H, Visse R and Murphy G: Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69: 562-573, 2006.
- Han KY, Dugas-Ford J, Seiki M, Chang JH and Azar DT: Evidence for the involvement of MMP14 in MMP2 processing and recruitment in exosomes of corneal fibroblasts. *Invest Ophthalmol Vis Sci* 56: 5323-5329, 2015.
- Siefert SA and Sarkar R: Matrix metalloproteinases in vascular physiology and disease. *Vascular* 20: 210-216, 2012.
- Klein T and Bischoff R: Active metalloproteases of the ADAM and metalloprotease (ADAM) family: Biological function and structure. *J Proteome Res* 10: 17-33, 2011.
- Ishida Y, Kuninaka Y, Nosaka M, Kimura A, Kawaguchi T, Hama M, Sakamoto S, Shinozaki K, Eisenmenger W and Kondo T: Immunohistochemical analysis on MMP-2 and MMP-9 for wound age determination. *Int J Legal Med* 129: 1043-1048, 2015.
- Tanriverdi-Akhisaroglu S, Menderes A and Oktay G: Matrix metalloproteinase-2 and -9 activities in human keloids, hypertrophic and atrophic scars: A pilot study. *Cell Biochem Funct* 27: 81-87, 2009.
- Gill SE and Parks WC: Metalloproteinases and their inhibitors: Regulators of wound healing. *Int J Biochem Cell Biol* 40: 1334-1347, 2008.
- Schultz GS and Wsocki A: Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 17: 153-162, 2009.
- Seah CC, Phillips TJ, Howard CE, Panova IP, Hayes CM, Asandra AS and Park HY: Chronic wound fluid suppresses proliferation of dermal fibroblasts through a Ras-mediated signaling pathway. *J Invest Dermatol* 124: 466-474, 2005.
- Chu X, Wang H, Jiang YM, Zhang YY, Bao YF, Zhang X, Zhang JP, Guo H, Yang F, Luan YC and Dong YS: Ameliorative effects of tannic acid on carbon tetrachloride-induced liver fibrosis in vivo and in vitro. *J Pharmacol Sci* 130: 15-23, 2016.
- Takawale A, Zhang P, Patel VB, Wang X, Oudit G and Kassiri Z: Tissue inhibitor of matrix metalloproteinase-1 promotes myocardial fibrosis by mediating CD63-integrin β 1 interaction. *Hypertension* 69: 1092-1103, 2017.
- Dong J and Ma Q: TIMP1 promotes multi-walled carbon nanotube-induced lung fibrosis by stimulating fibroblast activation and proliferation. *Nanotoxicology* 11: 41-51, 2017.
- Chen G, Wang R, Chen H, Wu L, Ge RS and Wang Y: Gossypol ameliorates liver fibrosis in diabetic rats induced by high-fat diet and streptozocin. *Life Sci* 149: 58-64, 2016.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Chapman SJ, Wood B, Drake TM, Young N and Jayne DG: Systematic review and meta-analysis of prophylactic mesh during primary stoma formation to prevent parastomal hernia. *Dis Colon Rectum* 60: 107-115, 2017.
- Funahashi K, Suzuki T, Nagashima Y, Matsuda S, Koike J, Shiokawa H, Ushigome M, Arai K, Kaneko T, Kurihara A and Kaneko H: Risk factors for parastomal hernia in Japanese patients with permanent colostomy. *Surg Today* 44: 1465-1469, 2014.
- Pilgrim CH, McIntyre R and Bailey M: Prospective audit of parastomal hernia: Prevalence and associated comorbidities. *Dis Colon Rectum* 53: 71-76, 2010.
- Murray BW, Cipher DJ, Pham T and Anthony T: The impact of surgical site infection on the development of incisional hernia and small bowel obstruction in colorectal surgery. *Am J Surg* 202: 558-560, 2011.
- Höer J, Lawong G, Klinge U and Schumpelick V: Factors influencing the development of incisional hernia. A retrospective study of 2,983 laparotomy patients over a period of 10 years. *Chirurg* 73: 474-480, 2002.
- Gurmu A, Matthiessen P, Nilsson S, Pahlman L, Rutegård J and Gunnarsson U: The inter-observer reliability is very low at clinical examination of parastomal hernia. *Int J Colorectal Dis* 26: 89-95, 2011.
- Hotouras A, Murphy J, Power N, Williams NS and Chan CL: Radiological incidence of parastomal herniation in cancer patients with permanent colostomy: What is the ideal size of the surgical aperture? *Int J Surg* 11: 425-427, 2013.
- Jänes A, Cengiz Y and Israelsson LA: Preventing parastomal hernia with a prosthetic mesh. *Arch Surg* 139: 1356-1358, 2004.
- Kanehisa H, Miyatani M, Azuma K, Kuno S and Fukunaga T: Influences of age and sex on abdominal muscle and subcutaneous fat thickness. *Eur J Appl Physiol* 91: 534-537, 2004.
- Henriksen NA, Yadete DH, Sorensen LT, Agren MS and Jorgensen LN: Connective tissue alteration in abdominal wall hernia. *Br J Surg* 98: 210-219, 2011.
- Brown SR, Melman L, Jenkins E, Deeken C, Frisella MM, Brunt LM, Eagon JC and Matthews BD: Collagen type I:III ratio of the gastroesophageal junction in patients with paraesophageal hernias. *Surg Endosc* 25: 1390-1394, 2011.
- Shoulders MD and Raines RT: Collagen structure and stability. *Annu Rev Biochem* 78: 929-958, 2009.
- Henriksen NA, Mortensen JH, Lorentzen L, Ågren MS, Bay-Jensen AC, Jorgensen LN and Karsdal MA: Abdominal wall hernias-A local manifestation of systemically impaired quality of the extracellular matrix. *Surgery* 160: 220-227, 2016.
- Basson MD: Invited research review: Cell-matrix interactions in the gut epithelium. *Surgery* 133: 263-267, 2003.

38. Jabłońska-Trypuć A, Matejczyk M and Rosochacki S: Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *J Enzyme Inhib Med Chem* 31 (Suppl 1): S177-S183, 2016.
39. Su Y, Wan D and Song W: Dryofragin inhibits the migration and invasion of human osteosarcoma U2OS cells by suppressing MMP-2/9 and elevating TIMP-1/2 through PI3K/AKT and p38 MAPK signaling pathways. *Anticancer Drugs* 27: 660-668, 2016.
40. Agren MS, Jorgensen LN, Andersen M, Viljanto J and Gottrup F: Matrix metalloproteinase 9 level predicts optimal collagen deposition during early wound repair in humans. *Br J Surg* 85: 68-71, 1998.
41. Bigg HF, Rowan AD, Barker MD and Cawston TE: Activity of matrix metalloproteinase-9 against native collagen types I and III. *FEBS J* 274: 1246-1255, 2007.
42. Van Doren SR: Matrix metalloproteinase interactions with collagen and elastin. *Matrix Biol* 44-46: 224-231, 2015.



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