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Research article

High expression COL10A1 promotes breast cancer progression and predicts poor prognosis

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ABSTRACT

Background: As a common malignant disease in females, breast cancer (BCa) causes increasing numbers of cancer-related death. Collagen X alpha 1 chain (COL10A1) plays a critical role in the oncogenesis and progression of malignant tumors. However, a systematic analysis of COL10A1 in BCa has not been conducted.

Methods: The COL10A1 expression level and prognostic value in BCa were defined through the Cancer Genome Atlas (TCGA) as well as the Kaplan-Meier plotter data respectively. The expression pattern of COL10A1 was subsequently confirmed on tissue microarray (TMA) by immunochemistry (IHC) staining. Moreover, cellular functional assays which aimed to evaluate cell proliferation, migration, invasion, and apoptosis, were conducted to investigate the oncogenic activity of COL10A1 in BCa. Then, Tumor Immune Estimation Resource (TIMER) was adopted to determine the association between COL10A1 expression and immune cell infiltration.

Results: Bioinformatics analysis revealed that COL10A1 was significantly overexpressed and had notable prognostic value, especially for distant metastasis-free survival (DMFS) in BCa. Moreover, IHC analysis of 140 BCa tissues on TMA chips exhibited the overexpression of COL10A1 was correlated to advanced clinical stage, poor overall survival (OS), and worse recurrence-free survival (RFS). Besides, knockdown of COL10A1 remarkably suppressed cell proliferation, migration, and invasion in BCa cells, and notably promoted cell apoptosis as well. Furthermore, COL10A1 was positively associated with immune cell infiltration including B cell, $CD8^+$ T cell, $CD4^+$ T cell, macrophage, neutrophil, and dendritic cell.

Conclusion: The results revealed that COL10A1 is a novel oncogene and could serve as a potential prognostic biomarker in BCa. Besides, the downregulation of COL10A1 could inhibit BCa progression, which could be a potential target for BCa therapy.

1. Introduction

BCa is common cancer and possesses the highest morbidity among female malignant tumors all over the world [1]. Recently, the American Cancer Society predicted that BCa may cause 41,760 cancer-related death in USA [1]. As a complex heterogeneous disease, BCa shows great differences in molecular characteristics and malignant degree [2]. The promising comprehensive therapies for BCa, including surgery, endocrine therapy, chemotherapy, radiotherapy, targeted therapy, and emerging immunotherapy, have improved the curative effect to a great extent and notably reduced the mortality of BCa, nevertheless, patients with advanced stages still facing huge threats to lives [3, 4, 5]. Therefore, it remains urgent to investigate the underlying mechanisms of the oncogenesis and progression of BCa and identify novel therapeutic targets for BCa treatment furtherly.

Type X collagen belongs to the macromolecular collagen superfamily [6]. It is a kind of short-chain collagen, which is only half the length of ordinary collagen fibers and is composed of three α1 chains. The two ends are spherical structures, and the large one-end spherical structure is called NC1, which is the main domain of X collagen to exert its physiological functions [7]. COL10A1 is a specific cleavage fragment of type X collagen [8]. Unlike general collagen fibers that exist as a form of fibers, type X collagen usually exists in the extracellular matrix in a liquid form [9]. Type X collagens are synthesized and secreted by hypertrophic chondrocytes at the ossification site in the cartilage, and then penetrate the extracellular matrix [10]. Therefore, it is found in the active parts of

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human cartilage ossification such as the sternum, long bones, and cartilage of the head and face. Generally, as bone development stops, the level of type X collagen also decreases [11, 12]. Therefore, in adults, its content increases abnormally, which may indicate the occurrence of malignant tumors. In recent years, plenty of studies have revealed that type X collagen has increased expression in a variety of solid malignant tumors (colorectal cancer, gastric cancer, esophageal squamous cell carcinoma, lung adenocarcinoma, etc.), and is related to tumor growth, proliferation, migration, and poor prognosis [13, 14, 15, 16, 17, 18]. However, the systematic analysis of COL10A1 in BCa has not been conducted.

In this research, we demonstrated that COL10A1 was overexpressed in BCa tissues. Moreover, COL10A1 was significantly associated with poor prognosis via systematic bioinformatics analysis and IHC staining. We also elucidated the functional role of COL10A1 in BCa cells, namely inhibition of COL10A1 suppresses cell malignant biological properties. Besides, COL10A1 was remarkably associated with immune cell infiltration. Overall, these data supported that COL10A1 could serve as a promising target in BCa therapy and improve the prognosis of patients furtherly.

2. Materials and methods

2.1. TIMER database analysis

TIMER, based on the data from TCGA, is a web server for investigating molecular characterization of tumor–immune interactions and also can explore differential gene expression between tumor and normal tissue [19]. In this study, human COL10A1 expression levels in different tumor types from the TCGA database were evaluated by TIMER (https://cistrome.shinyapps.io/timer/) in log2 transformed TPM form [19, 20]. TIMER tool also was adopted to analyze the association between COL10A1 and immune cells (B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cell) infiltration in BCa.

2.2. Kaplan-Meier plotter analysis

Kaplan-Meier plotter (http://kmplot.com) was adopted to determine the prognostic value of COL10A1 in BCa from the databases including GEO, EGA, and TCGA [21]. The cutoff point of COL10A1 (Affymetrix ID 217428_s_at) mRNA gene expression levels was determined by auto-select the best cutoff from the selected BCa samples. The probe set used for the detection of prognostic values of COL10A1 was the JetSet best probe set. The survival curves were generated using Kaplan-Meier and compared by log-rank test. Hazard ratio (HR), 95% confidence interval (95% CI), and log-rank P value were calculated and displayed in respective Kaplan-Meier plots.

2.3. Tissue microarray (TMA) and immunohistochemistry (IHC)

Two BCa TMA slides (HBreD140Su03 and HBreD077Su01) were purchased from Outdo Biotech (Shanghai, China). The HBreD140Su03 slide contained 140 BCa tissues which consist of 7 HER2-positive HR-negative, 15 HER2-positive HR-positive, 23 triple negatives, 44 luminal A, and 30 luminal B (according to the 2021 Breast Cancer Guidelines of the Chinese Society of Clinical Oncology) or 128 invasive ductal carcinomas, 6 mucinous adenocarcinomas, 3 invasive lobular carcinomas and

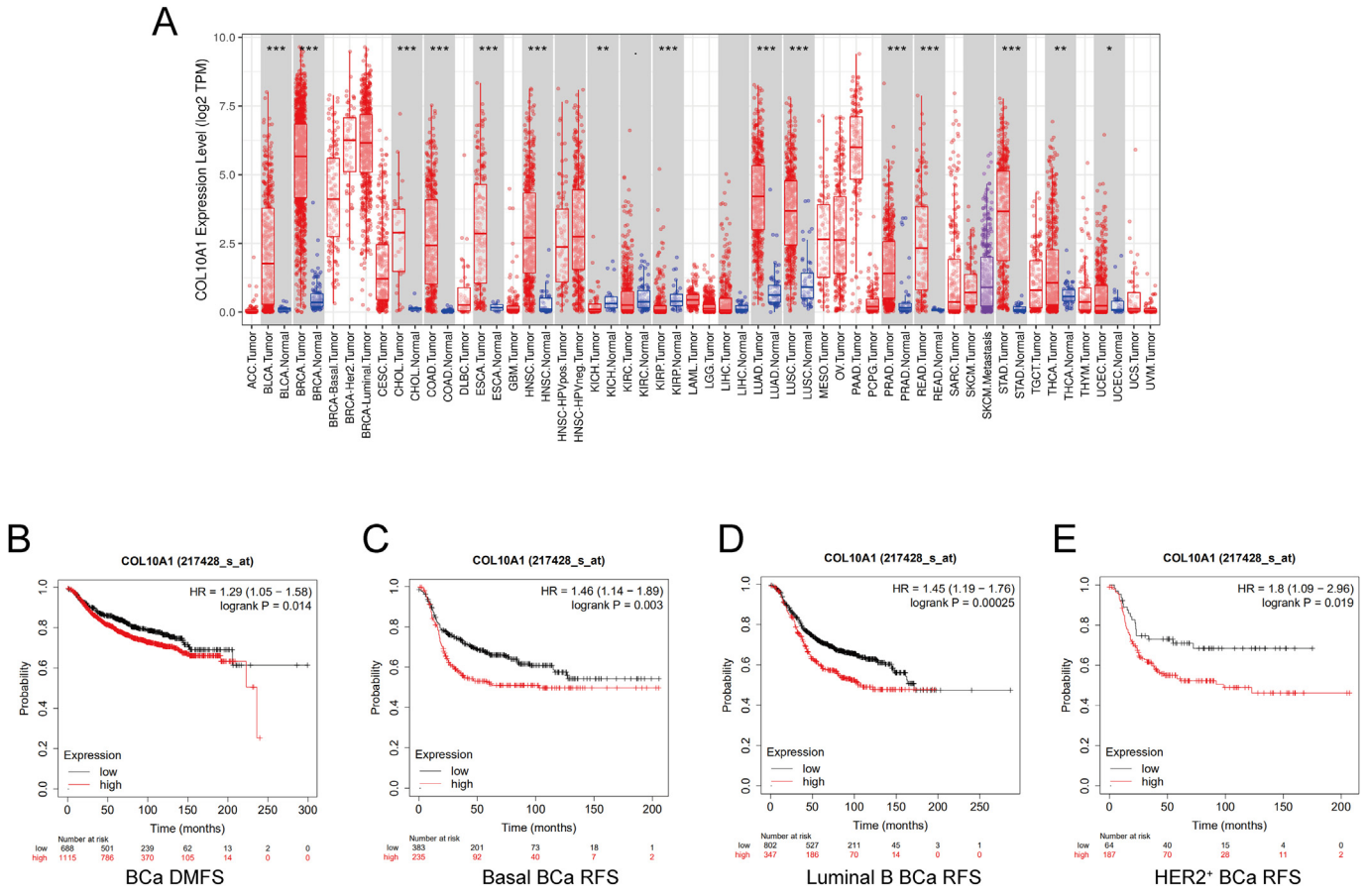

Figure 1. COL10A1 was up-regulated in mRNA level and associated with poor prognosis in BCa. (A) The mRNA expression of COL10A1 in BCa was detected by the TIMER database in log2 transformed TPM form. (B) DMFS curve of COL10A1 in BCa patients in Kaplan-Meier plotter. (C) RFS curve of COL10A1 in basal BCa patients in Kaplan-Meier plotter. (D) RFS curve of COL10A1 in luminal B BCa patients in Kaplan-Meier plotter. (E) RFS curve of COL10A1 in HER2^{+} BCa patients in Kaplan-Meier plotter.

1 intracapsular papillary carcinoma (based on the pathology of BCa). And the HBreD077Su01 slide contained 77 adjacent tissues paired with the tumor tissues on the HBreD140Su03 slide. Excluding spots that were peeled off during the staining process, 134 cases were retained for analysis with 74 paired adjacent tissues. Clinical stages were defined based on the 7th classification criteria from American Joint Committee on Cancer (AJCC). In this study, the use of tissue microarray slides was under ethical approval from the Clinical Research Ethics Committee, Outdo Biotech (Shanghai, China).

The TMA slides were dried, dewaxed, and rehydrated, followed by immersion in 3% hydrogen peroxide for 10 min. Subsequently, the slides were stained against COL10A1 antibody with 1:200 dilution (Cat. ab58632, Abcam). DAB and hematoxylin counterstain was used for antibody staining visualization. Furthermore, IHC staining and semi-quantitative analysis of COL10A1 expressions were performed as previously described [8]. The micrographs of immunostained sections were obtained by the use of a microscope (Olympus BX43).

2.4. Cell culture and transfection

Human BCa cell lines MCF-7 and MDA-MB-231 were purchased from KeyGEN BioTECH Inc. (Nanjing, China). MCF-7 cells were cultured in RPMI-1640 media (KeyGEN BioTECH Inc.) containing 10% fetal bovine serum (FBS). MDA-MB-231 cells were cultured in L15 media (KeyGEN BioTECH Inc.) containing 10% FBS. All cells were cultured in the condition of 37 °C with 5% CO_2. For subsequent assays, COL10A1-siRNA and siRNA control, synthesized in KeyGEN BioTECH Inc. (Nanjing, China), using Lipofectamine 3000 Reagent (Invitrogen) according to the manufacturer's instructions, was used to transfect incubated cells.

2.5. Quantitative real-time PCR

The total mRNA of BCa cells was extracted by the use of TRIzol reagent (Invitrogen). The primers for mRNA reverse transcription were synthesized by KeyGEN BioTECH Inc. (Nanjing, China). SYBR Green (One Step TB Green™ PrimeScript™ RT-PCR Kit II, TaKaRa) was used to label the amplified genes. The $2^{-\Delta\Delta Ct}$ method was used for GAPDH or COL10A1 expression analysis. Primers used for gene amplification and production length were as follows: GAPDH: 5′-CAAATTCCATGGCACCGTCA-3′ (forward), 5′-AGCATCGCCCCACTTGATTT-3’ (reverse), production length: 109 bp; COL10A1: 5′-GGATCAGGCTTCAGGGAGTG-3′ (forward) and 5′-GGCCATTTGACTCGGCATTG-3′ (reverse), production length: 86 bp.

2.6. Western blotting analysis

Cells were counted and seeded into 35-mm dishes (6×10^5 cells/dish) and then transfected with previously prepared COL10A1-siRNA and siRNA control. 48h after transfection, the proteins of cells were collected by the use of lysis buffer. And then proteins were separated with SDS-PAGE gel and transferred to PVDF membranes, which were incubated overnight with primary antibodies, followed by secondary antibodies for 1h, and then the chemiluminescent signals were detected by Enhanced chemiluminescence (ECL) kit (Millipore). The antibodies COL10A1 (1:200 dilution, Cat. ab58632, Abcam) and GAPDH (1:1000 dilution, rabbit. A19056, ABclonal) were used as primary antibodies. GAPDH was used as an internal control. The experiment was repeated three times and averaged for data analysis.

2.7. Cell proliferation, migration, and invasion analysis

Cell proliferation was assessed with the Cell Counting Kit-8 (CCK-8; KeyGEN BioTECH Inc., Nanjing, China) assay. Transfected cells were seeded in a 96-well plate at a concentration of 2000 cells/well, and then the CCK8 reagents were added to each well as depicted in the manufacturer's instructions. After incubating for 24 h, the absorbance was measured at 450 nm by using a microplate reader.

The wound-healing assay was adopted to evaluate cell migration. Transfected cells were seeded into a 24-well plate at a density of 1×10^5 cells/well, and then made a straight scratch with a pipette tip in each well as soon as cell concentration reached nearly 100%. An inverted microscope (Olympus, Japan) was used to record the scratch at 0 h and 24 h. The wound area was estimated by using the Image J software.

Transwell assay was adopted to evaluate cell invasion using Transwell chamber (Corning Incorporated, USA). 1×10^5 transfected cells which cultured without serum were suspended in the upper side of the Transwell chamber coated with Matrigel, and medium containing 10% FBS was added to the lower chamber. After 48 h, non-invasive cells were removed, while the invading cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. Finally, 5 random microscopic fields were scored to analyze the invasion of transfected cells. Each of the above experiments was repeated three times and averaged for data analysis respectively.

2.8. Apoptosis analysis

For the apoptosis analysis, transfected BCa cells were collected and washed once with PBS, and then centrifuged at 1500 rpm for 5 min. Subsequently, cells were resuspended in 1× annexin V (AV) binding

Table 1. Association between COL10A1 expression and clinical features in breast cancer.

Clinical Variables	Cases	COL10A1 expression		χ^2 value	*P* value
		Low	High		
Age(years)					
≤60	94	49	45	0.051	0.822
>60	40	20	20		
T stage					
T1	55	30	25	0.738	0.796
T2	77	38	39		
T3	2	1	1		
N stage					
N0	72	40	32	3.403	0.325
N1	19	11	8		
N2	35	16	19		
N3	8	2	6		
TNM stage					
1	32	16	16	3.821	0.148
2	58	35	23		
3	44	18	26		
Differentiation level					
High	93	54	39	4.145	**0.042**
Low	41	16	25		
Estrogen receptor					
Negative	40	17	23	0.043	0.837
Positive	90	40	50		
Unknown	4				
Progesterone receptor					
Negative	61	28	33	0.917	0.338
Positive	70	38	32		
Unknown	3				
Relapse status					
Relapsed	36	13	23	4.663	**0.031**
No relapse	98	56	42		
Survival status					
Alive	106	61	45	7.445	**0.006**
Dead	28	8	20		

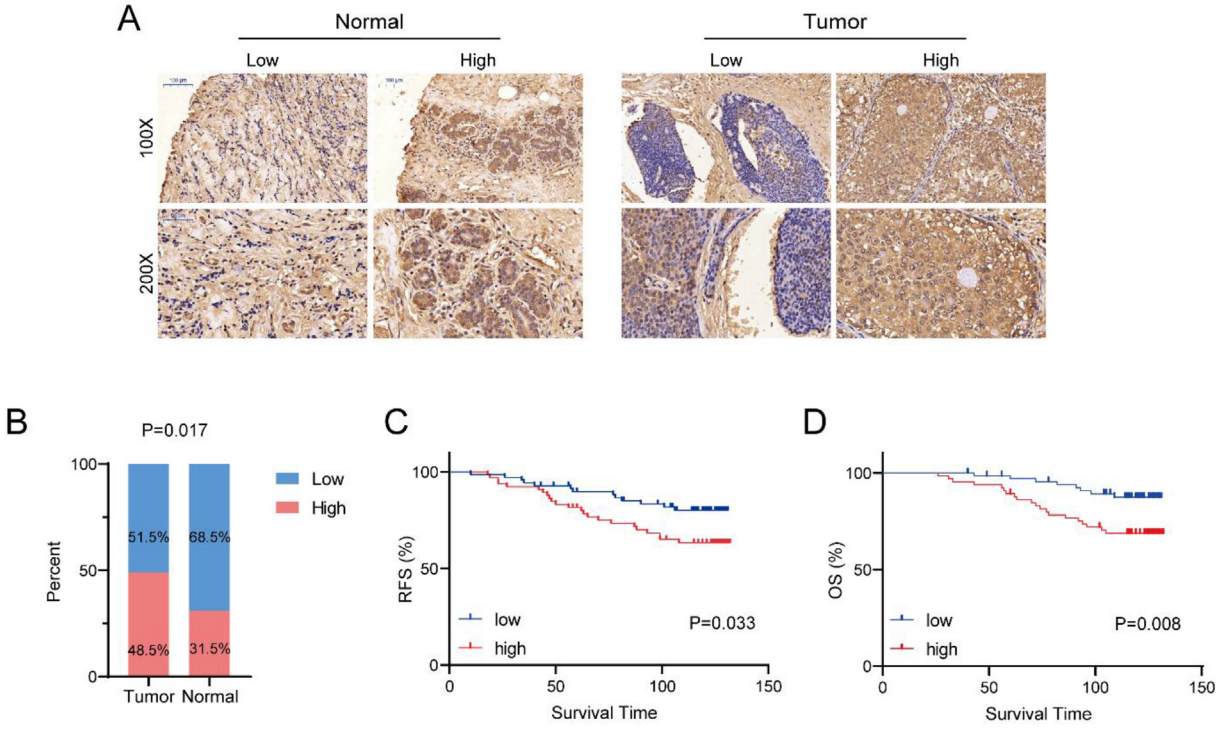

Figure 2. COL10A1 was overexpressed in protein level and associated with poor prognosis in BCa. (A) Representative IHC images of COL10A1 expression. (B) The staining score difference of COL10A1 expression in IHC. (C, D) Survival analysis of COL10A1 expression in BCa patients.

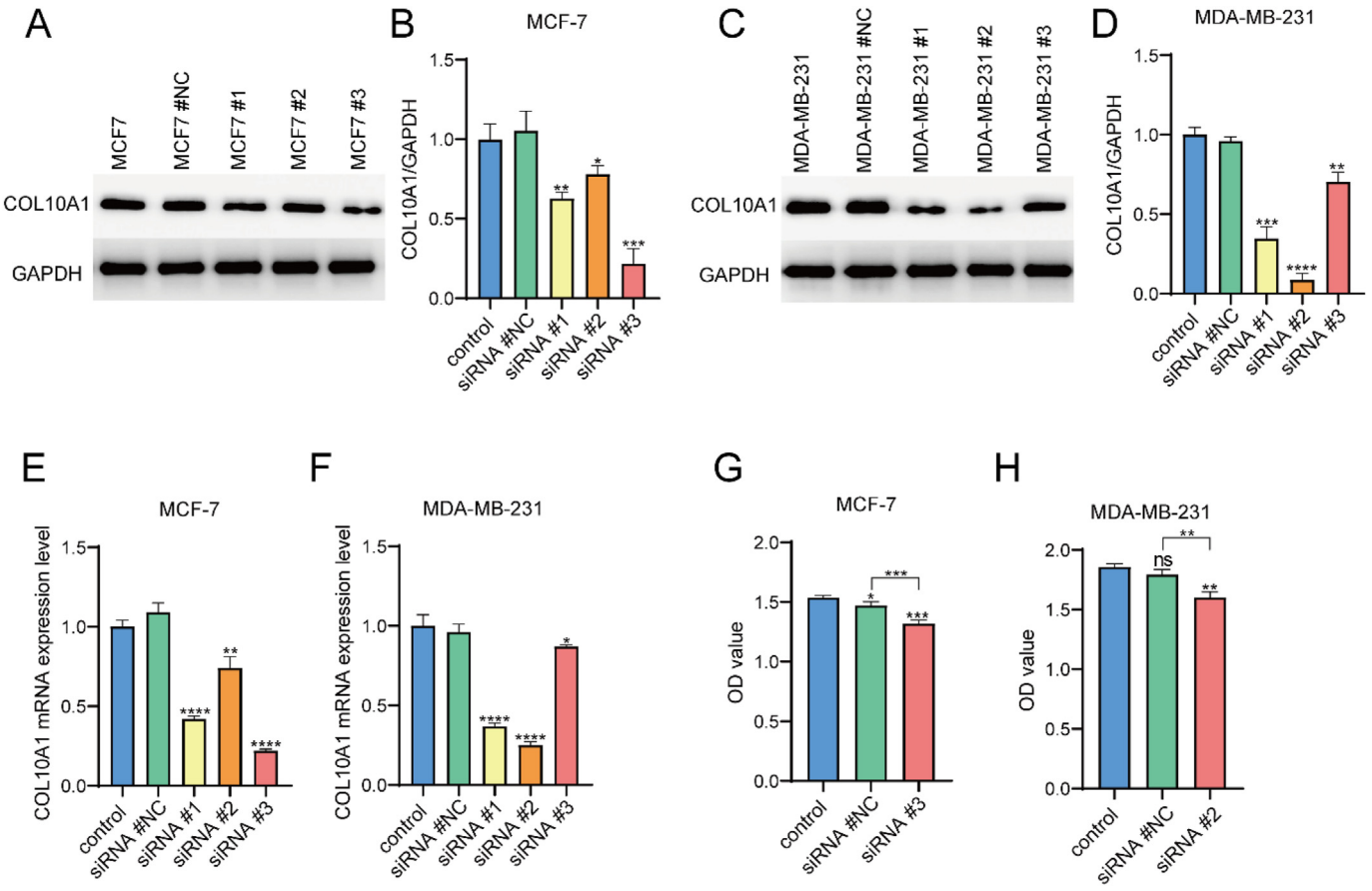

Figure 3. Inhibition of COL10A1 suppressed proliferation of BCa cells. (A, B) The transfection efficiency of MCF-7 was detected by Western blot assays at the protein level. (C, D) The transfection efficiency in MDA-MB-231 was detected by Western blot assays at the protein level. (E) The transfection efficiency in MCF-7 was detected by real-time PCR at the mRNA level. (F) The transfection efficiency in MDA-MB-231 was detected by real-time PCR at the mRNA level. (G) The cell proliferation in MCF-7 was detected by CCK8 assays. (H) The cell proliferation in MDA-MB-231 was detected by CCK8 assays. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

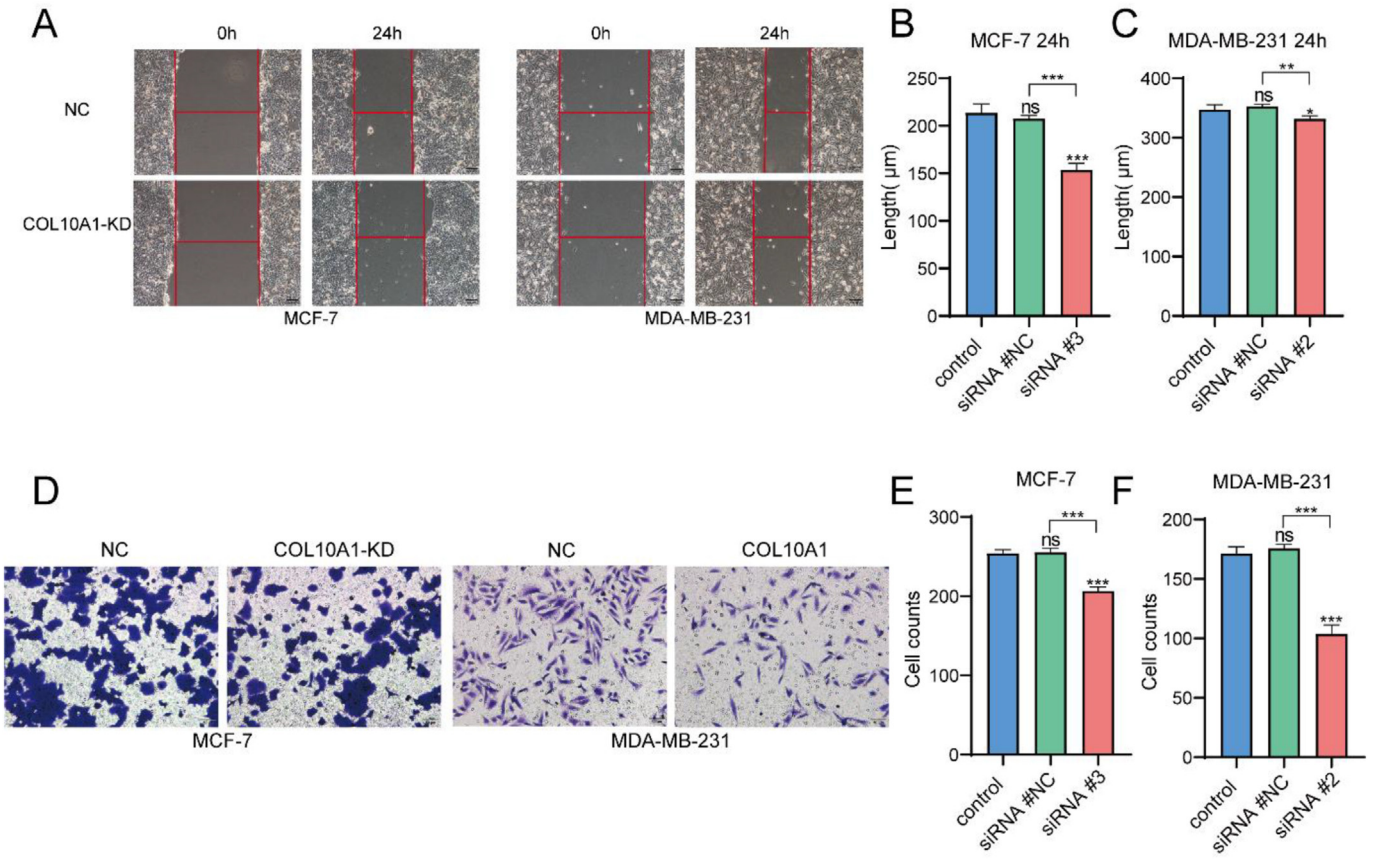

Figure 4. Inhibition of COL10A1 suppressed migration and invasion of BCa cells. (A, B, C) The cell migration rate was evaluated by wound healing assay. (E, D, F) The cell migration and invasion ability were detected by Transwell invasion assay. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

buffer and incubated with 5 μL of Annexin V and propidium iodide (PI) at 37 °C for 30 min. Flow cytometry (FACS Calibur) was adopted to evaluate cell apoptosis.

2.9. Statistical analysis

SPSS 25.0 software was used to calculate all statistical analyses. The data represent means ± SD for three independent experiments. Statistical differences between two groups and multiple groups were analyzed by Student's *t*-test and one-way ANOVA respectively, followed by Dunnett's multiple post-hoc tests. The association between COL10A1 expression level and clinicopathological characteristics was performed using Pearson's chi-squared test. The difference between the survival curves was measured using the Log-rank test. For all analyses, differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. COL10A1 is up-regulated in mRNA level and associated with poor prognosis in BCa

The mRNA expression of COL10A1 in BCa was analyzed based on the TCGA database by TIMER (https://cistrome.shinyapps.io/timer/) in log2 transformed TPM form. And the results revealed that COL10A1 was overexpressed in multiple cancer types, including BCa (Figure 1A). Then Kaplan-Meier plotter was applied to analyze the prognostic role of COL10A1 gene in BCa patients. Patients with up-regulated COL10A1 showed worse DMFS (HR = 1.29, P = 0.014) (Figure 1B). As for RFS, up-regulated COL10A1 was differently associated with different intrinsic sub-types. In basal (HR = 1.46, P = 0.003), luminal B (HR = 1.45, P < 0.001) and $HER2^+$ BCa (HR = 1.80, P = 0.019) group, patients with up-regulated COL10A1 had a higher risk of worse RFS (Figure 1C–E). Yet this effect was not significant in luminal A BCa patients.

3.2. COL10A1 is overexpressed in protein level and associated with poor prognosis in BCa

We further adopted TMA chips for IHC staining to validate the expression of COL10A1. The detailed information for TMA chips showed in *2.3* and Table 1. Representative IHC images were exhibited in Figure 2A. And then the expression level of COL10A1 was scored based on the intensity of the staining (from 1 to 12). The result showed the IHC staining of BCa tissues was notably deeper (higher scores) compared with corresponding normal tissues, suggesting that COL10A1 was stably high expressed in BCa tissues (P = 0.017) (Figure 2B). In addition, up-regulated COL10A1 expression was positively correlated with a lower differentiation level (P = 0.042), a higher chance of relapse (P = 0.031), and worse survival status (P = 0.006) (Table 1). Moreover, in our validated cohort, BCa patients with high COL10A1 expression exhibited poor RFS and OS than patients expressing low COL10A1 (P < 0.05) (Figure 2C and D).

3.3. Inhibition of COL10A1 suppressed proliferation, migration, and invasion of BCa cells

We chose two most widely used BCa cell lines, MCF-7 and MDA-MB-23, which were then transfected using COL10A1 siRNA lentivirus aimed to structure MCF-7-COL10A1-KD and MDA-MB-231-COL10A1-KD cell lines. Western blot (all P < 0.01; Figure 3A–D, Supplementary Figure) and real-time PCR (all P < 0.01; Figure 3E and F) were applied to detect the transfection efficiency. The two experiments concluded that MCF-7 siRNA #3 was the most effective siRNA to inhibit COL10A1 expression

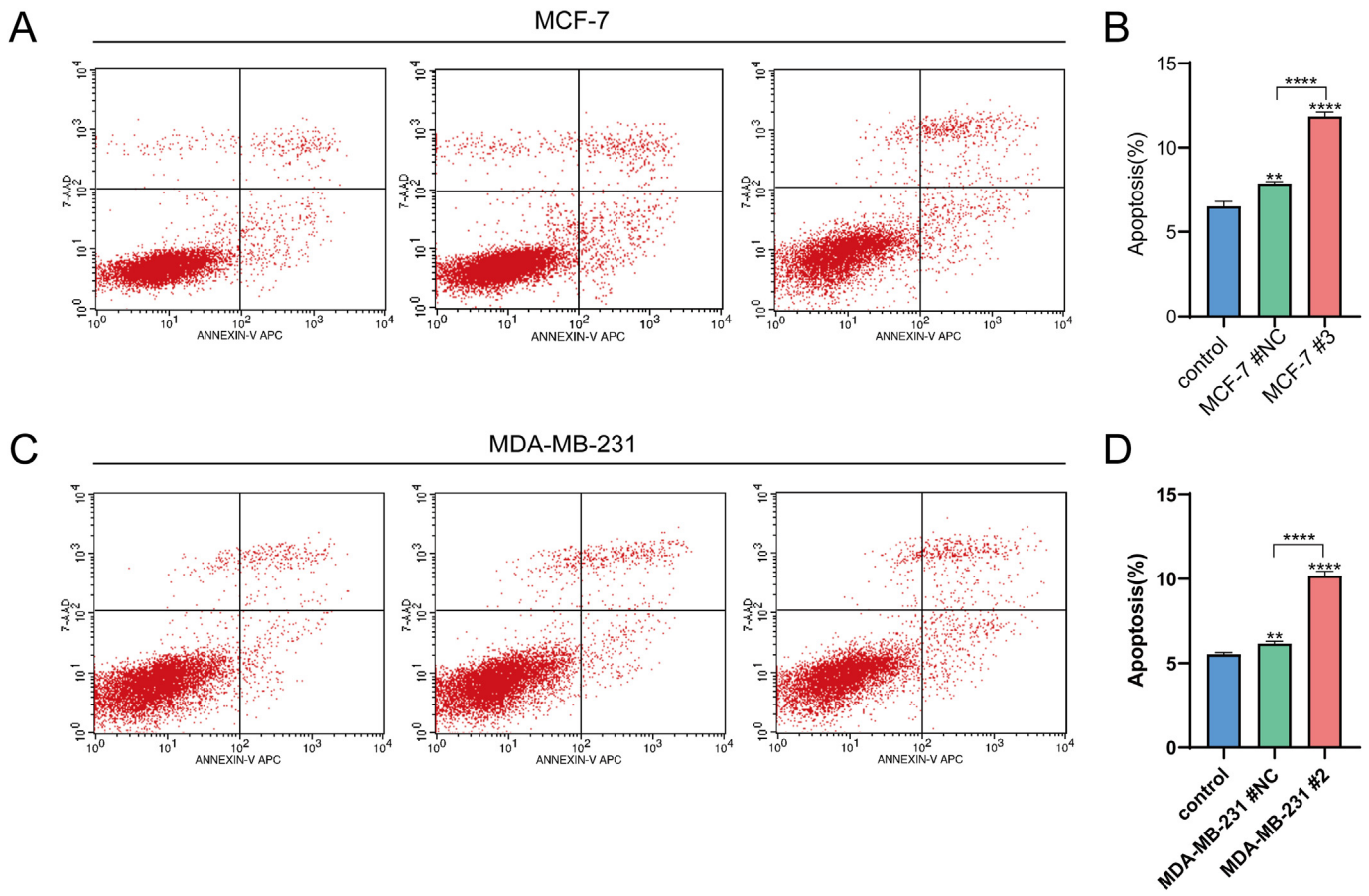

Figure 5. Deletion of COL10A1 induced cell apoptosis. (A, B) The cell apoptosis rate was up-regulated in MCF-7-KD cells compared with respective NC groups. (C, D) The cell apoptosis rate was up-regulated in MDA-MB-231-KD compared with respective NC groups. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

at both protein level and mRNA level. Likewise, siRNA #2 was the most effective siRNA to inhibit COL10A1 expression in MDA-MB-231. The CCK8 assay was adopted to detect cell proliferation. In MCF-7-KD and MDA-MB-231-KD cells, the cell proliferation was notably inhibited compared with respective NC groups (all $P < 0.01$; Figure 3G and H). The wound healing assay was adopted to measure cell migration. Representative images were exhibited in Figure 4A. The distance of migration was markedly reduced in MCF-7-KD ($P < 0.001$) and MDA-MB-231-KD ($P < 0.01$) cells in the condition of knocking down COL10A1 compared with respective NC groups (Figure 4B and C). In the invasion assay, the cell counts of MCF-7-KD and MDA-MB-231-KD cell lines were significantly lower than their respective NC groups (all $P < 0.001$, Figure 4D–F). Consequently, we concluded that COL10A1 was essential for the proliferation, migration, and invasion ability of BCa cells.

3.4. Deletion of COL10A1 induced cell apoptosis

The Annexin V-APC/7-AAD Apoptosis Detection Kit assay was applied to measure cell apoptosis. In MCF-7-KD ($P < 0.0001$) and MDA-MB-231-KD ($P < 0.0001$) cells, cell apoptosis rate was up-regulated compared with respective NC groups (Figure 5A–D). As a result, the apoptosis rate was highly up-regulated when COL10A1 was knocked down. These results showed that COL10A1 could decrease apoptosis in BCa cells.

3.5. COL10A1 association with immune cells infiltration

Moreover, the association between COL10A1 and immune cells was determined by the TIMER database. In BCa, it was shown that COL10A1 expression was positively correlated with B cell ($P < 0.05$), $CD8^+$ T cell ($P < 0.001$), $CD4^+$ T cell ($P < 0.001$), macrophage ($P < 0.001$), neutrophil ($P < 0.001$) and dendritic cell ($P < 0.001$) infiltration (Figure 6). Overall, the primary evidence implied that COL10A1 may impact the recruitment and modulation of immune cell infiltration within the BCa microenvironment.

4. Discussion

COL10A1 is liquid non-fibrous collagen mainly distributed in the intercellular substance and important components involved in the process of human endochondral ossification [22]. The lack or abnormal synthesis of type X collagen could cause dwarfism [23]. Increased research revealed the expression of COL10A1 was higher in tumors compared with normal tissues, and the overexpression of COL10A1 affected the oncogenesis and progression even the prognosis of diverse malignant tumors including but not limited to lung adenocarcinoma (LUAD), gastric cancer, and colorectal cancer (CRC) [13, 14, 15, 16, 17, 18]. Makoukji J et al. have also found that COL10A1 may be up-regulated in highly aggressive subtypes (HER-2 positive) BCa [24], while there are no reports about the effect of COL10A1 on BCa cell proliferation, invasion, and migration. Accordingly, we speculated that COL10A1 might function as an oncogene and correlate with the poor prognosis in patients with BCa, which is similar to its role in other cancers [25, 26]. First, we tried to systematically analyze the expression level and prognostic value of COL10A1 in BCa using the TCGA data and the Kaplan-Meier plotter. As shown in Figure 1, the COL10A1 was overexpressed in BCa at the mRNA level, and the up-regulated COL10A1 was associated with a poor prognosis in patients with BCa. Then, we applicated TMA chips to furtherly validate this finding (Figure 2). We found the intensity of COL10A1 positive staining was remarkably higher in BCa compared with normal

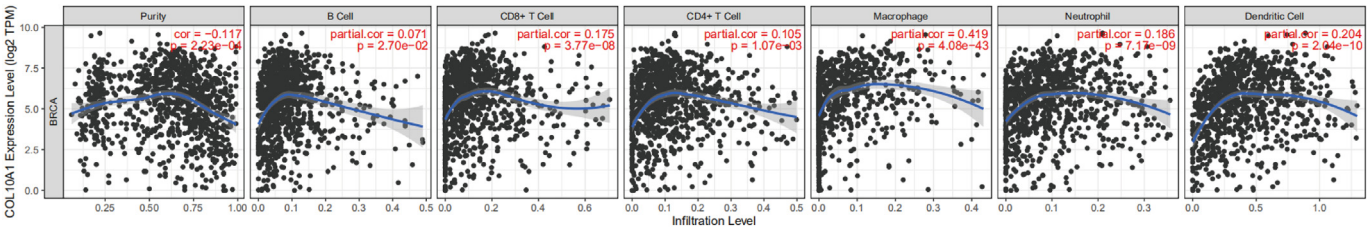

Figure 6. The TIMER database was used to determine the correlation between COL10A1 and immune cells in BCa.

breast tissues by IHC analysis. Furthermore, survival analysis suggested that RFS and OS were notably worse in BCa patients with high COL10A1 expression than those with low COL10A1 levels. Besides, up-regulated COL10A1 expression was correlated with differentiation level, relapse status, and survival status of BCa patients. These results accord with the previous research. For example, a significantly increased expression of COL10A1 was also found in the plasma of gastric adenocarcinoma patients and related to the early detection of gastric cancer [17]. It was also demonstrated the overexpression of COL10A1 was an independent risk factor of prognosis and overall survival in colorectal cancer (CRC) patients [14]. In addition, Li J et al. identified COL10A1 as a prognostic candidate gene by integrated bioinformatic analysis [27], which was consistent with our findings in the current research.

Subsequently, we focused on exploring the effect of COL10A1 on cellular functions including cell proliferation, apoptosis, and other potential biological functions in BCa cells by knockdown the COL10A1 expression with siRNA. In BCa cells, the results showed that inhibition of COL10A1 remarkably restrained cell proliferation, and cell apoptosis, and also notably suppressed the migration and invasion in vitro. The functional studies confirmed the oncogenic role of COL10A1 in BCa. The role of COL10A1 in tumor progression has also been reported in several cancers. A study showed that upregulated COL10A1 promotes the proliferation and invasion of lung adenocarcinoma (LUAD) cells in vitro and vivo. Another research reported that COL10A1 could promote EMT in CRC [14]. Thus, the overexpression of COL10A1 may facilitate the proliferation, and metastasis of BCa cells through the promotion of epithelial-mesenchymal transition (EMT) as well. In addition, COL10A1 can also accelerate tumor progression by regulating the downstream signaling pathway. Liang YK et al. demonstrated that COL10A1 promoted the proliferation and metastasis of LUAD cells through the COL10A1/DDR2/FAK axis [16]. In this study, our results accord with previous studies that COL10A1 has a cancer-promoting effect in diverse malignant tumors [13, 14, 15, 16, 17].

Collagen in cancer tissue accompanies various immune cells, such as macrophages, neutrophils, and lymphocytes [28]. For example, in pancreatic cancer, COL1 expression can influence the levels of $CD4^+$ T cells, $CD8^+$ T cells, macrophages, and neutrophils [29]. This study revealed that COL10A1 expression was positively correlated with B cell, $CD8^+$ T cell, $CD4^+$ T cell, macrophage, neutrophil, and dendritic cell, which indicated that COL10A1 may be a novel and potential target in BCa therapy.

Collectively, these findings revealed that the COL10A1 overexpressed in BCa, and COL10A1 down-regulation significantly inhibited BCa progression, which may be a potential therapeutic target in the future. However, there remained some limitations of our research including the lack of positive control in IHC staining, and more importantly, the specific molecular mechanism of COL10A1 in promoting BCa progression need to be further investigated.

5. Conclusions

In this research, we confirmed the expression of COL10A1 was up-regulated in BCa tissues by systematic analysis and IHC staining. Besides, up-regulated COL10A1 expression was correlated with the progression and poor prognosis of BCa. Moreover, COL10A1 played a crucial part in oncogenesis, and impacts on BCa cell growth, migration, invasion, and apoptosis. Overall, our findings reveal that COL10A1 acts as a potential molecular target and a novel prognostic indicator in BCa.

Declarations

Author contribution statement

Weijian Zhou; Yuting Li: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dingyi Gu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Junying Xu; Huiyu Wang; Chaoying Liu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Runjie Wang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e11083.

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