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RESEARCH ARTICLE

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Integrin α7 high expression correlates with deteriorative tumor features and worse overall survival, and its knockdown inhibits cell proliferation and invasion but increases apoptosis in breast cancer

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Abstract

Background: This study aimed to investigate the correlation of integrin α7 (ITGA7) expression with clinical/pathological characteristics and overall survival (OS), and its knockdown on inhibiting cell activities in breast cancer.

Methods: A total of 191 breast cancer patients underwent surgery were retrospectively reviewed, and ITGA7 expression in tumor tissues was determined by immunofluorescence and real-time quantitative polymerase chain reaction. Patients' clinical/pathological data were recorded, and OS was calculated. In vitro, control shRNA and ITGA7 shRNA plasmids were transfected into MCF7 cells to evaluate the influence of ITGA7 knockdown on cell proliferation, apoptosis, and invasion.

Results: Ninety-two (48.2%) patients presented with ITGA7 high expression, and 99 patients (51.8%) presented with ITGA7 low expression. ITGA7 expression was positively correlated with T stage, tumor-node metastasis (TNM) stage, and pathological grade. Kaplan-Meier curves showed that ITGA7 high expression was associated with shorter OS, and multivariate Cox's proportional hazards regression displayed that ITGA7 high expression was an independent predictive factor for poor OS. Moreover, in vitro experiments disclosed that cell proliferation (by Cell Counting Kit-8 assay) and cell invasion (by Matrigel invasion assay) were reduced, while cell apoptosis rate (by Annexin V/propidium iodide assay) was enhanced by ITGA7 knockdown in MCF-7 cells.

Conclusion: Integrin α7 high expression correlates with increased T stage, TNM stage, and pathological grade as well as worse OS, and its knockdown enhances cell apoptosis but inhibits cell proliferation and invasion in breast cancer.

KEYWORDS

apoptosis, breast cancer, integrin α7, proliferation, survival

1 | INTRODUCTION

Breast cancer has ranked the most common cancer and the leading cause of cancer death among females worldwide, which accounts for estimated 1 700 000 new cases and causes 520 000 deaths around the world according to 2015 global statistics.[1] In China, breast cancer occurs in 268 600 new cases and results in 69 500 deaths in females.[1,2] With the development in medical technology, various treatment options have been applied in breast cancer patients (such as surgery, chemotherapy, endocrine therapy, as well as targeted therapies).[3,4] Early breast cancer is considered potentially curable with these measurements, whereas the efficacy of current available treatments is still limited for the disease metastasis that is responsible for 90% of the deaths from breast cancer.[3-5] Thus, exploration of novel treatment target as well as convincing biomarker for prognosis is of great importance for management of breast cancer progression.

Integrins are a large family of heterodimeric cell surface receptors that regulated cell-cell and cell-extracellular matrix interactions.[6] Integrin α7 (ITGA7) is a gene localized on chromosome 12q13 and composed of at least 27 exons spanning a region of around 22.5 kb, which is the receptor for the extracellular matrix (ECM) protein laminin and forms heterodimer with integrin β1.[7] Recently, the functions of ITGA7 in cancers have attracted increasing attentions. Several previous studies have disclosed that ITGA7 is upregulated and correlates with adverse clinicopathological characteristics in some cancers (such as esophageal squamous cell carcinoma and clear cell renal cell carcinoma).[7,8] Moreover, some in vitro experiments have disclosed that ITGA7 serves as a tumor oncogene in different cancer cells (such as glioblastoma and pancreatic carcinoma) through affecting cell proliferation and invasion.[8,9] Considering these implications about the promotive effect of ITGA7 in different cancers, we speculated that ITGA7 also might contribute to the progression of breast cancer and might be a potential treatment target, while relevant evidence is still limited. Thus, we conducted this study to investigate the correlation of ITGA7 expression with clinical/pathological characteristics and overall survival (OS) in breast cancer patients and further explore its knockdown on inhibiting breast cancer cell activities in vitro.

2 | MATERIALS AND METHODS

2.1 | Patients

A total of 191 breast cancer patients underwent resection from January 2014 to December 2016 were reviewed in our study. The screening criteria were as follows: (a) diagnosed as primary breast cancer by clinical and histopathological examinations; (b) underwent surgical resection; (c) formalin-fixed, paraffin-embedded tumor tissue was appropriately preserved and available; and (d) clinical data were complete. Following patients were excluded: (a) relapsed or secondary cancer; (b) underwent neoadjuvant therapy; and (c) suffering from other malignancies. The approval for this study was obtained from the Ethics Committee of GanSu Provincial Cancer Hospital, and verbal (with recording) or written informed consents were collected from included patients or their guardians.

2.2 | Data collection

The screening and data retrieving were conducted in June 2018, and patients' clinical data including age, tumor size, T stage, N stage, M stage, tumor-node metastasis (TNM) stage, pathological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor-2 (HER-2) status, as well as survival records were collected. The pathological grade was classified as grade 1 (G1): well differentiation; grade 2 (G2): moderate differentiation; and grade 3 (G3): poor differentiation. The TNM stage was assessed in accordance with the American Joint Committee on Cancer (AJCC) Staging System for Breast Cancer (7th version). The last follow-up date was June 30, 2018, and OS was calculated from the date of surgical resection to the date of death or last visit.

2.3 | Sample collection and ITGA7 expression

All formalin-fixed, paraffin-embedded tumor tissues were collected from the Pathology Department after approval by the Hospital. Specimens were cut onto 5-μm slices, dried at 65℃ for 3 hours, deparaffinized in xylene (Catalog number: 95682; Sigma), followed by rehydration using gradient ethanol. Then, the slices were transparentized by polybutylene terephthalate and soaked in the solution of 1% bovine serum albumin (BSA) (Catalog number: A1933; Sigma) +0.1% Triton X-100 (Catalog number: T9284; Sigma) for 30 minutes. Next, slices were quenched with fresh 3% hydrogen peroxide (Catalog number: 323381; Sigma) to inhibit endogenous tissue peroxidase activity, and the antigen retrieval was performed using microwave. After blocked by 10% goat serum (Catalog number: 50062Z; Thermo), the slices were incubated with rabbit anti-ITGA7 antibody (Catalog number: ab203254; Abcam) at a dilution of 1:500 in buffer 4℃ overnight. Next day, slices were washed in buffer and then were incubated with Alexa Fluor® 488 conjugate-labeled antibody against rabbit IgG (Catalog number: #4412; CST) at dilution of 1:500. After that, the slices were washed with phosphate buffer saline (PBS) and followed by 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) (Catalog number: D3571; Invitrogen) staining and then were covered with coverslips. All slices were evaluated by 50i Nikon microscope in dark, and ITGA7 expression was semi-quantitatively assessed by using the following intensity categories: 0, no staining; 1, weak but detectable staining; 2, moderate or distinct staining; and 3, intense staining. A histological score (HSCORE) was derived from the formula HSCORE = $\Sigma Pi(i + 1)$, where i represents the intensity scores, and Pi is the corresponding percentage of the cells. According to the HSCORE, ITGA7 high expression was defined as HSCORE > 0.7, and the ITGA7 low expression was defined as HSCORE ≤ 0.7.[10] Furthermore, RNA was extracted from the

FFPE tissue and detected by real-time quantitative polymerase chain reaction (RT-qPCR).

2.4 | ITGA7 knockdown in breast cancer cell line

Control shRNA and ITGA7 shRNA plasmids were established by Shanghai GenePharma Bio-Tech Company using pEX-2 vectors. Then, plasmids were transfected into MCF7 cells as control group and ITGA7 knockdown group. ITGA7 protein and mRNA expressions were subsequently detected by Western blot and RT-qPCR at 24 hours post-transfection to determine the transfection success. Human breast cancer cell line MCF7 was purchased from Cell Resource Center of Shanghai Institute of Life Sciences, Chinese Academy of Sciences, and cultured in 90% MEM medium (Catalog number: 12571071; Gibco) with 10% fetal bovine serum (Catalog number: 10099141; Gibco) under 95% air and 5% CO_2 at 37℃. Sequences for ITGA7 shRNA were as follows: forward 5′-CACCGCTGCCCACTCTACAGCTTTTCGAAAAAAGCTGTAGAGTGGGCAGC-3′, reverse 5′-AAAAGCTGCCCACTCTACAGCTTTTTTCGAAAAGCTGTAGAGTGGGCAGC-3′, and sequences for control shRNA were as follows: forward 5′-CACCGTTCTCCGAACGTGTCACGTCGAAACGTGACACGTTCGGAGAA-3′, reverse 5′-AAAATTCTCCGAACGTGTCACGTTTCGACGTGACACGTTCGGAGAAC-3′.

2.5 | Detection of cell proliferation, cell apoptosis, and cell invasion

Cell proliferation, cell apoptosis, and cell invasion were measured by Cell Counting Kit-8 (CCK-8), Annexin V/propidium iodide (AV/PI), and Matrigel invasion assays, respectively. Cell viability was detected at 0, 24, 48, and 72 hours post-transfection using Cell Counting Kit-8 (Catalog number: CK04; Dojindo) according to the instructions of manufacturer. Cell apoptosis rate was detected at 24 hours post-transfection using FITC Annexin V Apoptosis Detection Kit II (Catalog number: 556547; BD) according to the instructions of manufacturer. Besides, cell invasive ability was detected by Matrigel invasion assay using Matrigel basement membrane matrix (Catalog number: 356234; BD), Transwell filter chamber (Catalog number: 3422; Coring), formaldehyde solution (Catalog number: 818708; Sigma), and crystal violet (Catalog number: 46364; Sigma) according to the method described previously.[11]

2.6 | Western blot

Western blot was performed as the following steps: (a) extraction of total protein was conducted with RIPA lysis and extraction buffer (Catalog number: 89901; Thermo); (b) concentration of total protein was measured by Pierce™ BCA Protein Assay Kit (Catalog number: 23227; Thermo), followed by electrophoresis and transfer to membranes; (c) membranes were blocked and then incubated with primary antibody (rabbit anti-ITGA7 antibody [Catalog number: ab203254; Abcam]) (1:1000 dilution) and horseradish peroxidase (HRP)-conjugated secondary antibody (goat anti-rabbit IgG H&L [HRP] [Catalog number: ab6721; Abcam]) (1:4000 dilution). Finally, the bands were visualized by Pierce™ ECL Plus western blotting substrate (Catalog number: 32132X3; Thermo).

2.7 | RT-qPCR

RT-PCR was performed as the following steps: (a) with TRIzol reagent (Catalog number: 15596018; Invitrogen), extraction of total RNA was performed; (b) the reverse transcription to cDNA was conducted using PrimeScript™ RT reagent Kit (Catalog number: RR037A; TAKARA); (c) qPCR was performed using QuantiNova SYBR Green PCR Kit (Catalog number: 208054; Qiagen), followed by qPCR amplification. Finally, the results of qPCR were calculated by $2^{-\Delta\Delta Ct}$ formula. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference. And information of primers used was as follows: ITGA7, forward (5′->3′): GCCACTCTGCCTGTCCAATG, reverse (5′->3′): GGAGGTGCTAAGGATGAGGTAGA; GAPDH, forward (5′->3′): GAGTCCACTGGCGTCTTCAC, reverse, ATCTTGAGGCTGTTGTCATACTTCT.

2.8 | Statistical analysis

Data were displayed as mean (standard deviation), median (interquartile range), or count (percentage). Difference between two groups was determined by Wilcoxon rank-sum test, *t* test, chi-square test, or log-rank test (for survival analysis). Survival curves were constructed with the Kaplan-Meier method. The influence of each variable on survival was examined by the univariate and multivariate Cox's proportional hazards regression analyses. All statistical analyses were performed with SPSS 19.0 software (IBM Corporation). A *P* value < .05 was considered statistically significant.

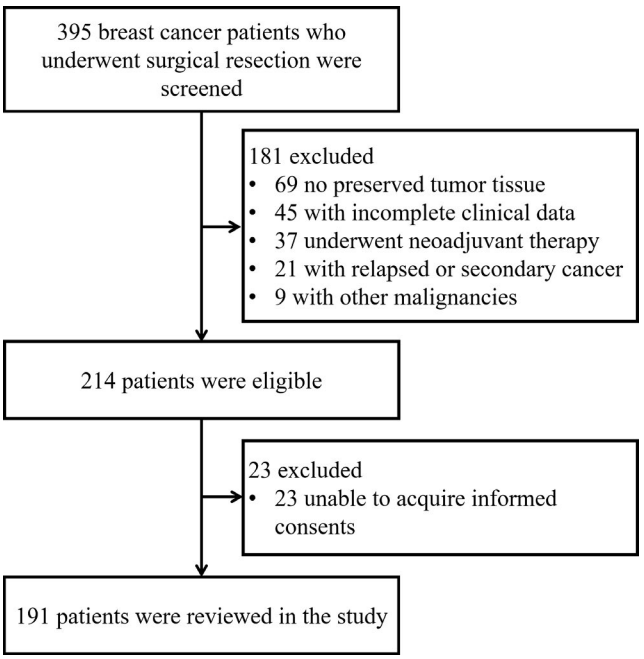

FIGURE 1 Study flow

3 | RESULTS

3.1 | Study flow

Totally 395 breast cancer patients who underwent surgical resection were retrospectively screened in this study, while 181 of them were excluded, including 69 patients with no preserved tumor tissue, 45 patients with incomplete clinical data, 37 patients underwent neoadjuvant therapy, 21 patients with relapsed or secondary cancer, and nine patients with other malignancies (Figure 1). Subsequently, in the 214 patients eligible, 23 patients who were unable to acquire informed consents were excluded, and finally 191 patients were reviewed and analyzed in this study.

3.2 | Baseline characteristics

A total of 191 breast cancer patients were enrolled, with the mean age of 54.3 ± 13.6 years and the median age of 53.0 (45.0-64.0) years (Table 1). For tumor size, the mean value was 3.2 ± 1.7 cm, and the median value was 3.0 (2.0-4.0) cm. Regarding the disease stage, the numbers of patients with TNM I, TNM II, as well as TNM III were 27 (14.1%), 119 (62.3%), and 45 (23.6%), respectively. As for pathological grade, the numbers of patients with grade G1, G2, and G3 were 42 (22.0%), 124 (64.9%), and 25 (13.1%), respectively. The detailed information about other baseline characteristics is presented in Table 1.

TABLE 1 Baseline characteristics of breast cancer patients

Characteristics	Breast cancer patients (N = 191)
Age (y)	
Mean (SD)	54.3 (13.6)
Median (IQR)	53.0 (45.0-64.0)
Tumor size (cm)	
Mean (SD)	3.2 (1.7)
Median (IQR)	3.0 (2.0-4.0)
T stage, No. (%)	
T1	61 (31.9)
T2	113 (59.2)
T3	17 (8.9)
N stage, No. (%)	
N0	95 (49.7)
N1	55 (28.8)
N2	38 (19.9)
N3	3 (1.6)
TNM stage, No. (%)	
I	27 (14.1)
II	119 (62.3)
III	45 (23.6)
Pathological grade, No. (%)	
G1	42 (22.0)
G2	124 (64.9)
G3	25 (13.1)
ER, No. (%)	
Negative	73 (38.2)
Positive	118 (61.8)
PR, No. (%)	
Negative	90 (47.1)
Positive	101 (52.9)
HER-2, No. (%)	
Negative	128 (67.0)
Positive	63 (33.0)

Abbreviations: ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2; IQR: interquartile range; N: node; PR: progesterone receptor; SD: standard deviation; T: tumor; TNM: tumor-node metastasis.

3.3 | ITGA7 expression in breast cancer patients

Examples of tumor ITGA7 high expression and ITGA7 low expression are shown in Figure 2A. In totally 191 patients, 92 (48.2%) patients presented with ITGA7 high expression, and 99 (51.8%) patients presented with ITGA7 low expression (Figure 2B).

3.4 | Correlation of ITGA7 expression with clinical characteristics in breast cancer patients

ITGA7 protein high expression was associated with elevated T stage (P = .004), increased TNM stage (P = .038), and raised pathological grade (P = .017) in breast cancer patients (Table 2), whereas no correlation of ITGA7 protein expression with age (P = .395), tumor size (P = .661), N stage (P = .131), ER (P = .584), PR (P = .442), and HER-2 (P = .915) was observed. Meanwhile, ITGA7 mRNA high expression was associated with increased T stage (P = .002), elevated TNM stage (P = .017), and higher pathological grade (P = .013). These data suggested that ITGA7 expression was positively correlated with T stage, TNM stage, and pathological grade in breast cancer patients.

3.5 | Correlation of ITGA7 expression with OS in breast cancer patients

K-M curves displayed that ITGA7 protein high expression was associated with shorter OS (P < .001) (Figure 3A); moreover, ITGA7 mRNA high expression was correlated with worse OS in breast cancer patients (P = .009) (Figure 3B).

3.6 | Analysis of factors affecting OS in breast cancer patients

Univariate Cox's regression displayed that ITGA7 high expression (P < .001) was associated with shorter OS, and larger tumor size (P < .001), higher T stage (P < .001), higher N stage (P < .001), higher TNM stage (P < .001), and higher pathological grade (P < .001) were also associated with worse OS in breast cancer patients (Table 3),

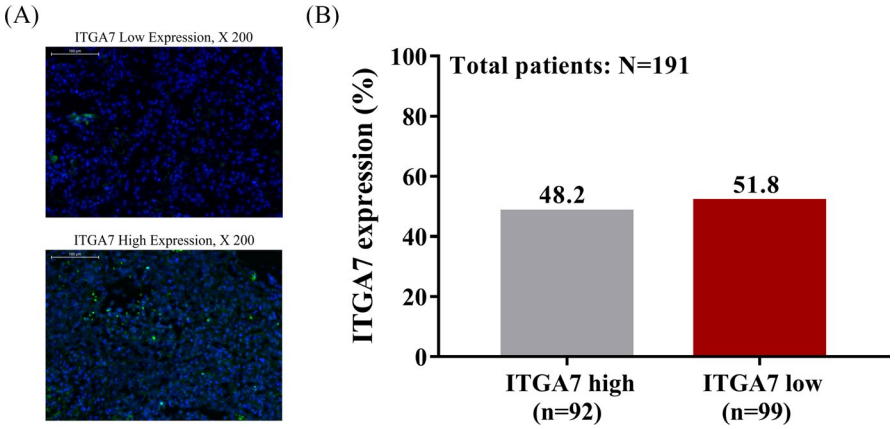

FIGURE 2 ITGA7 expression detected by IF. Examples of ITGA7 high expression and ITGA7 low expression detected by IF (A). There were 92 (48.2%) patients with ITGA7 high expression and 99 (51.8%) patients with ITGA7 low expression (B). ITGA7, integrin α7; IF, immunofluorescence

whereas ER (positive vs negative) (P = .010) and PR (positive vs negative) (P = .022) were correlated with longer OS in breast cancer patients. Furthermore, the multivariate Cox's regression analysis revealed that ITGA7 high expression was an independent predictive factor for poorer OS (P = .006) in breast cancer patients, and higher pathological grade also independently predicted unfavorable OS (P = .001) in breast cancer patients.

3.7 | Effect of ITGA7 knockdown on cell proliferation, cell apoptosis, and cell invasion in MCF7 cells

In order to assess the effect of ITGA7 knockdown on cell functions in breast cancer cells, control shRNA and ITGA7 shRNA plasmids were constructed and transfected into MCF7 cells. After transfection at 24 hours, the expressions of ITGA7 mRNA (P < .01) and ITGA protein were reduced in ITGA7 knockdown group compared to control group (Figure 4A,B). Cell proliferation was reduced in ITGA7 knockdown group at 48 hours (P < .05) and 72 hours (P < .05) compared to control group (Figure 4C). For cell apoptosis, its rate was elevated in ITGA7 knockdown group at 24 hours compared to control group (P < .01) (Figure 4D,E). Additionally, invasive cell count was lower in ITGA7 knockdown group compared to control group (P < .01) (Figure 4F,G). These data indicated that ITGA7 knockdown repressed cell proliferation and invasion, but enhanced cell apoptosis in MCF7 cells.

4 | DISCUSSION

Our results indicated that (a) ITGA7 high expression correlates with increased T stage, raised TNM stage, elevated pathological grade and worse OS, and it was an independent predictive factor for worse OS in breast cancer patients; (b) ITGA7 knockdown inhibited cell proliferation, cell invasion but enhanced cell apoptosis in breast cancer.

Integrins are transmembrane cell surface receptors, which comprises of 18 α and 8 β subunits.[7] ITGA7, encoding a subunit belonging to integrin, mediates a variety of cell-cell and cell-matrix interactions, and it is recently reported to play a role in cell migration, cell differentiation, and cell metastasis in cancers.[12,13] For instance, some studies disclose that ITGA7 knockdown inhibits Hsp27-mediated cell invasion in HCC cells and decreases S100P-mediated cell migration in lung cancer cells.[14,15] Besides, a study shows that ITGA7 represses cell apoptosis as well as promotes chemoresistance via activating focal adhesion kinase (FAK)/Akt signaling, but enhances cell metastasis via inducing epithelial-mesenchymal transition (EMT) in OSCC cells.[7] Another study illustrates that ITGA7 knockdown might inhibit cell proliferation via decreasing phosphorylated AKT and p38 in glioblastoma cells.[8] These previous data reveal that ITGA7 may be involved in the initiation and progression of some cancers, and it is able to affect some vital biological functions (such as cell apoptosis, cell invasion, and chemotaxis) of cancer cells via regulating multiple pathways (such as FAK/Akt signaling and phosphatidylinositol 3-kinase (PI3K)/Akt pathway).

In a few observational studies, the role of aberrant ITGA7 expression in some cancers has been disclosed.[7,8] For example, a study shows that ITGA7 overexpression correlates with increased disease grade in glioblastoma patients.[8] Another study displays that ITGA7 high expression is remarkably associated with poor differentiation and lymph node metastasis in esophageal squamous cell carcinoma patients.[7] These previous studies reveal that ITGA7 high expression correlates with aggravated disease progression in these cancer patients, while the correlation of ITGA7 with disease progression in breast cancer is still inconclusive. In this study, we enrolled 191 breast cancer patients to explore the correlation of ITGA7 with disease progression of breast cancer patients. We found that ITGA7 high expression was associated with raised T stage, increased TNM stage, and elevated pathological grade in breast cancer patients, which might due to the following reasons: (a) ITGA7 might increase cell proliferation via inactivating the phosphorylation of AKT and p38 and promote cell invasion through interacting with Hsp27 or S100P to facilitate tumor growth and tumor invasion; thus, it led to increased T stage as well as TNM stage; (b) ITGA7 drove cancer stem cell features through FAK/MAPK/ERK-mediated pathway, thereby enhanced abilities of self-renew and differentiation, which further led to increased pathological grade in breast cancer patients.[7] Furthermore, for the predictive value of ITGA7 on the treatment outcomes of cancer patients, ITGA7 high expression is

TABLE 2 Correlation of ITGA7 expression with clinicopathological characteristics

Items	ITGA7 protein expression			ITGA7 mRNA expression[a]		
	High (n = 92)	Low (n = 99)	*P* value	High (n = 95)	Low (n = 96)	*P* value
Age, No. (%)						
<60 y	63 (50.4)	62 (49.6)	.395	68 (54.4)	57 (45.6)	.105
≥60 y	29 (43.9)	37 (56.1)		27 (40.9)	39 (59.1)	
Tumor size, No. (%)						
<3 cm	38 (46.3)	44 (53.7)	.661	37 (45.1)	45 (54.9)	.268
≥3 cm	54 (49.5)	55 (50.5)		58 (53.2)	51 (46.8)	
T stage, No. (%)						
T1	25 (41.0)	36 (59.0)	.004	24 (39.3)	37 (60.7)	.002
T2	50 (44.2)	63 (55.8)		55 (48.7)	58 (51.3)	
T3	17 (100.0)	0 (0.0)		16 (94.1)	1 (5.9)	
N stage, No. (%)						
N0	43 (45.3)	52 (54.7)	.131	45 (47.4)	50 (52.6)	.295
N1	23 (41.8)	32 (58.2)		26 (47.3)	29 (52.7)	
N2	23 (60.5)	15 (39.5)		21 (55.3)	17 (44.7)	
N3	3 (100.0)	0 (0.0)		3 (100.0)	0 (0.0)	
TNM stage, No. (%)						
I	13 (48.1)	14 (51.9)	.038	9 (33.3)	18 (66.7)	.017
II	49 (41.2)	70 (58.8)		58 (48.7)	61 (51.3)	
III	30 (66.7)	15 (33.3)		28 (62.2)	17 (37.8)	
Pathological grade, No. (%)						
G1	19 (45.2)	23 (54.8)	0.017	18 (42.9)	24 (57.1)	.013
G2	52 (41.9)	72 (58.1)		57 (46.0)	67 (54.0)	
G3	21 (84.0)	4 (16.0)		20 (80.0)	5 (20.0)	
ER, No. (%)						
Negative	37 (50.7)	36 (49.3)	.584	39 (53.4)	34 (46.6)	.423
Positive	55 (46.6)	63 (53.4)		56 (47.5)	62 (52.5)	
PR, No. (%)						
Negative	46 (51.1)	44 (48.9)	.442	48 (53.3)	42 (46.7)	.348
Positive	46 (45.5)	55 (54.5)		47 (46.5)	54 (53.5)	
HER-2, No. (%)						
Negative	62 (48.4)	66 (51.6)	.915	64 (50.0)	64 (50.0)	.918
Positive	30 (47.6)	33 (52.4)		31 (49.2)	32 (50.8)	

Note: Difference between two groups was determined by Wilcoxon rank-sum test or chi-square test. *P* value < .05 was considered significant.
Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; IQR, interquartile range; ITGA7, integrin α7; N, node; PR, progesterone receptor; SD, standard deviation; T, tumor; TNM, tumor-node metastasis.
[a]The high or low expression was classified according to the median value of ITGA7 mRNA relative expression.

reported to be associated with reduced OS in both clear cell renal carcinoma patients and bladder urothelial carcinoma patients.[16,17] Also, ITGA7 expression is negatively correlated with OS in both low- and high-grade glioma patients.[8] These data reveal that ITGA7 high expression predicts unfavorable OS in some cancer patients, while limited studies show the predictive value of ITGA7 in breast cancer patients. In our study, we found that ITGA7 independently predicted poor OS in breast cancer patients, and the possible reasons might be (a) ITGA7 enhanced cell proliferation and invasion but repressed cell apoptosis via regulating some vital pathways (such as FAK/Akt and PI3K/Akt) to accelerate disease progression, thus resulted in shorter OS in breast cancer patients; (b) ITGA7 might enhance chemoresistance through activating FAK/Akt signaling, thus resulted in adverse treatment efficacy and further led to poor survivals in breast cancer patients. Additionally, there were still some limitations in our study: (a) sample size (N = 191) was relatively small, and the statistical power might be low; (b) this was a single-center study, which might lack wide representativeness; (c) this was a retrospective study, and

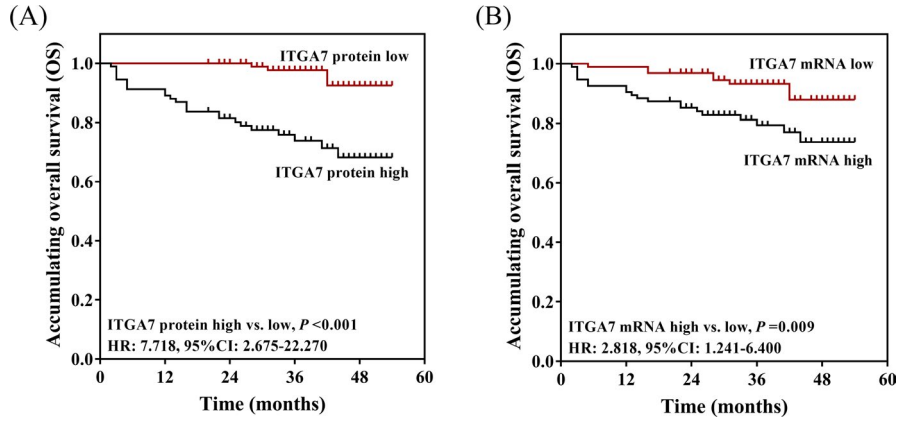

FIGURE 3 OS in ITGA7 high expression patients and ITGA7 low expression patients. OS in ITGA7 protein high expression patients was remarkably reduced than that in ITGA7 low expression patients (A). OS in ITGA7 mRNA high expression patients was decreased than that in IGTA7 low expression patients (B). K-M curves were used to display OS. Comparison of OS between two groups was determined by log-rank test. $P < .05$ was considered significant. OS, overall survival; ITGA7, integrin α7; K-M curves, Kaplan-Meier curves

assessment of ITGA7 expression was restricted to formalin-fixed and paraffin-embedded tissues; thus, further prospective study using fresh samples is needed to verify our results.

In order to explore the mechanisms about how ITGA7 affects cancer cell functions, several in vivo or in vitro experiments have been performed.[7-9] For example, ITGA7 is overexpressed in a highly metastatic human pancreatic carcinoma line (SW1990 HM) compared to the control human pancreatic carcinoma (SW1990) and enhances cell invasion.[9] In addition, ITGA7 knockdown reduces cell invasion in glioblastoma cells, and in vivo, the tumor growth is reduced in glioblastoma mice model treated by anti-ITGA7 compared to the control mice.[8] Moreover, ITGA7 promotes the ability of cell mobility, cell migration, and cell invasion but represses cell apoptosis in esophageal squamous cell carcinoma cells.[7] According to these data, ITGA7 functions as an oncogene in these cancer cells, while the role of ITGA7 in breast cancers cells is rarely reported. In order to investigate the effect of ITGA7 on cell activities, we conducted CCK-8, AV/PI, and Matrigel invasion assays in MCF-7 cells with ITGA7 knockdown, and we found that ITGA7 knockdown repressed cell proliferation, promoted cell apoptosis, and reduced cell invasion in MCF-7 cells. In addition, at 24 hours post-transfection, cell invasion rate was reduced in ITGA7 knockdown group

TABLE 3 Univariate and multivariate Cox's proportional hazards regression model analyses of factors affecting OS

	Univariate Cox's regression		Multivariate Cox's regression	
Items	*P* value	HR (95% CI)	*P* value	HR (95% CI)
ITGA7 expression (high vs low)	<.001	7.718 (2.675-22.270)	.006	5.450 (1.636-18.159)
Age (≥60 vs <60 y)	.675	1.176 (0.551-2.513)	.306	1.619 (0.644-4.071)
Tumor size (≥5 cm vs <5 cm)	<.001	10.589 (4.985-22.492)	.330	2.094 (0.473-9.273)
Higher T stage	<.001	7.112 (3.648-13.866)	.395	1.659 (0.517-5.325)
Higher N stage	<.001	2.673 (1.754-4.075)	.379	1.611 (0.557-4.658)
Higher TNM stage	<.001	6.855 (3.288-14.294)	.327	3.061 (0.326-28.693)
Higher pathological grade	<.001	4.995 (2.549-9.789)	.001	3.342 (1.675-6.665)
ER (positive vs negative)	.010	0.371 (0.174-0.793)	.803	1.245 (0.222-6.987)
PR (positive vs negative)	.022	0.396 (0.179-0.875)	.097	0.229 (0.040-1.306)
HER-2 (positive vs negative)	.626	1.208 (0.565-2.581)	.151	2.273 (0.741-6.971)

Note: P value < .05 was considered significant.

Abbreviations: CI, confidence interval; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; HR, hazard ratio; ITGA7, integrin α7; N, Node; OS, overall survival; PR, progesterone receptor; T, tumor; TNM, tumor-node metastasis.

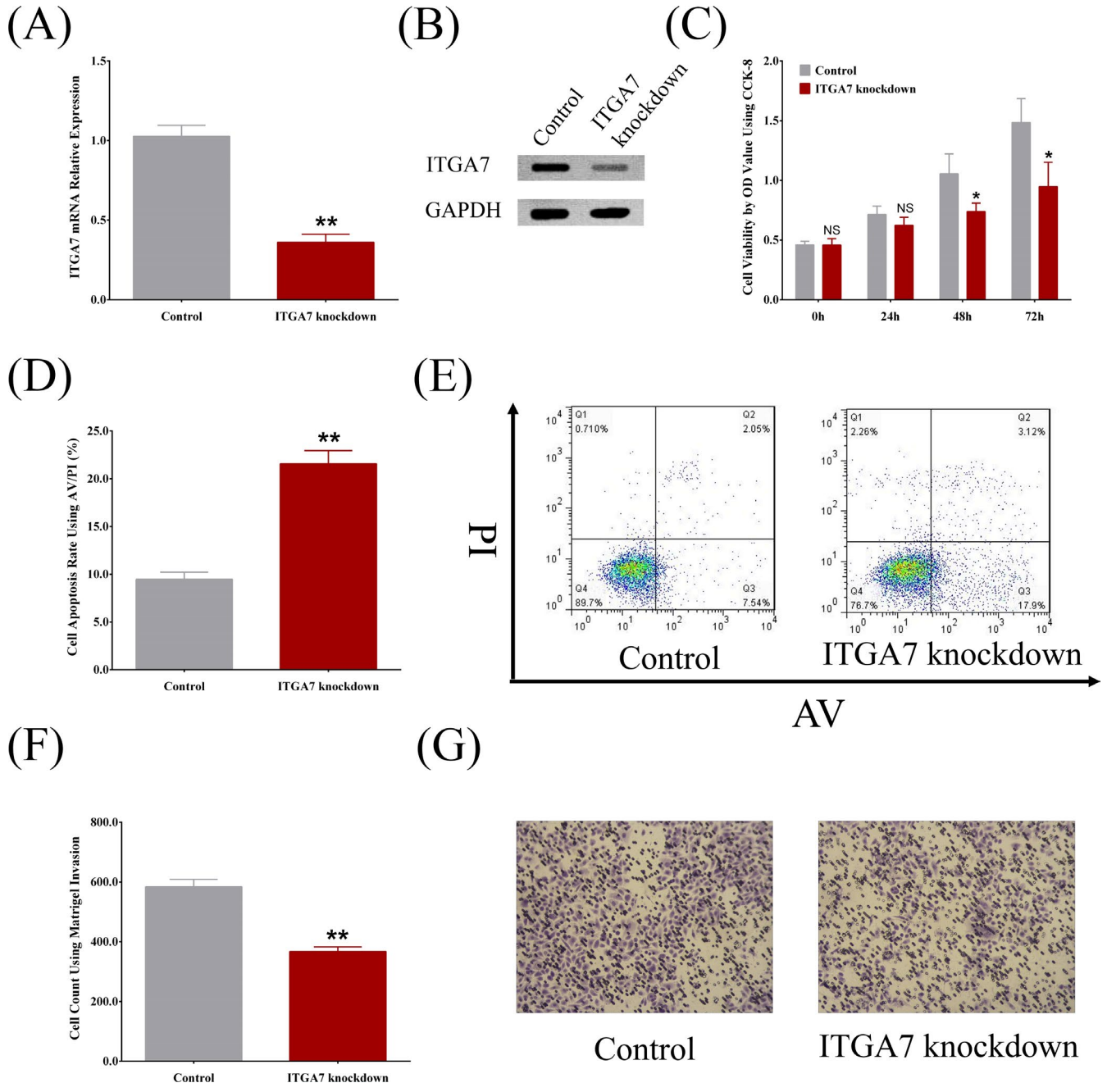

FIGURE 4 CCK-8, AV/PI, and Matrigel invasion assays. ITGA7 mRNA expression was decreased in ITGA7 knockdown group compared to control group (A). ITGA7 protein expression was lower in ITGA7 knockdown group compared to control group (B). Cell viability was reduced in ITGA7 knockdown group compared to control group at 48 h and 72 h (C). Cell apoptosis rate was increased in ITGA7 knockdown group compared to control group (D, E). Cell count using Matrigel invasion assay was reduced in ITGA7 knockdown group compared to control group (F, G). Comparison between two groups was determined by *t* test. $P < .05$ was considered significant. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. CCK-8, Cell Counting Kit-8; AV/PI, Annexin V/ propidium iodide; ITGA7, integrin α7

compared to control group, while no difference of cell viability existed between the two groups, indicating that the reduction in cell invasion was not due to the loss in viability by ITGA7 knockdown. Our results expanded the understanding about underlying mechanisms of ITGA7 in breast cancer cells and suggested that ITGA7 knockdown might serve as an anti-tumor approach through repressing cell proliferation and invasion but enhancing cell apoptosis.

In conclusion, ITGA7 high expression correlates with increased T stage, TNM stage, and pathological grade as well as worse OS, and its knockdown enhances cell apoptosis but inhibits cell proliferation and invasion in breast cancer.

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