ITGA7 functions as a tumor suppressor and regulates migration and invasion in breast cancer

Adheesh Bhandari*, Erjie Xia*, Yuying Zhou, Yaoao Guan, Jingjing Xiang, Lingguo Kong, Yinghao Wang, Fan Yang, Ouchen Wang, Xiaohua Zhang

Department of Thyroid and Breast Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, People’s Republic of China

*These authors contributed equally to this work

Background: Breast cancer is the most common malignancy in women and the underlying mechanism of breast cancer cell metastasis is still far from uncover. Integrin subunit alpha 7 (ITGA7) is a functioning protein. It has been detected in many malignancies. But the function of ITGA7 in breast cancer is not clear. Our aim is to explore ITGA7 expression and its role in breast cancer.

Methods: Real-time PCR was performed to determine ITGA7 expression in BC tissues and normal adjacent tissues. The specific functions of ITGA7 in breast cancer cell lines (MDA-MB-231 and BT-549) transfected with small interfering RNA were determined through migration, invasion assays. Western blot assays were performed to determine the expression of c-met and vimentin.

Results: ITGA7 was down-regulated in breast cancer tissues compared to the adjacent normal tissues (T:N =7.68±27.38: 41.01± 31.47, P<0.001) and this observation was consistent with the TCGA cohort (T:N =4.51±0.45:5.40±0.61, P<0.0001). In vitro experiments showed that knocking down ITGA7 significantly inhibited the migration and invasion of the breast cancer cell lines (MDA-MB-231 and BT-549). Meanwhile, knockdown of ITGA7 promoted c-met and vimentin expression, which may induce invasion and migration.

Conclusion: ITGA7 plays an important tumorigenic function and acts as a suppress gene in breast cancer. Our findings indicate that ITGA7 was the gene associated with breast cancer.

Keywords: breast cancer, ITGA7, migration, invasion

Introduction
Breast cancer is the most common malignancy in women. As the reason for cancer-related death, breast cancer ranks second after lung cancer.1,2 Many studies have proven that invasion of cancer cells was the major reason for cancer-related death.3,4 With acknowledging development of medical technology, doctors can treat breast cancer patients by surgery, chemotherapy, endocrine therapy, or targeted therapies.5 But the treatment is not always satisfactory. So, it is still important to explore the mechanism of breast cancer and find new biomarkers at an early stage.

C-met is a key regulator of cancer progression, and it has been reported that upregulated c-met is related with poor survival rates and malignant activities of breast cancer.6,7 During cancer progression, cancer cells can experience a feature change from an epithelial to a mesenchymal phenotype, which is called epithelial–mesenchymal transition (EMT).8,9 Hung et al10 found that osthole suppresses HGF-induced EMT via repression of the c-Met/Akt/mTOR pathway in human breast cancer cells.

Integrin subunit alpha 7 (ITGA7) belongs to the integrin alpha chain family, and the coding gene is located on chromosome 12q13.2.11 It has been found that ITGA7 is expressed in many cancers including malignant melanoma, prostate and liver.
carcinomas, and glioblastoma.\textsuperscript{12,13} Low levels of \textit{ITGA7} mediate cell adhesion migration on specific laminin isoforms and influence tumors growth and motility.\textsuperscript{14} Ziober et al\textsuperscript{12} found that upregulation of \textit{ITGA7} could reduce melanoma cell tumor growth, motility, and metastasis.\textsuperscript{12} Ren et al\textsuperscript{11} found that downregulation of \textit{ITGA7} expression increased the rate of migration in lung cancer cells. However, the relationship between \textit{ITGA7} and breast cancer is not exactly clear.

In order to clarify the expression and effect of \textit{ITGA7} in breast cancer, our study examined \textit{ITGA7} expression in breast cancer tissues and paired adjacent nontumor tissues by using real-time quantitative PCR (RT-qPCR), and we verified our clinical specimen data by using The Cancer Genome Atlas (TCGA) database. Besides, we characterized the function of \textit{ITGA7} in breast cancer cell lines. We found that downregulated \textit{ITGA7} could promote breast cancer cell migration and invasion.

\section*{Materials and methods}

\subsection*{Patients and breast tissue samples}

In this present study, we obtained 36 breast cancer tissues and paired adjacent nontumor tissues from the Department of Thyroid and Breast Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, People’s Republic of China. All patient-derived specimens and information were collected and recorded based on the protocols provided by the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University. These 36 fresh tissues were snap-frozen in liquid nitrogen immediately and stored at \(-80^\circ\text{C}\). Breast cancer mRNA expression data were downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). Gene expression data were available for 1,100 breast cancer samples compared to 113 normal samples.

\subsection*{Ethical approval}

Ethical approval for this study was obtained from the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

\subsection*{Cell cultures and growth conditions}

MDA-MB-231, BT-549, SK-BR-3, MDA-MB-468, MCF-7, and MCF-10A cells were used in this study. These cells were obtained from Shanghai Cell Biology, Institute of the Chinese Academy of Sciences (Shanghai, People’s Republic of China). MDA-MB-231, MCF-7, and SK-BR-3 were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (Gibco), Grand Island, NY, USA) supplemented with 10\% fetal bovine serum (FBS) (Gibco). BT-549 were cultured in Roswell Park Memorial Institute-1640 medium (Gibco) supplemented with 10\% FBS (Gibco). MDA-MB-468 cells were cultured in L-15 medium (Gibco) supplemented with 10\% FBS (Gibco). MCF-10A cells were cultured in DMEM-F12 (Gibco) supplemented with 100 U/mL of penicillin, 100 \(\mu\)g/mL of streptomycin, 2 mM of l-glutamine, 20 ng/mL of epidermal growth factor, and 10\% FBS (Gibco). MDA-MB-468 cells were incubated in a standard cell culture incubator (Thermo, Waltham, MA, USA) at 37\(^\circ\text{C}\) without CO\(_2\). The others were incubated in a standard cell culture incubator (Thermo) at 37\(^\circ\text{C}\) with 5\% CO\(_2\).

\subsection*{Cell transfection}

MDA-MB-231 and BT-549 were transfected using Lipo-fectamine RNAiMax transfection reagent (Invitrogen, Carlsbad, CA, USA) by following the manufacturer’s protocol. About 100,000 cells were plated 1 day before transfection. \textit{ITGA7} was silenced by transfecting 10 nM siRNA for 48 h. The siRNA sequences used in the study were the \textit{ITGA7} siRNAs that targeted the following sequences: \textit{ITGA7} siRNA-1, forward 5’S-GCAUCAAGAGCUCCGCUATT-3’ and reverse 5’S-UAGCGCAAGCCUUUGAUGCTT-3’; \textit{ITGA7} siRNA-2, forward 5’S-GCUGCCCAUCUCAGCUUUTT-3’ and reverse 5’S-AAGCUAGAGUGGGCAGCTT-3’; \textit{ITGA7} siRNA-3, forward 5’S-GUCAUCCUCUGCCUGUATT-3’ and reverse 5’S-UACAGCCAGAGGAUGACCTT-3’. Both siRNAs were provided by Genepharm (Shanghai, People’s Republic of China) company.

\subsection*{RNA extraction and RT-qPCR}

Total RNA was lysed using TRizol reagent according to the manufacturer’s instructions (Invitrogen). The purity of the isolated RNA was measured at 260/280 nm by spectrophotometry (Thermo). After measurement, RNA samples were stored at \(-80^\circ\text{C}\). Real-time reactions were run and analyzed by using a real-time PCR system (Applied Biosystems 7500, ThermoFisher Scientific). The relative expression of mRNA was calculated using the comparative cycle threshold (CT) (2\(^{-\Delta\Delta CT}\)) method with GAPDH as the endogenous control to normalize the data. The sequences of the primers used were as follows:

\textit{ITGA7} forward: 5’S-GCTGTTGAATCCTGGAAGTTGATT-3’ and reverse: 5’S-GCCTCTGGAGCACTCATTCTT-3’; GADPH forward: 5’S-GTCTCCTCTGACTTCA and reverse: 5’S-ACCACCCCTTTGCTGTA GCCAA-3’.

\subsection*{Invasion and migration assay}

For cell Transwell assays, cells were trypsinized with trypsin and collected in the medium containing 10\% FBS. Invasion of cells was measured in Matrigel (BD, Franklin Lakes, NJ, USA) by using the standard method with GAPDH as the endogenous control to normalize the data. The sequences of the primers used were as follows:

\textit{ITGA7} forward: 5’S-GCTGTTGAATCCTGGAAGTTGATT-3’ and reverse: 5’S-GCCTCTGGAGCACTCATTCTT-3’; GADPH forward: 5’S-GTCTCCTCTGACTTCA and reverse: 5’S-ACCACCCCTTTGCTGTA GCCAA-3’.
USA)-coated Transwell inserts (6.5 mm, Costar, Manassas, VA, USA) containing polycarbonate filters with 8 μm pores. The inserts were coated with 50 μL of 1 mg/mL Matrigel matrix according to the manufacturer’s recommendations. A total of 40,000 cells (~250 μL) were transferred into the upper chamber. About 700 μL medium containing 20% FBS was filled in the lower chamber. Then the plate was placed into the incubator. After 24 h, the membrane was fixed with 4% paraformaldehyde and stained with 0.4% crystal violet solution for 15 min. Motility assays were similar to invasion assay except that the Transwell insert was not coated with Matrigel. Cell migration and invasion ability were assessed by counting the cells that had migrated and invaded through the membrane. Five random fields of view were selected and images captured under a microscope at a magnification of 20×.

Western blot analysis
Whole cell lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gels electrophoresis (BioRad, Berkeley, CA, USA) and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk for 2 h at room temperature. According to the manufacturer’s protocol, the membranes were probed with polyclonal antibody overnight at 4°C. The membranes were then incubated with the anti-mouse IgG or anti-rabbit IgG as secondary antibody (Abcam, Cambridge, MA, USA) for 1 h at room temperature. The primary antibodies used were as follows: Vimentin (Abcam, ab92547), ITGA7 (Abcam, ab203254), and human GAPDH (Sigma, St. Louis, MO, USA).

Statistical analysis
All statistical analyses were performed using SPSS 23.0 software (IBM Corporation, Armonk, NY, USA). Data are presented as mean ± standard error. The differences were considered to be statistically significant at P<0.05. Student’s t-test (2-tailed) was performed to analyze differences between groups.

Result
ITGA7 was downregulated in breast cancer tissues
In order to investigate the function of ITGA7 in breast cancer tumorigenesis, the expression levels of ITGA7 were investigated in 36 breast cancer tissues and paired adjacent nontumor tissues by using RT-qPCR. We found that ITGA7 expression was significantly lower in breast cancer tissues, compared to the adjacent normal tissues (T:N = 7.68±27.38:41.01±31.47, P<0.001) (Figure 1A). TCGA also showed that ITGA7 was downregulated in breast cancer compared to the adjacent normal tissues (T:N = 4.51±0.45:5.40±0.61, P<0.001) (Figure 1B). In a word, these results implied that ITGA7 might function as a tumor suppressor in breast cancer.

The relationship between ITGA7 expression and clinical features
To understand the relation between ITGA7 and breast cancer, we investigated the relationship of ITGA7 with clinico-pathological features. In the TCGA cohort, we divided the patients into low-expression group and high-expression group.

**Figure 1** ITGA7 expression in breast cancer in validated cohort and TCGA cohort.

Notes: (A) ITGA7 expression was examined by RT-qPCR in 36 paired human breast cancer tissues and adjacent nontumorous tissues (paired t-test, P<0.001). A logarithmic scale of 2^ΔΔCt is used to represent the fold change in quantitative real-time PCR detection. (B) The TCGA cohort contained 1,100 breast tumor tissues and 113 normal tissues. RPKM is used to represent expression of ITGA7. The analysis was done using the Mann–Whitney U-test. ***P<0.001; ****P<0.0001.

Abbreviations: ITGA7, integrin subunit alpha 7; RPKM, reads per kilobases per million reads; RT-qPCR, real-time quantitative PCR; TCGA, The Cancer Genome Atlas.
group according to the median value. The results revealed that lymph node metastasis \((P=0.030)\) and tumor size \((P=0.024)\) were significantly related to the \(ITGA7\) expression (Table 1). In the validated cohort, we divided all patients into the low-expression group \((n=18)\) and high-expression group \((n=18)\) according to the median value as same. The results showed that age \((P=0.169)\) and lymph node metastasis \((P=0.075)\) were not related to the expression of \(ITGA7\) negatively (Table 2). These results indicated that low \(ITGA7\) expression may influence the ability of migration of breast cancer cells and was associated with unfavorable prognosis in breast cancer.

### ITGA7 regulates migration and invasion of breast cancer lines

To confirm the role of \(ITGA7\) in breast cancer, we assessed \(ITGA7\) expression level in several breast cancer cell lines and normal breast cell lines by using RT-qPCR. We found that expression level of \(ITGA7\) was higher in MDA-MB-231 and BT-549 than in other breast cell lines (Figure 2A). So, we selected MDA-MB-231 and BT-549 for further experiments. To further examine whether \(ITGA7\) functions in breast cancer progression, we knocked down \(ITGA7\) expression in MDA-MB-231 and BT-549 using siRNA. As can be seen in Figure 2B–D, both mRNA and protein levels of \(ITGA7\) were significantly reduced.

Previous studies have proven that a cancer cell’s abilities of migration and invasion were correlated with tumor progression.\(^{15,16}\) We next examined whether knocking down \(ITGA7\) affected the functions of breast cancer cell lines. Our results showed that downregulated \(ITGA7\) significantly enhanced migration capacity of MDA-MB-231 and BT-549 compared with the control groups (Figure 3A and B). The invasion assays also showed that downregulated \(ITGA7\) effectively enhanced invasion capacity of MDA-MB-231 and BT-549 (Figure 4A and B).

### ITGA7 regulates migration and invasion via addition to c-met-regulated vimentin

We found that knockdown of \(ITGA7\) led to significantly increased c-met in MDA-MB-231 and BT-549 (Figure 5A). In a previous study,\(^{10,28}\) repression of the c-Met/Akt/mTOR pathway was found to suppress EMT. Vimentin is important in EMT and tumor progression.\(^{17,18}\) In order to explore the

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**Table 1** The relationship between \(ITGA7\) and clinicopathologic characteristics in TCGA cohort

<table>
<thead>
<tr>
<th>Clinicopathologic characteristics</th>
<th>Low expression (%)</th>
<th>High expression (%)</th>
<th>(\chi^2)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 60)</td>
<td>296</td>
<td>296</td>
<td>0.001</td>
<td>0.973</td>
</tr>
<tr>
<td>(&gt;60)</td>
<td>243</td>
<td>242</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>7.475</td>
<td>0.024*</td>
</tr>
<tr>
<td>(&lt;2\ cm)</td>
<td>123</td>
<td>155</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2–5\ cm)</td>
<td>335</td>
<td>291</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;5\ cm)</td>
<td>81</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>8.951</td>
<td>0.030*</td>
</tr>
<tr>
<td>N0</td>
<td>256</td>
<td>274</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>195</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>60</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>28</td>
<td>47</td>
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<td></td>
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<tr>
<td>Distant metastasis</td>
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<td>0.356</td>
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<tr>
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<tr>
<td>Yes</td>
<td>9</td>
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<td></td>
<td></td>
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<tr>
<td>Clinical stage</td>
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<td></td>
<td>1.662</td>
<td>0.197</td>
</tr>
<tr>
<td>I–II</td>
<td>415</td>
<td>396</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III–IV</td>
<td>124</td>
<td>142</td>
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<td></td>
</tr>
</tbody>
</table>

**Note:** \(P\)-value < 0.05.

**Abbreviations:** \(ITGA7\), integrin subunit alpha 7; TCGA, The Cancer Genome Atlas.

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**Table 2** The relationship between \(ITGA7\) and clinicopathologic characteristics in the validated cohort

<table>
<thead>
<tr>
<th>Clinicopathologic characteristics</th>
<th>Low expression (%)</th>
<th>High expression (%)</th>
<th>(\chi^2)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>1.893</td>
<td>0.169</td>
</tr>
<tr>
<td>(\leq 60)</td>
<td>17</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;60)</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>20.029</td>
<td>0.598</td>
</tr>
<tr>
<td>(&lt;2\ cm)</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2–5\ cm)</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;5\ cm)</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>3.167</td>
<td>0.075*</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td>4.886</td>
<td>0.067</td>
</tr>
<tr>
<td>I–II</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III–IV</td>
<td>8</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** \(P\)-value < 0.05.

**Abbreviation:** \(ITGA7\), integrin subunit alpha 7.
ITGA7 is a gene associated with breast cancer

In our study, the main aim was to prove the potential role of ITGA7 in breast cancer. We found that ITGA7 in breast cancer tissues was downregulated compared to paired adjacent nontumor tissues by RT-qPCR. This result was further identified in TCGA cohort. We analyzed the clinical features of breast cancer patients from TCGA and found that lymph

Although much progress in medical research had been made in breast cancer, there is still much left unknown about the molecular mechanisms of breast cancer. Some studies have demonstrated that ITGA7 gene was associated with malignant melanoma, prostate and liver carcinomas, and glioblastoma. However, there is still known little about its function in breast cancer.

In our study, the main aim was to prove the potential role of ITGA7 in breast cancer. We found that ITGA7 in breast cancer tissues was downregulated compared to paired adjacent nontumor tissues by RT-qPCR. This result was further identified in TCGA cohort. We analyzed the clinical features of breast cancer patients from TCGA and found that lymph
node metastasis ($P=0.030$) and tumor size ($P=0.024$) were significantly related with the $ITGA7$ expression. These findings suggest that $ITGA7$ may play an important role in breast cancer and encouraged us to proceed with the next step to study the $ITGA7$ gene in cell lines. We found that $ITGA7$ was expressed at a higher level in MDA-MB-231 and BT-549 than in other breast cell lines. Then, using cellular and molecular technology, we found that knockdown of $ITGA7$ led to an increase in migration and invasion abilities, which is consistent with $ITGA7$ being associated with breast cancer.

In recent decades, the development of c-met has been increasingly recognized to play pivotal roles in promoting tumor process.22–24 Jia et al25 found that inhibiting c-MET could enhance the response of the colorectal cancer cells...
The influence of c-MET in MDA-MB-231 and BT-549 cells by Western blot.

**Notes:** These experiments were done at least 3 independent times. We defined ITGA7 siRNA-1 as S1 and ITGA7 siRNA-2 as S2. (A) The influence of ITGA7 expression on c-MET in MDA-MB-231 and BT-549 cells by Western blot. (B) The influence of ITGA7 expression on vimentin in MDA-MB-231 and BT-549 cells by Western blot. ††P < 0.01 and ‡‡P < 0.001 in comparison with the NC group using Student’s t-test. We defined ITGA7 siRNA-1 as S1 and ITGA7 siRNA-2 as S2.

**Abbreviations:** ITGA7, integrin subunit alpha 7; NC, negative control.

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The influence of c-MET in MDA-MB-231 and BT-549 cells by Western blot.

**Notes:** These experiments were done at least 3 independent times. We defined ITGA7 siRNA-1 as S1 and ITGA7 siRNA-2 as S2. (A) The influence of ITGA7 expression on c-MET in MDA-MB-231 and BT-549 cells by Western blot. (B) The influence of ITGA7 expression on vimentin in MDA-MB-231 and BT-549 cells by Western blot. ††P < 0.01 and ‡‡P < 0.001 in comparison with the NC group using Student’s t-test. We defined ITGA7 siRNA-1 as S1 and ITGA7 siRNA-2 as S2.

**Abbreviations:** ITGA7, integrin subunit alpha 7; NC, negative control.


