

# ILK promotes cell proliferation in breast cancer cells by activating the PI3K/Akt pathway

YIKUN QU<sup>1-4</sup>, CHUNFANG HAO<sup>1-3</sup>, JIAN XU<sup>4</sup>, ZHUOXIN CHENG<sup>4</sup>, WEIQUN WANG<sup>5</sup> and HONG LIU<sup>1-3</sup>

<sup>1</sup>The Second Department of Breast Cancer, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer; <sup>2</sup>Key Laboratory of Cancer Prevention and Therapy; <sup>3</sup>Key Laboratory of Breast Cancer Prevention and Therapy, Tianjin Medical University, Ministry of Education, Tianjin 300060;

<sup>4</sup>Department of General Surgery, The First Affiliated Hospital of Jiamusi University; <sup>5</sup>Department of Basic Medical College, Jiamusi University, Jiamusi, Heilongjiang 154002, P.R. China

Received August 21, 2016; Accepted May 15, 2017

DOI: 10.3892/mmr.2017.7180

**Abstract.** Breast cancer is a very common malignant tumor, whose incidence ranks the first among various types of cancer in women worldwide. An important hallmark of cancer is the activation of oncogenes, which lead to overgrowth of cancer cells. Therefore, it is necessary to identify the critical genes involved in regulating the progression of breast cancer and elucidate the corresponding molecular mechanisms. The present study demonstrated that integrin-linked kinase (ILK) overexpression promoted cell proliferation and growth in MCF-7 cells, while ILK knockdown led to growth arrest in MDA-MB-231 cells. In addition, activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway was positively regulated by ILK, suggesting that the regulatory effects of ILK on cell growth and proliferation may be at least in part mediated by PI3K/Akt signaling. These results indicated that ILK promoted cell proliferation and growth in breast cancer cells through activation of the PI3K/Akt pathway, suggesting that ILK may be considered to be a potential therapeutic target for the therapy of breast cancer in the future.

## Introduction

Breast cancer is the most common malignancy and its incidence ranks the first in women worldwide (1). Although the most common treatment strategy is surgical operation combined with radiotherapy or chemotherapy, molecular targeted therapies are also widely used, especially in patients where surgical removal is not indicated (2). However, the

molecular mechanisms underlying the progression of breast cancer remain largely unknown, resulting in a lack of effective therapy targets. Thus, it is necessary to explore the mechanisms of regulation of breast cancer cell growth in order to discover novel potential treatment targets and improve the efficiency of molecular targeted therapies.

Loss of adhesion between tumor cells and the extracellular matrix, which is required during the invasion and dissemination process of cancer cells, is closely controlled by integrins. Integrin-linked kinase (ILK) is a major serine-threonine kinase of integrins and widely expressed in a broad range of human tissues (3,4). Previous studies have reported that upregulation of ILK expression is frequently observed in multiple types of carcinomas (5,6). In addition, ILK participates in regulating various biological functions of cancer cells, including cell growth, cell cycle progression, invasion and migration, cell motility and angiogenesis (7-9). In breast cancer, ILK has been reported to serve an important role in regulating the stemness function of interleukin-6-abundant breast cancer cells via activating Notch1 (10). Furthermore, activation of ILK modulates cell motility and metastasis of breast cancer cells by binding to LIM domain containing 2 (11). However, the role of ILK in breast cancer cell growth and the underlying mechanisms remain unclear.

Based on the published literature, it was hypothesized that ILK possesses may affect the growth of breast cancer cells. The present results demonstrated that overexpression of ILK promoted cell growth, while ILK knockdown mitigated cell growth in breast cancer cell lines. In addition, the effects of ILK on cell proliferation were mediated by the phosphoinositide 3-kinase (PI3K)/Akt pathway. These findings provide useful insight into the role of ILK in breast cancer cell growth regulation and indicate that ILK may serve as a potential treatment target for breast cancer.

## Materials and methods

**Materials.** Primary antibodies against proliferating cell nuclear antigen (PCNA; cat. no. sc-25280; 1:500), Akt (cat. no. sc-1618; 1:500), phosphorylated (p-) Akt (Thr308) (cat. no. sc-135650; 1:500) and  $\beta$ -actin (cat. no. sc-1616; 1:5,000)

---

*Correspondence to:* Dr Hong Liu, The Second Department of Breast Cancer, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, 1 Huan-Hu-Xi Road, Tianjin 300060, P.R. China  
E-mail: hongliuh@tom.com

**Key words:** integrin-linked kinase, proliferation, breast cancer, phosphoinositide 3-kinase/Akt

were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Secondary antibodies of mouse anti-goat immunoglobulin (Ig)G-horseradish peroxidase (HRP) (cat. no. sc-2354; 1:5,000) and mouse anti-rabbit IgG-HRP (cat. no. sc-2357; 1:5,000) were also purchased from Santa Cruz Biotechnology, Inc. LY294002 (cat. no. S1737), the lactate dehydrogenase (LDH) assay kit and WST-1 Cell Proliferation Assay kit were purchased from Beyotime Institute of Biotechnology (Haimen, China).

**Cell lines and culture.** The human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal calf serum (Gibco; Thermo Fisher Scientific, Inc.), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in an incubator with 95% air and 5% CO<sub>2</sub>. HA-Akt plasmid (a constitutively active form of Akt) was obtained from Addgene, Inc. (Cambridge, MA, USA). A total of 1.5 µg plasmid, 7.5 µl Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) and 200 µl serum-free Opti-minimum essential medium (Gibco; Thermo Fisher Scientific, Inc.) were mixed together gently. Following 20 min incubation at room temperature, the mixture was added to the cells for 24 h transfection at 37°C, and cells were subsequently utilized for further experiments.

**Generation of ILK short hairpin (sh)RNA knockdown and overexpression lentivirus.** Human ILK-specific shRNA and a scrambled shRNA (used as a negative control) were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The resultant lentiviruses containing the ILK-specific shRNA sequence (forward, 5'-GATCCGACCCAAATTTGACATGATTCTCCTGTGAGAAATCATGTCAAATTTGGGTCTTTTG-3'; reverse, 5'-AATTCAAAAAGACCCAAATTTGACATGATTCTGACAGGAAGAATCATGTCAAATTTGGGTCCG-3') or the negative control sequence (forward, 5'-GATCCCGGCTCGAGGTTTTTGTCTTCCTGTCAGAACTCGAGTGCAGTACTCGATTTTGTG-3'; reverse, 5'-AATCAAAAACCGGCCTCGAGGTTTTTGTCTGACAGGAAGAACTCGAGTGCAGTACTCGAGG-3') were named ILK shRNA or sh Control, respectively. The ILK overexpression lentivirus (containing the whole coding sequence; no. NM\_004517) was also purchased from Shanghai GenePharma Co., Ltd. In the present study, the ILK overexpression lentivirus and the empty vector lentivirus were termed as ILK and vector, respectively. The vector used in the present study was the lentiviral expression vector pWPXL (Addgene, Inc.) and the multiplicity of infection that was used to generate the knockdown and the overexpressing cell lines was 1x10<sup>9</sup> transducing U/ml.

**Assessment of cell viability.** Cell viability was determined with the WST-1 Cell Proliferation Assay kit. According to the manufacturer's instructions, MCF-7 and MDA-MB-231 cells (3x10<sup>3</sup> cells/well) were plated in 96-well culture plates. After 24 h of incubation, the cells were subjected to growth arrest (cultured under conditions of serum-starvation) for 24 h. Cells were then treated with 20 µl WST-1 at 37°C for 2 h, and the absorbance was measured at 450 nm with a spectrophotometer.

The experiments were performed in triplicate and repeated three independent times.

**LDH assay.** LDH release into the culture medium was measured by a LDH assay kit. Briefly, 1x10<sup>5</sup> cells were seeded in a 6-well plate, incubated for 24 h, and then the culture medium of each well was collected separately. A total of 120 µl from each culture medium was mixed with 60 µl detection fluid from the kit into a new 96-well plate. Following incubation for 30 min in the dark at 25°C, absorbance was measured at 490 nm with a spectrophotometer.

**Western blotting.** The methodology has been described previously (12). A total of 1x10<sup>6</sup> cells were sonicated in radioimmunoprecipitation assay buffer [150 mmol/l NaCl, 1% Triton x-100, 1% Sodium deoxycholate, 0.1% SDS, 50 mmol/l Tris (pH 8.0), 1 mmol/l PMSF] on ice and homogenized. Debris was removed by centrifugation at 12,000 x g for 10 min at 4°C. Samples containing 50 µg protein were separated on 12% polyacrylamide SDS gels, and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk at 25°C for 1 h and incubated with primary antibodies at 4°C overnight. Subsequently the membranes were incubated with secondary antibodies of mouse anti-goat immunoglobulin (Ig)G-horseradish peroxidase (HRP) (Santa Cruz Biotechnology, Inc.; cat. no. sc-2354; 1:5,000) and mouse anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Inc.; cat. no. sc-2357; 1:5,000) at 25°C for 2 h. All membranes were developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Tiangen Biotech Co. Ltd., Beijing, China). β-actin was used as the internal control.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was extracted with TRI Reagent (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; cat. no. T9424) and synthesis of cDNA from total RNA was performed using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). qPCR was performed on an Applied Biosystems 7300 Fast Real-Time PCR system (Thermo Fisher Scientific, Inc.) using the SYBR Premix Ex Taq II kit (Takara Biotechnology Co., Ltd., Dalian, China). Each 20 µl qPCR reaction contained 1x SYBR Premix Ex Taq™ II master mix, 10 µM forward and reverse primers, 0.4 µl ROX reference dye, and 2 µl of cDNA. The reaction was performed as follows: 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec, and 60°C for 31 sec. Primers were specifically designed using the Applied Biosystems Primer Express 3.0 software (Thermo Fisher Scientific, Inc.). The primer sequences were as follows: ILK forward 5'-TCCCTGGATCACTCCACAGT-3', reverse 5'-TCGTTCTCCGTGTTGTCCAG-3'; β-actin forward 5'-TTGTTACAGGAAGTCCCTTGCC-3', reverse 5'-ATGCTATCACCTCCCCTGTGTG-3'. Relative quantitation of target gene expression was calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (13). The first step in the analysis is to normalize the target gene expression level to β-actin. The second step is to compare the difference between normalized target gene expression between different samples. The experiments were in triplicate in three independent experiments.

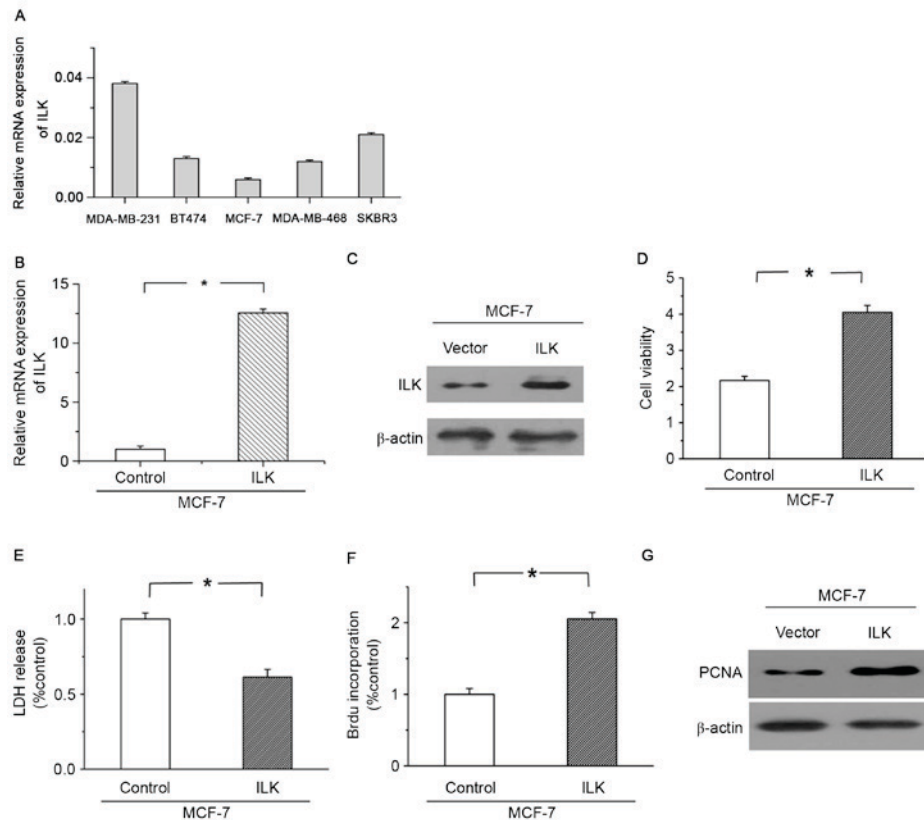


Figure 1. (A) MCF-7 breast cancer cells express low levels of ILK, while the MDA-MB-231 breast cancer cells express high levels of endogenous ILK protein. Cell growth and proliferation are promoted by ILK overexpression in breast cancer cells. MCF-7 cells were transduced with either an empty vector control (Control) or ILK-overexpressing (ILK) lentivirus, and efficiency of overexpression was confirmed by (B) reverse transcription-quantitative polymerase chain reaction and (C) western blotting. (D) Overexpression of ILK resulted in an increase of cell viability. (E) Overexpression of ILK inhibited cell death, as determined by LDH release assay. (F) Incorporation of BrdU was promoted by ILK overexpression. (G) Overexpression of ILK induced the protein expression levels of PCNA. Data are represented as the mean  $\pm$  standard error of the mean from three independent experiments. \* $P < 0.05$ . ILK, integrin-linked kinase; LDH, lactate dehydrogenase; BrdU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen.

**Bromodeoxyuridine (BrdU) incorporation.** Cells were seeded at a density of 5,000 cells/cm<sup>2</sup> in 96-well microplates. Cancer cell proliferation was evaluated by analyzing BrdU incorporation into newly synthesized DNA using a cell proliferation ELISA (Roche Applied Science, Penzberg, Germany). Optical density was measured at 450 nm using an ELISA plate reader (Sinergy HT; BioTek Instruments, Inc., Winooski, VT, USA). Proliferation was expressed as a % of control.

**Statistical analysis.** Data were presented as mean  $\pm$  standard error of the mean from at least three independent experiments. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Student's t-test was performed to compare the differences between two groups.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Overexpression of ILK promotes cell growth and proliferation in MCF-7.** We first examined the expression of ILK in a panel of breast cancer cells and found that the MCF-7 breast cancer cells express low levels of ILK, while the MDA-MB-231 breast cancer cells express high levels of endogenous ILK protein (Fig. 1A). To demonstrate the critical role of ILK in the progression of breast cancer, a stable cell line was established

in MCF-7 cells that overexpressed ILK by lentivirus transduction. RT-qPCR and western blot analysis were performed to ensure the efficiency of transduction (Fig. 1B and C). As demonstrated in Fig. 1D and E, overexpression of ILK in MCF-7 cells resulted in a significant increase of cell viability, and significant decrease of LDH release compared with cells transduced with empty vector control, suggesting that ILK overexpression promoted cell growth and inhibited cell death. Furthermore, overexpression of ILK resulted in a significant increase in BrdU incorporation and protein expression of the well-established proliferation marker PCNA, compared with cells transduced with empty vector control (Fig. 1F and G). These results indicate that cell growth and proliferation were facilitated by ILK overexpression in MCF-7 cells.

**Knockdown of ILK triggers growth inhibition and mitigates cell proliferation.** In order to further examine the role of ILK on tumor cell growth, its expression was silenced in the MDA-MB-231 cells by shRNA. The knockdown efficiency was verified by RT-qPCR and western blot analysis (Fig. 2A and B). Knockdown of ILK in MDA-MB-231 cells resulted in a significant decrease in cell viability (Fig. 2C), and a significant increase in cell death (Fig. 2D), compared with cells transduced with a scrambled control shRNA. In addition, incorporation of BrdU and PCNA protein expression were

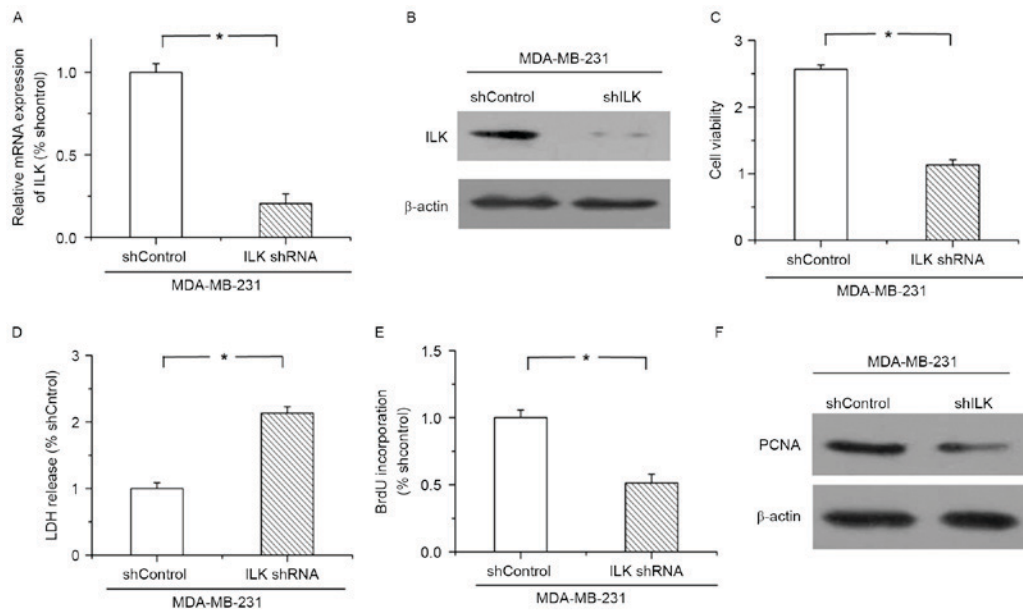


Figure 2. Knockdown of ILK inhibits cell growth and mitigates cell proliferation. MDA-MB-231 cells were transduced with either scrambled control (sh Control) or ILK-specific shRNA (ILK shRNA) lentivirus, and efficiency of knockdown was confirmed by (A) reverse transcription-quantitative polymerase chain reaction and (B) western blotting. (C) Knockdown of ILK led to a decrease in cell viability. (D) Cell death was enhanced by ILK silencing, as determined by LDH release assay. (E) Knockdown of ILK mitigated BrdU incorporation. (F) Protein expression levels of PCNA were decreased by ILK knockdown. Data are represented as the mean  $\pm$  standard error of the mean from three independent experiments. \* $P < 0.05$ . ILK, integrin-linked kinase; sh, short hairpin; LDH, lactate dehydrogenase; BrdU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen.

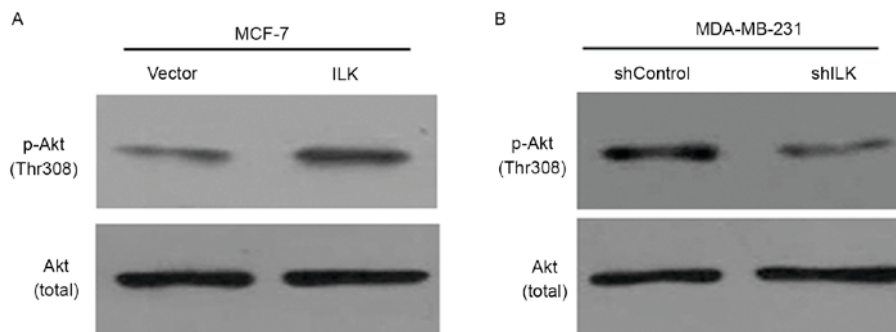


Figure 3. ILK positively regulates activation of the PI3K/Akt pathway in breast cancer cells. (A) Phosphorylation of Akt was enhanced by overexpression of ILK in MCF-7 cells. (B) Knockdown of ILK suppressed the phosphorylation levels of Akt in MDA-MB-231 cells. ILK, integrin-linked kinase; PI3K, phosphoinositide 3-kinase; p, phosphorylated; sh, short hairpin.

mitigated by ILK knockdown (Fig. 2E and F). These results indicate that ILK positively regulates the growth of breast cancer cells.

**Activation of Akt by ILK in breast cancer cells.** The PI3K/Akt pathway is one of the most important prosurvival pathways in cells. Because activation of the PI3K/Akt pathway is frequently observed in various types of cancer, including breast cancer, the effect of ILK on Akt phosphorylation was examined. The results demonstrated that overexpression of ILK in MCF-7 cells significantly enhanced the phosphorylation of Akt compared with control cells, but had no obvious effect on the levels of total Akt (Fig. 3A). By contrast, knockdown of ILK in MDA-MB-231 cells decreased the phosphorylation levels of Akt compared with control cells (Fig. 3B). These results suggest that activation of the PI3K/Akt pathway may be regulated by ILK in breast cancer cells.

*Promotive effects of ILK on cell proliferation are weakened by inhibition of the PI3K/Akt pathway.* In order to further test the involvement of the PI3K/Akt pathway in the ILK-promoted cell proliferation, the specific inhibitor LY294002 was used to block the PI3K/Akt pathway in MCF-7 cells. As illustrated in Fig. 4A, LY294002 treatment (10  $\mu$ M) for 24 h effectively inhibited phosphorylation of Akt in the ILK-overexpressing MCF-7 cells. WST-1 assay results demonstrated that the cell viability increase mediated by ILK overexpression was attenuated following LY294002 treatment in MCF-7 cells, compared with cells treated with DMSO vehicle control (Fig. 4B). In addition, the ILK-mediated decrease in the LDH release was reversed by the PI3K/Akt pathway inhibition (Fig. 4C). Finally, the promotive effects of ILK overexpression on BrdU incorporation and PCNA protein expression were partially reversed following treatment of MCF-7 cells with LY294002, compared with cells treated with DMSO vehicle control (Fig. 4D and E).



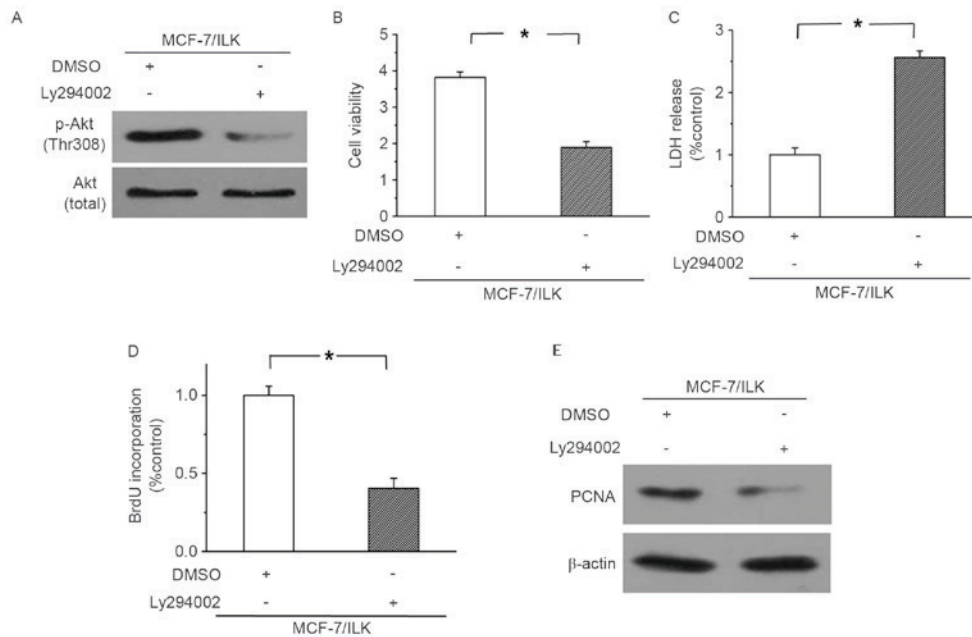


Figure 4. Cell proliferation promoted by ILK overexpression is attenuated by inhibition of the PI3K/Akt pathway. (A) Phosphorylation of Akt was effectively blocked by treatment with 10  $\mu$ M LY294002 in MCF-7 cells. (B) Results of WST-I assay demonstrating that the effects of ILK overexpression on cell viability were weakened by LY294002 treatment in MCF-7 cells. (C) Overexpression of ILK-inhibited cell death was mitigated by LY294002 treatment. (D) Promotive effects of ILK overexpression on BrdU incorporation were repressed by LY294002 treatment. (E) PCNA expression induced by ILK overexpression was attenuated by LY294002 treatment. Data are represented as the mean  $\pm$  standard error of the mean from three independent experiments. \* $P$ <0.05. ILK, integrin-linked kinase; PI3K, phosphoinositide 3-kinase; BrdU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen; DMSO, dimethyl sulfoxide; p, phosphorylated; LDH, lactate dehydrogenase.

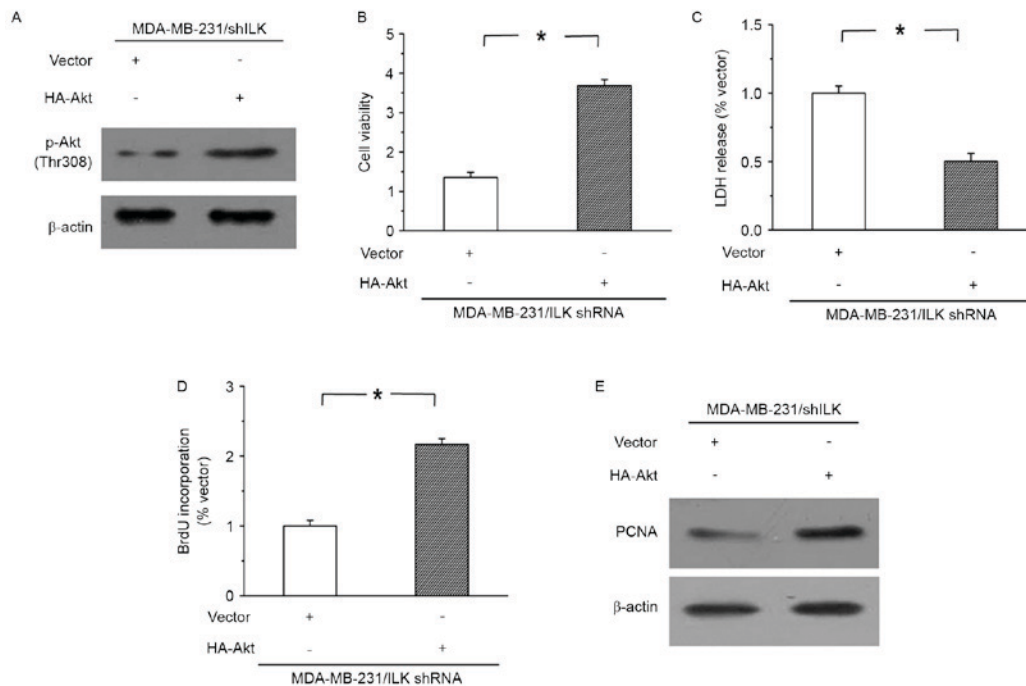


Figure 5. ILK knockdown-depressed cell proliferation is reversed by activation of the PI3K/Akt pathway. (A) Phosphorylation of Akt was increased by HA-Akt in MDA-MB-231 cells. (B) The inhibitory effects of ILK knockdown on cell viability were mitigated by HA-Akt. (C) Knockdown of ILK-induced cell death was reversed by HA-Akt. (D) BrdU incorporation depressed by ILK knockdown was reversed by HA-Akt. (E) ILK knockdown-inhibited PCNA expression was reversed by HA-Akt. Data are represented as the mean  $\pm$  standard error of the mean from three independent experiments. \* $P$ <0.05. ILK, integrin-linked kinase; PI3K, phosphoinositide 3-kinase; HA-Akt, constitutively active form of Akt; BrdU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen; p, phosphorylated; sh, short hairpin; LDH, lactate dehydrogenase.

These results imply that overexpression of ILK promoted cell proliferation through activation of the PI3K/Akt pathway.

*Inhibition of cell proliferation by ILK knockdown is attenuated by activation of the PI3K/Akt pathway. A constitutively*

active form of Akt (HA-Akt) was used in order to induce Akt phosphorylation and constitutive activation of the PI3K/Akt pathway in the ILK-knockdown MDA-MB-231 cells (Fig. 5A). As demonstrated in Fig. 5B and C, results from the WST-1 and LDH assays revealed that activation of the PI3K/Akt pathway led to increased cell viability and decreased cell death in the ILK-knockdown MDA-MB-231 cells. In addition, the inhibitory effects of ILK knockdown on BrdU incorporation and PCNA expression levels were attenuated by activation of the PI3K/Akt pathway (Fig. 5D and E). The above results further indicate that ILK potentiated the growth and proliferation of breast cancer cells via the PI3K/Akt pathway.

## Discussion

Overt proliferation is a well-known hallmark of the initiation and progression of cancer. In normal tissues, cell growth is strictly controlled to retain a dynamic balance between cell proliferation and cell apoptosis. Disturbing this balance, by either promoting cell proliferation or/and inhibiting cell apoptosis, can lead to overgrowth of cells. Multiple genes participate in regulating cell proliferation and are considered as potential treatment targets for the development of molecular targeted therapies. Thus, it is necessary to determine the genes and their upstream or downstream pathways that are critical in regulating the growth of cancer cells. In the present study, overexpression of ILK was demonstrated to promote cell proliferation and growth, while ILK knockdown mitigated cell proliferation in breast cancer cells. In addition, the results revealed that the effects of ILK on cell proliferation were at least in part mediated by the PI3K/Akt pathway.

An important finding of the present study is that ILK participates in regulating the progression of breast cancer by promoting cell proliferation in breast cancer cells. ILK is an integrin-associated, actin and tubulin cytoskeleton-interacting effector, which affects cell adhesion and other integrin-mediated and growth factor-regulated cell functions (14,15). Previous studies have demonstrated that ILK serves important roles in regulating the growth and metastasis in various types of cancer, such as ovarian cancer (5), but to date, no direct evidence existed on the role of ILK in the proliferation of breast cancer cells. The present research revealed that ILK overexpression facilitated cell proliferation and cell growth, while ILK knockdown had the opposite effects. The results suggest that ILK positively modulates the growth of breast cancer by enhancing cell proliferation.

Furthermore, in the present study, ILK was demonstrated to activate the PI3K/Akt pathway in the MCF-7 and MDA-MB-231 breast cancer cells. The PI3K/AKT pathway is an important and classic prosurvival pathway, and regulates multiple cellular events including cell growth, survival and metabolism (16-18). It has been reported that activation of the PI3K/AKT pathway is closely associated with the progression of breast cancer (19-21). In order to elucidate the molecular mechanism of ILK-regulated cell proliferation in breast cancer cells, the relationship between ILK and the PI3K/Akt pathway was examined in the present study. The results demonstrated that overexpression of ILK significantly increased Akt phosphorylation, while ILK knockdown blocked Akt phosphorylation. In addition, the cell growth and proliferation

increase mediated by ILK overexpression was reversed by inhibition of the PI3K/Akt pathway. By contrast, the ILK knockdown-attenuated cell proliferation was antagonized by activation of the PI3K/Akt pathway, induced by a constitutively active form of Akt. These results indicate that the promotive effects of ILK on cell growth and proliferation may be mediated by the PI3K/Akt pathway in breast cancer cells. The effect of ILK in regulating kinases/pathways relevant to cell growth in breast cancer other than PI3K/Akt remains unclear and should be determined in further studies.

In conclusion, the present study demonstrated that ILK facilitated cell proliferation in breast cancer cells through activating the PI3K/Akt pathway, suggesting a vital role of ILK in breast cancer cell growth. The present study implies that ILK and its downstream target signaling pathways may serve as potential therapeutic targets for improving the treatment of breast cancer.

## Acknowledgements

The present study was financially supported by grants from the National Nature Science Foundation of China (grant no. 81272854).

## References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. *CA Cancer J Clin* 61: 69-90, 2011.
2. Zeichner SB, Terawaki H and Gogineni K: A review of systemic treatment in metastatic Triple-Negative breast cancer. *Breast Cancer (Auckl)* 10: 25-36, 2016.
3. Mulrooney J, Foley K, Vineberg S, Barreuther M and Grabel L: Phosphorylation of the beta1 integrin cytoplasmic domain: Toward an understanding of function and mechanism. *Exp Cell Res* 258: 332-341, 2000.
4. Hannigan GE and Dedhar S: Protein kinase mediators of integrin signal transduction. *J Mol Med (Berl)* 75: 35-44, 1997.
5. McDonald PC, Fielding AB and Dedhar S: Integrin-linked kinase-essential roles in physiology and cancer biology. *J Cell Sci* 121: 3121-3132, 2008.
6. Mo JS, Kim MY, Han SO, Kim IS, Ann EJ, Lee KS, Seo MS, Kim JY, Lee SC, Park JW, *et al*: Integrin-linked kinase controls Notch1 signaling by down-regulation of protein stability through Fbw7 ubiquitin ligase. *Mol Cell Biol* 27: 5565-5574, 2007.
7. Tang MC, Chan LC, Yeh YC, Chen CY, Chou TY, Wang WS and Su Y: Thymosin beta 4 induces colon cancer cell migration and clinical metastasis via enhancing ILK/IQGAP1/Rac1 signal transduction pathway. *Cancer Lett* 308: 162-171, 2011.
8. Albasri A, Al-Ghamdi S, Fadhl W, Aleskandarany M, Liao YC, Jackson D, Lobo DN, Lo SH, Kumari R, Durrant L, *et al*: Cten signals through integrin-linked kinase (ILK) and may promote metastasis in colorectal cancer. *Oncogene* 30: 2997-3002, 2011.
9. Abboud ER, Coffelt SB, Figueroa YG, Zvezdaryk KJ, Nelson AB, Sullivan DE, Morris CB, Tang Y, Beckman BS and Scandurro AB: Integrin-linked kinase: A hypoxia-induced anti-apoptotic factor exploited by cancer cells. *Int J Oncol* 30: 113-122, 2007.
10. Hsu EC, Kulp SK, Huang HL, Tu HJ, Salunke SB, Sullivan NJ, Sun D, Wicha MS, Shapiro CL and Chen CS: Function of integrin-linked kinase in modulating the stemness of IL-6-Abundant breast cancer cells by regulating  $\gamma$ -Secretase-Mediated Notch1 activation in Caveolae. *Neoplasia* 17: 497-508, 2015.
11. Peng H, Talebzadeh-Farooji M, Osborne MJ, Prokop JW, McDonald PC, Karar J, Hou Z, He M, Kebebew E, Orntoft T, *et al*: LIMD2 is a small LIM-only protein overexpressed in metastatic lesions that regulates cell motility and tumor progression by directly binding to and activating the integrin-linked kinase. *Cancer Res* 74: 1390-1403, 2014.
12. Wang SJ, Gao Y, Chen H, Kong R, Jiang HC, Pan SH, Xue DB, Bai XW and Sun B: Dihydroartemisinin inactivates NF-kappaB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both in vitro and in vivo. *Cancer Lett* 293: 99-108, 2010.

13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>(Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
14. Azimifar SB, Böttcher RT, Zanivan S, Grashoff C, Krüger M, Legate KR, Mann M and Fässler R: Induction of membrane circular dorsal ruffles requires co-signalling of integrin-ILK-complex and EGF receptor. *J Cell Sci* 125: 435-448, 2012.
15. Chun SJ, Rasband MN, Sidman RL, Habib AA and Vartanian T: Integrin-linked kinase is required for laminin-2-induced oligodendrocyte cell spreading and CNS myelination. *J Cell Biol* 163: 397-408, 2003.
16. Zheng J, Koh X, Hua F, Li G, Larrick JW and Bian JS: Cardioprotection induced by Na(+)/K(+)-ATPase activation involves extracellular signal-regulated kinase 1/2 and phosphoinositide 3-kinase/Akt pathway. *Cardiovasc Res* 89: 51-59, 2011.
17. Koul D, Shen R, Bergh S, Sheng X, Shishodia S, Lafortune TA, Lu Y, de Groot JF, Mills GB and Yung WK: Inhibition of Akt survival pathway by a small-molecule inhibitor in human glioblastoma. *Mol Cancer Ther* 5: 637-644, 2006.
18. Liu Q, Turner KM, Alfred Yung WK, Chen K and Zhang W: Role of AKT signaling in DNA repair and clinical response to cancer therapy. *Neuro Oncol* 16: 1313-1323, 2014.
19. Hashimoto K, Tsuda H, Koizumi F, Shimizu C, Yonemori K, Ando M, Kodaira M, Yunokawa M, Fujiwara Y and Tamura K: Activated PI3K/AKT and MAPK pathways are potential good prognostic markers in node-positive, triple-negative breast cancer. *Ann Oncol* 25: 1973-1979, 2014.
20. Chang PH, Hwang-Verslues WW, Chang YC, Chen CC, Hsiao M, Jeng YM, Chang KJ, Lee EY, Shew JY and Lee WH: Activation of Robo1 signaling of breast cancer cells by Slit2 from stromal fibroblast restrains tumorigenesis via blocking PI3K/Akt/ $\beta$ -catenin pathway. *Cancer Res* 72: 4652-4661, 2012.
21. O'Brien NA, Browne BC, Chow L, Wang Y, Ginther C, Arboleda J, Duffy MJ, Crown J, O'Donovan N and Slamon DJ: Activated phosphoinositide 3-kinase/AKT signaling confers resistance to trastuzumab but not lapatinib. *Mol Cancer Ther* 9: 1489-1502, 2010.