SKA2 mediates invasion and metastasis in human breast cancer via EMT

ZHOUHUI REN[1,2*], TONG YANG[2*], PINGPING ZHANG[3], KAITAI LIU[4], WEIHONG LIU[1] and PING WANG[1]

[1]Zhejiang Provincial Key Laboratory of Pathophysiology, Ningbo University School of Medicine, Ningbo, Zhejiang 315211; [2]Department of Oncology Surgery, Ningbo No. 2 Hospital, Ningbo, Zhejiang 315010; [3]Department of Gynaecology, Ningbo Women and Children's Hospital, Ningbo, Zhejiang 315012; [4]Department of Oncology, Ningbo Medical Center, Li Huili Hospital, Ningbo, Zhejiang 315041, P.R. China

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Abstract. Spindle and kinetochore-associated protein 2 (SKA2) is essential for regulating the progression of mitosis. In recent years, SKA2 upregulation has been detected in various human malignancies and the role of SKA2 in tumorigenesis has received increasing attention. However, the expression and functional significance of SKA2 in breast cancer are not completely understood. To study the effects of SKA2 on breast cancer, the expression levels of SKA2 in breast cancer tissues and cell lines were evaluated by western blotting, reverse transcription-quantitative polymerase chain reaction and immunohistochemical staining. The results demonstrated that SKA2 expression was increased in breast cancer tissues and cells, and SKA2 overexpression was associated with clinical stage and lymph node metastasis. Functional investigations revealed that SKA2 knockdown in breast cancer cells significantly reduced migration and invasion, and resulted in the decreased expression levels of matrix metalloproteinase (MMP)2 and MMP9. Furthermore, the typical microtubule arrangement was altered in SKA2 small interfering RNA (siSKA2)-transfected cells. Reduced levels of SKA2 also downregulated the expression of epithelial-mesenchymal transition proteins, including fibronectin, N-cadherin and vimentin, whereas there were no alterations in the protein expression levels of E-cadherin. Conversely, upregulation of SKA2 decreased the expression levels of E-cadherin, and increased N-cadherin, fibronectin and vimentin levels. Notably, it was demonstrated that E-cadherin was translocated from the cytoplasm to the nucleus in siSKA2-transfected cells. These results demonstrated that SKA2 may be associated with breast cancer metastasis, and siSKA2 inhibited the invasion and metastasis of breast cancer via translocation of E-cadherin from the cytoplasm to the nucleus.

Correspondence to: Dr Ping Wang, Zhejiang Provincial Key Laboratory of Pathophysiology, Ningbo University School of Medicine, 818 Fenghua Road, Ningbo, Zhejiang 315211, P.R. China
E-mail: pinoav@hotmail.com

*Contributed equally

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Introduction

Spindle and kinetochore-associated (SKA)2 is located on chromosome 17 of the human genome and has been identified as a conserved protein involved in the kinetochore complex (1,2). SKA2, together with its cofactors SKA1 and SKA3, constitute the SKA complex, which maintains the metaphase plate and/or spindle checkpoint silencing (3-5). Checkpoint-dependent delays in a metaphase-like state are prolonged by RNA interference-mediated SKA2 depletion (6,7). In addition, SKA2 has been reported to serve a role in tumorigenesis. Aberrant patterns of SKA2 expression have been observed in several types of cancer, including lung cancer (8-10), kidney cancer (11), pancreatic cancer (12), gastric cancer (13), glioma (14) and osteosarcoma (15,16). However, to the best of our knowledge, the roles of SKA2 in breast cancer migration remain to be elucidated.

Breast cancer is common in women worldwide. The first highest incidence rates and second highest mortality rates have been demonstrated in the developed world (17,18). Furthermore, breast cancer is prone to metastasis and results in poor prognosis (19,20). Although marked progress has been achieved in breast cancer therapy due to modern technology, unknown molecular mechanisms of metastasis remain and require further investigation. As a fundamental process of migration, epithelial-mesenchymal transition (EMT) serves a crucial role in breast cancer progression (21-24). However, the specific regulatory mechanism mediating EMT and SKA2 in breast cancer progression remains to be investigated. In the present study, it was demonstrated that SKA2 was upregulated in human breast cancer cell lines and tissues by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and western blotting. Small interfering (si)SKA2 was used to knockdown the expression of SKA2 in MCF-7 and T47D cells, the results demonstrated that decreasing the level of SKA2 inhibited the metastasis of the breast cancer cells by wound healing assay and cell invasion assays. However, although the

results are promising, further studies are needed to confirm this. Therefore, the present study further investigated whether SKA2 regulates metastasis via EMT-associated proteins and matrix metalloproteinase (MMP)-2/9. The current study aimed to determine the potential role of SKA2 in breast cancer invasion, and its molecular mechanism.

Patients and methods

Patients. Tumor specimens and adjacent tissues were collected from patients who were diagnosed with breast cancer (stages I-IV) and underwent surgery at Ningbo No. 2 Hospital (Ningbo, China) between March 2015 and August 2017. None of these patients underwent local or systemic therapy prior to surgery. The clinical characteristics of these 160 patients are described in Table I. The present study was approved by the Research Ethics Committee of Ningbo No. 2 Hospital, and all patients provided written informed consent.

Cell lines and transfection. Human breast cancer cell lines MCF-7, T47D and MDA-MB-231, and normal MCF-10A cells were purchased from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; ExCell Bio, Shanghai, China) at 37˚C in an incubator containing 5% CO_2.

Cells (MCF-7 and T47D) were transfected with small interfering RNA (si)SKA2 (sense, 5'-GGCUGGAAUAUGAAAUCAATT-3' and antisense, 5'-UUGAUUUCAUAUUCCAGCCTT-3'), si-negative control (NC; sense, 5'-UCCUCCGAACGUGUCACGUTT-3' and antisense, 5'-ACGUGACACGUUCGGAGAATT-3') (both Shanghai GenePharma Co., Ltd., Shanghai, China), SKA2cDNA plasmid (Ref Seq: M_001100595.1, Atgg cctcggaggt ggggcacaat ttggagtcgc cggaaactcc gcggcgga ggctggacca gagtcgagtt ctcctcct gcaccaaagg gagccgccac tctggtgt ctaaaccgcc tcggttccag gctgagt ctgatctgga ttacattcaa caggctgg aatatgaaat caagactaat tcctgatt cagcaagtga gctgtcacca ctga) or NC plasmid (both Shanghai Genechem Co., Ltd., Shanghai, China) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. The cells were seeded in 6-well plates (siRNA transfection: 100 pmol siRNA and 5 μl Lipofectamine 2000, plasmid DNA transfection: 4 μg DNA and 10 μl Lipofectamine 2000, at room temperature) and were grown to 50% confluence prior to transfection. RNA and protein were extracted 24 h post-transfection. The MOCK was not transfected with any agent.

Extraction of cytoplasmic and nuclear proteins. A total of 24 h post-transfection, MCF-7 cells were harvested and washed three times with cold PBS. Cytoplasmic and nuclear protein fractions were extracted using a Mammalian Nuclear and Cytoplasmic Protein Extraction kit (Beijing Transgen Biotech, Co., Ltd., Beijing, China), according to the manufacturer's protocol; these proteins were then used for western blotting.

Western blot analysis. Total protein extracted from the cells by 1XSDS (Sigma-Aldrich; Merck KGaA) was quantified by bicinchoninic acid analysis (Beyotime Institute of Biotechnology, Shanghai, China). Cellular proteins (40 μg) were separated by 12% SDS-PAGE and were transferred onto polyvinylidende difluoride membranes (EMD Millipore, Billerica, MA, USA) for immunoblotting. After blocking with 5% bovine serum albumin (BSA; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 2 h at room temperature, the membranes were incubated with specific primary antibodies diluted in 1X Tris-buffered saline-0.1% Tween-20 overnight at 4˚C. The following antibodies were used: SKA2 (1:1,500; cat. no. ab91551), MMP2 (1:1,500; cat. no. ab7033), MMP9 (1:1,500; cat. no. ab137651), vimentin (1:2,000; cat. no. ab8978), fibronectin (1:2,000; cat. no. ab2413; all Abcam, Cambridge, UK), N-cadherin (1:2,000; cat. no. 14215), E-cadherin (1:2,000; cat. no. 3195), β-actin (1:1,500; cat. no. 8457), Histone H3 (1:1,500; cat. no. 9728) and GAPDH (1:1,500; cat. no. 5174; all Cell Signaling Technology, Inc., Danvers, MA, USA). Subsequently, membranes were incubated for 1 h at room temperature with goat anti-rabbit immunoglobulin (Ig)G-horseradish peroxidase (HRP) or goat anti-mouse IgG-HRP (1:5,000; cat. nos. BA1054/BA1050; Wuhan Boster Biological Technology, Ltd., Wuhan, China). The protein bands on the membrane were visualized using a chemiluminescence imaging system (LI-COR Biosciences, Lincoln, CA, USA) and detected by chemiluminescence. Densitometric analysis was performed by Tanon GIS version 4.1.2 software (Tanon Science and Technology Co., Ltd., Shanghai, China).

RT-qPCR. The mRNA expression levels of SKA2 were detected using RT-qPCR. Briefly, total RNA was isolated using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and 1 μg total RNA was subjected to first-strand cDNA synthesis for 15 min at 37˚C and 5 sec at 85˚C using a reverse transcription kit (Thermo Fisher Scientific, Inc.). cDNA was amplified by RT-qPCR using SYBR-Green PCR Master Mix (Roche Applied Science, Madison, WI, USA) on a LightCycler® 480 system (95˚C/10 min/1 cycle, 95˚C/10 sec/45 cycles, 60˚C/60 sec/45 cycles; Roche Applied Science) and fold-changes were calculated by relative quantification ($2^{-\Delta\Delta Cq}$) (25). The primers used were as follows: SKA2 forward, 5'-CTGAAACTATGCTAAGTGGGGGAG-3', reverse, 5'-TTCCAAACATCCTGACACTCAAAAG-3'; and GAPDH forward, 5'-AAGCCTGCCGGTGACTAAC-3' and reverse, 5'-GCATCACCCGGAGGAGAAAT-3'.

Wound healing assay. Cells were seeded in 6-well plates at a density of $1x10^5$ cells/well. Once cellular density reached ~70%, the cells were scraped in a straight line with a 1-ml blue micro-pipette tip. After rinsing the dislodged cells with PBS, the cell culture medium was replaced with fresh serum-free medium. The widths at 0 and 24 h were compared to assess the distance of migration using fluorescence microscopy.

Cell invasion assays. Cell invasion assays were conducted using a Matrigel-coated invasion chamber (24-well plates, 8-μm pore size; Corning Inc., Corning, NY, USA), in accordance with the manufacturer's protocol. A total of $5x10^4$ cells were seeded in the upper chambers of the wells in 100 μl FBS-free medium, and the lower chambers were filled with DMEM supplemented with 20% FBS, in order to stimulate cell invasion. After 24 h incubation at 37˚C in an incubator containing 5% CO_2, the cells on the filter surface were stained

Table I. SKA2 expression and clinicopathological characteristics of patients.

Characteristic	Number of patients	SKA2 expression levels		P-value
		Low (%)	High (%)	
Age (years)				0.839
≤50	72	24 (33.3)	48 (66.7)	
>50	88	28 (31.8)	60 (68.2)	
Tumor (cm)				0.077
<2	78	32 (41)	46 (59)	
2-5	55	14 (22.5)	41 (74.5)	
>5	27	6 (22.2)	21 (77.8)	
Tumor TNM staging				0.001
I	48	25 (52.1)	23 (47.9)	
II	40	14 (35)	26 (65)	
III	64	11 (17.2)	53 (82.8)	
IV	8	2 (25)	6 (75)	
Tumor histological grade				0.485
1	9	4 (44.4)	5 (55.6)	
2	96	28 (29.2)	68 (70.8)	
3	55	20 (36.4)	35 (63.6)	
Pathological type				0.052
Infiltrative	128	37 (28.9)	91 (71.1)	
Not infiltrative	32	15 (46.9)	17 (53.1)	
Molecular type				0.198
Luminal A				0.001
Lymphatic metastasis	27	5 (18.5)	22 (81.5)	
No lymphatic metastasis	18	12 (66.7)	6 (33.3)	
Luminal B				0.002
Lymphatic metastasis	50	10 (20)	40 (80)	
No lymphatic metastasis	21	12 (57.1)	9 (42.9)	
HER2				0.021
Lymphatic metastasis	16	2 (12.5)	14 (87.5)	
No lymphatic metastasis	9	5 (55.6)	4 (44.4)	
Basal				0.025
Lymphatic metastasis	13	2 (15.4)	11 (84.6)	
No lymphatic metastasis	6	4 (66.7)	2 (33.3)	
Lymphatic metastasis				<0.001
Yes	106	19 (17.9)	87 (82.1)	
No	54	33 (61.1)	21 (38.9)	

The P-value for molecular type includes analysis of Luminal A, Luminal B, HER2, Basal types. SKA2, spindle and kinetochore-associated protein 2.

with 0.1% crystal violet for 30 min and images were captured with a fluorescence microscope (Nikon Corporation, Tokyo, Japan). Absorbance was measured at 590 nm.

Immunohistochemical staining. Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue sections (4-μm; formalin fixation was performed at room temperature with no time limit set for fixation). All sections were blocked with 5% BSA for 30 min at room temperature and incubated with anti-SKA2 (1:500; cat. no. ab91551; Abcam) at 4˚C overnight. The sections were then incubated with a horseradish peroxidase-goat anti-rabbit IgG secondary antibody for 40 min at room temperature (1:100; cat. no. TA140003; OriGene Technologies Inc., Beijing, China). Finally, Myer's hematoxylin was used for background staining for 3 min at room temperature and photographs were taken using fluorescence microscopy. The appropriate positive (tumor tissues at different stages) and negative controls (normal tissue) were included. The positive

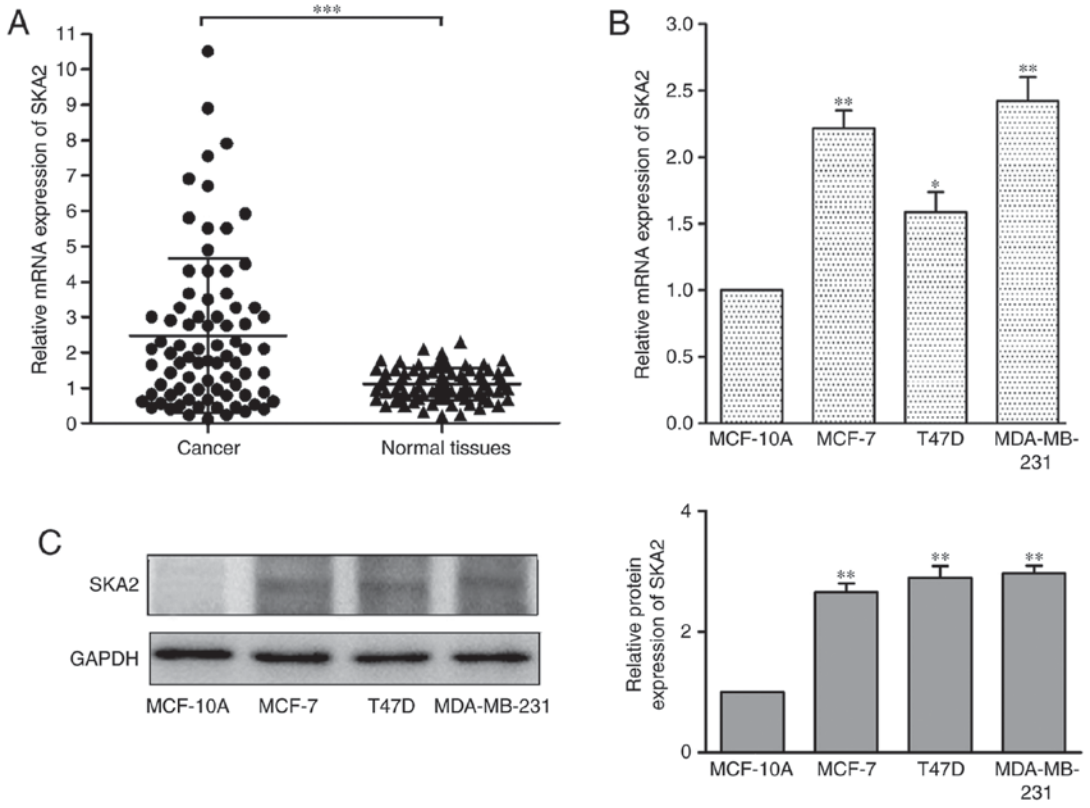

Figure 1. SKA2 expression is frequently upregulated in breast cancer tissues and cell lines. (A) Reverse transcription-quantitative polymerase chain reaction was performed to measure the levels of SKA2 in breast cancer tissues compared with in adjacent normal tissues. ***P<0.001 vs. normal tissues. Relative (B) mRNA and (C) protein expression levels of SKA2 were increased in breast cancer cells (MCF-7, T47D and MDA-MB-231) compared with in breast epithelial cells (MCF-10A). Data are presented as the means ± standard deviation from three independent experiments. *P<0.05 and **P<0.01 vs. MCF-10A cells. SKA2, spindle and kinetochore-associated protein 2.

cells and staining intensity of tumor tissues were scored respectively. According to the percentage of positive cells, <5% was 0, 5% ~<25% was 1, 25% ~<50% was 2, 50% ~<75% was 3, and <75% was 4. According to the coloring strength score, it could be divided into 4 grades: No coloring was 0, pale brown was 1, brown was 2, dark brown was 3. The result of the two products: 0 was negative, 1 was positive, in which <6 was low expression and ≥6 is high expression (26).

Fluorescent immunocytochemistry. MCF-7 cells were fixed in 4% paraformaldehyde for 30 min at room temperature and then permeabilized with 0.1% Triton X-100 in PBS at room temperature for 15 min. The cells were blocked with 1% BSA overnight and incubated overnight at 4˚C with Alexa Fluor® 488-conjugated β-tubulin rabbit monoclonal antibody to (1:500; cat. no. 2116) or E-cadherin antibody (1:1,000; cat. no. 3195; both Cell Signaling Technology, Inc.). Then all cells were counterstained with 4',6-diamidino-2-phenylindole (5:100; cat. no. C0060; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 15 min at room temperature and E-cadherin stained cells were counterstained with CY3-labeled goat anti-rabbit IgG (1:50; cat. no. BA1032; Wuhan Boster Biological Technology, Ltd.) for 1 h at room temperature. Finally, the coverslips were washed twice with PBS and images were captured using confocal scanning microscopy.

Statistical analysis. All experiments were repeated three times and data are expressed as the means ± standard deviation. The values between the two groups in Fig. 1A was compared with independent t-test. One-way analysis of variance and followed by Fisher's least significant difference tests was used to calculate P-values between the cell groups in the invasion and wound healing assays, and to compare measurements of the protein and mRNA expression levels in breast cancer cell lines. Data in Table I were analyzed using the χ^2 test of four by four table. Statistical analyses were performed using SPSS 15.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

SKA2 levels are upregulated in breast cancer and are associated with breast cancer metastasis. To investigate the role of SKA2 in breast cancer, SKA2 expression levels were measured in 80 patient samples by RT-qPCR and the levels were normalized to GAPDH. The results demonstrated that SKA2 mRNA was significantly upregulated in breast cancer tissues compared with in adjacent normal tissues (P<0.001; Fig. 1A). In addition, the mRNA (Fig. 1B) and protein (Fig. 1C) expression levels of SKA2 in three human breast cancer cell lines (MCF-7, T47D and MDA-MB-231) were evaluated and compared with the MCF-10A normal human mammary epithelial cell line. SKA2 was significantly increased in all three cell lines compared with in MCF-10A cells (P<0.05).

To investigate the clinical significance of SKA2 in patients with breast cancer, SKA2 immunohistochemical staining in

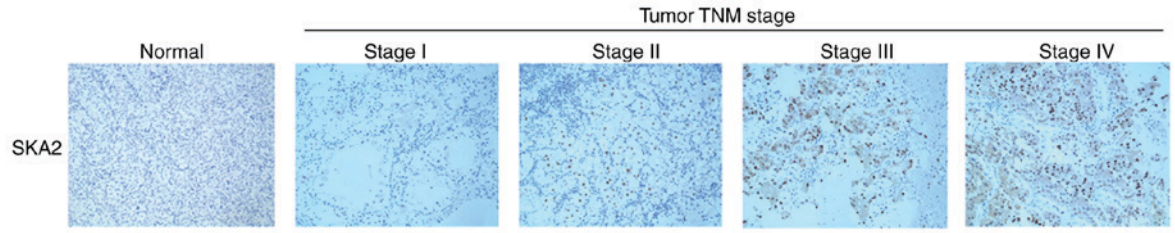

Figure 2. Representative immunohistochemical staining of SKA2. SKA2 levels were increased with clinical stage. Intense SKA2 signals were observed in the nuclei of tumor cells from TNM stages III and IV breast cancer tissues; the signal intensity was greater than that in stages I and II. Representative images were captured at magnification, x200. SKA2, spindle and kinetochore-associated protein 2.

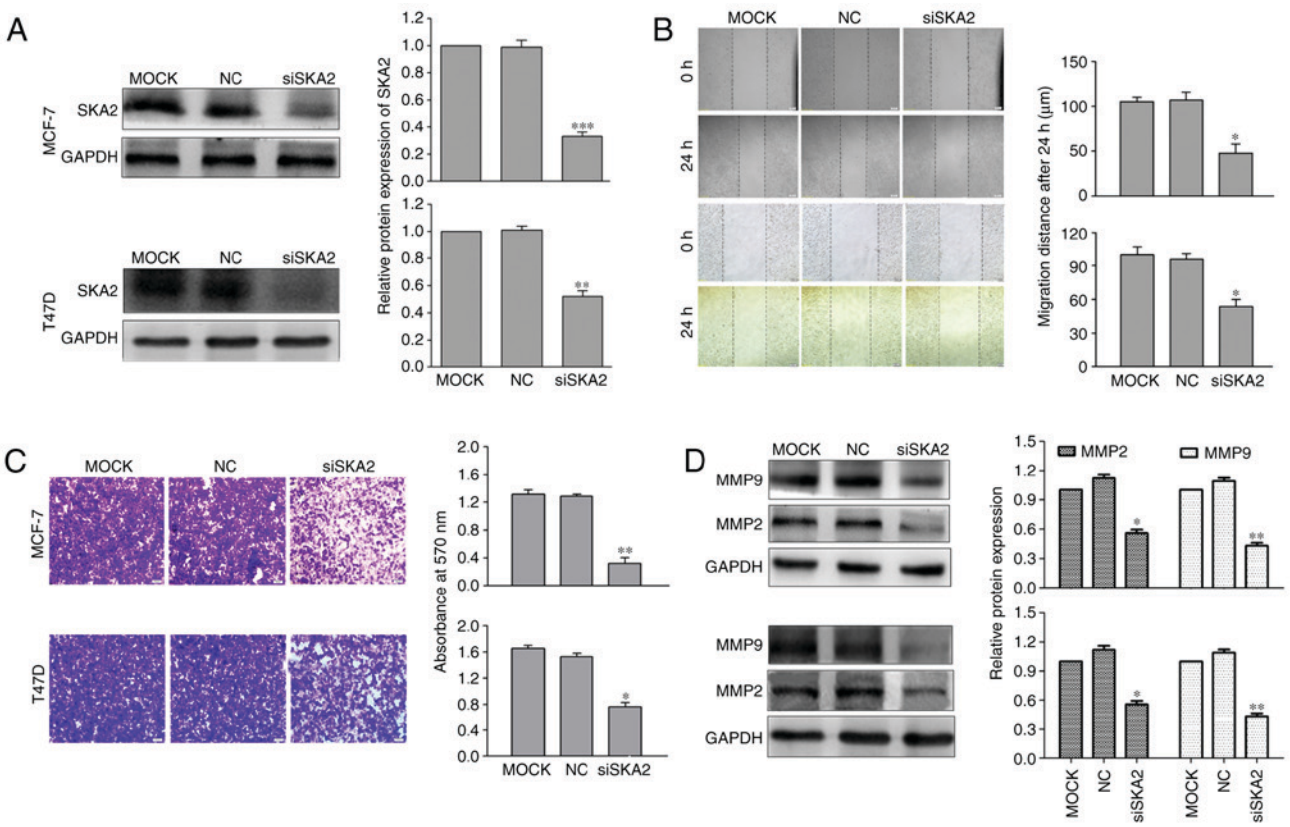

Figure 3. SKA2 promotes migration and invasion of breast cancer cells. (A) Once cells were transfected with siSKA2, the mRNA and protein expression levels of SKA2 were significantly decreased in MCF-7 and T47D cells compared with in MOCK cells. (B) Migration distance and (C) number of invasive cells were significantly decreased in the siSKA2 group compared with in the MOCK groups, magnification, x100. (D) Protein expression levels of MMP2 and MMP9 were significantly decreased in the siSKA2 groups compared with the MOCK group. Data are presented as the means ± standard deviation from three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. the MOCK group. MMP, matrix metalloproteinase; NC, negative control; si, small interfering RNA; SKA2, spindle and kinetochore-associated protein 2.

a cohort of 160 specimens was analyzed. As demonstrated in Table I, high intratumoral SKA2 levels were significantly associated with clinical stage (P=0.001) and all lymph node metastasis groups (Luminal A, B HER2 and basal; P<0.001) but not with patient age, tumor size, histological grade, pathological type or molecular typing (Table I). In addition, intratumoral SKA2 levels increased gradually with disease progression from TNM stage I to IV (Fig. 2).

SKA2 knockdown inhibits the migration and invasion of breast cancer cells. To investigate the function of SKA2 in breast cancer cell migration, siSKA2 specifically targeting SKA2 was used. Transfection with siSKA2 significantly decreased the protein expression levels of SKA2 in breast cancer cells (MCF-7 and T47D which were chosen as a result of their differential expression levels of SKA2; P<0.01; Fig. 3A). Following knockdown of SKA2 expression, its effects on cell migration and invasion *in vitro* were investigated using Transwell and scratch wound healing assays. Compared with the control cells, breast cancer cell migration and invasion were significantly inhibited in siSKA2-transfected cells (P<0.05; Fig. 3B and C).

It has previously been demonstrated that the extracellular matrix (ECM) serves a critical role in tumor invasion and metastasis (27). Since MMPs, particularly MMP2 and MMP9, destroy ECM processing, they are associated with tumor invasion and metastasis (28). The results of the present study demonstrated that siSKA2 transfection significantly decreased

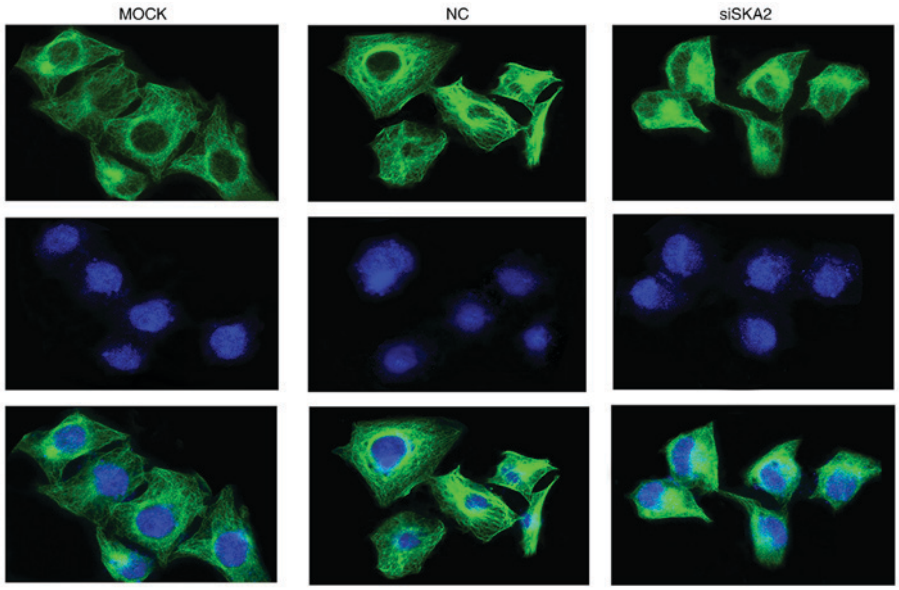

Figure 4. Effects of SKA2 on microtubules in the cellular cytoskeleton. MCF-7 cells were subjected to immunofluorescence analysis. β-tubulin was detected using an anti-β-tubulin antibody to determine the presence of microtubules (green); cell nuclei were counterstained with DAPI (blue). In siSKA2-transfected cells, the microtubules were polymerized, whereas there were no marked effects on NC cells. The cell images (magnification, x400) were merged. NC, negative control; si, small interfering RNA; SKA2, spindle and kinetochore-associated protein 2.

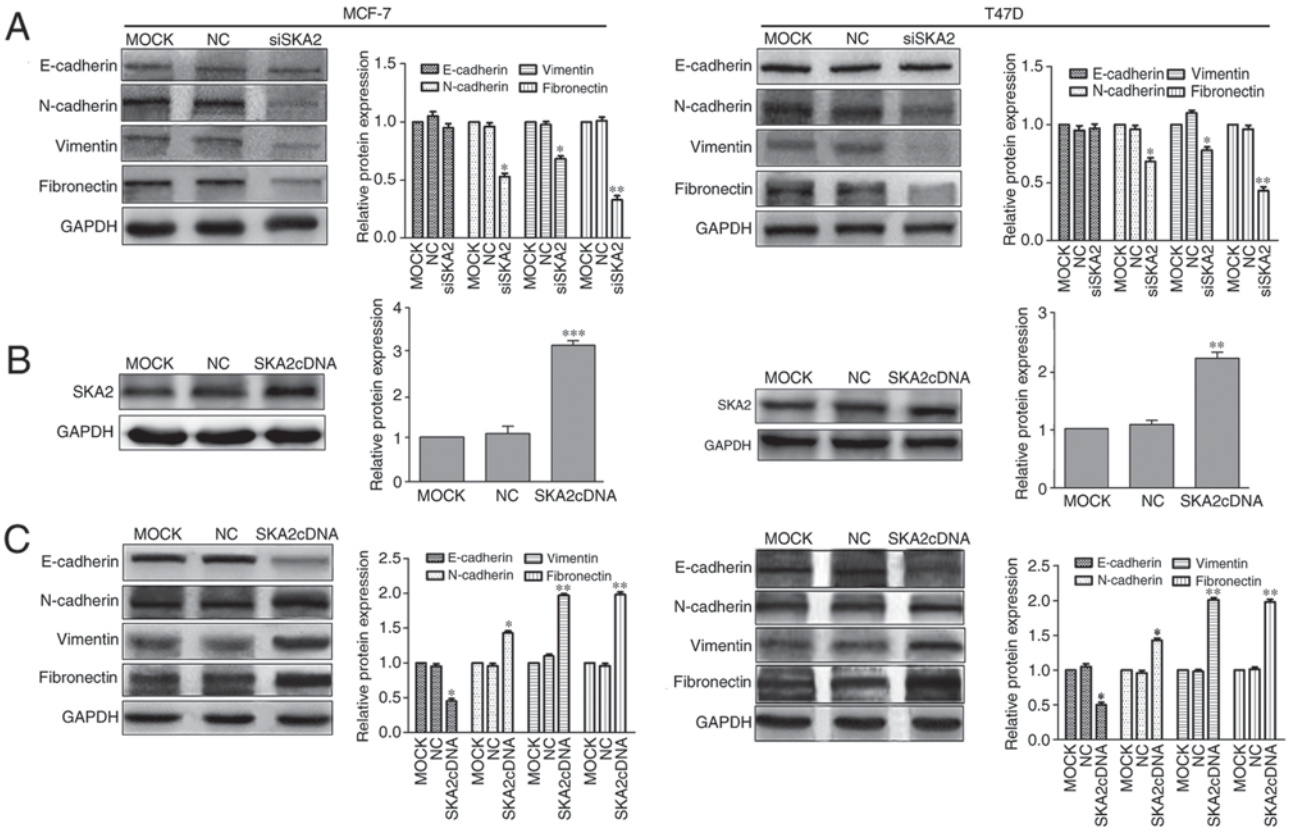

Figure 5. SKA2 mediates breast cancer metastasis via EMT. (A) After cells were transfected with siSKA2, the expression levels of proteins associated with EMT were determined by western blotting. Vimentin, fibronectin and N-cadherin were decreased in cells transfected with siSKA2 compared with in the MOCK group. However, E-cadherin levels were unaltered in siSKA2-transfected breast cancer cells. (B) SKA2 expression was upregulated with SKA2cDNA. (C) In SKA2cDNA-transfectd cells, the expression levels of E-cadherin were decreased, whereas N-cadherin, fibronectin and vimentin were increased. Data are presented as the means ± standard deviation from three independent experiments. *P<0.05, **P<0.01, ***P<0.001, vs. the MOCK group. EMT, epithelial-mesenchymal transition; si, small interfering RNA; SKA2, spindle and kinetochore-associated protein 2.

the expression levels of MMP2 and MMP9 in breast cancer cells (P<0.05; Fig. 3D).

SKA2 regulates microtubule organization in MCF-7 cells. MCF-7 cells were immunostained with anti-β-tubulin antibodies

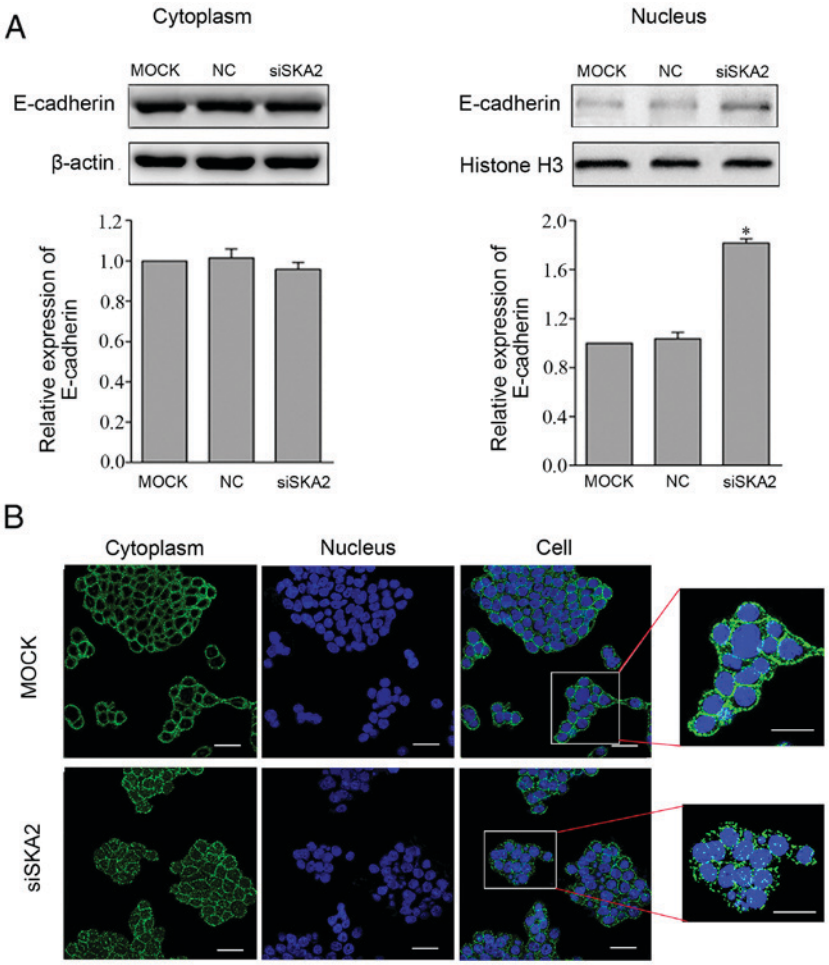

Figure 6. Effects of siSKA2 on translocation of E-cadherin from the cytoplasm to the nucleus. E-cadherin was stained green and blue was DAPI. A total of 24 h post-transfection with siSKA2, (A) fluorescent staining was clearly observed in the nucleus and (B) SKA2 nuclear protein expression was increased. Data are presented as the means ± standard deviation from three independent experiments. *P<0.05 vs. the MOCK group. Scale bar, 20 μm. NC, negative control; si, small interfering RNA; SKA2, spindle and kinetochore-associated protein 2.

to determine the presence of microtubules. NC-transfected cells exhibited clear and intact microtubules with well-formed dendrites. In siSKA2-transfected cells, the microtubules were polymerized and the structure was spindle-shaped, which may lead to inhibited cell migration (Fig. 4).

SKA2 promotes migration and invasion via EMT. EMT is a process in which epithelial cells lose epithelial characteristics and gain mesenchymal features, including motility and invasiveness (29). To identify the mechanisms underlying the decreased migration and invasion of siSKA2-transfected breast cancer cells (MCF-7 and T47D), the expression levels of E-cadherin, which is an epithelial marker that is downregulated during EMT, were measured. In addition, vimentin, fibronectin and N-cadherin, which are mesenchymal markers that are upregulated during EMT, were also measured (30). Notably, knockdown of SKA2 significantly decreased the expression levels of vimentin, fibronectin and N-cadherin (P<0.05); however, no significant effects were detected on the expression levels of E-cadherin (Fig. 5A). Subsequently, SKA2 expression was upregulated with SKA2cDNA, and the expression levels of E-cadherin were significantly decreased (P<0.05), whereas N-cadherin, fibronectin and vimentin levels were significantly increased (P<0.05; Fig. 5B and C). Therefore, SKA2 may mediate invasion and metastasis in human breast cancer via EMT.

SKA2 knockdown induces translocation of E-cadherin from the cytoplasm to the nucleus. To investigate the effects of SKA2 on the translocation of E-cadherin, cytoplasmic and nuclear proteins were isolated and extracted from MCF-7 cells (as SKA2 is highly expressed in this cell line and MCF-7 is frequently used in breast cancer studies this cell line was selected), and western blotting was performed. The results demonstrated that there were no obvious alterations in E-cadherin in the cytoplasm compared with in the MOCK group; however, E-cadherin levels were significantly increased in the nucleus following transfection with siSKA2 (P<0.05; Fig. 6A). Similar results were obtained by immunofluorescence (Fig. 6B). These findings indicated that the suppression of SKA2 facilitated the translocation of E-cadherin from the cytoplasm to the nucleus.

Discussion

The development and progression of malignant tumors constitutes a complex process that is affected by numerous factors, including the biological characteristics of the tumor cells themselves (31,32). The poor prognosis of breast cancer, which

is one of the most common and lethal malignant diseases, is due to its metastasis (33,34). The mechanism that induces and stimulates metastasis is complex and remains unknown. In recent years, although specific evidence (10) has confirmed that SKA2 may contribute to cancer metastases, the mechanism is far from clear. In the present study, the role of SKA2, a gene associated with various types of human cancer, was investigated. The results demonstrated that SKA2 participated in metastasis in breast cancer tissues; this finding may aid in achieving a breakthrough in breast cancer treatment.

To further evaluate the role of SKA2 in breast cancer metastasis, experiments were designed in which the expression levels of SKA2 were altered, in order to investigate migration and invasion of breast cancer cells. The results demonstrated that SKA2 expression was significantly upregulated in breast cancer cells compared with in normal cells. Knockdown of SKA2 significantly inhibited the ability of breast cancer cells to migrate and invade, and decreased the expression levels of MMP2 and MMP9. These results indicated that SKA2 may serve a crucial role in the progression of breast cancer metastasis and may be considered a novel biomarker for breast cancer prognosis.

Cytoskeletal function is associated with contraction and migration; therefore, the cytoskeleton serves a vital role in tumor metastasis. Microtubules are the backbone of the cytoskeleton, which control cell movement. It has been reported that anticancer drugs can promote the polymerization of microtubules, destroy the stable structure and normal functions of microtubules, and thus prevent cell division, movement and migration (35). Therefore microtubules serve an important role in cell migration. In cells depleted of SKA2, microtubules were polymerized and exhibited a spindle-shaped structure, which may lead to inhibition of cell migration.

To investigate the molecular mechanism underlying the effects of SKA2 on invasion and migration, the potential target of SKA2 was investigated in breast cancer cells. EMT, which refers to the biological phenomenon by which epithelial cells lose their epithelial characteristics and obtain a mesenchymal phenotype, is associated with embryonic development, tissue repair, fibrosis, tumor invasion and migration (29,30). During EMT, expression of the epithelial marker E-cadherin is lost, whereas the expression levels of mesenchymal markers, including vimentin, fibronectin and N-cadherin, are acquired. Furthermore, EMT has been demonstrated to be associated with tumor invasion and migration (36). Notably, siSKA2 decreased the protein expression levels of vimentin, fibronectin and N-cadherin, but had no effect on E-cadherin, in breast cancer cells. Nevertheless, in response to upregulation of SKA2, the expression levels of E-cadherin were decreased, whereas N-cadherin, fibronectin and vimentin were increased. Therefore, SKA2 may mediate invasion and metastasis in human breast cancer via EMT.

To further analyze the regulation of E-cadherin by SKA2, cytoplasmic and nuclear proteins were isolated, extracted and analyzed by western blotting. Notably, the results demonstrated no obvious alterations in E-cadherin in the cytoplasm; however, its expression was upregulated in the nucleus when cells were transfected with siSKA2. Therefore, it was hypothesized that E-cadherin exists primarily in the cytoplasm and rarely in the nucleus. When induced by siSKA2, translocation of E-cadherin to the nucleus may be promoted, thus resulting in an increase in E-cadherin levels in the nucleus. However, only a small amount of E-cadherin in the cytoplasm moved to the nucleus; therefore, no obvious alteration in E-cadherin levels in the cytoplasm was detected. Similar results were obtained by immunofluorescence. These results were similar to those reported previously (37,38). Nevertheless, the specific mechanism through which SKA2 acts upon E-cadherin remains unclear and requires further study.

In conclusion, the present study provided evidence to suggest that SKA2 serves a promoting role in breast cancer cell metastasis by regulating the expression levels of mesenchymal-associated proteins. These findings have laid the theoretical basis for further understanding the pathogenesis of breast cancer, and developing novel diagnostic and therapeutic strategies.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZR and TY collected and analyzed the data, wrote and revised the manuscript. PW conceived and designed the experiments. PZ, KL and WL conducted the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of Ningbo No. 2 Hospital and all patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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