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RESEARCH PAPER

High expression of IGHG1 promotes breast cancer malignant development by activating the AKT pathway

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ABSTRACT

This study researched the exact function of IgG1 heavy chain (IGHG1) on breast cancer (BC) progression. IGHG1 level within BC and paired normal tissues was acquired in Gene Expression Profiling Interactive Analysis dataset. Meanwhile, this work harvested tumor and paired healthy tissues in 42 BC cases. siRNA targeting IGHG1 was transfected into BC cells. SC79 was used to treat the transfected BC cells. CCK-8 assay, clone formation experiment, BrdU assay, Transwell experiment and flow cytometry were carried out to measure the viability, colony formation, proliferation, invasion, and apoptosis of BC cells. Paclitaxel and cisplatin sensitivity of BC cells was evaluated by MTT assay. Real-time quantitative reverse transcription-polymerase chain reaction and Western-blot were performed for measuring mRNA and protein expression. The overexpressed IGHG1 indicated dismal BC survival. IGHG1 silencing attenuated the viability, invasion, proliferation, epithelial-mesenchymal transition, but enhanced the apoptosis of BC cells. IGHG1 silencing enhanced the paclitaxel and cisplatin sensitivity of BC cells. IGHG1 silencing suppressed the activity of the MEK, AKT, and ERK pathways. AKT agonist partially reversed the inhibition of IGHG1 silencing on BC cell malignant phenotype and resistance to paclitaxel and cisplatin. IGHG1 promotes the malignant development of BC by activating the AKT pathway. It may be an effective target for BC treatment.

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Breast cancer; IGHG1; AKT; proliferation; invasion

Introduction

Breast cancer (BC) is a major factor resulting in cancer-related mortality among women globally [1]. With progresses in treatment methods, the prognosis of BC cases has been achieved tremendous improvement [2]. However, metastasis remains the main reason of death for BC cases. Its 5-year survival can be as high as 90% for overall BC patients, whereas is only about 26% for metastatic patients [3]. In addition, chemotherapy is a commonly used method to treat BC, while paclitaxel and cisplatin are commonly used to treat BC [4,5]. However, recurrence and death cause by drug resistance are major challenges for treatment failure [6]. Consequently, identifying the BC development mechanism is significant. The discovery of exact molecular mechanisms will develop important targets for BC treatment.

Immunoglobulins (IgG) are only generated via plasma cells and B lymphocytes [7]. However, some studies have shown that IgG can be produced by several tumor cells, such as prostate cancer cells, papillary thyroid cancer cells and BC cells [8–10]. Previous researches have reported that IgG produced by tumor cells was conductive to proliferation, advanced tumor grade [10]. On the contrary, IgG silencing suppressed tumor cell proliferation in vivo and in vitro [11]. IgG possesses 2 light chains and 2 heavy chains [12]. Studies have discovered that IgG1 heavy chain (IGHG1) shows high expression within some human tumors, such as pancreatic carcinomas [13]. IGHG1 mRNA showed high expression within BC, and it predicted dismal survival of high-grade patients and estrogen receptor negativity [14]. However, the detailed molecular

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mechanism of IGHG1 affecting the progression of BC remains unclear. Based on this, we speculated that IGHG1 might have oncogenic activity in BC. This work focused on exploring the role and molecular mechanism of IGHG1in regulating BC progression.

Previous studies have been reported that IGHG1 can be involved in the progression of human tumors by regulating several cancer-related signaling pathways [7,15]. As we know, the protein kinase B (AKT) signaling pathway is one of the most important cancer-related signaling pathways, which activation is a key hallmark of cancer progression, including breast cancer [16]. However, the effect of IGHG1 on the AKT signaling activity remains rarely reported. In our preliminary study, we discovered that IGHG1 silencing suppressed the activity of the AKT signaling activity. Thus, it was speculated that IGHG1 might promote the progression of BC by activating the AKT signaling pathway. This study was then designed to verify this speculation by performing a series of experiments. Findings in the present work might lay significant theoretical basis for IGHG1 as a therapeutic target for BC.

Methods

Gene Expression Profiling Interactive Analysis (GEPIA) dataset

Herein, we investigated IGHG1 levels within cancer (n = 1085) and paired healthy tissue samples (n = 291) from BC cases collected from GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=IGHG1) [17,18].

Patients and clinical samples

Informed consents were provided by all cases. Our present study protocols gained approval from Ethics Committee of Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. This work was conducted following Declaration of Helsinki.

This work obtained BC and matched non-carcinoma tissue samples in 42 surgical cases at Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. After collection, the tissue samples were preserved under −80°C. Our cases had the initial diagnosis of BC and did not receive any treatment for tumor-associated diseases before. After surgery, patients were followed up for 2000 days with death as the endpoint during this period. The clinical characteristics of all patients were recorded. The IGHG1 expression levels in clinical samples were analyzed through real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blot.

Cell lines and culture

Breast epithelial cell line (MCF-10A) and BC cells (MDA-MB-231, MCF-7, MDA-MB-453 and *T*-47D) were purchased in American Type Culture Collection (ATCC, Rockville, MA, USA). This work cultivated BC cells within Dulbecco's modified Eagle medium (DMEM) (Solarbio, Beijing, China) that contained 1% penicillin-streptomycin (Solarbio) as well as 10% fetal bovine serum (FBS, Solarbio) and incubated under 37°C and 5% CO_2 conditions. MCF-10A cells were cultivated in DMEM/Ham's F-12 (50:50) (Biolab Technology, Beijing, China) containing 5% horse serum (Biolab Technology), 20 ng/mL epidermal growth factor (Beyotime, Shanghai, China), hydrocortisone (Rongbai Biotechnology, Shanghai, China), insulin (Beyotime), cholera toxin 100 ng/mL (Beyotime) and 1% penicillin-streptomycin at 37°C and 5% CO_2.

Transfection and SC79 treatment

In brief, this work collected *T*-47D and MDA-MB-231 cells to prepare cell suspension (1×10^6 cells/mL) using serum-free DMEM. Using a sterile pipette, each well of the 6-well plates was added with cell suspension (1 mL). After the design and synthesis of siRNA-targeting IGHG1 together with negative control (NC) (Genechem, Shanghai, China), Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was utilized to transfect them in cells. Cells were considered as siIGHG1 group and siNC group. The transfection operation was completed strictly following manufacturer's instructions. After 8 h incubation of cells at 37°C and 5% CO_2, we

further cultivated cells for 48 h using DMEM that contained 10% FBS under 37°C and 5% CO_2 conditions. Cells were collected for determining transfection efficiency using Western blot assay.

Furthermore, the transfected *T*-47D and MDA-MB-231 cells were further cultivated within DMEM that contained 10% FBS and 10 μM SC79 (an AKT agonist) [19], which were regarded as siNC + SC79 group and siIGHG1 + SC79 group, followed by culture under 37°C and 5% CO_2 conditions for subsequent studies.

Cell counting kit-8 (CCK-8) assay

MDA-MB-231 and *T*-47D cells of siIGHG1 group and siNC group were cultivated within DMEM (100 μL) that contained 10% FBS into 96-well plates (1×10^4 cells/well). Simultaneously, DMEM (100 μL) that contained 10% FBS as well as 100 μM SC79 was utilized to culture *T*-47D and MDA-MB-231 cells of siNC + SC79 group and siIGHG1 + SC79 group (1×10^4 cells/well). Following 72 h culture at 37°C and 5% CO_2, every well was added with CCK8 regent (10 μL) for 2 h cell incubation at 37°C. This work employed the microplate reader (Molecular Devices, Sunnyvale, CA, USA) for measuring absorbance (OD) values of all wells at 450 nm. We set five replicate wells for cells in all groups.

Clone formation experiment

T-47D and MDA-MB-231 cells (1×10^4/well) in all groups were inoculated into the 6-well plates. Cells of siIGHG1 group and siNC group were cultured using DMEM (1 mL) that contained 10% FBS, whereas those in siIGHG1 + SC79 and siNC + SC79 groups were cultivated within DMEM (1 mL) that contained 10% FBS as well as 100 μM SC79. Medium within all wells were changed at 3-day intervals. Cells were cultured under 37°C and 5% CO_2 conditions for 2 weeks, followed by 15 min fixation using 4% paraformaldehyde (PFA) and 10 min staining using 0.1% crystal violet. Finally, an inverted microscope (Olympus, Tokyo, Japan) was employed for counting colonies (containing over 50 cells) formed within 5 randomly selected fields of view (FOVs).

BrdU assay

T-47D and MDA-MB-231 cells (1×10^5/well) from diverse groups were cultivated within the 6-well plates under 37°C and 5% CO_2 conditions for a 48 h period. Thereafter, all wells were added with 10 μg/mL of Brdu (Solarbio, Beijing, China) to achieve 30 min cell incubation under ambient temperature. Cells were rinsed by phosphate buffered saline (PBS) thrice, and then 4% PFA fixation, 10 min 0.5% Triton X-100 treatment, and another 1 h incubation using 10% goat serum under ambient temperature were conducted. Cells were later incubated with rat anti-BrdU monoclonal primary antibody (1:200, ab6326, Abcam, Cambridge, MA, USA) for a 12 h period, followed by additional 2 h incubation using Alexa FluorR® 594 goat anti-rat IgG secondary antibody (H+L, Invitrogen, Carlsbad, CA, USA). DAPI was adopted for cell nuclear staining. At last, the fluorescence microscope (Olympus, Tokyo, Japan) was employed to determine BrdU positive cell quantity from 5 randomly selected FOVs.

Transwell experiment

This work dispersed *T*-47D and MDA-MB-231 cells in all groups into the serum-free DMEM (with or without 100 μM SC79). Cell content was set at 1×10^4 cells/mL. Firstly, Matrigel-coated top chamber was added with cell suspension (500 μL), whereas bottom chamber was added with DMEM that contained 10% FBS, followed by 12 h cell maintenance under 37°C and 5% CO_2 conditions. Those invading cells were experienced fixation using 4% PFA as well as 0.1% crystal violet staining. Thereafter, this work utilized an inverted microscope for counting invading cell number within the 5 randomly selected FOVs.

Flow cytometry detection

T-47D and MDA-MB-231 cells were harvested and washed by pre-cooled PBS twice. After being fixed by pre-cooled 70% ethyl alcohol, cells were placed in the dark for the propidium iodide (PI) (Beyotime) staining for 15 min at 4°C. The distribution of cell cycle was detected by utilizing the flow cytometer (Beckman Coulter, Brea, CA,

USA). The ModFit LT 3.0 software (Verity Software house, Topsham, ME) was applied for the analysis of data. Regarding the apoptosis detection, the Annexin V-FITC/PI kit (Zeye Biotechnology, Shanghai, China) was used according to the instructions. The BD FACSCanto II instrument (BD Biosciences, San Jose, USA) was recruited to detect the apoptosis and data was analyzed by the FlowJo software (Tree Star, Ashland, OR, USA).

Drug sensitivity test

This work cultivated *T*-47D and MDA-MB-231 cells (1×10^4/well) in siIGHG1 group and siNC group using DMEM (100 μL) containing 10% FBS and different concentrations of paclitaxel (or cisplatin) into 96-well plates. *T*-47D and MDA-MB-231 cells in siNC + SC79 group and siIGHG1 + SC79 group were cultivated within DMEM that contained 10% FBS, 100 μM SC79 and different concentrations of paclitaxel (or cisplatin). After 48 h of culture, all wells were added with 5 mg/mL MTT reagent (20 μL) for 4 h cell incubation at 37°C. Thereafter, all wells were added with dimethyl sulfoxide (150 μL, DMSO) to replace remaining solution. This work adopted the microplate reader (Molecular Devices, Sunnyvale, CA, USA) for measuring OD values at 450 nm. Besides, the IC50 values of diverse groups were determined through LOGIT method.

qRT-pcr

This work utilized Trizol reagent (Invitrogen, Carlsbad, CA, USA) to extract total RNAs in line with specific protocols. Thereafter, ND-1000 NanoDrop Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) was utilized to determine total RNA content. A total of 5 μg total RNAs samples were subjected to reverse transcription using the PCR kit (A3500, Promega, Madison, WI, USA). Thereafter, the 7500 Real-Time PCR System (Applied Biosystems) was employed for qRT-PCR under the conditions below, 5 min under 94°C; 30 s under 95°C as well as 30 s under 58°C for 38 cycles; and eventual 5 min at 72°C. The primers used were listed as follows: IGHG1, forward, 5"-GCAGCCGGAGAACAACTACA-3", reverse, 5"-TGGTTGT-GCAGAGCCTCATG-3". β-actin, forward, 5"-ATAGCACAGCCTGGATAGCAACGTAC-3", reverse, 5"-CACCTTCTACAATGAGCTGCGTGTG-3". IGHG1 mRNA expression was measured through $2^{-\Delta\Delta CT}$ approach with β-actin as the control [20].

Western blot assay

This work inoculated cells on ice for a 30 min period using RIPA lysis buffer that contained protease inhibitor (Solarbio, Beijing, China) to isolate total proteins. The BCA kit (Beyotime, Shanghai, China) was later applied in detecting total protein content. Later, proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transfer onto PVDF membranes. Later, this work incubated membranes using 5% defatted-milk for a 2 h period. Primary (4°C, 12 h) as well as secondary (ambient temperature, 2 h) antibodies were used sequentially to probe the PVDF membranes. All primary antibodies utilized in the present work included: IGHG1 (1:1000, sc -515,946, Santa Cruz Biotechnology, Santa Cruz, CA, USA), E-cadherin (1:1000, ab15148, Abcam, Cambridge, UK), Vimentin (1:1000, ab137321, Abcam), N-cadherin (1:1000, ab18203, Abcam), ERK (1:1000, ab196883, Abcam), p-ERK (1:500, ybP003, Ybio, Shanghai, China), MEK (1:1000, abs120553, Absin, Shanghai, China), p-MEK (1:500, HK7245, Hushi Pharmaceutical Technology, Shanghai, China), AKT (1:1000, ab126811, Abcam), p-AKT (1:500, ab8805, Abcam), p-AKT (phosphorylation sites of Thr308) (1:500, AA331, Beyotime, Shanghai, China), p-AKT (phosphorylation sites of Ser473) (1:500, AA329, Beyotime, Shanghai, China) and β-actin (1:1000, ab8227, Abcam). The horseradish-peroxidase-conjugated secondary antibody (1:2000, ab205718) was purchased from Abcam (Cambridge, UK). β-actin served as the control for normalizing protein levels.

Statistical analysis

Data were displayed as mean ± standard deviation. SPSS19.0 was employed for statistical analysis. Kaplan-Meier survival analysis was performed to explore the effect of IGHG1 on the survival of BC

patients. Significance of differences was explored by two-tailed student's t-test between two groups. Tukey's *post hoc* test and one-way ANOVA were conducted to assess difference at least in three groups. Thresh hold was set as $P < 0.05$. Each assay was conducted in triplicate.

Results

IGHG1 showed overexpression within BC cells and tissues

IGHG1 levels within BC (n = 1085) as well as paired healthy tissues (n = 291) were acquired from GEPIA dataset. The result showed more IGHG1 expression within BC samples compared with matched non-carcinoma counterparts ($P < 0.05$) (Figure 1(a)). Consistently, tumor tissues from 42 BC patients exhibited markedly increased IGHG1 level relative to non-carcinoma samples ($P < 0.01$) (Figure 1(b)). Especially, the expression of IGHG1 in different molecular subtypes of BC patients was explored, including Luminal A, Luminal B, HER2 and Basal. In the four different molecular subtypes of BC patients, the expression of IGHG1 mRNA was higher in the tumor tissues than that in the corresponding adjacent normal tissues ($P < 0.05$or $P < 0.01$) (Figure 1(c)). Kaplan–Meier survival curve analysis showed that, in comparison to BC patients with low IGHG1 expression, those with high IGHG1 expression had lower 2000-day survival (Figure 1(d)). Western blot was performed to research the expression of IGHG1 protein in the clinical tissues of four BC cases. As a result, higher expression of IGHG1 protein was occurred in the tumor tissues when relative to the adjacent normal tissues ($P < 0.01$) (Figure 1(e)). This research explored the relation between IGHG1 expression and BC clinical features. According to Table 1, IGHG1 upregulation was linked with advanced clinical stage, lymph node metastasis (LNM) and positive estrogen receptor ($P < 0.01$). In addition, BC cells were cultured to verify the IGHG1 expression. According to Figure 1(f), aberrantly higher IGHG1 level was detected within BC cells (MDA-MB-231, MCF-7, MDA-MB-453 and *T*-47D) than that within breast epithelial cells (MCF-10A) ($P < 0.01$). These discoveries indicated that IGHG1 was abnormally overexpressed within BC cells and tissues.

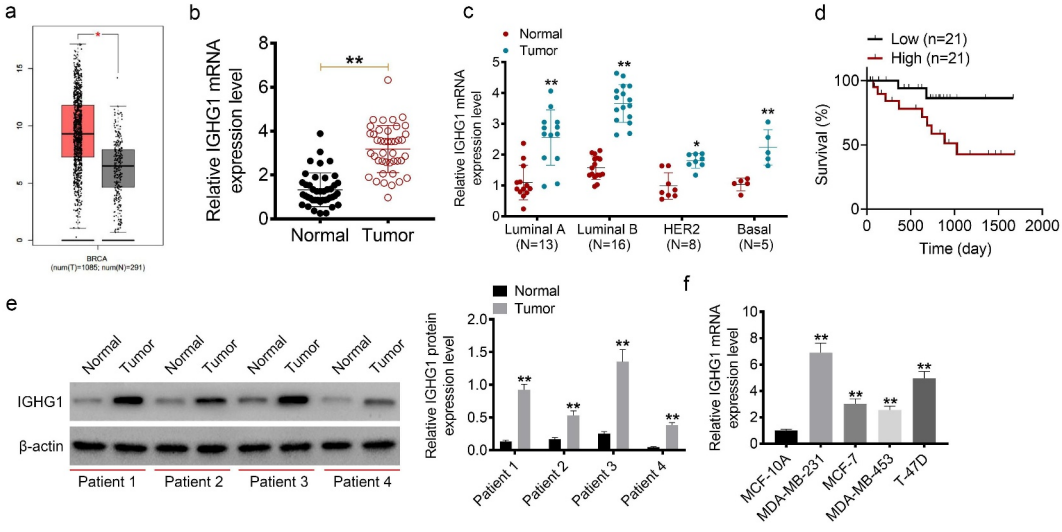

Figure 1. IGHG1 shows overexpression within BC cells and tissues.

Notes: (a) Data from GEPIA dataset indicated that IGHG1 was overexpressed in BC tissues compared with paired healthy samples. (b) qRT-PCR of 42 BC patients indicated that IGHG1 was overexpressed within cancer samples relative to paired healthy samples. (c) qRT-PCR indicated that IGHG1 mRNA was highly expressed within cancer samples of BC patients with different molecular subtypes compared to paired healthy samples. (d) Kaplan–Meier survival analysis revealed that high IGHG1 expression was associated with poor survival of BC patients. (e) Western blot displayed the higher expressed IGHG1 protein within cancer samples of BC patients relative to paired healthy samples. (f) qRT-PCR revealed IGHG1 overexpression within BC cells (MCF-7, MDA-MB-231, T-47D, MDA-MB -453) than that in breast epithelial cells. * $P < 0.05$. ** $P < 0.01$.

Table 1. The relationship between IGHG1 expression and BC patients clinical characteristics.

Characteristics	Number of patients	IGHG1 Low expression (< median)	IGHG1 High expression (≥ median)	*P value*
Number	42	21	21	
Ages(years)				0.525
<60	16	9	7	
≥60	26	12	14	
Tumor size				0.107
<2 cm	15	10	5	
≥2 cm	27	11	16	
Lymph node metastasis				*0.004***
Present	27	9	18	
None	25	12	3	
Distant metastasis				0.079
Yes	11	3	8	
No	31	18	13	
Clinical stage				*0.002***
I-II	22	16	6	
III-IV	20	5	15	
Progesterone receptor				0.259
Positive	33	15	18	
Negative	9	6	3	
Oestrogen receptor				*0.006***
Positive	30	11	19	
Negative	12	10	2	

IGHG1 silencing attenuated the proliferation and enhanced the apoptosis of BC cells

This work transfected the *T*-47D and MDA-MB -231 cells for evaluating how IGHG1 affected the development of BC. The transfection efficiency of *T*-47D and MDA-MB-231 cells was detected through Western blot analysis. In comparison with siNC group, *T*-47D and MDA-MB-231 cells in siIGHG1 group presented distinctly lower IGHG1 protein expression ($P < 0.01$) (Figure 2(a)). We conducted CCK-8 assay to measure cell viability. As a result, siIGHG1 group had much lower T-47D and MDA-MB-231 cell viability compared with siNC group ($P < 0.01$) (Figure 2(b)). Clone formation experiment and BrdU assay were responsible for the detection of T-47D and MDA-MB-231 cells proliferation (Figure 2(c,d)). siIGHG1 group had remarkably lower colony number and BrdU positive cell number within T-47D and MDA-MB-231 cells in comparison with siNC group ($P < 0.01$). Flow cytometry was utilized to investigate the effect of IGHG1 on the apoptosis and cell cycle of T-47D and MDA-MB-231 cells. IGHG1 silencing significantly enhanced the apoptosis of T-47D and MDA-MB-231 cells, as manifested by the higher cell apoptosis percentage in siIGHG1 group than siNC group ($P < 0.01$) (Figure 2(e)). However, IGHG1 silencing did not obviously affect the cell cycle when relative to siNC group (Figure 2(f)). Based on the above findings, IGHG1 silencing attenuated the proliferation and intensified the apoptosis of BC cells.

IGHG1 silencing inhibited BC cells invasion and epithelial-mesenchymal transition (EMT)

The function of IGHG1 on BC cell invasion and EMT was explored. Transwell experiment showed that siIGHG1 group had dramatically lower invasion cell number of *T*-47D and MDA-MB-231 cells relative to siNC group ($P < 0.01$) (Figure 3(a)). Based on Western blot assay, compared with siNC group, siIGHG1 group had significantly increased E-cadherin expression, but reduced Vimentin and N-cadherin expression within *T*-47D and MDA-MB-231 cells ($P < 0.01$) (Figure 3(b)). Therefore, IGHG1 silencing inhibited BC cells invasion and EMT.

IGHG1 knockdown promoted paclitaxel and cisplatin sensitivity of BC cells

The effect of IGHG1 on paclitaxel and cisplatin sensitivity of BC cells was investigated. In terms of paclitaxel, siIGHG1 group had an IC50 value of

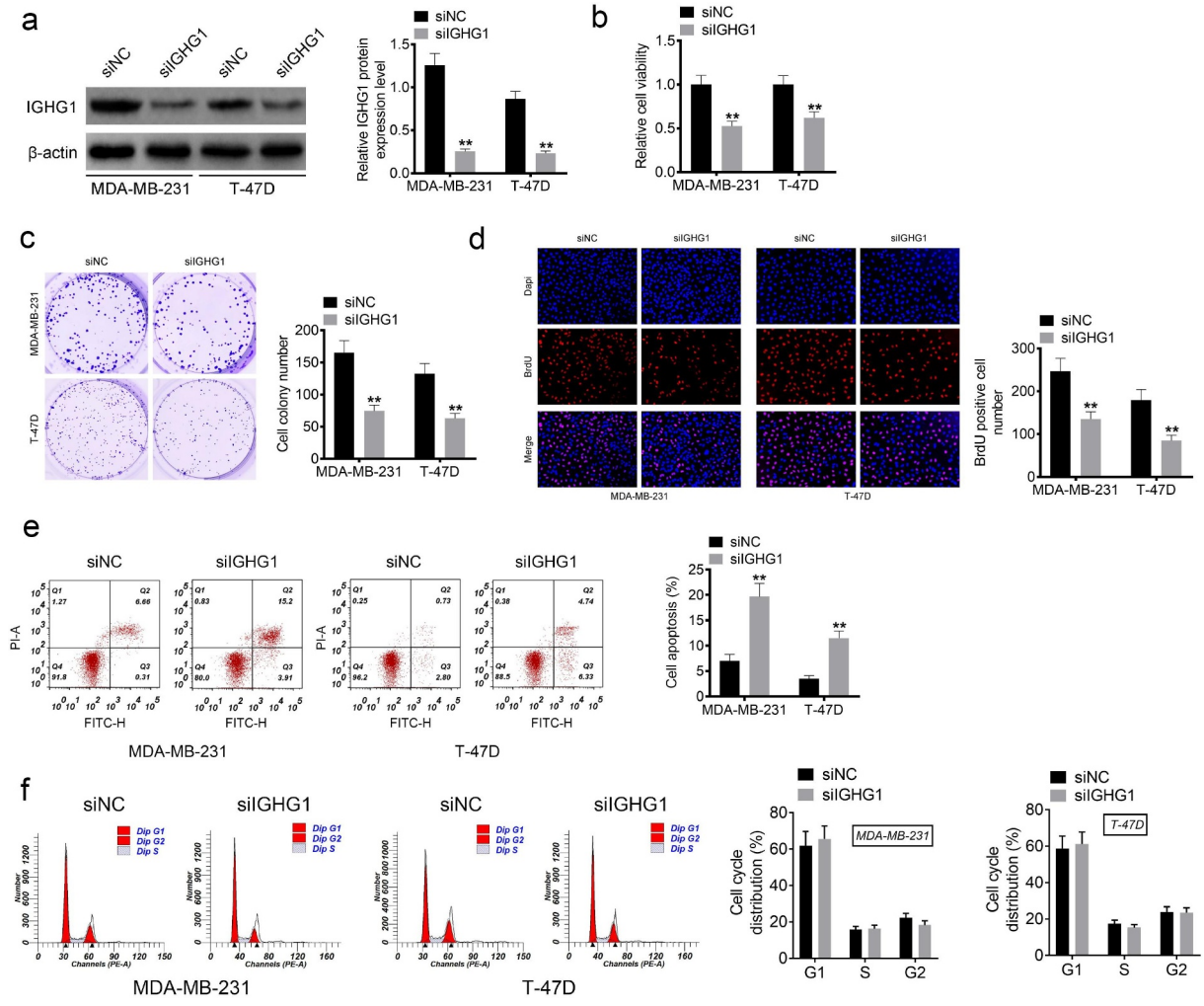

Figure 2. IGHG1 silencing attenuated BC cells proliferation.

Notes: (a) IGHG1 protein expression was successfully silenced by transfection. (b) CCK-8 assay indicated that IGHG1 silencing obviously attenuated BC cells viability. (c) Clone formation experiment revealed that IGHG1 silencing markedly reduced T-47D and MDA-MB-231 cell proliferation. (d) BrdU assay indicated that IGHG1 silencing significantly impaired T-47D and MDA-MB-231 cell proliferation. (e) Flow cytometry implied that IGHG1 silencing significantly enhanced the apoptosis of T-47D and MDA-MB-231 cells. (f) Flow cytometry showed that IGHG1 silencing did not obviously affect the cell cycle of T-47D and MDA-MB-231 cells. ** $P < 0.01$.

6.969 μM for MDA-MB-231 cells, markedly decreased in comparison with siNC group (23.08 μM). Similarly, *T*-47D cells of siIGHG1 group (29.54 μM) had much lower IC50 than that of siNC group (64.68 μM) (Figure 4(a)). However, regarding the sensitivity to cisplatin, the difference in IC50 was not obvious in MDA-MB-231 cells between siNC group (22.31 μM) and siIGHG1 group (21.58 μM). Interestingly, *T*-47D cells of siIGHG1 group (175.80 μM) exhibited obviously lower IC50 than that of siNC group (212.80 μM) (Figure 4(b)). Thus, IGHG1 silencing enhanced *T*-47D cell paclitaxel and cisplatin sensitivity, and MDA-MB-231 cell paclitaxel sensitivity.

IGHG1 silencing suppressed the MEK, AKT and ERK pathway activity

Phosphorylation levels of MEK, ERK and AKT within BC cells were determined through Western blot assay. The result was shown in Figure 5. Notably, relative to siNC group, *T*-47D and MDA-MB-231 cells of siIGHG1 group exhibited dramatically lower p-MEK/MEK, p-AKT/AKT and p-ERK/ERK proteins expression ($P < 0.01$) (Figure 5(a)). Moreover, the effect of IGHG on the AKT activity at the phosphorylation sites of Thr308 and Ser473 was explored. The results were presented in Figure 5(b). In comparison to MDA-

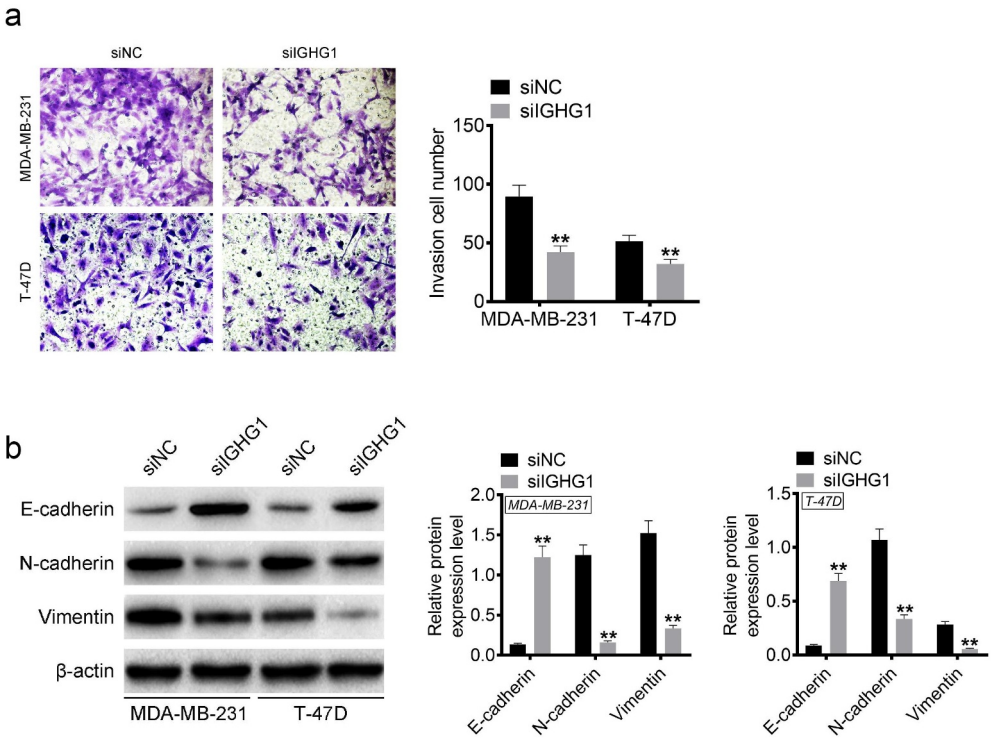

Figure 3. IGHG1 silencing suppresses BC cell invasion and EMT.

Notes: (a) Transwell experiment revealed that IGHG1 silencing inhibited BC cells invasion. (b) Western blot suggested that IGHG1 silencing inhibited the EMT in BC cells. ** $P < 0.01$.

MB-231 cells of siNC group, the p-AKT level at the phosphorylation sites of Thr308 and Ser473 was decreased in siIGHG1 group and increased in siNC + SC79 group ($P < 0.01$). Relative to siIGHG1 group, MDA-MB-231 cells of siIGHG1 + SC79 group had much increased p-AKT level at the phosphorylation sites of Thr308 and Ser473 ($P < 0.01$). Thus, IGHG1 silencing suppressed MEK, ERK, and AKT pathway activation.

AKT agonist partially abolished IGHG1 silencing's inhibition on BC progression

SC79 was an AKT agonist, which was used to treat the transfected *T*-47D and MDA-MB-231 cells. Thereafter, the mechanism of IGHG1 affecting BC progression was studied. CCK-8 assay showed much lower viability in siIGHG1 group and higher viability in siNC + SC79 group when compared with *T*-47D and MDA-MB-231 cells in siNC group ($P < 0.01$). Relative to siIGHG1 group, *T*-47D and MDA-MB-231 cells of siIGHG1 + SC79 group had much increased viability ($P < 0.01$) (Figure 6(a)). Colony forming and BrdU assays were performed to detect the growth of the above 2 cell lines. Consequently, these 2 cell lines in siIGHG1 group showed significantly decreased colony number and BrdU-positive cell quantity compared with siNC group ($P < 0.01$). On the contrary, siNC + SC79 group had markedly higher colony number and BrdU positive cell number within the above 2 cell lines compared with siNC group ($P < 0.01$). Similar to siIGHG1 group, siIGHG1 + SC79 group presented remarkably increased colony and BrdU-positive cell quantities within T-47D and MDA-MB-231 cells ($P < 0.01$) (Figure 6(b,c)).

Transwell experiment displayed less invasion cells of the above two cell lines of siIGHG1 group relative to siNC group ($P < 0.01$). Oppositely, siNC + SC79 group exhibited the increased invasion capacity of the above 2 cell lines compared with siNC group ($P < 0.01$). When relative to siIGHG1 group, invasion of the above 2 cell lines in siIGHG1 + SC79 group were much increased ($P < 0.01$) (Figure 6(d)). Regarded as EMT, siIGHG1 group displayed markedly

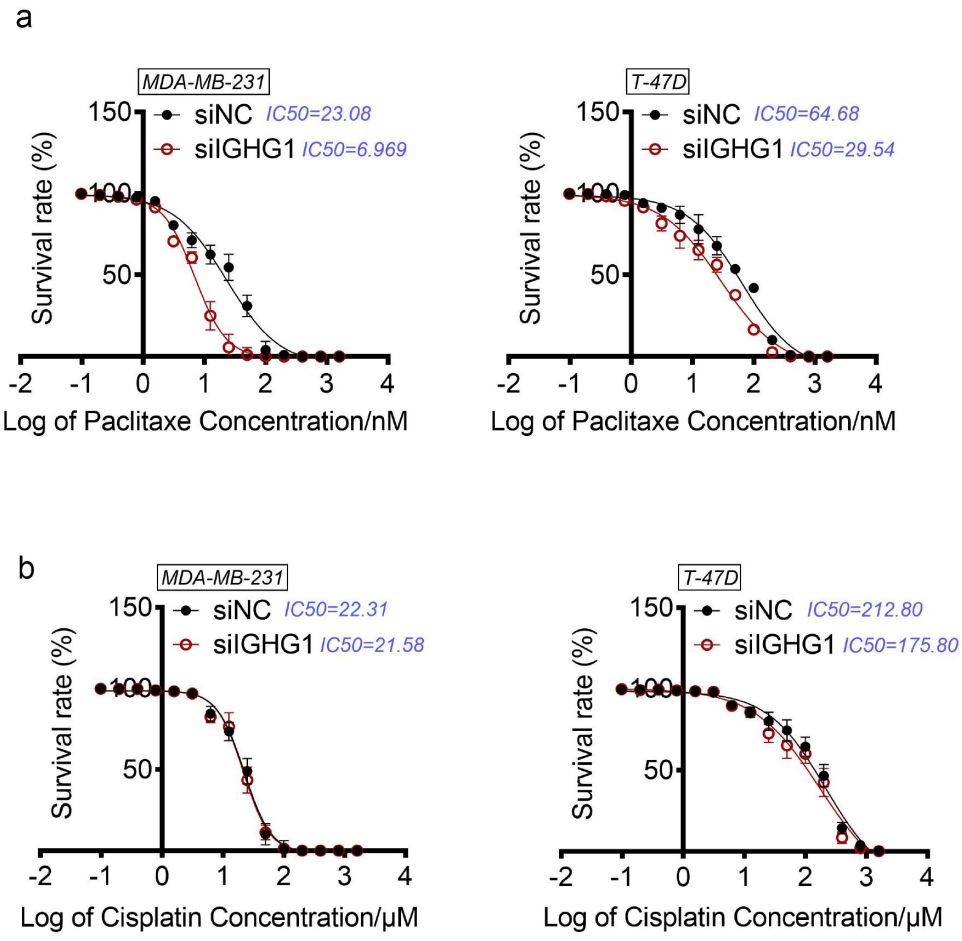

Figure 4. IGHG1 silencing enhanced paclitaxel and cisplatin sensitivity in BC cells.

Notes: (a) IGHG1 silencing enhanced paclitaxel sensitivity in T-47D and MDA-MB-231 cells. (b) IGHG1 silencing promoted cisplatin sensitivity in T-47D cells.

increased E-cadherin expression but reduced Vimentin and N-cadherin expression within *T*-47D and MDA-MB-231 cells relative to siNC group ($P < 0.01$). Conversely, relative to siNC group, siNC + SC79 group had markedly decreased E-cadherin expression but increased Vimentin and N-cadherin expression ($P < 0.01$). Relative to siIGHG1 group, siIGHG1 + SC79 group showed evidently decreased E-cadherin expression but increased Vimentin and N-cadherin expression within *T*-47D and MDA-MB-231 cells ($P < 0.01$) (Figure 6(e)).

According to paclitaxel sensitivity studies, siIGHG1 group had the IC50 values of 7.51 and 25.01 μM for MDA-MB-231 and *T*-47D cells, separately, which remarkably decreased relative to siNC group (21.96 and 63.14 μM for MDA-MB-231 and *T*-47D cells, separately). Simultaneously, much higher IC50 was found in siNC + SC79 group (53.47 and 184.8 μM for MDA-MB-231 and *T*-47D cells, separately) when compared with siNC group. In comparison with siIGHG1 group, the IC50 was obviously increased in siIGHG1 + SC79 group (38.58 and 101.6 μM for MDA-MB-231 and T-47D cells, separately). In terms of cisplatin sensitivity, the difference in IC50 was not obvious in MDA-MB-231 cells between siNC group (22.08 μM) and siIGHG1 group (21.62 μM). However, MDA-MB-231 cells of siNC + SC79 group (38.63 μM) had much higher IC50 than siNC group. Meanwhile, MDA-MB-231 cells of siIGHG1 + SC79 group (30.95 μM) showed obviously higher IC50 than siIGHG1 group. For T-47D cells, markedly lower IC50 in siIGHG1 group (147.40 μM) and higher IC50 in siNC + SC79 group (230.40 μM) was found when relative to siNC group (176.60 μM). In comparison with siIGHG1 group, the IC50 of T-47D cells to

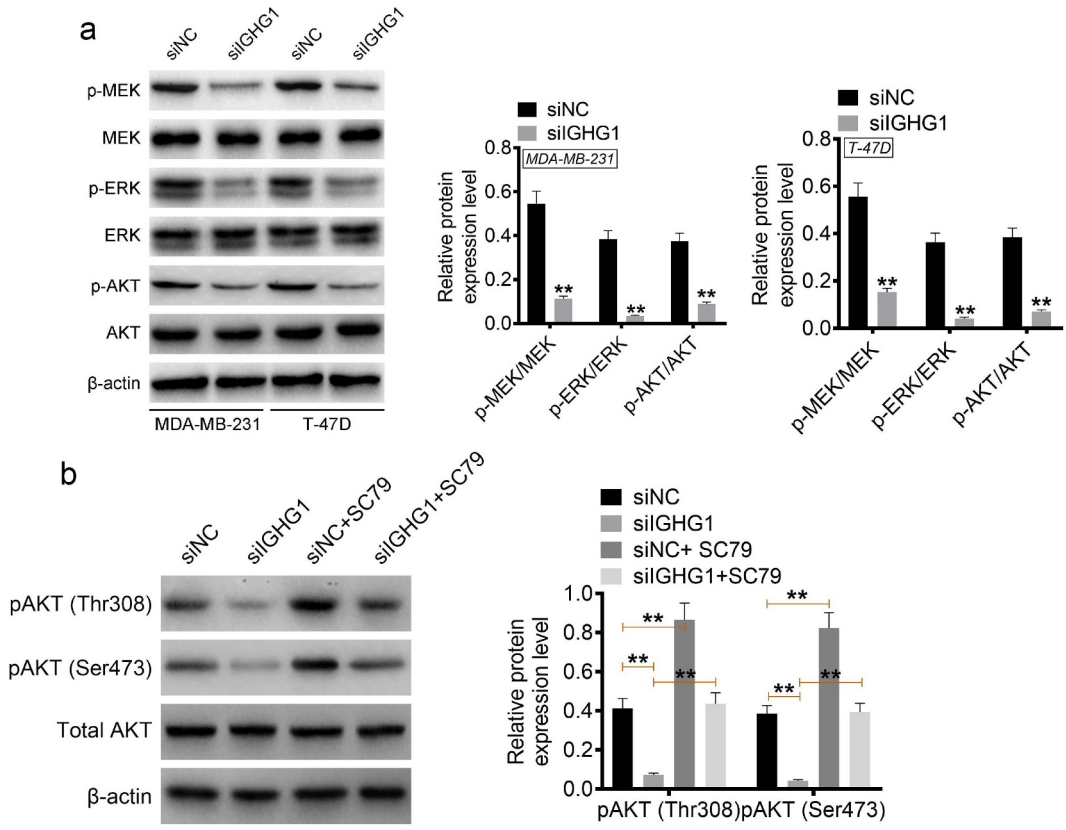

Figure 5. IGHG1 silencing suppressed the activity of the MEK, ERK and AKT pathways.

Notes: (a) Western blot revealed that IGHG1 silencing inhibited the activity of the MEK, ERK, and AKT pathways in MDA-MB-231 and T-47D and cells. (b) IGHG1 silencing reduced the AKT pathway activity at the phosphorylation sites of Thr308 and Ser473 in MDA-MB-231 cells. ** $P < 0.01$.

cisplatin was significantly increased (203.00 μM) (Figure 6(f)).

The activity of AKT pathway was assessed by Western blot. The results were presented in Figure 6(g). Notably, less p-AKT/AKT expression in *T*-47D and MDA-MB-231 cells in siIGHG1 group and more p-AKT/AKT expression in siNC + SC79 group were occurred relative to siNC group ($P < 0.01$). When matched with siIGHG1 group, the p-AKT/AKT expression in the above 2 cell lines in siIGHG1 + SC79 group significantly elevated ($P < 0.01$). Based on these findings, AKT agonist partly abolished IGHG1 knockdown's inhibition on BC development.

Discussion

This study investigated that IGHG1 facilitated the malignant development of BC. High expressed IGHG1 in BC indicated poor prognosis of patients. In vitro studies revealed that IGHG1 silencing reduced the viability, proliferation, invasion, EMT, but enhanced the apoptosis of BC cells. Recently, emerging evidence showed that IGHG1 was associated with malignant progression of human tumors. For instance, Qian et al [21]. investigated that IGHG1 expression showed positive relation to LNM in ovarian cancer. Ovarian cancer specimens expressed much higher IGHG1 than that in paired normal specimens. Simultaneously, IGHG1 facilitated ovarian cancer cells invasion and Vimentin and N-cadherin expression, but attenuated E-cadherin level. Moreover, IGHG1 showed overexpression within tongue squamous cell carcinoma tissues, which was closely linked with late clinical stage and LNM. The up-regulated IGHG1 expression augmented the proliferation and clone formation of tongue squamous cell carcinoma cells [22]. The up-regulated IGHG1 was also discovered in prostate cancer, while the inhibition of IGHG1 expression reduced growth and viability of tumor cells

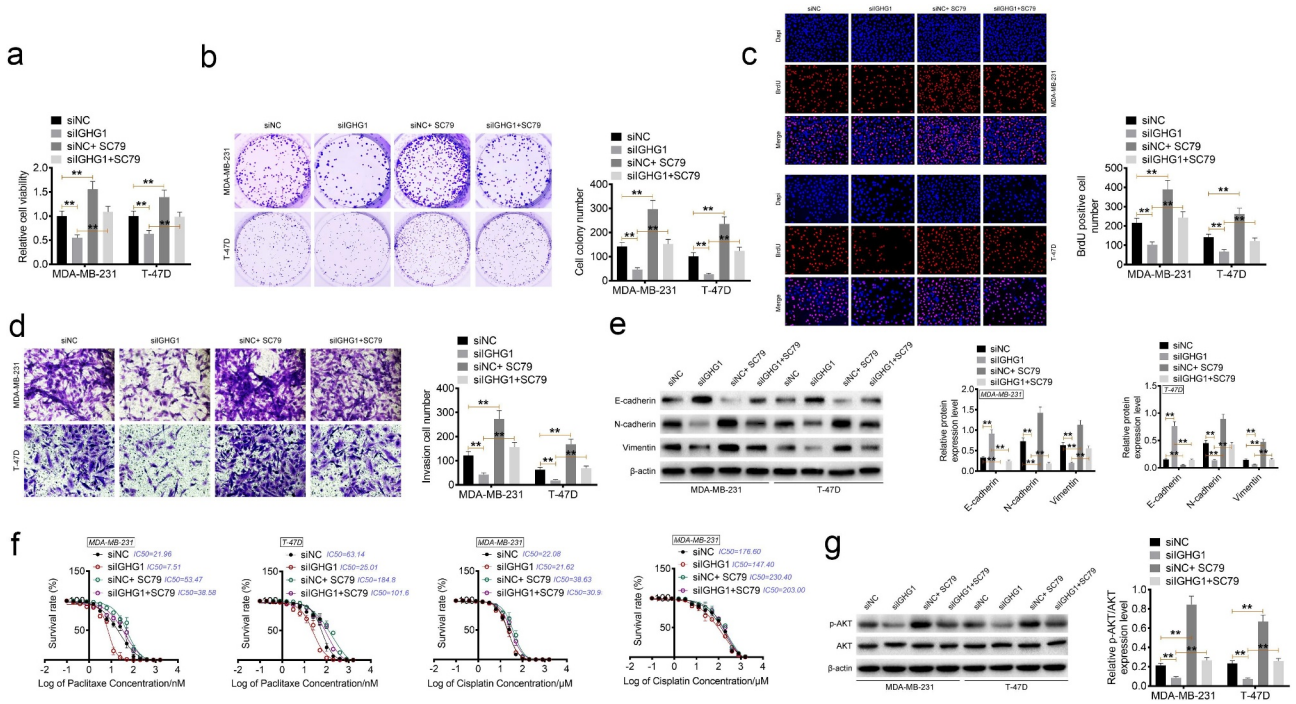

Figure 6. AKT agonist partly abolishes IGHG1 silencing's inhibition on BC progression.

Notes: (a) AKT agonist partially reversed IGHG1 silencing's impact on BC cell viability. (b) AKT agonist partially abolished IGHG1 silencing's inhibition on BC cell growth. (c) AKT agonist partially abolished IGHG1 silencing's impact on BC cell growth. (d) AKT agonist partially reversed IGHG1 silencing's inhibition on BC cell invasion. (e) AKT agonist partially reversed IGHG1 silencing's inhibition on BC cell EMT. (f) AKT agonist partly abolished IGHG1 silencing's promotion on T-47D cell paclitaxel and cisplatin sensitivity, and on MDA-MB-231 cell paclitaxel sensitivity. (g) AKT agonist partly abolished IGHG1 silencing's inhibition on AKT activity in BC cells. ** $P < 0.01$.

[7,8]. Results from this study were consistent with the above previous findings.

Previous study has reported that IGHG1 can promote the EMT in human tumor [21]. Metastasis is a major driver for death among cancer cases, while EMT is tightly associated with cancer cell metastasis and infiltration [23,24]. E-cadherin, Vimentin, and N-cadherin have been recognized to be the conventional canonical markers of EMT [25]. During EMT, the epithelial phenotype loss and the mesenchymal phenotype gain occurs, accompanying with the decreased E-cadherin expression and the enhanced Vimentin and N-cadherin expression [26]. E-cadherin accounts for a calcium-dependent molecule related to intercellular adhesion. The reduced E-cadherin protein during EMT will result in the improved cell motility and lost cell adhesion [23]. N-cadherin can reduce the adhesion among cell-to-cell, leading to aggressive phenotype as well as the ability to escape from primary tumor site to secondary site [27]. Vimentin can maintain the cellular integrity and promoted tumor cells resistance to stress [28]. The present work discovered that IGHG1 silencing elevated E-cadherin level and declined Vimentin and N-cadherin levels. Thus, IGHG1 silencing could inhibit BC cell invasion by suppressing EMT.

Drug resistance represents an important factor leading to treatment failure for cancers [29]. Paclitaxel and cisplatin are widely adopted for treating solid tumors, including BC. However, the resistance to paclitaxel and cisplatin is clinically major challenges in treating BC [30,31]. The present work firstly explored how IGHG1 affected paclitaxel/cisplatin sensitivity of BC cells. The results indicated that IGHG1 silencing enhanced paclitaxel/cisplatin sensitivity of *T*-47D cells and paclitaxel sensitivity of MDA-MB-231 cells. Thus, IGHG1 might be a promising candidate to alleviate BC cell resistance to paclitaxel and cisplatin. As previously reported, the suppression of IGHG1 could induce the apoptosis of tumor cells [7,8]. Similarly, this study implied that IGHG1

silencing augmented the apoptosis of BC cells. However, IGHG1 had negligible effect on the cell cycle of BC cells. Thus, triggering apoptosis was an important way for IGHG1 inhibition to promote the malignant development of BC.

Importantly, this article demonstrated that IGHG1 silencing declined the phosphorylation of MEK and ERK. In BC, the increased activity of the MEK/ERK pathway is identified to be associated with tumor metastasis [32,33]. Previous study has been found that the down-regulation of IGHG1 reduces human tumor progression by inhibiting the MEK/ERK pathway activity [7]. Similarly, our data illustrated that IGHG1 silencing inhibited the activity of the MEK/ERK pathway. The AKT pathway activation participates in the progression of human tumors containing BC [34,35]. This article researched that IGHG1 silencing suppressed the activity of the AKT pathway. Further research illustrated that AKT agonist partially abolished the inhibition of IGHG1 knockdown on BC development. Therefore, it might be that IGHG1 promoted BC malignant development by activating the AKT pathway. Previously study has been implied that the up-regulation of IGHG1 could intensify the phosphorylation of AKT to enhance the malignancy of gastric cancer [36]. This is similar to our results.

Of course, there are some limitations in our study. First, it should use the agonist of MEK and ERK pathways to treat the transfected BC cells, for investigating the effect of MEK and ERK pathways activation on the function of IGHG1 silencing on BC cell malignant phenotype. Secondly, it will be more meaningful to verify the effect of IGHG1 on the in vivo growth and metastasis of BC cells by animal model. Finally, the exact mechanism of IGHG1 in promoting the activity of the AKT pathway requires future exploration. However, no more experiments could be performed to explore the above three issues because of our limited laboratory conditions. These issues will be our future research focus.

This work identified IGHG1 overexpression within BC cases, which indicated poor prognosis of patients. In vitro studies indicated that IGHG1 silencing suppressed the malignant development of BC through inactivating the AKT pathway. The findings in this paper suggested that IGHG1 was a potential biomarker used to diagnose and treat BC.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Authors contributions

YMJ and LQ: conception, design, and analysis of data, performed the data analyses and wrote the manuscript; WQB: contributed to the conception of the study; wrote the manuscript; MHL, FC and HCN: contributed significantly to analysis and manuscript preparation; wrote the manuscript; BZ: performed the data analyses and wrote the manuscript; wrote the manuscript; All authors have read and approved the manuscript.

Ethical approval and consent to participate

Informed consents were provided by all cases. Our present study protocols gained approval from Ethics Committee of Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. This work was conducted following Declaration of Helsinki.

Data availability material

All data in the manuscript is available through the responsible corresponding author.

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