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Original Article

SIKs suppress tumor function and regulate drug resistance in breast cancer

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Abstract: Salt-inducible kinases (SIKs), belonging to an AMP-activated kinase (AMPK) family, have an evolving role in tumourigenesis and metastasis in many solid tumours. However, the function of SIKs in breast cancer is not fully established. Here, we systematically elucidated the function of SIK family members in breast cancer. In clinical cohort of breast cancer, the expression of SIK1, SIK2 and SIK3 increased expression of SIKs was associated with good clinical outcome in breast cancer cohort. *In vitro*, reduced expression of SIK2 and SIK3, by way of knockdown increased the proliferation of breast cancer cells. However, SIK2 and SIK3 had contrasting effects on adhesion in breast cancer cells. Knockdown of SIK2 only enhanced the adhesion of triple negative breast cancer cell, while knockdown of SIK3 can decrease the adhesion of both MDA-MB-231 and MCF-7 cells. Interestingly, knockdown of SIK1 and SIK3 was seen to increase the invasion of MDA-MB-231 cells. Furthermore, reduced SIKs, even triple knockdown of SIK1, SIK2 and SIK3 rendered the breast cancer cells to confer chemoresistance to paclitaxel and cisplatin. Collectively, the study reports that SIKs are actively involved in regulating the aggressive functions of breast cancer cells and influence the clinical course of the patients with breast cancer that they molecules are potential prognostic factors and chemotherapy biomarkers.

Keywords: Salt-inducible kinase, breast cancer, metastasis, drug resistance

Introduction

Breast cancer is the most frequently diagnosed cancer in the whole world. Over 270,000 new cancer cases are estimated to occur in the United States according to cancer statistics 2020 [1]. Mortality has been decreasing since the 1990s due to advances in the screening, diagnosis and treatment of breast cancer, but 20% patients still die from breast cancer [2]. Metastasis and drug resistance are major clinical problems for physicians when fighting cancer-related death. Thus, discovery of new relevant biomarker for prognosis and study of mechanisms in tumorigenesis and drug resistance is urgent need. Furthermore, understanding of the process of the cancer metastasis and the mechanism of cancer drug resistance may identify potential therapeutic targets, and improving clinical outcome.

Salt-inducible kinases (SIKs), first discovered in adrenal glands in high salt diet fed rats, are serine threonine protein kinase belonging to AMPK family. They comprise of SIK1, SIK2 and SIK3. SIKs have different functions in salt homeostasis, steroidogenesis and adipogenesis [3]. SIK1 is a regulator in maintaining intracellular ion concentration by targeting membrane transporters such as Na^+-K^+-ATPase [4], and also has play roles in modulating adrenocortical function with the stimulation of adrenocorticotrophic hormone (ACTH) or ion concentration. SIK2 has functions in modulating insulin regulation of adipose tissue [5], while SIK3 regulates gluconeogenic programs in the hepatocytes [6].

Previous studies have reported that SIKs play different roles in oncogenesis and metastasis in multiple solid cancers, such as prostate cancer, hepatocellular carcinoma (HCC) and ovari-

an cancer [7-11]. The function of SIKs in ovarian cancer has been particularly studied. Generally, SIK1 has been associated with a tumor suppressor function, which can inhibit cancer metastasis through suppressing the epithelial-mesenchymal transition (EMT) process. In ovarian cancer, SIK1 suppresses growth and aggressiveness through upregulation of liver kinase B1 (LKB1), indicating that the LKB1-SIK1 signal pathway is considered potential therapeutic target [12]. Similarly, SIK1 suppresses tumor growth and metastasis in hepatocellular carcinoma by inhibiting the EMT via repression of β-catenin transcriptional activity. It also phosphorylates the silences mediator activity of thyroid hormone receptor (SMRT) and retinoic acid and inhibit Twist1, which in turn negatively regulates SIK1 via a feedback loop [13].

In contrast to SIK1, high level of SIK2 expression is associated with poor prognosis in those patients with high-grade serous ovarian cancer [8]. It is found to be overexpressed in adipocyte-rich metastasis deposit of ovarian cancer compared with primary tumors [14]. SIK2 promotes tumor growth and metastasis of ovarian cancer by modulating glucolipid metabolism. SIK2 not only has a function in reprogramming glucose metabolism through PI3K/AKT/HIF-1α pathway, it also plays dual effects in enhancing AMRK-induced phosphorylation of acetyl-Coa carboxylase, which indicates the essential role of SIK2 in adipocyte-induced signaling cascades for ovarian cancer [14, 15]. Another study has shown that SIK2 upregulates the expression of sterol regulatory element binding protein 1c (SREBP1c) and SREBP2 pathway to enhance cholesterol and fatty acid synthesis, which promotes ovarian cancer growth [16]. Moreover, SIK2 is a critical player in initiating mitosis and regulates localization of centrosome linker protein, C-Nap1. Depletion of SIK2 results in cytoplasm residence and promotes the loss of centriole cohesion [8].

SIK3 has been suggested as having a possible oncogenic role by promoting cell cycle progression. In ovarian cancer, overexpression of SIK3 markedly promotes cell cycle progression and cancer cell growth [17]. It is a tumor-associated antigen with higher specificity to CA125, which is highly expressed in ovarian cancer compared with benign tumors, indicating SIK3 can be a potential diagnostic biomarker [17]. Previous studies show high expression of SIK3 is essential in aerobic glycolysis in breast cancer cell, therefore SIK3 is associated with tumor progression and tend to be a therapeutic target. Altogether, the molecular mechanism of SIKs in tumorigenesis is elusive and controversial [18].

As for the association of SIKs and drug resistance, repression of SIKs can sensitize cancer cells to drug resistance in some solid tumors [8, 9, 19, 20]. The delayed mitosis due to block of SIKs may be one of the mechanisms by which cytotoxic drug sensitization in tumors [8, 20]. Given that SIKs have functions in tumorigenesis and drug sensitivity, SIKs may be valuable therapeutic targets in solid tumors.

Compared with knowledge in HCC and ovarian cancer, the functions and roles of SIKs in breast cancer are rather less well defined. Previous study shows SIKs probably have different and sometime contrasting roles in breast cancer compared with that seen in say ovarian and HCC cancers. SIK1 is linked to p53-independent *anoikis* and suppresses metastasis in breast cancer [10]. The function of SIK2 in breast cancer is highly controversial. SIK2 inhibits breast tumorigenesis through PI3K/Akt and Ras/ERK signalling pathway blocking EMT especially in basal breast cancer in one study [21]. In contrast, SIK2 is reported to be essential for survival in triple-negative breast cancer in mechanism of autophagic flux [22]. For SIK3, it is a regulator in G0/G1-phase release mediated cell proliferation [23]. Other study reports SIK3 can regulate expression of store operated calcium entry proteins, and inhibition of SIK3 can result in P-glycoprotein mediated chemotherapy resistance [24].

Thus, the functions of SIK1, SIK2 and SIK3 in cancer have been demonstrated separately over different cancer types. It is vital to explore the combined function of SIKs and to investigate the connection between them. Furthermore, correlation of SIKs and drug sensitivity in breast cancer has seldom been reported. In our current study, we systematically elucidated the correlation of expression of all the members of the SIK family expression with tumor function, and resistance to chemotherapeutic drugs in breast cancer. Here, we report that the increased expression of SIKs is associated with good prognosis in breast cancer. *In vitro*,

Table 1. Primers used in study

PCR Primer name	Sense primers (5'-3')	Antisense primer (5'-3')
SIK1	CTGGAGGAGGTGCTAGAG	TGAGGTCACTCAGTGCAA
SIK2	AGTGAGCTTCAGAGAGGG	TAGGCCTGCAGTTGAGAC
SIK3	TCAGGCAGCCCAGTAATA	AGTGGAGGGACTTTCAGG
GAPDH	GGCTGCTTTTAACTCTGGTA	GACTGTGGTCATGAGTCCTT
QPCR Primer name	Sense primers (5'-3')	Antisense primer (5'-3')
SIK1	CTGGAGGAGGTGCTAGAG	ACTGAACCTGACCGTACAGATCACAAACGGGGCAG
SIK2	AGTGAGCTTCAGAGAGGG	ACTGAACCTGACCGTACATCCGGTCCTATTTGTTCA
SIK3	TCAGGCAGCCCAGTAATA	ACTGAACCTGACCGTACAGTTCAGTCCCAGAAACCC
GAPDH	AAGGTCATCCATGACAACTT	ACTGAACCTGACCGTACAGCCATCCACAGTCTTCTG

reduced expression of SIK2 and SIK3 promote proliferation of breast cancer cells whereas SIK2 and SIK3 exhibited different effects on adhesion in breast cancer cells. Knockdown of SIK2 only enhanced the adhesion of triple negative breast cancer cell, while knockdown of SIK3 can decrease the adhesion of both triple negative breast cancer cells and Luminal cancer cells. Reduction of SIK1 and SIK3 can increase the invasion of triple negative breast cancer. For chemotherapy resistance, reduced SIKs contribute to the resistance to paclitaxel and cisplatin in breast cancer cells. Triple knockdown of SIK1/SIK2/SIK3 enhanced the resistance to paclitaxel and cisplatin as well. We propose SIKs to be potential biomarkers of prognosis in breast cancer, with inhibition of SIKs being associated with disease progression and chemotherapy resistance.

Methods and materials

Breast cancer samples and cell lines

A total of 102 (female) patients diagnosed with breast cancer were included in this study. Tumor and adjacent normal tissues were collected immediately after surgical excision stored at -80°C until processed for further analysis. The anonymized breast tissue samples were obtained within the guidelines of the appropriate Ethics Committee (Bro Taf Health Authority 01/4303 and 01/4046). Informed patient consent was not applicable in this instance (as stated in the Human Tissue Act 2004, UK). RNA was extracted from the tissues and reversed transcribed to cDNA with an established method. Breast cancer cell lines MDA-MB-231 and MCF-7 were obtained from the ATCC (Manassas, Virginia, USA).

Extraction of RNA and polymerase chain reaction (PCR)

TRI reagent (Sigma-Aldrich, Dorset, UK) was used for the RNA extraction. A Go Script reverse transcription kit (Promega, Southampton, UK) was used to perform reverse transcription PCR. RT-PCR conditions used in this project were as follows: 94°C for 5 min, 30 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 40 sec, and a final extension of 72°C for 10 min. GoTaq Green Master Mix (Promega, Southampton, UK) was subsequently used for PCR, and primers sequence (5'-3') in this study are showed in **Table 1** (*SIK1, SIK2, SIK3, and a housekeeping gene control GAPDH*). The PCR conditions used in this project were as follows: 94°C for 5 min, 36 cycles of 94°C for 15 sec, 55°C for 25 sec and 72°C for 15 sec. The PCR products were separated on a 1% agarose gel containing SYBR Safe Gel Stain (Life Technologies, Paisley, UK) to stain the DNA. Precision FAST 2X qPCR MasterMix (Primerdesign Ltd., Chandler's Ford, UK) was used for quantitative-PCR based on StepOne Plus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and primers sequence (5'-3') in this project for QPCR are showed in **Table 1** (*SIK1, SIK2, SIK3, GAPDH*).

Tissue microarray analysis and immunohistochemical (IHC) staining for SIKs

A breast disease spectrum tissue array (TMA) was purchased from US Biomax, Inc. (Insight Biotechnologies, Inc., Wembley, England). The TMA was de-paraffinized followed by a 20-min incubation with TBS for rehydration. Briefly, immunohistochemistry was performed as described previously [25]. Primary antibodies (Santa-Cruz Biotechnologies Inc., Santa Cruz,

CA) were diluted to 1:100 and secondary antibody to 1:100 in buffer. The secondary staining kit was an ABC biotin-conjugate universal staining kit (Vector Laboratories, Nottingham, England). The staining of the proteins was analysed by percentage and intensity by three independent assessors and presented as the Klein score here.

Knockdown of SIK1, 2, 3 using si/shRNA

SIK1, 2 and 3 was knocked down using shRNA specific to the respective SIKs (SIK1 shRNA plasmid sc-91428-SH, SIK2 siRNA sc-44364, SIK3 siRNA sc-97056, purchased from Santa Cruz Biotechnologies Inc, California, USA).

The knockdown was performed using the specific protocol provided by the manufacturer. Solution A (containing si/shRNA plasmids) and Solution B (containing Plasmid Transfection Reagent) was mixed 1:1 with and incubate for 45 min at room temperature. 800 µl of Plasmid Transfection Medium (sc-108062, Santa Cruz, California, USA) was added to 200 µl of the mix to a total of 1 ml for each well. Cells were incubated with si/shRNA for 7 hours at 37°C, then 1,000 µl of media was added for further 24 hours. Transient transfected cells were cultured with selective media containing 1 µg/ml puromycin until monoclonal cells were obtained.

In this project, we use pEF-CT as the acronym to describe control cells containing blank plasmids with no si/shRNA; the acronyms, SIK1-KD, SIK2-KD, and SIK3-KD, are used to describe the cells containing SIK1, 2 and 3 si/shRNA respectively.

In vitro cell proliferation assay

Cells were seeded into 96-well plate (2×10^3 cells/well), and then incubated in 37°C, 5% CO_2 for 0, 1, 3 and 5 days. The cells were following fixed with 4% formalin for 30 minutes and stained with 0.5% crystal violet for 15 minutes at room temperature. The cells were extracted with 10% acetic acid after washing. The absorbance at 540 nm was measured using spectrophotometer (Elx800; BioTek Instruments, Inc., Winooski, VT, USA) to determine the cell density.

In vitro cell adhesion assay

Cells were seeded into 96-well plate (2×10^4 cells/well) pre-coated with 5 µg of Matrigel (BD Matrigel Basement Membrane Matrix, Cat Number 354234; BD Bio-Science, Oxford, UK). After incubation for 60 min, the non-adherent cells were washed and the adherent cells were fixed with 4% formalin and stained with 0.5% crystal violet for 15 minutes at room temperature. After washing, the cells were extracted with 10% acetic acid. Measuring the absorbance at 540 nm using spectrophotometer (Bio-Tek, Wolf laboratories, York, UK) to determine the cell density.

In vitro cell invasion assay

Transwell inserts with 8.0 µm pore size in 24 well plate (Greiner Bio-One Ltd., Gloucestershire, UK) were pre-coated with 100 µg of Matrigel. Cells were seeded into the insert (2×10^4 cells/insert). Incubation at 37°C, 5% CO_2 for 3 days. After removing the non-invading cells in the upper chamber. the remaining invaded cells on the bottom of the insert were then fixed with 4% formalin, stained with 0.5% crystal violet. After washing, the cells were extracted with 10% acetic acid. The absorbance was measure at 540 nm using spectrophotometer to determine the cell density.

Electric cell-substrate impedance sensing (ECIS) based cell assays

Electric Cell Substrate Sensing units, ECISθ (Applied BioPhysics, Inc., Troy, NY, USA) were used to measure the resistance of MCF-7 and MDA-MB-231 cells. 96W1E 96-well ECIS electrodes were used in the present study. After preparing the electrode with serum free medium for 1 h, the cells were seeded in to the 96-well plate (4×10^4 cells/well) and allowed to reach confluence. Electrical cell resistance changes continuously in multiple frequencies (1,000, 2,000, 4,000, 8,000, 16,000 and 32,000 Hz) for up to 24 h.

Transepithelial resistance (TER) and paracellular permeability (PCP) assays

Inserts with 0.4 µm pore size in 24 well plates (Greiner Bio-One Ltd., Gloucestershire, UK) were used. Cells were seeded into insert (5×10^4 cells/insert) and incubated at 37°C, 5% CO_2 until confluent. For the TER assay, an EVOM^2 Epithelial Volt/Ohm Meter (World Precision Instruments, Hitchin, Hertfordshire, UK) was employed to measure the resistance across the membrane for the TER assay. For PCP assay, 40 kDa molecular weight tetramethylrhodamine isothiocyante (TRITC)-dextran conjugate (42874, Sigma-Aldrich, Gillingham, Dorset, UK)

and 10 kDa molecular weight Fluorescein isothiocyanate (FITC)-dextran conjugate (FD10S, Sigma-Aldrich, Gillingham, Dorset, UK) was used. Dextran that passed through the paracellular space and entered the bottom chamber of the unit was monitored hourly, by measuring the fluorescence using the spectrophotometer with excitation at 520 nm and emission 580-640 nm for TRITC, and excitation at 940 nm and emission at 510-570 nm for FITC.

Cell viability assay with paclitaxel and cisplatin

MCF-7 and MDA-MB-231 cells were treated by the paclitaxel (Sigma-Aldrich, Dorset, UK) and cisplatin (Bio-Techne, UK) for the drug resistance assay. The concentration of the chemotherapy drug was respectively chosen based on their IC50 and cell line. Measuring the absorbance at 540 nm using spectrophotometer to determine the cell viability, using the same method given earlier.

Statistics analysis

SPSS software version 26.0 (SPSS, IBM Corp, Armonk, NY, USA) was employed for statistical analysis. Continuous variables were described using medians and interquartile range (IQR). Non-parametric distribution data was used with Mann-Whitney U test, while a two-sample t-test was assessed for normally distributed data. Disease-free survival (DFS) or relapse free survival (RFS) or was calculated from the day of surgery to first recurrence or distant metastasis, or censored at the last follow-up. Overall survival (OS) was calculated from the day of diagnosis to death or censored at the last follow-up. Kaplan-Meier method and log-rank test were used to calculate RFS and OS. The ROC analysis was performed using the data from TCGA database, which is accessible at https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga. And the analysis tool was at www.rocplot.org. The analysis protocol were as described in the previous study by Fekete et al. [26]. $P<0.05$ was considered to be statistically significant.

Results

Expression of SIKs in breast cancer tissues

The expression of SIKs was examined in breast cancer and normal tissues adjacent using RT-PCR. Expression of SIK2 was lower in breast cancer than normal tissue (P=0.0179), while expression of SIK1 and SIK3 had no significant difference between breast cancer and normal tissue. The relationship between SIKs expression and pathological factors in breast cancer cohort was also analysed. SIK3 expression was associated with ER status (P=0.02). The level of SIK2 expression was significantly different in Stage I and Stage III. While SIK3 expression was significantly higher in ER positive breast cancer than ER negative breast cancer. The relationship between SIKs expression and tumor characteristics are shown in **Table 2**.

The expression of SIKs and prognosis in breast cancer cohort

We analyzed the relationship between SIKs expression and prognosis in breast cancer cohort. Kaplan-Meier analysis showed patients with high levels of SIKs tend to have longer OS and FRS, although the links are yet to reach significance, due largely to relatively small cohort size (**Figure 1**). To further validate this link, we explored the larger TCGA cohort with an online resource (http://kmplot.com/analysis/index.php?p=background), we found that in line with our cohort, SIK2 and SIK3 expression was significantly associated with prognosis. The survival curves with significantly difference between SIKs expression and prognosis were showed in **Figure 2**. Both higher SIK1 and SIK2 were associated with a favorable RFS in all patients with breast cancer (**Figure 2A**), while higher SIK3 had a positive correlation with both better OS and RFS in all patients with breast cancer (**Figure 2A**). When the analysis carried for hormone receptor positive breast cancer, higher SIK3 expression had a positive connection with better OS and RFS (**Figure 2B** and **2C**). In addition, Higher SIK3 expression also indicated longer RFS in HER2 positive breast cancer (**Figure 2D**). Higher SIK3 expression indicated better OS in lymph node negative patients (**Figure 2E**). Meanwhile Higher SIK3 expression was associated with better RFS, regardless of lymph node status (**Figure 2E**, **2F**). These results indicated high levels of SIK2 and SIK3 were associated with better clinical outcomes. The only exception is higher expression of SIK2 showed negative correlation with lymph node positive patients (**Figure 2F**).

The relationship between SIK protein staining and clinicopathological features

We further investigate the protein expression of the SIK family members by ways immunohisto-

Table 2. The relationship between SIKs expression and tumor characteristics

Characteristic	SIK1		SIK2		SIK3	
	Median (IQR)	*p* value	Median (IQR)	*p* value	Median (IQR)	*p* value
Tissue						
Normal	0 (0-221)	Ref	161.4 (10-1810)	Ref	498 (16-5620)	Ref
Tumor	0 (0-1.2)	0.67	6.8 (0-373)	0.0179	207 (0-2740)	0.18
NPI						
NPI <2.5	0.1 (0-5)	Ref	4.8 (0-439)	Ref	197.9 (16-3785)	Ref
NPI 2.5-5.4	0 (0-1)	0.68	25 (0-373)	0.68	89.6 (0-2446)	0.53
NPI >5.4	0 (0-0)	0.43	7.3 (0-308)	0.92	1210 (0-5758)	0.63
Grade						
I	1.1 (0-8)	Ref	6.4 (0-651)	Ref	6.7 (0-1069)	Ref
II	0.1 (0-0.8)	0.72	7.4 (0.1-287.1)	0.75	96.2 (0-2740)	0.31
III	0.012 (0-0.013)	0.24	0.028 (0-0.28)	0.93	582.7 (0-3015)	0.056
TNM stage						
Stage I	0.1 (0-68)	Ref	22.7 (0-268)	Ref	73.6 (0-2511)	Ref
Stage II	0 (0-8)	0.78	7.3 (1-387)	0.83	423.1 (2.0-1971)	0.56
Stage III	0.012 (0-0.013)	0.05	0.028 (0.006-0.280)	0.02	0.3 (0-4074)	0.23
Stage IV	0.415 (0.095-0.692)	0.6	5.1 (0-595)	0.76	177.9 (30-14241)	0.56
Node status						
Node negative	0.1 (0-5)	Ref	4.8 (0-439)	Ref	198 (1.0-3785)	Ref
Node positive	0 (0-1)	0.52	10.7 (0-371)	0.71	125 (0-2511)	0.8
ER status						
ER negative	0 (0-1)	0.84	10.3 (0.2-377.1)	0.68	382.6 (3.0-4935)	0.02
ER positive	0.1 (0-1)		3 (0-167)		79.7 (0-1329)	
HER2 status						
HER2 negative	0.1 (0-3)	0.39	2.8 (0-383)	0.37	206.7 (2.0-2430)	0.76
HER2 positive	0.04 (0.01-0.27)		26.3 (1.3-331.3)		271.6 (0-2754)	
Clinical Outcome						
Alive and well	0.1 (0-1)	Ref	5.6 (0-352)	Ref	148.6 (0-2740)	Ref
Distant Metastasis	0 (0-1205)	0.8	0.5 (0-457)	0.47	124.6 (48-1567)	0.77
Died of breast cancer	0 (0-3)	0.42	5.6 (0-685)	0.75	755.6 (0-6037)	0.82

chemistry of TMAs, SIKs protein expression levels, presented here as Klein scores showed no significant difference between normal breast tissue and breast cancer in TMA (**Figure 3**). Furthermore, SIK1 Klein score was significantly higher in ER positive breast cancer than ER negative breast cancer. As for SIK3, higher Klein score was observed in breast cancer with higher Grade, node negative tumors and tumors with higher TNM stage (**Tables 3**-**5**).

Creation of SIK1, SIK2 and SIK3 knockdown breast cancer cells

We knockdown the SIK1, SIK2 and SIK3 in the breast cancer cell lines MDA-MB-231 and MCF-7 using the respective anti-SIK si/shRNA transgenes, control si/shRNA-A transgene (control siRNA sc-37007, Santa Cruz, California, USA. Control shRNA sc-108060, Santa Cruz, California, USA) into the cells for the control group. RT-PCR and quantitative-PCR showed the success in the knockdown (**Figure 4**).

Knockdown SIK2 and SIK3, but not SIK1, increased the proliferation of breast cancer cells

To analyze the effect of the knockdown of SIK1 on the proliferation of breast cancer cells, we performed *in vitro* growth assay both in MDA-MB-231 and MCF-7 cell lines. All the results showed that the knockdown of SIK1 did not change the proliferate ability of neither MDA-MB-231 nor MCF-7 cells through the normal-

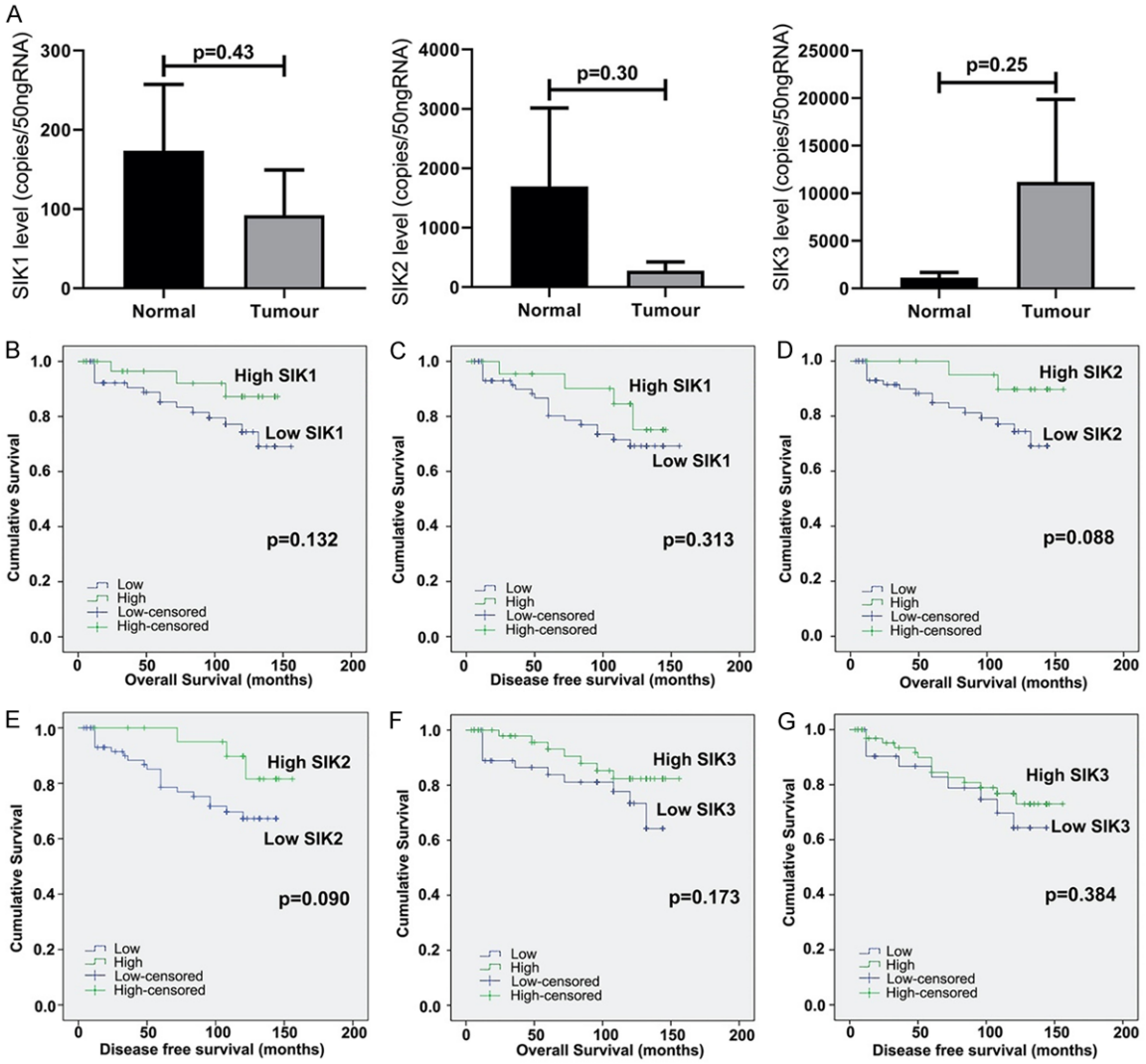


Figure 1. Correlation of SIKs expression with overall survival and disease free survival in breast cancer cohort. A: Expression of SIK1, 2 and 3 in normal breast tissue and breast tumor tissue from breast cancer cohort (error bars show SD.). B, C: The relation between SIK1 level and OS, DFS in breast cancer patients. D, E: The relation between SIK2 level and OS, DFS in breast cancer patients. F, G: The relation between SIK3 level and OS, DFS in breast cancer patients.

ized data in the day 3 (**Figure 4A**). Interestingly and in contrast to SIK1 knockdown, knockdown SIK2 increased the proliferative potential of MCF-7 cells, but not in the MDA-MB-231 cells. With both MDA-MB-231 and MCF-7 cells, knockdown of SIK3 increased the proliferation of the cells significantly (**Figure 5A**).

Knockdown SIK2 and SIK3 had different effect on the adhesiveness of breast cancer cells, while knockdown SIK1 had no effect

We performed the *in vitro* adhesion assay using the established SIK1, 2 and 3 knockdown cells together with transfection controls of MDA-MB-231 and MCF-7 cells. We found that knockdown SIK1 had no effect on the adhesion of MDA-MB-231 cells, same to the effect of SIK1 on the proliferation. Knockdown of SIK2 enhanced the adhesion of the MDA-MB-231 cells but had no impact on the MCF-7 cells. Knockdown of SIK3 decreased the adhesion of both MDA-MB-231 and MCF-7 cells (**Figure 5B**).

Knockdown of SIK1 and SIK3 increased the invasion of MDA-MB-231 cells

In vitro Matrigel-based invasion assay revealed that knockdown of SIK1 and SIK3 increased the invasion potential of MDA-MB-231 cells.

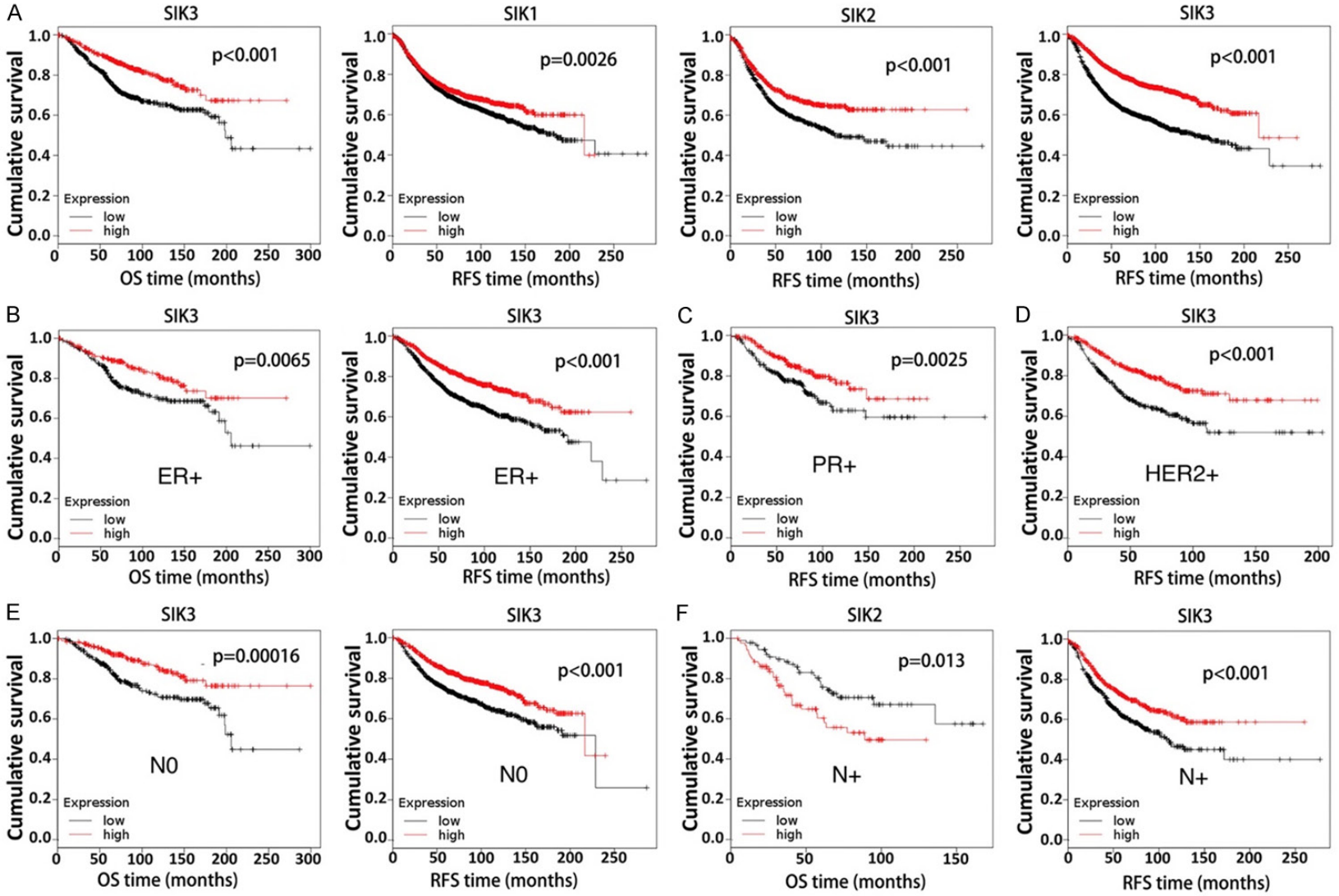


Figure 2. Correlation of SIKs expression with clinical outcomes in online database. A: The relation between SIKs expression and survival based on all breast cancer patients. From left to right: The relation between SIK3 expression and OS in all patients with breast cancer, and relation between SIK1, 2 and 3 expression and RFS in all patients with breast cancer. B: The relation between SIK3 expression and OS and RFS in ER positive patients. C: The relation between SIK3 and RFS in PR positive patients. D: The relation between SIK3 expression and RFS in HER2 positive patients. E: The relation between SIK3 expression and OS and RFS in node negative patients. F: The relation between SIK2 expression and OS in node positive patients, and the relation between SIK3 expression and RFS in node positive patients. (ER+: Estrogen receptor positive breast cancer; PR+: Progestogen receptor positive breast cancer; HER2+: HER2 positive breast cancer; N0: lymph node negative breast cancer; N+: lymph node positive breast cancer).

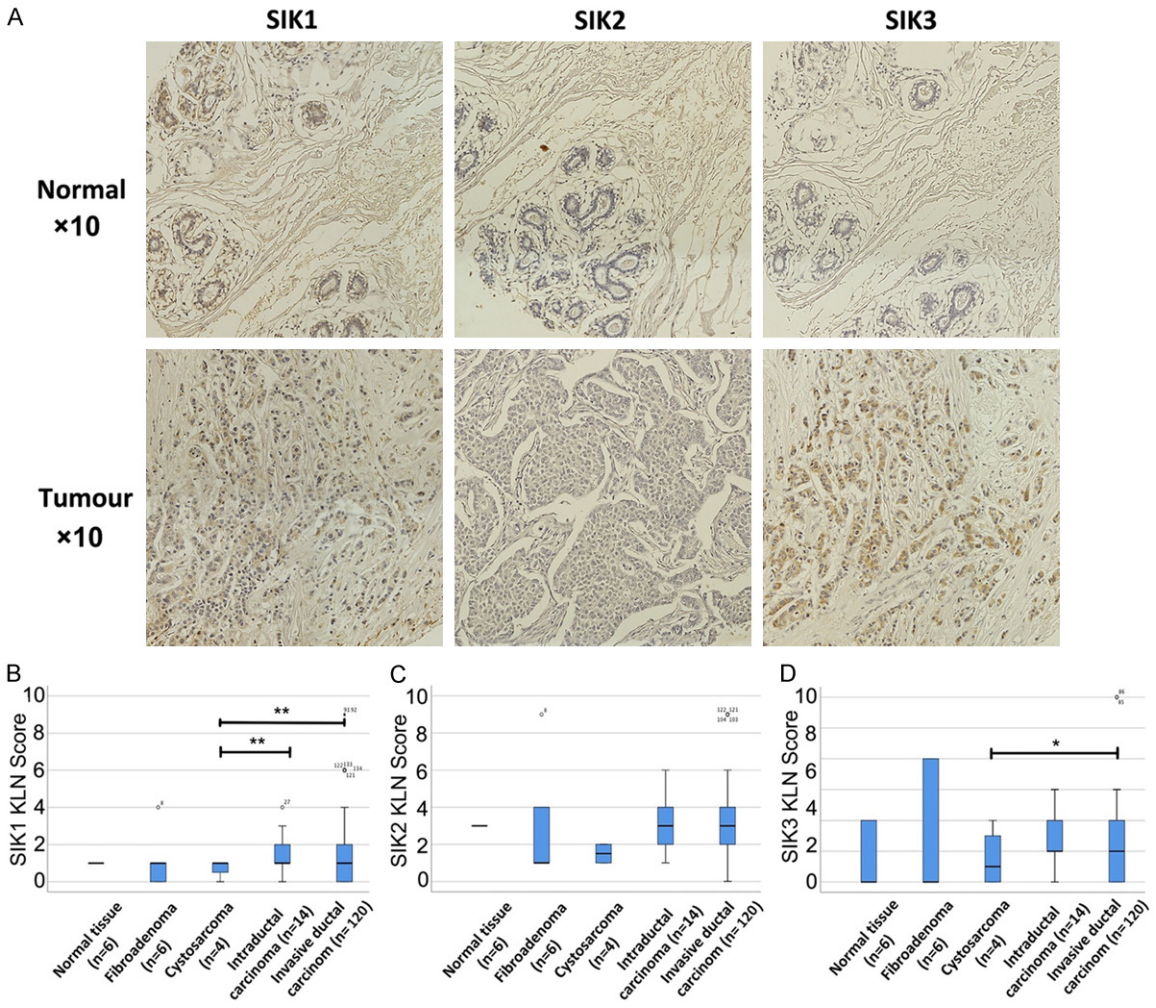


Figure 3. SIKs Immunochemical staining in normal breast tissue, fibroadenoma, cystosarcoma phyllodes, intraductal carcinoma and invasive ductal carcinoma (TMA). A: SIKs expression in normal breast tissue and invasive ductal carcinoma at 10× objective magnification. B-D: SIKs expression in different tissue. Respective boxplot representations show median, Q1 and Q3 staining intensity scores and whiskers represent minimum and maximum staining intensity scores. *P<0.05, **P<0.01.

Knockdown of SIK2 had no effect on invasion of neither MDA-MB-231 cells nor MCF-7 cells (**Figure 5C**).

Knockdown SIK1 increased the barrier integrity but knockdown of SIK2 and SIK3 decreased the barrier functions of the breast cancer cells

To investigate the tight junction-based barrier function of the breast cancer cells, we carried out the *in vitro* ECIS, TER and PCP assays. As revealed by ECIS assay, knockdown SIK1 tighten up the barrier of the MDA-MB-231 cells. Knockdown SIK2 and SIK3 had no effect on the tight junction of MDA-MB-231 ells but weakened the barrier of the MCF-7 cells (**Figure 6A**). In terms of the TER and PCP assay, we found only the knockdown of SIK2 could decreased the barrier of MCF-7 cells, which was same as the result of the ECIS (**Figure 6B** and **6C**).

Knockdown of SIK family increased the resistance of breast cancer cell to chemotherapeutic agents

We executed the cell proliferation assay using the cells treated with various concentration of chemotherapy drugs and placebo to investigate the effect of SIK family on the drug resistance of breast cancer cells. We found SIK1 and SIK2 knockdown in the MDA-MB-231 cells increased the resistance of cancer cells to paclitaxel

Table 3. IHC staining of SIK1 and clinicopathological features

Characteristic	SIK1 Klein Score								*p* value
	N	0	1	2	3	4	6	9	
T stage									0.068
T1	8	2	2	2	1	1	0	0	
T2	82	2	16	17	24	2	17	4	
T3	20	0	2	3	8	1	4	2	
T4	10	0	1	5	1	3	0	0	
Node metastasis									0.658
Negative	78	2	14	17	25	6	12	2	
Positive	42	2	7	10	9	1	9	4	
Grade									0.342
I	7	0	1	2	2	2	0	0	
II	96	2	17	21	25	5	20	6	
III	16	2	3	3	7	0	1	0	
TNM Stage									0.071
Stage I	8	2	2	2	1	1	0	0	
Stage II	82	2	14	18	25	3	16	4	
Stage III	30	0	5	7	8	3	5	2	
ER status									0.024
ER Negative	80	4	17	16	25	4	14	0	
ER Positive	40	0	4	11	9	3	7	6	
PR status									0.08
PR Negative	83	3	16	19	26	5	12	2	
PR Positive	34	1	5	6	8	2	8	4	
HER2 status									0.542
HER2 Negative	84	4	13	21	24	4	14	4	
HER2 Positive	36	0	8	6	10	3	7	2	
TNBC or not									0.057
TNBC	45	3	9	10	15	2	6	0	
Non-TNBC	75	1	12	17	19	5	15	6	
Molecular Type									0.003
Luminal A	28	1	2	12	7	0	4	2	
Luminal B	21	0	3	0	4	3	7	4	
TNBC	26	0	7	5	8	2	4	0	
HER2-enriched	45	3	9	10	15	2	6	0	

(IC50: SIK1-KD 18.62 μM vs pEF-CT 5.279 μM; SIK2-KD 32.99 μM vs pEF-CT 8.652 μM) (**Figure 7A**). Overall, we found the trend that knockdown SIK1, 2 and 3 could increase the resistance of both MDA-MB-231 and MCF-7 cells to the chemotherapy drugs paclitaxel and cisplatin (**Figure 7B**). When we knockdown SIK1, 2 and 3 together in the breast cancer cells, we found the same result: knockdown the three SIK family members together could increase the resistance of both the MDA-MB-231 and MCF-7 cells to the chemotherapy (**Table 6**).

Clinical drug resistance analysis on SIK family in breast cancer patients

We used the TCGA database to investigate the patients' responds to the chemotherapy agents. We found that patients who responded better to paclitaxel and cisplatin had higher expression of SIK1 and lower expression of SIK3 with statistically significant. However the trends of SIK2 is not clear: patients with lower SIK2 were more sensitive to paclitaxel according to the five-years' RFS; while the pathological

Table 4. IHC staining of SIK2 and clinicopathological features

Characteristic	SIK2 Klein Score								*p* value
	N	0	1	2	3	4	6	9	
T stage									0.292
T1	8	4	3	1	0	0	0	0	
T2	82	26	25	9	20	0	2	0	
T3	20	6	3	7	4	0	0	0	
T4	10	5	2	2	1	0	0	0	
Node metastasis									0.908
Negative	78	26	21	16	15	0	0	0	
Positive	42	15	12	3	10	0	2	0	
Grade									0.496
I	7	2	2	3	0	0	0	0	
II	96	31	27	13	23	0	2	0	
III	16	7	4	3	2	0	0	0	
TNM Stage									0.308
Stage I	8	4	3	1	0	0	0	0	
Stage II	82	26	22	15	17	0	2	0	
Stage III	30	11	8	3	8	0	0	0	
ER status									0.837
ER Negative	80	28	21	11	18	0	2	0	
ER Positive	40	13	12	8	7	0	0	0	
PR status									0.373
PR Negative	83	30	23	13	17	0	0	0	
PR Positive	34	10	9	6	8	0	1	0	
HER2 status									0.196
HER2 Negative	84	26	22	15	21	0	0	0	
HER2 Positive	36	15	11	4	4	0	2	0	
TNBC or not									0.789
TNBC	45	16	11	5	13	0	0	0	
Non-TNBC	75	25	22	14	12	0	2	0	
Molecular Type									0.349
Luminal A	28	10	8	7	3	0	0	0	
Luminal B	21	4	7	3	6	0	1	0	
TNBC	45	16	11	5	13	0	0	0	
HER2-enriched	26	11	7	4	3	0	1	0	

responds analysis showed higher SIK2 level resulting in better reaction to cisplatin but five-years' RFS analysis showed higher SIK2 caused higher resistance to cisplatin (**Figure 8**).

Discussion

Breast cancer is the most frequently malignant tumor in women. Despite advance in diagnosis and treatment, 47170 cases are estimated to die of breast cancer in United States, ranking the first cancer-related death reason in female [1]. Drug resistance and response to personalized therapy is unpredictable. It is vital to find new prognosis factor or therapeutic target to improve the clinical outcome of breast cancer. Previous study showed an increased Na^+ concertation in tumor compared to normal tissues [27], and Na^+ concertation decreased significantly in patients who achieved partial or complete response to neoadjuvant chemotherapy compared to non-responders [28], indicating voltage-gated sodium channels may have a role in tumorigenesis and drug sensitivity. Therefore, we have focused on the function of SIKs in tumorigenesis and explored the correla-

Table 5. IHC staining of SIK3 and clinicopathological features

Characteristic	SIK3 Klein Score								*p* value
	N	0	1	2	3	4	6	9	
T stage									0.33
T1	8	5	1	1	1	0	0	0	
T2	82	24	24	16	4	2	12	0	
T3	20	4	9	1	2	0	2	2	
T4	10	2	3	1	2	2	0	0	
Node metastasis									0.002
Negative	78	30	23	11	5	3	4	2	
Positive	42	5	14	8	4	1	10	0	
Grade									0.005
I	7	5	0	2	0	0	0	0	
II	96	22	29	16	9	4	14	2	
III	16	8	7	1	0	0	0	0	
NM Stage									0.003
Stage I	8	5	1	1	1	0	0	0	
Stage II	82	27	27	13	6	1	6	2	
Stage III	30	3	9	5	2	3	8	0	
ER status									0.678
ER Negative	80	22	24	15	8	2	7	2	
ER Positive	40	13	13	4	1	2	7	0	
PR status									0.618
PR Negative	83	25	25	16	5	4	6	2	
PR Positive	34	10	10	3	4	0	7	0	
HER2 status									0.093
HER2 Negative	84	26	31	8	7	4	6	2	
HER2 Positive	36	9	6	11	2	0	8	0	
TNBC or not									0.571
TNBC	45	12	17	8	4	2	0	2	
Non-TNBC	75	23	20	11	5	2	14	0	
Molecular Type									0.052
Luminal A	28	10	12	2	2	0	2	0	
Luminal B	21	4	4	2	2	2	7	0	
TNBC	45	12	17	8	4	2	0	2	
HER2-enriched	26	9	4	7	1	0	5	0	

tion between SIKs level and drug resistance in breast cancer.

Our findings suggest that the transcript levels of SIK1, 2 and 3 is expressed distinctly in breast cancer based on breast cancer clinical cohort. SIK1 and SIK2 have a decreased level in breast tumor tissue compared to normal breast tissue, while the SIK3 is highly expressed in tumor, and it has showed the same trend in previous study [18]. Little statistical significance was observed, which may be ascribed to the limited patients in cohort and few events occurred during follow-up. However, our smaller cohort, which in agreement with the large TCGA cohort clearly demonstrated that lower expression of SIK is related to worse prognosis and that RFS is more prone to this link with SIKs. The RFS of patients with low expression level of SIK1, 2 and 3 are all shorter than patients with higher SIK levels, in line with a recent report [10, 21]. Current researches show SIK1, 2 and 3 may suppress breast cancer growth and metastasis through different pathways in breast cancer. SIK1 is reported to be a key modulator of anoikis and control metastasis

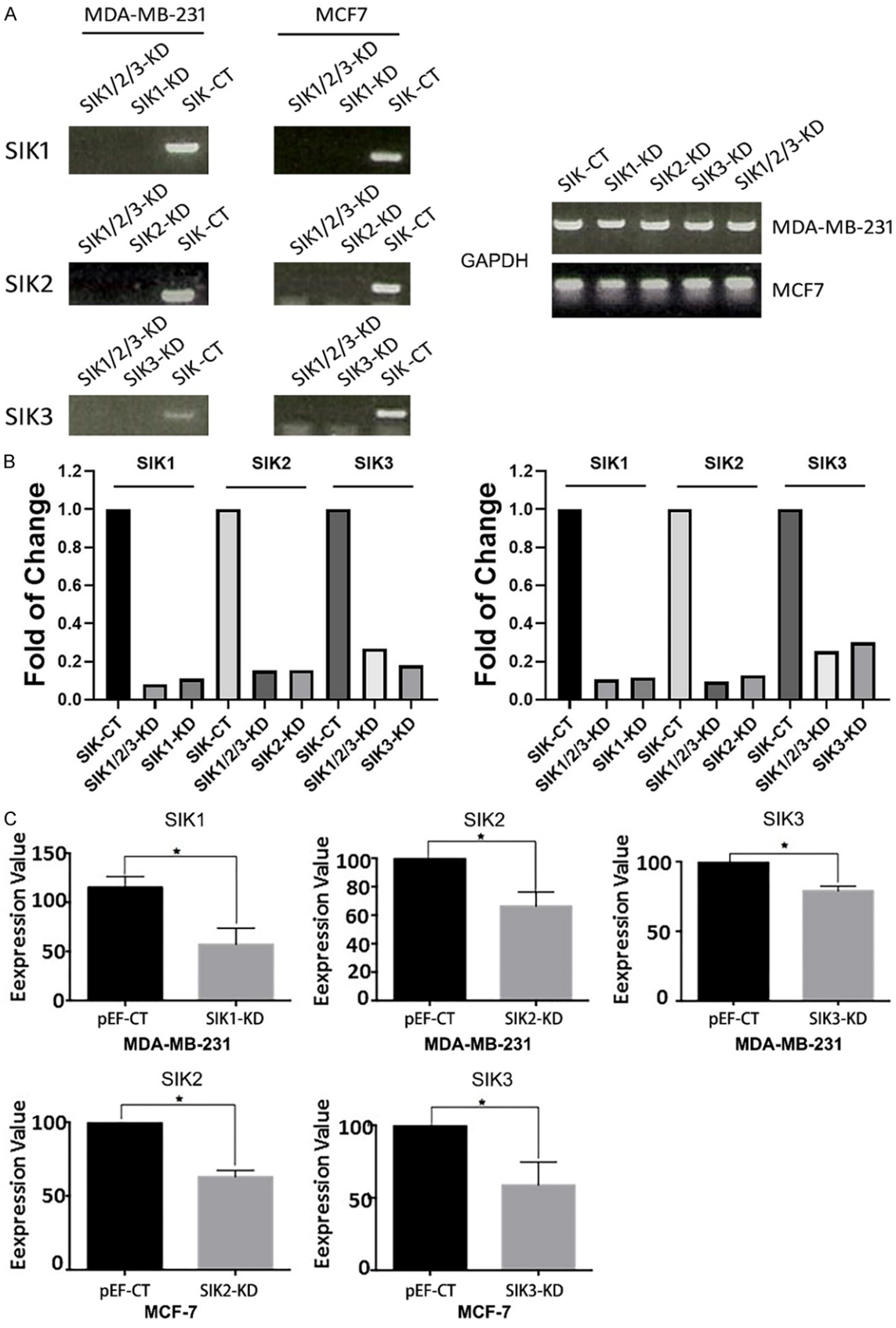

A
MDA-MB-231
MCF7
SIK1/2/3-KD
SIK1-KD
SIK-CT
SIK1
SIK2-KD
SIK2
SIK3-KD
SIK3
GAPDH
SIK-CT
SIK1-KD
SIK2-KD
SIK3-KD
SIK1/2/3-KD
MDA-MB-231
MCF7
B
SIK1
SIK2
SIK3
Fold of Change
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SIK-CT
SIK1/2/3-KD
SIK1-KD
SIK2-KD
SIK3-KD
C
SIK1
SIK2
SIK3
Eexpression Value
pEF-CT
SIK1-KD
SIK2-KD
SIK3-KD
MDA-MB-231
MCF-7

Figure 4. Knockdown of SIK family in the breast cancer cell lines. A. Confirmation of SIK knockdown in MDA-MB-231 and MCF-7 cell lines using conventional PCR. Left: confirmation of SIK1, 2 and 3 knockdown in the MDA-MB-231 cells. Right: confirmation of SIK2 and SIK3 knockdown in the MDA-MB-231 cells. B. Confirmation of SIK knockdown in MDA-MB-231 and MCF-7 cell lines using quantitative-PCR. Upper: confirmation of SIK1, 2 and 3 knockdown in the MDA-MB-231 cells. Lower: confirmation of SIK2 and SIK3 knockdown in the MDA-MB-231 cells. C. Shoes the difference in SIKs expression after knockdown using qPCR analysis. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (pEF-CT referring the cells containing blank plasmids as control group; and SIK1-KD, SIK2-KD, and SIK3-KD referring the cells containing SIK1, 2 and 3 si/shRNA respectively as experimental groups).

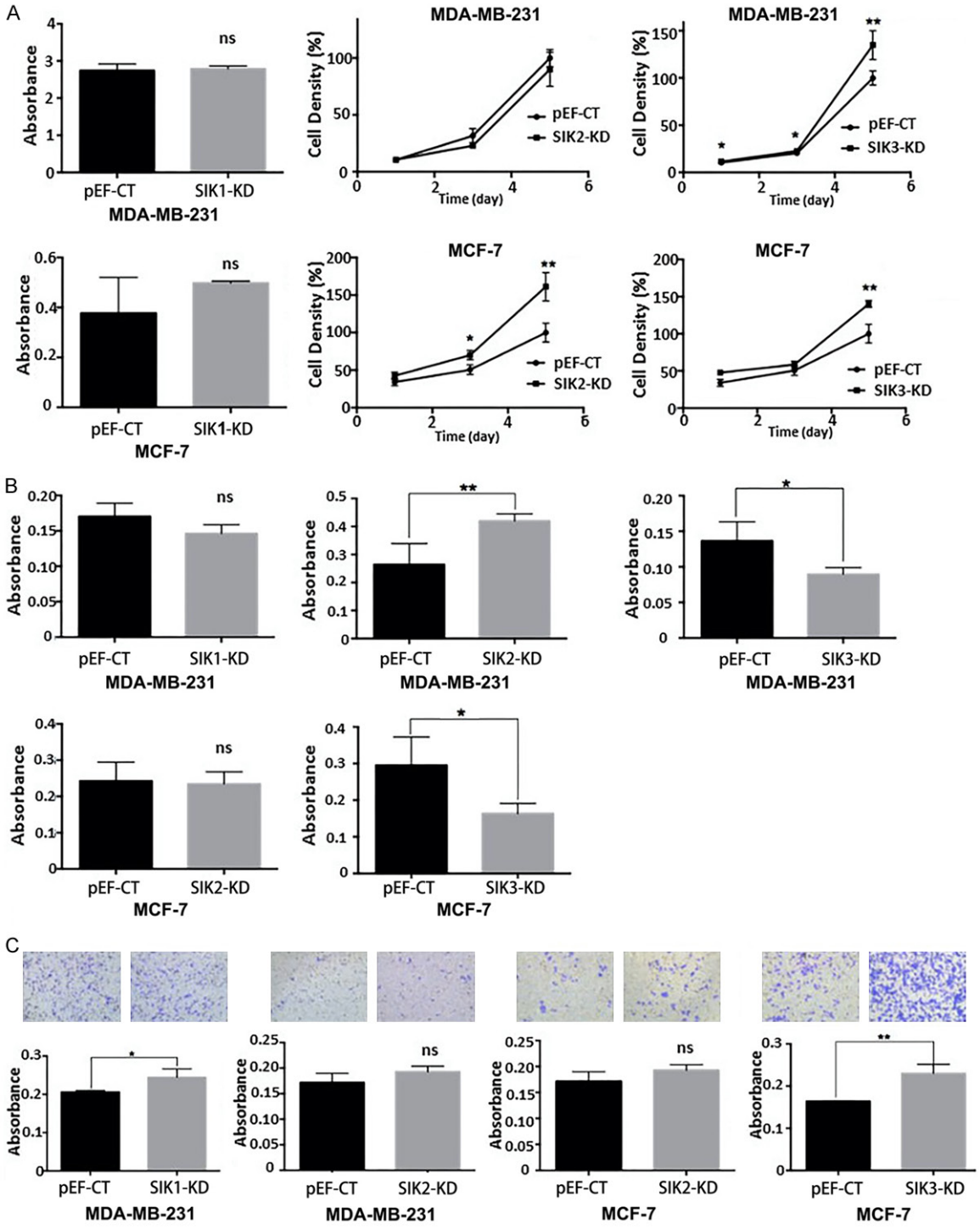


Figure 5. Effect of SIK knockdown on the functions of MDA-MB-231 and MCF-7 cell lines *in vitro* using cells which knockdown SIK (MDA-MB-231/SIK-KD and MCF-7/SIK-KD) compared with its control (MDA-MB-231/pEF-CT and MCF-7/pEF-CT). A. *In vitro* growth assay of breast cancer cells after knockdown of SIK1, 2 and 3. Left histogram: the effect of SIK1 knockdown on the MDA-MB-231 and MCF-7 cells based on the cell density grow to day 5. Right line charts: the effect of SIK2 and 3 knockdown on the MDA-MB-231 and MCF-7 cells with the cell density from day 1 to day 5. B. *In vitro* adhesion assay of breast cancer cells after knockdown of SIK1, 2 and 3. C. *In vitro* Matrigel-based invasion assay of breast cancer cells after knockdown of SIK1, 2 and 3. Representative images of the invades cells after incubation on Matrigel for 3 days and stained with crystal violet. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

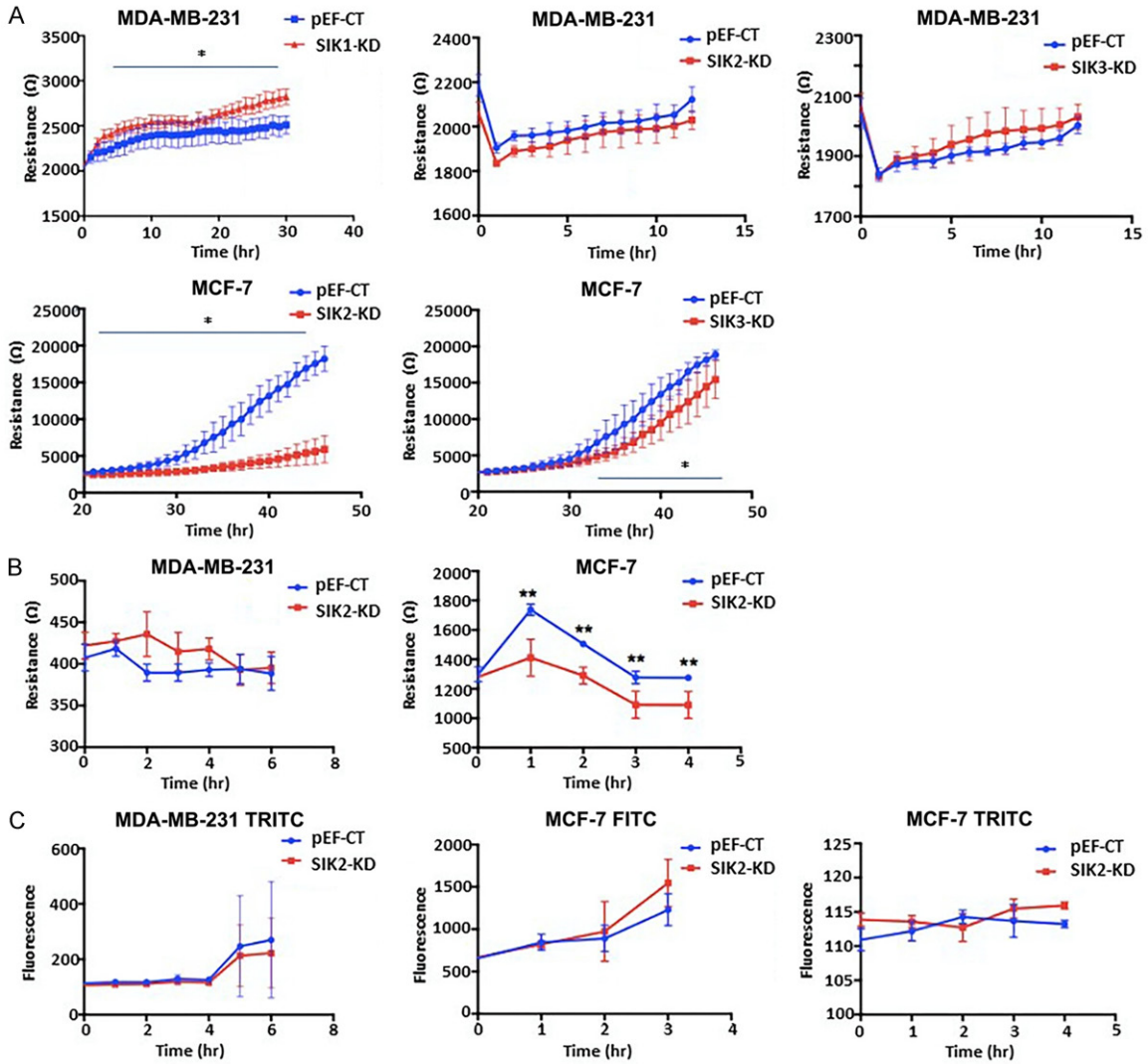


Figure 6. Effect of SIK knockdown on the barrier of MDA-MB-231 and MCF-7 cell lines *in vitro* using cells which knockdown SIK (MDA-MB-231/SIK-KD and MCF-7/SIK-KD) compared with its control (MDA-MB-231/pEF-CT and MCF-7/pEF-CT). A. ECIS assay showing the effect of knockdown of SIK1, 2 and 3 on the MDA-MB-231 cells (upper) and knockdown of SIK2 and 3 on the MCF-7 cells (lower). B. TER assay showing the effect of SIK2 knockdown on the MDA-MB-231 cells (left) and MCF-7 cells (right). C. PCP assay showing the effect of SIK2 knockdown on the MDA-MB-231 cells (left: using TRITC florescence) and MCF-7 cells (middle: using FITC florescence; right: using TRITC florescence). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

through modulating p53, and suppresses breast cancer growth and metastasis [10, 29]. Meanwhile, knocking down SIK1 expression promotes breast cancer invasiveness through activation of voltage-gated sodium channels, indicating a critical role for ion channels in

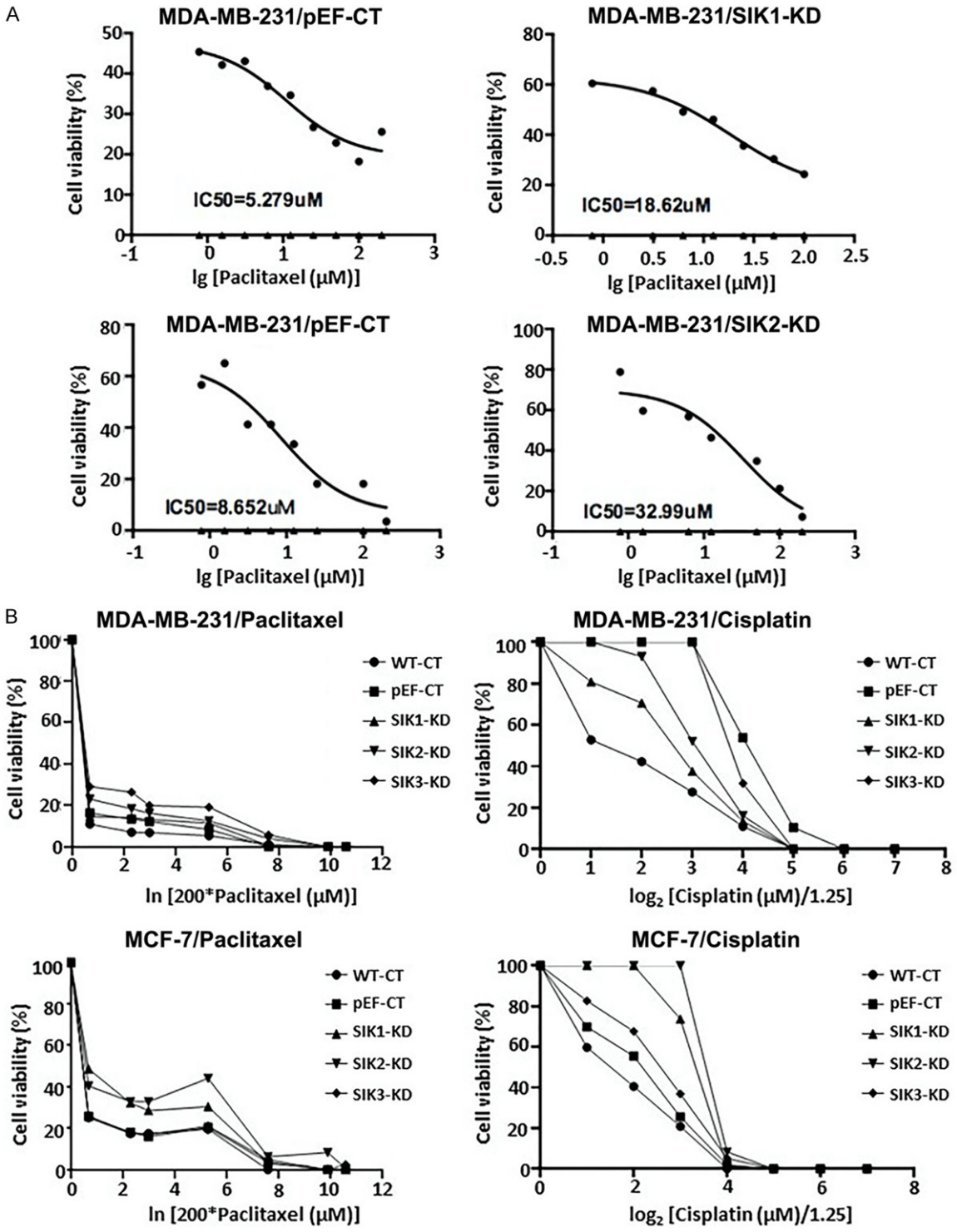


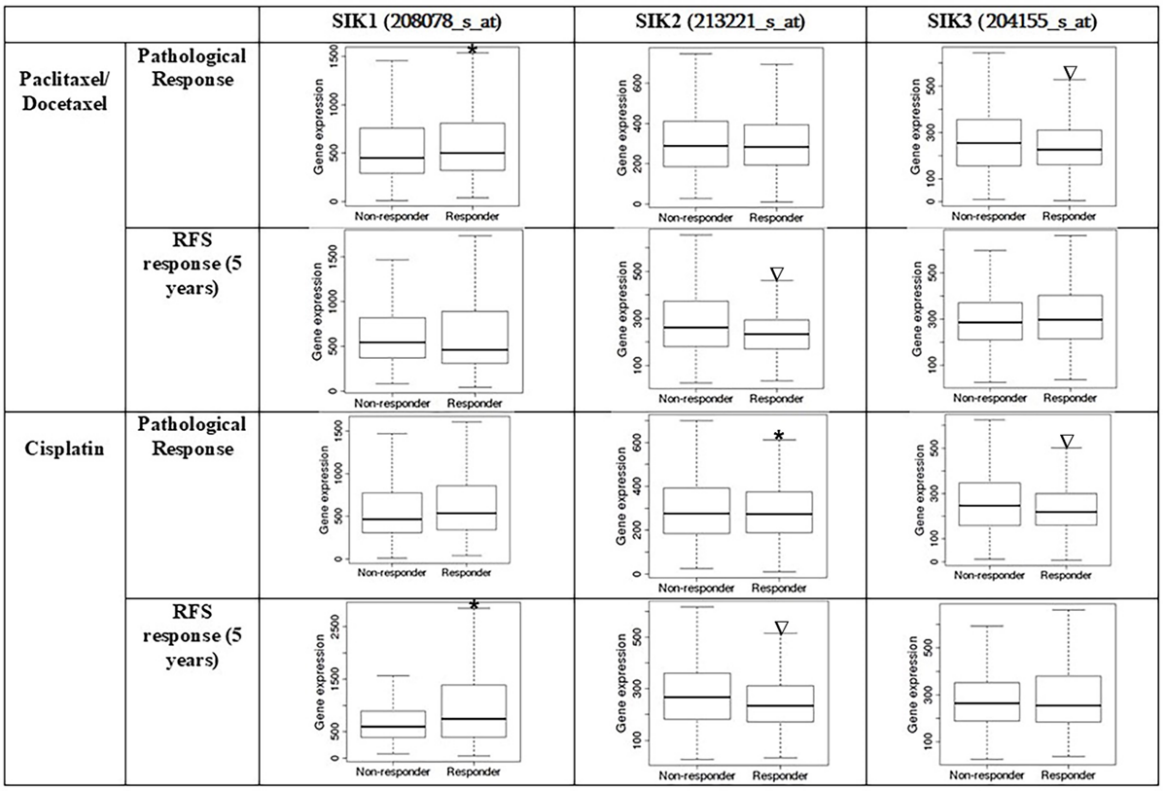
Figure 7. Effect of SIK knockdown on the chemotherapy resistance of MDA-MB-231 and MCF-7 cell lines *in vitro* using cells which knockdown SIK (MDA-MB-231/SIK-KD and MCF-7/SIK-KD) compared with its control (MDA-MB-231/pEF-CT and MCF-7/pEF-CT). A. Effect of SIK1 (upper) and SIK2 (lower) knockdown on the paclitaxel resistance of MDA-MB-231 cells. B. Effect of SIK knockdown on the resistance to paclitaxel (left) and cisplatin (right) of MDA-MB-231 and MCF-7 cells.

tumor metastasis. It's reported $Na_v1.5$ expression can modulate the epithelial-to-mesenchymal transition, thus promoting the invasiveness of breast cancer [30]. SIK2 may inhibits tumori-

Table 6. Percentage cell viability after knockdown with SIK1, SIK2, SIK3 or SIK 1/2/3 and treatment with Cisplatin or Paclitaxel

Cisplatin	Cell viability (%)				
	MCF-7-CT	MCF-7 SIK1-KD	MCF-7 SIK2-KD	MCF-7 SIK3-KD	MCF-7 SIK1/2/3-KD
200 μm	37.83242	35.51977	38.16793	35.12188	37.14973
100 μm	70.37485	87.58922	80.76371	92.35549	81.84645
50 μm	78.95753	100.1489	93.48407	97.11075	102.7223
25 μm	92.20578	104.2838	95.9981	89.94448	100.3598
12.5 μm	81.70201	93.51519	94.09528	93.55388	100.0973
6.75 μm	77.61237	94.37398	89.94372	96.99133	105.2713
3.375 μm	84.97141	96.06968	93.46303	93.1929	93.81606
0 μm	100	100	100	100	100
Paclitaxel	Cell viability (%)				
	MCF-7-CT	MCF-7 SIK1-KD	MCF-7 SIK2-KD	MCF-7 SIK3-KD	MCF-7 SIK1/2/3-KD
200 μm	35.31575	45.15218	46.72549	45.4456	41.6341
100 μm	27.29429	49.46108	59.21978	39.38767	41.34061
50 μm	62.31461	91.12916	86.95957	93.0813	95.80269
25 μm	73.42779	96.72346	87.47632	94.51171	88.85296
12.5 μm	77.32639	97.89073	94.57886	94.65889	93.13816
6.75 μm	78.00574	91.10169	93.33883	94.23561	97.22434
3.375 μm	72.42185	90.1108	91.20723	96.79966	96.52911
0 μm	100	100	100	100	100
Cisplatin	Cell viability (%)				
	MDA-MB-231-CT	MDA-MB-231 SIK1-KD	MDA-MB-231 SIK2-KD	MDA-MB-231 SIK3-KD	MDA-MB-231 SIK1/2/3-KD
200 μm	30.54597	48.65879	43.37078	42.80708	39.68384
100 μm	51.18091	56.86849	55.00427	61.55232	46.99156
50 μm	60.02509	76.22246	71.04096	74.80931	59.46459
25 μm	66.82706	77.1631	70.74142	79.26647	61.17217
12.5 μm	62.04829	77.03805	72.26244	81.25744	67.14237
6.75 μm	64.74549	76.75485	78.33908	81.18138	66.33589
3.375 μm	69.49182	81.38083	74.87894	78.43025	61.59574
0 μm	100	100	100	100	100
Paclitaxel	Cell viability (%)				
	MDA-MB-231-CT	MDA-MB-231 SIK1-KD	MDA-MB-231 SIK2-KD	MDA-MB-231 SIK3-KD	MDA-MB-231 SIK1/2/3-KD
200 μm	46.29602	62.16348	43.9928	39.67021	34.36813
100 μm	37.89596	55.53626	43.29313	56.64094	44.48662
50 μm	47.43355	59.8463	60.05016	68.19031	56.21459
25 μm	48.4959	57.03737	60.89593	65.50334	57.48575
12.5 μm	57.09747	60.72059	58.59096	67.14744	53.42212
6.75 μm	46.23206	55.3535	57.79193	57.39792	56.04128
3.375 μm	49.32616	61.95095	56.57353	59.29106	52.15618
0 μm	100	100	100	100	100

genesis of breast cancer through PI3K/Akt and Ras/ERK signaling pathway, especially in triple negative breast cancer [21], while other studies indicate SIK2 is essential for TNBC cell survival and growth. For SIK3, it mediates cell proliferation through regulating G0/G1-phase release and induces expression of tumor metastatic CXCR4 through MMP-9 in breast cancer, which tend to be a potential drug target [23]. Together, these findings may explain the clinical



		SIK1 (208078_s_at)		SIK2 (213221_s_at)		SIK3 (204155_s_at)	
		AUC	Chemoresponse significance (*p* value)	AUC	Chemoresponse significance (*p* value)	AUC	Chemoresponse significance (*p* value)
Paclitaxel/Docetaxel	Pathological Response	0.537	0.019*	0.053	NS	0.553	0.0093▽
	RFS response (5 years)	0.53	NS	0.574	0.022▽	0.519	NS
Cisplatin	Pathological Response	0.503	NS	0.552	0.0027*	0.549	0.0038▽
	RFS response (5 years)	0.562	0.019*	0.558	0.025▽	0.513	N.S.

Figure 8. The level of SIK expression and the response to chemotherapy. AUC: Area under the curve. *Significantly higher in responders. ▽Significantly lower in responders.

relevance revealed, that high SIKs expression are related to good clinical outcome, pointing out the direction of the following research.

Our findings that SIKs and clinical outcome in different molecular subtypes is connected is of particular interest. Low expression of SIK relates to worse prognosis, regardless of their nature as different Luminal type or HER-2 enriched breast cancer. Reduced expression of SIK3 is associated with poorer prognosis in patients with lymph node metastasis. The only exception is low SIK2 level in node metastasis positive patients is corelated to better overall survival. This may be due to the numbers of patients involved in the analysis of SIK2 and overall survival of node metastasis positive patients being less than any other groups, an area clearly warrants further investigation.

To explore the impact of SIK on cellular behavior, we created SIK1, 2 and 3 knockdown cell models from MDA-MB-231 cell line and SIK2 and 3 knockdowns from MCF-7 cell line. MCF-7 cell almost stoped growth after knockdown of SIK1. Knockdown of SIK2 enhanced the proliferation of MCF-7 cells and knockdown of both SIK2 and SIK3 increased the proliferation of MDA-MB-231 cells. The results of the SIKs on the regulation of proliferation of breast cells are able to correspond to the clinical prognosis analyzed previously. Growth of residual cancer cells is an important cause of tumor recurrence and progression and directly affect the patient's

RFS and OS. The *in vitro* adhesion assay showed that knockdown of SIK2 increased the adhesion of MDA-MB-231 cells and knockdown of both SIK3 increased the adhesion of MDA-MB-231 and MCF-7 cells. This trend is also the same as previous results, due to the adhesion of the cancer cells being conducive to distant metastasis of the tumor, leading to a worse long-term prognosis for patients. Knockdown of SIK1 and SIK3 enhanced the invasion of MDA-MB-231 cells. Tumor cell invasion is closely related to tumor metastasis and poor prognosis, the silence potential of SIK on invasion function of breast cancer proved that low SIK patients often have a poor prognosis.

Our findings that SIKs bear influence on the barrier functions of breast cancer cells are intriguing. Knockdown SIK1 enhances the tight junctions of MDA-MB-231 cells but knockdown SIK2 and SIK3 decreased the tight junctions of MCF-7 cells, and the influence of SIK2 on the MCF-7 cells is also confirmed by TER assay. In two different cell lines, different molecules may have different effects. Different SIK members have different effects on the tight junction function of two different breast cancer cell lines. A previous study suggests that SIK1 may contribute to the loss of epithelial polarity and regulate tight junction assembly through act on polarity protein Par3 [31]. The present study is the first time that SIK2 and SIK3 have been demonstrated to have a role in tight junction and would have a wider implication on the paracellular diffusion of drugs.

Drug resistance is one of the important reasons for tumor recurrence and death of patients with advanced breast cancer. We propose SIKs have a regulating role in chemotherapy sensitivity of breast cancer cell. The lower SIK leads to increased paclitaxel and cisplatin resistance of tumor cells, implicating a worse prognosis of tumor patients, which also echoes our prognostic analysis. In ovarian cancer, Ahmed et al. showed SIK2 was essential for centrosome separation in mitosis [8]. Depletion of SIK2 resulted in stalling in mitosis as well as mitosis-dependent synergy with paclitaxel and decreased ovarian cancer growth [8]. ARN-3236, SIK2 inhibitor, has showed promising in inhibiting ovarian cell growth and improving sensitivity to paclitaxel [32]. However, few studies have reported the correlation between SIK family expression with chemotherapy sensitivity in breast cancer. We have found that the trend of SIK expression and chemotherapy response is not the same as in ovarian cancer. Knockdown SIK1, 2 and 3 could increase the resistance to paclitaxel and cisplatin in breast cancer cells, so do triple knockdown of SIKs. It's reported high salt diet can induced breast cancer proliferation through SIK3 upregulation, which stimulated P-glycoprotein mediated paclitaxel resistance. This indicates ion influx maybe key mechanism in SIK3 regulation of chemo-drug resistance [24]. While we use the online database to investigate the patients' response to chemotherapy agents, we found the impact of SIKs on drug resistance and RFS response is not the all same as the in vitro result. The TCGA online cohort showed SIK1 was significantly higher in patients who were sensitive to paclitaxel/docetaxel and cisplatin, which had the same trend with breast cancer cell lines result. On the contrast, higher SIK3 expression seemed to be related to worse response to paclitaxel and cisplatin, and showed the opposite trend with the result in vitro. For SIK2, lower SIK2 tend to be related to better RFS survival. The difference of SIKs expression and drug response in cohort and in breast cancer cells may due to concomitant drugs or different systemic therapy that patients received. Further studies on mechanism are needed to explain how SIKs affect chemotherapy sensitivity of breast cancer and their combined impact on chemotherapy sensitivity. While in vivo experiments with durg-pretreated breast cancer cells and phosphoproteomics may provide convincing evidence to how SIKs have impact on drug response.

In conclusion, we characterized low expression of SIK1, 2 and 3 in breast cancer, and this was correlated with poor outcome and chemotherapy resistance of breast cancer. Reduced SIKs promote tumor metastasis by enhancing tumor function and tight junctions. Therefore, SIKs could be new potential biomarkers for prognosis and chemotherapy sensitivity in breast cancer.

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Disclosure of conflict of interest

None.

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