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Clinical significance of SPRY4-IT1 in efficacy and survival prediction in breast cancer patients undergoing neoadjuvant chemotherapy

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Summary. Breast cancer is the most frequent malignancy and the leading cause of cancer death among females. Long noncoding RNAs (lncRNAs) are under investigation as novel prognostic biomarkers in cancer. The aim of the study was to investigate the expression, clinical implications and prognostic significance of lncRNA SPRY4-IT1, and to identify the predictive value of SPRY4-IT1 on the outcome of chemotherapy in breast cancer patients undergoing neoadjuvant chemotherapy (NACT). Bioinformatics indicated SPRY4-IT1 was related to chemo-resistance in breast cancer. SPRY4-IT1 expression was assessed by qRT-PCR in breast cancer tissues and matched normal breast tissues (n=26 pairs). SPRY4-IT1 expression was also detected by *in situ* hybridization (ISH) in 60 paraffin slices with complete clinical datum. In this study, SPRY4-IT1 was significantly more expressed in cancer tissues than in normal tissues ($P<0.05$). Increased SPRY4-IT1 expression was significantly correlated with increased rates of lymph node metastasis ($P=0.002$) and recurrence ($P=0.017$). Both were independent factors of SPRY4-IT1 expression ($P<0.05$). High-SPRY4-IT1 patients had significantly lower overall survival and disease-free survival. High SPRY4-IT1 expression indicated poor clinical response in the whole group, luminal A subgroup and luminal B subgroup ($P<0.05$) and pathological complete response in the whole group. Overexpression of SPRY4-IT1 promoted chemo-resistance of MCF-7 and MDA-MB-231 cells to epirubicin. SPRY4-IT1 has the potential to be a biomarker to predict NACT efficacy and prognosis in breast cancer patients.

Key words: SPRY4-IT1, Neoadjuvant chemotherapy, Breast cancer, Clinical pathology factors, Prognosis

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Introduction

Globally, breast cancer is the most frequent malignancy and the leading cause of cancer death among females (Siegel et al., 2019). Some breast cancer patients are diagnosed at a locally advanced stage and 5-year survival rate is approximately 50% (Wang et al., 2015). In the field of therapy, many clinicians are striving for the goal, to identify areas where optimal care may be achieved with 'escalating' or 'de-escalating' treatment. As 'de-escalation' requires more valuable evidence and rigorous judgment, filtering novel biomarkers with prognostic function is considered to be an effective way (Zheng et al., 2019a,b).

Neoadjuvant chemotherapy (NACT) improves the clinical symptom and diminishes the volume of the tumors, which can make the clinical stage lower and has benefits for the radical operation (von Minckwitz et al., 2013). The application of NACT is not only for locally advanced breast cancer, but also for patients with T1N1M0, T2N0M0, T3N0M0 and T2N1M0 stages,

based on cTNM staging by American Joint Committee on Cancer (AJCC). Currently, anthracyclines (Doxorubicin/Epirubicin) is most commonly used in NACT (von Minckwitz et al., 2012). However, its efficacy is often affected by endogenous or acquired multidrug resistance. Therefore, the search for chemotherapy resistance-related factors are of great significance to guide the reasonable application of anthracyclines in clinical practice.

It is a long time since pathological complete response (pCR) represented a better prognosis in NACT patients (Liedtke et al., 2008). Molecular typing is also associated with NACT efficacy in breast cancer (Perou et al., 2000; Bear et al., 2012; Nabholtz et al., 2016). However, in an era of individualized treatment, these alone are far from enough. There are plenty of possible ways (such as multigene assays) to categorize breast cancer as well as by prognostic and by predictive parameters (Zheng et al., 2019a,b). To identify biomarkers to predict the clinical pathological response of NACT, seems to be an effective and feasible way (Chavez-MacGregor et al., 2017).

Long noncoding RNAs (lncRNAs) are a series of transcript RNA longer than 200 nucleotides without potential for protein-coding (Yuan et al., 2014). LncRNAs are involved in physiological and pathological processes, especially in development of malignant tumor (Prensner and Chinnaiyan, 2011; Batista and Chang, 2013; Fatica and Bozzoni, 2014; McHugh et al., 2015). LncRNAs can recruit transcription factors to regulate gene expression, and interact with mRNAs to influence the stability of mRNAs. SPRY4-IT1 gene locates at 5q31.3 (Yao et al., 2018). SPRY4-IT1 was highly-expressed in breast cancer tissues and cell lines. Another study reported that N-terminal polypeptide derived from viral macrophage inflammatory protein II (NT21MP) inhibited the biological functions of breast cancer cells through SPRY4-IT1 (Wu et al., 2018). It is unclear whether SPRY4-IT1 can act as a predictor for clinical pathological re-sponse in NACT.

Herein, we firstly reported the expression, clinical implications and prognostic significance of lncRNA SPRY4-IT1, and to identify the predictive value of SPRY4-IT1 on the outcome of chemotherapy in 60 breast cancer patients undergoing neoadjuvant chemotherapy (NACT) of epirubicin regimen. By bioinformatics and in vitro assays, SPRY4-IT1 expresssion influenced curative effect of NACT, which may be related to SPRY4-IT1 promoting chemo-resistance to epirubicin. This study provided theoretical support for finding a new biomarker in predicting NACT efficacy among breast cancer patients.

Materials and methods

TCGA analysis

All patient's data were downloaded the from TCGA-breast cancer (TCGA-BRCA) (https://cancergenome.nih.gov/). The analysis was based on processed RNA-Seq data. The edgeR package was performed to normalize gene expression. Multiple comparison correction was used. Differential expressions of genes between two groups (low/no- and high-expressing) were split by median value analyzed.

Patients, tissue samples and inclusion criteria

Breast cancer tissues and the matched normal tissues (n=60 pairs) were obtained from patients in China Medical University from 2011 to 2012. The inclusion criteria were as follows: I. Infiltrative ductal carcinoma. II. Patients with NACT indications. III. Avail-able tissues before NACT. IV. No previous treatment before NACT. V. Complete case records with follow-up information. The median age of the selected patients was 50.8 years old at diagnosis. Fresh cancer tissues and the matched adjacent normal tissues (n=22 pairs) were obtained from patients hospitalized in 2018, without follow-up information. Fresh tissues were snap frozen and stored in liquid nitrogen immediately after surgery. The study was supported by Ethics Committee of China Medical University. All procedures were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards.

Collection of clinical information

Data regarding age and tumor size were collected from Hospital Information System. The status of ER, PR, HER2, histological grade and lymph node metastases were collected from patient chart. The status of Ki67 could not be collected from patients directly, as Ki67 was not examined routinely before. Herein, pathologists were invited to do an extra detection of Ki67 in this study. OS (Overall survival) and DFS (Disease-free survival) were collected from patients or immediate family members through telephone follow-up twice a year. Patients who were lost to follow-up were excluded in this study. OS was defined from the date of diagnosis to breast cancer-related death. DFS was recorded from the date of diagnosis to the occurrence of local recurrence or distant metastasis. Follow-up continued for 80 months or until clinical outcome occurred. Among 60 patients, 17 patients (28.3%) experienced local recurrence or distant metastasis. 6 patients died of breast cancer (10.0%). The mean follow-up period was 79.3 months. Clinical stage relied on the clinical staging criteria set by the AJCC.

NACT regimen

Regimen: cyclophosphamide 1000 mg, epirubicin 80 mg/m2 every 3 weeks, capecitabine 1250 mg/m2/day twice daily for 2 weeks). The subjects received CEX regimen for a median of 4 cycles (range 4 cycles) before

surgery.

In situ hybridization (ISH)

The APES (3-Aminopropyl-Triethoxy Silane) glue was used to prevent slice escaping from glass slide. All liquid and experimental apparatus were sterilized by high temperature and high pressure, and washed with DEPC(diethyl pyrocarbonate)-treated water, to remove RNA enzymes. Slides were processed by 1 mL/L DEPC-treated water. The procedure follows manufacturers' protocol in lncRNAs ISH Kit (Boster). Firstly, the sections were de-waxed by xylene and rehydrated in graded alcohol series. Next, 3% hydrogen peroxide was used to block endogenous peroxidase activity and 3% fresh citric acid diluted pepsin was used to expose mRNA. Then, slides were incubated with 20μL preliminary hybrid liquid for 2 hours, followed by an overnight incubation with 20 μL digoxin-labeled oligonucleotide probe and hybrid liquid at 37°C. The probe for SPRY4-IT1 detection was 5'-Dig-AATTTATGTGGCTGACAAAGGA-Dig-3'. The final steps were to add blocking solution and biotinylated rat anti digoxin. The sections were visualized with DAB reagent.

Evaluation of ISH

DAB staining was evaluated by two professional pathologists who were blinded to the experiment separately. When discrepancies occured, we invited another senior pathologist who was blinded to the experiment to evaluate the scoring again. Then we came to a conclusion based on their diagnoses. SPRY4-IT1 expression was estimated by double score semi-quantitative analysis. The percentage of positive cells were scored as 0 (<5%), 1 (6-25%), 2 (26-50%), 3 (51-75%) and 4 (>76%). Staining intensity was recorded as 0 (negative), 1 (light), 2 (medium) and 3 (deep). In each slide we examined at least ten fields and 100 cells were observed each time at 400× magnification. The final score was determined by multiplying the two scores. Patients were categorized into two groups: SPRY4-IT1-high (score≥4) and SPRY4-IT1-low patients (score<4).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol reagent kit (CWBIO, China) was used to extract total RNA. RNA concentration was detected by NanoDrop 2000 spectrophotometer (Termo Scientific, USA). cDNA was compounded using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). SYBR® Green Realtime PCR Msater Mix (TOYOBO, Japan) was used. With $2^{-\Delta\Delta CT}$ method, concentration error could be eliminated. Primers used in this study were below: SPRY4-IT1 forward: 5'-AGCCACA TAAATTCAGCAGA-3', SPRY4-IT1 reverse: 5'-CGATGTAGTAGGATTCCTTTCA-3'. β-actin forward: 5'-CTGGCCGGGACCTGACT-3', β-actin reverse: 5'-TCCTTAATGTCACGCACGATTT-3'.

Evaluation of NACT response

According to Response Evaluation Criteria in Solid Tumors (RECIST1.0), the clinical response to NACT, including Complete Response (CR), Partial Response (PR), Progressive Disease (PD) and Stable Disease (SD), was evaluated by a combination of physical and imaging examinations. The clinical response after NACT was primary endpoint. The pCR, defined as the absence of invasive breast cancer cells in breast and nodes (ypT0/Tis ypN0), was the secondary endpoint. The baseline was calculated as the sum of the long diameters of primary breast tumors. CR meant all target lesions disappeared. PR represented the sum of axes decreased at least 30% from the baseline. When new lesions emerged or the sum of axes that acquired at least 20% increase on the baseline, it was called PD. SD meant the sum of axes variations between PR and PD. CR and PR were analyzed together as an efficacy group, whereas SD and PD were inefficacy group.

Cell lines and culture

Human breast carcinoma cell lines MCF-7 and MDA-MB-231 were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). MCF-7 and MDA-MB-231 cells were cultured in high-glucose (4.5 mg/ml) DMEM with 10% (v/v) FBS (HyClone), or L15 (Gibco) medium, at 37°C in a 5% CO_2 and 95% air incubator (Thermo Fisher Scientific Inc).

Cell transfection and virus infection

Lentiviral vectors (GV502) were purchased from Shanghai Genechem Co., Ltd. SPRY4-IT1 cDNA was cloned into a (polyA-MCS-UBI) RV-SV40-EGFP-IRES-puromycin vector (Genechem). MCF-7 and MDA-MB-231 were seeded into 6-well plates at a density of 1×10^5 cells per well. The culture medium was removed and the lentiviral vectors and polybrene were mixed with medium at MOI=20 for NC-cDNA or SPRY4-IT1-cDNA. The medium was replaced by fresh culture medium 24 hours after transfection. Stably transfected cells were selected by puromycin (1 μg/ml). The selection was repeated 2-3 times till green fluorescent protein (GFP) was observed in all cells under a fluorescence microscope (Nikon TE 2000-U, Japan).

Cell Counting Kit (CCK-8) assay

Cell viability was determined using a CCK-8 kit (Dojindo). Cells (5,000 cells/well) were plated in 96-well ultra-low adhesion plates. To determine IC50 values, MCF-7 and MDA-MB-231 cells were treated with different continuous concentrations of epirubicin for 48 hours. Next, the cells in each well were incubated

with 10 µl WST-8 for 4 hours at 37°C. The optical density (OD) was then measured at 450 nm using an Anthos 2010 microplate reader (Anthos Labtec Instruments GmbH, Austria).

Statistical analysis

Statistical analyses were performed using the SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). The data were analyzed by Pearson chi-square analysis or Fisher's exact test, and logistic regression analyses. Survival probabilities were judged by the Kaplan-Meier method and assessed by a log-rank test. All the statistical tests were two-sided, and a p-value of <0.05 was considered statistically significant.

Results

SPRY4-IT1 might be associated with drug-resistance of breast cancer in TCGA

To predict the association of SPRY4-IT1 and drug-resistance, we chose four recognized drug-resistance parameters, and downloaded their expression values in TCGA databases. HIF-2α (Hypoxia-inducible factor-2α) promotes stem-like phenotype in cancer stem cells (CSCs) and chemo-resistance of tumor cells. ABCG2 (ATP-binding cassette sub-family G member 2) encodes breast cancer resistance protein (Shannon et al., 2003). ABCC1 (ATP-binding cassette, sub-family C, member 1) leads to chemotherapy failure (Gao et al., 2019). ABCB1 (ATP-binding cassette subfamily B member 1) gene encodes the efflux transporter MDR1 (multidrug resistance protein 1) and variability in MDR1 transporter expression is closely tied to drug resistance. SPRY4-IT1 is associated with HIF-2α, ABCG2, ABCC1 and ABCB1 in the whole cohort in TCGA databases, as shown in Fig. 1A-D. These results suggested that the expression of SPRY4-IT1 might be associated with chemoresistance in breast cancer, adding to growing evidence that SPRY4-IT1 might be a predictor of NACT.

Expression of SPRY4-IT1 in breast cancer tissues

To elucidate whether SPRY4-IT1 contributed to breast cancer, we evaluated the expression levels of

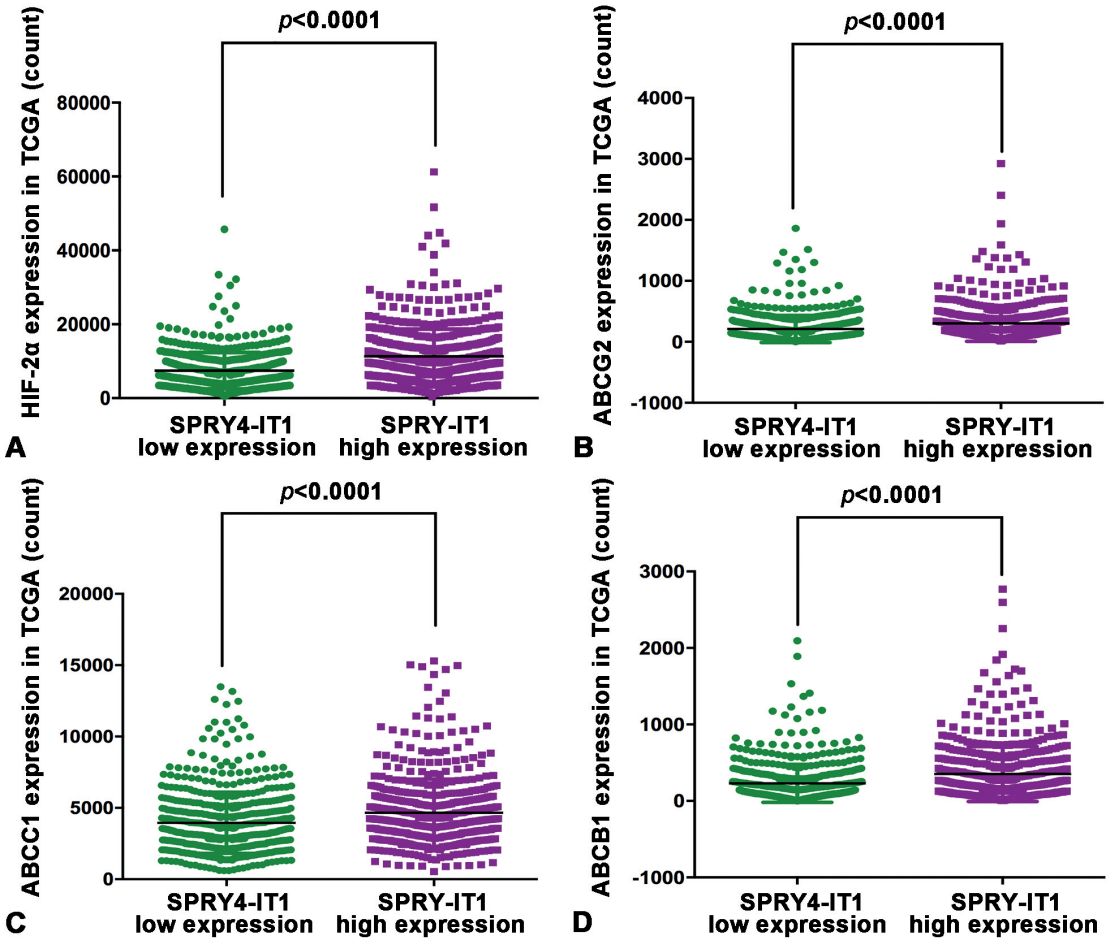

Fig 1. Differential expressions of drug-resistance parameters in low and high-expressing SPRY4-IT1 patients in the whole cohort of TCGA databases (P<0.05). **A.** HIF-2α. **B.** ABCG2. **C.** ABCC1. **D.** ABCB1.

SPRY4-IT1 by ISH. We observed a wide range of staining, including no staining, light staining, medium staining and deep staining, as shown in Fig. 2A-D. Breast cancer patients were categorized into two groups: 41 patients in SPRY4-IT1-high group (score≥4) and 19 patients in SPRY4-IT1-low patients (score<4). We defined the cut-off value of SPRY4-IT1 expression, as previously reported (Zheng et al., 2019a,b). We detected SPRY4-IT1 expression in 22 pairs of fresh specimens by qRT-PCR and found SPRY4-IT1 expression in cancer tissues was higher than matched adjacent normal tissues (P<0.01, Additional file 1: Fig. S1A). To verify the consistency of ISH and qRT-PCR result, we used 22 specimens to make paraffin sections and found that the ISH scoring had obviously positive correlation with relative expression of SPRY4-IT1 by qRT-PCR (r=0.8638, P<0.0001).

Association of SPRY4-IT1 with clinical pathology factors

To further elucidate how SPRY4-IT1 was involved in breast cancer development, we analyzed the correlation of SPRY4-IT1 expression with clinical pathology factors. Univariate analysis (Table 1) illustrated that increased SPRY4-IT1 expression was significantly correlated with increased positive rates of lymph node metastasis (P=0.002) and recurrence (P=0.017). Multivariate analysis showed lymph node metastasis and recurrence were independent factors of SPRY4-IT1 expression among breast cancer patients undergoing NACT (P<0.05).

Association of SPRY4-IT1 with prognosis

To elucidate the association of SPRY4-IT1 expression with OS and DFS in 60 cases of patients undergoing NACT, we performed Kaplan-Meier survival analysis. SPRY4-IT1-high patients had significantly lower overall survival (OS, P=0.039) and disease-free sur-vival (DFS, P=0.030) than SPRY4-IT1-low patients (Fig. 3A,B). To assess the influence of each clinical pathology variable on OS and DFS, we employed Cox regression analysis. Multivariate Cox regression analysis revealed that lymph node metastases (OS: P=0.036;

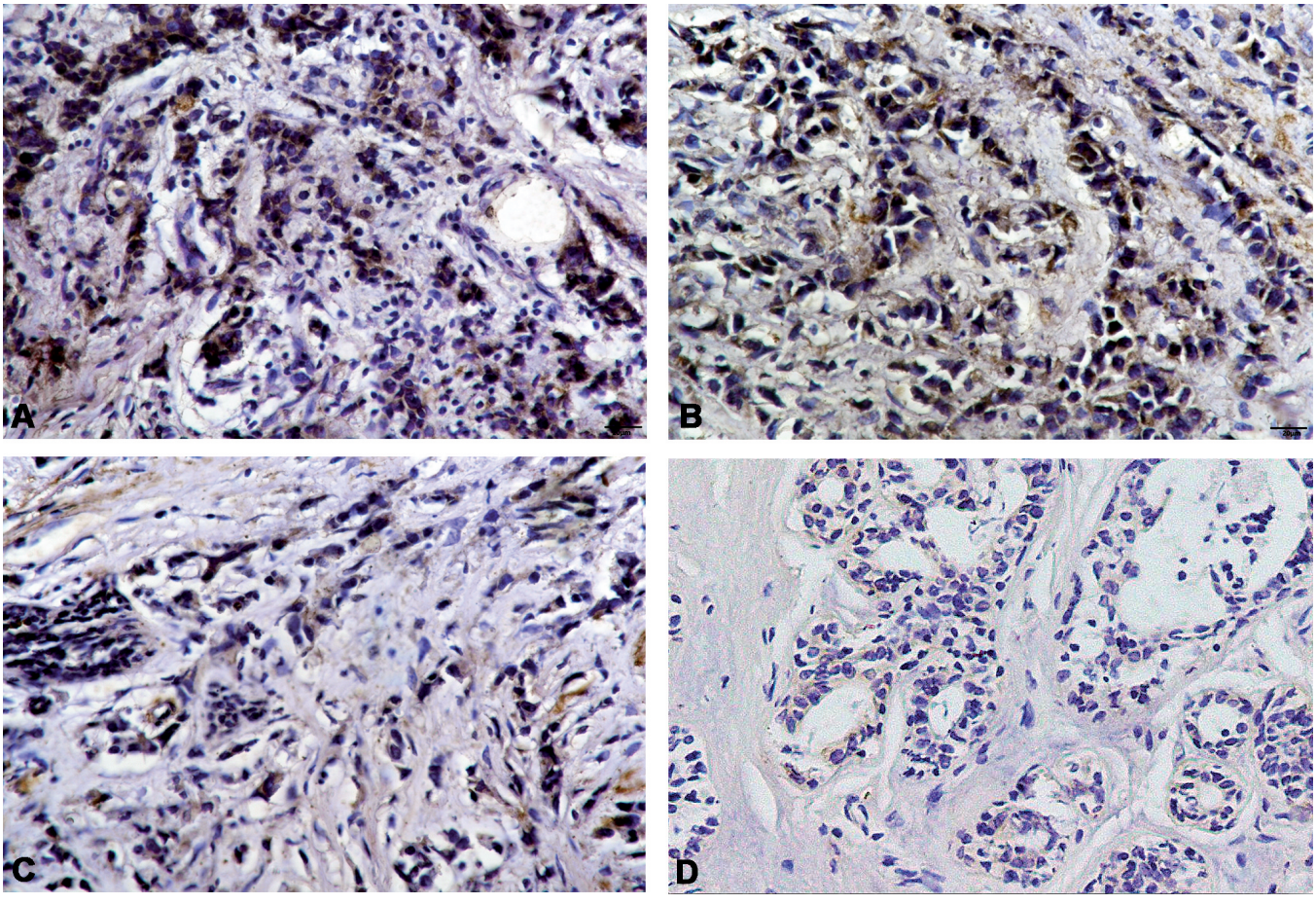

Fig 2. Staining range in ISH of SPRY4-IT1. **A.** Deep staining. **B.** Medium staining. **C.** Light staining. **D.** No staining. ISH stain. × 400.

DFS: P=0.032) and SPRY4-IT1 expression (OS: P=0.028; DFS: P=0.011) were independent prognostic factors to indicate a shorter OS and DFS in breast cancer patients.

Association of SPRY4-IT1 with the response to NACT

To illustrate the overall relationship between SPRY4-IT1 expression and NACT efficacy, we

Table 1. Univariate analysis of SPRY4-IT1 expression and clinical pathology factors.

Factors	Number (%)	SPRY4-IT1 expression		χ^2	P	Crude OR (95 CI)
		High (%)	Low (%)			
Age(years)				0.440	2.704[a]	
≤ 40	9(15.0)	5 (55.6)	4(44.4)		0.517[b]	0.536(0.081-3.533)
41-50	24(40.0)	19(79.2)	5(7.6)		0.568[b]	1.629(0.306-8.679)
51-60	17(28.3)	10(58.8)	7(41.2)		0.563[b]	0.612(0.116-3.226)
≥61	10(16.7)	7(70.0)	3(30.0)			Reference
Tumor size				0.629	0.428[a]	
≥ 3 cm	36(60.0)	26(72.2)	10(27.8)		0.429[b]	1.560(0.518-4.697)
< 3 cm	24(40.0)	15(62.5)	9(37.5)			Reference
LN Metastases				11.736	0.002[a]	
negative	12(20.0)	3(25.0)	9(75.0)			Reference
Level I metastasis	33(55.0)	26(78.8)	7(21.2)		0.002[b]	11.143(2.364-52.522)
Level II metastasis	15(25.0)	12(80.0)	3(20.0)		0.007[b]	12.000(1.947-73.971)
ER				0.372	0.542[a]	
positive	35(58.3)	25(71.4)	10(28.6)		0.543[b]	1.406(0.469-4.214)
negative	25(41.7)	16 (64.0)	9(36.0)			Reference
PR				1.371	0.242[a]	
positive	38(63.3)	28(73.7)	10(26.3)		0.245[b]	1.938(0.636-5.913)
negative	22(36.7)	13(59.1)	9(40.9)			Reference
Her2				0.144	0.704[a]	
positive	17(28.3)	11(64.7)	6(35.3)		0.704[b]	0.794(0.242-2.608)
negative	43(71.7)	30(69.8)	13(30.2)			Reference
Histological grade				1.383	0.545[a]	
2	46(76.7)	33(71.7)	13(28.3)			Reference
3	5(8.3)	3(60.0)	2(40.0)		0.588[b]	0.591(0.088-3.954)
unrated	9(15.0)	5(55.6)	4(44.4)		0.343[b]	0.492(0.114-2.127)
Molecular typing				3.987	0.272[a]	
Luminal A	27(45.0)	20(74.1)	7(25.9)		0.567[b]	1.714(0.323-9.109)
Luminal B	19(31.7)	14(73.7)	5(26.3)		0.563[b]	1.680(0.290-9.748)
Her-2	6(10.0)	2(33.3)	4(66.7)		0.288[b]	0.300(0.033-2.763)
TNBC	8(13.3)	5(62.5)	3(37.5)			Reference
Recurrence				5.743	0.017[a]	
negative	41(68.3)	24(58.5)	17(41.5)			Reference
positive	19(31.7)	17(89.5)	2(10.5)		0.027[b]	6.021(1.226-29.568)

P-value [a]: Pearson chi-square tests or Fisher's Exact Test. P-value [b]: logistic regression analyses

Table 2. Multivariate analysis of SPRY4-IT1 expression and clinical pathology factors.

Factors	Number (%)	SPRY4-IT1 expression		P	Adjusted OR (95 CI)
		High (%)	Low (%)		
Recurrence					
positive	41(68.3)	24(58.5)	17(41.5)		Reference
negative	19(31.7)	17(89.5)	2(10.5)	0.019	9.996(1.455-68.679)
LN Metastases					
negative	12(20.0)	3(25.0)	9(75.0)		Reference
Level I metastasis	33(55.0)	26(78.8)	7(21.2)	0.002	18.681(2.934-78.967)
Level II metastasis	15(25.0)	12(80.0)	3(20.0)	0.030	9.666(1.243-75.144)

evaluated the response to NACT in 60 patients. As to clinical response, patients with high SPRY4-IT1 expression obtained poor clinical response, in the whole group, luminal A subgroup and luminal B subgroup (P<0.05). As to pathological response, SPRY4-IT1-high patients obtained lower rates of pCR in the whole cohort

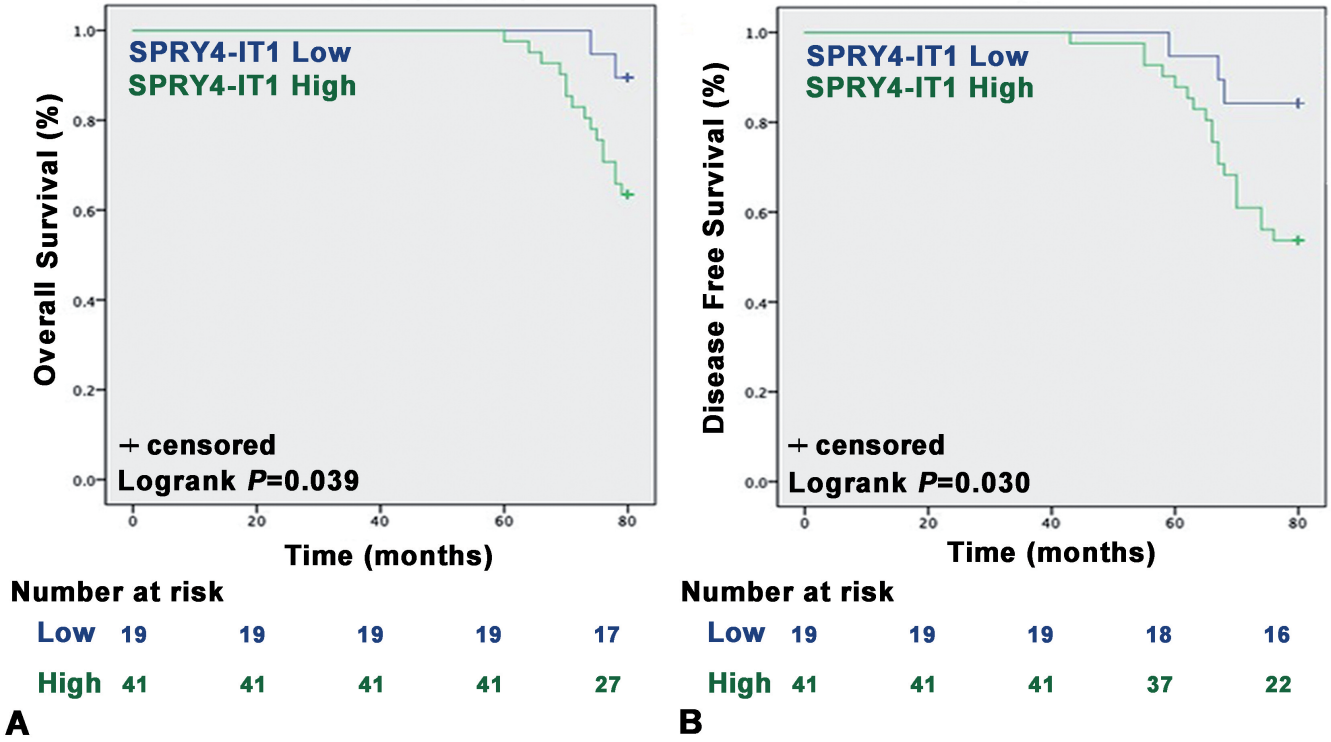

Fig 3. Correlation between SPRY4-IT1 expression and patient survival. **A.** Kaplan-Meier analysis of OS in 60 BC patients. **B.** Kaplan-Meier analysis of DFS in 60 BC patients.

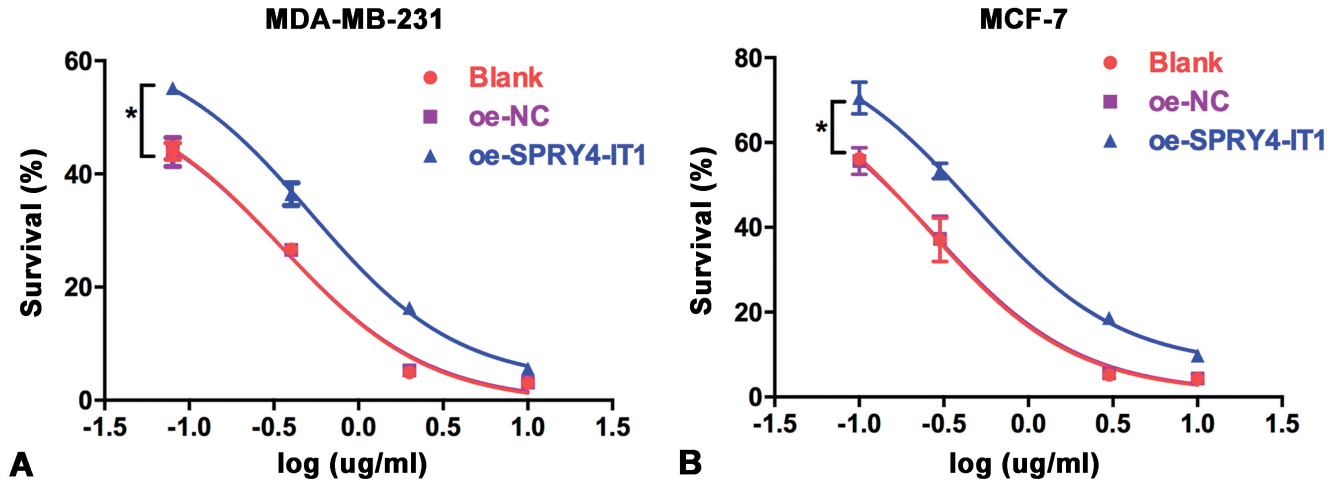

Fig 4. SPRY4-IT1 was related to epirubicin-resistance in breast cancer cells. **A.** CCK-8 assay indicated the survival rates of oe-SPRY4-IT1 MDA-MB-231 cells were higher than those of oe-NC cells. **B.** CCK-8 assay indicated the survival rates of oe-SPRY4-IT1 MCF-7 were higher than those of oe-NC cells.

Table 3. Analysis of NACT treatment response and SPRY4-IT1 expression.

Factor	N (%)	Clinical Response		P	Pathological Response		P
		SD/PD	CR/PR		No	Yes	
Total	60(100.0)	36(60.0)	24(40.0)		49(81.7)	11(18.3)	
low	19(31.7)	6(31.6)	13(68.4)	0.002	12(63.2)	7(36.8)	0.030
high	41(68.3)	30(73.2)	11(26.8)		37(90.2)	4(9.8)	
Luminal A	27(45.0)	18(66.7)	9(33.3)		22(81.5)	5(18.5)	
low	7(25.9)	2(28.6)	5(71.4)		4(57.1)	3(42.9)	
high	20(74.1)	16(80.0)	4(20.0)	0.024	18(90.0)	2(10.0)	0.174
Luminal B	19(31.7)	11(57.9)	8(42.1)		15(78.9)	4(21.1)	
low	5(26.3)	1(20.0)	4(80.0)		3(60.0)	2(40.0)	
high	14(73.7)	11(78.6)	3(21.4)	0.038	12(85.7)	2(14.3)	0.568

(P<0.05). SPRY4-IT1 expression level was significantly associated with clinical and pathological response, confirmed by Pearson Chi-square or Fisher's exact test (χ^2=9.358, P=0.002; P=0.030) (Table 3).

SPRY4-IT1 is related to epirubicin-resistance in breast cancer cells

To study the effects of SPRY4-IT1 on the sensitivity of breast cancer cell to epirubicin, we analyzed the cell survival rate via CCK-8 assay after culturing oe-NC and oe-SPRY4-IT1 MCF-7 and MDA-MB-231 cells with epirubicin for 48 hours. Epirubicin IC_{50} of MDA-MB-231 was lower than MCF-7 cells, which indicated MDA-MB-231 cells were more sensitive to epirubicin than MCF-7 cells (P<0.05). The survival rates of oe-SPRY4-IT1 MCF-7 and MDA-MB-231 cells were higher than those of oe-NC cells (P<0.05, Fig. 4A,B), which indicated that SPRY4-IT1 decreased the response of MCF-7 and MDA-MB-231 cells to epirubicin. These results indicated that SPRY4-IT1 promoted chemo-resistance to epirubicin in breast cancer and resulted in a poor clinical and pathological response.

Discussion

SPRY4-IT1 was firstly found to play a key role in apoptosis and invasion in melanoma (Khaitan et al., 2011). SPRY4-IT1 was reported to participate in the occurrence and development of pancreatic ductal adenocarcinoma (Yao et al., 2018), cholangiocarcinoma (Xu et al., 2018), ovarian cancer (Li et al., 2017), bladder cancer (Liu et al., 2017), hepatocellular carcinoma (Zhou et al., 2017), and esophageal squamous cell carcinoma (Tong et al., 2015). As to breast cancer, SPRY4-IT1 expression inhibited G1 phase arrest and apoptosis of cells, and promoted proliferation by targeting ZNF703 (Wu et al., 2018).

In this study, SPRY4-IT1 was more-expressed in breast cancer tissues than in normal breast tissues, which was consist with a previous study (Shi et al., 2015). As clinicians, we focused on the clinical application value of SPRY4-IT1. The association of SPRY4-IT1 with clinical pathology factors and prognosis has not been reported in breast cancer population undergoing NACT. Herein, SPRY4-IT1 expression was significantly correlated with increased positive rates of lymph node metastasis (P=0.002) and recurrence (P=0.017), which indicated SPRY4-IT1 might be involved in the postoperative recurrence and metastasis.

Due to non-availability of current biomarkers to precisely predict NACT efficacy, our results are a step in that direction, as we observed that SPRY4-IT1 expression had some relevance in forecasting the efficacy of NACT. This study showed that the sensitivity to chemotherapy of hormone receptor-negative cell line (MDA-MB-231) was significantly higher than that of hormone receptor-positive cell line (MCF-7) (P<0.05), as previously reported (Early Breast Cancer Trialists' Collaborative Group, 2005). Cells with high-SPRY4-IT1-expression were more resistant to epirubicin than those with low-SPRY4-IT1-expression, which indicated SPRY4-IT1 promoted chemo-resistance of breast cancer to epirubicin. Patients with high-SPRY4-IT1-expression obtained poor clinical response, in the whole group, luminal A subgroup and luminal B subgroup (P<0.05), which indicated that SPRY4-IT1 might exert its biological functions through hormone receptor signal transduction.

Although total sample size is enough, we have to acknowledge the statistical limitations of the small sample size in subgroups. We need multicenter, large sample, long-term clinical observation to confirm this conclusion. Since the molecular function of SPRY4-IT1 has not been fully explored, in-depth study is expected to explore the underlying mechanism of SPRY4-IT1 as a novel biomarker for breast cancer.

In conclusion, increased SPRY4-IT1 expression was significantly correlated with more lymph node metastasis, more recurrence and poor prognosis. SPRY4-IT1 promoted epirubicin resistance in breast cancer cells. SPRY4-IT1 might be a novel biomarker to predict NACT efficacy and prognosis in breast cancer.

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