Current Problems in Cancer 44 (2020) 100559

Contents lists available at ScienceDirect

Current Problems in Cancer

journal homepage: www.elsevier.com/locate/cpcancer

Roles and correlation of FOXA1 and ZIC1 in breast cancer

Yu-wei Zhang[a,1], Jun Ma[b,1], Chun-tao Shi[c], Wei Han[a], Xiao-jiao Gao[d], Ming-hui Zhou[e], Hou-zhong Ding[a], Hao-nan Wang[f,*]

[a] Department of General Surgery, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan, Jiangsu, China
[b] Department of Urology, Kunshan Hospital of Traditional Chinese Medicine, Kunshan, Jiangsu, China
[c] Department of General Surgery, Wuxi Xishan People's Hospital, Wuxi, Jiangsu, China
[d] Department of Pathology, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan, Jiangsu, China
[e] Centralab, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan, Jiangsu, China
[f] Oncology Department, Wuxi Fifth People's Hospital, Wuxi, Jiangsu, China

ABSTRACT

The aim of this study was to evaluate the prognostic role of Forkhead box A1 (FOXA1) in breast cancer and determine the relationship between FOXA1 and zinc finger of the cerebellum 1 (ZIC1). BCIP, GEPIA, and Oncomine databases were used to detect expression of FOXA1 and assess prognostic roles of FOXA1 and ZIC1 in invasive breast tumors. A total of 113 female invasive breast cancer cases were collected to investigate FOXA1 and ZIC1 expression via immunohistochemistry. Twenty pairs of frozen-thawed tumors were used to select reliable indicators via western blotting and real-time quantitative polymerase chain reaction. In addition, Kaplan-Meier curves and Cox regression analysis were performed to analyze the overall survival (OS) and relapse-free survival. Multiple databases showed that FOXA1 expression was elevated in invasive breast cancer and negatively related to ZIC1. BCIP database also displayed a poor prognosis of high FOXA1 and low ZIC1. FOXA1 was positively associated with tumor size, grading, lymph node metastasis, and Tumor Node Metastasis (TNM) staging, while ZIC1 expression was negatively related to grading, lymph node

☆ Conflict of Interest: None.

☆☆ This study was supported by the Foundation from Jiangsu University Science and Technology Program of Clinical Medicine (major project, grant no. JLY20180013), Wuxi Youth Projection of Health and family planning commission (No. Q201756), Wuxi Scientific and Technological Development of Medical and Health Guidance Programs (No. NZ2019014) and Kunshan Major Project of Social Research and Development (No. KS19038).

* Correspondence to: Hao-nan Wang, Oncology Department, Wuxi Fifth People's Hospital, Wuxi, Jiangsu, 214000, China.

E-mail address: whn15066940731@163.com (H.-n. Wang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.currproblcancer.2020.100559

metastasis, and TNM staging. In Kaplan-Meier and Cox regression analysis, FOXA1 negative group and ZIC1 positive group had better OS rate and recurrence-free survival rate. In addition, a joint evaluation showed that "FOXA1- ZIC1+" had the highest OS and relapse-free survival, but "FOXA1+ ZIC1-" had the lowest ones. FOXA1 was negatively related to ZIC1 in breast cancer and they had different roles in clinicopathology and prognosis. Combined examination of FOXA1 and ZIC1 could bring more benefit to breast cancer patients.

ARTICLE INFO

Keywords: FOXA1; ZIC1; Invasive breast cancer; Prognosis

Introduction

As one of the most common malignancies in women, breast cancer (BC) is severely threatening the human health and social development.[1] However, rapid progress, recurrence, and drug resistance are still the obstacles in the process of anticancer treatment, in the result of the pathogenesis of BC remains obscure.[2] Thus, further researches should be conducted to explore mechanisms of BC carcinogenesis in order to improve the prognosis of BC patients.

Forkhead box A1 (FOXA1) belongs to the forkhead box family of transcription factors, containing a forkhead DNA-binding domain.[3] FOXA1 is not only essential for development and functional integrity of human various tissues, but also crucial for the development of carcinomas.[4-7] Recent studies have shown that FOXA1 can become a potential oncogene in the development of tumors, like hepatocellular carcinoma, non–small cell lung cancer and glioma.[8-10] In BC, researchers demonstrated that FOXA1 was a promising candidate as a therapeutic and prognostic target.[11-12] However, they disagreed with the prognostic role of FOXA1.[13-14]

Zinc finger of the cerebellum 1 (ZIC1), a member of ZIC family, is essential for development of human nervous system, in the result of its 5 Cys2His2 zinc-finger domains interacting with the Gli family proteins.[15] Recently, reports have argued that ZIC1 is putative tumor-suppressor gene in various neoplasms, including BC.[16,17] Its downregulation has been associated with worse prognosis in patients with BC.[18]

In public databases, a significantly negative relationship between FOXA1 and ZIC1 can be found (Supplementary 1). This indicates that overexpressed FOXA1 may be related to development of BC. One study also found a higher expression of FOXA1 in BC compared with matched adjacent normal breast tissues.[19] Therefore, we designed this study to assess the prognostic role of FOXA1 in BC and determine the relationship between FOXA1 and ZIC1.

Matedals and methods

Data mining of datasets

BCIP (http://www.omicsnet.org/bcancer/database), GEPIA (http://gepia.cancer-pku.cn/), and Oncomine (https://www.oncomine.org/) databases were all used to analyze differences of FOXA1 expression between invasive breast tumors and normal breast tissues. BCIP and GEPIA databases were also used to evaluate the relationship between FOXA1 and ZIC1. In addition, to assess prognostic roles of FOXA1 and ZIC1 in invasive breast tumors, BCIP database were performed again.

Patients and tissue samples

A total of 113 female invasive BC patients were enrolled, with a mean age of 51.56 ± 9.23 years. All of them underwent modified radical mastectomy or mastectomy (only for TNM staging

Table 1
Clinicopathological parameters of 113 patients with invasive breast cancer.

Parameters	N/mo
Total	113
Year	
≤51	62
>51	51
Location	
Left	55
Right	58
Tumor size	
≤2 cm	44
>2 cm, ≤5 cm	61
>5 cm	8
Grading	
G1	35
G2	57
G3	21
Lymph node metastasis	
Positive	68
Negative	45
TNM staging	
I	16
II	64
III	30
IV	3
ER	
Positive	53
Negative	60
PR	
Positive	65
Negative	48
HER2	
Positive	44
Negative	69
Hormonal treatment	
Yes	80
No	33
Targeted therapy	
Yes	35
No	78
Mean follow-up time	51.50±15.05

IV) in Wuxi Xishan People's Hospital from January, 2010 to December, 2013, with complete clinicopathologic data. All patients' breast tumors and corresponding normal tissues were available for paraffin sectioning. None had received radiotherapy or chemotherapy before surgery. Follow-up data were available for all cases, with a duration ranged from 3 to 60 months. More details of clinicopathologic data were listed in Table 1. In addition, 20 pairs of fresh-frozen breast tumors and matched normal tissues (stored at −80°C) were collected from Kunshan First People's Hospital. Every patient signed the informed consent form. This research achieved the ethical approval of Wuxi Xishan People's Hospital Ethics Committee and Kunshan First People's Hospital Ethics Committee.

Immunohistochemistry (IHC)

A SP Rabbit & Mouse horse radish peroxidase (HRP) Kit (CWBIO, China) was used for IHC. Rabbit antihuman FOXA1 monoclonal antibodies and rabbit antihuman ZIC1 polyclonal antibodies (Bioss, China) were used as the primary antibodies diluted at 1:100 in phosphate-buffered

saline. Deparaffinized slides were boiled in a citrate buffer solution with a concentration of 10 mmol/L. Then these chips were treated with blocking buffers and incubated with the primary antibodies for 12 hours. Next, slides marked by streptavidin with horseradish peroxidise were developed by diaminobenzidine (DAB), and were counterstained by hematoxylin. Finally, these chips were dehydrated and mounted for storing and observing.

Evaluation of IHC staining

Scores of percentages of positive cells were measured by ImageJ 1.52r ($P = 0\%$-100%). Two pathologists (XJG and FC) assessed the staining intensity of FOXA1 and ZIC1 expression ($I = 0$, no staining of cells; 1, mild staining; 2, moderate staining; and 3, marked staining). A total score, called immunoreactivity score ($IRS = P \times I$), ranging from 0 to 300, was used for the evaluation of expression levels of FOXA1 and ZIC1. The mean IRS of FOXA1 or ZIC1 was defined as the cut-off value of FOXA1 or ZIC1, which would be used for the assessment of prognosis. estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (Her-2) statuses were considered as positive if more than 10% of tumor cells showed staining.[20] Any disagreement of IRS was resolved by discussion.

Western blotting

Twenty pairs of fresh-frozen invasive BC tumors and corresponding normal tissues were used for western blotting analysis. Total proteins were extracted from representative tumor regions and normal breast tissues by a RIPA Lysis Buffer (Beyotime Biotechnology, China). Then supernatants in which concentrations of proteins were measured by Bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology, China) were collected and mixed with SDS-PAGE Loading Buffer (Beyotime Biotechnology, China). By boiling for 8-10 minutes, they were resolved on ExpressPlus PAGE Gel (Genscript, China) and transferred to PVDF membranes (Millipore, Bedford, MA). After blocking membranes in Tris Buffered Saline + Tween confining liquid, with 5% nonfat dry milk for 2 hours at room temperature, the primary antibodies, including FOXA1 (diluted at 1:800), ZIC1 (diluted at 1:500), and GAPDH (Rabbit Monoclonal antibody, diluted at 1:2000, Abcam, Eugene, OR), were used to incubate them in 4°C overnight. Then corresponding secondary antibodies with HRP were used to incubate these membranes for 2 hours at 37°C. Finally, protein bands were detected through an Enhanced Chemiluminescence Detection System (Beyotime, China). The formula of relative expression levels of proteins quantified with Image J was relative protein expression = Gray Value (FOXA1/ZIC1)/Gray Value (GAPDH).

RT-qPCR

Frozen-thawed tissues were also used to isolate total RNA through Trizol regent (Thermo Fisher Scientific). Two microgram RNA of each sample was reverse transcribed using the SuperScript II RNase-Reverse Transcriptase system (Thermo Fisher Scientific). cDNA was subjected to quantitative PCR using primers specific for FOXA1, ZIC1, and GAPDH. PCR primers were designed as follows: FOXA1 forward primer, 5′-AACTGTGAAGATGGAAGGGCA-3′ and reverse primer, 5′-GGGTTGGCATAGGACATGTTG-3′ (204 bp); ZIC1 forward primer, 5′-GCGTCCTTTTGTGGATCTTTAA-3′ and reverse primer, 5′-AGTAATCACATCTGCTTCTGGG-3′ (178 bp); and GAPDH (internal control) forward primer, 5′-GAAGGTGAAGGTCGGAGT-3′ and reverse primer, 5′-GAAGATGGTGATGGGATTTC-3′ (226 bp). The PCR cycling conditions were as follows: 94°C for 4 minutes, followed by 40 cycles of 95°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute. Amplifed DNA was measured by the SYBR Premix Ex Taq kit (Takara Bio, Japan), and quantitative polymerase chain reaction (qPCR) was performed using an iQ5 real-time PCR

detection system (Bio-Rad). $2^{-\Delta\Delta Ct}$ value was used to calculate the relative expression and $\Delta\Delta Ct = (Ct_{Tumor\text{-}FOXA1/ZIC1} - Ct_{Tumor\text{-}GAPDH}) - (Ct_{Normal\text{-}FOXA1/ZIC1} - Ct_{Normal\text{-}GAPDH})$. A higher level of $2^{-\Delta\Delta Ct}$ meant a higher mRNA expression.

Statistical analysis

All experiments were performed in triplicate. Continuous variables were expressed as the $\bar{x} \pm$ SD and the differences of FOXA1 and ZIC1 expression between breast tumors and matched normal tissues were analyzed by a paired-*t* test. The relationship between FOXA1 and ZIC1 was assessed by Pearson correlative analysis. A $P < 0.05$ was considered statistically significant and results were analyzed by SPSS 20.0 software or GraphPad Prism 6.0. In addition, Kaplan-Meier curves with Log Rank test and the Cox univariate and multivariate regression analyses were performed to evaluate prognostic roles of factors in overall survival (OS) and relapse-free survival (RFS).

Results

FOXA1 expression was elevated in invasive breast cancer in databases

In BCIP database, TCGA data showed that FOXA1 expression in breast tumors was significantly higher than in adjacent normal tissues (Supplementary 1A). GEPIA database also displayed a higher level of FOXA1 in breast tumors compared with normal breast tissues (Supplementary 1B). Furthermore, the same results could be found in Oncomine database (Supplementary 1C).

Relationships between FOXA1 and ZIC1 expression in invasive breast cancer in databases

BCIP and GEPIA databases were also used to identify coexpression genes. As a result, a strikingly negative relationship between FOXA1 and ZIC1 was established in databases ($P < 0.001$, Supplementary 1D and 1E). Besides, survival curves of OS performing in BCIP database showed a poor prognosis of high FOXA1 expression and low ZIC1 expression (Supplementary 1F).

Associations of FOXA1 and ZIC1 expression with clinicopathologic factors

To analyze clinicopathologic roles of FOXA1 and ZIC1 in invasive BC, 113 samples were examined via IHC. FOXA1 were detected in the nucleus with higher expression in tumors compared with adjacent normal tissues (93.5 ± 61.3 vs 38.5 ± 21.4, $P < 0.001$, Fig 1A), while ZIC1 were investigated in the nucleus and cytoplasm with lower expression than normal (65.0 ± 46.5 vs 98.4 ± 47.9, $P < 0.001$, Fig 1B). In breast tumors, there was also a significantly negative correlation between FOXA1 and ZIC1 ($P = 0.01$, Fig 1C). In addition, higher expression of FOXA1 and lower expression of ZIC1 in breast tumors were then identified by western blotting and qRT-PCR (Fig 1D and E).

Next, their clinicopathologic roles were assessed. FOXA1 expression was positively associated with tumor size, grading, lymph node metastasis, and TNM staging, while ZIC1 expression was negatively related to grading, lymph node metastasis, and TNM staging (Fig 2). They were both independent of age, location, ER, PR, HER2, hormonal treatment and targeted therapy (Supplementary 2).

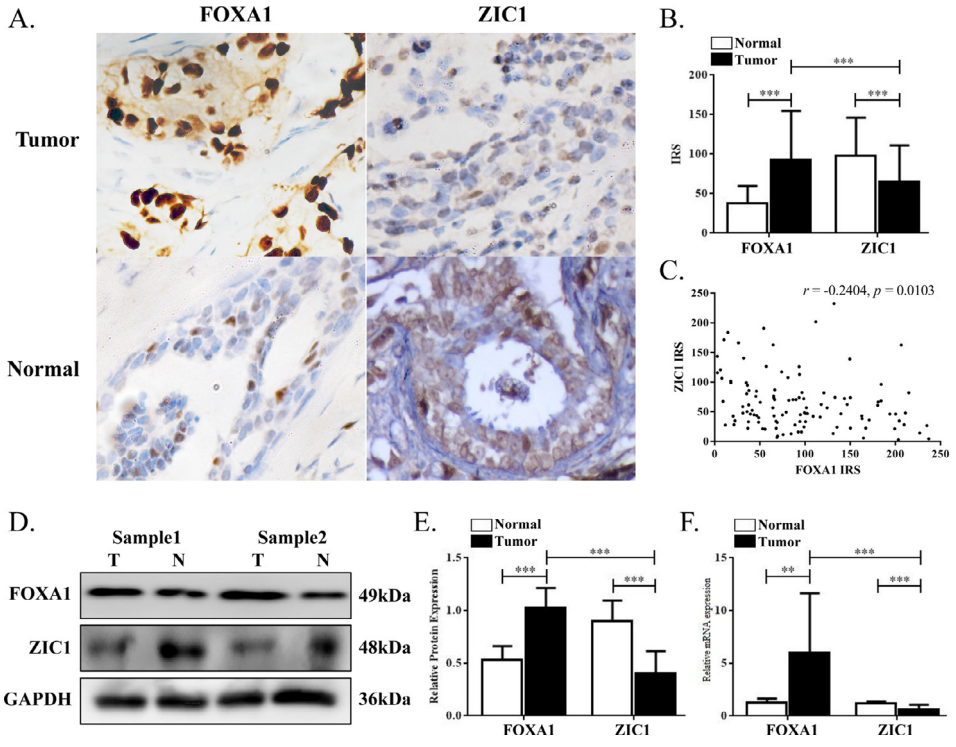

Fig. 1. Expression of FOXA1 and ZIC1 in breast tumors and corresponding normal tissues. (A) Immunohistochemical staining of FOXA1 and ZIC1 in breast tumors and corresponding normal tissues (×400 magnification); (B) Comparison of FOXA1 and ZIC1 between breast tumors and matched normal tissues; (C) Relationship between FOXA1 and ZIC1 expression by Pearson correlative analysis; (D) Western blotting analysis of FOXA1 and ZIC1 in 20 pairs of breast tumors and matched normal tissues. (E) Relative protein expression of FOXA1 and ZIC1; (F) RT-qPCR analysis of FOXA1 and ZIC1 in 20 pairs of breast tumors and matched normal tissues. "N": normal tissue, "T": breast tumor. ** $P < 0.01$. *** $P < 0.001$.

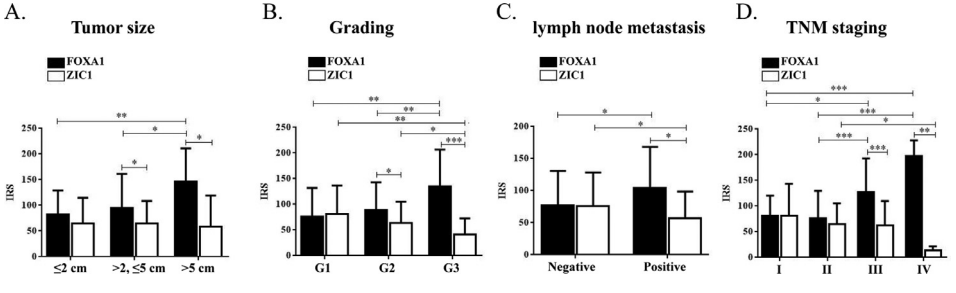

Fig. 2. Associations of FOXA1 and ZIC1 expression with various clinicopathological factors of 113 patients with invasive breast cancer. (A) tumor size; (B) Grading; (C) lymph node metastasis; (D) TNM staging. * $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$.

Associations of FOXA1 and ZIC1 expression with prognosis

According to the average IRS, we set the cut-offs: 93.5 for FOXA1 and 65.0 for ZIC1. One case with a score > cut-off would be included into the positive group. Thus, the FOXA1 positive group

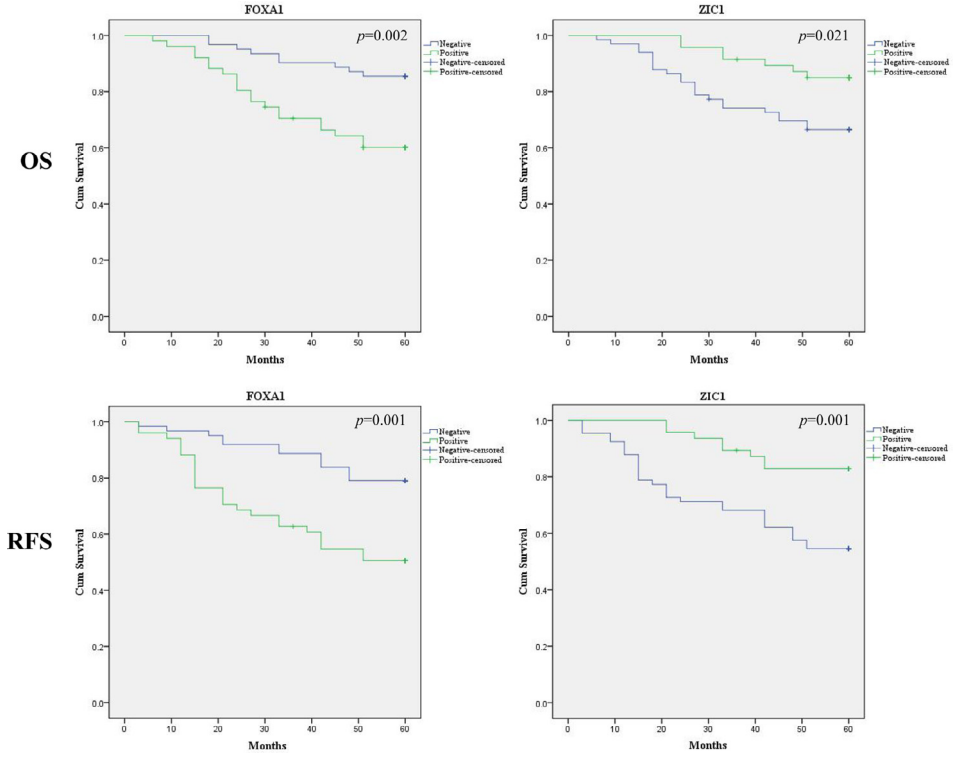

Fig. 3. Kaplan-Meier survival curves of overall survival (OS) and relapse-free survival (RFS) for FOXA1 and ZIC1 expression in invasive breast cancer.

had 51 cases and ZIC1 positive group involving 47 cases. Then, to evaluate the prognostic roles of FOXA1 and ZIC1, Kaplan-Meier analysis and Cox regression analysis were both performed.

In Kaplan-Meier analysis, FOXA1 negative group and ZIC1 positive group had better overall survival rate and recurrence-free survival rate (Fig 3). Similar results could be found in Cox regression analysis. Univariate analysis showed that FOXA1 (HR = 3.274, 95%CI: 1.489-7.199, P = 0.003), ZIC1 (HR = 0.384, 95%CI: 0.164-0.900, P = 0.028), lymph node metastasis (HR = 2.939, 95%CI: 1.196-7.221, P = 0.019), TNM stage (HR = 2.850, 95%CI: 1.374-5.912, P = 0.005), and Targeted therapy (HR = 0.334, 95%CI: 0.116-0.961, P = 0.042) were significantly associated with OS (Table 2). However, only FOXA1 (HR = 3.194, 95%CI: 1.449-7.040, P = 0.004), ZIC1 (HR = 0.393, 95%CI: 0.167-0.922, P = 0.032), and lymph node metastasis (HR = 3.195, 95%CI: 1.295-7.879, P = 0.012) were independent factors for OS in multivariate analysis (Table 2).

Besides, FOXA1 (HR = 2.965, 95%CI: 1.514-5.807, P = 0.002), ZIC1 (HR = 0.308, 95%CI: 0.141-0.672, P = 0.003), lymph node metastasis (HR = 2.568, 95%CI: 1.214-5.430, P = 0.014), and TNM stage (HR = 2.642, 95%CI: 1.390-5.020, P = 0.003) were related to RFS in the univariate analysis (Table 3). Also, FOXA1 (HR = 2.871, 95%CI: 1.461-5.642, P = 0.002), ZIC1 (HR = 0.316, 95%CI: 0.144-0.691, P = 0.004), and lymph node metastasis (HR = 2.855, 95%CI: 1.345-6.062, P = 0.006) were 3 independent factors for RFS in multivariate analysis (Table 3).

To assess the combined role on prognosis of invasive BC patients, a joint evaluation composed of FOXA1 and ZIC1 expression was used in survival analysis. In Kaplan-Meier curves, "FOXA1- ZIC1+" had the highest OS and RFS, while "FOXA1+ ZIC1-" earned the lowest ones (Fig 4). In addition, "FOXA1- ZIC1+" had a hazard ratio (HR) being 0.276 (95%CI: 0.083-0.912, P = 0.035) for OS and a HR being 0.193 (95%CI: 0.059-0.628, P = 0.006) for RFS; while "FOXA1+ ZIC1-" had a

Table 2
Prognostic value of FOXA1, ZIC1, and clinicopathological factors for the overall survival of patients with invasive breast cancer by univariate and multivariate analyses with Cox regression.

Parameters	HR	95%CI	*P*
Univariate analysis			
FOXA1 expression: positive vs negative	3.274	1.489-7.199	0.003
ZIC1 expression: positive vs negative	0.384	0.164-0.900	0.028
Year: ≤51 vs >51 y	1.596	0.768-3.319	0.210
Location: right vs left	0.714	0.343-1.484	0.367
Tumor size: ≤2 vs >2 cm	1.138	0.537-2.410	0.736
Histological grade: 3 and 2 vs 1	1.080	0.492-2.373	0.848
Lymph node metastasis: yes vs no	2.939	1.196-7.221	0.019
TNM stage: III and IV vs I and II	2.850	1.374-5.912	0.005
ER: positive vs negative	1.257	0.607-2.605	0.538
PR: positive vs negative	1.085	0.518-2.272	0.828
Her-2: positive vs negative	0.488	0.208-1.142	0.098
Hormonal treatment: yes vs no	0.790	0.367-1.700	0.547
Targeted therapy: yes vs no	0.334	0.116-0.961	0.042
Multivariate analysis			
FOXA1 expression: positive vs negative	3.194	1.449-7.040	0.004
ZIC1 expression: positive vs negative	0.393	0.167-0.922	0.032
Lymph node metastasis: yes vs no	3.195	1.295-7.879	0.012

Table 3
Prognostic value of FOXA1, ZIC1, and clinicopathological factors for the relapse-free survival of patients with invasive breast cancer by univariate and multivariate analyses with Cox regression.

Parameters	HR	95%CI	*P*
Univariate analysis			
FOXA1 expression: positive vs negative	2.965	1.514-5.807	0.002
ZIC1 expression: positive vs negative	0.308	0.141-0.672	0.003
Year: ≤51 vs >51 y	1.327	0.702-2.506	0.384
Location: right vs left	0.782	0.414-1.479	0.450
Tumor size: ≤2 vs >2 cm	1.211	0.626-2.341	0.570
Histological grade: 3 and 2 vs 1	1.035	0.522-2.052	0.921
Lymph node metastasis: yes vs no	2.568	1.214-5.430	0.014
TNM stage: III and IV vs I and II	2.642	1.390-5.020	0.003
ER: positive vs negative	1.034	0.547-1.955	0.918
PR: positive vs negative	0.936	0.494-1.774	0.839
Her-2: positive vs negative	0.603	0.299-1.217	0.158
Hormonal treatment: Yes vs No	0.621	0.324-1.191	0.152
Targeted therapy: yes vs no	0.541	0.248-1.182	0.123
Multivariate analysis			
FOXA1 expression: positive vs negative	2.871	1.461-5.642	0.002
ZIC1 expression: positive vs negative	0.316	0.144-0.691	0.004
Lymph node metastasis: yes vs no	2.855	1.345-6.062	0.006

HR being 3.774 (95%CI: 1.812-7.863, $P < 0.001$) for OS and a HR being 3.588 (95%CI: 1.893-6.802, $P < 0.001$) for RFS.

Discussion

Though with chemotherapy and targeted therapy followed by operation, recurrence, and metastasis remain the major risk of death from BC. Novel biomarkers appearing provides new research orientation and prospect for wiping out cancer. Therefore, determining the significance of these biomarkers is pivotal for the exploration of potential anticancer drugs or micromolecules and the assessment of therapy and prognosis in BC patients.

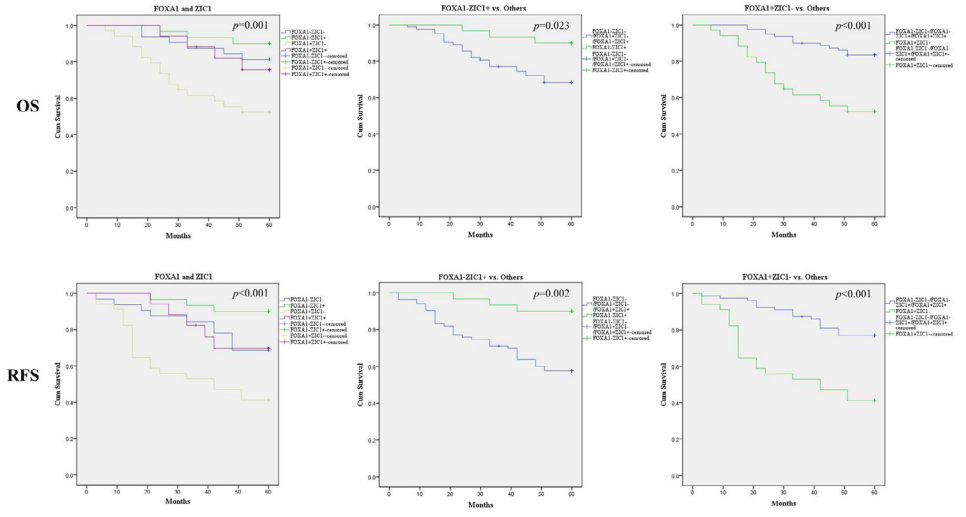

Fig. 4. Kaplan-Meier survival curves of overall survival (OS) and relapse-free survival (RFS) for combined examination of FOXA1 and ZIC1 in invasive breast cancer.

Previous studies demonstrated that overexpressed FOXA1 was involved in the development of various carcinomas.[8-10] However, the roles of FOXA1 in BC were contradictory in previous studies.[13,14,21] One research showed that FOXA1 inhibited BC cell proliferation and migration via downregulating SOD2 and IL6 expression.[21] But another one reported that high expression of FOXA1 indicated a good prognosis.[14]

In this research, FOXA1 as well as ZIC1 was identified to be a potential BC biomarker through a series of bioinformatics analysis. We investigated these 2 molecules to analyze their relationship and evaluate their prognostic roles in BC. First, FOXA1 expression in tumors was higher than in paired adjacent normal breast tissues and its expression level was negatively correlated with ZIC1. Besides, they owned opposite clinicopathologic and prognostic roles in BC. Based on results of public databases and previous studies, we argue that FOXA1 is an indicator of poor prognosis, while high ZIC1 predicts a good prognosis. In addition, according to Kaplan-Meier curves and Cox regression analyses, patients with "FOXA1- ZIC1+" had the highest OS and RFS in all groups, while those with "FOXA1+ ZIC1-" had the lowest survival rates. Therefore, the combined examination of FOXA1 and ZIC1 could enhance the precision of prognostic assessment, and BC patients could retrieve more benefit from this evaluation method.

Several studies demonstrated that FOXA1 is indispensable for the expression of ER in BC.[21,22] However, our research showed that there was no difference of FOXA1 expression between "ER positive" group and "ER negative" group as well as ZIC1 expression. Previous studies also showed ZIC1 expression not related to ER, PR, and HER-2 in BC.[17,18] Though without any correlation with ER, FOXA1 expression was inversely related to ZIC1. Abnormal expression of FOXA1 and ZIC1 may be crucial for the development of BC. The major role of FOXA1 is to open up the tightly coiled chromatins and modulate activation of transcription factors.[23] As a transcription factor, ZIC1 acts as a tumor suppressor in BC via the regulation of Akt/mTOR/P70S6K pathway.[24] We therefore speculated that the mutual regulation between FOXA1 and ZIC1 might play a pivotal in the development of BC. Further researches should be performed to confirm the interrelation of FOXA1 and ZIC1 in vitro and in vivo.

Due to no relationship of FOXA1/ZIC1 with ER, PR, or HER-2, cases should be analyzed according to different BC subtypes after collection of more specimens. Shi et al demonstrated that lower expression of ZIC1 indicated worse prognosis in Triple-Negative Breast Cancer (TNBC) patients.[17] Recently, TNBC has been divided into several subtypes, including "AR positive," in order

to explore novel therapeutic targets.[25] Among 349 nonmetastatic TNBC patients, patients with androgen receptor + /FOXA1 + had the shortest RFS and OS.[14] However, Guiu et al dicovered that there was lower expression of FOXA1 in TNBC than in other subtypes, and high level of FOXA1 was significantly associated with better patient outcome in clinics.[21] Though with different views, these discoveries revealed that FOXA1 and ZIC1 was involved in the TNBC tumorigenesis. Further studies should be conducted to identify roles of FOXA1 and ZIC1 in TNBC and other subtypes.

In conclusion, FOXA1 was upregulated in BC compared with matched adjacent normal tissues and was negatively related to ZIC1. In addition, these 2 biomarkers had opposite roles in clinicopathology and prognosis. Therefore, the combined examination of FOXA1 and ZIC1 could bring more benefit to BC patients. Further research is still required to confirm mutual regulation ad functions of FOXA1 and ZIC1 in BC subtypes.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2020.100559.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424.
2. Peart O. Breast intervention and breast cancer treatment options. *Radiol Technol*. 2015;86:535M–558M.
3. Parolia A, Cieslik M, Chu SC, et al. Distinct structural classes of activating FOXA1 alterations in advanced prostate cancer. *Nature*. 2019;571:413–418.
4. Gasser E, Johannssen HC, Rülicke T, Zeilhofer HU, Stoffel M. Foxa1 is essential for development and functional integrity of the subthalamic nucleus. *Sci Rep*. 2016;6:38611.
5. Li S, Zheng K, Pei Y, Wang W, Zhang X. Long noncoding RNA NR2F1-AS1 enhances the malignant properties of osteosarcoma by increasing forkhead box A1 expression via sponging of microRNA-483-3p. *Aging (Albany NY)*. 2019;11:11609–11623.
6. Liu BL, Qin JJ, Shen WQ, et al. FOXA1 promotes proliferation, migration and invasion by transcriptional activating KRT7 in human gastric cancer cells. *J Biol Regul Homeost Agents*. 2019;33:1041–1050.
7. Park YL, Kim SH, Park SY, et al. Forkhead-box A1 regulates tumor cell growth and predicts prognosis in colorectal cancer. *Int J Oncol*. 2019;54:2169–2178.
8. Wang Y, Yang L, Chen T, et al. A novel lncRNA MCM3AP-AS1 promotes the growth of hepatocellular carcinoma by targeting miR-194-5p/FOXA1 axis. *Mol Cancer*. 2019;18:28.
9. Li J, Zhang S, Zhu L, Ma S. Role of transcription factor FOXA1 in non-small cell lung cancer. *Mol Med Rep*. 2018;17:509–521.
10. Li Q, Lu J, Xia J, Wen M, Wang C. Long non-coding RNA LOC730100 enhances proliferation and invasion of glioma cells through competitively sponging miR-760 from FOXA1 mRNA. *Biochem Biophys Res Commun*. 2019;512:558–563.
11. Fu X, Pereira R, De Angelis C, et al. FOXA1 upregulation promotes enhancer and transcriptional reprogramming in endocrine-resistant breast cancer. *Proc Natl Acad Sci U S A*. 2019;116:26823–26834 pii: 201911584.
12. Forma E, Jóźwiak P, Ciesielski P, et al. Impact of OGT deregulation on EZH2 target genes FOXA1 and FOXC1 expression in breast cancer cells. *PLoS One*. 2018;13.
13. BenAyed-Guerfali D, Dabbèche-Bouricha E, Ayadi W, et al. Association of FOXA1 and EMT markers (Twist1 and E–cadherin) in breast cancer. *Mol Biol Rep*. 2019;46:3247–3255.
14. Guiu S, Mollevi C, Charon-Barra C, et al. Prognostic value of androgen receptor and FOXA1 co-expression in non-metastatic triple negative breast cancer and correlation with other biomarkers. *Br J Cancer*. 2018;119:76–79.
15. Aruga J, Hatayama M. Comparative genomics of the Zic Family Genes. *Adv Exp Med Biol*. 2018;1046:3–26.
16. Nakakido M, Tamura K, Chung S, et al. Phosphatidylinositol glycan anchor biosynthesis, class X containing complex promotes cancer cell proliferation through suppression of EHD2 and ZIC1, putative tumor suppressors. *Int J Oncol*. 2016;49:868–876.
17. Shi CT, Ma J, Shi QF, Zhang Y, Wang HN. High survivin and low zinc finger of the cerebellum 1 expression indicates poor prognosis in triple-negative breast carcinoma. *J Breast Cancer*. 2019;22:248–259.
18. Han W, Zhang C, Gao XJ, et al. Clinicopathologic and prognostic significance of the zinc finger of the cerebellum family in invasive breast cancer. *J Breast Cancer*. 2018;21:51–61.
19. Jing X, Liang H, Hao C, Hongxia L, Cui X. Analyses of an epigenetic switch involved in the activation of pioneer factor FOXA1 leading to the prognostic value of estrogen receptor and FOXA1 co-expression in breast cancer. *Aging (Albany NY)*. 2019;11:7442–7456.
20. Idirisinghe PK, Thike AA, Cheok PY, et al. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer. Pathologic correlations and clinical significance. *Am J Clin Pathol*. 2010;133:416–429.
21. Dai X, Cheng H, Chen X, et al. FOXA1 is prognostic of triple negative breast cancers by transcriptionally suppressing SOD2 and IL6. *Int J Biol Sci*. 2019;15:1030–1041. doi:10.7150/ijbs.31009.eCollection 2019.

22. Carroll JS, Liu XS, Brodsky AS, et al. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell*. 2005;122:33–43.
23. Li J, Wang W, Chen S, et al. FOXA1 reprograms the TGF-β-stimulated transcriptional program from a metastasis promoter to a tumor suppressor in nasopharyngeal carcinoma. *Cancer Lett*. 2019;442:1–14.
24. Han W, Cao F, Gao XJ, et al. ZIC1 acts a tumor suppressor in breast cancer by targeting survivin. *Int J Oncol*.. 2018;53:937–948.
25. Bhattarai S, Klimov S, Mittal K, et al. Prognostic role of androgen receptor in triple negative breast cancer: a multi-institutional study. *Cancers (Basel)*. 2019;11:995.