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Original Article

Overexpression of enhancer of zeste homolog 2 (EZH2) and focal adhesion kinase (FAK) is associated with cancer metastasis and poor prognosis in breast cancer

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Abstract: Enhancer of zeste homolog 2 (EZH2), a polycomb histone methyltransferase, is a key epigenetic modifier implicated in the metastasis of various cancers. Focal adhesion kinase (FAK), a protein tyrosine kinase, modulates signaling and fundamental functions to facilitate cancer progression and metastasis. However, the link between EZH2 and FAK expression and their mechanisms in breast cancer (BC) remain ambiguous. In the present study, EZH2 and FAK expression was examined in two tissue microarrays containing specimens from 300 patients with breast cancer using immunohistochemistry. Kaplan-Meier survival analysis demonstrated that high expression of EZH2 (56.6%) and FAK (74.7%) was associated with poor prognosis ($P < 0.05$). In addition, univariate and multivariate Cox regression analyses suggest that FAK is an independent prognostic factor (ER status: hazard ratio (HR) = 0.776, 95% Confidence Interval (CI): 0.603-1.972; PR status: HR = 0.790, 95% CI: 0.338-1.061; AR status: HR = 0.934, 95% CI: 0.751-1.162; EZH2 status: HR = 1.376, 95% CI: 0.845-2.242; FAK status: HR = 2.117, 95% CI: 1.147-3.909, $P = 0.016$). In the nuclear grade II, negative Her2, negative EGFR status, or positive P53 status subgroups, patients expressing high EZH2 and FAK levels also presented with poor survival. Furthermore, invasion of MDA-MB-231 cells decreased after combined inhibition of EZH2 and FAK. Moreover, FAK protein levels were significantly downregulated in EZH2 knocked down cell lines *in vitro*. In conclusion, EZH2 and FAK expression is correlated and associated with BC prognosis.

Keywords: Breast cancer, enhancer of zeste homolog 2, focal adhesion kinase, prognosis

Introduction

Breast cancer (BC) is the most common cancer in females, representing 1,676,600 new cases and causing 521,900 deaths worldwide in 2012 [1]. In spite of many advances in surgical treatment, radiotherapy, chemotherapy, and targeted therapy used for the treatment of breast carcinoma, it is still the leading cause of cancer death in females aged 20 to 59 years in the USA [2]. Most of these treatments are based on the four BC subtypes classified per the 13th St. Gallen International Expert Consensus recommendations (Luminal A, Luminal B, HER2+, and Triple negative) [3]. However, not every patient can benefit from these therapeutic strategies, due to lack of suitable markers for early detection, failure to respond to available drugs, advanced disease stages, and lack of knowledge of the mechanisms underlying the poor survival risk factors. This resulted in the emergence of studies about prognostic biomarkers of BC, which aimed to explore new therapeutic targets [4-7].

Enhancer of zeste homologue 2 (EZH2) is a catalytic subunit of the polycomb repressive complex 2 (PRC2), located on chromosome 7q35. EZH2 is a highly conserved histone methyltransferase that takes part in regulating chromatin structure and repressing gene expression epigenetically by methylating lysine 27 of histone 3 (H3K27) [8]. In addition, recent studies suggest that EZH2 is required in fundamental cellular processes and tumorigenesis such as cell fate decision, differentiation, can-

cer cell proliferation, progression, metastasis, stem cell maintenance, and drug resistance [9-11]. EZH2 overexpression, which has been detected in various invasive tumors, including breast, prostate, lung, gastric, bladder, and renal cell cancer, is suggested to be associated with poor outcomes [12-19].

Focal adhesion kinase (FAK) is a cytoplasmic non-receptor tyrosine kinase that regulates several cellular signaling pathways activated by growth factors binding to their receptors or integrins in various tumors [20, 21]. Overexpression and activation of FAK have been investigated in various carcinomas and are correlated with poor clinical outcomes, highlighting FAK as an anticancer target and potential prognostic marker [22-24]. Studies showed that E-cadherin-mediated cell-cell adhesion can be deregulated by the Src/FAK signaling axis and overexpression of EZH2 [21, 25, 26]. However, little is known about the association between EZH2 and FAK in BC and their diagnostic value. This study was designed to determine the clinical significance of EZH2 and FAK and their relationships to identify the mechanisms underlying malignant progression and therapeutic targets of BC.

Materials and methods

Patient selection and tissue microarray

This study was approved by the Ethics Committee of Taizhou Hospital of Zhejiang Province for use of human tissues and informed consent was obtained from all subjects from whom 300 paraffin-embedded BC tissue specimens were obtained for two tissue microarrays. The methods were carried out in accordance with the approved guidelines. This study was conducted and patients were recruited in 2010. The clinical data included age, distant metastasis, and TNM stage were obtained from their medical records with a range from the day they operated to 2015. As for the follow up of the patients two of our authors had access to information that could identify individual participants during data collection. BC was classified into the four molecular subtypes according to the expression of molecular markers [3]: luminal A: ER+ and/or PR+, with HER2- and Ki67-; luminal B (HER2-): ER+ and/or PR+, with Ki67+ and/or HER2-; luminal B (HER2+): ER+ and HER2+, with any PR, Ki67, or HER2 overexpression; HER2- type: ER-, PR-, and HER2+; and basal-like/triple negative: ER-, PR-, and HER2-.

Mastectomy and/or axillary dissection (based on magnetic resonance imaging, ultrasonography, and mammography) were performed on these patients between 2001 and 2008. Patients who received preoperative neoadjuvant hormone therapy and/or chemotherapy were excluded from the study. Standard chemotherapy, endocrine therapy, and radiotherapy after surgical treatment were performed on all these patients according to the therapeutic strategies from the National Comprehensive Cancer Network. In term of endocrine therapy, tamoxifen was prescribed to premenopausal patients for 5 years, while aromatase inhibitors were prescribed to postmenopausal patients for 5 years.

Immunohistochemistry

Expression of EZH2, FAK, ER, PR, Ki-67, HER2, CK5/6, AR, P53, and EGFR was evaluated by using immunohistochemistry (IHC), and the results were analyzed by three different pathologists. EZH2 and FAK levels were evaluated with monoclonal antibodies to EZH2 (GTX825-03, GeneTex, Irvine, CA, USA) and FAK (#3285, CST, Beverly, MA, USA) at a 1:50 dilution. ER and PR expression was defined as negative (< 1% positive nuclei) and positive (≥ 1% positive nuclei), Ki-67 expression was defined as positive (> 14% positive nuclei) or negative (≤ 14% positive nuclei), while CK5/6, AR, P53, and EGFR expression was defined as negative (< 10% positive nuclei or membrane) and positive (≥ 10% positive nuclei or membrane) according to the current Swedish clinical guidelines. HER2 expression was semi-quantitatively assessed using a standard protocol (HercepTest; Dako, Carpinteria, CA, USA) [27]. Positive expression was defined as strong membranous staining (3+); negative expression was defined as membranous staining of 0 or 1+. In addition, for cases with membranous staining of 2+, evaluation of HER2 amplification using fluorescence *in situ* hybridization was required to determine the level of expression.

Scoring

IHC data were evaluated by three experienced pathologists who were blinded to the patients' clinical information. EZH2 and FAK expression was graded according to the proportion of positive cells (0 = 0-5%, 1 = 6-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100%), and the inten-

Table 1. Correlations between pathological information of patients with BC and EZH2 and FAK status

Characteristic	EZH2		X^2	*p*	FAK		X^2	*p*
	Negative (n = 125)	Positive (n = 163)			Negative (n = 73)	Positive (n = 215)		
Age at diagnosis								
< 50	43	55	0.014	0.503	25	73	0.002	1.000
≥ 50	82	108			48	142		
Tumor size(cm)								
≤ 2	39	33	4.636	0.098	22	50	1.983	0.160
> 2, ≤ 5	72	111			45	138		
> 5	14	19			6	27		
Nuclear grade								
1	28	11	23.687	0.000*	17	22	8.708	0.013*
2	94	129			52	171		
3	3	23			4	22		
Lymph node status								
0	54	75	0.392	0.942	34	95	0.631	0.889
1-3	35	46			18	63		
4-9	26	31			15	42		
≥ 10	10	11			6	15		
Tumor stage								
0/1	20	18	2.377	0.305	12	26	0.903	0.637
2	65	98			40	123		
3	40	47			21	66		
ER								
Negative	31	73	12.248	0.001*	21	83	2.286	0.159
Positive	94	90			52	132		
PR								
Negative	50	98	11.468	0.001*	32	116	2.233	0.139
Positive	75	65			41	99		
HER2								
Negative	106	115	8.045	0.005*	64	157	6.550	0.010*
Positive	19	48			9	58		
Ki-67								
< 14	103	88	25.568	0.000*	61	130	15.000	0.000*
≥ 14	22	75			12	85		
Molecular subtypes								
Luminal A	76	45	33.108	0.000*	45	76	16.097	0.001*
Luminal B	22	49			10	61		
HER2	7	26			7	26		
Triple-negative	20	43			11	52		
CK5/6								
Negative	102	122	1.867	0.199	57	167	0.005	1.000
Positive	23	41			16	48		
AR								
Negative	17	33	2.178	0.159	10	40	0.914	0.377
Positive	108	130			63	175		
P53								
Negative	52	26	23.568	0.000*	29	49	7.915	0.006*

Positive	73	137			44	166		
EGFR								
Negative	97	90	15.569	0.000*	57	130	7.428	0.007*
Positive	28	73			16	85		
FAK								
Negative	48	25	19.886	0.000*				
Positive	77	138						
EZH2								
Negative					48	77	19.886	0.000*
Positive					25	138		

*P < 0.05.

sity of the EZH2 and FAK staining was graded as 0-3. The final score for EZH2 and FAK expression (positive or negative) was calculated as the sum of both grades (negative: total grade 0-3, positive: total grade 4-7).

Cell line and siRNA transfection

MDA-MB-231 cells (American Type Culture Collection, Manassas, VA, USA) were grown in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS (Gibco) in a 5% CO_2 atmosphere at 37°C. Twenty-four hours before transfection, MDA-MB-231 cells were seeded (3×10^5 cells per well) onto a six-well plate containing 1 mL of Opti-MEM (Gibco). Ten microliters of 20 mM EZH2 siRNA (AACCATGTTTACAACTATCAA), FAK siRNA (GUAUUGGACCUGCGAGGGA-TT), or a siRNA negative control with 10 mL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added and gently mixed. The cells were then incubated for 48 h before the assays were performed.

Matrigel invasion assay

The migration assay was performed by using the BD biocoat Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) and invasion chamber (Corning, Corning, NY, USA) according to the manufacturer's protocol. MDA-MB-231 cells were starved for 12 h in DMEM. Transfected and untransfected cells were plated in the upper chamber in serum-free medium with or without the small molecular inhibitor of EZH2 (DZNeP, 4703, TOCRIS bioscience, Bristol, UK) and FAK (PND-1186, S7653, Selleck Chemicals, Houston, TX, USA). After 24 h of incubation, the cells on the bottom of the filter were fixed and stained with 2% crystal violet solution in ethanol (Beyotime Biotechnology, Shanghai, China) and counted using an IX70 inverted microscope (Olympus, Tokyo, Japan). These Matrigel invasion assays were conducted at least 3 times.

Western blot analysis (WB)

MDA-MB-231 cells were grown in a six-well plate (3×10^5 cells per well). For cell lysis, cells were washed twice with ice-cold phosphate-buffered saline (PBS) and then incubated in radioimmunoprecipitation assay buffer (Beyotime Biotechnology) for 30 min on ice. Protein concentration was measured by using a BCA Protein Assay Kit following the manufacturer's instructions (Goodbio Biotechnology, Wuhan, China). Cell extracts were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Goodbio Biotechnology) under reducing conditions with a MiniPROTEAN® Tetra Cell (Bio-Rad, Hercules, CA, USA), using a 4% stacking gel and a 10% separating gel. Antibodies against EZH2, FAK, and GAPDH (#5174; CST) were used in WB. A goat anti-rabbit IgG horseradish peroxidase (HRP) conjugated antibody (#7074; CST) was used for immunodetection according to the manufacturer's instructions. Enhanced chemiluminescence (Goodbio Biotechnology) was used to visualize the immunodetected proteins. Molecular Imager ChemiDoc™ XRSþ (Bio-Rad) was used to image the chemiluminescent blots. ImageLab software version 4.1 (Bio-Rad) was used to select and determine the appropriate background-subtracted density of the bands in all WB.

Statistical analysis

The tissue microarrays were scanned using Aperio Scan Scope slide scanner and Image

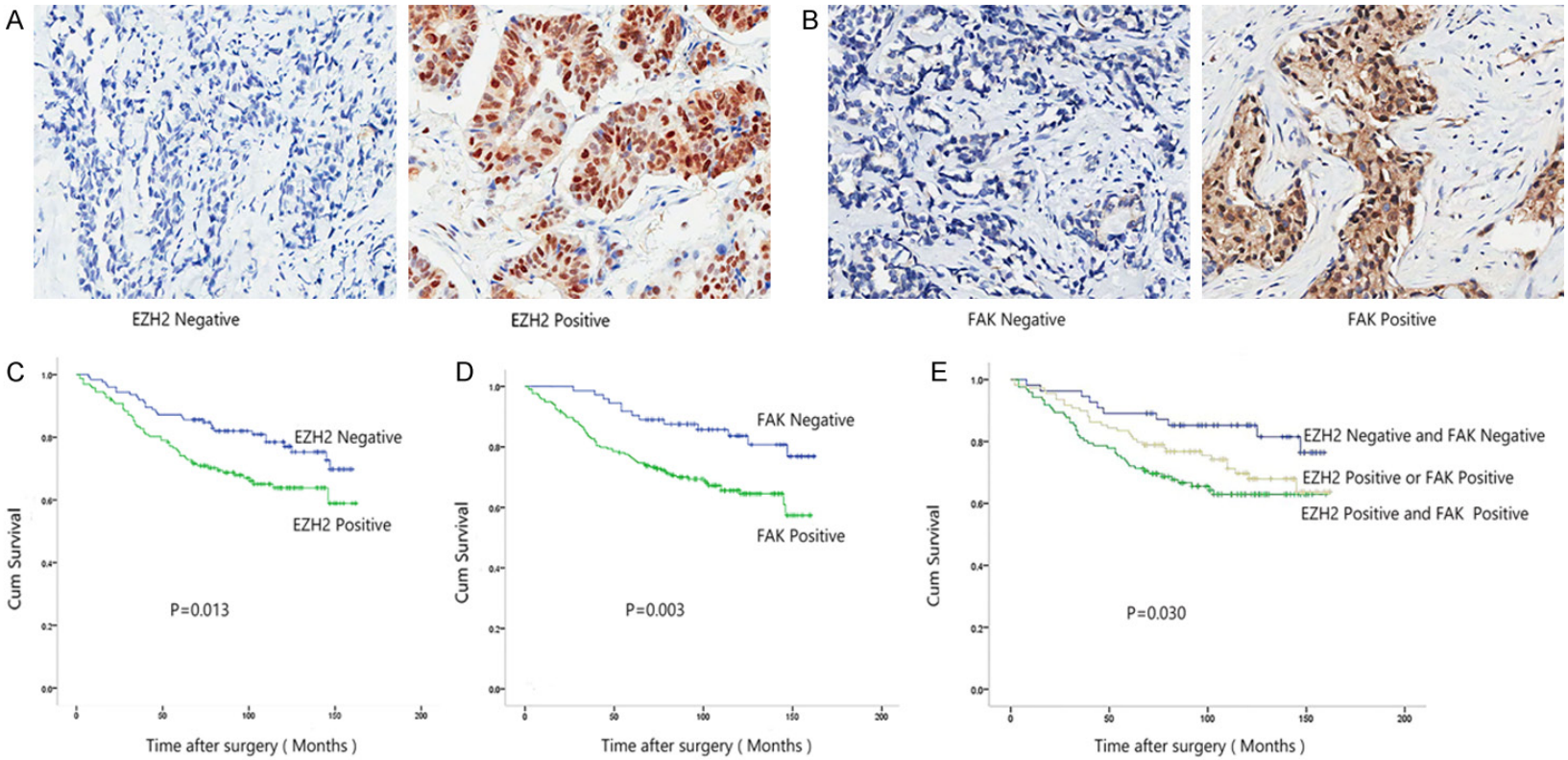

Figure 1. Tissue microarray and IHC staining of EZH2 and FAK in BC tumor samples and association between EZH2 and FAK expression and cumulative survival of patients with BC. A, B: IHC staining and evaluation examples of EZH2, FAK (200 ×); C-E: Association of EZH2, FAK expression with cumulative survival of patients with BC determined by Kaplan-Meier survival curve. *P* values shown in the figures were calculated from the Log-rank test.

Table 2. Univariate and multivariate analyzes of prognostic factors in BC for overall survival

Variable	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
ER (Positive vs Nagetive)	0.649	0.422-0.999	0.049*	0.776	0.603-1.972	0.776
PR (Positive vs Nagetive)	0.545	0.351-0.848	0.007*	0.790	0.338-1.061	0.079
Ki-67 (Positive vs Nagetive)	1.154	0.739-1.801	0.530			
Her2 (Positive vs Nagetive)	1.217	0.743-1.994	0.435			
CK5/6 (Positive vs Nagetive)	1.275	0.777-2.092	0.337			
EGFR (Positive vs Nagetive)	1.135	0.730-1.767	0.574			
P53 (Positive vs Nagetive)	1.054	0.657-1.690	0.827			
AR (Positive vs Nagetive)	0.552	0.334-0.912	0.020*	0.934	0.751-1.162	0.539
FAK (Positive vs Negative)	2.386	1.317-4.324	0.004*	2.117	1.147-3.909	0.016*
EZH2 (Positive vs Negative)	1.756	1.119-2.755	0.014*	1.376	0.845-2.242	0.200

*P < 0.05.

Scope software (Aperio, Buffalo Grove, IL, USA) and Adobe Illustrator were used to obtain images of representative areas. EpiData software (version 3.1; EpiData Association, Odense, Denmark) was used to analyze the primary clinical and histopathological data. SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The means and group differences of the data were statistically analyzed using the Student's t-test and the correlation between variables were analyzed by the Pearson's X^2 test. Kaplan-Meier survival curves and the log-rank test were used to evaluate overall survival (OS). Univariate and multivariate regression analyses were performed using a Cox proportional hazards model. All tests were two-sided and *P*-values of < 0.05 were considered statistically significant [28].

Results

Patient characteristics

This study included specimens from 300 patients with BC, 12 patients were excluded because the IHC failed. Detailed pathological information of the tissue microarrays is listed in **Table 1**. Among all patients, the mean age was 56.91 ± 13.15 (range, 29-88 years). About half of the patients had been diagnosed at a tumor size of T2, with no metastasis to the lymph nodes (N0), or at a TNM stage of II. In addition, based on the expression of ER, PR, HER2, and Ki67, of the 288 patients, 42% were Luminal A, 24.7% were Luminal B, 11.5% were HER2 overexpression, 21.9% were basal-like/triple negative. Positive P53, CK5/6, AR, and EGFR expression was detected in 72.9%, 22.2%, 82.6%, and 35.1% patients, respectively. For EZH2 and FAK expression, 56.6% and 74.7% of the patients were positive.

High expression of EZH2 and FAK is tightly associated with the poor prognosis of patients with BC

Among the 300 patients, the mean OS was 95.20 ± 42.45 months (95% confidential interval (CI): 89.98-99.64 months). As shown in **Table 1**, among the 300 tumor specimens, 163 tumors presented a high expression of EZH2, while 215 tumors were strongly stained for FAK (**Figure 1A**, **1B**). To further understand the role of EZH2 and FAK in the prognosis of patients with BC, we performed Kaplan-Meier survival curves and used the log-rank test to evaluate the association of EZH2 and FAK expression with OS (**Figure 1C-E**). The results showed that the expression of both EZH2 and FAK was significantly associated with OS. Furthermore, patients with high expression of EZH2 and FAK presented a poor prognosis compared to patients without EZH2 and FAK expression (P = 0.013, P = 0.003, and P = 0.030, respectively).

In addition, univariate and multivariate Cox regression models were used to analyze the association of OS with the canonical prognostic factors (ER, PR, Ki-67, Her2, CK5/6, EGFR, P53, and AR), and EZH2 and FAK expression (**Table 2**). The survival rate for FAK positive patients was much lower than that determined by using the other prognostic factors (ER status: hazard ratio (HR) = 0.776, 95% CI: 0.603-1.972; PR status: HR = 0.790, 95% CI: 0.338-1.061; AR status: HR = 0.934, 95% CI: 0.751-

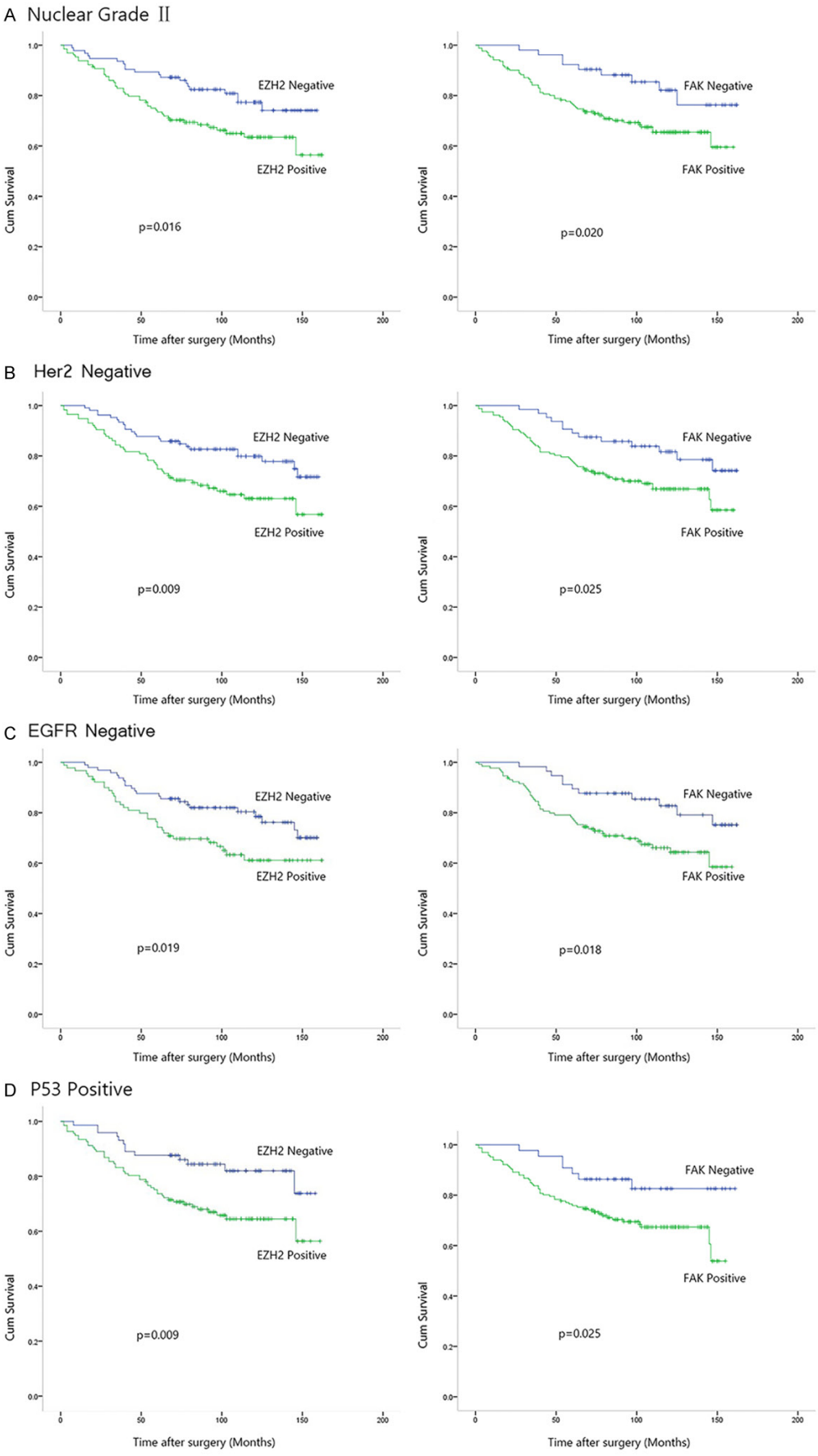
A Nuclear Grade II
EZH2 Negative
EZH2 Positive
p=0.016
FAK Negative
FAK Positive
p=0.020
B Her2 Negative
EZH2 Negative
EZH2 Positive
p=0.009
FAK Negative
FAK Positive
p=0.025
C EGFR Negative
EZH2 Negative
EZH2 Positive
p=0.019
FAK Negative
FAK Positive
p=0.018
D P53 Positive
EZH2 Negative
EZH2 Positive
p=0.009
FAK Negative
FAK Positive
p=0.025
Cum Survival
Time after surgery (Months)

Figure 2. Association between EZH2 and FAK expression and nuclear grade, status of Her2, Ki67, P53, and EGFR, and molecular subtypes of BC analyzed by Kaplan-Meier survival curve. *P* values shown in the figures were calculated from the Log-rank test.

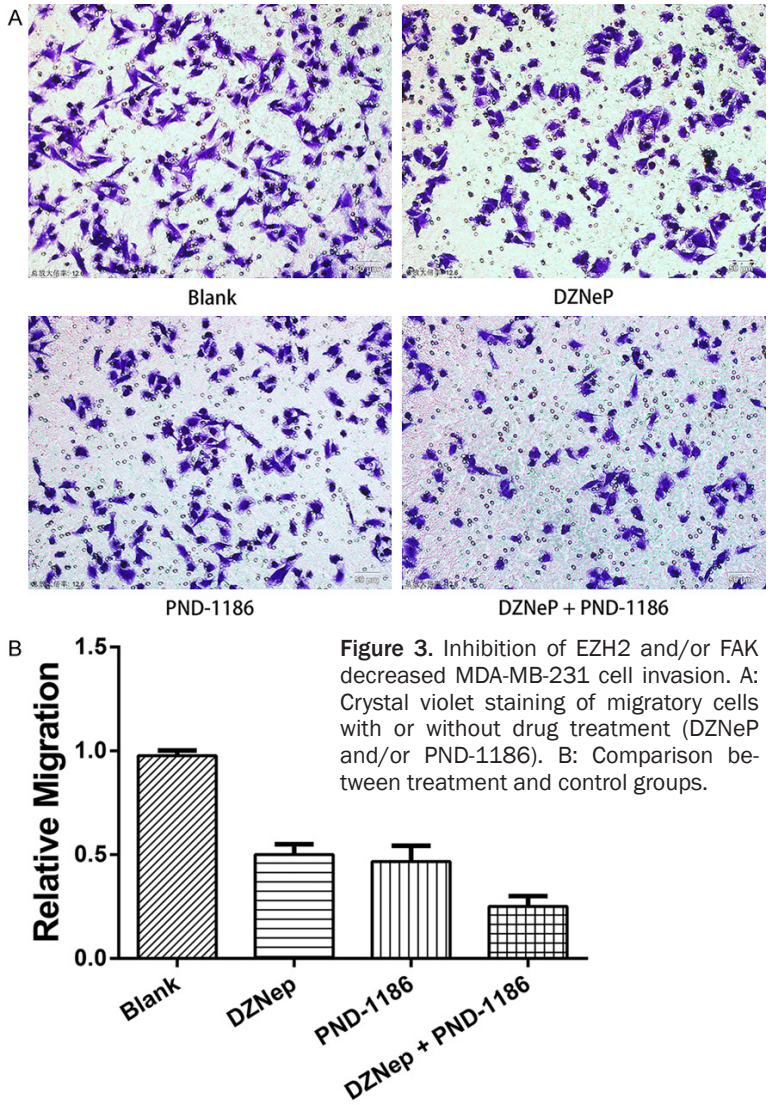

Figure 3. Inhibition of EZH2 and/or FAK decreased MDA-MB-231 cell invasion. A: Crystal violet staining of migratory cells with or without drug treatment (DZNeP and/or PND-1186). B: Comparison between treatment and control groups.

1.162; EZH2 status: HR = 1.376, 95% CI: 0.845-2.242; FAK status: HR = 2.117, 95% CI: 1.147-3.909, P = 0.016). All these data clearly indicated that FAK expression may be a predictor of poor prognosis for BC.

EZH2 and FAK are significantly associated with nuclear grade and several canonical prognostic factors

The results presented in **Table 1** indicate that the expression of both EZH2 and FAK is significantly associated with nuclear grade, status of Her2, Ki67, P53, EGFR, and molecular subtypes of BC. Furthermore, we evaluated the association between EZH2 and FAK expression with nuclear grade and status of Her2, Ki67, P53, and EGFR using a Kaplan-Meier survival curve and the log-rank test (**Figure 2**). In patients with nuclear grade II tumors, the expression of both EZH2 and FAK was associated with poor OS at the follow-up after surgery (**Figure 2A**, $P < 0.05$). Similar results were obtained in patients whose tumor showed negative Her2, EGFR status, or positive P53 status (**Figure 2B-D**, $P < 0.05$). These results indicate a significant relationship between the expression of EZH2 and FAK with nuclear grade and expression of Her2, Ki67, P53, and EGFR. Furthermore, when the survival time was taken into consideration, this significance remained.

Combined inhibition of EZH2 and FAK is more effective at decreasing MDA-MB-231 cell invasion than inhibiting FAK or EZH2 alone

In the matrigel invasion assay, DZNeP and PND-1186 were used to inhibit the expression of EZH2 and FAK in MDA-MB-231 cells, respectively. Treatment with DZNeP effectively decreased MDA-MB-231 cell motility ($P < 0.05$, **Figure 3**), as did PND-1186 ($P < 0.05$, **Figure 3**). However, combined inhibition of EZH2 and FAK had dramatic effects on MDA-MB-231 cell invasion ability ($P < 0.05$, **Figure 3**).

EZH2 knockdown inhibits the invasion of MDA-MB-231 cells by downregulating FAK activity

SiRNA transfection was used to knockdown EZH2 and FAK in MDA-MB-231 cells to examine

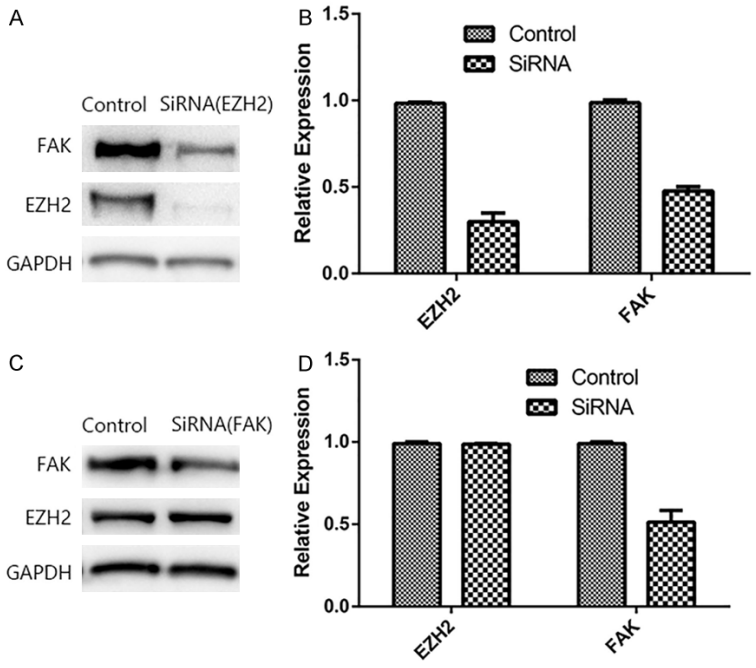

Figure 4. Expression of EZH2 and FAK in MDA-MB-231 cells *in vitro* after siRNA transfection. A, B: Expression of EZH2 and FAK after knockdown of EZH2. C, D: Expression of EZH2 and FAK after knockdown of FAK.

their association. Expression of EZH2 and FAK was detected by WB after siRNA transfection (**Figure 4**). EZH2 and FAK expression was significantly inhibited by transfection using their respective siRNA (**Figure 4A**, **4B**). In EZH2 siRNA transfected cells, FAK expression was significantly decreased. However, FAK siRNA transfection did not affect EZH2 expression (**Figure 4C**, **4D**). These results indicated that FAK may be a downstream molecule of EZH2 and helps regulating the invasion ability of breast cancer cells.

Discussion

In the present study, we investigated the relationship between EZH2 and FAK expression and the outcome of patients with BC. We also demonstrated the association between EZH2, FAK, and several classical prognostic markers. To our knowledge, this is the first study that describes a relationship between EZH2 and FAK in BC. Similarly, Jun Zhou *et al*. [29] demonstrated that overexpression of EZH2, FAK, and pFAK correlates with well-established pathologic risk factors and may predict a more aggressive biologic behavior in endometrial cancer. However, this conclusion was based solely on immunohistochemical analysis of a tissue microarray. The study was lacking *in vitro* and *in vivo* experiments explaining the molecular mechanisms underlying carcinogenesis. Therefore, our study was designed to explore the role of EZH2 and FAK in BC.

Recent studies showed that EZH2 and FAK overexpression could serve as a marker for advanced or metastatic disease in a variety of malignancies, including breast, prostate, bladder, osteosarcoma, and renal cell cancer [12, 20, 25, 30]. Similarly, our results suggest that high expression of EZH2 and FAK is tightly associated with the poor prognosis of patients with BC. Furthermore, our results indicate that FAK could serve as an independent prognosis marker (**Tables 1** and **2**; **Figure 1**). Additionally, the expression of EZH2 and FAK was significantly related to nuclear grade, Her2, Ki67, P53, EGFR, and molecular subtypes of BC (**Tables 1** and **2**; **Figure 2**). Taken together, these results suggest that high expression of EZH2 and/or FAK is associated with BC neoplasia and aggressive tumor behavior.

EZH2 acts as a key component of the PRC2 complex, playing an important role in genetic and epigenetic modifications. It appears to be a promising tumor biomarker and may contribute to tumor progression as a potential oncogene. EZH2 downregulation by si/shRNA or inhibitors decreased cell proliferation, inhibited tumor metastasis *in vitro*, and significantly decreased breast xenograft growth *in vivo* [31-33]. Our results are consistent with these findings. EZH2 knockdown by siRNA and inhibition by DZNeP resulted in a decrease of MDA-MB-231 cell motility. Many studies showed that FAK is activated in many cancers, including BC, and promotes cancer progression and metastasis [34, 35]. In the present study, FAK knockdown by siRNA and inhibition by PND-1186 inhibited cell invasion.

Moreover, EZH2 expression and FAK expression were significantly correlated in BC. In MDA-MB-231 cells *in vitro,* FAK expression was significantly inhibited after EZH2 downregulation, but EZH2 expression was not affected by FAK downregulation. Furthermore, downregulation of both FAK and EZH2 induced a significant decrease in the cell invasion ability of MDA-MB-231 cells. These results demonstrated that FAK is truly downregulated in EZH2 downregulated cells, although the underlying molecular mechanisms need further investigation. Numerous studies suggested that EZH2 overexpression plays crucial roles in epigenetic modifications by upregulating H3K27me3 levels, increasing tumor-initiating cells, and inducing the epithelial-mesenchymal transition (EMT) in various cancers [31, 32, 36]. In this study, we identified another signaling pathway regulated by EZH2, the FAK pathway, suggesting the possible molecular role of EZH2 in promoting BC metastasis. Taken together, the molecular mechanisms of EZH2 and FAK in BC progression and metastasis need further characterization.

However, our study has several limitations. First, we only analyzed the overall survival of the patients, the disease-free survival data are missing. In addition, all patients were from a single center. Additionally, different sensitivities of antibodies, different cutoffs defined to discriminate positive *vs.* negative EZH2 and FAK samples may lead to different results. These issues will be addressed in future studies. In fact, a multicenter study with a detailed long-term follow-up and using accepted standards of cutoffs is warranted to verify our conclusions.

Conclusions

In conclusion, the current evidence suggests that high expression of EZH2 and FAK correlates with aggressive tumor phenotypes and poor survival of patients with BC. In addition, FAK may be a prognostic factor of BC after adjusting for nuclear grade, status of Her2, Ki67, P53, EGFR, and EZH2, and molecular subtypes in multivariate Cox regression analysis. In addition, our findings establish a previously unrecognized link between EZH2 and FAK signaling activation in BC. Although a thorough exploration of the roles of EZH2 and FAK in BC is definitely required, these data advance our understanding of the mechanisms of EZH2 and FAK in BC.

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Disclosure of conflict of interest

None.

Authors' contribution

Chunping Liu and Tao Huang conceived the experiments, Pan Yu, Zeming Liu, Chen Chen, Yunke Zhang, Xingjie Yin, Shan Wang, Nie Xiu and Huang Bangxing conducted the experiments, Yawen Guo and Yusufu Maimaiti analysed the results and wrote the main manuscript text. All authors reviewed the manuscript.

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