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Retrospective Study

Expression Profiles and Clinical Implications of FOXM1, COX-2, and GRP78 in Breast Invasive Ductal Carcinoma

FOXM1, COX-2, and GRP78 in Breast Cancer

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Abstract

BACKGROUND

Breast infiltrating ductal carcinoma (BIDC) represents the largest heterotypic tumor group, and an in-depth understanding of the pathogenesis of BIDC is key to improving the prognosis of BIDC.

AIM

This study aims to analyze the expression profiles and clinical implications of forkhead box M1 (FOXM1), cyclooxygenase-2 (COX-2), and glucose-regulated protein 78 (GRP78) in BIDC.

METHODS

A total of 65 BIDC patients and 70 healthy controls who presented to our hospital between August 2019 and May 2021 were selected for analysis. The peripheral blood FOXM1, COX-2, and GRP78 Levels in both groups were measured and the association between their expression profiles in BIDC was examined. Additionally, we investigated the diagnostic value of FOXM1, COX-2, and GRP78 in patients with BIDC

and their correlations with clinicopathological features. Furthermore, BIDC patients were followed up for 1 year to identify factors influencing patient prognosis.

RESULTS

The levels of FOXM1, COX-2, and GRP78 were significantly higher in BIDC patients compared to healthy controls ($P<0.05$), and a positive correlation was observed among them ($P<0.05$). ROC analysis demonstrated the excellent diagnostic value of FOXM1, COX-2, and GRP78 in predicting the occurrence of BIDC ($P<0.05$). Subsequently, we found significant differences in FOXM1, COX-2, and GRP78 Levels among patients with different histological grades and metastasis status (with vs. without) ($P<0.05$). COX analysis revealed that FOXM1, COX-2, GRP78, increased histological grade, and the presence of tumor metastasis were independent risk factors for prognostic death in BIDC ($P<0.001$).

CONCLUSION

FOXM1, COX-2, and GRP78 exhibit abnormally high expression in BIDC, promoting malignant tumor development and closely correlating with prognosis. These findings hold significant research implications for the future diagnosis and treatment of BIDC.

Key Words: Excellent diagnostic value; FOXM1; COX-2; GRP78; Clinical implications

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Core Tip: FOXM1, COX-2, and GRP78 exhibit elevated expression in breast invasive ductal carcinoma (BIDC) and are associated with poor prognosis. Their diagnostic value suggests potential as biomarkers for BIDC detection. Understanding their clinical

implications can aid in the diagnosis and treatment of BIDC, contributing to improved patient outcomes.

INTRODUCTION

Breast cancer (BC) is the most prevalent malignancy in women, and its incidence has been increasing in recent years[1]. Among the various types of invasive BC, breast infiltrating ductal carcinoma (BIDC) represents the largest heterotypic tumor group, lacking distinct tissue characteristics and therefore classified as a non-special type cancer[2]. BIDC is also the most common type of invasive BC, accounting for over 80% of cases[3]. While advancements in medical standards have contributed to a decrease in the overall mortality rate of BIDC, the prognosis for advanced BIDC patients remains unsatistifactory[4,5]. Researchers believe that a comprehensive understanding of the pathogenesis of BIDC and the discovery of new diagnostic and treatment methods are key to improving patient prognosis[6].

Forkhead box M1 (FoxM1), a member of the Fox protein family, plays a vital role in modulating the cell cycle[7]. Numerous studies have demonstrated that FOXM1 is overexpressed in various human tumors, promoting oncogenic transformation and participating in tumor occurrence and development[8,9]. Cyclooxygenase-2 (COX-2), a subtype of cyclooxygenase, has been identified as an essential component in the pathogenesis of malignant tumors and is closely associated with the occurrence and progression of BC based on previous data[10,11]. Additionally, glucose-regulated protein 78 (GRP78), a signature stress protein of the endoplasmic reticulum, has been found to induce tumor development, contribute to drug resistance, and facilitate tumor cell survival[12]. Although some studies have shown abnormal expression of FOXM1, COX-2, and GRP78 in BC[13-15], their specific roles in BIDC have yet to be characterized.

To develop new diagnostic and treatment protocols and identify targets for the prevention of BIDC, this study aims to analyze the expression profiles of FOXM1, COX-2, and GRP78 in BIDC and investigate their clinical implications in the disease. The

objective is to establish the correlation between these markers and BIDC, thus laying the foundation for further research.

MATERIALS AND METHODS

Specimen collection

This study obtained approval from the Medical Ethics Committee and enrolled 65 BIDC patients (research group, RG) and 70 healthy controls (control group, CG), who presented between August 2019 and May 2021. Informed consent was obtained from all participants.

Eligibility criteria

Eligible patients had histopathologically confirmed BIDC[16] and exhibited normal organ function, complete clinical data, cooperation with treatment and follow-up, no contraindications to chemotherapy, and a life expectancy of ≥3 mo. Pregnant and lactating women were excluded from the study. Additionally, individuals with immune deficiency, inflammatory diseases, severe hematopoietic injury, a history of other malignant tumors, cardio-cerebrovascular diseases, and poor treatment compliance were excluded.

Sampling and testing

Fasting venous blood samples were collected from all participants upon admission. Total RNA was isolated from the blood using Trizol, followed by reverse transcription into cDNA for PCR detection. The PCR reaction was conducted for 40 cycles under the following conditions: 95°C/30s, 95°C/5s, 60°C/30s, and 72°C/30s. The design and construction of primer sequences (Table 1) were performed by Tsingke Biotechnology Co., Ltd. The relative expression of the target genes to β-actin was calculated using the $2^{-\Delta\Delta CT}$.

Follow-up for patient prognosis

BIDC patients were followed up for 1 year through regular hospital reexaminations, with reexamination intervals not exceeding 2 mo. Death was considered a termination event, and the patient's prognosis and survival were recorded.

Outcome measures

The expression profiles of FOXM1, COX-2, and GRP78 and their correlations in BIDC were analyzed. The diagnostic value of the three markers in BIDC patients and their correlations with clinicopathological features were also investigated. Finally, the factors influencing patient prognosis were analyzed based on the follow-up results.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 software. Count data, such as previous medical history, were presented as (%), and intergroup differences were assessed using the Chi-square test. Expression levels of OXM1, COX-2, and other measurement data were presented as (`c±s). Inter-group and multi-group differences were identified using independent sample t-test and analysis of variance (ANOVA) with Bonferroni posthoc tests, respectively. Correlation analysis was conducted using the Pearson correlation coefficient, the diagnostic value was determined using ROC analysis, and related factors were identified using COX regression analysis. A significance level of $P<0.05$ was used to indicate statistical significance.

RESULTS

Comparison of clinical baseline data

Clinical baseline data, including age, family history, and smoking habits, were collected at admission. The analysis revealed no significant intergroup differences ($P>0.05$, Table 2), confirming the comparability of the study groups.

Comparison of FOXM1, COX-2, and GRP78 expression levels

The mRNA expression of FOXM1 was (4.63±0.76) in the RG and (3.94±0.73) in the CG, indicating significantly higher FOXM1 Levels in BIDC patients (P<0.05, Figure 1a). Similarly, COX-2 mRNA expression in the RG was (3.40±0.77), which was also higher compared to the CG (P<0.05, Figure 1b). Finally, the inter-group comparison showed a higher GRP78 mRNA level in the RG compared to the CG (P<0.05, Figure 1c).

Diagnostic value of FOXM1, COX-2, and GRP78 in BIDC

ROC analysis revealed that when the peripheral blood mRNA level of FOXM1 was >4.05, it had a sensitivity of 81.54% and specificity of 55.71% for diagnosing BIDC (P<0.05, Figure 2a). Similarly, COX-2 had a sensitivity of 69.23% and a specificity of 70.00% for BIDC diagnosis when its mRNA level was >3.05 (P<0.05, Figure 2b). GRP78 exhibited a sensitivity of 63.08% and specificity of 74.29% for diagnosing BIDC when its mRNA level was >2.76 (P<0.05, Figure 2c).

Relationship between the expression levels of FOXM1, COX-2, and GRP78

The Pearson correlation coefficient demonstrated a positive correlation between FOXM1 and both COX-2 and GRP78 (P<0.05, Figure 3a and b), as well as a positive association between COX-2 and GRP78 (P<0.05, Figure 3c), in the peripheral blood of patients in the RG (P<0.05, Figure 3c).

Correlation of FOXM1, COX-2, and GRP78 with clinicopathological features of BIDC

There were no significant differences in FOXM1, COX-2, and GRP78 Levels among patients of different age groups (P>0.05). However, significant differences in their levels were observed in patients with different histological grades and metastasis (with vs. without) (P>0.05, Table 3), indicating a close relationship between FOXM1, COX-2, and GRP78, and the above indexes.

Univariate analysis of prognostic mortality in BIDC

During the follow-up period, 12 patients died. Deceased patients exhibited higher age, FOXM1, COX-2, and GRP78 levels compared to the surviving patients, with a higher proportion of histological grade III and tumor metastasis ($P<0.05$, Table 4). These findings suggest that age, FOXM1, COX-2, GRP78, histological grade, and tumor metastasis were individual factors affecting the prognostic death of BIDC.

Multivariate analysis of prognostic mortality in BIDC

Univariate indicators of BIDC (Age: ≤60 assigned to 0, >60 assigned to 1; Histological grade: grade I assigned to 0, grade II assigned to 1, grade III assigned to 2; Tumor metastasis: No assigned to 0, yes assigned to 1; FOXM1, COX-2, GRP78 were analyzed using raw data) were input as covariates into SPSS for COX regression analysis, with patient death used as the dependent variable. Age was not found to be an independent factor for the prognostic death of BIDC ($P>0.05$). However, FOXM1, COX-2, and GRP78, increased histological grade, and tumor metastasis were identified as significant factors ($P<0.001$, Table 5).

DISCUSSION

The incidence of breast cancer (BC) has been increasing in recent years, and it is affecting women at a younger age, posing significant risks to their health and well-being[17]. Early-stage BIDC often goes undetected, leading to a diagnosis at advanced stages with tumor metastasis, contributing to the poor prognosis of BIDC[18]. Therefore, analyzing the expression profiles and clinical implications of FOXM1, COX-2, and GRP78 in BIDC is essential for advancing research in this field.

This study confirmed the high expression levels of FOXM1, COX-2, and GRP78 in BIDC, which is consistent with previous research on BC[19-21]. These findings suggest the involvement of FOXM1, COX-2, and GRP78 in the onset and progression of BIDC. Furthermore, correlation analysis revealed positive associations between the expression levels of FOXM1, COX-2, and GRP78 indicating a synergistic relationship in their abnormal expressions. Previous studies have shown that FOXM1 regulates tumor

cell activity, including promoting liver cancer cell growth through KIF4A and influencing the quiescence and survival state of leukemia stem cells by regulating MLL[22,23]. Additionally, FOXM1 plays a role in angiogenesis, endothelial cell proliferation, migration, and endothelial cell channel formation[24]. Vascular endothelial growth factor (VEGF), secreted by tumor tissues, induces tumor neovascularization, promoting tumor cell invasion and metastasis[25]. The close correlation between VEGF and tumor-distant metastasis has been well documented[26]. FOXM1 can bind to the VEGF promoter and transcriptionally regulate its expression[27]. Therefore, it is speculated that this may be the mechanism by which FOXM1 is involved in BIDC. COX-2, a subtype of COX, is minimally expressed under normal physiological conditions but is overexpressed in pathological states. By promoting prostaglandin synthesis, COX-2 increases the risk of tumor growth, proliferation, vascular permeability, and metastasis[28]. Furthermore, COX-2 enhances the anti-apoptotic abilities of tumor cells by upregulating the expression of the proto-oncogene Bcl-2. It also inhibits lymphokine production, reduces T and B cell proliferation through the production of prostaglandin E2, weakens the immune surveillance function, and makes tumor cells prone to immune escape by inducing the expression of the immunosuppressive enzyme 3-dioxygenase[29]. Increased COX-2 represents an increased risk of cancer cell growth, proliferation, and metastasis, a stronger anti-apoptotic ability, and an increased risk of adverse prognosis in patients undergoing chemotherapy. GRP78, a molecular chaperone, assists in protein folding and transport in the endoplasmic reticulum, cytoplasm, and cell membrane. It also participates in the activation of unfolded protein response signals[30]. Studies have shown that GRP78 promotes cell proliferation and migration, and its mechanism involves inhibiting Bcl-2 and binding to Caspase-7 to prevent apoptosis[31,32]. Previous studies on FOXM1, COX-2, and GRP78 have all indicated their association with tumor invasion or migration, thus suggesting that their abnormal expression in BIDC is expected. Moreover, ROC analysis revealed that the three are effective in diagnosing the occurrence of BIDC, which may significantly contribute to promoting early diagnosis in the future.

Clinical data analysis demonstrated that FOXM1, COX-2, and GRP78 were closely related to the histological grade and metastasis of BIDC. This reinforced the relationship between the three markers and the pathological progression of BIDC, indicating that abnormally elevated levels of FOXM1, COX-2, and GRP78 can promote the malignant development of BIDC. Finally, the prognosis analysis revealed that FOXM1, COX-2, GRP78, histological grade, and tumor metastasis were independent factors affecting the prognostic outcomes of patients. Histological grade and tumor metastasis, being typical pathological features, naturally influence the malignant progression of BIDC[33]. The analysis results of FOXM1, COX-2, and GRP78 also support the above viewpoint, confirming their role in promoting the malignant development of BIDC. Conversely, these results suggest that targeted silencing of FOXM1, COX-2, and GRP78 may inhibit BIDC progression and facilitate the treatment of BIDC.

However, the exact mechanism by which FOXM1, COX-2, and GRP78 contribute to BIDC still needs to be further confirmed through experiments. Additionally, this study did not analyze tissue samples from BIDC patients, and the short follow-up time limits the evaluation of long-term prognosis These areas should be addressed and supplemented in future research.

CONCLUSION

The findings of this study indicate that FOXM1, COX-2, and GRP78 exhibit abnormally high expression levels in BIDC, contributing to the malignant development of the tumor and significantly impacting the prognosis of BIDC patients. These results hold significant research implications for the future diagnosis and treatment of BIDC.

ARTICLE HIGHLIGHTS

Research background

Reast infiltrating ductal carcinoma (BIDC) represents the largest heterotypic tumor group, and this study analyzed the clinical significance of FOXM1, COX-2, and GRP78 in BIDC, which could provide a reliable foundation for subsequent studies.

Research motivation

Forkhead box M1 (FOXM1), Cyclooxygenase-2 (COX-2), and Glucose-regulated protein 78 (GRP78) are closely related to breast cancer development and progression, but their roles in BIDC remain unclear. They may play equally important roles and hold promise for future diagnosis and treatment of BIDC.

Research objectives

To analyze the clinical significance of FOXM1, COX-2, and GRP78 in BIDC, and to provide references and new research directions for future diagnosis and treatment of BIDC.

Research methods

In this study, we analyzed the clinical significance of FOXM1, COX-2, and GRP78 in BIDC by detecting the expression levels of FOXM1, COX-2, and GRP78 in the peripheral blood of patients with BIDC and healthy people.

Research results

FOXM1, COX-2, and GRP78 were elevated in BIDC and demonstrated excellent diagnostic and prognostic assessment of BIDC.

Research conclusions

FOXM1, COX-2, and GRP78 exhibit abnormally high expression in BIDC, promoting malignant tumor development and closely correlating with prognosis.

Research perspectives

This study demonstrated the clinical significance of FOXM1, COX-2, and GRP78 in BIDC, and in the future, they can be used in the clinic as reference indexes for the diagnosis, disease evaluation, and prognosis assessment of BIDC. In addition, they can also be used as targets to study new targeted therapeutic options for BIDC in the future.

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