

Expressions of FHIT, BCRP and Bcl-2 Proteins in Breast Invasive Ductal Carcinoma

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Abstract: Objective: To explore the expressions of FHIT (Human fragile histidine triad), BCRP (breast cancer resistance protein) and Bcl-2 proteins in breast invasive ductal carcinoma (IDC) and its relationship with clinical pathological factors. Methods: The expressions of FHIT, BCRP and Bcl-2 proteins in 84 cases of intraoperative resection of primary breast IDC and 36 cases of normal breast tissue about 3 cm away from the neoplastic foci were detected with the method of immunohistochemical streptavidin-peroxidase (SP) to analyze the relationship between the three proteins and the pathological features of breast IDC. Results: The positive expression rates of FHIT, of BCRP and Bcl-2 were 42.86%, 72.62% and 61.90% respectively in the breast IDC group and 72.22%, 36.11% and 38.89 % respectively in the normal breast tissue control group, between which the differences were statistically significant ($P < 0.05$). In the IDC group, the expression of the three proteins was related to the lymph nodes metastasis and tumor recurrence of breast IDC, but was not significantly correlated with the expression of estrogen receptor (ER), progesterone receptor (PR) and proto-oncogene human epidermal growth factor receptor 2 (Her-2). The expression of FHIT was negatively correlated with the expression of and Bcl-2 ($r = -0.322, -0.389$), but BCRP and Bcl-2 expression were positively correlated ($r = 0.276$). Conclusion: The abnormal expressions of FHIT, BCRP and Bcl-2 proteins in breast IDC may play an important role in its onset and development.

Keywords: Breast Cancer, Invasive Ductal Carcinoma, Human Fragile Histidine Triad, Bcl-2

1. Introduction

Breast cancer has become the malignant tumor with the highest incidence in females, which keeps on increasing. The metastasis to other vital organs leads to death. FHIT, which is one of the most important apoptosis performers in the FHIT family, has been of great concern in recent years. Most of the factors triggering apoptosis kill cells ultimately through signal transduction pathway mediated by FHIT [1]. BCRP is an acidic secretory calcium-binding phosphorylated glycoprotein with negative charge and hydrophilicity discovered recently, with the biological functions of cell adhesion, migration, calcium metabolism, vascular remodeling and immune regulation, etc. BCRP expression is elevated in a variety of malignant cells and its high expression is more prone to

invasion and metastasis [2, 3]. Bcl-2 protein, which can be involved in intracellular and extracellular signal transduction, may play an important role in cell proliferation, cytoskeleton reorganization, adhesion, invasion and other important activities. FHIT, BCRP and Bcl-2 proteins may be correlated with tumor occurrence and development [4]. In this study, the expressions of the three proteins in breast IDC were detected with immunohistochemical streptavidin-peroxidase (SP) to explore their functions in the occurrence and development of breast IDC.

2. Materials and Methods

2.1. Clinical Information

84 primary breast IDC patients who received breast surgical

resection in our hospital from January 2017 to December 2017 were selected, of whom the samples were located in the center of cancerous tissue and central necrotic tissue was avoided. In addition, 36 cases of normal breast tissue pathologically diagnosed as about 3 cm away from the neoplastic foci were randomly selected and used as the control group. All samples were female patients, aged between 34 and 68 years old, with the median age of 51 years old, who did not receive preoperative radiotherapy, chemotherapy or endocrine therapy. Clinical staging was conducted according to the TNM staging standards specified in the Union for International Cancer Control (UICC). There were 27 cases in phase I, 38 cases in phase II and 19 cases in phase III. 62 cases had axillary lymphatic metastasis and 22 cases no lymphatic metastasis. Histological grading was performed according to the standards of the World Health Organization (WHO). There were 22 cases in G1, 29 cases in G2 and 33 cases in G3. In the 84 patients, 62 (phase I in 17 cases, II in 31 cases and III in 14 cases as per TNM staging) had complete follow-up data for 3 years or above, and the other patients could not be contacted. In the 62 patients, metastasis and recurrence were found in 38 patients, which were not found in other 24 patients. All patients underwent mastectomy or modified radical mastectomy of breast cancer, all of which were confirmed IDC according to intraoperative fast frozen section and conventionally pathological examination after surgery.

2.2. Reagents

Rabbit anti-human FHIT monoclonal antibody was purchased from Wuhan Boster Biological Engineering Co., Ltd., mouse anti-human Bcl-2 monoclonal antibody (3C12) from Lab-vision / NeoMarkers and mouse anti-human BCRP monoclonal antibody, SP immunohistochemical hypersensitive kit and color kit (DAB kit) from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

2.3. Detection of FHIT, BCRP and Bcl-2 Expressions

FHIT positive film and liver cancer BCRP positive film confirmed and Bcl-2 positive control film purchased were taken as the positive control. The primary antibody was replaced with phosphate-buffered saline (PBS) as the negative control. The steps were performed strictly in accordance with the kit instructions. After fixation in 4% formaldehyde solution, ethanol dehydration and paraffin embedding, all samples resected intraoperatively were subjected to conventional serial sections at 3 μ m and immunohistochemical SP staining to detect the expressions of FHIT, BCRP and Bcl-2 in all samples. In this study, the working concentrations of rabbit anti-human FHIT monoclonal antibody, mouse anti-human BCRP monoclonal antibody and mouse anti-human Bcl-2 monoclonal antibody were 1:200, 1:50 and 1:100 respectively.

2.4. Results Determination

FHIT coloring was located in the nuclear and/or cytoplasm and BCRP and Bcl-2 coloring in the cell membrane and/or

cytoplasm. The specific coloring sites stained brownish yellow or dark brown fine particles were positive. The sections were read with the counting method and the double-blind method. At first, the sections were observed fully at low magnification ($\times 100$, $\times 200$) and then 5 visual fields were randomly selected at high magnification ($\times 400$), in which positive cells $\geq 30\%$ were positive and $<30\%$ were negative.

2.5. Statistical Analysis

The data were analyzed by SPSS13.0. The differences between groups and the relationship between the three indicators and all pathological parameters of breast IDC were analyzed by χ^2 test. χ^2 test and Pearson coefficient of association were adopted to analyze whether FHIT, BCRP and Bcl-2 indicators were correlated or not with the test level of $\alpha = 0.05$.

3. Results

3.1. Expressions of FHIT, BCRP and Bcl-2 Proteins in Cell

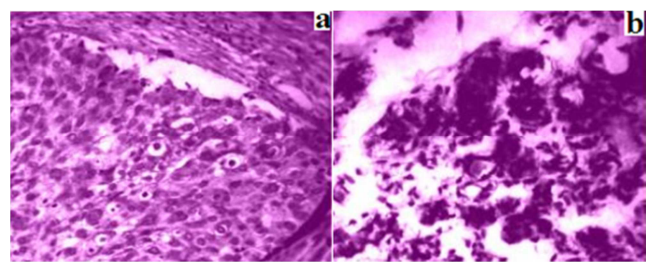


Figure 1. Staining of FHIT (SP, $\times 200$). a: breast cancer tissue; b: normal breast tissue.

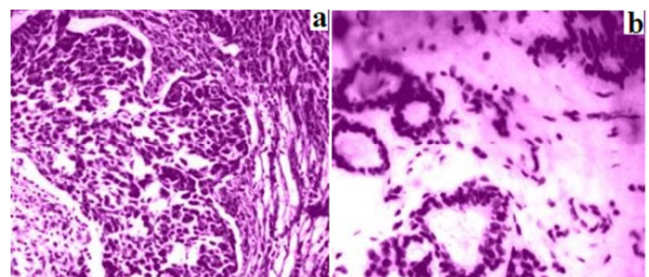


Figure 2. Staining of BCRP (SP, $\times 200$). a: breast cancer tissue; b: normal breast tissue.

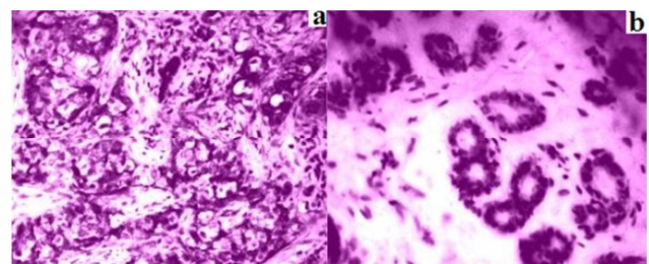


Figure 3. Staining of Bcl-2 (SP, $\times 200$). a: breast cancer tissue; b: normal breast tissue.

The positive expression rates of FHIT, of BCRP and Bcl-2 were 42.86% (36/84), 72.62% (61/84) and 61.90% (52/84)

respectively in the breast IDC group and 72.22% (26/36), 36.11% (13/36) and 38.89% (14/36) respectively in the normal breast tissue control group, between which the differences were statistically significant ($P < 0.05$). The immunohistochemical results are shown in Figure 1 to 3. Figure 1 shows that FHIT was mainly expressed in the nuclear and/or cytoplasm, with the positive particles stained brownish yellow. In normal breast tissue, FHIT expression exhibits a nest-like distribution (Figure 1b), which is significantly stronger than that in breast IDC tissue (Figure 1a). Figure 2-3 show that BCRP and Bcl-2 were expressed in the nuclear and/or cytoplasm, with the positive particles stained dark brown, and their expressions in breast IDC tissue (Figure 2a, Figure 3a) were significantly stronger than those in normal breast tissue (Figure 2b, Figure 3b).

3.2. Relationship Between FHIT, BCRP and Bcl-2 Expression and Breast IDC Clinical Pathology Parameters

The expressions of FHIT, BCRP and Bcl-2 were not significantly correlated with patients' age and menopause. FHIT expression was not significantly correlated with TNM staging but was correlated with lymphatic metastasis, histological grade and tumor recurrence ($P < 0.05$). BCRP expression was not significantly correlated with TNM staging and histological grade, but may be correlated with lymphatic metastasis and tumor recurrence ($P < 0.05$). Bcl-2 expression and TNM staging, lymphatic metastasis, histological grade and tumor recurrence were correlated ($P < 0.05$) (Table 1).

Table 1. Relationship between FHIT, BCRP and Bcl-2 expression and breast IDC clinical pathology parameters.

Clinical parameter		Case No.	FHIT		P	BCRP		P	Bcl-2		P
			Case No.	%		Case No.	%		Case No.	%	
Age	<50	49	36	73.47	0.647	28	57.14	0.137	23	46.94	0.187
	≥50	35	26	74.29		19	54.29		16	45.71	
Menstruation status	Premenopause	51	34	66.67	0.541	31	60.78	0.288	22	43.14	0.216
	Postmenopause	33	21	63.64		20	60.61		15	45.45	
TNM staging	I	27	13	48.15	0.115	10	37.04	0.083	8	29.63	0.035
	II	38	18	47.37		15	39.47		16	42.11	
	III	19	8	42.11		6	31.58		7	36.84	
Lymphatic metastasis	No	22	15	68.18	0.018	13	59.1	0.011	14	63.64	0.008
	Yes	62	37	59.68		30	48.39		29	46.77	
Histological grade	G1	22	16	72.73	0.004	12	54.55	0.091	11	50	0.014
	G2	29	18	62.07		15	51.72		17	58.62	
	G3	33	17	51.52		19	57.58		23	69.7	
Recurrence	No	24	14	58.33	0.024	13	54.17	0.018	11	45.83	0.023
	Yes	38	18	47.37		16	42.11		14	36.84	

3.3. Correlation Analysis

Correlation between FHIT, BCRP and Bcl-2 expressions and those of ER, PR and Her-2.

In the breast IDC group, the positive expression rates of ER, PR and Her-2 were 67.86% (57/84), 75.00% (63/84) and 46.42% (39/84) respectively. The statistical analysis reveals that the expressions of FHIT, BCRP and Bcl-2 were not significantly correlated with the expressions of ER, PR and Her-2 (Table 2).

Table 2. Correlation between FHIT, BCRP and Bcl-2 expressions and those of ER, PR and Her-2.

Immunohistochemical index	FHIT	BCRP	Bcl-2
ER	$X^2=2.974$	$X^2=1.602$	$X^2=1.743$
	$P=0.116$	$P=0.241$	$P=0.230$
PR	$X^2=3.045$	$X^2=1.464$	$X^2=2.544$
	$P=0.107$	$P=0.423$	$P=0.142$
Her-2	$X^2=2.681$	$X^2=1.357$	$X^2=2.153$
	$P=0.152$	$P=0.384$	$P=0.251$

Correlation between FHIT, BCRP and Bcl-2 expressions.

FHIT expression was negatively correlated with those of BCRP and Bcl-2 ($r = -0.322, -0.389, P = 0.025, 0.009$), but BCRP and Bcl-2 expressions were positively correlated ($r = 0.276, P = 0.018$).

4. Discussion

FHIT is the main enzyme that induces cell apoptosis by successively activating FHIT protein. FHIT is a key effect enzyme located in the downstream of cascade reaction. Normally, FHIT exists in the cells as inactivated zymogen, which generates a series of complex reactions and is activated under the stimulation of various apoptosis signals to degrade various proteins, thereby inducing cell apoptosis [5, 6]. When the FHIT activity is inhibited, the cell apoptosis will be blocked, which cannot dynamically balance cell apoptosis and cell proliferation that results in tumor [7, 8]. In this study, compared to the positive expression rate (72.22%) of the normal breast tissue, the positive expression rate (42.86%) of FHIT protein in the breast IDC was obviously down-regulated ($P < 0.05$). The results indicate that there may be some apoptosis mechanism abnormalities in which FHIT participate during the generation of breast IDC. Further analysis on the relationship between the FHIT expression in the IDC group and clinical pathological parameters reveals that the positive expression of FHIT is closely related to the lymphatic metastasis of breast IDC and tumor recurrence ($P < 0.05$), but had no significant relationship with patients' age and menopause. In the patients with lymphatic metastasis and tumor recurrence, the positive expression probability of FHIT

rose, and the FHIT expression was down-regulated in case of higher histological grading of breast cancer (Table 1). The results suggest that FHIT plays a key role in the malignant progression of breast IDC, which references the prognosis of breast IDC forecast. FHIT is involved in cell changes related to apoptosis, and is closely related to the occurrence and development of breast IDC. It affects the assessment of breast IDC grade malignancy, metastasis prediction and prognosis judgment [9].

BCRP, which is a secretory extracellular substrate protein, is secreted by macrophage, osteoclast, osteocyte, vascular smooth muscle cell and various tumor cells. BCRP facilitates many physiological processes such as cell chemokine, adhesion and migration, etc. Studies have indicated that the expression of BCRP protein is increased during the occurrence and development of various malignant cells, and its high expression is more prone to cancer invasion and metastasis [10, 11]. Bhatia *et al.* implanted the breast cancer cells transfected by wide-type BCRP gene in vitro into nude mouse, and found that these cells' lymphatic vessel invasion, lymph gland and pulmonary metastasis rates increased significantly [12]. Hamdan conducted a prospective study on 158 cases of metastatic breast cancer. He found that higher BCRP concentration shortened the progression-free survival and the overall survival of the patients, and easily led to organic metastasis in vivo [13].

In this study, the positive expression rate (72.62%) of BCRP protein in breast IDC group was significantly higher than that in the control group (36.11%), implying that the BCRP expression up-regulation may play a role in the generation of breast IDC by inhibiting the apoptosis of breast cancer cells. BCRP expression in breast IDC tissue was closely related to the lymphatic metastasis and tumor recurrence ($P < 0.05$). The high expression of BCRP in breast IDC tissue indicates that the tumor is inclined to lymphatic metastasis and recurrence. This study on the expression plays an important role in predicting the prognosis of breast cancer. There was no significant correlation between the BCRP expression and patients' age, menopause, pathology TNM staging and histological grading in this study ($P > 0.05$). This may be attributed to the complex apoptotic pathways, multiple factors involved and the comprehensive action.

Bcl-2, one of the ERM family members, is a membrane-cytoskeleton linking protein. Under physiological conditions, Bcl-2 is engaged in the movement, migration, adhesion and mitosis of cells, and is associated with cell senescence and apoptosis. Bcl-2 simulates FAS passage to launch cell apoptosis by linking the actin cytoskeleton and FAS protein [14, 15]. When the FAS-Bcl-2 link is abnormal, it will influence the reactivity of the FAS-induced cell apoptosis to induce anti-apoptosis. Therefore, Bcl-2 may affect the generation of the malignant tumor in this way [16]. This study also reveals that the high expression of Bcl-2 is also associated with the invasion and metastasis of various malignant tumors. The over-expression and activation of Bcl-2 may result in abnormal intercellular signal transmission, and facilitate the tumor metastasis, thereby determining tumor invasion and

metastasis [17, 18]. In this study, the positive expression rate (61.90%) of Bcl-2 in the breast IDC group was higher than that (38.89%) in the control group, between which the difference was statistically significant. This indicates the high expression of Bcl-2 is closely related to the generation of breast IDC. Meanwhile, the statistical analysis reveals that with the increase of clinical TNM staging, lymphatic metastasis and histological grading, the expression of Bcl-2 was increased significantly. In the recurrence cases, Bcl-2 expression was also increased distinctly (Table 1), suggesting that the breast IDC patients with highly expressed Bcl-2 have a relatively high histopathologic staging, histologic staging and the potential of recurrence and lymphatic metastasis. To some extent, the detection of Bcl-2 expression assists to determine the degree of malignancy and the invasive and metastatic potential of breast IDC.

Numerous factors influence the occurrence and development of tumor cells with sophisticated interaction mechanisms. Studies have found that BCRP and Bcl-2 proteins may be engaged in the interaction between cells and between cells and substrate through adjusting the cell membrane receptor and the cellular signal transduction pathway. This will loosen the connection between cells and make the intercellular signal transmission abnormal, which down-regulates the expression of FHIT protein, thus preventing the apoptosis of the infiltrating ductal cancer cells and promoting the occurrence and development of invasive ductal carcinoma [19]. The results of this study show that in breast IDC, the expression of FHIT protein was negatively correlative with those of BCRP and Bcl-2, but the expression of BCRP protein was positively correlative with that of Bcl-2, indicating that they independently and cooperatively participated in the occurrence and development of breast IDC.

In addition, the measurement and evaluation of ER, PR and Her-2 in breast cancer has been widely used in clinic. The expressions of ER and PR provide theoretical basis for the postoperative endocrine therapy. Her-2 also has become an important indicator in predicting tumor prognosis. However, this study does not find that there is a significant correlation between the expressions of FHIT, BCRP and Bcl-2 and those of ER, PR and HER-2 (Table 2), which is consistent with previous researches, surmising that the occurrence and development of breast IDC results from multiple factors. As each factor has phenotypic advantage and signal-regulated network, FHIT, BCRP and Bcl-2 in the breast IDC generation and development may not be directly associated with ER, PR and HER-2, and the specific mechanism cannot be explained clearly by genetic expression in a stationary phase, and needs to be further explored by functional morphology.

5. Conclusion

In summary, the abnormal expressions of FHIT, BCRP and Bcl-2 proteins predominated the occurrence and development of breast IDC. Their expressions may be correlated with breast IDC lymphatic metastasis and tumor recurrence, which can be used as a reference for clinicians to estimate the degree of

malignancy of breast cancer and infiltrating metastatic potential to provide a basis for the selection of therapeutic programs for patients.

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