Correlated expression of Fas, NF-κB, and VEGF-C in infiltrating ductal carcinoma of the breast

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Summary

Objective: To investigate the expressions of Fas, NF-κB, and vascular endothelial growth factor-C (VEGF-C) in infiltrating ductal carcinoma of the breast and provide scientific basis for early diagnosis and prognosis of breast cancer. *Materials and Methods:* The immunohistochemical technique (SP method) was used to detect expression of Fas, NF-κB, and VEGF-C in 137 cases of breast-infiltrating ductal carcinoma, 17 cases of intraductal carcinoma of the breast, and 20 cases of normal breast tissues, and analyze its relationship with clinicopathologic factors of breast cancer and patients' survival rate, as well as the correlation among their expression, clinicopathologic factors, and survival rate. *Results:* Fas expression was less commonly detected in infiltrating ductal carcinoma than in intraductal carcinoma and normal tissue. In contrast, both NF-κB and VEGF-C were more commonly detected in infiltrating ductal carcinoma than in intraductal carcinoma and normal tissue. Fas expression was correlated with tumor size, histological grade, and clinicopathological stage; NF-κB expression was correlated with tumor size, histological grade, lymph node metastasis; VEGF-C expression was correlated with lymph node metastasis and clinical and pathological stages of breast cancer ($p \leq 0.05$). Spearman rank correlation analysis revealed a negative correlation between Fas expression and both NF-κB and VEGF-C expression in infiltrating breast cancer ($p < 0.05$) Additionally, Kaplan-Meier survival analysis demonstrated that five-year survival was higher for patients with Fas-positive samples but lower for those with VEGF-C-positive samples. *Conclusions:* The present results demonstrate that Fas and NF- B play a role in the initiation and development of breast cancer, while VEGF-C appears to promote lymph node metastasis. Thus, these proteins may serve as useful diagnostic and prognostic markers of invasive breast cancer.

Key words: Breast-infiltrating ductal carcinoma; Fas; NF-κB; VEGF-C; Immunohistochemistry,

Introduction

Breast cancer is the most common malignant tumor among women. Approximately 1.3 million new cases of breast cancer arise worldwide each year, and 460,000 women die annually from this disease [1]. Indeed, breast cancer is predicted to account for 29% of malignant tumors in women in 2012 [2]. Both the occurrence and progression of breast cancer are caused by interactions of genetics, hormones, and immune function, as well as various environmental factors. Additionally, cancer cell invasion and early metastasis are closely related to disease prognosis.

Given the high incidence of breast cancer and its prognosis, much research has been devoted to identifying and employing biological indicators of breast cancer for aiding in diagnosis and treatment of the disease. Several different molecules have received some attention as potential markers of breast cancer development. First, apoptosis inhibitor (Fas antigen/Apo-1/CD95), one of the members of the death receptor superfamily [3], regulates apoptosis by combining with specific ligand FasL and initiating signaling through the death-inducing signal cascade (DISC) [4]. Absence of Fas is related to tumorigenesis, and indeed, Fas expression can reflect the biological behavior of tumors, including breast cancer [5-7]. Another potential marker is nuclear factor-kappa B (NF-κB), a transcription factor that binds DNA response elements and regulates expression of many genes. This protein specifically binds with the immunoglobulin κ light chain gene enhancer κB sequence and is involved in immune response, inflammation, cell proliferation, and apoptosis [8, 9]. NF-κB is dysregulated in many tumor types, with inappropriate activation stimulating tumor cell growth and inhibiting tumor cell apoptosis [10-12]. In fact, NF-κB is constitutively active in some breast tumors, making it potentially useful as a breast cancer biomarker [13, 14]. Finally, human vascular endothelial growth factor-C (VEGF-C), a platelet-derived growth factor, promotes angiogenesis and lymphangiogenesis [15]. Specifically, VEGF-C can induce formation and expansion of lymph vessels in and around solid tumors and increase the contact area of tumor cells and lymphatic vessels, contributing to lymphatic metastasis of tumors [16]. In fact, VEGF-C is overexpressed in some breast and many other tumors and as such, is used as an indicator of lymph node metastasis and long-term prognosis [17, 18].

While many studies have investigated expression of Fas, NF-κB, and VEGF-C in various tumors, little correlation has been observed between their expression. The authors assessed in the present study the expression of Fas, NF-κB, and VEGF-C in infiltrating ductal breast carcinoma. Statistical correlations were used to establish a link between expression of these proteins and lay a foundation for understanding their contribution to breast cancer metastasis.

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Materials and Methods

Specimens

Archived paraffin blocks were collected from January to December 2004 in the Pathology Division, The Third People's Hospital of Yancheng. Blocks included 137 specimens of infiltrating ductal breast carcinoma, 17 specimens of intraductal carcinoma, and 20 specimens of normal breast tissues removed due to benign lesions. All infiltrating ductal carcinoma specimens were obtained from females who ranged in age from 34 to 75 years (mean age 50.95 ± 4.7 years). Prior to surgery, patients had not received any radiotherapy, chemotherapy, or biological therapy. Paraffin blocks were serially sectioned at four μm, and sections were placed onto slides and stained with hematoxylin and eosin (H&E) using conventional methods. Cases were classified according to criteria for *Histopathological Diagnosis of Tumors* [19]: 21 specimens were grade I, 63 were grade II, and 53 were grade III; and 75 cases had lymph node metastasis, while 62 cases had no metastasis. Clinical tumor, node, metastases (TNM) staging was performed according to the criteria established by both Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) [20]: 19 specimens had a carcinoid tumor diameter ≤ two cm, 95 cases had a diameter of two cm to five cm, and 23 cases had a diameter greater than five cm; additionally, 33 cases were TNM grade I, 51 were grade II, 37 were grade III, and 16 cases were grade IV. Follow-up was completed on December 31, 2009, and the survival rate was calculated from surgery date to follow-up cutoff date or death date due to recurrence, metastasis, or other reasons.

Immunohistochemistry

Sections were dewaxed and rehydrated. Antigens were retrieved at room temperature in citric acid (pH 6.0). Endogenous peroxidase was blocked with 0.3% H_2O_2, then sections were washed in distilled water followed by 0.1 M phosphate-buffered saline (PBS). Sections were sealed with ten percent goat serum and incubated at room temperature. Primary antibodies against Fas, NF-κB, or VEGF-C (Santa Cruz Biotechnology, CA, USA) were applied and slides were placed at 4°C overnight. Sections were washed with PBS, then secondary antibodies (Zhongshan-Golden Bridge Biotechnology Co., Ltd, Beijing, China) were applied for incubation at 37°C. DAB chromogen (Zhongshan-Golden Bridge Biotechnology Co., Ltd, Beijing, China) was used according to manufacturer's instructions to develop staining color. Sections were washed with distilled water, lightly re-stained with hematoxylin, dehydrated in an alcohol gradient, treated with dimethylbenzene, and sealed with neutral resins. PBS was used as a negative control in place of primary antibody, and known positive sections were used as a positive control.

Determination of staining results

Staining criteria for Fas, NF-κB, and VEGF-C were based on criteria modified from the Shimizu method [21]: for each protein, brownish-yellow particles observed in the cytoplasm under a light microscope indicated positive staining. Semi-quantitative assessment was performed by two blinded pathologists using a combination of staining proportion and intensity. Five fields were randomly selected under 400× magnification. Staining proportion was scored as the percentage of positively-stained cells from all cells in the field and was scored as follows: ≤ 1% of cells were positive was designated a 0; 2%-20% positive, 1; 21%-50% positive, 2; ≥ 51% positive, 3. Staining intensity reflected the general degree of staining within the field, as follows: no staining was scored as 0, light staining as 1, and dark staining as 2. After combining these two scores for each specimen, total scores were then translated to a negative (–) and positive (+) system, as follows: total score ≤ 1.9 as negative (–), 2-2.9 as weak positive (+), 3-3.9 as positive (++), and ≥ 4 as strong positive (+++).

Statistical analysis

Statistical analysis was performed with SPSS15.0 statistical software, and 2 test was performed to detect the correlation between expression of Fas, NF-κB, VEGF-C, and clinicopathological parameters of infiltrating ductal carcinoma. Correlation of Fas, NF-κB, and VEGF-C expression in tissues was analyzed by Spearman method. Kaplan-Meier survival analysis was performed on the relationship of each variable and five-year survival, and survival curves were generated. Meanwhile, log-rank test was performed for inter-group survival test, and $p < 0.05$ was considered as statistically significant.

Results

Expression of Fas, NF-κB, and VEGF-C in breast tissues

Fas, NF-κB, and VEGF-C proteins were each detected using immunohistochemistry in infiltrating ductal breast cancer specimens (Figures 1, 2, and 3). For Fas, expression was detected in 50.4% (n = 137) of infiltrating ductal carcinoma samples, 64.7% (n = 17) of intraductal carcinoma samples, and 85.0% (n = 20) of normal breast tissues. In contrast, NF-κB expression was detected in 62.0% of infiltrating ductal carcinoma samples, 41.2% of intraductal samples, and just 10.0% of normal breast samples; similarly, VEGF-C was expressed in 68.6%, 47.1%, and 0%, of infiltrating ductal carcinoma, intraductal carcinoma, and normal breast samples, respectively. Expression of Fas, NF-κB, and VEGF-C were significantly different among infiltrating ductal carcinoma, intraductal carcinoma and normal breast ($p < 0.05$, Table 1).

Correlation of Fas, NF-κB, and VEGF-C expression with clinicopathological characteristics of infiltrating ductal carcinoma

Fas expression was correlated with tumor diameter, histological grade, and TNM stage (Table 2). NF-κB expression was related to tumor size, histological differentiation degree, and lymph node metastasis (Table 3). VEGF-C expression was related to lymph node metastasis, TNM stage, and degree of malignancy; furthermore, its expression rate increased with increasing tumor diameter (Table 4).

Correlation of Fas, NF-κB, and VEGF-C expression in infiltrating ductal carcinoma tissues

To determine whether expression of these proteins is related to one another, the authors assessed correlation of expression of one protein within a sample with expression of the other proteins. Twenty (14.6%) of 137 infiltrating ductal carcinoma samples were negative for both Fas and NF-κB, and 37 samples (27.0%) were positive for both Fas and NF-κB. Of the 68 samples lacking Fas expres-

Table 1. — *Positive expression rates of Fas, NF-κB, and VEGF-C in infiltrating ductal breast carcinoma, intraductal carcinoma, and normal breast tissues (%).*

Tissues	n	Fas[a] Positive (%)	NF-κB[b] Positive (%)	VEGF-C[c] Positive (%)
Infiltrating ductal carcinoma	137	50.4	62.0	68.6
Intraductal carcinoma	17	64.7	41.2	47.1
Normal breast tissue	20	85.0	10.0	0

[a] $p = 0.002$, $\chi^2 = 9.099$; [b] $p = 0.034$, $\chi^2 = 6.301$; [c] $p = 0.031$, $\chi^2 = 11.162$.

Table 2. — *Correlation between Fas expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.*

Clinicopathological characteristics	n	Fas Pos. cases	Fas Neg. cases	Positive expression rate (%)	χ^2 values	*p* values	
Age (years)							
≤ 45	46	21	25	45.6	0.019	0.892	> 0.05
> 45	91	48	43	52.7			
Carcinoma diameter (cm)							
≤ 2	19	17	2	89.5	5.094	0.03	< 0.05
2-5	95	40	45	42.1			
> 5	23	2	21	8.7			
Histologic grade							
Grade I	21	14	7	66.7			< 0.05
Grade II	63	38	25	60.3	5.992	0.02	
Grade III	53	17	26	32.0			
Lymph node metastasis							
Yes	75	39	36	52.0	0.177	0.073	> 0.05
No	62	30	32	48.4			
TNM Staging							
Stage I-II	84	48	36	57.1	3.990	0.046	< 0.05
Stage III-IV	53	21	32	39.6			

Table 3. — *Correlation between NF-κB expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.*

Clinicopathological characteristics	n	NF-κB Pos. cases	NF-κB Neg. cases	Pos. expression rate (%)	χ^2 values	*p* values	
Age (years)							
≤ 45	46	25	21	54.3	1.742	0.190	> 0.05
> 45	91	60	31	65.9			
Carcinoma diameter (cm)							
≤ 2	19	9	10	47.4	4.346	0.038	< 0.05
2-5	95	58	37	61.1			
> 5	23	18	5	78.3			
Histologic grade							
Grade I	21	8	13	38.1			< 0.05
Grade II	63	41	22	65.1	6.139	0.04	
Grade III	53	36	17	67.9			
Lymph node metastasis							
Yes	75	53	22	70.7	5.233	0.022	< 0.05
No	62	32	30	51.6			
TNM Staging							
Stage I-II	84	49	35	58.3	1.269	0.263	> 0.05
Stage III-IV	53	36	17	73.5			

Table 4. — *Correlation between VEGF-C expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.*

Clinicopathological characteristics	n	VEGF-C Pos. cases	VEGF-C Neg. cases	Pos. expression rate (%)	χ^2 values	*p* values	
Age (years)							
≤ 45	46	29	17	63.0	0.530	0.470	> 0.05
> 45	91	63	28	69.2			
Carcinoma diameter (cm)							
≤ 2	19	10	9	52.6	3.955	0.08	> 0.05
2-5	95	66	29	69.5			
> 5	23	18	5	78.3			
Histologic grade							
Grade I	21	12	9	57.1			> 0.05
Grade II	63	43	20	68.3	1.895	0.185	
Grade III	53	39	14	73.6			
Lymph node metastasis							
Yes	75	58	17	77.3	5.852	0.015	< 0.05
No	62	38	26	58.0			
TNM Staging							
Stage I-II	84	51	33	60.7	6.291	0.012	< 0.05
Stage III-IV	53	43	10	81.1			

Table 5. — *Correlation between Fas and NF-κB expression in nfiltrating ductal breast carcinoma.*

Fas	NF-κB positive	NF-κB negative	*p* value	*r* value
Positive	37	32	0.041	-0.175
Negative	48	20		

Table 6. — *Correlation between Fas and VEGF-C expression in infiltrating ductal breast carcinoma.*

Fas	NF-κB positive	NF-κB negative	*p* value	*r* value
Positive	39	30	0.002	-0.262
Negative	55	13		

Table 7. — *Correlation between NF-κB and VEGF-C expression in infiltrating ductal breast carcinoma.*

NF-κB	VEGF-C positive	VEGF-C negative	*p* value	*r* value
Positive	64	21	0.031	0.184
Negative	30	22		

sion, 48 (70.6%) were positive for NF-κB expression. Alternatively, of the 69 samples with Fas expression, 37 (53.6%) were negative for NF-κB expression. Spearman correlation analysis ($r = -0.175$) indicated that expression of Fas and NF-κB were negatively correlated (Table 5).

Similar results were found for Fas and VEGF-C expression. Thirteen (9.5%) of 137 samples were negative for both Fas and VEGF-C expression, while 39 (28.5%) were positive for both proteins. However, in the 68 samples lacking Fas expression, VEGF-C was expressed in 55 (80.9%) of them. Furthermore in 69 samples positive for Fas expression, VEGF-C was detected in 39 (56.5%) of them. Thus, expression of Fas was also negatively correlated with VEGF-C expression (r = -0.262) (Table 6).

Fig. 1

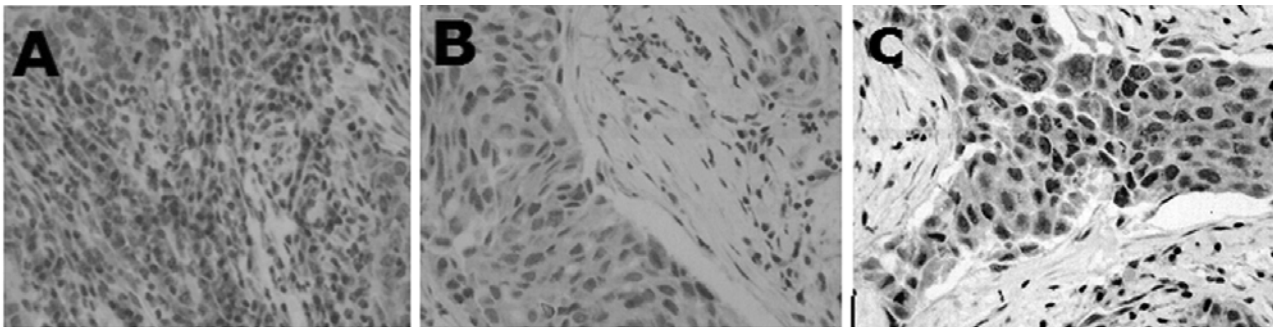

Fig. 2

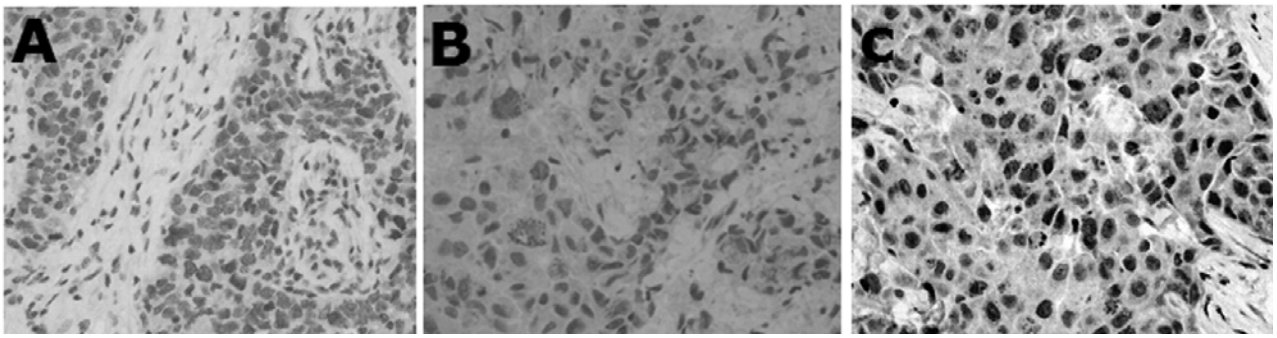

Fig. 3

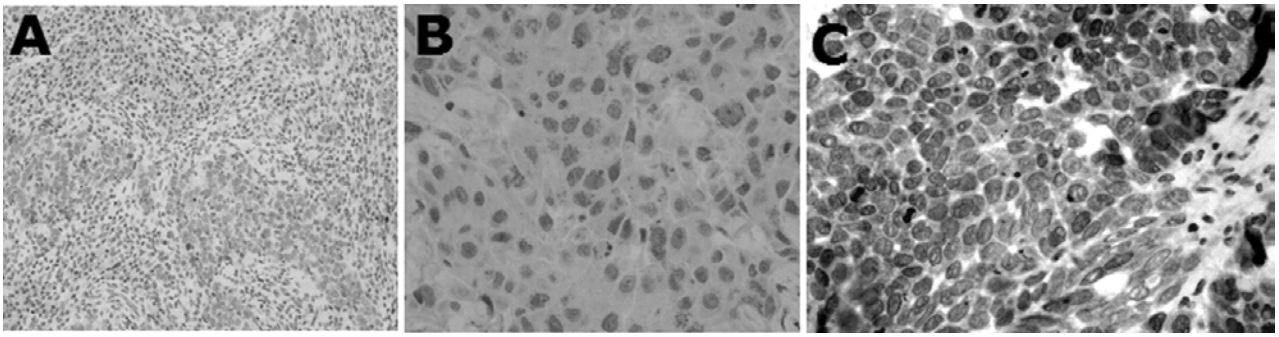

Figure 1. — Detection of Fas expression in infiltrating ductal breast carcinoma tissue by immunohistochemistry. A) grade 1 (×200), B) grade II (×200), and C) grade III (×100) infiltrating ductal carcinoma.
Figure 2. — Detection of NF-kB expression in infiltrating ductal breast carcinoma tissue by immunohistochemistry. A) grade 1 (×200), B) grade II (×200), and C) grade III (×100) infiltrating ductal carcinoma.
Figure 3. — Detection of VEGF-C expression in infiltrating ductal breast carcinoma tissue by immunohistochemistry. A) grade 1 (×200), B) grade II (×200), and C) grade III (×100) infiltrating ductal carcinoma.

Finally, 22/137 (16.1%) samples were negative for both NF-κB and VEGF-C expression, while 64/137 (46.7%) were positive for both proteins. In samples negative for NF-κB, 57.7% (30/52) expressed VEGF-C; in samples positive for NF-κB, 75.3% (64/85) also expressed VEGF-C. Expression of NF- B and VEGF-C were positively correlated ($r = 0.184$) (Table 7).

Correlation between expression of Fas, NF-κB, and VEGF-C and infiltrating ductal carcinoma prognosis

To determine whether expression of these three potential markers of invasive breast cancer correlated with disease outcomes, the authors assessed five-year follow-up data for the patients. Of the 137 cases of infiltrating ductal carcinoma, 18 patients were lost to follow-up and five patients died of accidental causes, leaving 114 cases completing follow-up. By December 31, 2009, 33 patients had died from breast cancer recurrence or metastasis; 81 patients had survived. The five-year survival rate was 71.1%, with a mean survival period of 51.47 ± 2.01 months. Kaplan-Meier survival analysis revealed that five-year survival was significantly higher for patients with Fas-positive tumor samples. Further, log-rank test showed a significant difference in five-year survival rates between patients with Fas-positive and Fas-negative samples ($\chi^2 = 4.448$, $p = 0.035$), indicating that Fas expression was positively correlated with five-year survival. In contrast, NF-κB expression resulted in a significantly decreased five-year survival rate, but log-rank test found no statistical difference in five-year survival rates between patients with and without NF-κB expression (χ^2

= 2.695, p = 0.101). However, survival time of patients with NF-κB-positive samples was shorter than those with NF-κB-negative samples. Similarly, five-year survival rate was significantly lower in patients with VEGF-C-positive samples; log-rank test found no significant difference in five-year survival rates between patients with and without VEGF-C expression (χ^2 = 4.721, p = 0.030), indicating that positive expression of VEGF-C was negatively correlated with five-year survival.

Discussion

Breast cancer prognosis is closely related to early diagnosis and the biological characteristics of the tumor. In the present study, the authors through using immunohistochemistry, detected Fas, NF-κB, and VEGF-C protein expression in samples of infiltrating ductal breast carcinoma to explore the correlation between their expression and clinicopathological characteristics of breast cancer. The authors sought to establish the value of these proteins as clinical indicators for diagnosis and prognosis of the disease.

Expression of the apoptosis-inducing Fas antigen is reportedly down-regulated or lost in breast cancer, liver cancer, ovarian cancer, and other tumor cells, and, indeed, Fas expression is more likely to decrease with increasing degree of malignancy [22-24]; the present results corroborate these observations. It was also found that Fas expression was highest in infiltrating ductal carcinoma of the lowest malignancy grade, with significantly decreasing expression rates from grade I to grade III tissues. Additionally, Fas negative expression was positively correlated with tumor diameter and TNM stage of infiltrating ductal carcinoma, and patients with Fas-negative samples had a lower five-year survival rate than those with Fas-positive samples, consistent with previous reports [25, 26]. Thus, down-regulation of Fas expression in malignant tumors may promote tumor progression. Decreased Fas expression may allow tumor cells to escape immune surveillance, and during subsequent tumor progression, selective proliferation of cells may occur: that is, breast cancer cells may make the immune system attack tumors cells with a lower malignancy degree and positive Fas expression through selective cell apoptosis, while tumor cells with a higher malignancy degree and negative Fas expression survive, lead to further development of tumors. Loss of Fas expression in cancer cells may prevent attack by tumor-infiltrating lymphocytes (TIL) and cytotoxic T lymphocytes (CTL), which are usually initiated by FasL expression. When FasL is expressed in cancer cells, apoptosis signaling is initiated through the Fas system, allowing TIL and CTL to kill tumor cells. When Fas expression is lost in tumors, apoptosis is evaded. Therefore, tumor cells lacking Fas expression are more likely to grow, leading to larger carcinoma diameter and higher malignancy degree, making loss of Fas expression an important potential cause of tumorigenesis [27, 28].

NF-κB refers to the most abundantly expressed p50/RelA (p50/p65) heterodimer in the Rel/NF-kB family. The protein is a core regulation and control member in the apoptotic pathway, controlled by death receptor signaling and able to regulate downstream anti-apoptotic genes via trans-activation [29]. NF-κB is constitutively activated in many tumors. As a transcription factor, constitutive expression of NF-κB can lead to increased transcriptional activity, particularly of downstream gene products like VEGF (including VEGF-C). Increased VEGF activity can result in increased tumor vascularization and promote infiltration of surrounding tissues [30].

The authors found that NF-κB expression was higher in infiltrating ductal carcinoma than in intraductal carcinoma and normal breast tissues. Furthermore NF-κB expression was correlated to carcinoma diameter, histological grade, and lymph node metastasis. Higher NF-κB expression has been reported for patients with lymph node metastasis compared to those without lymph node metastasis [31]. A previous study [32] suggested that NF-B is related to cell apoptosis induced by DNA damage that is caused by chemotherapy; indeed, NF-κB expression is not only related to occurrence and development of tumors, but also influences the sensitivity of tumor cells to chemotherapy and disease prognosis. However, the present study did not find a difference in five-year survival for patients with and without NF-κB expression in their tumors. Further studies are needed to find the cause of these differences.

Current hypotheses [33] suggest that abnormal activation of NF-κB leads to uncontrolled regulation of the cell cycle, manifested as unlimited cell proliferation and division, and tumor formation. NF-κB dysfunction can not only initiate the development of breast cancer, but may also influence the invasion, growth, and progression of breast cancer. NF-κB may regulate cellular adhesions and induce transcription of other proteins to promote tumor invasion and metastasis. Thus, NF-κB may represent an appropriate clinical target for tumor therapy.

VEGF-C, a member of the VEGF family of growth factors, is expressed in both embryos and mature tissues. In adults, VEGF-C is mainly expressed in heart, placenta, ovary, small intestine, and thyroid. During embryonic development, VEGF-C is involved in formation of the lymphatic network. However, VEGF-C is also expressed in malignant tumors, and tumor cells with VEGF-C expression have a higher rate of local lymph node metastasis [34]. In the present study VEGF-C was detected more commonly in infiltrating ductal carcinoma than in intraductal carcinoma or normal breast tissues. Additionally, expression was correlated with lymph node metastasis and TNM stage. Five-year survival was also reduced in patients with VEGF-C-positive samples, suggesting that VEGF-C expression may predict breast cancer recurrence, consistent with the literature [35, 36].

VEGF-C expression can promote growth, invasion, and metastasis of tumors. Over-expression in tumors can induce lymphatic endothelial cell proliferation through its receptor, VEGFR-3, located in lymphatic endothelial cells. Additionally, lymph-vessel extension, development

of lymph networks, and formation of lymph vessels around the tumors can be induced. Meanwhile, existing lymph vessels can also proliferate and increase in diameter, merge with newly-formed lymph vessels, and transfer tumor cells into lymph nodes, causing lymph node metastasis [36, 37]. Lymph node metastasis is the most common method by which breast cancer spreads. Thus, VEGF-C, by reflecting the degree of lymph node involvement, may help determine staging of breast cancer and its treatment options.

While many studies have focused on the pairwise correlations between Fas, NF-κB [38], and VEGF-C, few have focused on the correlation among all three, particularly in breast cancer. The present results suggest that a possible pathway to promote tumor lymph node metastasis may contain Fas, NF-κB, and VEGF-C. This mechanism would involve loss of Fas expression, subsequent NF-κB activation, and downstream activation of VEGF-C, promoting lymphangiogenesis and lymphatic metastasis.

Briefly, in infiltrating ductal breast carcinoma, Fas expression was negatively correlated with the expression of both NF-κB and VEGF-C. A loss of Fas expression was correlated to increasing tumor severity, while NF-κB and VEGF-C expression reflected more severe disease. Additionally, loss of Fas but positive expression of NF-κB and VEGF-C were each correlated with lower five-year survival rates. Thus, loss of Fas and increased expression of NF-κB and VEGF-C can promote breast cancer progression. NF-κB and VEGF-C may serve as both diagnostic and prognostic indicators of invasive breast cancer.

References

[1] Siegel R., Naishadham D., Jemal A.: "Cancer statistics, 2012". CA Cancer Clin., 2012, *62,* 10.

[2] Olszewski W.P., Szumera-Cieckiewicz A., Piechocki J., Towpik E., Olszewski W.T.: "The characteristics of the sentinel lymph node metastasis in predicting the axillary lymph node status in patients with breast carcinoma". *Pol. J. Pathol*., 2009, *60,* 138.

[3] Krammer P.H., Arnold R., Lavrik I.N.: "Life and death in peripheral T cells". *Nat. Rev. Immunol*., 2007, *7*, 532.

[4] Lavrik I.N., Krammer P.H.: "Regulation of CD95/Fas signaling at the DISC". *Cell. Death Differ*., 2012, *19,* 36.

[5] Liang Q.L., Li Z.Y., Chen G.Q., Lai Z.N., Wang B.R., Huang J.: "Prognostic value of serum soluble Fas in patients with locally advanced unresectable rectal cancer receiving concurrent chemoradiotherapy". *J. Zhejiang Univ. Sci. B*, 2010, *11*, 912.

[6] Boroumand-Noughabi S., Sima H.R., Ghaffarzadehgan K., Jafarzadeh M., Raziee H.R., Hosseinnezhad H. *et al*.: "Soluble Fas might serve as a diagnostic tool for gastric adenocarcinoma". *BMC Cancer*, 2010, *10*, 275.

[7] Sjöström-Mattson J., Von Boguslawski K., Bengtsson N.O., Mjaaland I., Salmenkivi K., Blomqvist C.: "The expression of p53, bcl-2, bax, fas and fasL in the primary tumour and lymph node metastases of breast cancer". *Acta Oncol.,* 2009, *48,* 1137.

[8] Gao H., He S., Tang W.X. *et al*.: "Inhibition of NF-kappa B activity enhanced apoptosis induced by matrine in hepatocellular carcinoma cells". *Zhonghua Gan Zang Bing Za Zhi,* 2007, *15,* 914.

[9] Ruiz-Ortega M., Esteban V., Egido J.: "The regulation of the inflammatory response through nuclear factor-kappab pathway by angiotensin IV extends the role of the rennin angiotensin system in cardiovascular diseases". *Trends Cardiovasc. Med.,* 2007, *17,* 19.

[10] Rushworth S.A., Murray M.Y., Barrera L.N., Heasman S.A., Zaitseva L., Macewan D.J.: "Understanding the role of miRNA in regulating NF-κB in blood cancer". *Am. J. Cancer Res*., 2012, *2*, 65.

[11] Chen D., Niu M., Jiao X., Zhang K., Liang J., Zhang D.: "Inhibition of AKT2 enhances sensitivity to Gemcitabine via regulating PUMA and NF-κB signaling pathway in human pancreatic ductal adenocarcinoma". *Int. J. Mol. Sci.,* 2012, *13*, 1186.

[12] Li J., Wang K., Chen X., Meng H., Song M., Wang Y., Xu X., Bai Y.: "Transcriptional activation of microRNA-34a by NF-kappa B in human esophageal cancer cells". *BMC Mol. Biol*., 2012, *13,* 4.

[13] Prasad S., Ravindran J., Aggarwal B.B.: "NF-kappaB and cancer: how intimate is this relationship". *Mol. Cell. Biochem.,* 2010, *336,* 25.

[14] Ahmed A.: "Prognostic and therapeutic role of nuclear factor-kappa B (NF-kappaB) in breast cancer". *J. Ayub. Med. Coll. Abbottabad,* 2010, *22*, 218.

[15] Raica M., Cimpean A.M., Ceausu R., Ribatti D.: "Lymphatic microvessel density, VEGF-C and VEGFR-3 expression different molecular types of breast cancer". *Anticancer Res.,* 2011, *31,* 1757.

[16] Acs G., Paragh G., Rakosy Z., Laronga C., Zhang P.J.: "The extent of retraction clefts correlates with lymphatic vessel density and VEGF-C expression and predicts nodal metastasis and poor prognosis in early-stage breast carcinoma. Mod Pathol, 2012, 25(2):163-77.

[17] Watanabe O., Kinoshita J., Shimizu T., Imamura H., Hirano A., Okabe T. *et al*.: "Expression of a CD44 variant and VEGF-C and the implications for lymphatic metastasis and long-term prognosis of human breast cancer". *J. Exp. Clin. Cancer Res*., 2005, *24,* 75.

[18] Tanaka T., Ishiguro H., Kuwabara Y., Kimura M., Mitsui A., Katada T. *et al*.: "Vascular endothelial growth factor C (VEGF-C) in esophageal cancer correlates with lymph node metastasis and poor patient prognosis". *J. Exp. Clin. Cancer Res*., 2010, *29*, 83.

[19] Tavassoli F.A., Devilee P.: "World Health Organization classification of tumors". Pathology and genetics, tumors of the breast and female genital organs. Lyon, IARC Press, 2003.

[20] Edge S.B., Byrd D.R., Compton C.C., Fritz A.G., Greene F.L., Trotti A. (eds.). AJCC Cancer Staging Manual. 7th ed. New York, Springer, 2009.

[21] Shimizu M., Saitoh Y., Itoh H.: "Immunohistochemical staining of ha-ras oncogene product in normal, benign, and malignant human pancreatic tissues". *Hum. Pathol.,* 1990, *21*, 607.

[22] Razmkhah M., Jaberipour M., Ghaderi A.: "Bcl-2 and Fas expressions correlate with proliferative specificity of adipose-derived stem cells (ASCs) in breast cancer". *Immunol. Invest.,* 2011, *40,* 290.

[23] Kountouras J., Zavos C., Chatzopoulos D.: "Apoptotic and antiangiogenic strategies in liver and gastrointestinal malignancies". *J. Surg. Oncol*., 2005, *90*, 249.

[24] Kim R., Inoue H., Toge T.: "Bax is an important determinant for radiation sensitivity in esophageal carcinoma cells". *Int. J. Mol. Med*., 2004, *14,* 697.

[25] Ryan B.M., Konecny G.E., Kahlert S., Wang H.J., Untch M., Meng G. *et al*.: "Survivin expression in breast cancer predicts clinical outcome and is associated with HER-2, VEGF, Urokinase plasminogen activator". *Ann. Oncol*., 2006, *17*, 597.

[26] Jaberipour M., Habibagahi M., Hosseini A., Abbasi M., Sobhani-Lari A., Talei A., Ghaderi A.: "Detection of B cell lymphoma 2, tumor protein 53, and FAS gene transcripts in blood cells of patients with breast cancer". *Indian Cancer,* 2010, *47*, 412.

[27] Rosell R., Cuello M., Cecere F., Santarpia M., Reguart N., Felip E. *et al*.: "Usefulness of predictive tests for cancer treatment". *Bull. Cancer,* 2006, *93*, E101.

[28] Zemzoum I., Kates R.E., Ross J.S., Dettmar P., Dutta M., Henrichs C. *et al*.: "Invasion factors Upa/PAI-1 and HER2 status provide independent and complementary information on patient outcome in node-negative breast cancer". *J. Clin. Oncol*., 2003, *21,* 1022.

[29] Lin X., Zhang X., Wang Q., Li J., Zhang P., Zhao M., Li X.: "Perifosine downregulates MDR1 gene expression and reverses multidrug-resistant phenotype by inhibiting P13K/Akt/NF-κB signaling pathway in human breast cancer cell line". *Neoplasma,* 2012, *59,* 248.

[30] Tsai P.W., Shiah S.G., Lin M.T., Wu C.W., Kuo M.L.: "Up-regulation of vascular endothelial growth factor C in breast cancer cells by heregulin-beta 1. A critical role of p38/nuclear factor-kappa B signaling pathway". *J. Biol. Chem.,* 2003, *278*, 5750.

[31] Lee S.T., Li Z., Wu Z., Aau M., Guan P., Karuturi R.K. *et al*.: "Context-specific regulation of NF-κB target gene expression by EZH2 in breast cancers". *Mol*. *Cell*., 2011, *43,* 798.
[32] Zerbini L.F., Tamura R.E., Correa R.G., Czibere A., Cordeiro J., Bhasin M. *et al*.: "Combinatorial effect of non-steroidal anti-inflammatory drugs and NF-κB inhibitors in ovarian cancer therapy". *PLoS One,* 2011, *6,* e242285. Epub 2011 Sep 12.
[33] Hagan S., Al Mulla F., Mallon E., Oien K., Ferrier R., Gusterson B. *et al*.: "Reduction of Raf-1 kinase inhibitor protein expression correlates with bread cancer metastasis". *Clin*. *Cancer Res*., 2005, *11,* 7392.
[34] Herrmann E., Eltze E., Bierer S., Köpke T., Görge T., Neumann J. *et al*.: "VEGF-C, VEGF-D and Flt-4 in transitional bladder cancer relationships to clinicopathological parameters and long-term survival". *Anticancer Res*., 2007, *27,* 3127.
[35] Wu Q.W., She H.Q., Liang J., Huang Y.F., Yang Q.M., Yang Q.L., Zhang Z.M.: "Expression and clinical significance of extracellular matrix protein 1 and vascular endothelial growth factor-C in lymphatic metastasis of human breast cancer". *BMC Cancer*, 2012, *12,* 47. doi: 10.1186/1471-2407-12-47.
[36] Acs G., Paragh G., Rakosy Z., Laronga C., Zhang P.J.: "The extent of retraction clefts correlates with lymphatic vessel density and VEGF-C expression and predicts nodal metastasis and poor prognosis in early-stage breast carcinoma". *Mod*. *Pathol*., 2012, *25,* 163.
[37] Gjini E., Hekking L.H., Küchler A., Saharinene P., Wienholds E., Post J.A. *et al*.: "Zebrafish Tie-2 shares a redundant role with Tie-1 in heart development and regulates vessel integrity". *Dis*. *Model*. *Mech*., 2011, *4,* 57.
[38] Lee S.M., Kim E.J., Suk K., Lee W.H.: "Stimulation of Fas (CD95) induces production of pro-inflammatory mediators through ERK/JNK-dependent activation of NF-κB in THP-1 cells". *Cell*. *Immunol*., 2011, *271,* 157.

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