Review Article
Expression of VEGF, COX-2 and MMP-9 in breast cancer and their relationship with ultrasound findings

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Abstract: Objective: We wished to ascertain the relationship between expression of vascular endothelial growth factor (VEGF), cyclo-oxygenase (COX)-2, and matrix metallopeptidase (MMP)-9 and various features of ultrasound images in breast cancer (BC) patients. Methods: Eighty-nine breast lesions were confirmed to have BC by ultrasound, surgery and pathology. According to the Breast Imaging Reporting and Data System classification method published by the American College of Radiology, six groups were created: spiculation and non-Burr sign; calcification and non-calcification; vascular anomaly syndrome and non-vascular anomaly syndrome; lymph node metastasis (LNM) and non-LNM. In each case, the expression of VEGF, COX-2 and MMP-9 was detected by immunohistochemistry. Results: Expression of VEGF, COX-2 and MMP-9 was higher in BC patients with the Burr sign than in those without it (P<0.05). There was no significant difference in expression of VEGF, COX-2, and MMP-9 between the calcification group and non-calcification group of BC patients (P>0.05). Expression of VEGF, COX-2, and MMP-9 was higher in those with vascular abnormalities than in those without them (P<0.05), as well as in the LNM group compared with the non-LNM group (P<0.05). Conclusions: The high expression of VEGF, COX-2, and MMP-9 could have an influence on the Burr sign, abnormal blood vessels, and LNM in BC patients using ultrasound.

Keywords: Breast cancer, ultrasound features, VEGF, COX-2, MMP-9

Introduction

Cancer statistics released in 2012 revealed that the worldwide prevalence of female breast cancer (BC) had reached 29% [1]. In 2012, approximately 1.7 million people worldwide were diagnosed with breast cancer and 500,000 people died of breast cancer, which is one of the leading causes of death in developing countries [2, 3]. The prognosis for early BC is quite good, but that of advanced BC is poor. Hence, the early diagnosis of BC is extremely important. Ultrasound of the breast is a simple, reliable and non-invasive method for BC diagnosis. The occurrence and development of BC is a multi-factor, multi-stage, multi-step process. Studies [4] have suggested that angiogenesis and lymphangiogenesis have important roles in the growth of BC and other tumors. Some researchers [5-7] have pointed out that vascular endothelial growth factor (VEGF) pro-

motes angiogenesis during BC progression. Studies have demonstrated that cyclo-oxygenase (COX)-2, prostaglandin E2 (PGE2) and thromboxane A2 inhibit the apoptosis of endothelial cells and promote tumor angiogenesis [8, 9]. Matrix metallopeptidase (MMP)-9 leads to membrane damage and creates the conditions for tumor-cell invasion into surrounding tissue [10]. We wished to study the relationship between the expression of VEGF, COX-2 and MMP-9 to more accurately assess the prognosis of BC and to provide a theoretical basis to formulate a rational treatment plan.

Materials and methods

Patients

A total of 89 women with invasive ductal carcinoma of the breast underwent ultrasound examination and resection from March 2014 to June 2017 at the Affiliated Hospital of Tai'an
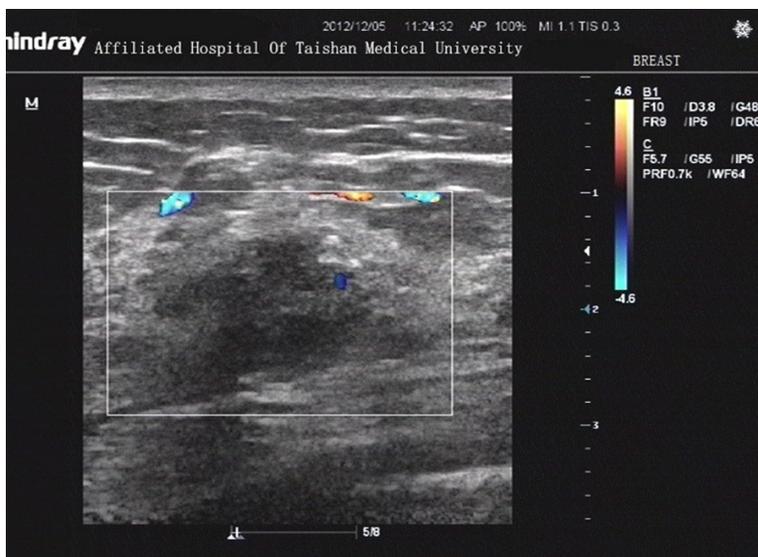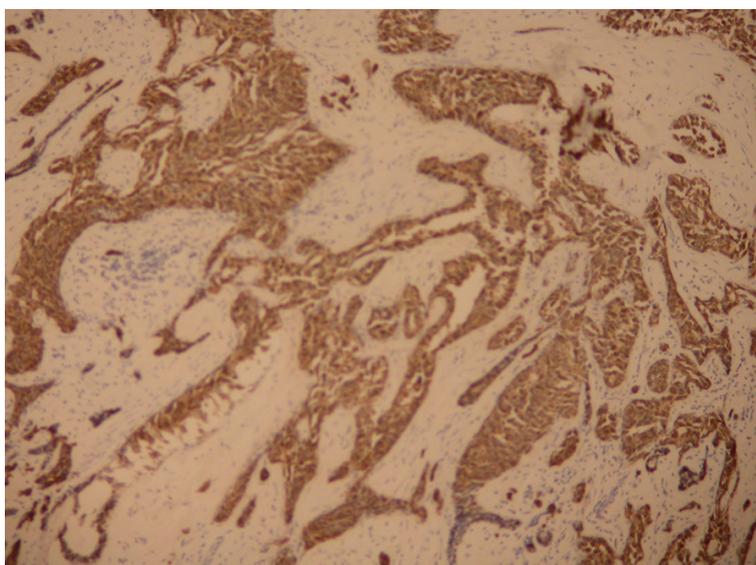
Figure 1. Ultrasound of the breast.

Figure 2. VEGF showed strong expression in BC patients with the Burr sign (×400 magnification).

Table 1. Expression of VEGF, COX-2 and MMP-9 in BC patients with and without the Burr sign

	N	VEGF	COX-2	MMP-9
Burr	60	90.00%	93.33%	100%
Non-Burr sign	29	72.41%	65.52%	58.62%
P		0.033	0.001	<0.001
χ^2		4.561	11.411	28.697

Medical College (Shandong, China). The age range was 42-66 (mean, 42.5) years.

Ultrasound of the breast

Ultrasound of the breast was undertaken by two very experienced physicians (deputy chief physician and attending physician).

Immunohistochemical (IHC) analyses

Immunohistochemistry was used to detect the expression of VEGF, COX-2 and MMP-9 proteins in 89 specimens using the streptavidin peroxidase conjugated method.

The results of IHC staining were evaluated using phosphate-buffered saline as a blank control. Under microscope observation, the positive signals of VEGF, COX-2 and MMP-9 in normal cells were located in the cytoplasm and expressed in brown granules. No staining denoted a negative result. The proportion of positive cells in cancer cells was counted according to the method of Miyake et al., the proportion of positive cells were ≤30% indicated negative outcome, and >30% indicated positive.

Imaging

The two ultrasonographers were blinded to the final pathologic findings of breast lesions and expression of IHC markers. A double-blind imaging diagnosis was carried out. According to the Breast Imaging Reporting and Data System classification method published by the American College of Radiology, six groups were created: spiculation and non-Burr sign; calcification and non-calcification; vascular anomaly syndrome and non-vascular anomaly syndrome; lymph node metastasis (LNM) and non-LNM.

Statistical analyses

Statistical analyses were undertaken using SPSS v13 (IBM, Armonk, NY, USA). The chi-

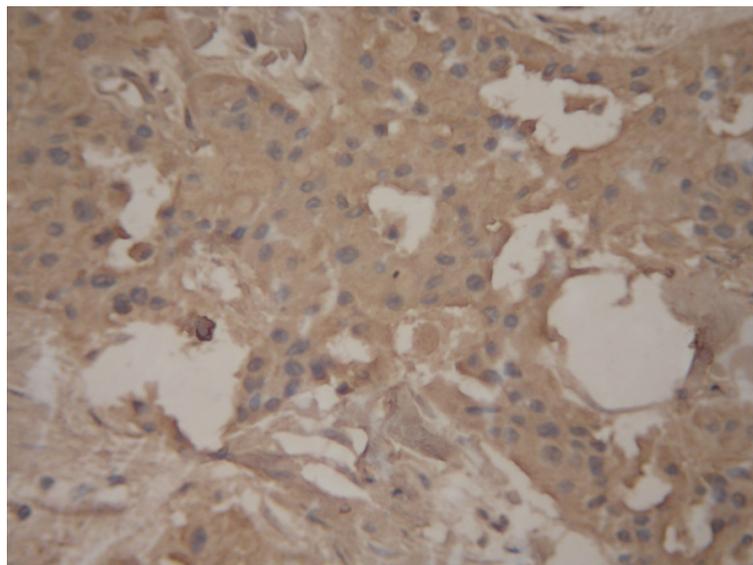**Figure 3.** Ultrasound of a BC patient with multiple calcifications.

Figure 4. A BC patient with calcification showed high expression of COX-2 (×400 magnification).

Table 2. Expression of VEGF, COX-2 and MMP-9 in BC patients with and without calcification

	N	VEGF	COX-2	MMP-9
Calcification	40	75.00%	85.00%	65.00%
Non-calcification	49	73.47%	85.71%	65.31%
P		0.870	0.924	0.976
X^2		0.027	0.009	0.001

cal variables. P<0.05 was considered significant.

Results

Expression of VEGF, COX-2 and MMP-9 in BC and its relationship with the Burr sign

The Burr sign is an important feature in an ultrasound diagnosis of BC, and is used to differentiate benign and malignant breast tumors (**Figures 1**, **2**). Expression of VEGF, COX-2 and MMP-9 was higher in BC patients with the Burr sign compared with BC patients without the Burr sign, and the difference was significant (**Table 1**).

Relationship between expression of VEGF, COX-2, MMP-9 and calcification

Calcification of breast tissue is important in BC diagnosis. Calcification of breast tissue was made by pathology in 60-83 of patients, and by ultrasound in 40-60 of patients. Calcification within a tumor can be used as an important index to judge benign and malignant tumors [6]. Expression of VEGF, COX-2, and MMP-9 showed no obvious correlation with calcification (**Figures 3**, **4**). There was no significant difference between expression of VEGF, COX-2, and MMP-9 in the group with calcification and in those without calcification (**Table 2**).

Expression of VEGF, COX-2 and MMP-9 and its correlation with vascular abnormalities

Another important ultrasound feature of BC is vascular abnormalities (**Figures 5**, **6**). Expression of VEGF, COX-2 and MMP-9 in BC patients with a vascular anomaly group was significantly higher than that in BC patients without a vascular abnormality (**Table 3**).
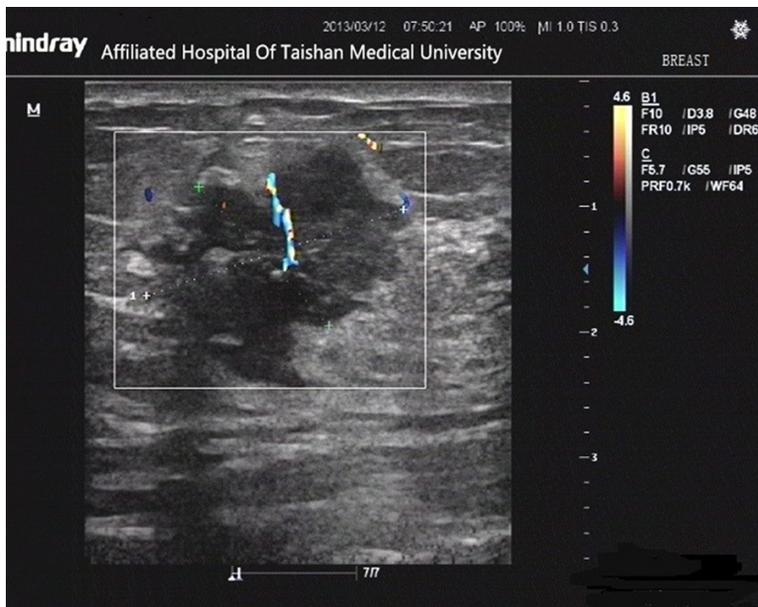
square test was used to ascertain if there was a significant relationship between two categori-

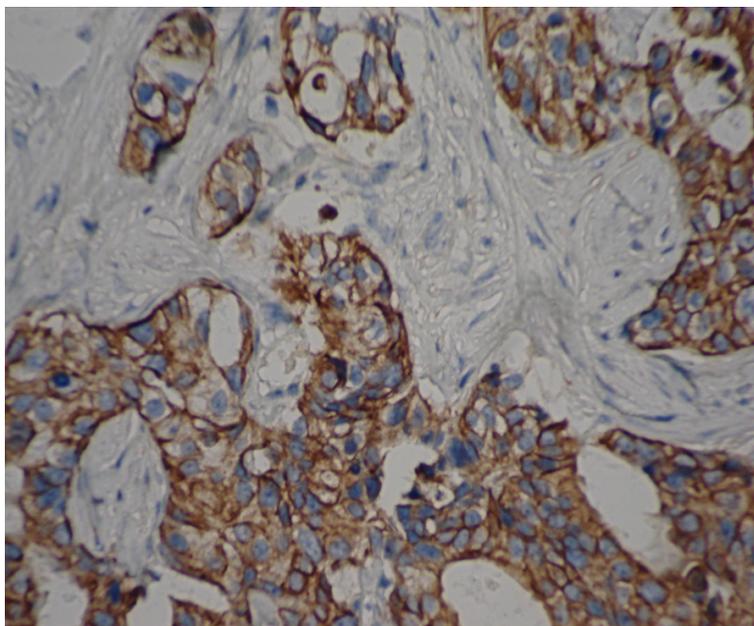**Figure 5.** Ultrasound of a BC patient with vascular abnormalities.

Figure 6. High expression of COX-2 in a BC patient with a vascular anomaly (×400 magnification).

Table 3. Expression of VEGF, COX-2 and MMP-9 in BC patients with and without a vascular anomaly

Group	N	VEGF	COX-2	MMP-9
Vascular anomaly	31	100%	100%	90.32%
Non-vascular anomaly	58	65.52%	55.17%	67.24%
P		<0.001	<0/001	0.021
X^2		13.788	14.349	5.301

Expression of VEGF, COX-2 and MMP-9 and its relationship with LNM

Accurate preoperative staging of BC is the key to treatment success. Checking for LNM from BC is an important component of preoperative staging (**Figures 7**, **8**). Expression of VEGF, COX-2, and MMP-9 in the LNM group was significantly higher than that in the non-LNM group (**Table 4**).

Discussion

Breast tumors can secrete many vasoactive substances. VEGF can enhance angiogenesis and vascular and lymphatic permeability, and aids the proliferation and metastasis of tumor cells [5-7]. COX-2 is an inducible enzyme which, under normal physiologic conditions, is not detected in most tissues. However, if cells receive the corresponding stimulus after PG synthesis, COX-2 expression can increase and promote the formation of tumor vessels [8, 9]. MMP-9 can dissolve in type-IV collagen, leading to damage to basement membranes, and has a very important role in the metastasis of cancer cells [10]. Neovascularization of malignant tumors is affected by interactions between stimulatory and inhibitory factors. VEGF, COX-2, and MMP-9 have important roles in the migration and proliferation of endothelial cells, degradation of basement membranes, angiogenesis, induction of tumor metabolism and increase in the blood supply of tumors [11].

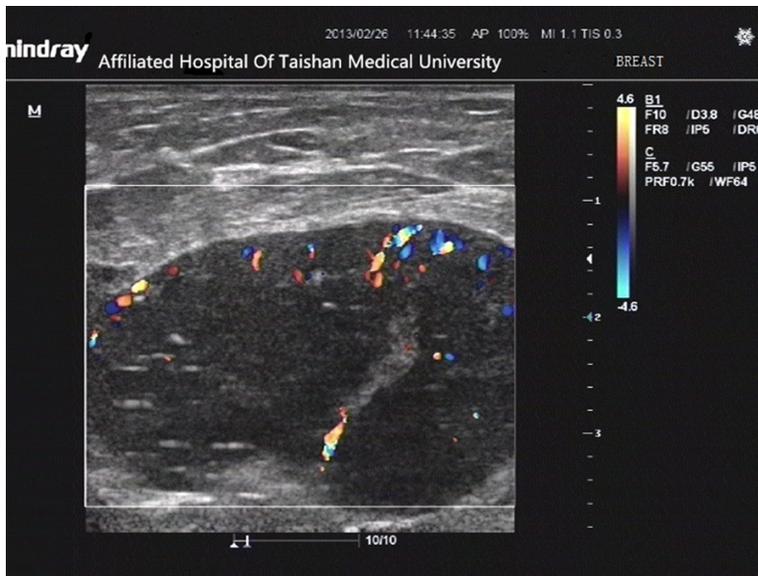Our results showed that high expression of VEGF, COX-2,

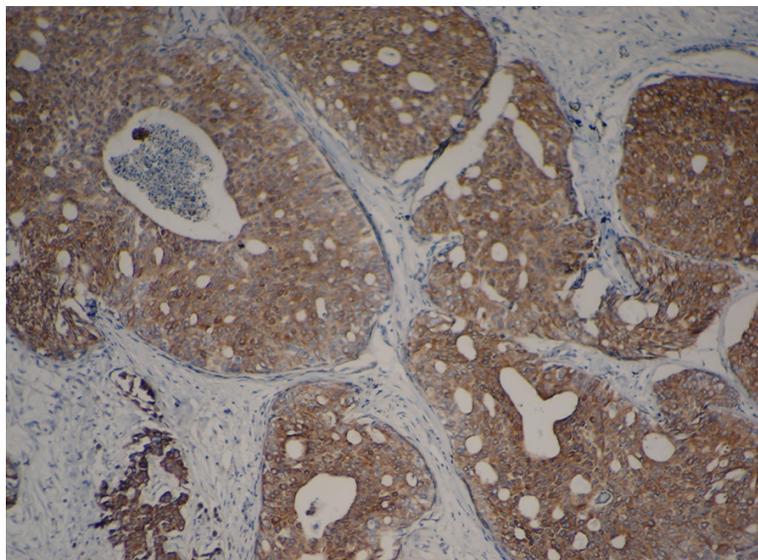**Figure 7.** Ultrasound of a BC patient with lymph node metastasis.

Figure 8. MMP-9 expression in a BC patient with lymph node metastasis (×200 magnification).

Table 4. Expression of VEGF, COX-2 and MMP-9 in BC patients with and without lymph node metastasis

	N	VEGF	COX-2	MMP-9
Lymph node metastasis	39	87.18%	82.05%	100%
Non-lymph node metastasis	50	64.00%	62.00%	76.00%
P		0.013	0.049	0.001
X^2		6.143	4.260	11.510

high expression may be correlated with a poor prognosis in BC patients. Due to increased expression of VEGF and COX-2 and accelerated microvessel formation in tumors, the risk of tumor proliferation is increased.

Calcification is another feature of BC and is different in different parts of the breast. The central area of tumor calcification may be due to tumor metabolism and blood supply, which can lead to necrosis and calcinosis. Calcification in tissues adjacent to the tumor may be due to secretion of substances from cells. VEGF expression in the calcification group was higher than that in the non-calcification group, but the difference was not significant. This finding may have been because of slight calcification in tumor tissue. Secretion of vasoactive substances without calcification foci is weak, so the ability to induce the formation of new blood vessels will be relatively poor [12]. Whether there is a correlation between tumor calcification and expression of COX-2 and MMP-9 merits further study.

Calcification, the Burr sign, and the release of cytokines with biologic activity can be used to ascertain the degree of malignancy, and predict microvessel formation and the prognosis of BC. However, the occurrence and development of BC is a complex process involving many factors, stages and interactions. There are several types of markers, but we chose those used widely in clinical trials: VEGF COX-2, MMP-9. More IHC markers and imaging signs could provide a theoretical basis for the evaluation, treatment and prognosis of BC.

and MMP-9 reflected hyperplasia, proliferation of BC tissue, and vascular abnormalities. Such

Disclosure of conflict of interest

None.

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References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Nadia H, Michael G. Breast cancer. Lancet 2016; 389: 1134-50.

[3] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics 2012. CA Cancer J Clin 2015; 65: 87-108.

[4] Kurmyshkina OV, Belova LL, Kovchur PI, Volkova TO. Remodeling of angiogenesis and lymphangiogenesis in cervical cancer development. Biomed Khim 2015; 61: 579-597.

[5] Zhang L, Wang H, Li C, Zhao Y, Wu L, Du X, Han Z. VEGF-A/Neuropilin 1 pathway confers cancer stemness via activating Wnt/β- catenin axis in breast cancer cells. Cell Physiol Biochem 2017; 44: 1251-1262.

[6] Chen Y, Liu Y, Wang Y, Li W, Wang X, Liu X, Chen Y, Ouyang C, Wang J. Quantification of STAT3 and VEGF expression for molecular diagnosis of lymph node metastasis in breast cancer. Medicine (Baltimore) 2017; 96: e8488.

[7] Soukup V, Ĉapoun O, Peŝl M, Sobotka R, Vávřová L, Hanuš T, Zima T, Kalousová M. Placental growth factor in bladder cancer compared to the diagnostic accuracy and prognostic performance of vascular endothelial growth factor A. Anticancer Res 2018; 38: 239-246.

[8] Tian J, Hachim MY, Hachim IY, Dai M, Lo C, Raffa FA, Ali S, Lebrun JJ. Cyclooxygenase-2 regulates TGFB-induced cancer stemness in triple-negative breast cancer. Sci Rep 2017; 7: 40258.

[9] Tury S, Becette V, Assayag F, Vacher S, Benoist C, Kamal M, Marangoni E, Bièche I, Lerebours F, Callens C. Combination of COX-2 expression and PIK3CA mutation as prognostic and predictive markers for celecoxib treatment in breast cancer. Oncotarget 2016; 7: 85124-41.

[10] Puzovic VJ, Brcic I, Ranogajec I, Jakic-Razumovic J. Prognostic values of ETS-1 MMP-2 and MMP-9 expression and CO expression in breast cancer patients. Neoplasma 2014; 61: 439-446.

[11] Hall FM, Storella JM, Silverstone DZ, et al. Nonpalpable breast lesion: recommend at sonography 2000; 167: 356-359.

[12] Fondrinier E, Lrimier G, Guerin-Boblet V. Breast microcalcification: multivariate analysis of radiologic and clinical factors for carcinoma. World J Surg 2002; 26: 290-297.