Correlation analysis between expression of PCNA, Ki-67 and COX-2 and X-ray features in mammography in breast cancer

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Abstract. This study investigated expression of proliferating cell nuclear antigen (PCNA), proliferation-associated nuclear antigen (Ki-67) and cyclooxygenase-2 (COX-2) in tissues of breast invasive ductal carcinoma, and analyzed the correlations between these indexes and X-ray features in mammography. A total of 90 patients who were admitted to Huangshi Central Hospital and diagnosed as breast invasive ductal carcinoma from January 2014 to January 2016 were selected. The expression of PCNA, Ki-67 and COX-2 in cancer tissues and cancer-adjacent normal tissues of patients were detected by immunohistochemical staining, and X-ray features in mammography of patients were observed. By using Spearman correlation analysis, the correlations between expression of PCNA, Ki-67 and COX-2 and X-ray features in mammography in breast cancer were investigated. As a result, the positive expression rates of PCNA, Ki-67 and COX-2 in cancer tissues of the patient groups were respectively 42.2, 45.6 and 51.1%, which were significantly higher than those in cancer-adjacent normal tissues of the control group ($p<0.05$). PCNA, Ki-67 and COX-2 expression in cancer tissues of the patient group was associated with clinical staging and lymphatic metastasis ($p<0.05$), but had no correlation with age and tumor size ($p>0.05$). PCNA, Ki-67 and COX-2 expression in cancer tissues of the patient group had no correlation with the existence of lumps and localized density-increased shadows ($p>0.05$), but were associated with manifestations of architectural distortion, calcification as well as skin and nipple depression ($p<0.05$). Spearman correlation analysis revealed that there was a significantly positive correlation between the expression of PCNA and COX-2 in cancer tissues of the patient group ($r=0.676$, $p<0.05$); there was a significantly positive correlation between the expression of Ki-67 and COX-2 ($r=0.724$, $p<0.05$); PCNA expression had no obvious correlation with the expression of Ki-67 ($p>0.05$). In conclusion, PCNA, Ki-67 and COX-2 expression is of great significance in the occurrence, invasion and metastasis of breast invasive ductal carcinoma. There is a strong correlation between PCNA, Ki-67 and COX-2 expression levels and X-ray features in mammography in breast invasive ductal carcinoma. The application of X-ray features in mammography can evaluate the expression levels of PCNA, Ki-67 and COX-2 in tissues of breast invasive ductal carcinoma, thereby prospectively predicting biological behavior of these cancer cells and patient prognosis.

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Key words: breast cancer, proliferating cell nuclear antigen, proliferation-associated nuclear antigen (Ki-67), breast X-ray photography technique, cyclooxygenase-2

Introduction

Breast cancer is a common, worldwide malignant tumor of women. The number of female breast cancer patients in China remains stubbornly high, and the mortality rate is also relatively high; moreover, the occurrence of this disease increasingly tends to affect the young population. Due to the occult feature of breast cancer, most patients are at an advanced stage when they are diagnosed. Therefore, how to accurately diagnose breast cancer at an early stage without treatment delay for the treatment to improve patient prognosis has become a hot issue in the diagnosis and treatment of breast cancer in recent years (1). The technique of breast X-ray photography is an important imaging examination method, which plays an auxiliary role in the early diagnosis of breast cancer (2). Studies have shown that (3-5) related cancer factors affect the biological behavior of tumor cells to a certain extent, which is closely associated with the recurrence and metastasis of cancer cells. Thus, this investigation focuses on whether there is a correlation between the clinical features of breast X-ray photography techniques and related cancer factors, how to predict the biological behavior of breast cancer cells and patient's prognosis by using X-ray features in mammography combined with related cancer factors. This study mainly aims to investigate proliferating cell nuclear antigen (PCNA),

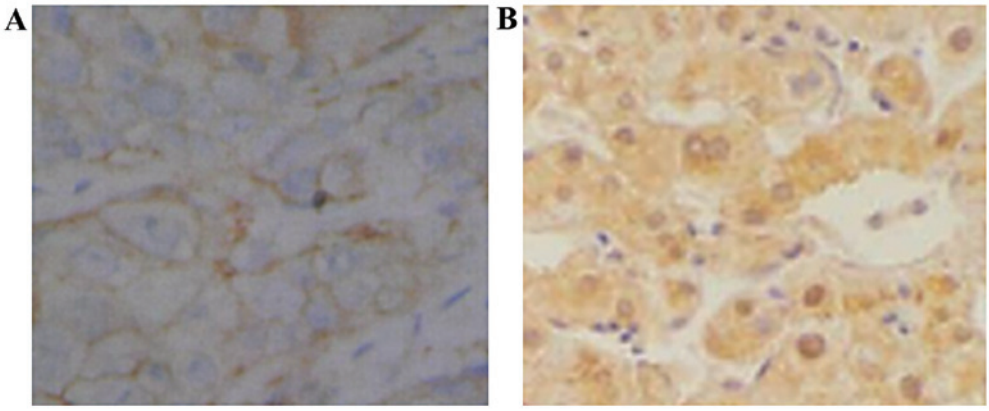

Figure 1. PCNA expression (magnification, x400). (A) Positive expression of PCNA in cancer tissues (magnification, x400). (B) Negative expression of PCNA in cancer-adjacent tissues (magnification, x400). PCNA, proliferating cell nuclear antigen.

proliferation-associated nuclear antigen (Ki-67) and cyclo-oxygenase-2 (COX-2) expression in breast invasive ductal carcinoma tissue, and to analyze the correlations between these indexes and X-ray features in mammography, in order to provide more valuable reference for the growth, recurrence, metastasis and prognosis of breast cancer, thereby benefiting clinical diagnosis and treatment in such patients.

Materials and methods

General data. A total of 90 patients who were admitted to Huangshi Central Hospital with confirmed breast invasive ductal carcinoma from January 2014 to January 2016 were selected as the patient group. All patients were women with single unilateral invasive breast cancer lesions. Patients were aged from 29 to 73 years, with an average of 56.7±1.6 years; for clinical staging, there were 60 cases in stages I+II and 30 cases in stages III+IV; for lymphatic metastasis, there were 40 cases with metastasis and 50 cases without metastasis; for tumor diameter, there were 33 cases with the tumor size <2 cm and 57 cases with the size ≥2 cm; there were 48 cases with cancer in the left breast and 42 cases with disease in the right breast. The selected patients did not receive any intervention therapy before operation, and breast invasive ductal carcinoma was confirmed by pathology after operation (6). Additionally, 40 patients who had cancer-adjacent normal breast tissues being more than 5 cm far away from cancer tissues confirmed by pathology were selected as the normal control group. All patients were women, aged from 30 to 72 years, with an average of 56.2±1.4 years. This study was approved by the Ethics Committee of Huangshi Central Hospital. Signed written informed consents were obtained from all participants before the study.

Methods. i) The expressions of PCNA, Ki-67 and COX-2 in cancer-adjacent normal breast tissues and cancer tissues were detected by immunohistochemical staining SP method. The above staining procedures were operated, respectively according to the instructions of EnVision kits (Biosharp, Hefei, China) of rabbit polyclonal PCNA antibody (dilution, 1:100; cat. no. ab18197); rabbit polyclonal Ki-67 antibody (dilution, 1:100; cat. no. ab92742) and rabbit polyclonal COX-2 antibody (dilution, 1:100; cat. no. ab52237) (all purchased from Abcam, Cambridge, MA, USA), followed by observing staining results and ii) patients were routinely examined by full-field digital mammography (MAMMOMAT 3000; Siemens AG, Munich, Germany) before operation. Images were jointly read and diagnostically analyzed by two radiologists who were engaged in the X-ray diagnosis of breast cancer. The occurrence of lump lesion of patients including burr, calcification, asymmetric dense shadow and gland structure disorder were mainly observed, and the above results were compared with the corresponding pathological indexes for analysis.

Evaluation criteria. Using a semi-quantitative scoring method combined with the Berry grading method (7): the positive expression of PCNA, Ki-67 and COX-2 was stained in the cytoplasm; the scores were recorded, respectively, according to the positive cell rate and positive cell staining intensity; the expression level was determined according to the staining degree scores: staining the same as negative control, score 0; staining light yellow, score 1; staining brown yellow, score 2; staining reddish brown, score 3. The scores were recorded according to the proportion of positive cells in the observed cells: positive cell number ≤10%, score 1; ≥11 - ≤50% positive, score 2; ≥51 - ≤75% positive, score 3; positive cell number >75%, score 4. The product of two item scores: 0-3 scores (-), 4-5 scores (+), 6-7 scores (++), >8 scores (+++); the score ≤3 represented negative, and the score >3 represented positive.

Statistical analysis. SPSS 20.0 software (IBM Corporation, Armonk, New York, NY, USA) was used for statistical analysis. For enumeration data, the Chi-square test was adopted for the comparison of positive rate between groups; for measurement data, t-test was utilized for the comparison between groups. Spearman correlation test was used for correlation analysis, and the test level was α=0.05.

Results

Expression of PCNA, Ki-67 and COX-2 in breast cancer tissues. The positive expression products of PCNA, Ki-67 and COX-2 were brown yellow particles, mainly located in the cytoplasm (Figs. 1-3). The positive expression rate of PCNA

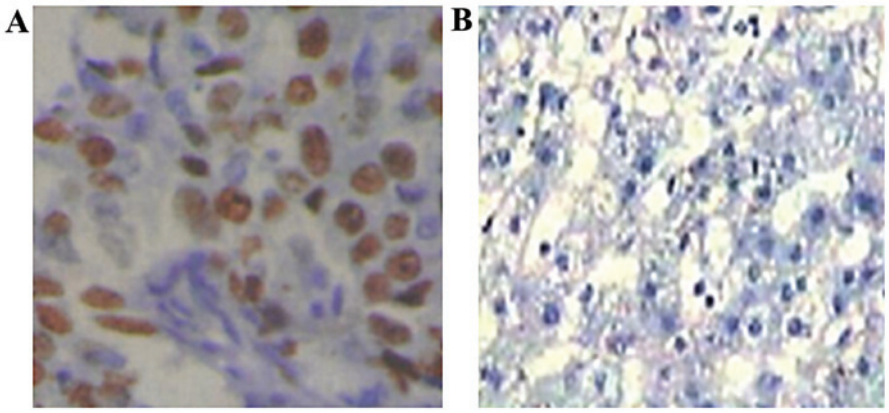

Figure 2. Ki-67 expression (magnification, x400). (A) Positive expression of Ki-67 in cancer tissues (magnification, x400). (B) Negative expression of Ki-67 in cancer-adjacent tissues (magnification, x400).

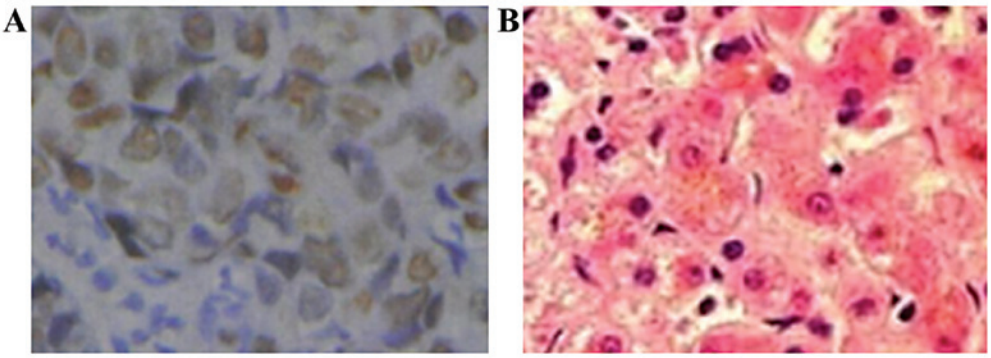

Figure 3. COX-2 expression (magnification, x400). (A) Positive expression of COX-2 in cancer tissues (magnification, x400). (B) Negative expression of COX-2 in cancer-adjacent tissues (magnification, x400). COX-2, cyclooxygenase-2.

Table I. Expression of PCNA in cancer tissues and cancer adjacent normal tissues (case).

Groups	n	+	++	+++	Positive rate (%)	-
Patient	90	9	14	15	42.2	52
Control	40	1	0	0	2.5	39
P-value						<0.05

PCNA, proliferating cell nuclear antigen.

Table II. Expression of Ki-67 in cancer tissues and cancer adjacent normal tissues (case).

Groups	n	+	++	+++	Positive rate (%)	-
Patient	90	10	15	16	45.6	49
Control	40	1	0	0	2.5	39
P-value						<0.05

Table III. Expression of COX-2 in cancer tissues and cancer adjacent normal tissues (case).

Groups	n	+	++	+++	Positive rate (%)	-
Patient	90	12	16	18	51.1	44
Control	40	1	0	0	2.5	39
P-value						<0.05

COX-2, cyclooxygenase-2.

in cancer tissues of the patient group was 42.2%, which was significantly higher than 2.5% in cancer-adjacent normal tissues of the control group ($p<0.05$, Table I). The positive expression rate of Ki-67 in cancer tissues of the patient group was 45.6%, which was significantly higher than 2.5% in cancer-adjacent normal tissues of the control group ($p<0.05$, Table II). The positive expression rate of COX-2 in cancer tissues of the patient group was 51.1%, which was significantly higher than the 2.5% in cancer-adjacent normal tissues of the control group ($p<0.05$, Table III).

Relationships between PCNA, Ki-67 and COX-2 and clinico-pathological features of breast cancer patients. PCNA, Ki-67 and COX-2 expression in cancer tissues of the patient group was associated with clinical staging and lymphatic metastasis

Table IV. Relationships between PCNA, Ki-67 and COX-2 and clinicopathological features of breast cancer patients.

Clinicopathological feature	n	Positive case of PCNA	P-value	Positive case of Ki-67	P-value	Positive case of COX-2	P-value
Age			>0.05		>0.05		>0.05
≤60 years	46	20		21		24	
>60 years	44	18		20		22	
Tumor size (cm)			>0.05		>0.05		>0.05
<2	33	19		19		22	
≥2	57	19		22		24	
Clinical staging			<0.05		<0.05		<0.05
I+II	60	13		15		17	
III	30	25		26		29	
Lymphatic metastasis			<0.05		<0.05		<0.05
Yes	40	28		27		30	
No	50	10		14		16	

PCNA, proliferating cell nuclear antigen; COX-2, cyclooxygenase-2.

Table V. Relationships between X-ray photography features and PCNA, Ki-67 and COX-2 expressions in breast invasive ductal carcinoma.

Clinicopathological feature	n	Positive case of PCNA	P-value	Positive case of Ki-67	P-value	Positive case of COX-2	P-value
Lump			>0.05		>0.05		>0.05
Yes	70	19		22		23	
No	20	19		19		23	
Calcification			<0.05		<0.05		<0.05
Yes	80	35		36		42	
No	10	3		5		4	
Architectural distortion			<0.05		<0.05		<0.05
Yes	63	28		32		34	
No	27	11		9		12	
Localized density-increased shadow			>0.05		>0.05		>0.05
Yes	41	18		22		23	
No	49	20		19		23	
Skin and nipple depression			<0.05		<0.05		<0.05
Yes	68	31		36		37	
No	21	7		5		9	

PCNA, proliferating cell nuclear antigen; COX-2, cyclooxygenase-2.

($p<0.05$), but had no correlation with age and tumor size ($p>0.05$, Table IV).

Digital mammography X-ray photography features of the patient group. Among 90 cases in the patient group, 70 cases manifested as lumps with different sizes; there were 8 cases of mass shape calcification, 6 cases of cluster calcification, 14 cases of vascular calcification, 18 cases of sand-like calcification, 14 cases of thick rod-shaped calcification and 20 cases of line like branching calcification (Fig. 4); there were 63 cases with manifestations of architectural distortion, 41 cases with localized density-increased shadow and 68 cases with skin and nipple depression.

Relationships between X-ray photography features and PCNA, Ki-67 and COX-2 expression in breast invasive

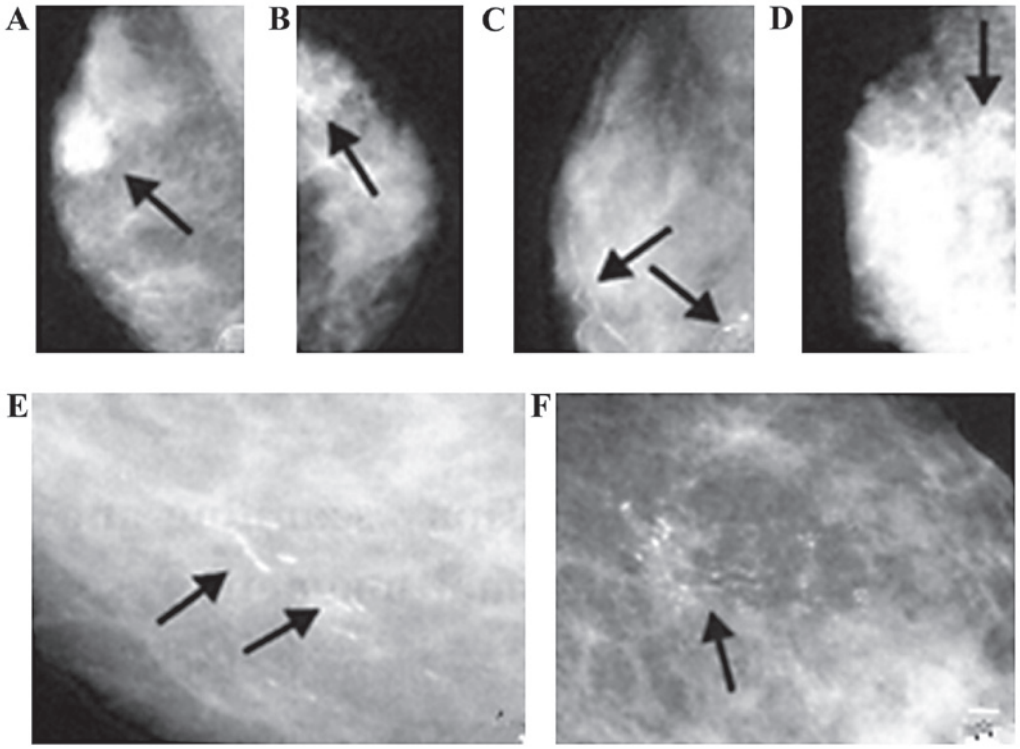

Figure 4. Various calcified morphologies. Black arrows indicated: (A) Mass shape calcification. (B) Cluster calcification. (C) Vascular calcification. (D) Sand-like calcification. (E) Thick rod-shaped calcification. (F) Line like branching calcification.

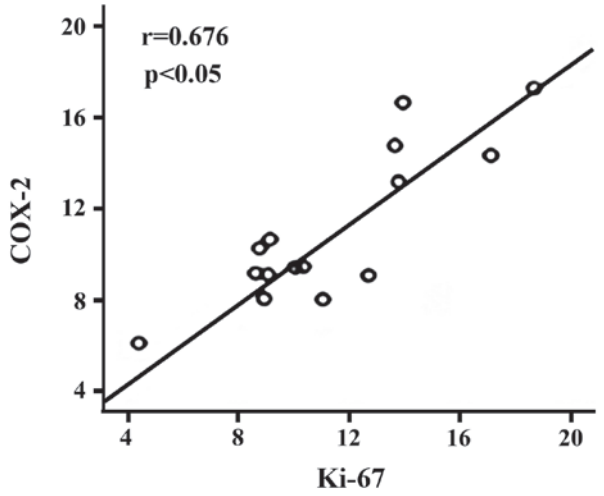

Figure 5. Correlation between the expression of PCNA and COX-2. PCNA, proliferating cell nuclear antigen; COX-2, cyclooxygenase-2.

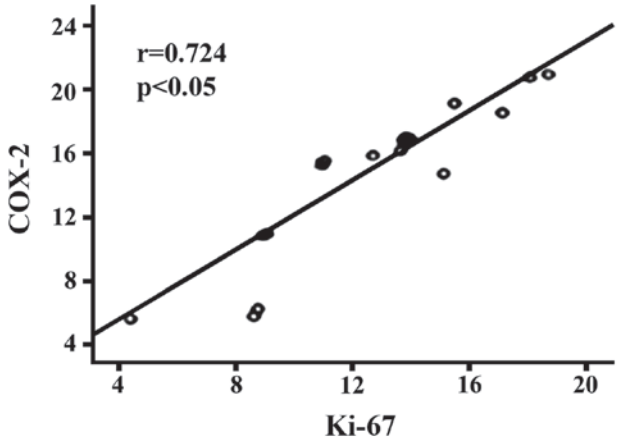

Figure 6. Correlation between the expression of Ki-67 and COX-2. COX-2, cyclooxygenase-2.

ductal carcinoma. PCNA, Ki-67 and COX-2 expression in cancer tissues of the patient group had no correlation with the existence of lumps and localized density-increased shadows ($p>0.05$), but were associated with manifestations of architectural distortion, calcification as well as skin and nipple depression ($p<0.05$, Table V).

Correlations among PCNA, Ki-67 and COX-2 expression. Spearman correlation analysis revealed that there was significantly positive correlation between the expression of PCNA and COX-2 in cancer tissues of the patient group ($r=0.676$, $p<0.05$, Fig. 5); there was significantly positive correlation between the expression of Ki-67 and COX-2 ($r=0.724$, $p<0.05$, Fig. 6); PCNA expression had no obvious correlation with the expression of Ki-67 ($p>0.05$).

Discussion

The occurrence and development of breast cancer is associated with multiple factors, especially for some patho-biological factor indicators including HER-2 and P53 (1,8,9). With the rapid development of modern biological techniques, many biological factor indexes have been found (10,11). As one kind of acidic protein in the nucleus, PCNA is an indispensable coenzyme for eukaryotic cells to synthesize DNA. PCNA can be used to measure cell proliferation activity; the higher the

proliferation activity is, the larger the tension between cells, resulting in lower adhesion, which promotes tumor cells being separated from primary lesions, and metastasizing (12). The results of this study indicated that the positive expression rate of PCNA in cancer tissues of the patient group was 42.2%, which was significantly higher than the 2.5% in the cancer-adjacent normal tissues of the control group ($p<0.05$). PCNA expression in cancer tissues of the patient group were associated with clinical staging and lymphatic metastasis ($p<0.05$), but had no correlation with age and tumor size ($p>0.05$), suggesting that PCNA expression is closely related to the occurrence and development of breast cancer, which is consistent with the conclusions of other international medical researchers (13,14).

Ki-67 is a kind of nuclear antigen, the quantity of which generated from cell proliferation is closely associated with mitosis (15). Previous studies have confirmed that (16) Ki-67 is highly expressed in gastric, prostate and liver cancer and other multiple tumor cells, and there are close correlations between it and the development and prognosis of the above tumors. This study revealed that the positive expression rate of Ki-67 in cancer tissues of the patient group was 45.6%, which was significantly higher than the 2.5% in the cancer adjacent normal tissues of the control group ($p<0.05$). Ki-67 expression in cancer tissues of the patient group were associated with clinical staging and lymphatic metastasis ($p<0.05$), but had no correlation with age and tumor size ($p>0.05$), suggesting that Ki-67 expression is closely related to the clinical staging and lymphatic metastasis of breast cancer, thus, the more severe the clinical staging is, the higher the positive expression rate of Ki-67 will be.

As an important antigen that can promote malignant tumor angiogenesis and cell proliferation, COX-2 greatly affects prognosis of tumor patients (17). COX-2 belongs to an inducible enzyme, which is not easily detected in normal tissue, but under the stimulation of oncogenes and other stimuli, its inducible expression can occur rapidly (18,19). This study showed that the positive expression rate of COX-2 in cancer tissues of the patient group was 51.1%, which was significantly higher than the 2.5% in cancer-adjacent normal tissues of the control group ($p<0.05$). COX-2 expression in cancer tissues of the patient group was associated with clinical staging and lymphatic metastasis ($p<0.05$), but had no correlation with age and tumor size ($p>0.05$), suggesting that COX-2 can promote the occurrence and development of breast tumors, which involves inducing tumor cell proliferation, inhibiting tumor cell apoptosis, suppressing anti-tumor immune response *in vivo*, promoting malignant tumor angiogenesis and other multiple mechanisms.

Further analysis indicated that there was a significantly positive correlation between the expression of PCNA and COX-2 in cancer tissues of the patient group ($r=0.676$, $p<0.05$); there was significantly positive correlation between the expression of Ki-67 and COX-2 ($r=0.724$, $p<0.05$); PCNA expression had no obvious correlation with the expression of Ki-67 ($p>0.05$). The results further demonstrated the relationships among PCNA, Ki-67 and COX-2.

Breast X-ray mammography is characterized by simple operation and low cost in examining benign and malignant breast diseases, which also has a relatively high sensitivity and accuracy in the detection of microcalcifications. The results of breast X-ray photography for the 90 patients showed that the typical X-ray in patients with breast cancer was manifested by lumps in different sizes, malignant calcification, manifestations of architectural distortion, localized density-increased shadow, skin and nipple depression. The relationships between X-ray photography features and PCNA, Ki-67 and COX-2 expression in breast invasive ductal carcinoma were primarily investigated in this study, which showed that PCNA, Ki-67 and COX-2 expression in cancer tissues of the patient group had no correlation with the existences of lump and localized density-increased shadow ($p>0.05$), but were associated with manifestations of architectural distortion, calcification as well as skin and nipple depression ($p<0.05$). Therefore, it can be speculated that the infiltration of breast cancer with manifestations of architectural distortion, calcification as well as skin and nipple depression is stronger, and the malignancy degree is also higher, which shows poor clinical prognosis.

In conclusion, expression of PCNA, Ki-67 and COX-2 is a significant predictor for the occurrence, invasion and metastasis of breast invasive ductal carcinoma. There is a correlation between PCNA, Ki-67 and COX-2 expression levels and X-ray features in mammography in breast invasive ductal carcinoma. The application of X-ray features in mammography can evaluate the expression levels of PCNA, Ki-67 and COX-2 in tissues of breast invasive ductal carcinoma, thereby prospectively predicting biological behavior of these cancer cells and patient's prognosis.

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