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The expression of osteopontin in breast cancer tissue and its relationship with p21ras and CD44V6 expression

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Summary

Objective: This study aimed to investigate the expression of osteopontin (OPN), p21ras, and CD44V6 in breast cancer tissues, and to analyze the relationships between their expression and a patient's clinicopathological characteristics and five-year survival rate. *Materials and Methods:* Streptavidin-peroxidase immunohistochemistry was used to detect the expression of OPN, p21ras, and CD44V6 in tissue samples from 96 breast cancer patients, and the multivariate Cox proportional hazards model (mCOX-PHM) was used to analyze the factors that affect prognosis. *Results:* Among the 96 breast cancer patients studied, positive staining for OPN, CD44V6, and p21ras was observed in 54.2%, 58.3%, and 43.8% of samples, respectively. The expression of OPN and CD44V6 were positively correlated (r = 0.58), and the expression of OPN and p21ras were also positively correlated (r = 0.25). Coexpression OPN, CD44V6, and p21ras was negatively correlated with a patient's five-year survival rate ($p < 0.05$). Kaplan-Meier analysis indicated that a patient without OPN, CD44V6, or p21ras expression had an improved survival ($p < 0.05$). Results from the mCOX-PHM analysis indicated that CD44V6 expression, the degree of tumor differentiation, and lymph node metastasis were all independent factors that indicate prognosis. The combined detection of OPN, CD44V6, and p21ras could contribute to a more accurate assessment of the biological behavior of breast cancers, and could help to indicate the prognosis of breast cancer patients.

Key words: OPN; P21ras; CD44V6; Breast cancer.

Introduction

Invasive breast cancer is one of the dominant female malignant tumors, and the most common pathological subtype is invasive ductal carcinoma. Worldwide, 1.2 million women are diagnosed with breast cancer every year, and 500,000 of them die from the disease [1]. Approximately 70–80% of breast cancer patients with lymph node metastasis undergo recurrence or distant metastases, which are major causes of patient death. Osteopontin (OPN) is a phosphorylated acidic secreted glycoprotein, which is highly expressed in a variety of malignant tumors, such as breast [2], prostate [3], liver [4], and lung cancers [5]. It is widely believed that the expression of OPN is correlated with the metastatic potential of tumor cells. OPN can bind to the αVβ3 integrin receptor [6] via an arginine-glycine-aspartic acid (RGD)-peptide and can promote non-specific cell migration through extracellular matrix components [7-9]. It has been shown that tumor metastasis is closely related to plasma OPN levels [10]. There is also an intimate relationship between *OPN* and Ras gene expression. OPN can bind to the C-terminus of three types of the CD44 family of cell adhesion molecule receptors [11]. The v6-containing CD44 splice variant (CD44V6) is frequently expressed in pancreatic cancer, colon cancer, breast cancer, and lymphoma cells. The correlation between abnormal CD44V6 expression and breast cancer has been the subject of much interest in recent years. CD44V6 expression is closely correlated with the occurrence, invasion, and metastasis of breast cancer cells; however, reports regarding its relationship with breast cancer prognosis are inconsistent. In this study, the expression of OPN, p21ras, and CD44V6 in breast cancer patients was quantified, and the correlation between their expression and the patient's five-year survival rate was investigated. The clinical significance of these expression data is discussed. The principle objective was to provide a reliable means for accurately assessing the biological behavior of breast cancers, and to aid in the identification of breast cancer patient prognosis.

Materials and Methods

General information

Included in the study were 96 female breast cancer patients who underwent pathological confirmation and surgical resection at the First Affiliated Hospital of Henan University of Science and Technology from 1999 to 2002. All of the patients had follow-up results. The patients were aged 25–83 years, with a mean age of 50.23 ± 12.02 years. None of the patients had received any treatment prior to surgery. There were 41 patients who had axillary lymph node metastases. The Ellis and Eiston semi-quantitative method was used to evaluate the cancer histological grade; 24 cases were Grade I, 42 cases were Grade II, and 30 cases were

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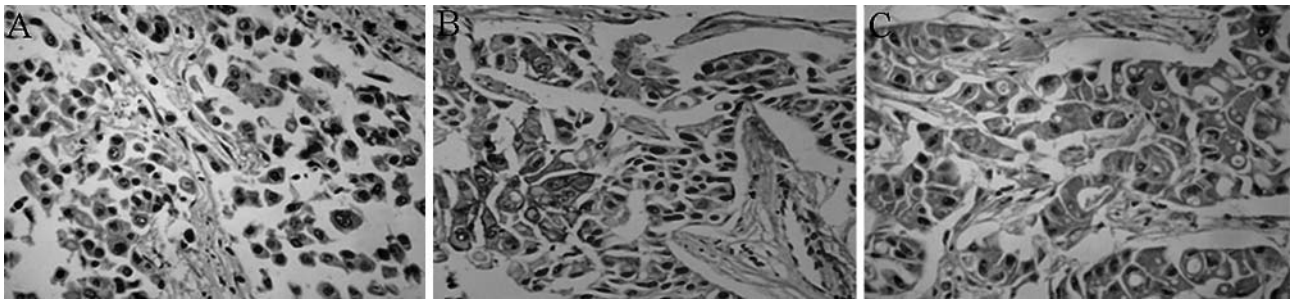

Figure 1. — The positive expression of OPN (SP ×200, located in cytoplasm. A) CD44V6 (SP ×400, located in cell membrane, B) P21ras (SP ×400, located in cytoplasm, and C) in breast carcinoma.

Table 1. — *The expression of OPN, CD44V6, P21ra, and their relationship with the clinical parameters.*

Group	N	OPN(+)	X^2	*p*	CD44V6(+)	X^2	*p*	P21ras(+)	X^2	*p*
Age			0.23	0.40		0.77	0.49		0.86	0.36
≤ 50 years	44	25			27			25		
> 50 years	52	27			29			17		
Histological grade			6.68	0.03		3.37	0.19		1.57	0.46
Grade I	24	10			10			8		
Grade II	42	20			24			19		
Grade III	30	22			22			15		
Lymph node transfer			7.91	0.004		5.14	0.02		1.62	0.20
Positive	41	29			29			21		
Feminine	55	23			27			21		
Lifetime			17.63	0.000		16.8	0.00		9.33	0.00
≤ 5 years	64	25			28			21		
> 5 years	32	27			28			21		

Grade III. A patient's survival period was calculate from the surgery date to the end of follow-up, with five-year survival as a dividing line; 64 cases exhibited > five years survival (including five years), and 32 cases exhibited < five years survival. As a control, 20 cases of benign breast hyperplasia were used. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Zhengzhou University. Written informed consent was obtained from all participants.

Immunohistochemistry and hematoxylin-and-eosin (HE) staining

Streptavidin-peroxidase (SP) immunohistochemistry was used, in accordance with the SP kit instructions. Tumor samples were taken from the center of all tumors, and fixed in 10% formalin prior to embedding in paraffin wax. Continuous four-μm sections were cut from the specimens; HE staining was performed in four sections and immunohistochemical staining was performed in three sections. A known positively-staining biopsy sample was used as a positive control, and phosphate-buffered saline was used in place of the primary antibody as a negative control.

Result determination

Each section was scored using a double-blind method. Positive staining was judged using the following two points: (1) scoring according to the number of stained cells, < 5% was allocated 0 points, > 5% but < 25% was allocated 1 point, > 25% but < 50% was allocated 2 points, and > 50% was allocated 3 points; (2) scoring according to the intensity of cancer cell staining, no staining was allocated 0 points, weak pale-yellow staining was allocated 1 point, moderate brownish-yellow staining was allocated 2 points, and strong tan staining was allocated 3 points. If the combined score from (1) and (2) was > 3, the section was defined as positive; a score of < 3 was classified as negative. CD44V6 staining was primarily located in the cell membrane, although some cytoplasmic staining was also observed, and cells with a yellow or brown membrane were defined as positive. OPN and p21ras were stained in the cytoplasm of breast cancer cells.

Statistical analysis

SPSS11.0 statistical software was used for statistical analyses. The relationship between OPN, p21ras, and CD44V6 expression and clinical indicators was calculated using the chi-squared (χ^2) test, with a p-value < 0.05 considered as statistically significant. Correlation analysis was undertaken using the Spearman method, with a p-value < 0.05 considered as statistically significant. The follow-up survival rate was calculated using the Kaplan-Meier method. Non-parametric tests of multiple groups were performed using the log-rank method, and the multivariate Cox proportional hazards model (mCOX-PHM) was used to analyze the factors affecting prognosis.

Results

Expression of OPN

Positive staining for OPN expression was primarily located in the cytoplasm of breast cancer cells (Figure 1A). Positive OPN staining was observed in 52 cases, and negative staining was observed in 44 cases. The rate of OPN

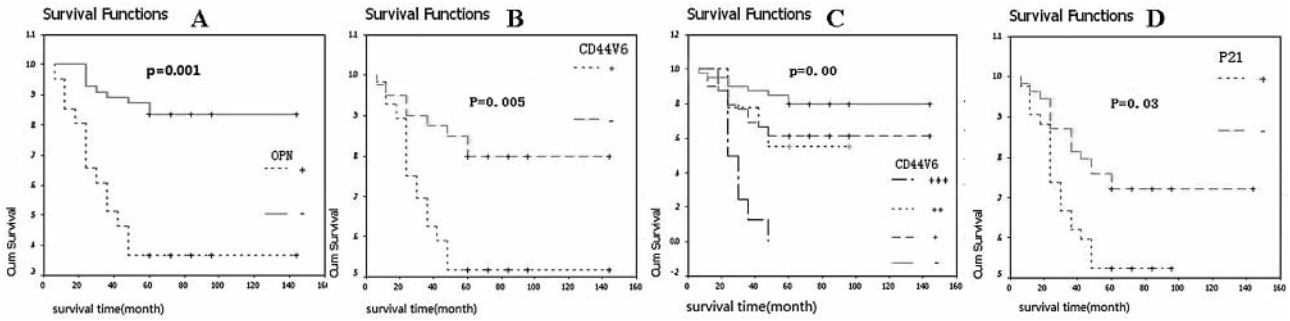

Figure 2. — The overall survival curves of breast cancer patients (A) In OPN-negative and OPN-positive groups; B) in CD44V6-negative and CD44V6-positive groups; C) different expression of CD44V6; D) in P21ras-negative and P21ras-positive groups).

positive staining was 54.2%, which was significantly higher than the 15% (3/20) positive staining that was observed in the 20 benign breast hyperplasia cases ($p < 0.05$). OPN expression was closely correlated with the histological grade, lymph node metastasis, and patient survival period ($p < 0.05$), but was not correlated with the patient's age (Table 1). Kaplan-Meier analysis was used to calculate the overall survival curves for the OPN-negative and OPN-positive groups. The results indicated that the survival of the OPN-negative group was significantly better than the OPN-positive group, with the log-rank test indicating that $p = 0.001$ (Figure 2A).

Expression of CD44V6

Positive staining for CD44V6 expression was primarily located in the cell membrane of breast cancer cells, with a small amount of staining in the ductal myoepithelial cells (Figure 1B). Among the 96 patients, 56 cases showed positive staining, and 40 cases showed negative staining. The overall rate of CD44V6 positive staining was 58.3%, which compared with 20% (4/20) in the 20 control benign breast hyperplasia cases ($p < 0.05$). CD44V6 expression was closely correlated with lymph node metastasis and a patient's five-year survival rate ($p < 0.05$), but was not correlated with the patient's age or histological grade (Table 1).

Kaplan-Meier analysis was used to calculate the overall survival curves for the CD44V6-negative and CD44V6-positive groups. The results indicated that the survival of the CD44V6-negative group was significantly better than the CD44V6-positive group ($p < 0.05$), with the log-rank test indicating that $p = 0.005$ (Figure 2B). When analyzed according to CD44V6 staining intensity, a negative correlation between the level of CD44V6 expression and the patient's survival was identified; as CD44V6 expression increased, the overall patient survival decreased. The differences observed between the CD44V6 (-), (+), (+ +), and (+ + +) groups were significant ($p = 0.00$; Figure 2C).

Expression of p21ras

Positive staining for p21ras was primarily located in the cytoplasm of breast cancer cells (Figure 1C). Among the 96 patients, 42 cases showed positive staining, and 54 cases showed negative staining. The overall rate of p21ras-positive staining was 43.8%, which compared with 10% (2/20) of the 20 control benign breast hyperplasia cases ($p < 0.05$). Kaplan-Meier analysis indicated that the survival of the p21ras-negative patients was significantly better than the p21ras-positive patients, with the log-rank test indicating that $p = 0.03$ (Figure 2D).

Correlation between OPN and p21ras expression

Among the 96 patients, 27 exhibited positive staining for both OPN and p21ras, 25 were OPN positive and p21ras negative, 15 were OPN negative and p21ras positive, and 29 were negative for both OPN and p21ras ($r = 0.25$, $p = 0.01$). The analysis indicated that the expression of OPN and p21ras were significantly correlated in the breast cancer tissues samples.

Correlation between OPN and CD44V6 expression

Among the 96 cases, 44 exhibited positive staining for both OPN and CD44V6, eight were OPN positive and CD44V6 negative, 12 were OPN negative and CD44V6 positive, and 32 were negative for both OPN and CD44V6 ($r = 0.58$, $p < 0.05$). The analysis indicated that the expression of OPN and CD44V6 were significantly correlated in the breast cancer tissues samples.

mCOX-PHM analysis

The factors that might affect breast cancer patient prognosis, such as age, degree of tumor differentiation, lymph node metastasis, and OPN, CD44V6, and p21ras expression, were included in the mCOX-PHM analysis, which employed a forward stepwise analysis method. The results indicated that lymph node metastasis, the degree of tumor differentiation, and CD44V6-positive staining were independent prognostic factors; age, p21ras expression, and OPN expression were not independent prognostic factors (Table 2).

Table 2. — *Multivatiate Cox prognostic analysis for breast carcinoma.*

Step	Item	B	SE	Wald	Df	Sig	Exp(B)	95% CI for Exp (B)	
								Lower	Upper
Step1	Transfer	1.558	0.379	15.368	1	0.000	4.750	2.180	10.353
Step2	Transfer	1.333	0.409	10.616	1	0.001	3.793	1.701	8.459
	CD44V6	.530	0.182	8.514	1	0.004	1.698	1.190	2.424
Step3	Transfer	1.141	0.418	7.460	1	0.006	3.131	1.380	7.103
	CD44V6	0.446	0.183	5.928	1	0.015	1.561	1.091	2.235
	Differentiated degree	-0.624		4.650	1	0.031	0.536	0.304	0.945

Discussion

OPN is a secreted protein which was originally isolated from the bone matrix. OPN binds to CD44 via its integrin receptor, and plays an important role in several processes, including the promotion of tumor cell migration and invasion. Collins *et al.* [12] have studied the expression of OPN in pancreatic adenocarcinoma and have shown that the median survival and two-year survival rate of patients with low OPN expression are better than for patients with high OPN expression. As such, OPN is considered an independent prognostic indicator for pancreatic cancer; tumor grade and tumor size have shown not to be independent prognostic indicator for pancreatic cancer. Thorat *et al.* [13] have found that the simultaneous overexpression of OPN and HER2 is indicative of a poor prognosis in breast cancer patients. Furthermore, Li *et al.* [14] have shown that OPN can activate the serine phosphorylation of the twist pathway, thus accelerating tumor cell epithelial-mesenchymal transition.

Wang *et al.* [15] have analyzed the relationship between OPN expression and breast cancer patient prognosis, and found that OPN expression is correlated with tumor size, histological grade, and lymph node metastasis. This is similar to the results of the present study. However, Tókés *et al.* [16] believe that OPN expression is not correlated with the clinicopathological features of breast cancer. Among other factors, this discrepancy could be due to the specific patients, test reagents, and detection methods employed. Hedley *et al.* [17] have found that OPN expression is significantly higher than breast cancer metastasis suppressor-1 expression in the human metastatic breast cancer cell line, MDA-MB-435, and that the expression of OPN is related to disease progression; higher OPN expression is correlated with a worse patient prognosis. In the present study, the rate of OPN-positive staining in breast cancer patients was 53.5%, which is lower than the 66% observed in the study by Rudland *et al.* [18]. Research on OPN overexpression in malignant tumor tissue [19] suggests that OPN may play an important role in the development and progression of malignant tumors. Ortiz-Martínez *et al.* [20] have shown that high OPN expression is closely correlated with a poor prognosis and an elevated risk of recurrence. Borges *et al.* [21] have studied the mechanisms of hypoxia induced *OPN* transcription and found that under hypoxic conditions *OPN* mRNA and protein expression are clearly increased. In the *OPN* promoter region, an RAE, which is located at 731–732 base pairs upstream of the transcription start site, is responsible for the hypoxia induced transcription of *OPN*, which may be regulated by Akt.

In this study the authors found that OPN expression is significantly different between grade I, II, and III carcinomas. They found that the level of OPN expression significantly increases as the degree of cancer differentiation decreases ($p < 0.05$), indicating that the expression of OPN is closely correlated with tumor progression. Furger *et al.* [22] have found that αVβ3 expression is high in the non-metastatic human breast cancer cell line, 21NT, following β3 transfection. The αVβ3 protein can combine with OPN to improve the invasiveness of cells. The rates of αVβ3 expression in patients with and without lymph nodes metastasis are reported to be 72.2% and 40%, respectively. Breast cancer cells with high OPN expression have a greater invasive and metastatic potential. This indicates that OPN may play a role in promoting breast cancer metastasis. Lymph node metastasis is recognized as a significant indicator of a poor prognosis, and the expression of OPN, therefore, has the potential to be used in the clinic for predicting the prognosis of breast cancer patients.

In this study the expression of OPN in patients with a survival period > five years and < five years was 78.1% and 38.9%, respectively, which was shown to be a significant difference. This indicates that the level of OPN expression is related to patient prognosis. The mCOX-PHM prognostic analysis indicated that lymph node metastasis and the degree of tumor differentiation are independent prognostic indicators for breast cancer patients. In this analysis, OPN was not shown to be an independent indicator of prognosis ($p = 0.058$). It can, therefore, be speculated that OPN indirectly affects patient prognosis by promoting lymph node metastasis or influencing the histological grade.

Ras oncogenes are involved in the occurrence and development of many human tumors. The product encoded by the Ras oncogene is p21ras, a guanosine triphosphate-binding protein and membrane protein. OPN is able to bind to the αVβ3α integrin to stimulate cellular signal transduction, which inhibits activated macrophages and endothelial

cell proliferation. In addition to directly stimulating tumor cell migration, OPN can also induce anti-apoptotic and anti-migratory signaling in endothelial and vascular cavity forming cells by activating nuclear factor kappa-light-chain-enhancer of activated B cells p50 and p65 signaling. This process may require the participation of Ras and Src. Research in colorectal adenoma and adenocarcinomas has shown that the rate of mutation in the *KRAS* gene, which causes p21ras protein overexpression, is higher than in the p53 encoding *TP53* gene. Different mutations and degrees of protein overexpression have been identified in para-cancerous mucosa and adenoma, indicating that Ras gene mutation and p21ras protein overexpression are early events in the occurrence of colorectal cancer, and play a key role in the process of adenoma to adenocarcinoma transformation. Adenomas with Ras gene mutations may have a greater tendency to malignant transformation. Gao *et al.* [23] have shown that OPN may regulate Stat1 protein degradation via the ubiquitin-proteasome pathway, and may alter the expression levels of the growth inhibiting γ-interferon protein and p21ras. Thorat *et al.* [13] have shown that in the breast cancer patients, OPN overexpression is frequently accompanied by HER2 overexpression; OPN expression is significantly increased in the breast cancer patients with high HER2 expression.

Results from this study indicate that the rate of p21ras positive staining in breast cancer tissues is 43.8%, significantly higher than that found in benign breast hyperplasia samples (10%, $p < 0.05$). This indicates that abnormal p21ras expression is frequently correlated with tumor occurrence and development. The role of p21ras in breast cancer may be similar to that observed in colorectal cancer, namely, it may be involved in the early stages of malignant cell transformation and carcinogenesis. The present authors found that the rate of p21ras positive staining gradually increased in grade I, II, and III cancerous tissues. However, this difference was not statistically significant, suggesting that the link between p21ras expression and malignant cell transformation is not direct. The rates of p21ras positive staining in patients with or without the lymph node metastasis were 51.2% and 38.2%, respectively ($p > 0.05$). This suggests that p21ras expression may not be closely associated with cancer cell proliferation. In patients with a survival period of > five years or < five years, the rates of p21ras positive staining were 32.8% and 65.6%, respectively ($p < 0.05$). This suggests that p21ras expression is closely correlated with patient prognosis. However, mCOX-PHM analysis indicated that p21ras is not an independent prognostic factor, although it may indirectly influence prognosis through a number of other mechanisms.

In this study, the p21ras expression in breast cancer tissue was not significant different in patients with or without lymph node metastasis or with different histological grades. However, p21ras expression was related to a patient's five-year survival rate. This suggests that mutation of the gene encoding p21ras, resulting in altered protein expression, may be an early event in the occurrence of breast cancer. As such, quantification of p21ras expression may be useful for assessing a patient's prognosis.

CD44 is a widely expressed single-chain transmembrane glycoprotein, which is an important cell surface adhesion molecule. CD44V6 can bind to the extracellular matrix and to hyaluronic acid of the basement membrane, and participates in the tumor invasion mechanisms of different tumor types. CD44V6 is regarded as a protein marker of metastasis in lymphoma, liver cancer, breast cancer, lung cancer, pancreatic cancer, colorectal cancer, and gastric cancer [24]. It has been shown that the RGD sequence of OPN is the same as the sequence recognized by adhesion molecules on many extracellular matrix proteins. Krishnamachary *et al.* [25] have shown that expression of the CD44 variants, V6 and V7/8, is higher in breast cancer cells under hypoxic conditions. However, some reports suggest that the expression of CD44V6 is not significantly associated with the overall survival rate of patients with breast cancer [26, 27]. It has also been shown [28] that CD44V6 is not expressed in normal breast tissue. In the present study, the authors found that in grade I, II, and III invasive breast cancer, the expression of CD44V6 gradually increases, suggesting that CD44V6 may play an important role in the metastasis and progression of breast cancer. The results of this study also indicate that CD44V6 is expressed in some breast benign hyperplasia tissue, and expressed at a low level in some breast ductal myoepithelial cells. These findings are similar to those of Heider *et al.* [29, 30].

Results from this study indicate that the rate of CD44V6-positive staining in breast cancer is 58.3%, significantly higher than that observed in benign breast hyperplasia tissue (10%; $p < 0.05$). This indicates that abnormal expression of CD44V6 may play a role in the process of mammary epithelial cell carcinogenesis. The authors found that the level of CD44V6 expression increased with the degree of tissue malignancy. The rates of CD44V6-positive staining in patients with or without lymph node metastasis were 70.7% and 48.2%, respectively ($p < 0.05$). In this study, the authors also found that lymph node metastasis was an independent prognostic factor. They hypothesize that higher CD44V6 expression results in higher breast cancer cell proliferation, which leads to a worse patient prognosis. In grade I, II, and III patients, the levels of CD44V6 expression were significantly different. As the histological grade increased, CD44V6 expression gradually increased, indicating that the CD44V6 expression is closely correlated with the degree of breast cancer differentiation. They also found that CD44V6 expression is closely correlated with the histological grade and lymph node metastasis of breast cancer, indicating that CD44V6 may play an important role in the invasion and metastasis of breast cancer. In patients with a survival period > five years and < five years, the rates of CD44V6-positive staining were 43.8% and 87.5%,

respectively ($p < 0.05$), indicating that CD44V6 expression is significantly associated with patient prognosis. When CD44V6 expression increases in breast cancer, namely from (+)–(+ + +), patient survival significantly decreases. In patients with (+ + +) CD44V6 expression, the survival curve has no plateau and the patients had a very poor prognosis. As such, a patient's prognosis is negatively correlated with CD44V6 expression levels.

The mCOX-PHM analysis indicated that CD44V6 is an independent prognostic factor for breast cancer, indicating that CD44V6 can be used to predict the prognosis of breast cancer patients. Preliminarily, patients with strong CD44V6 expression can be expected to have a poor disease prognosis. Ma *et al.* [31] have suggested that CD44V6 expression in the breast cancer is related to tumor size, TNM staging, axillary lymph node metastasis, and a patient's probability of five-year survival. However, the present multivariate analysis indicated that CD44V6 expression cannot be used as an independent prognostic factor for a patient's prognosis. Lian *et al.* [32] have shown that the survival of breast cancer patients with CD44V6 expression is significantly higher than those without CD44V6 expression, and that CD44V6 expression is significantly associated with the TNM stage, tumor size, and lymph node metastasis ($p < 0.05$). As such, CD44V6 expression can be considered as an independent prognostic factor. Shah *et al.* [33] have reported that the levels of CD44V6 protein expression are not closely correlated with patient survival or prognosis, but do closely relate to CD44V6 RNA levels, which can be used as an independent prognostic factor in breast cancer patients. However, the prognosis of breast cancer patients cannot be definitively determined by a single factor. The present study demonstrates that the occurrence and development of breast cancer is the result of combined effects from multiple factors. Therefore, further studies are required in order to comprehensively analyze and understand the role of CD44V6 in breast cancer prognosis.

Results from this study indicate that the expression of OPN and p21ras are significantly positively correlated in breast cancer ($r = 0.25$, $p < 0.05$), indicating that there is an intrinsic link between OPN and p21ras expression, which may play a role in the occurrence and development of breast cancer. Teramoto *et al.* [18] have studied the H-Ras-transformed, mouse NIH3T3 cells, and have found that OPN expression is significantly increased in the fibroblasts. Transfection of NIH3T3 cells with antisense OPN siRNAs have indicated that OPN is induced by p21ras and is involved in a cells migratory capacity. As such, it can be considered that p21ras expression not only directly affects the five-year survival rate of breast cancer patients, but also indirectly induces OPN expression to enhance tumor cell migration. As such, p21ras can be seen to exert an effect on the pathological features and prognosis of breast cancer patients. Although mutation of the Ras oncogene alone is insufficient to induce breast cancer, Ras mutation may elevate OPN gene expression, and overexpression of OPN may, in turn, promote the adhesion of cells to the basement membrane, create an acidic microenvironment, and, thus, further promote the destruction of the local basement membrane and adjacent cellular matrix. Increased proteolytic enzyme activity (e.g., uPA) will accelerate the extracellular matrix degradation and induce angiogenesis, thereby creating favorable conditions for the growth and migration of tumor cells.

Previous studies [34, 35] have shown that the OPN-CD44V6 axis plays an important role in the metastasis of various tumors, such as papillary thyroid cancer, colon cancer, and liver cancer. The results of this study indicate that the expression of OPN and CD44V6 are significantly positively correlated ($r = 0.58$, $p < 0.05$). In the context of breast cancer, it can be assumed that OPN can bind to the CD44V6 receptor and promote the invasion and metastasis of tumor cells, thus affecting a patient's prognosis.

Results from this study have also indicated that when OPN, p21ras, and CD44V6 are all coexpressed, the patients have a very poor prognosis. Only 10/29 patients with OPN, p21ras, and CD44V6 expression had a survival period > five years. Among the patients without OPN, p21ras, or CD44V6 expression, 23/27 had a survival period > five years. This difference was shown to be significant, and indicates that the expression of OPN, p21ras, and CD44V6 are closely correlated with a patient's prognosis. Currently, the mechanism underlying the OPN-CD44-Ras axis mediated effects on the invasion and metastasis of breast cancer is not clear. This study suggests that there may be a common mechanism between OPN, p21ras, and CD44V6, which enables them all to influence an invasive breast cancer patient's prognosis, although the details of this pathway require further study.

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