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Prognostic evaluation of oral squamous cell carcinoma based on pleiotrophin, urokinase plasminogen activator, and glycoprotein nonmetastatic melanoma protein B expression

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Abstract

This study investigated the expression of pleiotrophin (PTN), urokinase plasminogen activator (uPA), and glycoprotein nonmetastatic melanoma protein B (GPNMB) in oral squamous cell carcinoma (OSCC) tissues and their correlation with prognosis. From February 2017 to January 2020, PTN, uPA, and GPNMB expression in cancer tissues and adjacent tissues of 93 patients with OSCC was determined using immunohistochemistry. The diagnostic value of the combined detection of OSCC and its relationship with clinicopathological characteristics were analyzed, as well as the prognostic potential of PTN, uPA, and GPNMB. Cancer tissues from patients with OSCC exhibited high expression of PTN, uPA, and GPNMB. The AUC for the combined detection of PTN, uPA, and GPNMB for diagnosis and prognosis was greater than that of each index alone. The rates of expression of PTN, uPA, and GPNMB were higher in the death group than in the survival group. Patients with PTN, uPA, and GPNMB expression had lower 3-year survival rates. PTN expression was a risk factor affecting the prognosis of patients with OSCC. The rate of PTN, uPA, and GPNMB expression in OSCC tissues was high, and their expression was related to clinicopathological features such as lymph node metastasis and tumor invasion depth. The combined detection of each index has a predictive value for the prognosis of patients.

Abbreviations: OSCC = oral squamous cell carcinoma, PTN = pleiotrophin, uPA = urokinase plasminogen activator.

Keywords: clinicopathological features, GPNMB, oral squamous cell carcinoma, prognosis, PTN, uPA

1. Introduction

Oral squamous cell carcinoma (OSCC) ranks sixth in terms of incidence of oral and maxillofacial tumors. Clinical data have shown that the 5-year survival rate of patients with OSCC is still low.[1] Tumor location, size, and stage, as well as cervical lymph node invasion, are closely associated with the prognosis of OSCC.[2,3] Tumor invasion and metastasis is a highly organized and organ-specific process involving various factors.[4]

Pleiotrophin (PTN) is a heparin-binding protein isolated and purified from the brain of adult bovines. PTN is rich in basic amino acids and cysteine and can bind and interact with heparin to be involved in cell growth, differentiation and migration, wound repair, tumor growth, and inflammatory response.[5] PTN expression is closely associated with tumor growth, differentiation, and metastasis.[6] GPNMB is a type I transmembrane glycoprotein that mediates intracellular signaling pathways and participates in cellular growth, proliferation, and differentiation; its abnormal expression is related to the pathological and physiological processes of various diseases.[7] Urokinase plasminogen activator (uPA) is a serine proteolytic enzyme that can specifically dissolve proteins in the extracellular matrix and basement membrane, enabling tumor cells to break through the barrier formed by connective tissue after detachment from the primary lesion and grow locally infiltratively, leading to distant metastasis into the vascular system or lumen.[8] Based on this, this study aimed to explore the expression of PTN, uPA, and GPNMB in OSCC and their relationship with prognosis, to provide a reference for disease assessment and early prognosis evaluation.

2. Materials and methods

2.1. Clinical information

All 93 patients selected between February 2017 and January 2020 were diagnosed with OSCC.[4] The included patients

YM and YL contributed equally to this work.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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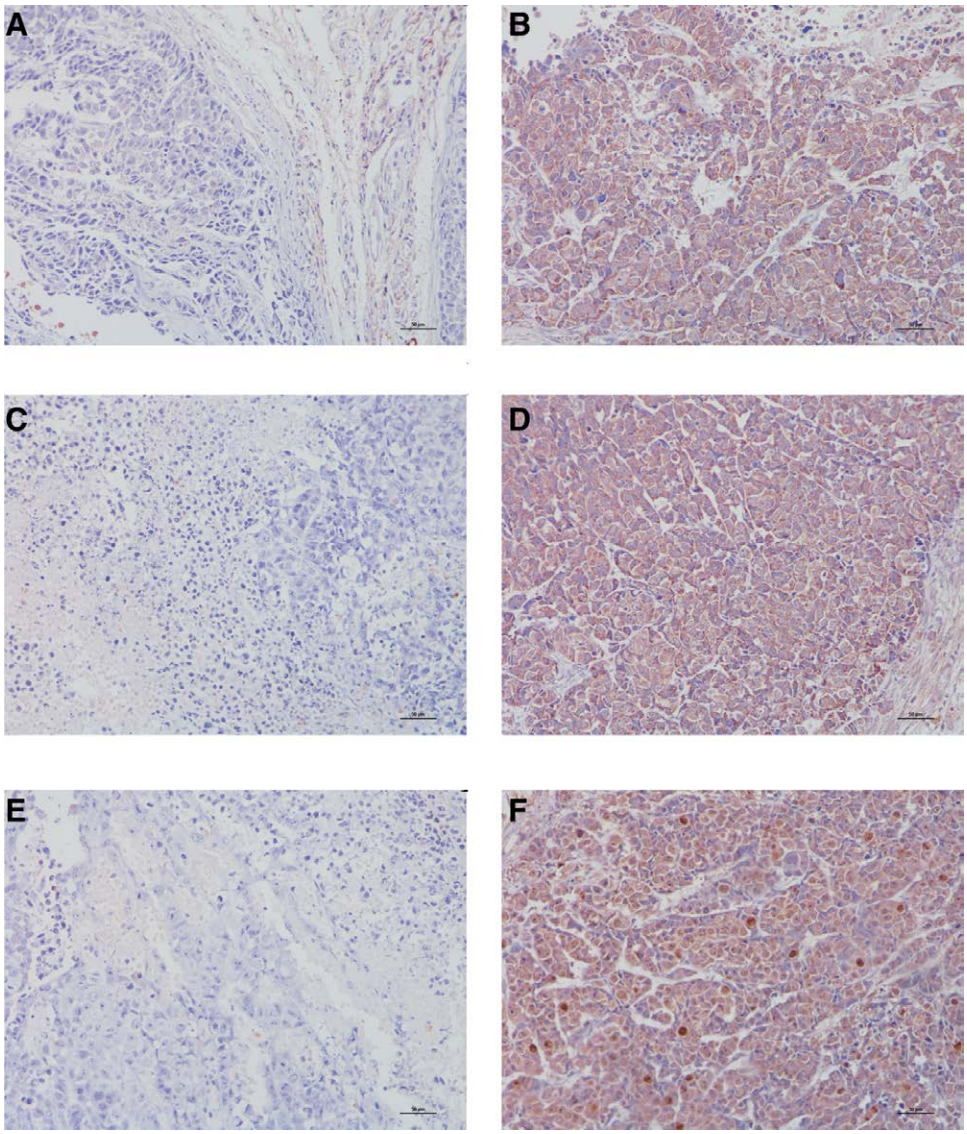

Figure 1. Typical images of the immunohistochemistry of PTN, uPA, and GPNMB. (A) PTN, (C) uPA, and (E) GPNMB expression in normal tissue. (B) PTN, (D) uPA, and (F) GPNMB expression in OSCC tissue. PTN = pleiotrophin, uPA = urokinase plasminogen activator.

were diagnosed by pathological examination, had complete clinical data, and underwent surgical treatment. Patients with severe organ dysfunction, other malignant tumors, expected survival time ≤ 3 months, autoimmune diseases, other oral tumors, or those who underwent radiotherapy and chemotherapy were excluded. This study followed the principles of the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of Xianning Central Hospital.

Table 1

Comparison of PTN, uPA and GPNMB expression in cancer tissue and adjacent tissue.

Tissues	n	PTN	uPA	GPNMB
Cancer tissue	93	53 (56.99)	61 (65.59)	65 (69.89)
Adjacent tissue	93	19 (20.43)	30 (32.26)	27 (29.03)
χ^2		26.196	20.676	31.057
P		<.001	<.001	<.001

2.2. Methods

2.2.1. Clinical data collection. The general information and clinical data of the patients were collected using the hospital information system, including sex, age, tumor differentiation, and invasion depth.

2.2.2. Immunohistochemistry. Cancer tissue and adjacent tissues (more than 3 cm away from the cancer tissue and confirmed as noncancerous tissue by intraoperative pathological examination) were cut into paraffin-embedded slices (3 μm) for immunohistochemical staining using an S-P immunohistochemical kit, rabbit antihuman PTN monoclonal antibody, mouse antihuman uPA monoclonal antibody (ZSGB-Bio, Beijing, China), and mouse antihuman GPNMB monoclonal antibody (R&D company). PTN and uPA are expressed in the cytoplasm, whereas GPNMB is expressed in both the cytoplasm and nucleus. Typical immunohistochemistry images are shown in Figure 1.

2.3. Observation indicators

(1) The diagnostic value of the combined detection of OSCC and its relationship with clinicopathological features was analyzed. (2) The patients were divided into survival and death groups according to their survival status within 3 years of enrollment, and the relationship between PTN, uPA, and GPNMB expression, the survival status of patients, and their predictive value for the prognosis of patients were analyzed.

Table 2

Analysis of the diagnostic value of PTN, uPA, and GPNMB expression in OSCC.

Index	AUC	SEN	SPE	95%CI
PTN	0.683*	0.42	0.69	0.605–0.760
uPA	0.667*	0.41	0.67	0.588–0.745
GPNMB	0.704*	0.49	0.73	0.628–0.780
Combined	0.786	0.54	0.86	0.720–0.853

*$P < .05$ compared with combined.

2.4. Statistical analysis

SPSS software (version 22.0) was used to process the data. Continuous variables are expressed as mean ± standard deviation (SD), and categorical variables are expressed as n (%). When the data of different groups had a normal distribution and the variance was homogeneous, analysis of variance was used, and the chi-square test was used to compare the classification data of different groups. ROC curves were used to analyze the diagnostic value of PTN, uPA, and GPNMB expression in OSCC, and multivariate logistic regression analysis was used to assess the relationship between PTN, uPA, and GPNMB expression and patient prognosis. Survival curves were drawn

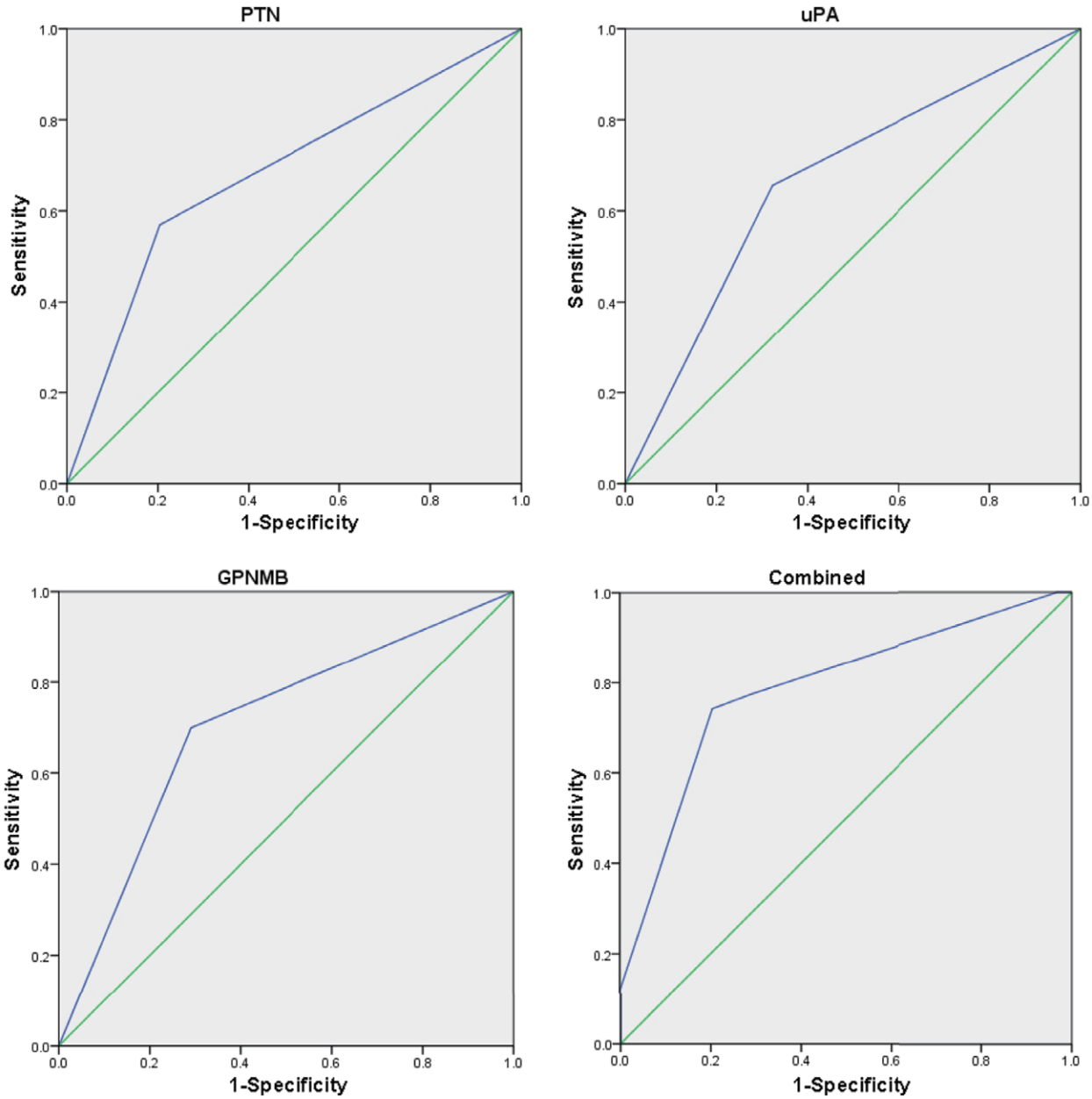

Figure 2. ROC analysis of the diagnostic value of PTN, uPA, and GPNMB. PTN = pleiotrophin, uPA = urokinase plasminogen activator.

Table 3

The relationship between the expression of PTN, uPA and GPNMB and clinicopathological features in OSCC cancer tissue.

Variables	n	PTN positive (n = 53)	χ^2	uPA positive (n = 61)	χ^2	GPNMB positive (n = 65)	χ^2
Gender							
Male	51	29 (54.72)	0.001	35 (57.38)	0.461	40 (61.54)	3.913*
Female	42	24 (45.28)		26 (42.62)		25 (38.46)	
Age			0.155		0.058		0.558
≥60 years	51	30 (56.60)		34 (55.74)		34 (52.31)	
<60 years	42	23 (43.40)		27 (44.26)		31 (47.69)	
TNM stage			17.485*		14.058*		5.916*
I-II	42	14 (26.42)	17.485*	19 (31.15)	14.058*	24 (36.92)	5.916*
III-IV	51	39 (73.58)		42 (68.85)		41 (63.08)	
Tumor site			3.133		3.098		0.76
Tongue squamous cell carcinoma	30	14 (26.42)		21 (34.43)		21 (32.31)	
Lip squamous cell carcinoma	25	15 (28.30)		13 (21.31)		19 (29.23)	
Buccal squamous cell carcinoma	20	11 (20.75)		15 (24.59)		13 (20.00)	
Gingival squamous cell carcinoma	18	13 (24.53)		12 (19.67)		12 (18.46)	
Lymph node metastasis			21.003*		15.966*		13.943*
Yes	44	36 (67.92)		38 (62.30)		39 (60.00)	
No	49	17 (32.08)		23 (37.70)		26 (40.00)	
Differentiation			15.372*		10.685*		6.299*
Low	45	35 (66.04)		37 (60.66)		37 (56.92)	
Moderate/high	48	18 (33.96)		24 (39.34)		28 (43.08)	
Invasion depth			12.123*		13.131*		9.305*
T1–T2	58	25 (47.17)		30 (49.18)		34 (52.31)	
T3–T4	35	28 (52.83)		31 (50.82)		31 (47.69)	

* Indicated $P < .05$.

by GraphPad Prism software, and the cumulative survival rate was compared by log-rank χ^2. $P < .05$ was considered statistically significant.

3. Results

3.1. Immunohistochemistry staining

Cancer tissues from patients with OSCC exhibited higher expression rates of PTN, uPA, and GPNMB than adjacent tissues (Table 1).

3.2. Diagnostic value of PTN, uPA, and GPNMB expression

The AUC for the combined detection of PTN, uPA, and GPNMB expression for OSCC diagnosis was greater than that of each index alone (Table 2 and Figure 2).

3.3. Clinicopathological analysis

The proportion of PTN-positive patients with TNM stages (III and IV), lymph node metastasis, poor differentiation, and invasion depth (T3–T4) was higher than that of PTN-negative patients. The proportion of patients with poor differentiation and invasion depth (T3–T4) was higher than that of uPA-negative patients. GPNMB-positive patients had a higher proportion of men, TNM stages III and IV, lymph node metastasis, poor differentiation, and invasion depth (T3–T4) than uPA-negative patients (Table 3).

3.4. PTN, uPA, and GPNMB expression in survivors and deaths

The expression rates of PTN, uPA, and GPNMB in cancer tissues of the death group were higher than those in the survival group (Table 4).

Table 4

Comparison of the expression of PTN, uPA and GPNMB in cancer tissue between the survival group and the death group.

Groups	n	PTN	uPA	GPNMB
Death	30	25 (83.33)	27 (90.00)	26 (86.67)
Survival	63	28 (44.44)	34 (53.97)	39 (61.9)
χ^2		12.539	11.691	5.922
P		<.001	<.001	<.05

Table 5

Comparison of cumulative survival rates of patients with different expressions of PTN, uPA, and GPNMB.

Groups	n	Survival rate	Log-rank $\chi 2$	*P*
PTN				
Positive	53	28 (52.83)	11.96	<.001
Negative	40	35 (87.50)		
uPA				
Positive	61	34 (55.74)	10.62	<.001
Negative	32	29 (90.63)		
GPNMB				
Positive	65	39 (60.00)	5.33	.021
Negative	28	24 (85.71)		

3.5. Survival curve analysis

Patients with PTN, uPA, or GPNMB expression had a lower 3-year survival rate than those with negative expression (Table 5 and Figure 3).

3.6. Prognosis analysis

The combined detection of PTN, uPA, and GPNMB expression had a greater AUC for predicting OSCC prognosis than each index alone (Table 6 and Figure 4).

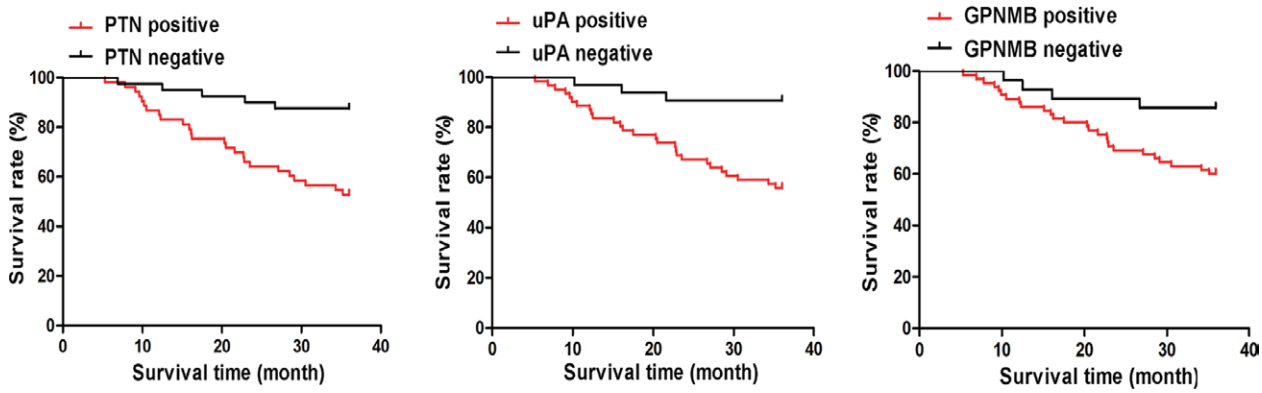

Figure 3. Survival curve analysis of patients.

Table 6

Predictive value of PTN, uPA, and GPNMB expression in cancer tissue for prognosis of OSCC.

Index	AUC	SEN	SPE	95%CI
PTN	0.694*	0.57	0.85	0.584–0.805
uPA	0.680*	0.56	0.81	0.570–0.790
GPNMB	0.624*	0.6	0.79	0.507–0.740
Combined	0.781	0.49	0.88	0.685–0.878

* $P < .05$ compared with combined.

3.7. Logistic regression analysis

PTN expression was a risk factor in the prognosis of patients with OSCC (Table 7).

4. Discussion

OSCC is a squamous cell carcinoma of the oral mucosa. It has the characteristics of strong local invasiveness and easy metastasis to the cervical lymph nodes, which in turn affects the prognosis of patients.[9] The occurrence of OSCC is a complex process, from normal mucosal epithelium and leukoplakia to malignant lesions, involving abnormal changes in various genes, proteins, and signaling pathways.[10,11] PTN is one of the growth factors closely related to the proliferation, angiogenesis, and metastasis of lung cancer, and it can be secreted into extracellular body fluids and blood.[12] PTN can promote the growth of blood vessels, the nervous system, and bone, and it can stimulate cell proliferation and migration. uPA, an activator of the exogenous fibrinolytic pathway, participates in cell migration and tissue repair under physiological and pathological conditions through enzymatic reactions, mediates the hydrolysis of extracellular matrix proteins, lyses type IV collagenase, activates plasminogen to fibrinolytic enzymes, directly degrades the extracellular matrix and basement membrane, participates in the clearance of normal and pathological tissues, and stimulates angiogenesis. GPNMB on the surface of antigen-presenting cells can inhibit T cell activity, reduce the secretion of proinflammatory cytokines, prevent T lymphocytes from entering the cell cycle, and participate in autophagy.[13] GPNMB is highly expressed in glioma and breast cancer cells.[14] The study results demonstrated that PTN, uPA, and GPNMB expression in the cancer tissues of patients with OSCC was increased, indicating that PTN, uPA, and GPNMB may be related to the occurrence of OSCC. In addition, the AUC of the combined detection of PTN, uPA, and GPNMB expression in the diagnosis of OSCC was greater than that of each index alone. However, related studies have shown that the sensitivity of tumor markers in the diagnosis of OSCC is high, but the specificity is low, which may be affected by other factors;[15] therefore, their value in the clinical diagnosis of OSCC requires further research.

Angiogenesis is a prerequisite for tumor growth and reproduction, and tumor blood vessels provide oxygen and nutrients for tumor cell growth. Various factors are involved in tumor angiogenesis.[16] PTN has multiple signaling functions that promote angiogenesis and stimulate cell proliferation and migration. Some studies have shown that when PTN is dominantly inactive, the malignant phenotype of glioma cells can be reversed and the cell cycle slowed.[17] A recent report suggested that the greater the expression of uPA, the stronger the tumor invasion in patients with esophageal squamous cell carcinoma, and this is related to the tumor growth pattern, invasion, and lymph node metastasis.[18] Relevant studies have indicated that uPA may be involved in tumor angiogenesis.[19] Previously, some scholars have found that GPNMB expression is high in liver cancer tissues, and the reduction of GPNMB expression in liver cancer cells with a faster proliferation rate only slows down cell proliferation without causing cell death.[20] It has also been reported that GPNMB induces tumor metastasis, reduces apoptosis, and increases vascular density.[21] This study found that PTN, uPA, and GPNMB expression was related to clinical pathology, such as lymph node metastasis and tumor invasion depth. This is because uPA binds to its receptor, which can promote tumor invasion and metastasis by regulating signaling pathways in cells, and enhances the ability to degrade the extracellular matrix and basement membrane. Cells are more likely to infiltrate deep tissues and lymphatic vessels and metastasize.

uPA was the first proteolytic enzyme to be used as a prognostic marker for malignant tumors. uPA can be released from tumor cells in an inactive form and converted into active uPA after binding to cell surface receptors. The degradation of fibronectin, collagen, and other components of the matrix regulates tumor invasion and metastasis.[22] GPNMB overexpression can promote the migration and infiltration of breast cancer cells and is accepted as an independent prognosis predictor of patients.[23] The study results highlighted that patients with PTN, uPA, and GPNMB expression had a low 3-year survival rate, indicating that the PTN, uPA, and GPNMB expression may increase patient mortality. Further analysis revealed that PTN expression is a risk factor affecting the prognosis of patients with OSCC. However, this study showed that uPA and GPNMB expression is unrelated to the prognosis of patients, which is inconsistent with a previous study[24] and may be related to the small sample size of this study. Therefore, it is

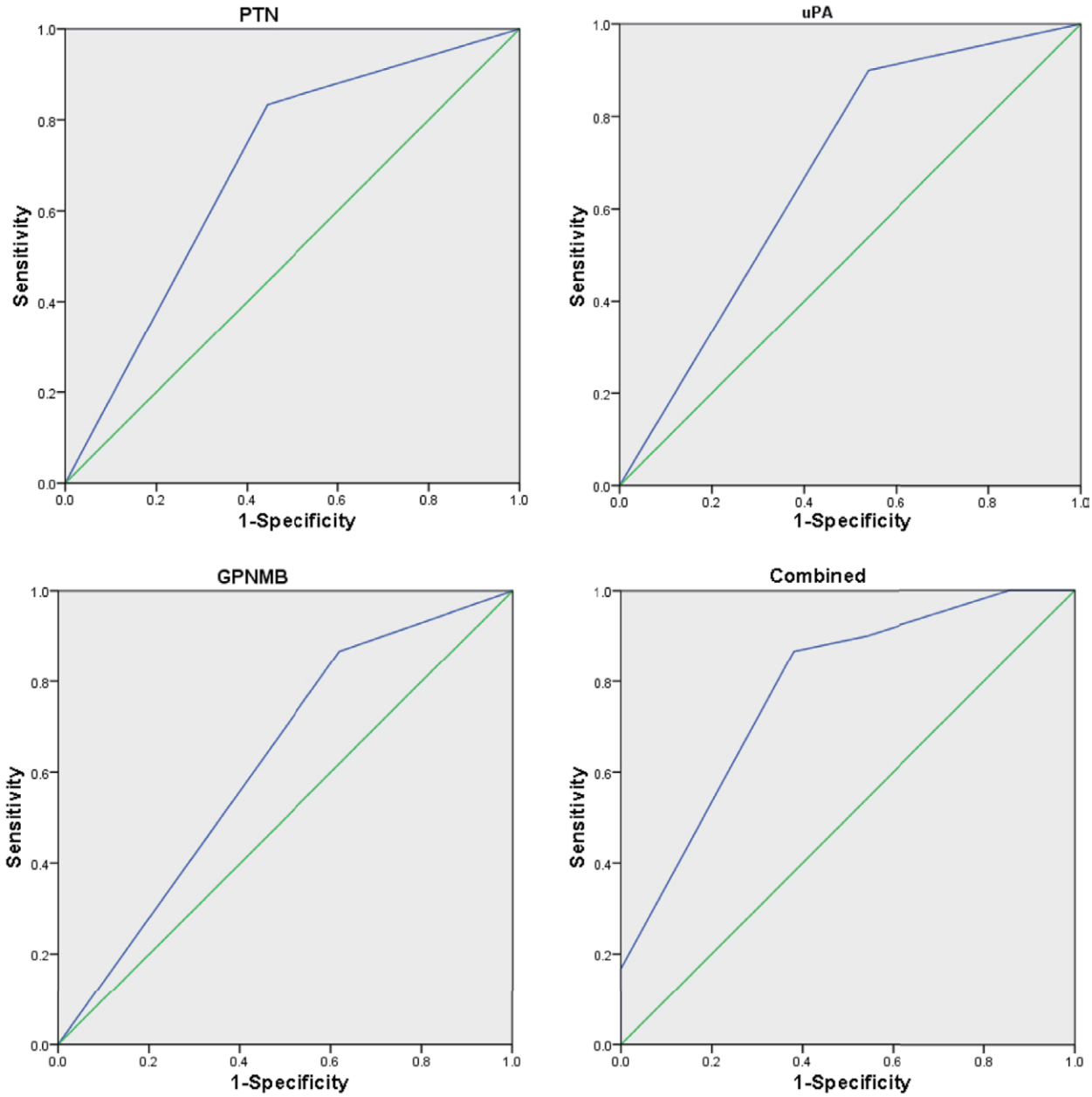

Figure 4. ROC analysis of the prognostic value of PTN, uPA, and GPNMB expression. PTN = pleiotrophin, uPA = urokinase plasminogen activator.

Table 7

Logistic regression analysis of the expression of PTN, uPA, and GPNMB in cancer tissue and the prognosis of patients.

Index	β	SE	Wald χ^2	OR	95%CI	*P*
PTN	0.585	0.249	5.52	1.795	1.102–2.924	.019
uPA	0.793	0.526	2.273	2.21	0.788–6.196	.132
GPNMB	1.678	1.115	2.265	5.355	0.602–47.627	.133

necessary to increase the sample size in future studies to further explore the relationship with the prognosis of patients with OSCC. In addition, the combined detection of PTN, uPA, and GPNMB had a predictive value for the prognosis of patients with OSCC.

In conclusion, the rate of PTN, uPA, and GPNMB expression in OSCC tissues is high, and their expression is related to clinicopathological features such as lymph node metastasis and tumor invasion depth, and the combined detection of each index has prognostic value for patients.

Author contributions

Conceptualization: Yuxin Ma, Yue Liu, Han Meng.
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Supervision: Yuxin Ma, Yue Liu.
Validation: Yuxin Ma, Han Meng.
Visualization: Yuxin Ma, Yue Liu.
Writing – original draft: Yuxin Ma.
Writing – review & editing: Han Meng.

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