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Original Article

Prognostic and clinicopathologic significance of PAQR3 and VEGF-A expression in pulmonary adenocarcinoma

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Abstract: Progesterone and adipoQ receptor family member 3 (PAQR3) and vascular endothelial growth factor (VEGF)-A are associated with tumorigenesis and progression. The aim of this study is to investigate the expression of PAQR3 and VEGF-A in pulmonary adenocarcinoma (PA) and explore their clinical and pathologic significance. The expressions of PAQR3 and VEGF-A protein were detected in 86 cases of human PA and 26 cases of tumor-adjacent tissue by immunohistochemistry. The positive rate of PAQR3 was 39.5% in PA, which was lower than that in tumor-adjacent tissues (80.8%), P=0.001. Negative expression of PAQR3 was obviously linked to tumor TNM stage, differentiation, and lymphatic metastasis; and *P* values were 0.013, 0.025, and 0.034, respectively. The positive expression rate of VEGF-A was 68.6% in human PA whichwas higher than that of tumor-adjacent tissues (11.5%), P<0.001. The positive expression of VEGF-A was correlated with tumor TNM stage, differentiation, and lymphatic metastasis, and *P* values were 0.026, 0.001 and P=0.001, respectively. The expression of PAQR3 was negatively correlated with the expression of VEGF-A (r=-0.698, P<0.001). Log-rank test statistical analysis suggested that patients with negative expression of PAQR3 or positive expression of VEGF-A had shorter overall survival. Cox multivariate analysis indicated that tumor TNM stage, differentiation, and lymphatic metastasis, and PAQR3 and VEGF-A expression were independent factors for prognosis of PA, and *P* values were 0.021, 0.017, 0.006, 0.018 and P=0.007 respectively. In conclusion, negative expression of PAQR3 and positive expression of VEGF-A are markedly correlated with tumor TNM classification, histologic grade, and lymphatic metastasis. Tumor TNM stage, differentiation, and lymphatic metastasis, negative expression of PAQR3, and positive expression of VEGF-A are risk factors for prognosis of patients with PA. Detection of PAQR3 and VEGF-A may be helpful to evaluate prognosis and infiltrative capability of PA.

Keywords: PAQR3, VEGF-A, pulmonary adenocarcinoma, survival, prognosis

Introduction

The mortality of lung cancer ranks first in China [1]. From 2009 to 2011, the prevalence of lung cancer in China was 733.3 (1/100000). Among them, 509.3 (1/100000) were males, ranking first among malignant tumors, and female 224 (1/100000), ranking second among malignant tumors [1].

Lung cancer includes 2 main types: non-small cell lung cancer (NSCLC, 85% of cases), and small cell lung cancer (SCLC) according to the World Health Organization (WHO) [2]. Adenocarcinoma is the most common NSCLC, comprising 50% of cases and it has increased over the past decades [3]. Surgical treatment is the first choice for lung cancers, but 57% of the patients have distant metastasis at the initial diagnosis and are not candidates for surgery [4, 5]. Therefore, in-depth study of the mechanism of lung cancers occurrence, development, and the discovery of new tumor markers for early treatment are particularly important for increasing the efficiency of treatment and prolonging survival.

PAQR3 also is named Raf kinase trapping to Golgi (RKTG), and is a type III seven-transmembrane protein [6, 7]. Many studies have found that PAQR3 is down-regulated in cancer tissues such as esophageal squamous cell carcinoma,

prostate cancer, glioma cells, breast cancer, and laryngeal squamous cell carcinoma, which can inhibit cell proliferation, neovascularization and invasion [6-10].

VEGF is a vital regulatory factor for tumor angiogenesis. When combined with its receptor, it can promote the division of vascular endothelial cells, increasing vascular permeability, which promotes tumor invasion and metastasis [11, 12].

VEGF-A is an important member of VEGF family, which is highly expressed in a variety of tumors, and is closely related to tumor invasion and metastasis [13, 14].

However, the role of PAQR3 and VEGF-A in lung cancer has rarely been studied. In this research, we detected the PAQR3 and VEGF-A protein in 82 cases of pulmonary adenocarcinoma by immunohistochemistry. The purpose is to find a convenient marker for the diagnosis and prognosis of the PA.

Materials and methods

Patients

This research plan was approved by the ethics committee of *First Affiliated Hospital of Soochow University*. From July 2014 to July 2019, 86 cases of PA patients and 26 tumor adjacent tissue samples were collected with proper informed consent. 86 cases were operated on, and their average age was 66.2 years old, ranging from 47-84 years old. TNM staging was carried out according to American Joint Committee on Cancer (AJCC) in 2010 [15]. Two pathologists confirmed that all sections were PA. All patients were followed up 3-60 months by telephone. None received preoperative radiotherapy or chemotherapy.

Immunohistochemistry analysis

Immunohistochemical analysis was similar to the previous literature [16]. In short, all specimens were cut into 4 μm slices and baked, then were dewaxed and hydrated. Specimens were treated with 3% H_2O_2 for 10 min, then immersed in citric acid buffer in pressure cooker, and boiled. Then we incubated the sections with rabbit anti-human PAQR3 antibody or VEGF-A antibody (1:120, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), 4°C overnight, washed three times with phosphate buffer saline (PBS), then we added secondary antibody. The specimens were incubated with streptavidin horseradish peroxidase, and DAB was added for visualization. Hematoxylin was used to stain the specimens and they were washed to colorless. PAQR3 or VEGF-A antibody was replaced by PBS as negative control.

Evaluation of PAQR3 and VEGF-A staining

Positive PA cells were scored as follows: 0 (1-5%), 1 (5-50%), 2 (51-100%). The staining intensity score was 0 (no PA cell was stained), 1 (PA cells were stained light yellow), 2 (PA cells were stained yellow brown), and 3 (PA cells were stained brown). The total score was the sum of the intensity score and the proportional score. When score was more than 4, the expression was defined as positive (+) and when score was 0-4, the expression was defined as negative (-) [6].

Statistical analysis

The study used SAS 9.2 software for statistical analysis. Chi square test was used for classified variables. Graphpad prism version 5.0 was used for survival curves. Survival data were determined by Kaplan Meier method. We used Cox proportional hazards model for multivariate analysis. $P<0.05$ was considered significant.

Results

PAQR3 and VEGF-A expression in PA and tumor-adjacent tissue

PAQR3 positive staining was largely observed in the cytoplasm of PA cells (**Figure 1A**). VEGF-A positive staining was largely observed in the cytoplasm of PA cells, as hown in **Figure 1B**. PAQR3 expression was much lower in PA than in adjacent tissue (P=0.001), as shown in **Table 1**. VEGF-A expression in PA was much higher than that in adjacent tissue (P<0.001), as shown in **Table 1**.

PAQR3 and VEGF-A expression are associated with clinicopathologic characteristics of PA

Positive expression rate of PAQR3 was 39.5% in PA, which was lower than that in adjacent tissues (80.8%), P=0.001. Negative expression of PAQR3 was linked to tumor TNM stage, differ-

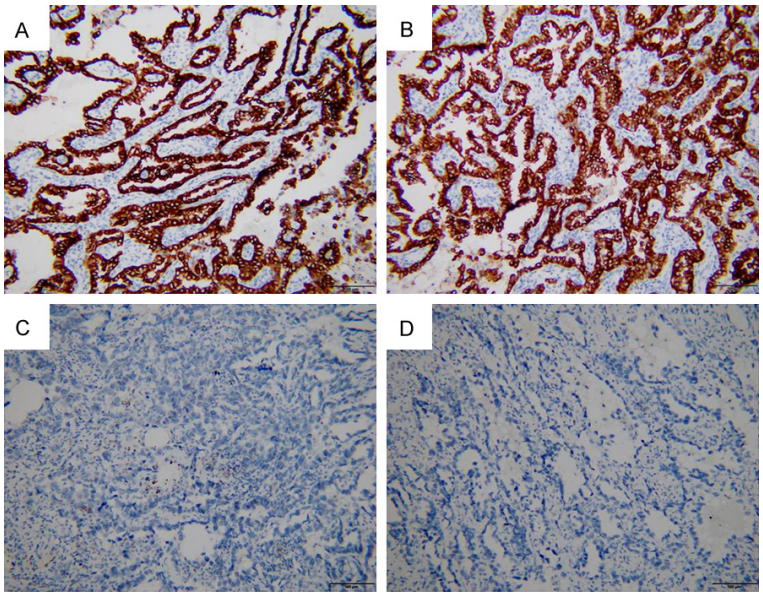

Figure 1. PAQR3 positive expression in PA tissues; cytoplasm was stained brown (200×) (A). PAQR3 negative expression in PA tissues (200×) (C). VEGF-A positive expression in PA tissues; cytoplasm was stained yellow (200×) (B). VEGF-A was not expressed in PA (200×) (D).

Table 1. Expression of PAQR3[a] and VEGF-A[b] in PA and tumor-adjacent tissue

Marker	Pulmonary adenocarcinoma tissue		Tumor-adjacent tissue		*P* Value
	Negative (-)	Positive (+)	Negative (-)	Positive (+)	
PAQR3	52 (60.5)	34 (39.5)	5 (19.2)	21 (80.8)	0.001
VEGF-A	27 (31.4)	59 (68.6)	23 (88.5)	3 (11.5)	<0.001

[a]Progesterone and adipoQ receptor family member 3 (PAQR3), [b]Vascular endothelial growth factor)-A.

entiation, and lymphatic metastasis, and *P* values were 0.013, 0.025 and 0.034, respectively. However, PAQR3 expression had nothing to do with age, sex, and tumor size, as shown in **Table 2**. The positive expression rate of VEGF-A was 68.6% in human PA which was higher than that in tumor-adjacent tissues (11.5%), P<0.001. The positive expression of VEGF-A was correlated with tumor TNM stage, differentiation, and lymphatic metastasis, and *P* values were 0.026, 0.001 and P=0.001, respectively. However, VEGF-A protein expression was not relevant to age, sex, and tumor size, as shown in **Table 2**.

PAQR3 and VEGF-A expression correlate with survival

The overall survival of patients with PAQR3 negative expression was much lower than that of positive patients (P=0.014, log-rank test), (**Figure 2A**). The overall survival of patients with VEGF-1 positive expression was much higher than that of patients with negative expression (P=0.001, log-rank test), (as shown in **Figure 2B**). Cox multivariate analysis indicated that tumor TNM stage, differentiation, and lymphatic metastasis, PAQR3, and VEGF-A expression were independent factors for prognosis of PA, and *P* values were 0.021, 0.017, 0.006, 0.018 and P=0.007 respectively, as shown in **Table 3**. Tumor TNM stage, differentiation, and lymphatic metastasis, negative expression of PAQR3, and positive expression of VEGF-A were risk factors for prognosis of PA.

Correlations between PAQR3 and VEGF-A expression

In PA tissues, the expression of PAQR3 was negatively correlated with the expression of VEGF-A (r=-0.698, P<0.001), **Table 4**.

Discussion

Studies have shown that PAQR3 activates different pathways that control the development of tumors. First, PAQR3 can bind Raf kinase competitively, anchor it in Golgi body, block the signal transmission between Raf kinase and upstream and downstream, inhibit the activation of Ras/Raf/MEK/ERK pathway, and eventually inhibit the proliferation, differentiation, invasion and metastasis of tumor cells [10, 17, 18].

Second, PAQR3 regulates the cell cycle. It was found that its overexpression could up regulate the expression of $P27^{KiPI}$ protein and down regulate the expression of cyclin D1 protein, thus affecting the cell cycle, reducing the proportion of tumor cells entering S phase, increasing the proportion of cells in G0 phase/G1 phase, and eventually inhibiting the proliferation of tumor cells [19]. Third, it controls the PI3K/AKT pathway to inhibit cell invasion [20]. In addition, PAQR3 could enhance Twist1 degradation

Table 2. Analysis of PAQR3[a] and VEGF-A[b] positive expression and related factors

Variable	n	PAQR3 expression		*P* Value	VEGF-A expression		*P* Value
		Negative (-)	Positive (+)		Negative (-)	Positive (+)	
Age (year)							
≥60	48	28 (58.3)	20 (41.7)	0.650	14 (29.2)	34 (70.8)	0.722
<60	38	24 (63.2)	14 (36.8)		13 (34.2)	25 (65.8)	
Sex							
Male	32	16 (50.0)	16 (50.0)	0.127	8 (25.0)	24 (75.0)	0.325
Female	54	36 (66.7)	18 (33.3)		19 (35.2)	35 (64.8)	
Tumor size (cm)							
<3	35	25 (71.4)	10 (28.6)	0.085	12 (34.3)	23 (65.7)	0.632
≥3	51	27 (52.9)	24 (47.1)		15 (29.4)	36 (70.6)	
TNM stage							
I-II	39	18 (46.2)	21 (53.8)	0.013	17 (43.6)	22 (56.4)	0.026
III-IV	47	34 (72.3)	13 (27.7)		10 (21.3)	37 (78.7)	
Differentiation							
Well + moderate	56	29 (51.8)	27 (48.2)	0.025	25 (44.6)	31 (55.4)	0.001
Poor	30	23 (76.7)	7 (23.3)		2 (6.7)	28 (93.3)	
Lymphatic metastasis							
no	41	20 (48.8)	21 (51.2)	0.034	21 (51.2)	20 (48.8)	0.001
yes	45	32 (71.1)	13 (28.9)		6 (13.3)	39 (86.7)	

[a]Progesterone and adipoQ receptor family member 3 (PAQR3), [b]Vascular endothelial growth factor)-A.

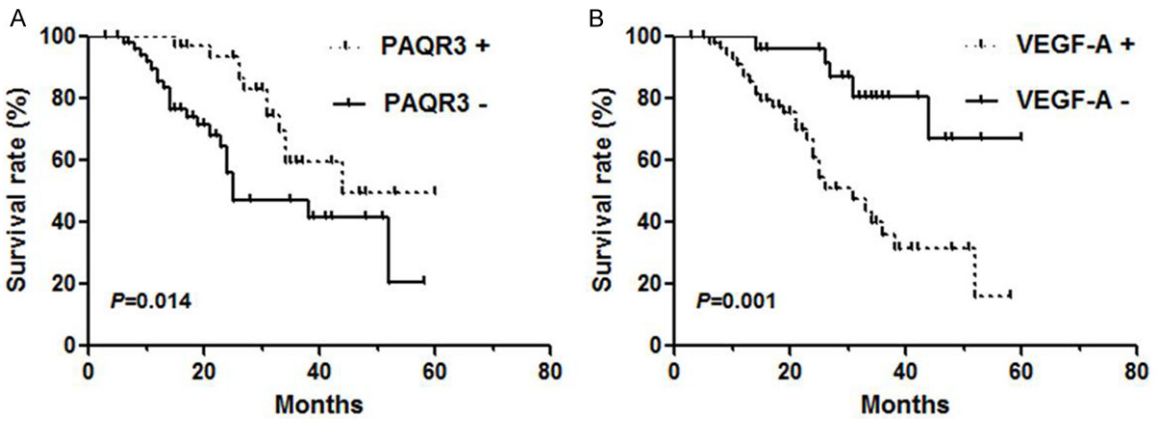

Figure 2. Kaplan-Meier survival curves of PA patients based on PAQR3 expression (A) or VEGF-A expression (B).

to suppress epithelial-mesenchymal transition and metastasis in gastric cancer cells [21].

The expression of PAQR3 in PA cells was significantly lower than that in the tumor-adjacent tissue in the study, (P<0.05). Its positive rate was relatively lower in the patients who had lymph node metastasis than that in patients without lymph node metastasis, and it was inversely proportional to histologic grade and TNM stage. Thus, the loss of PAQR3 appears important to PA development, and could be used for the auxiliary diagnosis in lung cancer.

Compared with patients of PAQR3 positive expression, the overall survival time of negative patients was shorter in the study [4, 7]. Our follow-up results also suggest a poor prognosis in patients with negative expression. Cox multivariate analysis also showed that its negative expression was a risk factor for prognosis of lung cancer.

Table 3. Cox analyses of different clinicopathologic variables and PAQR3[a] and VEGF-A[b] expression status as predictors for overall survival in pulmonary adenocarcinoma tissues

Variable	Hazard Ratio	95% Hazard Ratio	Confidence Limits	*p* value
Age (≥65 vs. <65)	0.863	0.516	2.264	0.973
Sex (Male vs. Female)	1.127	0.558	2.167	0.792
Tumor size (<3 vs. ≥3))	2.456	0.894	5.788	0.084
TNM stage (I-II vs. III-IV)	3.728	1.437	10.416	0.021
Differentiation (well-moderate vs. poor)	2.165	1.256	5.262	0.017
Lymphatic metastasis (no vs. yes)	4.314	1.871	8.525	0.006
PAQR3 (positive vs. negative)	0.189	0.072	0.923	0.018
VEGF-A (positive vs. negative)	5.841	1.522	16.624	0.007

[a]Progesterone and adipoQ receptor family member 3 (PAQR3), [b]Vascular endothelial growth factor)-A.

Table 4. Correlations between PAQR3[a] and VEGF-A[b] expression in pulmonary adenocarcinoma tissues

VEGF-A	PAQR3		Contingency coefficient (r)	χ^2	P
	+	-			
+	10	49	-0.698	40.102	<0.001
-	24	3			

[a]Progesterone and adipoQ receptor family member 3 (PAQR3), [b]Vascular endothelial growth factor)-A.

PAQR3 protein is a good prognostic marker. With the development of biology technology, many scholars have found that PAQR3 overexpression could suppress the invasion and metastasis of tumor cells [8, 19], suggesting that it is a gene therapeutic target.

Many studies have found that VEGF is overexpressed in cancer tissues, and patients with overexpression of VEGF had shorter overall survival time compared with those with negative expression of VEGF [14, 22].

In this study, the positive rate of VEGF-A protein in PA was much higher than that in the paracancerous tissue (P<0.05). The positive rate of VEGF-A was relatively higher in patients who had lymph node metastasis than those who did not and it was inversely proportional to grade and TNM stage. Thus, VEGF-A is vital to the occurrence and development of the PA, and can be used for an auxiliary marker. Compared with patients who had VEGF-A negative expression, the overall survival time of VEGF-A positive patients was shorter [22]. Our follow-up results also showed that patients who were positive expression of VEGF-A had a poor prognosis. Expression of VEGF-A was an independent risk factor for prognosis of lung cancer. Our study also found that PAQR3 had low expression while VEGF protein in the lung cancer was overexpressed, and they were negatively correlated. This is similar to Xiu's study that PAQR3 negatively regulates VEGF by activating Raf/MEK/ERK signaling [23].

However, the factors and mechanism of lung cancer development are very complex. We studied PAQR3 and VEGF-A expression by immunohistochemistry only, which is a drawback of our study. In the future, we can up-regulate the expression of PAQR3 and study its role in the occurrence and development of lung cancer.

Conclusion

Negative expression of PAQR3 or overexpression of VEGF-A were important biomarkers for progression, metastasis, and prognosis in lung cancer. Patients with negative of PAQR3 or positive expression of VEGF-A had poor survival. PAQR3 negatively regulates VEGF-A protein. Negative expression of PAQR3 or positive expression of VEGF-A correlates with histologic grade, TNM stage, and lymph node metastasis. Further work is needed on these targets in lung cancer.

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Disclosure of conflict of interest

None.

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