The expression tendency and prognostic value of PDGFRβ in oral cancer

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Abstract

Background Oral cancer is a common malignant tumor in head and neck with poor prognosis. This study aimed to determine the expression tendency and prognostic value of PDGFRβ in oral cancer. Methods The mRNA expression level of PDGFRβ in the oral cancer tissues and adjacent normal tissues of oral cancer patients were detected by quantitative real-time polymerase chain reaction (qRT-PCR). And the association of PDGFRβ expression with clinicopathological characteristic was analyzed via chi-square test. Then we used Kaplan-Meier analysis to analyze the effects of PDGFRβ expression on the overall survival of oral cancer patients. The multivariate cox analysis was used to evaluate its prognostic value. Results The results indicated that the mRNA expression level of PDGFRβ was significantly increased in oral cancer tissues compared with that in the adjacent normal tissue ($P < 0.001$). And its expression is positively associated with clinical stage, T stage, lymph node metastasis and histological grade. Kaplan-Meier analysis revealed that patients with high expression of PDGFRβ had markedly worse overall survival than those with low expression of PDGFRβ (log rank test, $P < 0.05$). Additionally, cox regression analysis revealed that the high expression of PDGFRβ was an independent prognostic maker in oral cancer patients. Conclusion PDGFRβ is up-regulated and involved in the development of oral cancer. Moreover, it could be an independent prognostic bio-marker for oral cancer.

Background

Oral cancer is the most common malignant tumor in head and neck, most of which are characterized by invasive growth, invasion of surrounding tissue and easy to occur cervical lymph node metastasis [1]. Approximately 90% of oral neoplasms are oral squamous cell carcinoma (OSCC) [2]. Although surgical resection with chemotherapy and radiotherapy act as the most effective therapeutic method for treatment oral cancer, the prognosis for patients suffering OSCC remain poor [3]. Therefore, it is of important to identify the molecular markers that are associated with oral malignancy; which may further improve the clinical management and therapeutic development.

Platelet-derived growth factor receptors (PDGFRs) are part of the class III receptor tyrosine kinase family, including *PDGFRα* and *PDGFRβ* two subtypes [4, 5]. The receptor for PDGF-BB, *PDGFRβ*, located on chromosome 5q33.1, has an important role in regulating mesenchymal cells development, including pericytes, fibroblasts, and vascular smooth muscle cells [6, 7]. *PDGFRα* and *PDGFRβ* are important biological markers of cancer-associated fibroblast (CAFs) that can promote the occurrence, growth, invasion and metastasis of tumor, which are involved in the process of tumor cell proliferation and migration [8–10]. *PDGFRβ* has been found aberrantly expressed in many diseases and in tumor progression. However, the clinical significance of *PDGFRβ* in the prognosis of oral cancer was few reported.

In this study, we investigated the expression level of *PDGFRβ* in clinical oral cancer tissues and adjacent normal tissues. And we also investigated the relationship between *PDGFRβ* expression and clinicopathological characteristics of patients. Besides, the prognostic value of *PDGFRβ* was estimated.

Methods

Patients and specimens

A total of 156 patients with oral cancer, who underwent surgery at Chinese PLA General Hospital were included in this study. Among them, there were 74 females and 82 males. They were all histopathologically confirmed and without a prior history of cancer or previous chemo- or radiotherapy. And all the cancer tissues and adjacent normal tissues were extracted from patients and immediately put into liquid nitrogen after surgical resection and then stored at -80 °Cuntil RNA extraction, respectively. The clinicopathology of the cases were summarized in Table 1. A 5-years' follow-up was conducted and patients who were died from unexpected events or other diseases were excluded from our study.

Table 1
The relationship between *PDGFRβ* expression and clinicopathological features in oral cancer patients

Characteristics	No. (n = 156)	*PDGFRβ* expression		χ^2	*P* values
		Low (n = 75)	High (n = 81)		
Age (year)				0.605	0.437
< 60	82	37	45		
≥ 60	74	38	36		
Gender				0.256	0.613
Male	82	41	41		
Female	74	34	40		
Clinical stage				4.394	0.036
I-II	80	45	35		
III-IV	76	30	46		
Histopathological grade				4.340	0.037
G1-G2	78	44	34		
G3	78	31	47		
T stage				6.679	0.010
T1-T2	81	47	34		
T3-T4	75	28	47		
Lymph node metastasis				6.179	0.013
negative	88	50	38		
positive	68	25	43		
Differentiation				0.814	0.367
Moderate + well	92	47	45		
poor	64	28	36		

All the participants provided written, informed consent prior to surgery. The experiment was approved by the Medical Ethics Committee of Chinese PLA General Hospital.

Rna Extraction And Quantitative Real-time Rt-pcr (qrt-pcr)

The *PDGFRβ* gene was amplified via qRT-PCR. Total RNA was extracted using Trizol reagent (Invitrogen, Carisbad, CA, USA) according to the manufacturer's instructions. Then the first-strand complementary DNA (cDNA) was synthesized using the PrimeScript RT reagent kit (Takara, Dalian, China). QRT-PCR reaction was performed using a SYBR Green Premix Ex Taq (Takara, Dalian China) on the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *β-actin* was used as internal control for normalization of data and the expression of *PDGFRβ* at mRNA level was calculated via the comparative cycle threshold (CT) method. QRT-PCR primer sequences for *PDGFRβ* were as follows: forward, 5'-GTGGTGAGCACACTGCGTCTG-3' and reverse, 5'-GTAACGT GGCTTCTTCTGCCA-3' [11]. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 statistical software (SPSS, Inc., Chicago, IL, USA). All data were presented as mean ± standard deviation (SD). The associations between protein expression and clinicopathological factors were assessed using the χ^2 test. The mRNA level of *PDGFRβ* between tumor and normal groups was compared by Student's t test. The association between *PDGFRβ* and overall survival was examined using the Kaplan-Meier method and log-rank test. Multivariate analysis was performed to identify the independent risk factor for oral cancer. *P* values less than 0.05 were regarded as statistically significant.

Results

The expression of PDGFRβ was increased in oral cancer

To test role of *PDGFRβ* in the progression of oral cancer, we measured the expression levels of *PDGFRβ* in a total 156 oral cancer tissues and adjacent normal tissues specimens. As shown in Fig. 1, the mRNA expression level of *PDGFRβ* was significantly higher in oral cancer tissues compared with adjacent normal tissues ($P < 0.001$).

Relationship between PDGFRβ and clinicopathological characteristics of oral cancer

The correlation between *PDGFRβ* and clinicopathological features was analyzed via chi-square test. To explore whether *PDGFRβ* was involved in the development of oral cancer, oral cancer samples were classified into the low expression group (n = 75) and high expression group (n = 81) according to the median expression level of all the samples. As shown in Table 1, the expression of *PDGFRβ* was positively associated with clinical stage ($P = 0.036$), T stage ($P = 0.010$), lymph node metastasis ($P = 0.013$) and histological grade ($P = 0.037$). However, there was no significant association was observed between *PDGFRβ* expression and other parameters including age, gender and differentiation ($P > 0.05$).

Association of PDGFRβ expression with prognosis in oral cancer patients

To investigate the prognostic role of *PDGFRβ* in oral cancer, we performed a 5 years' follow-up. As shown in Fig. 2, our results suggested that patients with high expression of *PDGFRβ* had a shorter overall survival than those with low expression (log rank test, $P = 0.000$). The multivariate analysis using the cox regression analysis adjusted for all variables was performed which manifested that the high expression of *PDGFRβ* (Table 2, HR = 2.467, 95% CI = 1.340–4.544, $P = 0.004$) was an important factor for predicting poor outcome and it might be an independent prognostic bio-marker.

Table 2
Multivariate analysis adjusted for clinical variables for the prognostic value of *PDGFRβ* in oral cancer patients

Variable	HR	95% CI	*P* value
PDGFRβ expression High vs. low	2.467	1.340–4.544	0.004
Lymph node metastasis Positive vs. negative	1.859	1.095–3.156	0.022
Differentiation Poor vs. moderate + well	1.813	1.067–3.082	0.028

Discussion

The occurrence of oral cancer is related to many factors, such as smoking, alcohol use, smokeless tobacco products and HPV infections [12, 13]. The 5-year survival rate of patients with early stage oral cancer ranges from 69–82%, but the 5-year overall survival rate of patients with nodal metastases remain unsatisfactory when compared to the early stage patients [14–16]. The accurate prognostic bio-markers are meaningful for the prediction of the prognosis for oral cancer. Therefore, finding effective prognostic factors is crucial to improve the prognosis and therapy of oral cancer patients.

Recently, many researchers have been devoted to examine specific biological markers with prognosis in head and neck cancer, including oral cancer [17–19]. RUMI YOSHIHAMA et al. indicated that SOX2, KLF4 and brachyury serve important roles in tumor progression, and these transcription factors may thus represent clinically useful prognostic markers for OSCC [20]. Acidic leucine-rich nuclear phosphoprotein-32A (ANP32A) was found commonly increased in OSCC and ANP32A protein could act as a potential biomarker for prognosis assessment of oral cancer patients with lymph node metastasis [21].

In this study, we detected the expression of *PDGFRβ* at mRNA level using qRT-PCR analyses. And *PDGFRβ* was found to be up-regulated in oral cancer tissues compared to that in adjacent non-cancerous tissues which indicated that it might be a tumor oncogene in oral cancer. Then we further explored the role of *PDGFRβ* in the development of oral cancer via investigating the relationship between its expression and

clinical factors. The high expression of *PDGFRβ* was involved in the progression of oral cancer due to its tightly correlation with clinical stage, T stage, lymph node metastasis and histological grade.

PDGFRβ was found high expressed and involved in cancer progression in many types of cancer [22–25]. GONG et al. studied the fusion gene PDGFRβ-CEV14 in myelodys-plastic and myeloproliferative neoplasms using the methods of FISH and RT-PCR and proven to be of significant value in improving diagnosis, guiding treatment and increasing the cure rate of MDS/MPN patients [11]. Anne S. Tsao et al. evaluated the incidence of PDGFRB gene copy number gain (CNG) by fluorescence in situ hybridization (FISH) and found PDGFRB CNG > 40% of MPM tumor cells is a potential prognostic biomarker for surgery and may identify a unique population of mesothelioma patients [26]. Sayaka Yuzawa et al. suggested the prognostic potential of cancer stroma via PDGF-B signaling and indicated *PDGFRβ* expression as a reliable prognostic marker and a possible therapeutic target in tumor stroma of pancreatic adenocarcinoma. Accumulated studies had verified *PDGFRβ* as bio-marker in the prognosis of cancers. So we subsequently evaluated the prognostic value of *PDGFRβ* in oral cancer.

Kaplan-Meier analysis with the data from follow-up showed that patients with a high *PDGFRβ* expression had worse overall survival compared to patients with low expression, which suggested that *PDGFRβ* expression was related to the prognosis of patients with oral cancer. According to cox regression analysis, the result showed high *PDGFRβ* expression was an independent and significant prognostic factor for oral cancer.

Conclusion

In conclusion, *PDGFRβ* expression is increased in oral cancer patients and its expression is influenced by some clinical factors. The present evidence provides support for *PDGFRβ* to be a potential prognostic marker. However, it still remains to be further warrant its prognostic utility.

Declarations:

Declarations

Ethics approval and consent to participate: This study was supported by the Ethics Committee of Chinese PLA General Hospital, Beijing and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication: The patients provided written informed consent for the publication of any associated data and accompanying images

Availability of data and materials: All data generated or analysed during this study are included in this article.

Competing interests: The authors declare that they have no competing interests.

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Figures

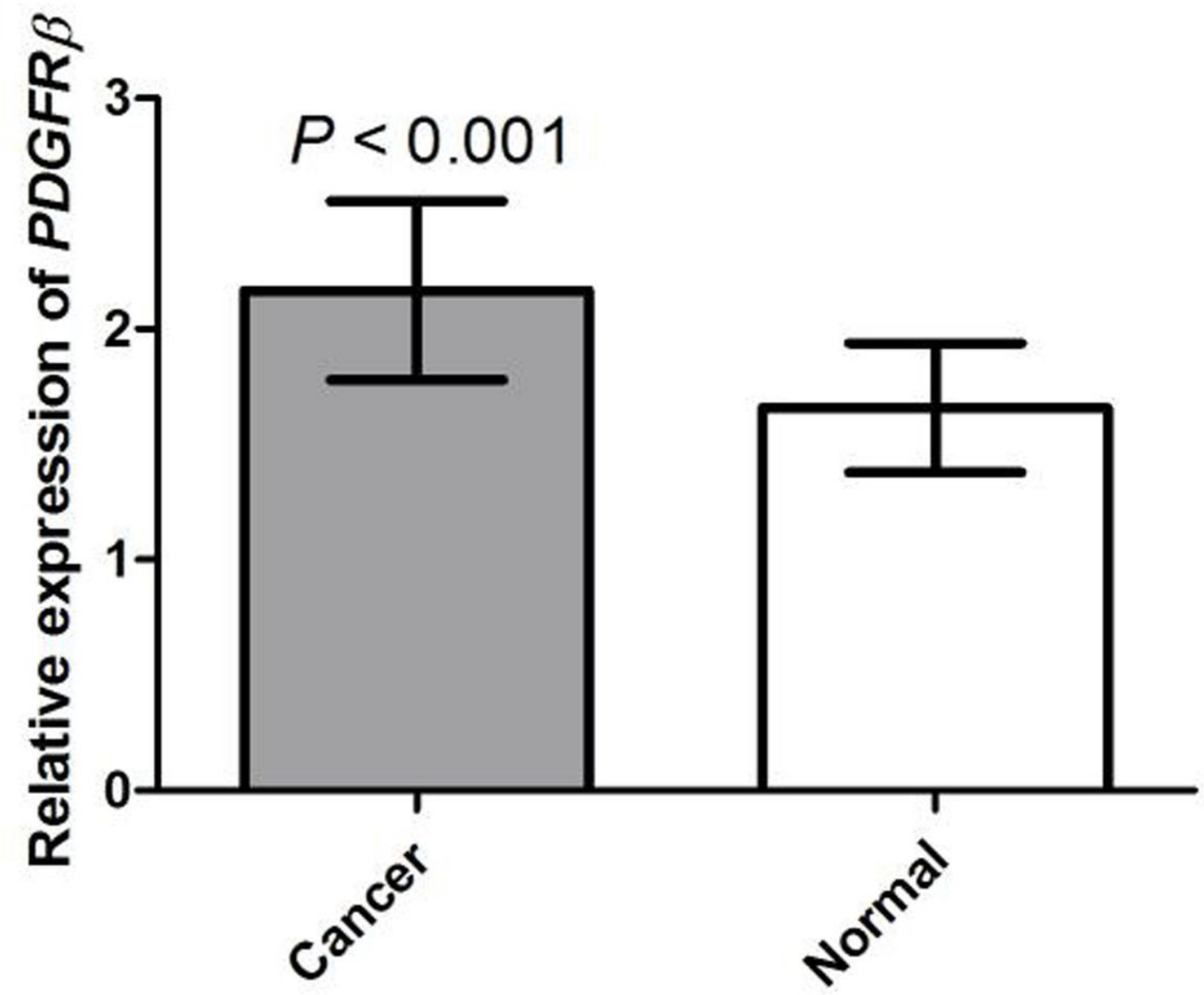

Figure 1

The mRNA expression of PDGFRβ in oral cancer tissues and normal tissue. The expression level of PDGFRβ in oral cancer tissues was higher than in the adjacent normal specimens (P < 0.001).

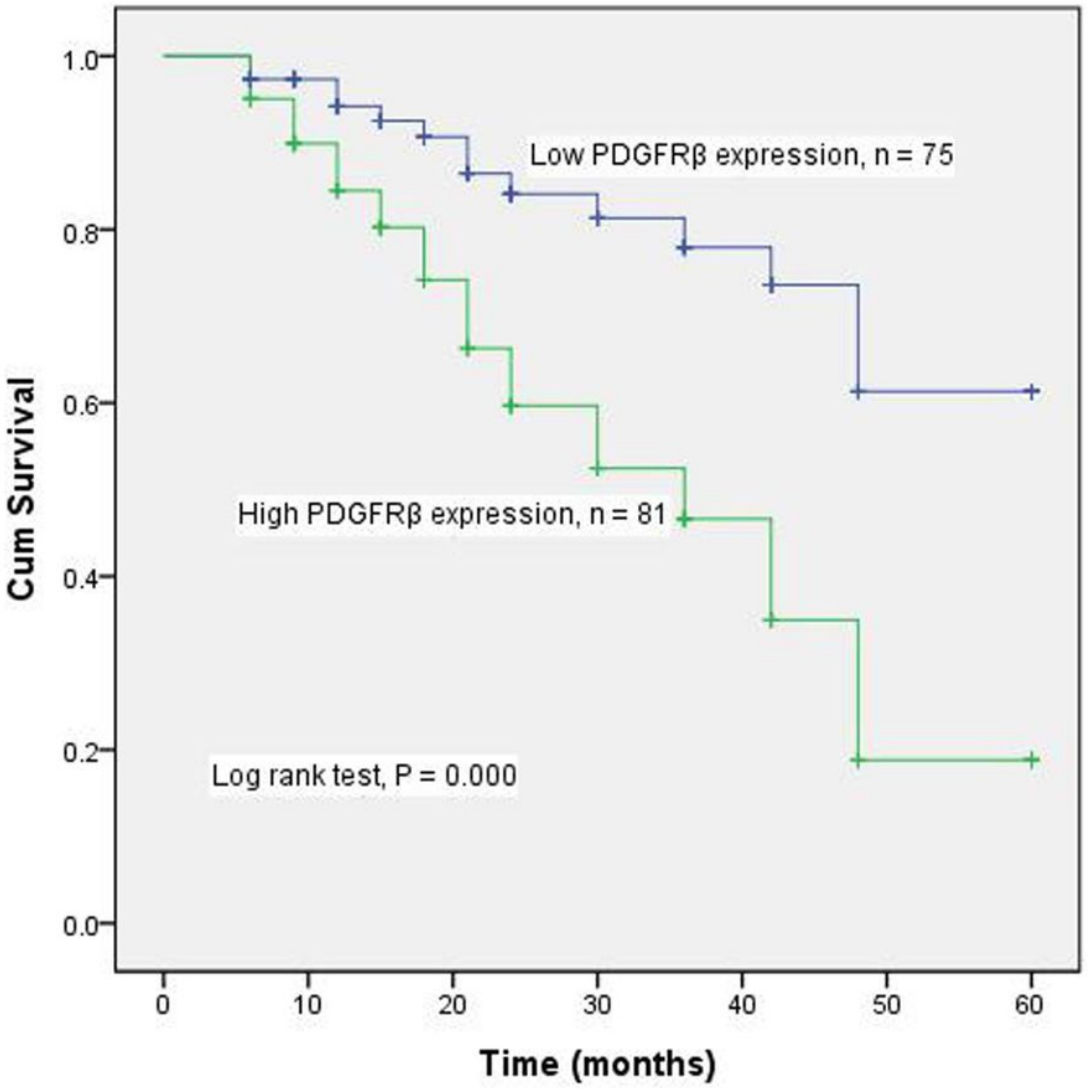

Figure 2

Kaplan-Meier analysis for the overall survival of patients with oral cancer. Patients with high PDGFRβ expression had worse overall survival than those low expression (log rank test, P = 0.000)