Reduced expression of polymeric immunoglobulin receptor (*pIgR*) in nasopharyngeal carcinoma and its correlation with prognosis

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Abstract Polymeric immunoglobulin receptor (*pIgR*) is a key component of the mucosal immune system that mediates epithelial transcytosis of immunoglobulins. The expression of *pIgR* was reported to be up-regulated and related to the prognosis of several human cancers. However, the clinical significance of *pIgR* in nasopharyngeal carcinoma (NPC) remains unclear. The purpose of this study was to detect the *pIgR* expression and its prognostic value in NPC. The expression of serum *pIgR* was measured in NPC patients and healthy controls by real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and western blotting analyses. The relationship between its expression and clinical factors was analyzed by chi-square test. Then, the overall survival of patients was assessed by Kaplan-Meier analysis while the prognostic value of serum *pIgR* was estimated using univariate and multivariate analyses with cox regression analysis. Serum *pIgR* was down-regulated in NPC patients compared to that in healthy controls both at messenger RNA (mRNA) and protein levels. Especially, its expression was also significantly lower in patients at advantage stages (III–IV) than those at early stages (I–II). And, the low *pIgR* expression was strongly associated with advanced clinical stages, T stage, N stage, and distant metastasis. Kaplan-Meier analysis demonstrated that patients with low *pIgR* expression had a significantly shorter overall survival than those with high expression at

any stages. Cox regression analysis suggested that *pIgR* was closely related to the prognosis of NPC. Serum *pIgR* expression was reduced in NPC, and it could be an independent prognostic predictor for patients with this cancer.

Keywords Nasopharyngeal carcinoma · Polymeric immunoglobulin receptor · Prognosis

Introduction

Nasopharyngeal carcinoma (NPC) is a rare malignant tumor in the word, and its average incidence is less than 1/105 population worldwide [1]. However, it is considered to be an endemic carcinoma in southern China [2, 3]. The pathogenesis of NPC may be related to Epstein-Barr virus (EBV) infection, genetic or environmental factors [4–6]. As it is difficult to detect because of lack of clinical manifestations and the hidden location at the early stages, the majority of NPCs are diagnosed with lymph node metastasis which is the major cause of death in patients with NPC [7, 8]. Although the treatments of NPC have great achievements, approximately 20 % of patients will suffer from recurrence which is the leading cause of treatment failures, and lead to the 5-year overall survival rate remains 50–60 % [9, 10]. Therefore, it is necessary to detect a new bio-marker that could predict the prognosis of NPC patients.

Polymeric immunoglobulin receptor (*pIgR*), a member of the immunoglobulin superfamily, is expressed in mucosal surfaces and can mediate polymeric IgA (pIgA) transcytosis from the basolateral pole to the apical surface of epithelial cells [11, 12]. Due to proinflammatory cytokines, responding to viral or bacterial infection, thus linking innate and adaptive immunity as well as leading to *pIgR*, it is often overexpressed

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[13–16]. In previous studies, *pIgR* had been confirmed to be up-regulated in various cancers such as colon cancer, epithelial ovarian cancer, esophageal and gastric adenocarcinoma, and hepatocellular carcinoma while it was down-regulated in lung cancer, pancreatic and periampullary adenocarcinoma, and NPC [11, 17–23]. However, its clinical significance in NPC was never clarified so far.

In this study, we detected the expression of *pIgR* and analyzed its relationship with clinical factors. We also estimated the prognostic value of *pIgR* via analyzing its influence on overall survival of patients with Kaplan-Meier analysis as well as cox regression analysis. To our knowledge, this was the first time for the evaluation of the prognostic performance of *pIgR* in human NPC.

Materials and methods

Patient samples

Blood samples were collected from 126 patients (including 61 at early stages (I–II) and 65 at advanced stages (III–IV) according to the tumor-node-metastasis (TNM) classification of the sixth American Joint Committee on Cancer [24]) who were newly histologically diagnosed with NPC at the The Affiliated Hospital of Weifang Medical College. Forty healthy blood donors in the hospital were selected as healthy controls. Blood samples of healthy controls with empty stomach were also obtained. Then, all the blood samples were centrifuged for 10 min at 3000 rpm within 1 h and stored at −80 °C till use. The subjects with cancer history were excluded. No NPC patients had received any form of specific therapy before the study. All the patients received radiotherapy treatment in this study. Clinical information is summarized in Table 1, and the complete follow-up information was obtained by a telephone to the patients or their relatives. Patients who were died from unexpected events or other diseases were excluded from our study.

The study protocol was approved and supervised by the Ethics Committee of The Affiliated Hospital of Weifang Medical College, and written informed consent was obtained from each participant.

RNA extraction and qRT-PCR analysis

Total RNA from serum samples was extracted using the TRIzol LS Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol, respectively. Then, the first chain of cDNA was synthesized by reverse transcription with a kit (TaKaRa, Japan). RT-PCR was conducted in the 7300 Real-Time PCR System (Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as endogenous control. The primer sequences were as

Table 1 Correlation between *pIgR* expression and clinicopathological characteristics of NPC patients

Characteristic	Cases ($n = 126$)	*pIgR* expression		*P* value
		High ($N = 60$)	Low ($N = 66$)	
Gender				0.388
Male	54	27	27	
Female	72	33	39	
Age (years)				0.235
≥50	64	33	31	
<50	62	27	35	
Clinical stage				0.001
Early (I–II)	61	38	23	
Advanced (III–IV)	65	22	43	
T stage				0.007
T_1–T_2	73	42	31	
T_3–T_4	53	18	35	
N stage				0.001
N_0–N_1	72	23	49	
N_2–N_3	54	37	17	
Distant metastasis				0.036
Yes	47	17	30	
No	79	43	36	
Smoking				0.497
Yes	62	29	33	
No	64	31	33	

follows: *pIgR*: forward-5′-CTCTCTGGAGGACCACCGT-3′, reverse-5′-CAGCCGTGACATTCCC- TG-3′; GAPDH: forward-5′-CTCCTCCTGTTCGACAGTCAGC-3′, reverse-5′-CC- CAATACGACCAAATCCGTT-3′. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative messenger RNA (mRNA) expression of *pIgR*. Each sample was in triplicate.

Western blotting

The total protein was extracted from the serum of patients with NPC and healthy controls using TPER Protein Extraction Reagent (Pierce, Rockford), respectively. Then, the protein was separated by SDS-PAGE gel and the brands were transferred onto polyvinylidene fluoride membranes (PVDF). After blocking with 5 % non-fat milk (Bio-Rad), the membrane was incubated with anti-*pIg*R antibody (1:200) at 4 °C overnight. Secondary antibody HRP-conjugated anti-rabbit IgG antibody was then added and incubated with membranes. The immunoreactive signal of antibody-antigen pairs was visualized using the Chemiluminescence Plus Western Blot analysis kit (Santa Cruz Biotechnology). β-Actin was used as internal control. Experiments were performed at least three times.

Statistical analysis

All statistical analyses were performed using SPSS 13.0 software (Chicago, USA), and the figures were designed by GraphPad prism 5. The continuous data were presented as the mean ± SD. Student's *t* test was used to identify the differences between NPC patients and healthy controls or early stages and advanced stages in *pIgR mRNA* and protein expression. The relationship between *pIgR* expression and clinical factors was analyzed via chi-square test. Kaplan-Meier analysis with the log-rank test was used for estimating the overall survival of patients with NPC. Univariate and multivariate analyses were performed to evaluate the prognostic value of *pIgR* with cox regression analysis. The difference was considered to be statistically significant when $P < 0.05$.

Results

The expression of *pIgR* was down-regulated in patients with NPC

The mRNA expression of serum *pIgR* was detected by qRT-PCR assay in 126 NPC patients and 40 healthy controls. As shown in Fig. 1a, the *pIgR* mRNA level in NPC serum was significantly lower than that in healthy controls (2.04 ± 0.79 vs 4.28 ± 1.08, $P < 0.001$). Additionally, in contrast with the *pIgR* expression at early stages, *pIgR* expression at advanced

stages of NPC was significantly decreased (1.77 ± 0.80 vs 2.46 ± 0.56, $P < 0.001$). Meanwhile, the protein expression of *pIgR* was measured with western blotting analysis. And, the result showed that pIgR protein was also decreased in patients with NPC compared to that in healthy controls (Fig. 1b, $P < 0.001$).

Relationship between *pIgR* expression and clinical features of patients with NPC

To further investigate whether *pIgR* was involved in the development of NPC, the relationship between its expression and clinicopathological features was assessed and is summarized in Table 1. One hundred twenty-six patients were divided into two groups according to the median expression of *pIgR*. Sixty patients were attributed into high *pIgR* expression group while the others were low *pIgR* expression group. The outcome demonstrated that the down-regulation of *pIgR* was closely associated with clinical stages ($P = 0.001$), T stage ($P = 0.007$), N stage ($P = 0.001$), and distant metastasis ($P = 0.036$). However, there was no significant association between *pIgR* expression and age, gender, and smoking ($P > 0.05$).

The prognostic value of *pIgR* in patients with NPC

To assess the prognostic value of pIgR in NPC patients, a 5-year follow-up was performed. During the follow-up, the

Fig. 1 Relative expression of *pIgR* in patients with NPC and healthy controls. The relative mRNA expression (**a**) and protein expression (**b**) of *pIgR* in serum of patients with NPC were both significantly lower than that in healthy controls ($P < 0.001$)
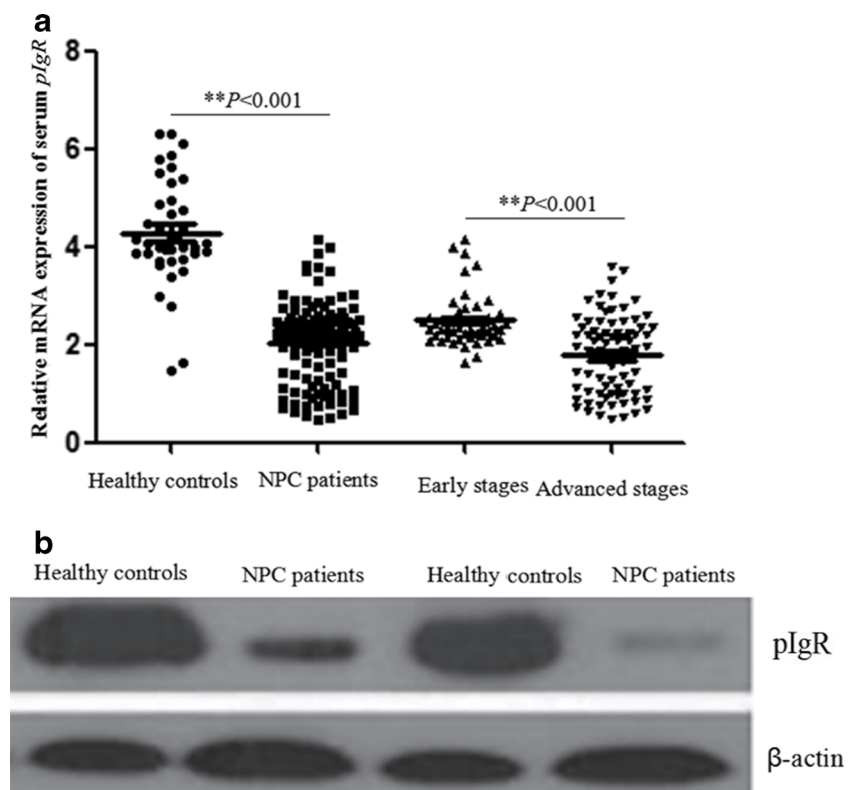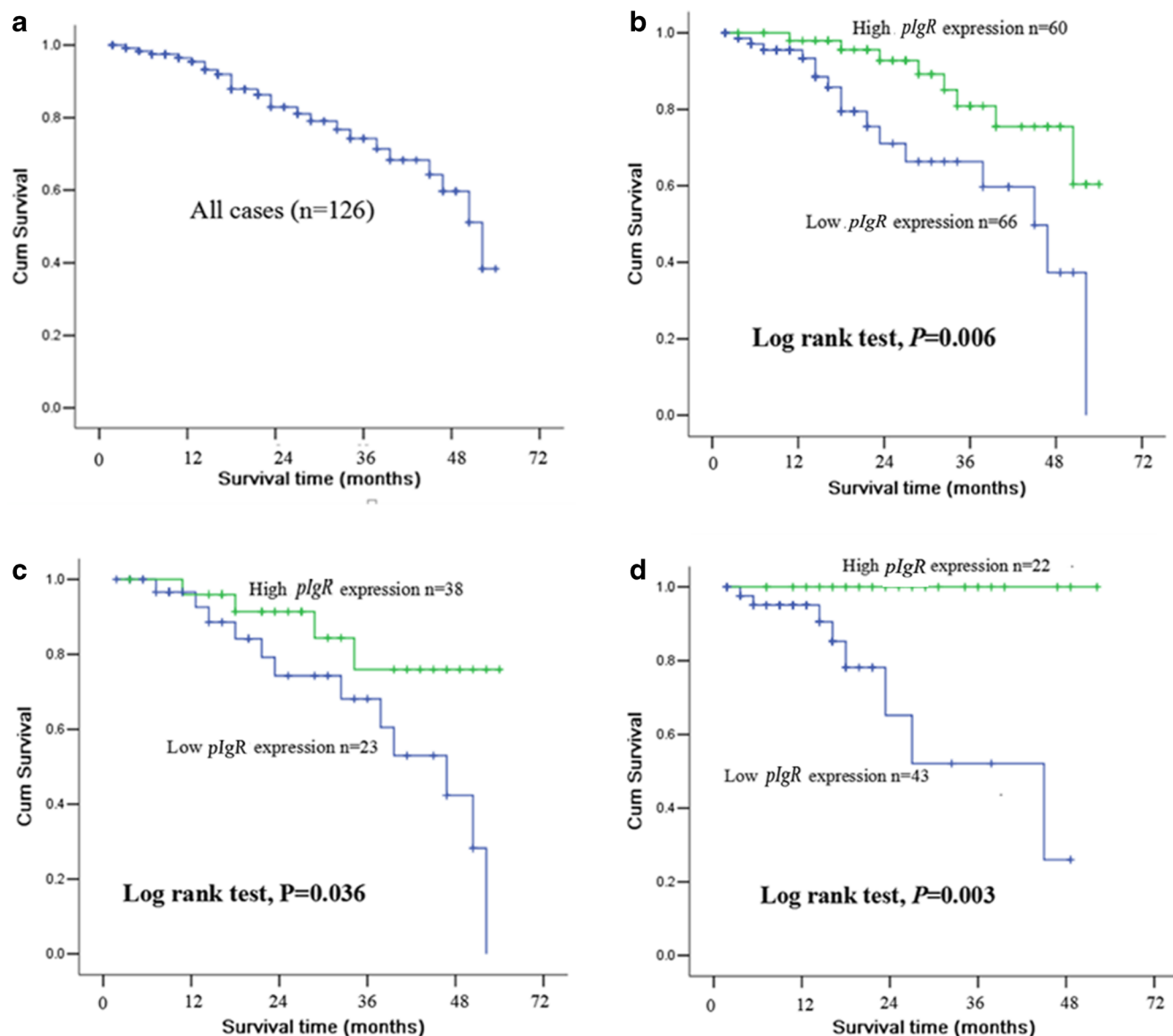
Fig. 2 Kaplan-Meier analysis for the overall survival of patients with NPC according to the expression of *pIgR*. The 5-year overall survival rate was 74.2 % in 126 NPC patients (**a**). Patients with low *pIgR* expression had a worse overall survival than those with high expression (log-rank test, $P = 0.006$, **b**). Patients with high *pIgR* expression had better overall survival compared with those with low *pIgR* expression at both early stages (I–II, **c**) and advanced stages (III–IV, **d**)

Table 2 Univariate and multivariable analyses adjusted for clinical factors for estimating the prognostic value of *pIgR* in NPC

Variables	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Gender (female vs male)	1.494 (0.667–3.348)	0.329	–	–
Age (>50 vs ≤50)	1.110 (0.492–2.504)	0.802	–	–
Clinical stage (advanced vs early)	2.533 (1.114–5.760)	0.027	2.450 (1.008–5.954)	0.048
T stage (T_3, T_4 vs T_1, T_2)	2.494 (1.060–5.868)	0.036	3.014 (1.202–7.558)	0.019
N stage (N_2, N_3 vs N_0, N_1)	0.844 (0.385–2.033)	0.772	–	–
Distant metastasis (present vs absent)	0.808 (0.355–1.840)	0.612	–	–
Smoking (yes vs no)	0.655 (0.290–1.479)	0.309	–	–
pIgR expression (low vs high)	3.145 (1.331–7.434)	0.009	4.886 (1.876–12.724)	0.001

5-year overall survival (OS) rate of 126 NPC patients was 74.2 % (Fig. 2a). Kaplan-Meier analysis with log-rank test manifested that patients with low expression of *pIgR* had a significantly shorter OS than those with high expression of *pIgR* expression group (log-rank test, *P* = 0.006, Fig. 2b). In view of the difference of *pIgR* expression in different clinical stages, we further assessed the OS patients at early and advanced stages. The results showed that patients with low *pIgR* expression had worse OS not only at early stages (I, II; log-rank test: *P* = 0.036; Fig. 2c) but also at advanced stages (III, IV; log-rank test: *P* = 0.003; Fig. 2d) compared to those with high expression of *pIgR*. These indicated that *pIgR* was related to the prognosis of NPC.

Univariate and multivariate analyses adjusted for clinical factors were further carried out to estimate the prognostic value of *pIgR* with cox regression analysis (Table 2). In univariate analysis, clinical stages, T stage, and *pIgR* expression turned out to be prognostic indicators of NPC (HR = 2.533, 95 % CI 1.114–5.760, *P* = 0.027; HR = 2.494, 95 % CI 1.060–5.868, *P* = 0.036; HR = 3.145, 95 % CI 1.331–7.434, *P* = 0.009). The multivariate analysis exhibited that *pIgR* expression (HR = 4.886, 95 % CI 1.876–12.724, *P* = 0.001), clinical stages (HR = 2.450, 95 % CI 1.008–5.954, *P* = 0.048), and T stage (HR = 3.014, 95 % CI 1.202–7.558, *P* = 0.019) were correlated with the prognosis of patients with NPC and they might be independent prognostic bio-markers for NPC patients.

Discussion

To date, the NPC is mostly prevalent with a high incidence and still remains unsatisfactory prognosis. Therefore, it is necessary to identify potential prognostic bio-markers and expected to provide a new therapy target for NPC patients.

During the past several decades, a lot of molecule bio-markers for the prognosis of NPC had been confirmed. For instance, acylglycerol kinase was increased in patients with NPC and could act as a potential prognostic marker [25]. Ooft et al. found that the overexpression of EGFR could predict a poor prognosis of NPC [26]. You et al. considered that the up-regulated ADAM10 was not only a promoter for the progression and migration but also a potential therapeutic target for the treatment of NPC [27]. The overexpression of long non-coding RNA AFAP1-AS1 was also proved to be related to the prognosis of NPC [28]. To detect the expression of interleukin-35 and relative survival analyses, Zhang et al. had confirmed the prognostic value of it in NPC [29]. Nevertheless, the prognostic role of *pIgR* in NPC was never reported.

PIgR is a transporter of dimeric IgA (dIgA) and pentameric IgM which are the first-line antibodies produced in response to infection. It has been reported to be associated with many

cancers. Jonna et al. found that *pIgR* was overexpressed and related to the prognosis of epithelial ovarian cancer [18]. Wang et al. detected the expression of *pIgR* in osteosarcoma and confirmed that it was an important prognostic marker in this cancer [30]. *pIgR* was positively expressed and could predict the prognosis of patients with glioma [31]. On the contrast, *pIgR* was considered to be down-regulated in some other cancers [11, 21]. In this study, we detected the expression of *pIgR* both at mRNA and protein levels. The outcome showed that serum *pIgR* was decreased in NPC patients compared to that in healthy controls which revealed that *pIgR* might be a tumor suppressor in NPC. This was consistent with previous studies.

Subsequently, we explored the association between serum *pIgR* expression and clinical factors of patients with NPC. As a result, we found that its expression was significantly influenced by some clinical factors which suggested that *pIgR* was involved in the development of NPC. Based on the result above, we further investigated the prognostic value of serum *pIgR* in NPC. According to the Kaplan-Meier analysis which demonstrated that the expression of *pIgR* was related to the OS of patients, we concluded that *pIgR* was correlated with the prognosis of NPC. Then, cox regression analysis proved our view and confirmed that *pIgR* could be a potential and independent prognostic indicator for patients with NPC.

In conclusion, our results prove that *pIgR* is down-regulated in NPC and it is linked with the development and progression of this cancer. Besides, it has a high prognostic value in predicting the clinical outcome of NPC.

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