Original Paper

Role of the TGFβ/PDCD4/AP-1 Signaling Pathway in Nasopharyngeal Carcinoma and Its Relationship to Prognosis

Jie Ma^a Shu-Hong Xuan^b Yan Li^a Zhi-Ping Zhang^c Xin-Hua Li^d

^aDepartment of Oncology, Jining No. 1 People's Hospital, Jining, ^bDepartment of Oncology, Jining No. 1 People's Hospital, Jining, ^cDepartment of Thyroid Breast Surgery, Jining No. 1 People's Hospital, Jining, ^dInternal Medicine Department, Jining No. 1 People's Hospital, Jining, P.R. China

Key Words

Nasopharyngeal carcinoma • TGFβ/PDCD4/AP-1 signaling pathway • Transforming growth factor-β receptor I • Programmed cell death 4 • Activator protein-1 • Prognosis

ABSTRACT

Background: The objective of the present study was to evaluate the role of the TGFβ/PDCD4/AP-1 pathway in nasopharyngeal carcinoma (NPC) and its relationship to NPC prognosis. **Methods:** NPC tissues collected from 66 NPC patients were compared to 17 nasopharyngeal mucosa biopsy specimen collected from normal tissues. Immunohistochemical staining was performed to assess expression of transforming growth factor-β receptor I (TGFβRI), programmed cell death 4 (PDCD4) and activator protein-1 (AP-1). The Kaplan-Meier method was applied to evaluate NPC patient overall survival (OS) and progression-free-survival (PFS). Cox regression analysis was used to estimate independent prognostic factors for NPC. The human NPC cell line CNE2 was selected and treated with SB431542, an inhibitor of TGFβRI; expression of TGFβRI and PDCD4 in CNE2 cells was determined by western blotting. NPC tissues showed high expression of TGFβRI and AP-1 but lower expression of PDCD4 than normal tissues (all $P < 0.05$). **Results:** The results of Kaplan-Meier analysis showed that TGFβRI-positive patients and AP-1-positive patients had shorter OS and PFS than TGFβRI-negative patients and AP-1-negative patients; additionally, PDCD4-positive patients had higher OS and PFS than PDCD4-negative patients. Cox regression analysis revealed that advanced tumor stage, overexpression of TGFβRI and AP-1, and low expression of PDCD4 were unfavorable factors influencing OS and PFS in NPC patients. Compared with the control group, expression of TGFβRI decreased and that of PDCD4 increased significantly in CNE2 cells treated with the inhibitor (all $P < 0.05$). These findings indicate that the TGFβ/PDCD4/AP-1 pathway may be associated with NPC development and progression. **Conclusion:** High expression of TGFβRI and AP-1 and low expression of PDCD4 may be unfavorable prognostic factors for NPC.

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J. Ma and S.-H. Xuan contributed equally to this work

Xin-Hua Li

Internal Medicine Department, Jining No. 1 People's Hospital
No. 6, Jiankang Road, Jining, Shandong Province, (P.R. China)
Tel. +86-0537-2253145, E-Mail majie2316@163.com

Introduction

According to the latest global cancer statistics, the highest incidence of nasopharyngeal carcinoma (NPC) occurs in China [1], where there were 41,503 newly diagnosed NPC cases in 2010 alone, comprising 1.34% of the total cancer cases nationwide that year [2]. As a disease that arises from the epithelial lining of the nasopharynx, NPC has a multifactorial etiology that includes genetic factors as well as smoking and alcohol consumption [3, 4]. Radiotherapy has become the standard radical treatment for patients with NPC, with reported long-term effects [5]. For example, NPC patients treated with radiation therapy and adjuvant chemotherapy have an approximate 70% 5-year overall survival (OS) rate [6]. However, treating these patients with radiotherapy is often difficult because some tumors are located adjacent to important organs such as the brain stem, optic nerve, cranial nerves and spinal cord [7]. As microarray technology has become increasingly used to identify differentially expressed genes in NPC, various associations between NPC pathogenesis and gene function have been discovered [8].

Transforming growth factor- β (TGF β), which belongs to a superfamily of structurally and functionally related cytokines, regulates a broad array of cellular responses, including proliferation, migration, apoptosis and differentiation [9]. TGF β is produced in a latent form (L-TGF β) and is then activated by a variety of factors, including matrix metalloproteases, plasmin protease, hypoxia and thrombospondin [11]. TGF β plays a dual part in cancers: it inhibits the proliferation of early-stage cancer cells while promotes the progression and metastasis of late-stage cancer cells [12]. Deficiency in anti-proliferative responsiveness to TGF β is a feature of multiple tumor types, suggesting that TGF β and its associated components have crucial roles as tumor suppressors [13, 14]. TGF β plays a role in NPC carcinogenesis by regulating survival and apoptosis in NPC cells [14–16]. Activator protein-1 (AP-1), a dimer typically formed by combination of structurally and functionally related members of the Jun protein family (c-Jun, JunB and JunD) and Fos protein family (c-Fos, FosB, Fra-1 and Fra-2) [17], is a transcription factor with important roles in many biological processes, including cell proliferation, survival, differentiation, apoptosis and stress responses [18]. It has been reported that Epstein-Barr nuclear antigen 1 (EBNA1), c-Jun and activating transcription factor 2 (ATF2) improve the activity of AP-1 as well as other transcription factors in NPC cells and that therapies targeting AP-1 may be a viable treatment option for NPC [19]. Another important tumor suppressor, programmed cell death 4 (PDCD4), which was found to inhibit neoplastic transformation in JB6 mouse epidermal cell line [20], has been proven to modulate transcription and translation of multiple genes [21]. MicroRNA-374a (miR-374a) targets PDCD4 and promotes the proliferation, migration, and invasion of NPC cells, indicating that PDCD4 may be a new target for NPC treatment [22]. Based on the above findings, we performed the current study to determine the role of the TGF β /PDCD4/AP-1 signaling pathway in NPC and its association with the prognosis of NPC.

Materials and Methods

Ethics statement

This research was carried out in strict accordance with the protocols established by the Ethics Committee of Jining No. 1 People's Hospital. All experimental procedures were performed in accordance with the Declaration of Helsinki [23]. Informed consent was obtained from all subjects participating in this study or their families, and the study was approved by the local institutional review board.

Subjects

A total of 66 NPC patients admitted to Jining No. 1 People's Hospital between 2007 and 2011 were enrolled. The cohort included 52 males and 14 females ranging in age from 25–73 years (median age, 49 years). Before treatment, tumor tissues were collected by needle biopsy, fixed in 10% regular formalin, embedded in paraffin, and then serially sectioned (4 μ m/section). At diagnosis, the patients were classified

according to the tumor-node-metastasis (TMN) classification system published by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) in 2003 [24] (9 patients were stage I, 14 were stage II, 28 were stage III, and 15 were stage IV; 10 were Tx-T1, 17 were T2, 24 were T3, and 15 were T4; 8 were T0, 17 were N1, 12 were N2, and 29 were N3; 66 were M0). No patient showed distant metastasis, and the diagnosis of non-keratinizing squamous cell carcinoma was according to the well-experienced pathologists using complete pathological data. The exclusion criteria were as follows: NPC patients with other tumors and NPC patients with serious diseases affecting other organ systems, including cardiovascular and cerebrovascular diseases, diabetes, uremia, severe hepatic and renal dysfunction, myasthenia gravis and malnutrition. As controls, 17 biopsy specimens of nasopharyngeal mucosa were selected from the ear-nose-throat department.

Immunohistochemical staining

Immunohistochemical staining was performed using a commercial kit according to the manufacturer's instructions (Zymed Company). The paraffin sections were dewaxed with xylene, passed through an alcohol gradient and then immersed in 3% H₂O₂ for 5 min to remove endogenous peroxidase. After high-temperature antigen retrieval in ethylene diamine tetraacetic acid (EDTA), after cooling, the sections were rinsed with phosphate-buffered saline (PBS, 5 min × 3 times) and incubated with normal goat serum (room temperature, 30 min) to block non-specific antigens. Antibodies against TGFβRI (No. BA0294, BOSTER Biological Technology Co., Ltd., Wuhan, China), PDCD4 (No. MT1015, MT-Biotechnology Co., Ltd., Shanghai, China) and c-Jun (1:50; No. A02038; BOSTER Biological Technology Co., Ltd., Wuhan, China) were diluted and incubated with the sections (first at 4°C overnight and then at room temperature for 30 min). Following three rinses with PBS, biotin-labeled secondary antibodies were added; the sections were then incubated at 37°C for 20 min, rinsed again three times with PBS, and stained with diaminobenzidine (DAB). The staining reaction was halted with tap water until the appearance of a clear positive result under the microscope. Subsequently, the sections were counterstained with hematoxylin and then washed with running water, followed by baking (60°C, 1 h) and sealing with resinene. A microscope was used for observation. Staining was performed using the method proposed by Masunaga et al [25]. Each section was observed in 5 randomly selected, high-power fields, and each field contained 100 cells. The sections were scored based on the proportion of positive cells as follows: < 5% positive cells, 0 points; 5 – 25% positive cells, 1 point; 26 – 50% positive cells, 2 points; > 50% positive cells, 3 points. The color intensity of the stained cells was also scored, as follows: colorless, 0 points; light yellow, 1 point; yellow or brownish yellow, 2 points; brown, 3 points. The score for each section was calculated as the sum of the two scores. High expression (positive expression, +) referred to a sum 3 points, whereas low expression (negative expression, -) referred to a sum < 3 points.

Follow-up

After the end of treatment, 66 patients were randomly followed up through outpatient services, hospitalization and telephone contacts, with a duration varying from 6 to 60 months (median, 42 months). During the follow-up period, 20 patients experienced local recurrence, and 23 experienced distant metastasis. The prognosis was evaluated according to OS and progression-free-survival (PFS). PFS was defined as the duration from the day treatment ended to the day tumor progression or death occurred. OS referred to the duration from the day treatment ended to the day death occurred.

Cell culture

The human NPC cell line CNE2 (No. ZY-H362, Shanghai Ze Ye Biological Technology Co., Ltd, Shanghai, China) was routinely cultured in 6-well plates in a 95% air/5% CO₂ incubator at 37°C using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). When the cells reached the logarithmic growth phase, the medium was replaced with serum-free DMEM. The cells were then assigned to either a control group (no treatment) or an inhibitor group (treated with 50 nM SB431542, a TGFβRI inhibitor; No. HPBIO181007, Hepeng Biological Technology Co., Ltd, Shanghai, China). After 24 hours, the cells were collected at 4°C, and total protein was extracted.

Western blotting

Cells were collected and mixed with 100 μl radio-immunoprecipitation assay (RIPA) lysate (No. R0020, Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) with 1 mmol/l phenylmethylsulfonyl fluoride

(PMSF). The cells were homogenized at 3000 r/min until sufficiently lysed and placed on ice for 30 min, after which they were centrifuged at 12,000 $\times$ g for 4 min. The supernatant was collected and stored at -80°C. The protein concentration was assessed using a commercial bicinchoninic acid (BCA) kit according to the manufacturer's instructions (BOSTER Biological Technology Co., Ltd., Wuhan, China), and the concentration of each sample was adjusted to 3 μ g/ μ l. The extracted protein was mixed with sample buffer and boiled at 100°C for 10 min, and 30 μ g was separated by 10% polyacrylamide gel electrophoresis (PAGE). The proteins were then transferred onto polyvinylidene fluoride (PVDF) membranes (No. P2438, Sigma-Aldrich, St. Louis, MO, USA) using a semi-dry electroblotting technique, and the membranes were incubated with 5% bovine serum albumin (BSA) at room temperature for 1 h. Anti-rabbit primary antibodies against TGF β RI (1:5000; ab180619, Abcam, Inc., MA, USA), PDCD4 (1:2000, ab51495, Abcam, Inc., MA, USA) or AP-1 (1:1000; ab32137, Abcam, Inc., MA, USA) were added, and the membranes were incubated at 4°C overnight. The membranes were rinsed in Tris-buffered saline (TBS)/Tween-20 (TBST) 3 times for 5 min. The corresponding goat anti-rabbit secondary antibody (ab150077, Abcam, Inc., MA, USA) was added and incubated for 1 h, after which the membranes were again washed 3 times for 5 min each. A fluorescent reagent (No. NCI 5080, Yanhui Biology Ltd., Shanghai, China) was applied to develop the protein bands. Glyceraldehyde-phosphate dehydrogenase (GADPH) was used as an internal reference. A Bio-Rad Gel Doc EZ imager (Bio-Rad, Richmond, California, USA) was used for visualization, and gel value analysis was performed using Image J software (National Institutes of Health).

Statistical analysis

Data analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Categorical data are expressed as frequencies or percentages, and comparisons among multiple groups were carried out using the chi-square test. Correlations were analyzed using Spearman rank correlation analysis. Survival rates were calculated using the Kaplan-Meier method. Comparisons between two groups were achieved using the log-rank test, and prognostic factors were screened using a Cox regression model. $P < 0.05$ indicated statistical significance.

Results

Localization of TGF β RI, PDCD4, and AP-1 (c-Jun) determined by immunohistochemical staining in NPC and normal tissues

Immunohistochemical staining (Fig. 1) revealed positive TGF β RI expression in plasma membranes and cytoplasm. Positive PDCD4 and AP-1 (c-Jun) expression was found in the nucleus and partially in the cytoplasm, appearing as brownish yellow granules.

NPC tissues showed increased expression of TGF β RI and AP-1 and decreased expression of PDCD4 compared with normal tissues

In comparison with normal tissues, NPC tissues showed increased expression of TGF β RI (73.33% vs. 23.53%, $P < 0.01$) and AP-1 (77.27% vs. 11.76%, $P < 0.05$) and reduced expression of PDCD4 (39.40% vs. 76.47%, $P < 0.05$) (Table 1).

Fig. 1. TGF β RI, PDCD4, and AP-1 (c-Jun) protein expression determined by immunohistochemical staining in NPC and normal tissues ($\times 200$). Note: AP-1, activator protein-1; PDCD4, programmed cell death 4; TGF β RI, transforming growth factor- β receptor I.

Table 1. Increased expression of TGFβRI and AP-1 and decreased PDCD4 expression in NPC tissues compared with normal tissues

	cases	TGFβRI		PDCD4		AP-1	
		+	-	+	-	+	-
Normal tissues	17	4	13	13	4	2	15
NPC tissues	66	48	18	26	40	51	15
P value		< 0.001		< 0.006		< 0.001	

Table 2. Associations between TGFβRI, PDCD4, and AP-1 and clinicopathological features of NPC. Note: AP-1, activator protein-1; PDCD4, programmed cell death 4; TGFβRI, transforming growth factor-β receptor I; OS, overall survival; NPC, nasopharyngeal carcinoma

	n	TGFβRI		P	PDCD4		P	AP-1		P
		+	-		+	-		+	-	
Gender										
Male	52	38(73.08%)	14(26.92%)	0.902	19(36.54%)	33(63.46%)	0.36	40(76.92%)	12(23.08%)	0.096
Female	14	10(71.43%)	4(28.57%)		7(50%)	7(50%)		11(78.57%)	3(21.43%)	
Age										
< 50	45	33(73.33%)	12(26.67%)	0.871	17(37.78%)	28(62.22%)	0.694	35(77.78%)	10(22.22%)	0.886
≥ 50	21	15(71.43%)	6(28.57%)		9(42.86%)	12(57.14%)		17(80.95%)	4(19.05%)	
Tumor staging										
Tx-T2	27	13(48.15%)	14(51.85%)	< 0.001	15(55.56%)	12(44.44%)	0.05	13(48.15%)	14(51.85%)	< 0.001
T3-T4	39	35(89.74%)	4(10.26%)		11(28.21%)	28(71.79%)		38(97.44%)	1(2.56%)	
Node staging										
N0	8	2(25.00%)	6(75.00%)	0.001	7(87.50%)	1(12.50%)	0.003	7(87.5%)	1(12.5%)	< 0.001
N1-N3	58	46(79.31%)	12(20.69%)		19(32.76%)	39(67.24%)		46(79.31%)	12(20.69%)	
OS										
< 5	29	28(96.55%)	1(3.45%)	< 0.001	5(17.24%)	24(82.76%)	0.003	27(93.1%)	2(6.9%)	0.007
≥ 5	37	20(54.05%)	17(45.95%)		21(56.76%)	16(43.24%)		24(64.86%)	13(35.14%)	

Association between TGFβRI, PDCD4, and AP-1 expression levels and clinicopathological features of NPC

As shown in Table 2, the expression levels of TGFβRI, AP-1 and PDCD4 varied in patients with different tumor and node stages. Compared with

those who were T1-T2 and N1-N3, significantly reduced expression of TGFβRI and AP-1 and significantly high expression of PDCD4 was observed in patients at Tx-T2 and N0 (all $P < 0.05$). Additionally, PDCD4 expression was significantly decreased and TGFβRI and c-Jun expression significantly increased in patients with < 5 years survival than those with > 5 years survival (all $P < 0.05$). However, no significant differences in TGFβRI, AP-1 or PDCD4 expression was found when comparing patients based on gender or age (all $P > 0.05$).

Correlations among expression levels of TGFβRI, AP-1 and PDCD4 in NPC

Spearman analysis revealed (Table 3) that TGFβRI expression positively correlated with AP-1 expression ($r > 0$, $P < 0.05$) and that both TGFβRI and AP-1 expression negatively correlated with PDCD4 expression ($r < 0$, $r < 0$, both $P < 0.05$).

Association between TGFβRI, PDCD4, and AP-1 expression and NPC patient survival

The 5-year OS and 5-year PFS were calculated using the Kaplan-Meier method (Fig. 2). Compared with TGFβRI-negative patients, TGFβRI-positive patients had significantly shorter 5-year OS and 5-year PFS (5-year OS: 41.67% (20/48) vs. 94.44% (17/18), $P < 0.05$; 5-year PFS: 39.58% (19/48) vs. 61.11% (11/18), $P < 0.05$). Similarly, AP-1-positive patients exhibited significantly shorter survival than AP-1-negative patients (5-year OS: 80.77% (21/26) vs. 40.00% (16/40), $P < 0.05$; 5-year PFS: 76.92% (20/26) vs. 25.00% (10/40),

Fig. 2. Kaplan-Meier survival curves. Note: A, association between TGF β RI expression and OS of NPC patients; B, association between TGF β RI expression and PFS of NPC patients; C, association between PDCD4 expression and OS of NPC patients; D, association between PDCD4 expression and PFS of NPC patients; E, association between AP-1 expression and OS of NPC patients; F, association between AP-1 expression and PFS of NPC patients. Note: AP-1, activator protein-1; PDCD4, programmed cell death 4; TGF β RI, transforming growth factor receptor I; OS, overall survival; PFS, progression-free survival; NPC, nasopharyngeal carcinoma.

$P < 0.05$). Conversely, PDCD4-positive patients presented markedly longer 5-year OS and 5-year PFS than PDCD4-negative patients (5-year OS: 47.06% (24/51) vs. 86.67% (13/15), $P < 0.05$; 5-year PFS: 37.25% (19/51) vs. 73.33% (11/15), $P < 0.05$).

High expression of TGF β RI and AP-1 and low expression of PDCD4 were risk factors for OS and PFS

Cox regression analysis demonstrated (Table 4) that advanced tumor and node stage, TGF β RI over-expression (OS: relative risk = 1.255, $P < 0.05$; PFS: relative risk = 1.870, $P < 0.05$), AP-1 over-expression (OS: relative risk = 1.251, $P < 0.05$; PFS: relative risk = 1.223, $P < 0.05$) and PDCD4 under-expression (OS: relative risk = 1.162, $P < 0.05$; PFS: relative risk = 1.160, $P < 0.05$) were unfavorable risk factors for OS and PFS.

Table 4. High expression of TGF β RI and AP-1 and low PDCD4 expression related to risk factors for OS and PFS. Note: AP-1, activator protein-1; PDCD4, programmed cell death 4; TGF β RI, transforming growth factor- β receptor I; NPC, nasopharyngeal carcinoma; OS, overall survival; PFS, progression-free survival; Exp (B), relative risk; 95% CI, 95% confidence interval

	OS/PFS	B	SE	Wald	Exp (B)	95% CI	P
Tumor staging	OS	1.139	0.431	10.398	1.249	1.107 ~ 1.580	0.001
	PFS	1.373	0.377	13.288	1.253	0.121 ~ 2.530	<0.001
Node staging	OS	1.762	0.394	2.648	1.882	0.483 ~ 4.944	0.036
	PFS	0.635	0.813	0.609	1.53	0.108 ~ 2.608	0.035
AP-1	OS	1.951	0.389	3.006	1.251	1.077 ~ 1.443	0.032
	PFS	0.201	0.616	0.107	1.223	0.366 ~ 4.090	0.001
	OS	1.611	0.39	3.005	1.255	1.037 ~ 1.495	0.001
	PFS	1.054	0.797	1.748	1.87	0.601 ~ 5.691	0.014
PDCD4	OS	1.822	0.743	6.02	1.162	0.377 ~ 1.801	0.014
	PFS	1.831	0.564	10.545	1.16	0.123 ~ 1.484	0.001

Fig. 3. Expression of TGF β RI, AP-1 (c-Jun), and PDCD4 determined by western blotting. Note: A, grey value of TGF β RI, AP-1 (c-Jun), and PDCD4 protein bands; B, protein levels of TGF β RI, AP-1 (c-Jun), and PDCD4; *, $P < 0.05$ compared with the control group; AP-1, activator protein-1; PDCD4, programmed cell death 4; TGF β RI, transforming growth factor- β receptor I.

Decreased TGF β RI expression up-regulated PDCD4 expression, as shown by western blotting

Western blotting (Fig. 3) revealed that TGF β RI and AP-1 (c-Jun) expression decreased and PDCD4 expression increased in CNE2 cells treated with inhibitor compared with the control group, and these changes were significant (all $P < 0.05$). These results indicate that down-regulation of TGF β RI expression increases PDCD4 expression, thus reducing AP-1 (c-Jun) expression.

Discussion

NPC is an Epstein Barr virus (EBV)-associated malignancy with higher metastatic tendency than other head and neck cancers due to persistent EBV infection and the presence of immune cell infiltration around tumor lesions [26]. NPC is poorly differentiated and characterized by invasive growth into surrounding tissues and frequent metastasis [27]. Recent reports of the causes of NPC are helping to improve the cure rate.

In the present study, we found that NPC tissues show higher expression of TGF β RI and AP-1 and lower expression of PDCD4 compared with normal tissues. A previous study demonstrated that TGF β expression in tumor cells is promoted by surrounding cells in the tumor microenvironment [28]. It has been reported that TGF- β 1 was overexpressed in several cancers such as hepatocellular carcinoma and it was related to the progression of tumor cells [29, 30]. AP-1 helps to regulate host immune responses and is up-regulated in many types of cancer, including breast cancer and pancreatic cancer [31–33]. AP-1 activation in patients with NPC is mediated by latent membrane protein 1 (LMP-1), which is encoded by EBV. LMP-1 induces transcription of P53 by regulating AP-1 protein expression, and increased P53 may in turn lead to activation and increased expression of AP-1 [34]. A study conducted by Gao et al. showed that loss of PDCD4 expression is common in human glioma

and may partially contribute to tumor development [35]. Another previous study suggested that TGF β 1 activity may be activated by AP-1 and that TGF β , the receptor of which is TGF β RI, plays an important role in the progression of chronic renal disease [36]. Over-expression of PDCD4 in RT101 cells has been reported to inhibit AP-1 transactivation, possibly resulting from inhibition of Jun or Fos protein synthesis, sequestration of Jun or Fos proteins, suppression of Jun or Fos protein activation [37]. Furthermore, PDCD4 has been shown to impede tumor progression in human colon carcinoma cells by down-regulating mitogen-activated protein kinase 1 (MAP4K1) transcription, with consequent inhibition of AP-1 dependent transcription and c-Jun activation [38].

Our study also revealed that TGF β RI-positive patients and AP-1-positive patients significantly reduced 5-year OS and 5-year PFS and that over-expression of TGF β RI and AP-1 and decreased PDCD4 expression are unfavorable risk factors for NPC. A prior study reported that advanced NPC is often associated with high expression of TGF β 1, which promotes tumor proliferation. Additionally, over-expression of TGF β 1 may overcome factors affecting the negative regulation of TGF β 1 and thereby further accelerate disease progression, leading to a poor prognosis in patients with advanced NPC [16]. Moreover, the transcription factor activity of AP-1 might play an essential role in mediating NPC metastasis, and AP-1 is a novel and effective target for the treatment or prevention of NPC [39]. Chen et al. proposed that a lack of PDCD4 protein expression is associated with a poor prognosis in patients with lung cancer, with a mean survival time of 22 months for patients with PDCD4-positive tumors compared to only 22 months for those with PDCD4-negative tumors [40]. Additional research showed that one possible mechanism by which PDCD4-positive expression improves patient prognosis occurs through PDCD4's activity as a suppressor of tumorigenesis, transformation and invasion. PDCD4 also plays a role in suppressing tumor intravasation, and inhibition of PDCD4 can be achieved by regulating *u-PAR* gene expression [41].

In conclusion, our study provided evidence that TGF β RI and AP-1 expression levels are elevated in NPC, whereas PDCD4 expression is decreased. These results suggest that the TGF β /PDCD4/AP-1 pathway is associated with NPC development and progression. Furthermore, high expression of TGF β RI and AP-1 and low expression of PDCD4 may be unfavorable prognostic factors for patients with NPC. Spearman rank correlation analysis showed TGF β RI expression to be positively correlated with AP-1 expression and TGF β RI and AP-1 expression to be negatively correlated with PDCD4 expression. However, resource limitations prevented us from further exploring the mechanism underlying how TGF β RI targets PDCD4. Future studies investigating this mechanism and aimed at identifying intermediate targets responsible for TGF β RI-mediated regulation of PDCD4 should prove worthwhile.

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Disclosure statement

The authors have declared that no competing interests exist.

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