PD-L1 Associates with The Expression of Cancer Cell-Intrinsic PD-1 and p-S6 Proteins and Predicts A Good Prognosis in Nasopharyngeal Carcinoma

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Abstract

Aims: programmed cell death ligand 1 (PD-L1) is the ligand of programmed death 1 (PD-1), which is a host immunity inhibitory receptor. Expression of PD-L1 in diverse tumor types has been widely discussed, while there is little research about tumor intrinsic-PD-1. P-S6 is an important downstream effector in the PI3K/AKT/mTOR pathway. Our study was focus on investigating PD-L1/PD-1/ p-S6 protein expression and aimed to illustrate their relationship in nasopharyngeal carcinoma (NPC).

Methods: the expression of PD-1, PD-L1 and p-S6 proteins in tissues of NPC, non-cancerous nasopharyngeal epithelia, primary lesions and matching metastases was detected by immunohistochemistry.

Results: PD-1, PD-L1 and p-S6 expression and co-expression of PD-1 and PD-L1 proteins were significantly higher in NPC than in the non-cancerous control tissue, respectively (all $P<0.05$). Furthermore, there was evidently elevated PD-1 expression and co-expression of PD-1 and PD-L1 in matched metastasis of NPC compared to their primary lesions (all $P<0.01$). NPC patients with positive expression of PD-L1 showed significantly higher overall survival rate than others ($P=0.035$). Multivariate Cox proportional hazard regression analysis confirmed that positive expression of PD-L1 and p-S6 were independent prognostic factors for NPC patients.

Conclusions: positive expression of PD-L1 associates with expression of cancer cell-intrinsic PD-1 and p-S6, PD-L1 might serve as a good prognostic biomarker and p-S6 could be a valuable independent poor prognostic biomarker for NPC patients.

1. Background

As one of the most common cancers in Asia, especially in southern China, Nasopharyngeal carcinoma (NPC) has been deeply studied [1]. Epstein-Barr virus (EBV) is a recognized cause of NPC. Infection, genetic susceptibility, high nitrite food and smoking are independent risk factors for NPC [2]. Nowadays, treatments of NPC are mainly radiotherapy and chemotherapy. Patients with early-stage of NPC could benefit from radiotherapy or/and chemotherapy, thereby obtaining a longer survival time [3, 4]. Unfortunately, most NPC patients, at the time of diagnosis, are already in the advanced stage, and chemotherapy, radiotherapy, targeted therapy or immune treatment still cannot significantly effectively extend the survival time of them. [5–7]. In recent years, studies have found that immune escape of tumor cells and abnormal activation of signaling pathways play important roles in the occurrence and development of NPC [1]. Finding new targets of NPC will provide new clues for exploring more effective treatment of NPC patients.

Programmed death 1 (PD-1), a cell membrane protein with 288 amino acids, is a protein from the CD28 superfamily. The expression of tumor cell-intrinsic programmed death 1 (PD-1) played an important role in melanoma tumorigenesis [8]. As one of major ligands of PD-1, programmed death ligand 1 (PD-L1) also takes part in the tumor progression [9]. Ribosomal protein S6 (S6) could be activated by phosphorylated p70S6K which is a downstream effector of the AKT/mTOR pathway. Activated S6 is related to poor prognosis of NPC via the messenger RNA translation machinery [10–11]. Recent research found that the cancer cell-intrinsic PD-1 activated by PD-L1 would promote phosphorylation of S6 and, initiate the translation process, which played a vital role in tumor occurrence, development, invasion and metastasis [12]. Besides, the cytoplasmic domain of PD-1 interacts with the S6 to promote phosphorylation of the mTOR effector protein, initiate the translation process of messenger RNA, and promote tumorigenesis, development, invasion and metastasis [9, 12–13]. However, whether there is abnormal activation of the cell-intrinsic PD-1/PD-L1 axis in NPC, and the relationship between it and S6/p-S6 expression have not been studied yet.

In this study, we evaluated expression of PD-L1, cancer cell-intrinsic PD-1 and p-S6 proteins in 281 cases of NPC and 51 cases of non-cancerous nasopharyngeal epithelia, as well as in 24 primary NPC and their matched metastases, to illustrate the relationship between PD-L1, cancer cell-intrinsic PD-1 or p-S6 proteins expression and clinicopathological features and prognosis in NPC.

2. Methods

2.1 Ethics Statement

All experimental protocols were approved by the Ethics Review Board, the Second Xiangya Hospital of Central South University, and informed consent was applied to all samples.

2.2 Tissue samples and clinical data

All samples were paraffin-embedded tissue, including 281 NPC tissues, 24 primary NPC and their matched metastases, and 51 non-cancerous control nasopharyngeal epithelia, from Department of pathology, the Second Xiangya Hospital of Central South University during January 2008 to December 2017. All clinical record and the follow-up data were obtained. All cases were pathologically diagnosed and classified according to the latest WHO (February 2017) stage category of head and neck tumors. These NPC patients did not receive radiotherapy or chemotherapy prior to biopsy. The time period from first diagnosis to the date of death or the last known date alive was defined as the overall survival time. All NPC samples were divided into different clinically stages according to the standard in UJCC/AJCC staging system [14, 15]. Characteristics of patient were presented in supplementary Table 1.

2.3 IHC and scores

PD-L1, PD-1 and p-S6 proteins staining was employed by ready-to-use MaxVisionTM^{+} HRP-Polymer anti-Mouse IHC Kit (Dako; Carpintrria, CA) on 4 μm tissue sections. As described in our previous publication [11], 1:100 dilution of primary antibody to PD-1 (Mouse polyclonal antibody, Catalog #MX033, MXB Biotechnologies, China), PD-L1 (Rabbit monoclonal antibody, Catalog #ab228462, abcam, UK), and Phospho-S6(p-S6)$^{Ser235/236}$ (Rabbit polyclonal antibody,

Catalog #4857, Cell Signaling Technology, USA) were used to detected the expression of those three proteins in all samples. Each experiment included positive and negative control slide. To confirm the specificity of the antibody, we used the matched IgG isotype antibody as a negative control.

Immunohistochemical staining was independently evaluated under a light microscope at a magnification of x 200 by Y Zhang and Y Zhan blinded to patients' information. The score calculation method was: cancer cell-intrinsic PD-1 [16] was assessed as positive for NPC cells with a greater than 5 positive percentage. PD-L1 [17] scored NPC by calculating combined positive scores (CPS). The CPS criterion is the ratio of the sum of positive tumor cells and positive infiltrating lymphocytes/macrophages relative to total tumor cells. PD-L1 was regarded as positive when the score was higher than 5. Staining scores ≥ 2 was regarded as positive expression for an optimal cut-off value for p-S6 [18]. The two reviewers scored a concordance rate of 95%, and the discordance were resolved by looking at microscopic slides and discussion again.

2.4 Statistical analysis

The relationship between expression of cancer cell-intrinsic PD-1, PD-L1, p-S6 and PD-L1/PD-1 proteins and clinicopathological features in NPC was analyzed using chi-square test. The pairwise association between PD-1, PD-L1, p-S6 and PD-L1/PD-1 expression in NPC was approached through the Spearman's rank correlation coefficient. KaplanMeier analysis was hired to draw the overall survival curves, and the logrank test was the tool to evaluate statistical significance. Cox comparative hazards model was performed to assess the independent prognostic factors of NPC with PD-1, PD-L1, p-S6 and PD-L1/PD-1 expression. All the above analysis was completed by SPSS (IBM SPSS Statistics 24.0) software. Based on two-sided statistical analysis, $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Association between PD-1, PD-L1, p-S6 and combined PD-1 and PD-L1 expression and clinicopathological features in NPC cases

The expression and subcellular localization of PD-L1, PD-1 and p-S6 proteins in NPC and non-cancerous control nasopharyngeal epithelia were marked by IHC. PD-L1 protein staining was located in the membrane of NPC (Figure-1A), while PD-1 protein staining was in the cytoplasm of NPC (Figure-1B). Staining of p-S6 protein was discovered in the cytoplasm in both NPC (Figure-1C) and the control normal nasopharyngeal epithelia (Figure-1D). No staining of PD-L1 protein was found in the normal nasopharyngeal epithelia (Figure-1E). No staining showed up in negative control in the NPC (Figure-1F) (IHC, DAB staining, X200).

The positive expression of PD-1, PD-L1, p-S6 and co-expression of PD-1 and PD-L1 in NPC was 22.1% (62/281), 62.3% (175/281), 87.2% (245/281), and 17.8% (50/281), respectively. However, the positive expression of these proteins in non-cancerous nasopharyngeal epithelia was dramatically lower (7.8% (4/51), 33.3% (17/51), 51.0% (26/51) and 3.9(2/51), respectively) (Fig. 2A).

We further investigated PD-L1, PD-1, p-S6 and combined PD-1 and PD-L1 expression in the primary NPC and their matched lymph node metastases. Results in Fig. 2B showed that the positive expression percentage of PD-1 in the primary lesions (25.0%, 6/24) was significantly lower than that in matched metastasis (62.5%, 15/24) ($P = 0.009$), as well as the co-expression of PD-1 and PD-L1 ($P = 0.009$) (Fig. 2B). It's worth noting that in these primary and matched metastatic samples, all samples with PD-1 positive also acquired PD-L1 positive. No obvious difference in the expression of PD-L1 and p-S6 protein between primary NPC and their matched metastasis yet ($P > 0.05$).

We then explored the relationship between PD-1, PD-L1, p-S6 or the co-expression of PD-L1/PD-1 proteins and clinicopathological features of NPC patients including gender, age, T/N/M stage category, clinical stages, histological type and lymph node metastasis status. These results were displayed in Table 1. The positive percentage of PD-L1 ($P = 0.002$) was statistically lower in NPC patients with clinic T1 than those in T2, T3 and T4, but the patients with N0, N1 and N2 stage category was evidently higher than that N3 ($P = 0.015$). However, the positive percentages of PD-1 and co-expression of PD-L1 and PD-1, and p-S6 were not associated with gender, N or M stage category, clinical stage, histological type and lymph node status (all $P > 0.05$).

Table 1
Association between expression of PD-L1、PD-1 and p-S6 proteins and NPC clinicopathological features (n = 281)

Parameter(n)	PD-L1		*P-values*	PD-1		*P-values*	p-S6		*P-values*	PD-1/PD-L1[#]		*P-values*
	P (%)	N (%)		P (%)	N (%)		P (%)	N (%)		H (%)	L (%)	
Gender												
Male (203)	130(64.0)	73(36.0)	0.326	46(22.7)	157(77.3)	0.697	181(89.2)	22(10.8)	0.110	38(18.7)	165(81.3)	0.513
Female (78)	45(57.7)	33(42.3)		16(20.5)	62(79.5)		64(82.1)	14(17.9)		12(15.4)	66(84.6)	
Age (yr)[##]												
50 (146)	100(62.1)	61(37.9)	0.947	29(18.0)	120(82.0)	0.058	136(84.5)	25(15.5)	0.115	25(15.5)	136(84.5)	0.250
≥ 50 (135)	75(62.5)	45(37.5)		33(27.5)	99(72.5)		109(90.8)	11(9.2)		25(20.8)	95(79.2)	
Histological type												
DNC (12)	7(58.3)	6(41.7)	0.773	2(16.7)	10(83.3)	0.645	9(75.0)	3(25.0)	0.197	2(16.7)	10(83.3)	0.917
UDNC (269)	168(62.5)	101(37.5)		60(22.3)	209(77.7)		236(87.7)	33(12.3)		48(17.8)	221(82.2)	
T-classification												
T1 (25)	7(28.0)	18(72.0)	0.002**	5(20.0)	20(80.0)	0.921	19(76.0)	6(24.0)	0.357	2(8.0)	23(92.0)	0.317
T2 (103)	65(63.1)	38(36.9)		23(22.3)	80(77.7)		91(88.3)	12(11.7)		16(15.5)	87(84.5)	
T3 (78)	51(65.4)	27(34.6)		19(24.4)	59(75.6)		68(87.2)	10(12.8)		18(23.1)	60(76.9)	
T4 (75)	52(69.3)	23(30.7)		15(20.0)	60(80.0)		67(89.3)	8(10.7)		14(18.7)	61(81.3)	
N-classification												
N0 (46)	29(63.0)	17(37.0)	0.015*	14(30.4)	32(60.6)	0.416	43(93.5)	3(6.5)	0.180	10(21.7)	36(78.3)	0.729
N1 (82)	49(59.8)	33(40.2)		16(19.5)	66(80.5)		72(87.8)	10(12.2)		13(15.9)	69(84.1)	
N2 (122)	85(69.7)	37(30.3)		24(19.7)	98(80.3)		101(82.8)	21(17.2)		23(18.9)	99(81.1)	
N3 (31)	12(38.7)	19(61.3)		8(25.8)	23(74.2)		29(93.5)	2(6.5)		4(12.9)	27(87.1)	
M-classification												
M0 (272)	171(62.9)	101(37.1)	0.262	59(21.7)	213(78.3)	0.407	237(87.1)	35(12.9)	0.887	48(17.6)	224(82.4)	0.7249
M1 (9)	4(44.4)	5(55.6)		3(33.3)	6(66.7)		8(88.9)	1(11.1)		2(22.2)	7(77.8)	
Clinical stage												
and (n = 57)	34(59.6)	23 (40.4)	0.647	10(17.5)	47(82.5)	0.357	52(91.2)	5(8.8)	0.307	7(12.3)	50(87.7)	0.223
and (224)	141(62.9)	83(37.1)		52(23.2)	172(76.8)		193(86.2)	31(13.8)		43(19.2)	181(80.8)	
Lymph node status												
LNM (235)	146(62.1)	89(37.9)	0.907	48(20.4)	187(79.6)	0.134	202(86.0)	33(14.0)	0.163	40(17.0)	195(83.0)	0.444
No LNM (46)	29(63.0)	17(37.0)		14(30.4)	32(69.6)		43(93.5)	3(6.5)		10(21.7)	36(78.3)	

Abbreviations: *NPC* nasopharyngeal carcinoma; *DNPC* differentiated non-keratinizing nasopharyngeal carcinoma; *UDNPC* undifferentiated non-keratinizing nasopharyngeal carcinoma; *LNM* lymph node metastasis; *N* negative; *P* positive. [#] Co-expression of PD-1 and PD-L1; [##] the average age of all subjects was 49.8 years; *Correlation is significant at the p < 0.05 level (two tailed). **Correlation is significant at the p < 0.01 level (two tailed).

3.2 Correlations of PD-1, PD-L1, p-S6 and co-expression of PD-L1 and PD-1 proteins expression in NPC

There was a notable phenomenon that in primary and matched metastatic NPC tissues, samples with positive PD-1 expression were accompanied by positive PD-L1, which attracted our attentions (Fig. 2B). Therefore, we investigated whether there were some correlations among these proteins. The relationship between PD-1, PD-L1, p-S6 and co-expression of PD-L1/PD-1 proteins in 281 NPC patients was shown in Table 2. PD-L1 expression was positively associated with PD-1 (r = 0.219, $P < 0.001$), p-S6 (r = 0.273, $P < 0.001$) or co-expression of PD-L1 and PD-1 (r = 0.366, $P < 0.001$) in NPC. In addition, p-S6 was also positively

related to PD-1 (r = 0.127, *P* = 0.033) and co-expression of PD-L1 and PD-1 in NPC (r = 0.153, *P* = 0.01). Consistent with the notable phenomenon, expression of PD-1 was strongly related to combined PD-L1 and PD-1 expression (r = 0.885, *P* < 0.001).

Table 2
The pairwise correlation between expression of PD-L1, PD-1 and p-S6 proteins in the 281 cases of NPC

	PD-L1	PD-1	p-S6	PD-1/PD-L1#
PD-L1				
Values	-	0.219	0.273	0.366
Significant		0.000**	0.000**	0.000**
PD-1				
Values	-	-	0.127	0.885
Significant			0.033*	0.000**
p-S6				
Values	-	-	-	0.153
Significant				0.010*

Values are Spearman's rank correlation coefficient. # Co-expression of PD-1 and PD-1; *Correlation is significant at the p < 0.05 level (two tailed); **Correlation is significant at the p < 0.05 level (two tailed).

3.3 The impact of PD-L1, PD-1, p-S6 and combined PD-1and PD-L1 proteins expression on prognosis in NPC patients.

Survival status of NPC patients with differentially expressed PD-L1, PD-1, p-S6 and combined PD-L1 and PD-1 proteins was studied through Kaplan-Meier survival curves (Fig. 3). As to NPC patients, overall survival rate was significantly higher in cases with positive expression of PD-L1, compared to ones with negative PD-L1 expression (*P* = 0.035, Fig. 3A). On the contrary, NPC patients with positive expression of PD-1 (*P* = 0.031, Fig. 3B), p-S6 (*P* = 0.044, Fig. 3C) or combined PD-1 and PD-L1 (*P* = 0.042, Fig. 3D) had shorter survival time than others by univariate analysis.

Furthermore, we investigated whether PD-1, PD-L1, p-S6 and combined PD-L1 and PD-1 proteins expression could be used as independent prognostic factors for NPC patients. Data in Table 3 revealed that positive expression of PD-L1 protein was identified as an independent good prognostic factor (*P* = 0.002), while positive expression of p-S6 protein (*P* = 0.003), lymph node metastasis (LNM) status (*P* = 0.004), N-stage category (*P* < 0.001), M-stage category (*P* < 0.001) and clinical stage (*P* = 0.005) were identified as independent poor prognostic factors for overall survival of NPC patients. However, patients with positive PD-1 expression and co-expression of PD-L1 and PD-1 had no significant impact on the overall survival of NPC patients (*P* > 0.05, respectively). Other factors including gender, age, histological type and T/N stage category also have no obvious impacts on the prognosis in NPC (all P > 0.05).

Table 3
Summary of multivariate Cox proportional hazard regression analysis used to evaluate overall survival in 281 cases of NPC patients.

Parameter	SE	Wald	Significance	Exp(B)	95.0% CI for Exp(B)	
					Lower	Upper
Gender	0.305	0.626	0.429	0.785	0.432	1.429
Age	0.251	2.736	0.098	1.516	0.926	2.481
Histological type	1.017	0.759	0.384	0.412	0.056	3.024
LNM status	0.412	8.504	0.004**	3.329	1.483	7.470
T-classification	0.169	1.090	0.297	1.193	0.856	1.662
N-classification	0.167	12.792	0.000**	1.817	1.310	2.521
M-classification	0.452	16.961	0.000**	6.426	2.651	15.575
Clinical stages	0.176	7.830	0.005**	1.637	1.159	2.312
PD-L1	0.301	9.434	0.002**	0.397	0.220	0.716
PD-1	0.501	1.626	0.202	1.895	0.709	5.064
p-S6	0.481	8.665	0.003**	4.115	1.604	10.556
PD-L1/PD-1	0.607	0.170	0.680	1.284	0.391	4.217

Abbreviations: SE, standard error (SE); Exp (B), exponentiation of the B coefficient; CI, confidence interval.; LNM lymph node metastasis; NPC nasopharyngeal carcinoma. Note: multivariate analysis of Cox proportional hazard regression, *Correlation is significant at the p<0.05 level (two tailed). **Correlation is significant at the p<0.01 level (two tailed).

4. Discussion

As a ligand for PD-1, PD-L1 is a transmembrane protein expressed on immune cells and tumor cells encoded by the Cd274 gene. It is not only a cancer-promoting factor in some certain malignant tumors such as hepatocellular carcinoma, gastric cancer and esophageal cancer; also as protective factor in some other tumors including Merkel cell carcinoma and breast cancer [19–24]. However, the roles of PD-L1 action in NPC is not clear. It has been reported that short overall survival time of NPC patients was related to high expression of PD-L1 [25, 26]. But other research results drew a opposite conclusion that patients with high PD-L1 expression obtained a better prognosis [27–29], which was in line with our results. We found that there was significant higher positive expression of PD-L1, PD-1, p-S6 and combined PD-1 and PD-L1 in NPC than that of in non-cancerous nasopharyngeal epithelia. Furthermore, PD-L1 expression had effects on T-stage category of NPC. In conclusion, overexpression of PD-1, PD-L1, combined PD-L1 and PD-1 and p-S6 were all related with tumorigenesis.

Cox multivariate regression analysis showed that positive expression of PD-L1 in NPC patients had a lower death risk. In fact, the association between the expression of PD-L1 and tumor immune evasion is not directly proportional, and may just represent the persistence of anti-tumor response [30]. Here's more evidence that PD-L1 could take part in the regulation of anti-tumor immunity. It was found that AhR (Aryl hydrocarbon receptor) activation could mediate the expression of PD-1 in CD8 + T. Maybe their co-stimulators AhR induced the correlation expression between PD-1 and combined PD-L1 and PD-1 expression [31, 32]. The secretion of interferon gamma (IFNγ) and the enhancement of tumor PD-L1 expression promote each other, resulting in CD8 + and CD3 + T cells enrichment around NPC tissues, and the accumulation of these immune cells promotes the immune response to tumor tissue [33–35].

As ligands of PD-1, PD-L1 and PD-L2 are mostly found in tumor cells and antigen presenting cells. And they will inhibit the tumor cell death through down-regulating the T cell response when they bind to PD-1. The transmembrane receptor PD-1 is a prominent checkpoint primarily expressing on T cells [36, 37]. PD-1 plays vital roles in promoting NPC growth and is related to the short overall survival time in NPC patients [38]. Studies focused on PD-1 protein in NPC have primarily examined PD-1 expression in lymphocytes [39–40]. Kleffel's team firstly reported that PD-1 protein was also presented in cancer cells and promoted tumor occurrence and development in melanoma [8]. Other studies have also shown cell-intrinsic PD-1 promotes the development of liver cancer and pancreatic cancer, and shortens the survival rate [41, 42]. Tumor cell-intrinsic PD-1 promotes tumor occurrence such as hepatocellular carcinoma and melanoma by activating the mTOR signaling [12]. In pancreatic cancer, cell-intrinsic PD-1 promoted tumor growth through the Hippo signaling pathway outside the immune system [4, 20]. However, Du et al found that cell-intrinsic PD-1 was presented in NSCLC as a tumor suppressor [43]. In current study, we proved that patients with positive expression of PD-1 in NPC tissues had shorter survival time, which is consistent with findings in most malignancies. We also demonstrated that the positive expression rate of cancer cell-intrinsic PD-1 in metastatic NPC lesions was obviously higher than that in primary lesions. Although we haven't discovered statistical difference of the expression of PD-1 in primary NPC with or without lymph node metastasis, it was worth noting that the immune morphology of NPC cells altered during distant metastasis. Consequently, cell-intrinsic PD-1 might, we speculated, participate in the distant metastases of NPC. However, the number of matched primary/metastatic and lymph node metastases is limited, so further experimentation with larger size is needed to confirm the association between PD-1 expression and the NPC metastasis.

The PI3K signaling pathway participates PD-L1 expression regulation. S6 is one of the downstream targets in the PI3K/AKT/mTOR signaling pathway. Overexpression of p-S6 results in mTOR signaling pathway dysregulation [44–46]. PD-1 promoted S6 phosphorylation and tumor proliferation in melanoma [12]. Our results showed that positive p-S6 expression shortened overall survival in NPC patients, and there was an association between p-S6 expression and PD-1 and PD-L1 expression. At the same time, the p-S6 positive rate in patients with co-expression of PD-1 and PD-L1 was significantly higher than other patients. Hence, we speculated that PD-1 might also play an encouraging role in S6 phosphorylation in NPC. In conclusion, expression of PD-1, PD-L1,

combined PD-L1 and PD-1 and p-S6 are correlational, especially between PD-1 and combined PD-L1 and PD-1 expression. But, these need to be further verified by in vitro and in vivo study in the future experiments.

In summary, in our study, there was significantly higher positive expression of PD-1, PD-L1, p-S6 and combined PD-1 and PD-L1 proteins in NPC. Furthermore, PD-1 might also be involved in the metastatic spread of NPC. Positive PD-L1 expression associated with PD-1 and p-S6 expression, positive expression of PD-L1 and p-S6 could serve as valuable independent prognostic biomarkers for NPC patients.

5. Conclusion

Positive expression of PD-L1 associates with expression of cancer cell-intrinsic PD-1 and p-S6, PD-L1 might serve as a good prognostic biomarker and p-S6 could be a valuable independent poor prognostic biomarker for NPC patients.

Abbreviations

PD-L1
programmed cell death ligand 1; PD-1:programmed death 1; NPC:nasopharyngeal carcinoma; IFNγ:interferon gamma.

Declarations

Ethics approval and consent to participate

Written Informed consent was legally obtained and all research protocols were approved by the Ethics Review Committee of the Second Xiangya Hospital of Central South University (Scientific and Research Ethics Committee, No. Y202/2014).

Consent for publication

All authors have consented for publication.

Availability of data and material

All data and material were available.

Competing interests

There is no conflicts of interest to declare.

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Authors' contributions

Experiment design and manuscript editing were done by Dr. SF. Y Zhang wrote the manuscript and made the tables. HZ and Y Zhan collected clinical statistics. YY, YN and HW participated in the experiment. WW checked the manuscript and gave the suggestions.

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Figures

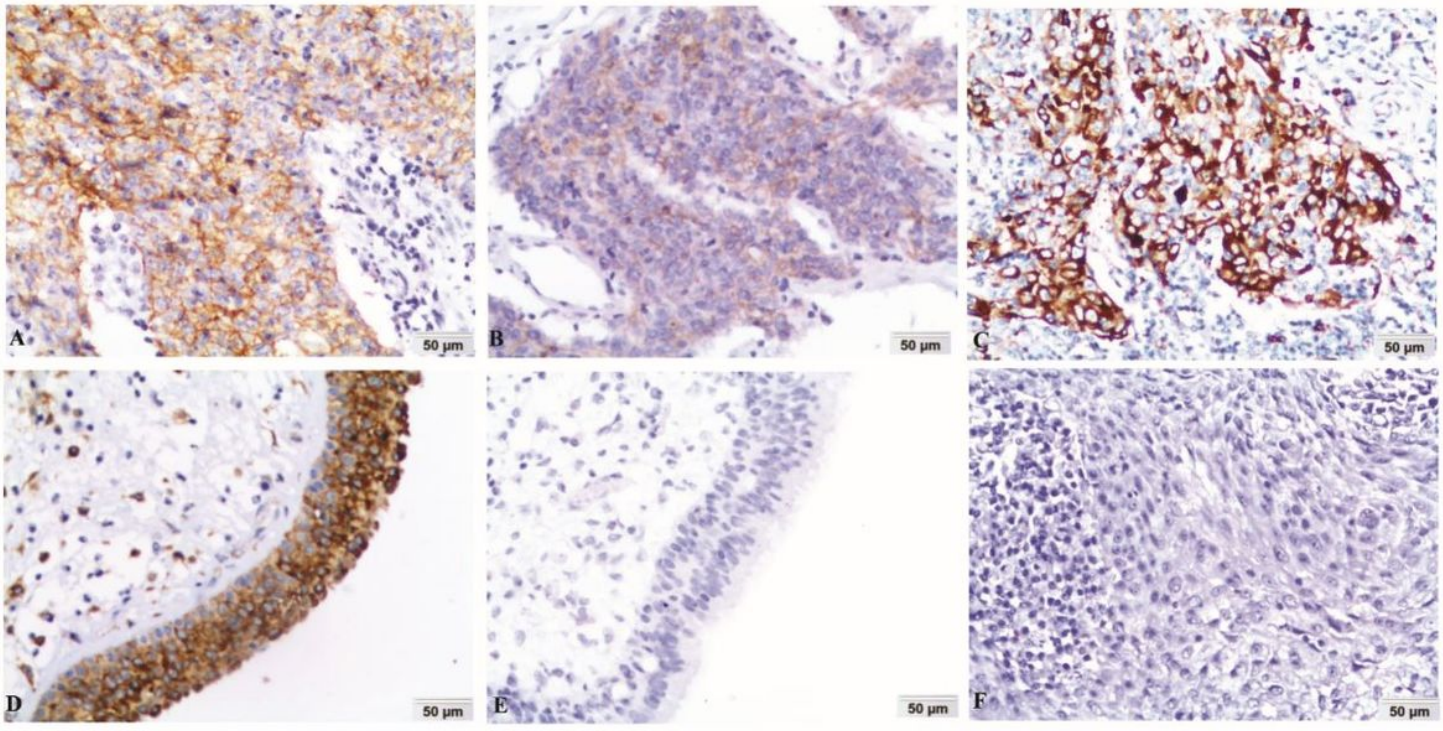

Figure 1

PD-L1, PD-1 and p-S6 proteins expression in NPC and non-cancerous nasopharyngeal epithelia was detected using immunohistochemistry (IHC). A: PD-L1 protein staining was located in the membrane of NPC. B: PD-1 protein staining was in the cytoplasm of NPC. C and D: Staining of p-S6 protein was discovered in the cytoplasm in both NPC and the control normal nasopharyngeal epithelia. E: No staining of PD-L1 protein was found in the control normal nasopharyngeal epithelia. F: No staining showed up in negative control in the NPC (IHC, DAB staining, x200).

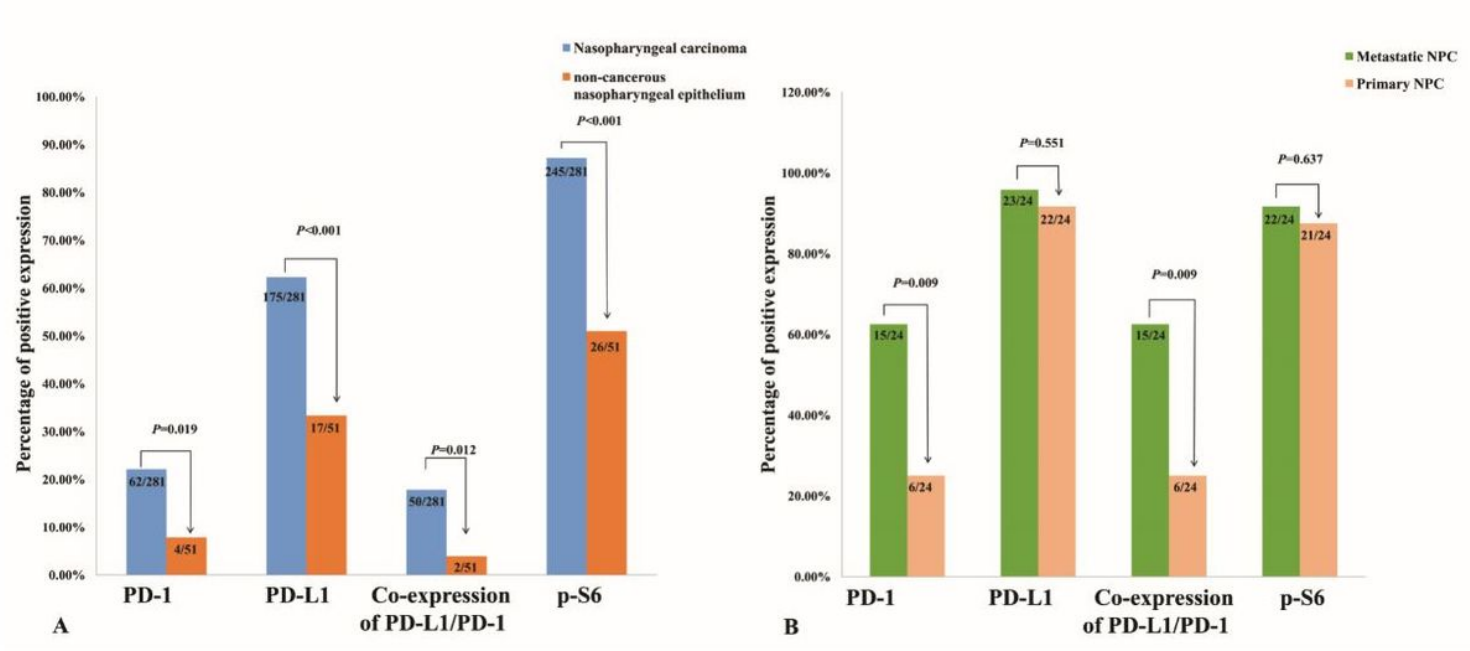

Figure 2

Positive expression of PD-1, PD-L1, p-S6 and common expression PD-1/PD-L1 in 281 NPC and 51 non-cancerous control nasopharyngeal epithelia, 24 pairs of primary NPC and their corresponding metastases were analyzed by χ2 test. A: The percentages of positive expression of PD-1, PD-L1, p-S6 and common expression PD-1/PD-L1 in NPC were evidently higher than those in the non-cancerous nasopharyngeal epithelia (all P<0.05). B: There were significantly lower expression of PD-1 and co-expression of PD-1/PD-L1 in primary NPC compared to the matched metastatic NPC (all P<0.01).

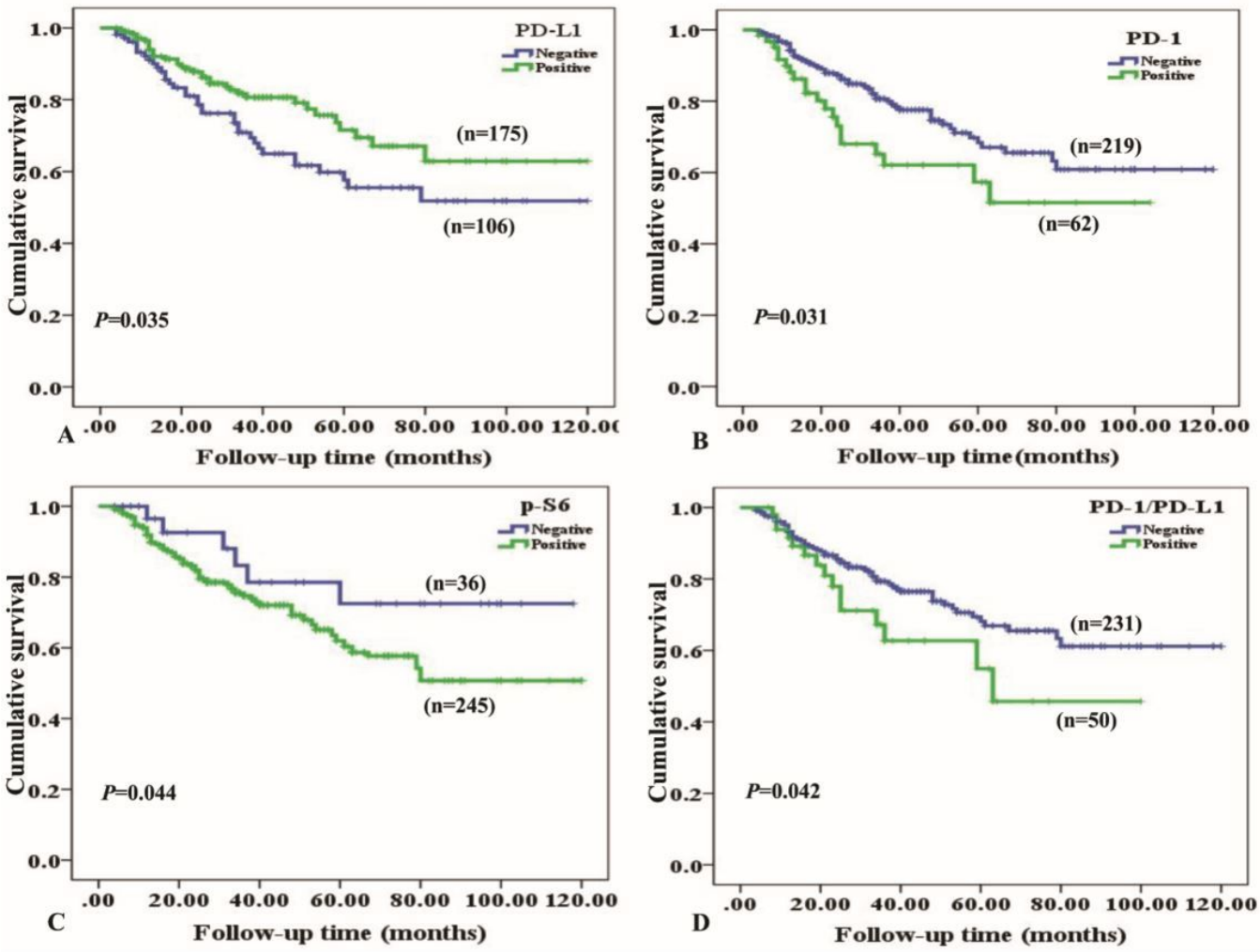

Figure 3

Kaplan-Meier curves for overall survival of NPC patients with PD-L1, PD-1, p-S6 proteins expression and co-expression of PD-L1/PD-1. A NPC patients with positive expression of PD-L1 caught a longer OS (overall survival) (P=0.035); B: NPC patients with negative PD-1 staining showed a longer OS (P=0.031); C: NPC patients with negative p-S6 revealed a longer OS (P=0.044); D: NPC patients with common negative PD-L1/PD-1 had a longer OS (P=0.042). All tests were 2-sided.

Supplementary Files

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- TableS1.docx