Heliyon 9 (2023) e18130

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Role of β-catenin in PD-L1 expression of nasopharyngeal carcinoma

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ARTICLE INFO

Keywords:
β-catenin
p-β-catenin^{Tyr654}
PD-L1(22c3)
Nasopharyngeal carcinoma

ABSTRACT

Nasopharyngeal carcinoma (NPC) is a particular type of tumor connected to Epstein-Barr virus infection, genetic, and environmental factors. It is typically discovered late, with few therapeutic options and poor clinical outcomes. Cellular immune responses can be attenuated when programmed death ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1) are combined. Although PD-1 inhibitors have a different anti-tumor response rate than chemotherapy alone, they can nevertheless considerably outperform chemotherapy in patients with metastatic or recurrent NPC. The nuclear β-catenin can bind to the CD274 promoter region, promoting transcription and upregulating the expression of tumor-specific PD-L1. Separation of β-catenin from E-cadherin and translocation it into nucleus were both aided by β-catenin phosphorylates at the Tyr654 site. Its function in NPC and the expression of PD-L1 have not yet been investigated. This study investigated the predictive significance of PD-L1 and p-β-catenin^{Tyr654} expressions in NPC. Our findings indicated that patients with distant metastases or poor prognoses exhibited higher levels of PD-L1 and p-β-catenin^{Tyr654} expressions. According to Cox multivariate prognostic analysis, PD-L1 was also an effective indicator for predicting the survival status of patients with NPC. We subsequently demonstrated that PD-L1 transcription and protein production could be downregulated by targeting inhibition of the level of β-catenin in NPC cells. This is for developing the β-catenin or TCF4 inhibitor as a potential new option for immune checkpoint immunosuppression in NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a squamous cell tumor originating from the lining of the nasopharyngeal mucosa. Epstein-Barr virus (EBV) infection and genetic, and environmental factors contribute to the pathogenesis of NPC [1]. Patients with early-stage NPC can experience a significantly improved clinical outcome when receiving intensity-modulated radiotherapy (IMRT) alone or in combination with chemotherapy. However, it is typically discovered when the disease is already advanced and when there are limited

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https://doi.org/10.1016/j.heliyon.2023.e18130
Received 21 January 2023; Received in revised form 5 July 2023; Accepted 7 July 2023
Available online 8 July 2023

treatment options and poor clinical outcomes. Additional treatment challenges include local or distant recurrence, affecting 20%–30% of patients, and treatment resistance [2].

An immune checkpoint called programmed cell death protein 1 (PD-1), is expressed on the surface of activated T cells to limit their proliferation and activation [3]. The programmed death ligand 1 (PD-L1), the primary ligand of PD-1 is expressed in activated T cells, B cells, dendritic cells, macrophages, endothelial cells, and some tumor cells. The combination of PD-L1 and PD-1 can attenuate the cellular immune response by reducing regulatory T-cell apoptosis or promoting T-cell failure [4]. Several malignant tumors have been shown to benefit significantly from the clinical benefits of inhibiting PD-1/PD-L1 signal transduction [2]. Gemcitabine and cisplatin, the first-line standard treatment, combined with *anti*-PD-1, may yield therapeutic outcomes significantly better than chemotherapy alone in patients with metastatic or recurrent NPC [5]. However, the anti-tumor response rate of PD-1 inhibitor was restricted, as evidenced by the cumulative data of clinical trials conducted on a few solid tumors [6]. Therefore, it is critical to understand how PD-1/PD-L1 expression is regulated in cancers.

Across cancer types, the Wnt/β-catenin signal transduction influences cancer immunosurveillance [7]. For example, tumor-induced β-catenin signal transduction inhibits the cross-sensitization of anti-tumor cytotoxic T cells dependent on dendritic cells. It switches the invading immune effector cells into an "immune tolerance" state [2,8]. The activation of β-catenin promotes PD-L1 blockade resistance by decreasing the recruitment of $CD103^{+}$ dendritic cells mediated by chemokines and triggering T-cell rejection in the melanoma mouse model [9]. Additional reports suggest that PD-L1-mediated immunosuppression in tumors can be regulated by β-catenin. According to Du et al. [10], the binding of β-catenin and LEF1 to the CD274 promoter region stimulated transcription. It upregulated the expression of endogenous tumor PD-L1 in glioblastoma cells continuously elevated in an AKT-dependent manner. When β-catenin is phosphorylated at the Tyr654 site, it helps the β-catenin detach from E-cadherin and translocate into the nucleus [11]. Its function in NPC and the expression of PD-L1 have not yet been investigated.

In this study, we used an immunohistochemical technique to identify the expression of p-β-catenin^{Tyr654} and PD-L1 (22c3) proteins. Also, we highlighted the correlation between the clinicopathological characteristics and prognosis of patients with NPC and the expression of the patients mentioned above. We subsequently investigated the association between the expression of the two proteins. We established that targeting inhibition of the nuclear level of β-catenin in NPC cells might downregulate the PD-L1 transcription level and protein expression.

2. Material and methods

2.1. Ethical statement

The Xiangya Hospital of Central South University Ethics Review Board (Scientific and Research Ethics Committee, No. 201803326) approved all protocols, and all research was carried out following relevant guidelines/regulations. All study samples were obtained with written informed consent. Caretakers or guardians signed written consent for minors who participated in this study.

2.2. Patient cohorts

We gathered 425 patients with NPC who had received a diagnosis at The Xiangya Hospital, Central South University, between 2011 and 2017. Expert pathologists evaluated each tumor according to the WHO nasopharyngeal carcinoma histological classification. The staging classification of the current analysis was completed based on the standards of the 8th edition of the AJCC/UICC TNM staging system of NPC. No patients had radiation or chemotherapy at the time of the initial procedure, and throughout the follow-up period, none had received treatment that targeted EGFR and PD1/PDL1. The overall survival time was calculated as the interval between diagnosis and death, or the last known moment of survival.

2.3. Inclusion and exclusion criteria

Patients who meet the following inclusion criteria are eligible for inclusion in this article: (1) Nasopharyngeal carcinoma; (2) Complete follow-up data; (3) Clinicopathological data. The table shows the specific clinic parameters for the enrolled patients.

Following were the exclusion criteria: (1) Other treatments used following the procedure; (2) Missing follow-up visits or clinic parameters.

2.4. Immunohistochemistry and scores

The immunohistochemistry experiment was carried out following the guidelines of our previous study. The primary antibody to p-β-catenin^{Tyr654} was diluted to 1:50 (Rabbit polyclonal antibody, Catalogue ab59430; abcam Cambridge, UK). PD-L1 (anti-human PD-L1 antibody, clone 22C3; pharmDx assay Agilent Technologies, Santa Clara, CA). Slides serving as positive controls were used in each experiment. A matching immunoglobulin G (IgG) isotype antibody was used as a negative control to assess the specificity of the antibody. FSQ and WWY, blinded to the clinicopathological data, independently assessed the expression using 200 × magnification light microscopy. p-β-catenin^{Tyr654} was divided into positive and negative expressions. Stainings of PD-L1 were assessed for tumor proportion score (TPS). PD-L1 expression $<50\%$ was considered low, whereas $\geq 50\%$ was considered high [12].

2.5. Cell lines and cell culture

The Cancer Research Institute, Central South University, provided the cell lines 5–8F, 6–10B, and HK1. Integrated Hospital of Traditional Chinese Medicine, Southern Medical University, provided the SUNE1 used in this study. Short tandem repeat profiling using Microread Gene Technology (Beijing, China) was recently used to verify the authenticity of all cell lines. The human NPC cell lines (5–8F, 6–10B, and SUNE1) were cultured in Dulbecco's Modified Eagle Medium (Biological Industries, Israel) supplemented with 10% fetal bovine serum (Gibco, USA), and HK1 was cultured in RPMI-1640 medium (Biological Industries, Israel). A 37 °C incubator with 5% carbon dioxide was used to keep culture dishes.

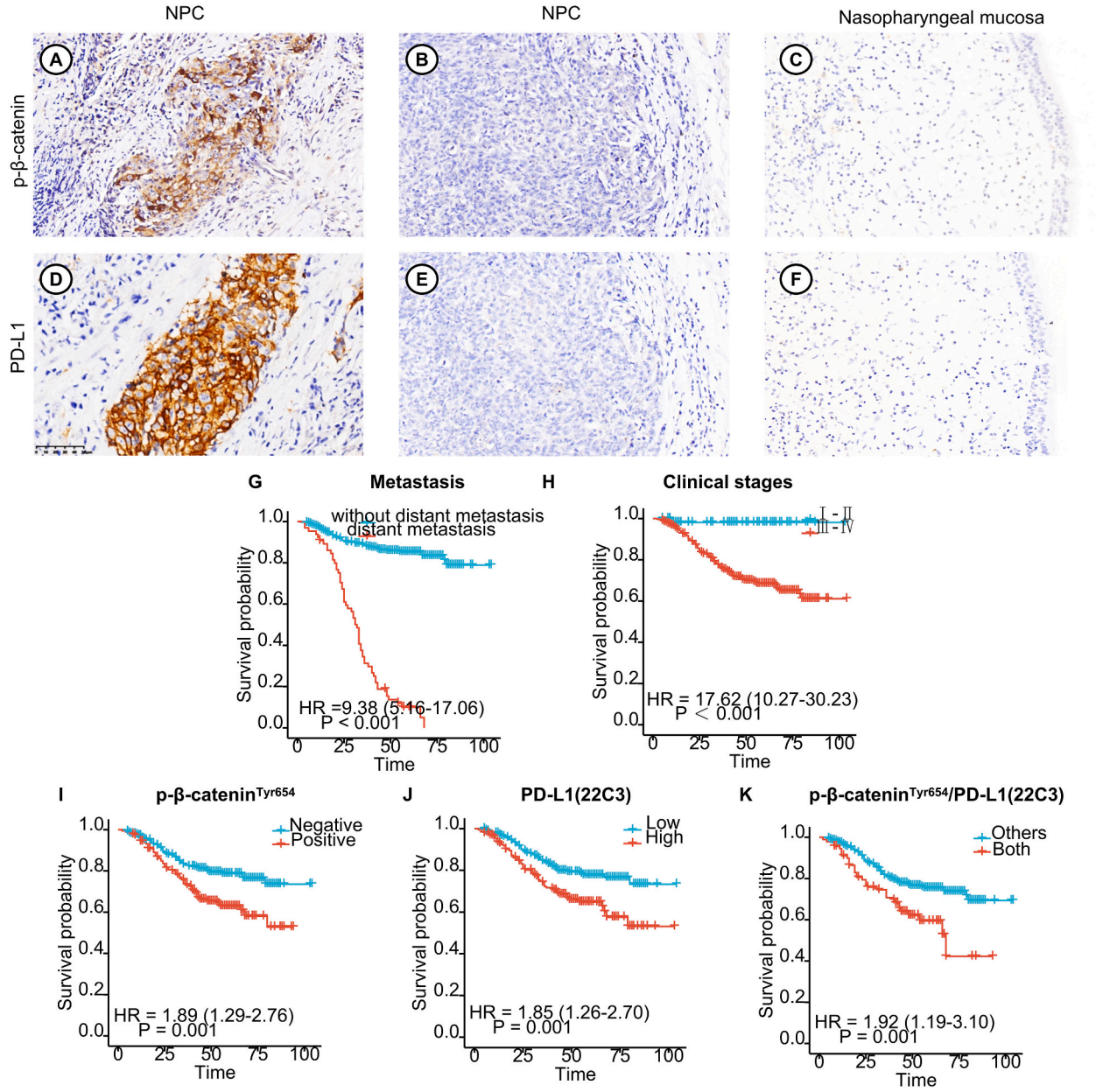

Fig. 1. Expression of p-β-catenin^{Tyr654} and PDL1(22C3) predicted overall survival of patients with NPC. (A) Positive expression of p-β-catenin^{Tyr654} protein was presented in the NPC. (B) Negative expression of p-β-catenin^{Tyr654} was indicated in the NPC. (C) Negative expression of p-β-catenin^{Tyr654} was indicated in the columnar epithelial cells of non-cancerous nasopharyngeal tissue. (D) Positive expression of PD-L1 protein was presented in the NPC. (E) Negative expression of PD-L1 was indicated in the NPC. (F) Negative expression of PD-L1 was indicated in the columnar epithelial cells of non-cancerous nasopharyngeal tissue. The overall survival rates were significantly lower for NPC patients with metastasis (G, $P < 0.001$), advanced stages (H, $P < 0.001$), positive expression of p-β-catenin^{Tyr654} (I, $P = 0.001$), high expression of PD-L1 (J, $P = 0.001$), and both expression of positive p-β-catenin^{Tyr654} and high PD-L1 (K, $P = 0.001$).

2.6. Cell counting Kit-8 (CCK8) assay

The CCK-8 assay (Bimike, USA) was used to evaluate cell survival rates. In 96-well plates with 100 μl of the appropriate medium in each well, about 1000 cells were counted and seeded. Each well was incubated with 10 μl of CCK-8 solutions for 2 h away from light before measuring the absorbance at 450 nm with a Multilabel Plate Reader (SpectraMax iD3, Molecular Devices).

2.7. Real-time quantitative polymerase chain reaction (qRT-PCR)

TRIzol RNA reagent (Thermo Fisher Scientific, USA) was used to extract total RNA. Reverse transcription was carried out with the help of the PrimeScript™ RT reagent kit with genomic DNA (gDNA) eraser (Thermo Fisher Scientific, USA). SYBR Green™ Premix Ex Taq™ II (Biomaker, USA) was used for qRT-PCR in the Applied Biosystems 7500 (AB, USA). The primers were as follows: β-actin, forward 5′ CTGGGACGACATGGAGAAAA 3′; reverse 5′ AAGGAAGGCTGGAAGAGTGC'; CD274, forward 5′ TGGCATTTGCTGAACG-CATTT 3′; reverse 5′ TGCAGCCAGGTCTAATTGTTTT 3′.

2.8. Western blotting analysis

Western blotting was performed according to the standard protocol using the following antibodies: p-β-catenin^{Tyr654} (Rabbit polyclonal antibody, Catalogue ab59430; abcam, Cambridge, UK) and PD-L1(Mouse monoclonal antibody, Catalogue 66248-1-Ig; Proteintech Group, Chicago, USA).

2.9. Statistical methods

Statistical analyses were carried out using the Chi-square test, multivariate Cox regression analysis, and log-rank test when necessary, using Statistical Package for Social Sciences (SPSS) for Windows (18.0; SPSS, Inc.) and GraphPad Prism (Prism 8.0; GraphPad Software Inc.) software packages. $P < 0.05$ was considered statistically significant. Error bars indicate the standard deviation in all the Figures. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ using two-tailed *t*-test.

3. Results

3.1. Association between the expressions of p-β-catenin^{Tyr654} and PD-L1(22c3) and clinicopathological characteristics of NPC

We discovered that using an immunohistochemical technique, p-β-catenin^{Tyr654} (Fig. 1A and B) and PD-L1 (22c3) (Fig. 1D and E) were positively expressed and localized within NPC, rather than in nasopharyngeal mucosa (Fig. 1C and F). The cytoplasm included the

Table 1
Analysis of the association between expression of p-β-catenin^{Tyr654} and PD-L1 (22c3) and clinicopathological features of NPC (n = 425).

Clinicopathological features (n = 425)	p-β-catenin^{Tyr654}			PD-L1 (22c3)			p-β-catenin Tyr654/PD-L1 (22c3)#		
	Ne (%)	Po (%)	*P*-value	Low (%)	High (%)	*P*-value	Others (%)	C^+ (%)	*P*-value
Age (years)									
≤55 (n = 325)	194 (59.7)	131 (40.3)	0.418	181 (55.7)	144 (44.3)	1.000	257 (79.1)	68 (20.9)	0.889
>55 (n = 100)	55(55.0)	45 (45.0)		56 (56.0)	44 (44.0)		78 (78.0)	22 (22.0)	
Gender									
Male (n = 304)	177 (58.2)	127(41.8)	0.828	172 (56.6)	132 (43.4)	0.665	241 (79.3)	63 (20.7)	0.793
Female (n = 121)	72 (59.5)	49 (40.5)		65 (53.7)	56 (46.3)		94 (77.7)	27 (22.3)	
T stages									
1-2 (n = 219)	134 (61.2)	85 (38.8)	0.279	128 (58.4)	91 (41.6)	0.283	178 (81.3)	41 (18.7)	0.235
3-4 (n = 206)	115 (55.8)	91 (44.2)		109 (52.9)	97 (47.1)		157 (76.2)	49 (23.8)	
LNM									
No (n = 34)	23 (67.6)	11 (32.4)	0.283	19 (55.9)	15 (44.1)	1.000	27 (79.4)	7 (20.6)	1.000
Yes (n = 391)	226 (57.8)	165 (42.2)		218 (55.8)	173 (44.2)		308 (78.8)	83 (21.2)	
Metastasis									
No (n = 360)	221 (61.4)	139 (38.6)	0.009**	209 (58.1)	151 (41.9)	0.030*	290 (80.6)	70 (19.4)	0.048*
Yes (n = 65)	28 (43.1)	37 (56.9)		28 (43.1)	37 (56.9)		45 (69.2)	20 (30.8)	
Clinical stages									
I-II (n = 56)	38 (67.9)	18 (32.1)	0.147	33 (58.9)	23 (41.1)	0.666	45 (80.4)	11 (19.6)	0.862
III-IV (n = 369)	211 (57.2)	158 (42.8)		204 (55.3)	165 (44.7)		290 (78.6)	79 (21.4)	
Survival status									
Alive (n = 315)	200 (63.5)	115 (36.5)	0.001**	189 (60.0)	126 (40.0)	0.004**	259 (82.2)	56 (17.8)	0.006**
Dead (n = 110)	49 (44.5)	61 (55.5)		48 (43.6)	62 (56.4)		76 (69.1)	34 (30.9)	

Chi-square test, statistically significant difference ($P < 0.05$, **$P < 0.01$).
Abbreviations: NPC, Nasopharyngeal carcinoma; Po, Positive expression; Ne, Negative expression; LNM, lymph node metastasis; p-β-catenin ^{Tyr654}/PD-L1 (22c3)$^{\#}$, C^+, the positive p-β-catenin combined with high expression of PD-L1 (22c3), others, other combination of expression of these two factors.

positive staining for p-β-catenin^{Tyr654} (Fig. 1A). PD-L1 (22c3) was primarily found in the cell membrane (Fig. 1D). We further investigated the relationship between the expression of p-β-catenin^{Tyr654}, PD-L1 (22c3) protein, and the clinicopathological characteristics of NPC using univariate Chi-square test, including age, gender, T stage, lymph node metastasis status, distant metastasis status, clinical stages, and survival status. The results are shown in Table 1. The positive expression of p-β-catenin^{Tyr654} ($p = 0.009$, $p = 0.001$), the high level of PD-L1(22c3) ($p = 0.030$, $p = 0.004$), and the positive p-β-catenin^{Tyr654} combined with high PD-L1(22c3) ($p = 0.048$, $p = 0.006$) were all associated to distant metastases and poor survival status in patients with NPC. However, there was no significant correlation with age, gender, T stage, lymph node metastasis status, and clinical stages.

3.2. Pairwise association between expression of p-β-catenin^{Tyr654} and PD-L1(22c3) in NPC

The pairwise correlation between abnormal p-β-catenin^{Tyr654} expression and PD-L1 (22c3) protein is shown in Table 2. The positive expression of p-β-catenin^{Tyr654} was positively correlated ($r = 0.117$, $P = 0.016$) with the increased expression of PD-L1 in NPC.

3.3. Impact of the expression of p-β-catenin^{Tyr654} and PD-L1(22c3) proteins on the prognosis of patients with NPC

In univariate survival analysis, the relationship between the survival status and the abnormal expression of p-β-catenin^{Tyr654}, PD-L1 (22c3), both of them, were analyzed using Kaplan-Meier survival curves and log-rank test. Additionally, we conducted the classical indicators, such as distant metastasis and clinical stages, to predict the prognosis of patients with NPC to verify the randomness and objectivity of the selected samples. The Kaplan-Meier survival curves for patients with NPC with the various factors indicated above are shown in Fig. 1G–K. Patients with NPC without distant metastasis (Fig. 1G, $P < 0.001$) or with clinical stages I and II (Fig. 1H, $P < 0.001$) had higher survival times than patients with distant metastasis or clinical stages III and IV. Patients with NPC with positive expression of p-β- catenin^{Tyr654} had significantly lower total survival times than those with negative expression (Fig. 1I, $P = 0.001$) and having the higher expression of PD-L1(22c3) (Fig. 1J, $P = 0.001$) and shared impaired expression of p-β-catenin^{Tyr654} and PD-L1(22c3) (Fig. 1K, $P = 0.001$).

Cox multivariate regression analysis that revealed advanced age ($P = 0.048$), distant metastasis ($P < 0.001$), higher clinical stages ($P = 0.030$), and advanced expression of PD-L1(22c3) ($P = 0.016$) protein were independent predictors of poor prognosis for patients with NPC, as shown in Table 3. However, neither the p-β-Catenin^{Tyr654} protein nor its co-expression with p-β-catenin^{Tyr654} and PD-L1 (22c3) significantly impacted the prognosis of patients with NPC. Additionally, the prognosis of patients with NPC was unaffected by gender, T stage, and lymph node metastases (all $P > 0.05$).

3.4. Suppression of β-catenin downregulates the transcription and expression of endogenous PD-L1 protein in NPC cells

After discovering a good correlation between the expression of PD-L1(22c3) and p-β-catenin^{Tyr654} in prior work, we studied this relationship further in NPC cell lines. First, we found that, except for the NPC cell line SUNE1, all other NPC cell lines, including 5–8F, 6–10B, and HK1, had higher levels of β-catenin than PD-L1 alone (Fig. 2A and S1). The staining of PD-L1 and β-catenin in NPC cell lines by immunocytochemistry was basically consistent with the results of western blotting (Fig. 2B). Therefore, for further research, we selected SUNE1 and HK1 cell lines with considerably higher β-catenin expression. The level of PD-L1 was evaluated using the β-catenin inhibitor XAV939, a small molecule that inhibits transcriptional activity. The IC_{50} values of HK1 and SUNE1 cell lines were approximately 0.9 μM and 46 μM, respectively (Fig. 2C). We found that XAV939 inhibited PD-L1 at both the mRNA (Fig. 2D and E) and protein (Fig. 2F-G and S2-3) levels. This was consistent with the findings that XAV939 increased E-cadherin while decreasing cyclinD1, meaning that β-catenin would be more stable on the cell membrane and that transcriptional activity would be inhibited.

4. Discussion

In recent years, there has been a lot of interest in the process and molecular mechanism of PD-1/PD-L1 mediated immune escape in tumor genesis and development. Different types of human cancer cells overexpress PD-L1. Additionally, it is widely expressed in patients with NPC [13]. When tumor cells overexpress PD-L1, their interaction with immunocytes expressing PD-1 can either help tumor cells escape the immune system or promote their malignant biological function. Subsequently, the prognosis of patients worsened [14]. For example, the overexpression of PD-L1 indicated that patients with pancreatic cancer [15] and gastric adenocarcinoma [16] would have shorter survival times. Conversely, it was demonstrated that patients with HPV-positive oropharyngeal squamous cell carcinoma [17] and non-small cell lung squamous cell carcinoma [18] would have higher survival times. It has

Table 2
The pairwise association between expression of p-β-catenin^{Tyr654} and PD-L1 (22c3) in 425 cases of NPC.

	PD-L1 (22c3)		
	Low (%)	High (%)	*P*-value
p-β-catenin^{Tyr654}			
Ne (%)	151(60.6)	98(39.4)	0.016*
Po (%)	86(48.9)	90(51.1)	(r =0.117)

Spearman's rank correlation test, statistically significant difference ($P < 0.05$).

Table 3
Summary of multivariate of Cox proportional regression for overall survival in 425 cases of NPC.

Parameter	B	SE	Wald	Sig.	Exp (B)	95.0% CI for Exp (B)	
						Lower	upper
Age	.415	.210	3.901	.048*	1.514	1.003	2.284
Gender	−.404	.219	3.412	.065	.668	.435	1.025
T stages	.079	.202	.151	.697	1.082	.728	1.607
LNM status	−.917	1.025	.801	.371	.400	.054	2.978
Metastasis	2.189	.203	115.748	.000***	8.925	5.990	13.299
Clinical stages	2.195	1.010	4.722	.030*	8.980	1.240	65.024
p-β-catenin^{Tyr654}	.192	.199	.929	.335	1.212	.820	1.791
PD-L1 (22c3)	.469	.195	5.797	.016*	1.599	1.091	2.342
p-β-catenin/PD-L1 (22c3)$^{\#}$	−.491	.391	1.583	.208	.612	.284	1.316

Abbreviations: CI, confidence interval; LNM, lymph node metastasis; NPC: Nasopharyngeal carcinoma.
Note: multivariate analysis of Cox regression, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

previously been considered uncertain and controversial as to the significance of PD-L1 overexpression in the clinical prognosis of patients with NPC [13,19]. In our study, 425 patients with NPC have examined for PD-L1(22C3) expression. The findings showed that while the overall survival of patients with NPC with overexpression of PD-L1 decreased, the expression of PD-L1 was maintained at a high level in most patients with NPC. This suggests that overexpression of PD-L1 may serve as an independent molecular marker of poor prognosis.

Among the 425 nasopharyngeal carcinoma patients included in this study, staining of PD-L1 (22c3) was assessed for TPS. After differentiation of the tumors by a ≥50% cut off, the rate of high expression of PD-L1 is 44%, which is higher than in HNSCC [12]. There is significant heterogeneity in staining intensity of PD-L1. Some cases show TPS ≥50%, but the staining intensity is weak, while some cases show TPS<50%, but the staining is strong (supplementary, Table S). This reminds us to be vigilant to avoid missing some weakly positive expression cases, which may lead to incorrect evaluation of TPS. In addition, we used two pathologists for double-blind interpretation based on a TPS of ≥50%, the consistency between them was as high as 90% (pathologist1:188/425, pathologist2:174/425). This difference is mainly reflected in the judgment of the 50% threshold, which suggests that the TPS ≥50% is easy to generalize. For the interpretation of PD-L1%, there is no statistical difference between two pathologists (pathologist 1: 37.86 ± 34.224, pathologist 2: 37.86 ± 32.964). We also found that for NPC patients, co-expressed PD-L1 and p-β- catenin^{Tyr654} has a shortest survival time, indicating that the combined expression of the two markers serve as an evaluation indicator for poorer prognosis in patients. Furthermore, we found that the correlation between PD-L1 or p-β-catenin^{Tyr654} and the clinical pathological characteristics of patients showed good consistency. Both are associated with distant metastasis and poor prognosis in patients.

The model of Wnt signal transduction includes the phosphorylation of β-catenin at the Tyr654 site. β-catenin is phosphorylated at Tyr654 after the activation of growth factor receptor tyrosine kinase (RTK). This adds a negative charge, changing the conformation of the C-terminal of β-catenin, impacting the protein-protein interaction. E-cadherin and β-catenin interaction are one of the interactions. The cytoplasmic concentration of free β-catenin increases as the affinity of E-cadherin and β-catenin decreases. The recruitment of transcription mechanism proteins like TCF4, TBP, and CBP is the outcome of the C-terminal phosphorylation of β-catenin. Finally, the Wnt signal increases with higher metastasis and activation of other carcinogenic pathways [20]. This is consistent with our findings. We found that distant metastasis and a poor prognosis are the main causes of the expression of p-β-catenin^{Tyr654} in NPC.

Several complex processes, including gene transcription, post-transcriptional and post-translational modifications, and exosome transport, control the expression of PD-L1. In various cancer types, MYC enhances the expression of PD-L1 by binding to its promoter [21]. Anaplastic lymphoma kinase (ALK), whose overactivated signal pathway is caused by NPM-ALK gene fusion, is another factor contributing to the overexpression of PD-L1 [22]. In addition to MYC and ALK, mutant or excessively activated HIF1/2α, NF-κB, MAPK, PTEN/PI3K, and EGFR carcinogenic pathways can also enhance PD-L1 mRNA expression [23]. In fibroblasts, activation of the Wnt/β-catenin pathway can upregulate PD-L1 expression [24]. AKT participates in the production of PD-L1 expression in glioblastoma cells by the β-catenin/TCF/LEF complex, which binds to its promoter [8]. These findings are consistent with blocking AKT and PD-L1 in GBM [25]. Additionally, the histone demethylase inhibitor (5-carboxy-8-hydroxyquinoline (IOX1)) led to the downregulation of β-catenin, which in turn led to the downregulation of PD-L1 [26]. In the mouse lung cancer model, Wnt/β-catenin inhibition enhanced the tumor response to PD-L1 blockade [27]. According to our study, PD-L1 and p-β-catenin^{Tyr654} were positively correlated in NPC. And when XAV939 targeted β-catenin, the expression of PD-L1 was also inhibited.

We also noticed that when the concentration of XAV939 reached at 5 μM in HK1 or 75 μM in SUNE1 cell lines, the inhibitory effect of XAV939 on PD-L1 seemed to rebound. We considered that this might be the adaptive anti-cancer drug resistance mechanisms. Targeted therapies often cease to be effective due to adaptive mechanisms triggered in response to treatment. For instance, resistance to oncogene-targeted drugs is frequently due to the upregulation of parallel signaling cascades [28]. XAV939 is representative TNKS inhibitor that abrogate WNT/β-catenin signaling and tumorigenesis [29]. Therefore, when using the overload dose of XAV939 to inhibit the transcriptional activity of β-catenin may activate other transcriptional mechanisms of PD-L1, which reminds us to pay attention to the dosage of the drug in the use of targeted therapy to avoid the occurrence of adaptive resistance.

This study aimed to investigate the prognostic significance of PD-L1 and p-β-catenin^{Tyr654} in NPC cells. Our findings indicated that patients with distant metastases or poor prognoses exhibited higher levels of PD-L1 and p-β-catenin^{Tyr654} expressions. According to Cox multivariate prognostic analysis, PD-L1 was a useful signal for predicting the survival status of patients with NPC. Furthermore, we

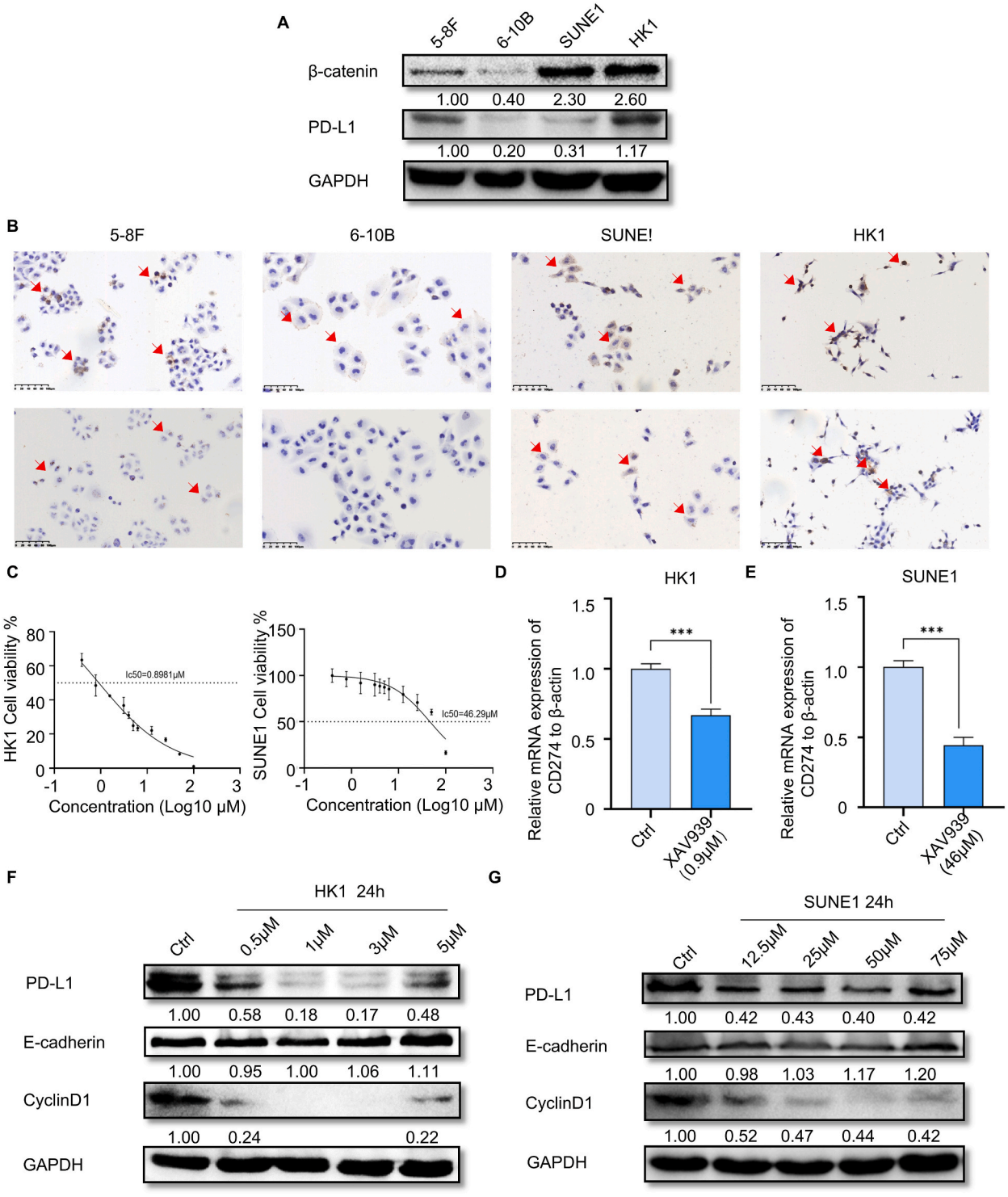

Fig. 2. Suppression of nuclear β-catenin down-regulates the transcription and expression of endogenous PD-L1 protein in NPC cells. (A)Expression of β-catenin in NPC cell lines. (B) The staining of β-catenin and PD-L1 in NPC cell lines by immunocytochemistry. (C) The IC_{50} of XAV939 in SUNE1 and HK1 cell lines. XAV939 significantly inhibited the mRNA of CD274 in HK1(D) and SUNE1(E) cell lines. XAV939 significantly suppressed the expression of PD-L1 and cyclin D1, but induced the expression of E-cadherin in HK1(F) and SUNE1(G) cell lines.

demonstrated that β-catenin might engage directly or indirectly in the regulation of PD-L1 transcription. This is for developing the β-catenin or TCF4 inhibitor as a potential new option for immunosuppression of the immune checkpoint in NPC.

Data availability statement

Data included in article/supplementary material/referenced in article.

Competing interests

The authors declare that they have no conflicts of interest.

Funding/Support

This work was supported by the National Nature Science Foundation of China (grant number: 81802791, 81972838) and the Natural Science Foundation of Hunan Province (2021JJ40854, 2022JJ30966).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Haihua Wang; Weiyuan Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yuting Zhan; Kaiju Luo: Performed the experiments; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Shuping Peng; Songqing Fan: Conceived and designed the experiments; Analyzed and interpreted the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18130.

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