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Clinicopathological and prognostic significance of p16 protein in nasopharynx cancer patients
A PRISMA-compliant meta-analysis

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Abstract

Background: p16 protein is significantly down-regulated in several cancers, which reveals that it may be a potential biomarker for cancers. However, the clinicopathological and prognostic value of p16 protein in nasopharynx cancer patients remains unclear. Therefore, we performed a meta-analysis to assess the relationships of p16 protein expression with the clinicopathological features and prognosis of nasopharynx cancer.

Methods: PubMed, Web of Science, Embase, and Chinese CNKI were searched to obtain eligible data. The relationships of p16 protein expression with risk, clinicopathological features, and prognosis of nasopharynx cancer were analyzed with stata 14.0 software. The pooled odds ratio (OR) with 95% CI (confidence interval) and hazards ratio (HR) with 95% CI were calculated to evaluate the association between p16 protein expression and nasopharynx cancer.

Results: A total of 28 studies with 2612 nasopharynx cancer patients were included in the meta-analysis. p16 protein expression was significantly associated with the risk, lymph node metastasis, TNM-stage (tumor-node-metastasis), distant metastasis, and T stage of nasopharynx cancer (Risk, OR = 17.82, 95% CI = 11.20–28.35; Lymph node metastasis, OR = 2.11, 95% CI = 1.42–3.14; TNM-stage, OR = 2.25, 95% CI = 1.54–3.28; Distant metastasis, OR = 3.43, 95% CI = 1.55–7.58; T-stage, OR = 1.72, 95% CI = 1.27–2.33). The negative rate of p16 protein expression in control group was 8.77%, while the negative rate of p16 protein expression in the nasopharynx cancer tissue was 63.78%. However, no significant associations of p16 expression with the overall survival and progression-free survival of nasopharynx cancer were found.

Conclusion: The meta-analysis revealed that downregulated p16 expression was significantly associated with the risk, lymph node metastasis, TNM-stage, distant metastasis, and T stage of nasopharynx cancer. No significant association between p16 protein expression and prognosis of nasopharynx cancer was found. However, additional high-quality and multicenter studies should be conducted to validate these findings in the future.

Abbreviations: CDKN2A = cyclin dependent kinase inhibitor 2A, CDKN2B = cyclin dependent kinase inhibitor 2B, CHFR = checkpoint with forkhead and ring finger domains, CI = confidence interval, GABBR1 = gamma-aminobutyric acid type B receptor subunit 1, HCG9 = HLA complex group 9, HLA = human lymphocyte antigen, HR = hazards ratio, MDM2 = MDM2 proto-oncogene, MECOM = MDS1 and EVI1 complex locus, MMP2 = matrix metallopeptidase 2, NOS = Newcastle–Ottawa Scale, OR = odds ratio, OS = overall survival, PFS = progression-free survival, RAD51L1 = RAD51 paralog B, RASSF1A = ras association domain family member 1, RIZ1 = PR/SET domain 2, TNFRSF19 = TNF receptor superfamily member 19, TNM = tumor-node-metastasis, TP53 = tumor protein p53, WIF1 = WNT inhibitory factor 1.

Keywords: meta-analysis, nasopharynx cancer, p16, prognosis

1. Introduction

Nasopharyngeal carcinoma is a rare cancer with an incidence of 0.4 cases per 100,000 people in United States. Most new cases are

found in Central Asia, South Asia, Southeast Asia, the Arctic, Middle East, and North Africa. In some parts of China, the incidence of nasopharyngeal carcinoma is up to 21 cases per 100,000 population.[1] In addition, the incidence of nasopharyngeal carcinoma in some races is much higher such as: the Bidayuh in Borneo, Nagas in northern India, and Inuits in the Artic. Men also seem to have a more significant predisposition for nasopharyngeal carcinoma than women.[2] Several studies have discussed the influence of EBV virus infection in the development of nasopharyngeal carcinoma. And EBV virus RNA is often detected in nasopharyngeal carcinoma cells but not in normal nasopharyngeal epithelium with in-situ hybridization techniques, which indicated that EBV virus infection plays an important role in the pathogenesis of nasopharyngeal carcinoma.[3] Even EBV virus infection is found in initial phases of malignant transformation of nasopharyngeal carcinoma.[4] However, EBV virus RNA is not detected in the nasopharyngeal tissue samples of high-risk individuals, which reveals that other factors may be involved in the development of nasopharyngeal carcinoma. Therefore, recent studies were extensively performed to seek other important factors, which affected the EBV infection and development of

nasopharyngeal cancer cells. For instance, many susceptibility loci of *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DQ*, *HLA-DR*, *HLA-F* genes were found in genome-wide association studies of large cohorts of nasopharyngeal carcinoma with high-throughput whole-genome sequencing technology.[5] Other non-HLA susceptibility loci of some genes such as *GABBR1*, *HCG9*, *TNFRSF19*, *MECOM*, *CDKN2A*, *RAD51L1*, *MDM2*, *TP53*, *MMP2*, and *CDKN2B* were also identified in several case-control studies.[6] Additionally, relevant epidemiological studies found some other environmental factors such as: dietary and social practices, which were significantly associated with the risk of nasopharyngeal carcinoma.[11] The long history of salted fish consumption and long-term of exposure in N-nitrosamine increased the risk of nasopharyngeal carcinoma.[7] However, the changing of cancer cell genome and signal pathway of nasopharyngeal carcinoma were still research hotspot.

p16 gene, an important tumor suppressor gene, is frequently inactivated in nasopharyngeal cancer biopsy. p16 protein inactivation promotes the transition from low grade nasopharyngeal cancer to higher grade lesions and affects the stability of virus RNA.[8] *p16* tumor suppressor gene is located on 9p21, and its encoded protein – p16 blocks the G1-S phase of the cell cycle and inhibits the abnormal proliferation of cancer cells.[9] Furthermore, p16 protein inhibits the activation of cyclin-dependent kinase 4 and the phosphorylation of pRb, and further restrains the cell cycle.[10] Mutation, deletion, and abnormal methylation of *p16* gene were also found in the head and neck carcinoma tissue, which had significant correlations with the risk, development, and prognosis of head and neck carcinoma.[11] Thus, the associations of p16 protein expression with nasopharyngeal cancer development and progression were analyzed and discussed in the present study. According to the results of literature searching, no systematic meta-analysis was performed to evaluate the relationship between p16 protein and nasopharyngeal cancer. Thus, we conducted the present meta-analysis to assess the potential value of p16 protein in the development of nasopharyngeal cancer.

2. Materials and methods

2.1. Study strategy

According to the PRISMA guideline, literature searching was performed in Web of science, PubMed, Embase, and CNKI databases, while the following key words were used: "nasopharyngeal carcinoma", "p16", "CDKN2A", "p16INK4A", "nasopharyngeal cancer", "expression", "nasopharynx cancer", and "p16 protein".[12] The literature retrieval was completed on June 8, 2018. References of included studies and reviews were carefully scanned to obtain eligible studies. Moreover, the present study was meta-analysis and did not involve the collection of samples. Therefore, ethical approval was not required.

2.2. Inclusion criteria and exclusion criteria

The following inclusion criteria of the literatures were as follows:

1. accurate diagnosis of nasopharyngeal carcinoma;
2. associations of p16 protein expression with risk, clinical features, and overall survival of nasopharyngeal cancer;
3. immunohistochemistry (IHC) and western blotting were applied to detect the p16 protein expression;
4. articles were published in English and Chinese language with full texts.

The exclusion criteria were as follows:

1. overlapped studies and studies with repeated data;
2. articles with insufficient data to calculate or extract the HR, OR, and 95% CI;
3. review, meta-analysis, letters, and case reports.

2.3. Data extraction

Two researchers independently extracted relevant information from eligible studies. The information included first author's name, year of publication, study country, race, methods of p16 expression detection, number of p16 protein positivity and negativity in cases and controls, TNM classification, T grade, distant metastasis, lymph node metastasis, cut-off value, data of HR and 95% CI, and sample type. Moreover, if data of HR and 95% CI were not found in the included studies, the Kaplan–Meier curve was extracted to calculate the HR and 95% CI with Engauge Digitizer software 4.1 (http://digitizer.sourceforge.net).

2.4. Quality assessment

According to the Newcastle–Ottawa scale (NOS) table (http://www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf), all included studies were evaluated from 3 parts: selection (3 stars), comparability (3 stars), and outcomes (3 stars). Studies with scores of ≥6 were considered to be of high quality. The quality of included studies was independently assessed by 2 investigators.

2.5. Statistical analysis

HR and its 95% CI were applied to evaluate the relationship between p16 protein expression and overall survival and progression-free survival of nasopharynx cancer, while OR and 95% CI were used to assess the association of p16 protein expression with risk and clinical features of nasopharynx cancer. If data of HR and its 95% CI were not found in the included studies, survival curve was used to calculate the HR and 95% CI. Then, the extracted HR and 95% CI were used to calculate the accumulated HR and 95% CI to evaluate the associations between p16 protein expression and overall survival and progression-free survival of nasopharynx cancer. The associations of p16 protein expression with risk and clinical features of nasopharynx cancer were evaluated with pooled OR and 95% CI. If the lower limit and upper limit were both >1 or <1, it implied a signification association. Heterogeneity among studies was tested by I^2 statistics.[13] $I^2 > 50\%$ or $P < .05$ indicated that there was significant heterogeneity among studies, and thus random-effects model was used; otherwise, fixed-effects model was employed. Furthermore, sensitivity analysis was also used to evaluate the stability of the overall results. Begg test and Egger test were conducted to assess potential publication bias of included studies.[14–15] Data analysis was conducted with stata 14.0 software (version 14.0, Stata Corporation, College Station, TX). Finally, all P values were two-sided, and P value of <.05 was considered as significant.

3. Result

3.1. Characteristics of included studies

In total, 307 literatures were retrieved from electronic databases including Web of science, PubMed, Embase, and CNKI databases. After duplication was removed, 283 papers were

Table 1

Main characteristics of included studies which assessed the association of p16 protein expression with the risk and clinical features of nasopharyngeal carcinoma.

Author	Time	Country	Ethnicity	Method	Control			Case			Cut-off value	NOS
					Sample type	p16 +	p16 -	Sample type	p16 +	p16 -		
Lei[16]	1999	China	Asians	IHC	NTNPT	15	0	NPC	75	41	0%	6
Wang[17]	1999	China	Asians	IHC	NPMCIT	10	0	NPC	28	45	0%	6
Mao[18]	1999	China	Asians	IHC	NNPE	38	2	NPC	22	42	0%	6
Dai[19]	2000	China	Asians	IHC	NNPE	20	0	NPC	14	46	10%	6
Mo[20]	2000	China	Asians	IHC	NPMCIT	20	0	NPC	32	42	0%	6
Huang[21]	2001	China	Asians	IHC	NTNPT	20	0	NPC	43	31	10%	6
Liu[22]	2001	China	Asians	IHC	NPMCIT	20	0	PDNPC	26	40	10%	6
Wang[23]	2001	China	Asians	IHC	NTNPT	30	0	NPC	8	22	NR	6
Zhang[24]	2002	China	Asians	IHC	NNPE	18	0	NPC	31	210	NR	6
Chen[25]	2002	China	Asians	IHC	NPMCIT	34	1	NPC	29	26	0%	6
Liang[26]	2002	China	Asians	IHC	NNPE	16	4	PDNPC	14	46	10%	6
Li[27]	2003	China	Asians	IHC	NPMCIT	25	0	NPC-NKCT	45	21	5%	6
Wei[28]	2003	China	Asians	IHC	NPMCIT	67	13	NPC	22	68	0%	6
Yang[29]	2004	China	Asians	IHC	NPMCIT	20	2	NPC	28	32	NR	6
Fan[30]	2004	China	Asians	IHC	NPMCIT	16	4	NPC	10	29	10%	6
Huang[31]	2004	China	Asians	IHC	NPMCIT	15	0	NPC	11	25	NR	6
Li2[32]	2004	China	Asians	IHC	NPMCIT	26	5	NPC	17	53	10%	6
Fan[33]	2006	China	Asians	IHC	NNPE	49	3	NPC	34	114	0%	8
Li[34]	2006	China	Asians	IHC	ACT	16	2	NPC	19	28	5%	8
Wu[35]	2006	China	Asians	IHC	NPMCIT	17	3	NPC	16	34	5%	6
Zhong[36]	2006	China	Asians	IHC	NPMCIT	26	3	NPC-DNKCT	41	4	5%	6
Li[37]	2007	China	Asians	IHC	NPMCIT	20	0	NPC	31	69	5%	6
Liang[38]	2007	China	Asians	IHC	NPMCIT	21	10	NPC-PDSCC	20	29	25%	6
Qu[39]	2007	China	Asians	IHC	NNPE	18	2	NPC	8	22	0%	6
Ji[40]	2010	China	Asians	IHC	NNPE	47	6	NPC-PDSCC	62	89	5%	8
Chen[41]	1999	China	Asians	IHC	–	–	–	NPC	19	31	0%	–
Zhang[42]	2001	China	Asians	IHC	–	–	–	NPC	50	75	0%	–
Hwang[43]	2002	China	Asians	IHC	–	–	–	NPC	23	42	5%	–
Chen[44]	2005	China	Asians	IHC	–	–	–	NPC	16	49	5%	–
Gu[45]	2005	China	Asians	IHC	–	–	–	NPC	20	48	5%	–
Zheng[46]	2000	China	Asians	IHC	–	–	–	NPC	27	42	0%	–
Xiang[47]	2005	China	Asians	IHC	–	–	–	NPC	48	42	10%	–
Jiang[48]	2015	China	Asians	IHC	–	–	–	NPC	40	46	NR	–

ACT = adjacent cancer tissue, IHC = immunohistochemistry, NPC = nasopharyngeal carcinoma, NTNPT = non-tumour nasopharyngeal tissue, NNPE = normal nasopharyngeal epithelia, NPMCIT = nasopharyngeal mucosa chronic inflammatory tissue, NR = no report,, NPC-NKCT = nasopharyngeal carcinoma (non-keratinized cancer tissue), NPC-DNKCT = nasopharyngeal carcinoma (differentiated non-keratinized cancer tissue), NPC-PDSCC = nasopharyngeal carcinoma (poorly differentiated squamous cell carcinoma), PDNPS = poorly differentiated nasopharyngeal carcinoma.

remained. Then, retrieved articles were evaluated by reading titles and abstracts, 218 articles were removed because they were not related with p16 protein expression or nasopharyngeal cancer. In remained 65 literatures, full texts were read carefully and 25 articles were eliminated. Finally, 5 papers were further removed because of repeated data, and 35 articles with 2612 nasopharyngeal cancer patients were included.[16–50] 6 articles were English language and 29 literatures were Chinese language in the eligible studies. (Table 1, Table 2; Fig. 1)

3.2. Association of p16 protein expression with survival of nasopharynx cancer patients

The forest plot was performed to evaluate the associations of p16 protein expression with the overall survival and progression-free survival of nasopharynx cancer. The results indicated that no significant associations of p16 protein expression with overall survival and progression-free survival of nasopharynx cancer was found (Overall survival, HR = 0.65, 95% CI = 0.06–1.23; progression-free survival, HR = 0.16, 95% CI = −0.14 to 0.45).

Table 2

Main characteristics of included studies which assessed the association of p16 protein expression with the prognosis of nasopharyngeal carcinoma.

Author	Time	Country	Ethnicity	Tumor stage	Detected sample	Num.	Follow-up median	Method	Survival analysis	Source of HR	HR	95%CI	HR	95%CI	Cut-off value
Wang[17]	1999	China	Asians	I-IV	Tumor tissue	73	3 years	IHC	p16+ vs p16-	Curve	0.99	0.20-4.93	–	–	25%
Makitie[49]	2003	Canada	Caucasians	I-IV	Tumor tissue	84	8.4 years	IHC	p16+ vs p16-	Curve	0.64	0.27-1.52	–	–	5%
Jiang[48]	2016	USA	Caucasians	I-IV	Tumor tissue	44	3 years	IHC	p16+ vs p16-	Data	0.44	0.04-4.77	0.11	0.02-0.64	60%
Saito[50]	2016	Japan	Asians	I-IV	Tumor tissue	61	3.9 years	IHC	p16+ vs p16-	Data	–	–	0.59	0.17–2.03	70%

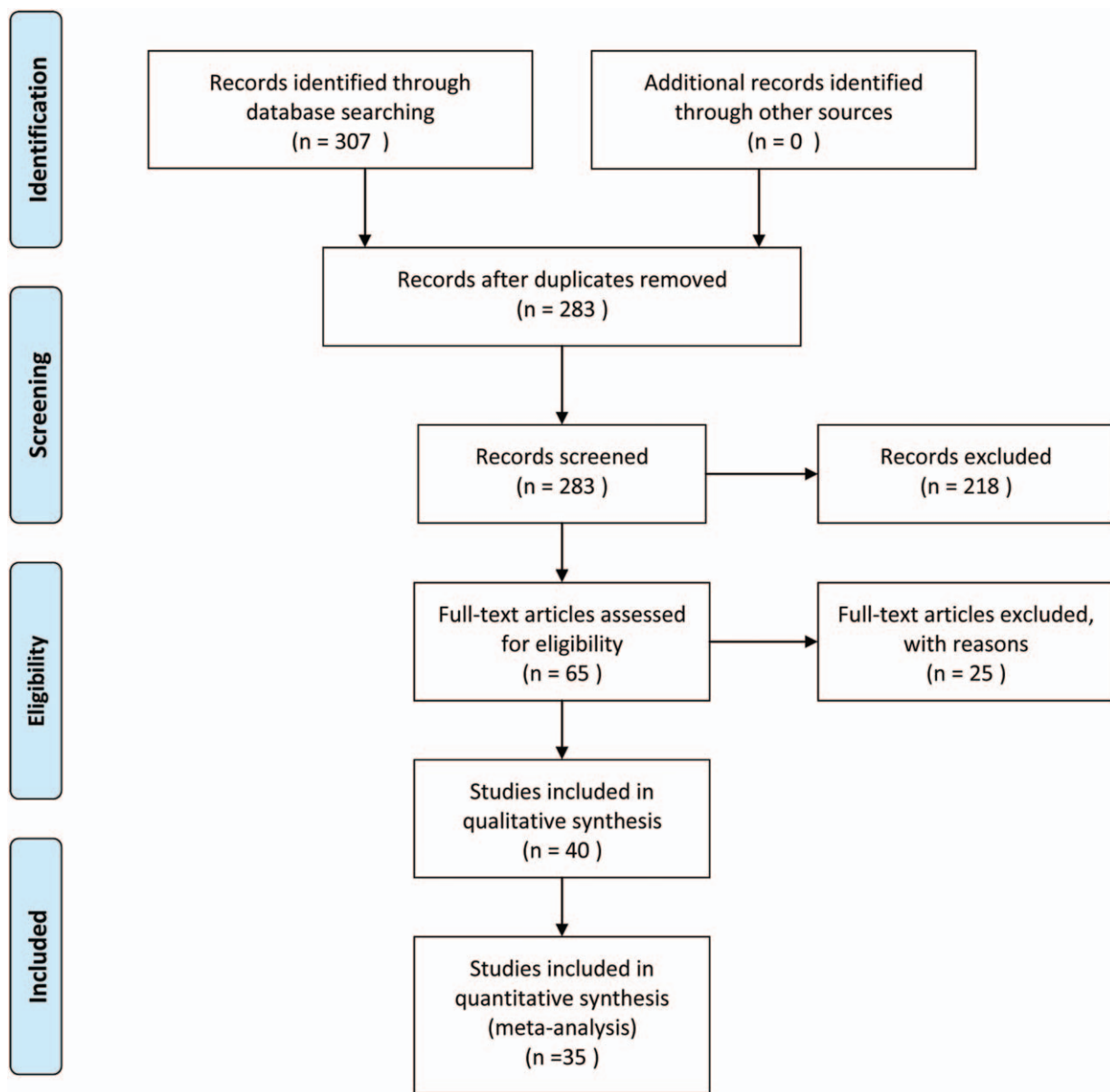IHC = immunohistochemistry, OS = overall survival, PFS = progression-free survival.

Figure 1. Flow diagram of studies selection.

Furthermore, random-effects model was employed due to significant heterogeneity among studies. Subgroup analysis was not performed because only 2 studies or 3 studies were included to discuss the relationship of p16 protein expression with survival of nasopharynx cancer. (Table 3; Fig. 2, Fig. 3, Figs. 5–8)

Table 3

Main results of meta-analysis.

| Group | HR | 95%CI | Publication bias | | Begg test (P value) | Egger test (P value) |
			I^2	P		
Overall survival (p16$^+$ vs p16$^-$)	0.65	0.06–1.23	0.00%	.95	.60	.62
Progression-free survival (p16$^+$ vs p16$^-$)	0.16	−0.14 to 0.45	0.00%	.34	.32	—
	OR					
Risk (Control vs Case; p16$^+$ vs p16$^-$)	17.82	11.20–28.35	47.90%	.004	.01	.002
TNM ((I-II) vs (III-IV); p16$^+$ vs p16$^-$)	2.25	1.54–3.28	42.50%	.04	.30	.79
Lymph node metastasis (N0 vs (N1-N2); p16$^+$ vs p16$^-$)	2.11	1.42–3.14	54.00%	.004	.51	.50
Distant metastasis (M0 vs (M1-M2); p16$^+$ vs p16$^-$)	3.43	1.55–7.58	0.00%	.49	1.00	.52
T stage ((T1-T2) vs (T3-T4); p16$^+$ vs p16$^-$)	1.72	1.27–2.33	46.70%	.06	.10	.55

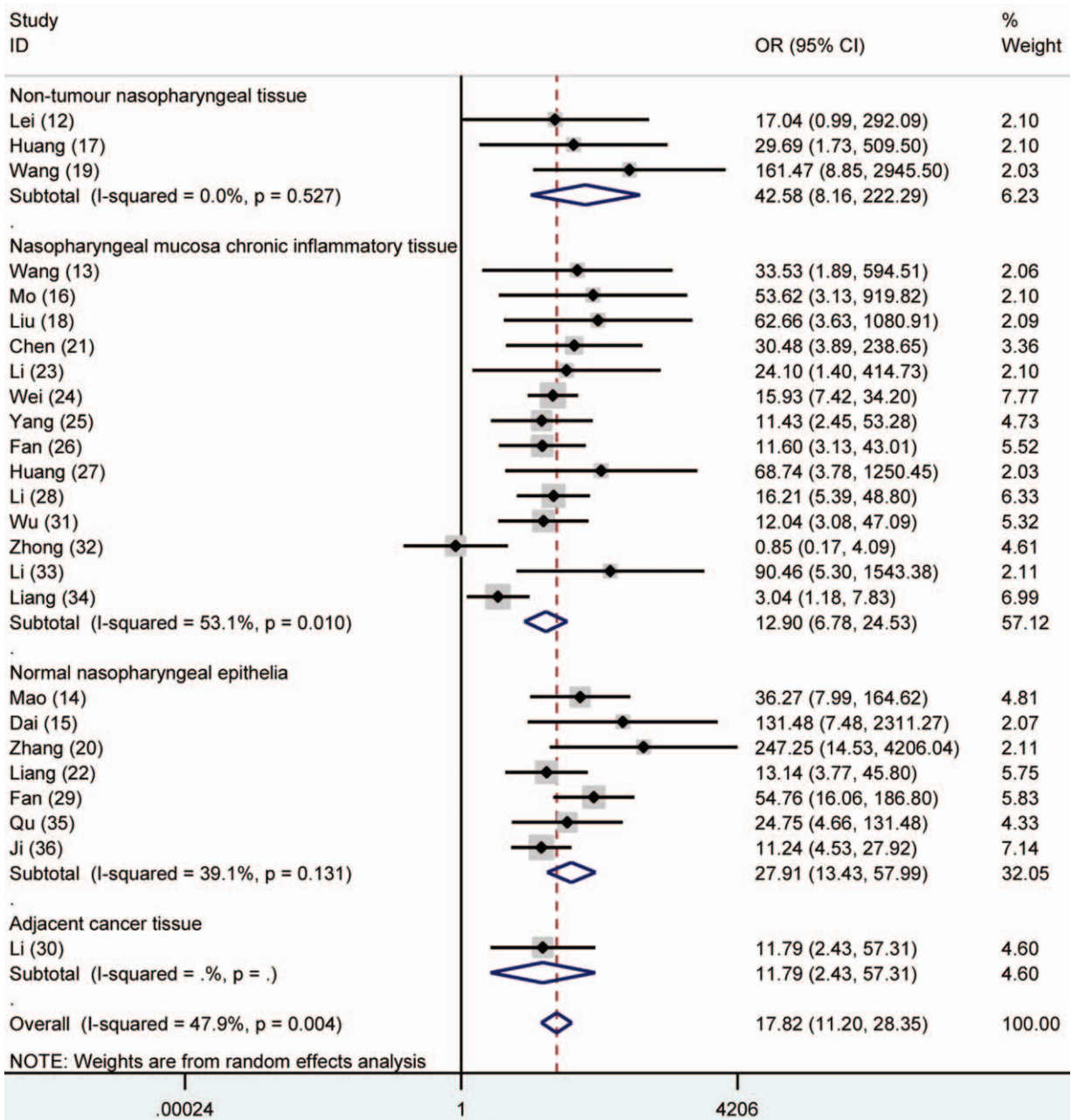HR = hazard ratio, TNM = tumor-node-metastasis.

Figure 2. Forest plot of the association between p16 protein expression and risk of nasopharynx cancer. OR = odds ratio, CI = confidence interval.

3.3. Association of p16 protein expression with clinical features of nasopharynx cancer patients

A total of 25 studies were pooled to evaluate the role of p16 protein expression in the risk of nasopharynx cancer. The results showed that downregulated p16 protein expression was an increased risk factor to nasopharynx cancer (OR = 17.82, 95% CI = 11.20–28.35). The negative rate of p16 protein expression was 8.77% in control group, 63.78% in nasopharynx cancer tissue. However, significant inter-study heterogeneity was found and random-effects model was applied. In order to lower the heterogeneity among studies, subgroup analysis based on control group type was performed, and the results indicated that significant inter-study heterogeneity was reduced. Furthermore, there were significant associations of p16 protein expression with risk, lymph node metastasis, TNM-stage, distant metastasis, and

T stage of nasopharynx cancer (Risk, OR = 17.82, 95% CI = 11.20–28.35; Lymph node metastasis, OR = 2.11, 95% CI = 1.42–3.14; TNM-stage, OR = 2.25, 95% CI = 1.54–3.28; Distant metastasis, OR = 3.43, 95% CI = 1.55–7.58; T-stage, OR = 1.72, 95% CI = 1.27–2.33). Additionally, included studies were mainly performed in Chinese population. So genetic differences were not discussed in the present study and no subgroup analysis based on ethnicity was conducted. The results of stratified analysis based on control sample type suggested that heterogeneity among studies decreased. (Table 3; Fig. 4)

3.4. Publication bias and sensitivity analysis

In the pooled analysis regarding the risk, TNM-stage, and lymph node metastasis of nasopharynx cancer, significant heterogeneity among studies was found. Therefore, random-effects model was
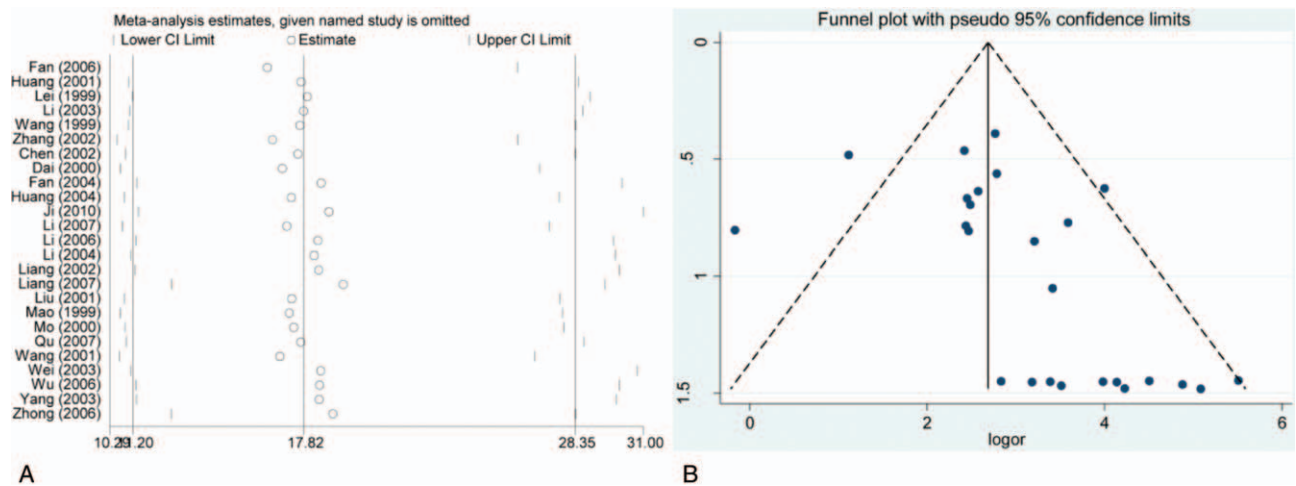
Figure 3. Sensitivity analysis and funnel plot of the association between p16 protein expression and risk of nasopharynx cancer. A, sensitivity analysis, B; funnel plot. CI = confidence interval, OR = odds ratio.

applied to improve the accuracy of results. In addition, $P < .05$ of Begg test and Egger test for the analysis about risk of nasopharynx cancer was found. So, some potential factors might contribute to the significant publication bias. We speculated that different stage cancer sample might lead to the significant publication bias, because no significant heterogeneity and publication bias was found in the analysis about lymph node metastasis, distant metastasis, and T stage of nasopharynx cancer. In addition, sensitivity analysis revealed that the overall results were stable. (Figs. 3,Figs. 5–8)

4. Discussion

In the present study, the associations of p16 protein expression with risk, clinical features, and prognosis of nasopharynx cancer were comprehensively evaluated with 35 studies. Previous studies have found significant decreased expression of p16 protein in the tissue of nasopharynx cancer.[16–40] The negative rate of p16 protein expression in the tissue of late stage nasopharynx cancer was much higher.[17,20,22,24,26,27,29,30,32,39,40,42,43,46] In addition, published study simultaneously detected the status of *p16*,

RASSF1A, *WIF1*, *CHFR*, and *RIZ1* gene promoter in the nasopharyngeal carcinoma and normal nasopharyngeal tissue, and the study have found the higher methylation in the promoter of these genes in nasopharynx cancer tissue.[51] So the result might imply that the expression of the tumor suppressor gene was significantly associated with the development of nasopharynx cancer. Previous study has found that the knockout of *p16* gene in vivo and in vitro significantly affected the growth of normal cell and increased the incidence of cancer.[52]

The present study has shown that downregulated p16 protein expression significantly increased the risk of nasopharynx cancer. The negative rate of p16 protein expression in nasopharynx cancer was much higher compared to normal nasopharynx tissue or nasopharyngeal mucosa chronic inflammatory tissue. The p16 protein expression level of normal nasopharynx tissue and nasopharyngeal mucosa chronic inflammatory tissue might have an obvious difference, since significant heterogeneity between the 2 studies was found. Subgroup analysis based on control sample type showed that heterogeneity was lowered and no significant heterogeneity was found. And the overall results were consistent with the study of Fan et al and Huang et al.[21,33] Other report
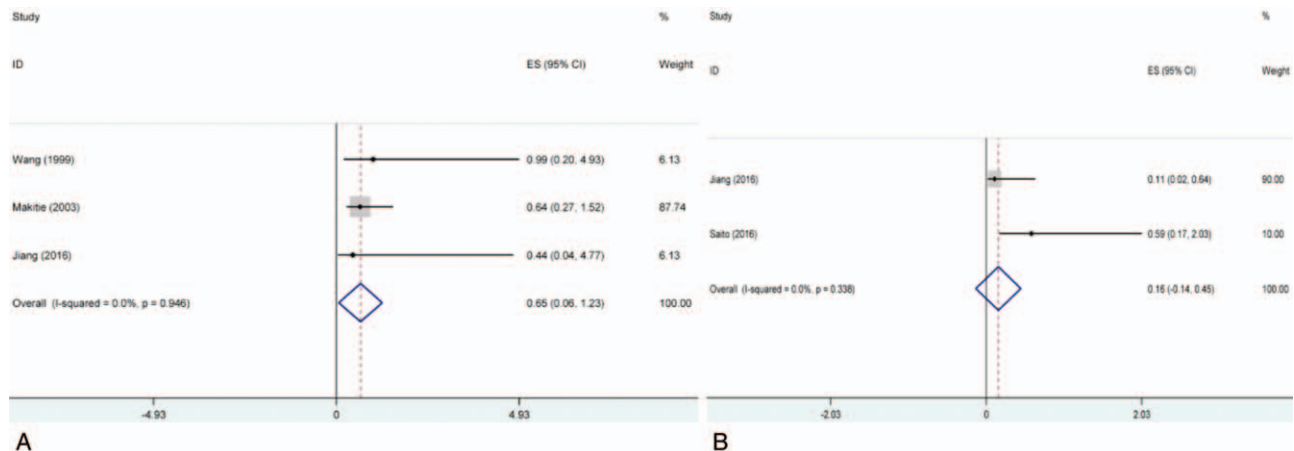

Figure 4. Forest plot of the association between p16 protein expression and prognosis of nasopharynx cancer. A, overall survival; B, Progression-free survival. CI = confidence interval, HR = hazard ratio.
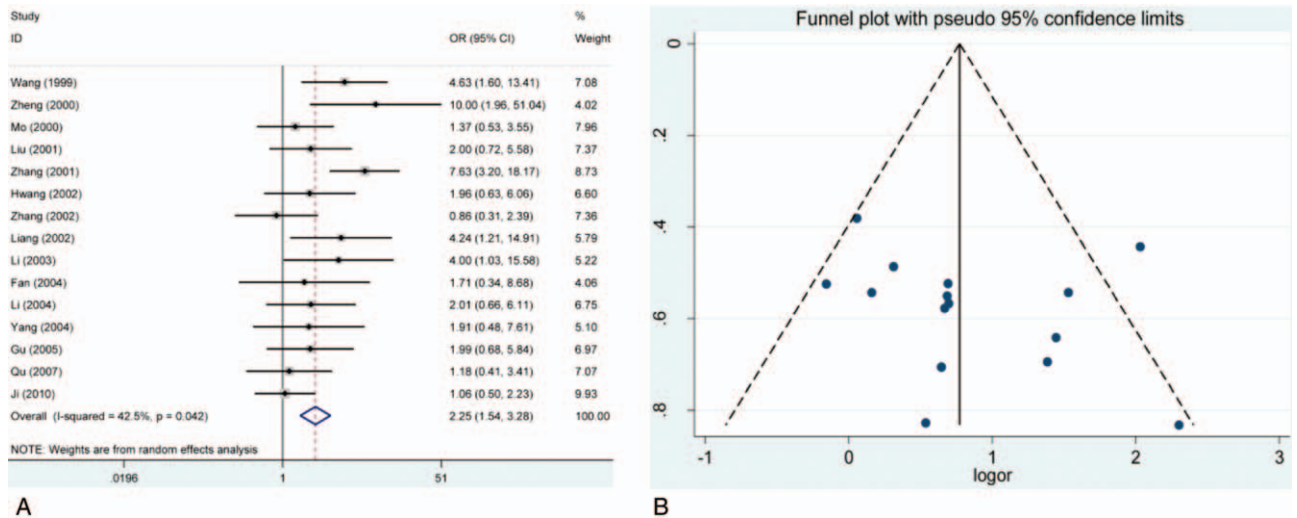
Figure 5. Forest plot and funnel plot of the association between p16 protein expression and TNM-stage of nasopharynx cancer. A, forest plot; B, funnel plot. CI = confidence interval, OR = odds ratio, TNM = tumor-node-metastasis.

also found loss of p16 expression and *p16* gene deletion in the tissue of nasopharyngeal cancer.[53] In order to further explore the role of p16 protein expression in the development of nasopharynx cancer, we investigated the relationship between p16 protein expression and TNM-stage, lymphatic metastasis, distant metastasis, and T-stage of nasopharynx cancer. As expected, downregulated p16 protein expression was significantly associated with the TNM-stage, lymphatic metastasis, distant metastasis, and T-stage of nasopharynx cancer. These results demonstrated that p16-positive lesions had a crucial role in the progression of nasopharynx carcinoma. In these included original studies of the meta-analysis, 4 studies have obtained positive results, while other eleven reports did not find the significant association about TNM-stage. In published reports about other cancers, p16 protein negative status also was significantly related with multiple myeloma and colorectal cancer.[54,55] However, significant heterogeneity was found in

the analysis regarding TNM-stage and lymph node metastasis of nasopharynx carcinoma. As we all known, nasopharynx carcinoma has 3 pathological subtypes according to WHO criteria, including differentiated tumors with surface keratin, non-keratinising differentiated, and undifferentiated tumors.[56] In addition, EBV infection and other gene expression status also affected the progression of nasopharynx carcinoma.[5,7] Therefore, selection of nasopharynx carcinoma sample and status of other gene expression might have some relationships with the significant heterogeneity. And no significant associations of p16 protein expression status with the distant metastasis and T-stage of nasopharynx carcinoma were found.

In order to explore the potential value of p16 expression in the prognosis of nasopharynx carcinoma, we merged the HR and 95% CI of survival curve of nasopharynx cancer patients. The pooled overall HR and 95% CI suggested that p16 protein did not significantly influence the prognosis of nasopharynx
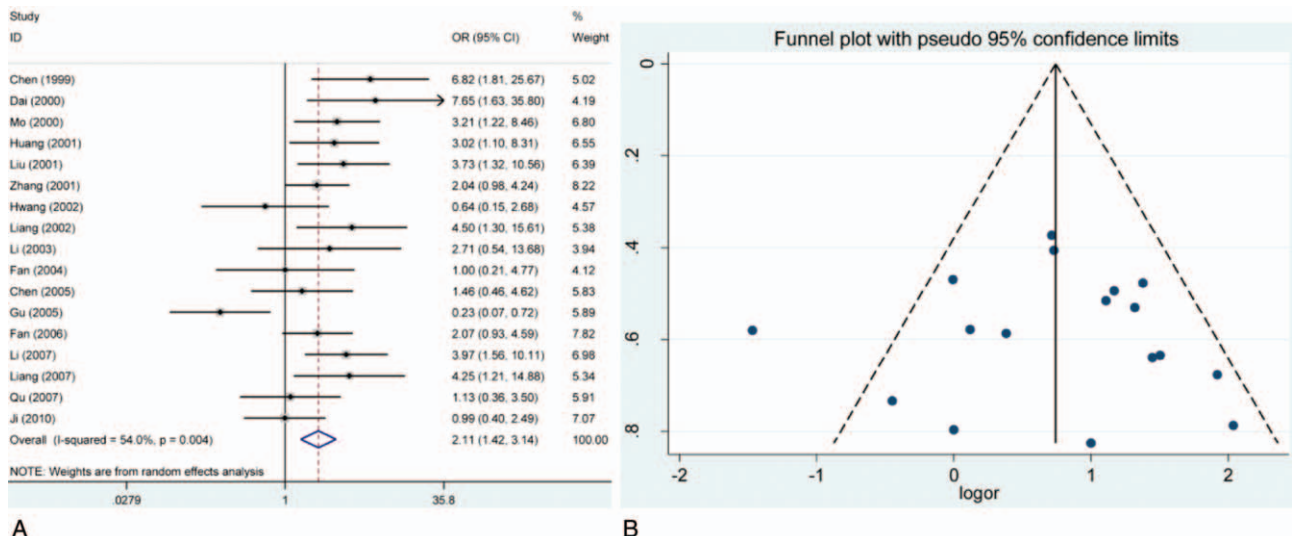

Figure 6. Forest plot and funnel plot of the association between p16 protein expression and lymph node metastasis of nasopharynx cancer. A, forest plot; B, funnel plot. CI = confidence interval, OR = odds ratio.
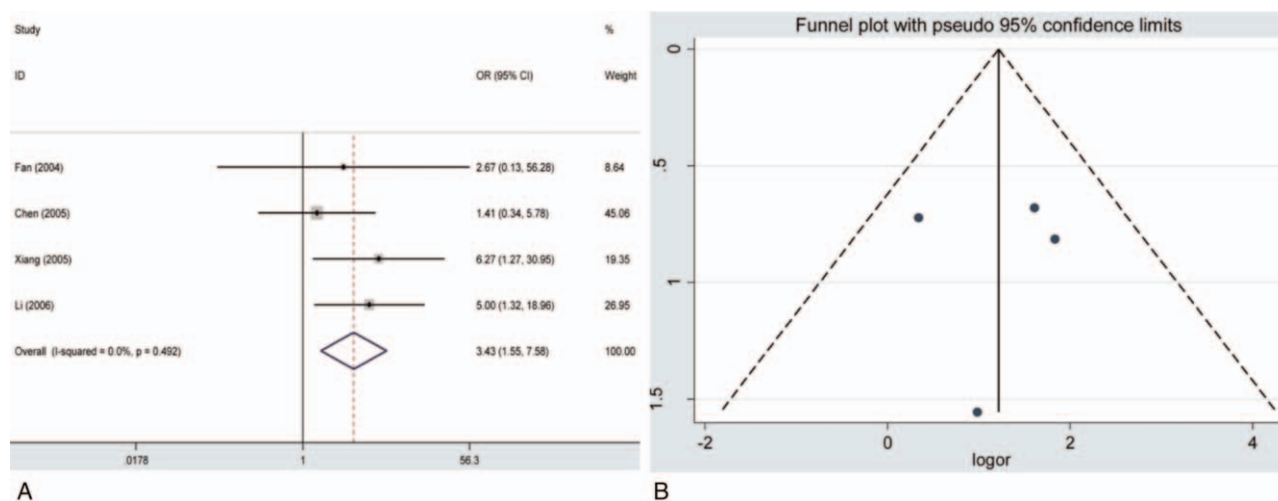
Figure 7. Forest plot and funnel plot of the association between p16 protein expression and distant metastasis of nasopharynx cancer. A, forest plot; B, funnel plot. CI = confidence interval, OR = odds ratio.
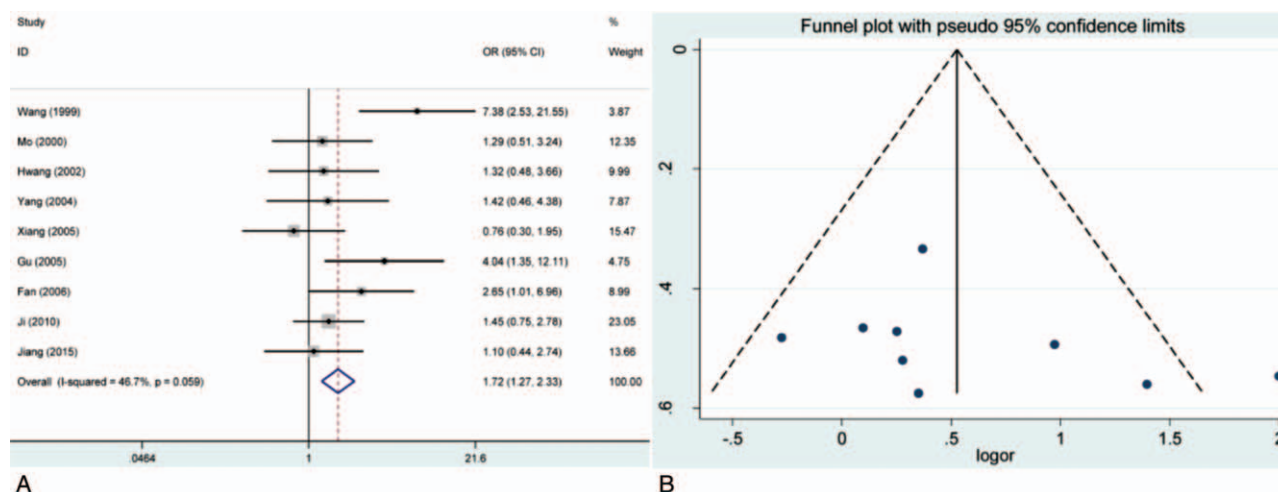

Figure 8. Forest plot and funnel plot of the association between p16 protein expression and T stage of nasopharynx cancer. A, forest plot; B, funnel plot. CI = confidence interval, OR = odds ratio.

carcinoma. In the eligible studies, only Jiang report has found significant correlation of p16 protein expression and prognosis of nasopharynx carcinoma.[48] Similar result was also found in colorectal cancer.[54] However, significant association between p16 expression and prognosis of cancer was found in many other cancers such as prostate cancer, esophageal squamous cell carcinoma, and breast cancer.[57–59] Given the function of p16 protein in cell growth and the association between p16 protein expression and development of nasopharynx carcinoma, false negative results might be found. Thus, more multicenter, large-scale studies were urgent to be performed to validate these findings.

Several limitations should be acknowledged in the present study. First, the studies evaluating the associations of p16 protein with risk and clinical features of nasopharynx carcinoma were all about Chinese population. So, it might only reflect the role of p16 protein expression in the progression of nasopharynx carcinoma in Chinese population. Second, the number of sample about nasopharynx carcinoma prognosis was too few, and only 262

nasopharynx carcinoma patients were enrolled in the meta-analysis. In addition, the follow-up time of patients was inconsistent. Third, the status of other genes and EBV virus infection was unclear, which might affect the accuracy of the results.

Despite these limitations, we have demonstrated that p16 protein expression was significantly associated with the risk, TNM-stage, distant metastasis, lymph node metastasis, and T-stage of nasopharynx cancer. In addition, further studies with more confounding factors such as: large-scale sample, multi-ethnic, and more clinical information should be conducted to investigate the role of p16 protein expression in the prognosis of nasopharynx cancer.

Author contributions

Methodology: Qingli Huang.
Resources: Lingling Sun.
Software: Lingling Sun.
Validation: Qingli Huang, Jingjing Song.
Visualization: Qingli Huang.
Writing – original draft: Lingling Sun, Jingjing Song.

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