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Expression of vimentin in nasopharyngeal carcinoma and its possible molecular mechanism: A study based on immunohistochemistry and bioinformatics analysis

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ABSTRACT

Background: Although previous researchers have analyzed the expression level of vimentin in nasopharyngeal carcinoma (NPC), the sample size of each study was too small, and there was no further in-depth study utilizing microarray and RNA-sequencing data. More importantly, the role and molecular mechanism of vimentin in NPC have not yet been addressed comprehensively. Accordingly, the aim of the present research was to conduct a full exploration of the clinical significance of vimentin in NPC in a large sample size.

Materials and methods: Immunohistochemistry was used to test the expression of vimentin in clinical samples. Data from relevant microarray and RNA-sequencing datasets were screened and extracted to explore the clinical role of vimentin in NPC. Subsequently, vimentin-related signaling pathways were investigated via in-silico approaches.

Results: The clinical immunohistochemistry detection showed the positive expression ratio of vimentin was 24.6% (14/57) of the NPC specimens, whereas vimentin expression was negative in nasopharyngitis (NPG) tissues (0/20, P = 0.016). The mRNA and protein levels of vimentin were both remarkably up-regulated in NPC based on 196 and 1566 cases, respectively. The protein level of vimentin was also a risky factor for the prognosis prediction of NPC with the hazard ratios (HR) being 3.831. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analyses, the localization of vimentin was in both the cytoplasm and the cytoskeleton, and vimentin was involved in the regulation of molecular function, the execution phase of apoptosis, and the regulation of cellular component organization.

Conclusion: The high expression of vimentin plays a pivotal role in the development and poor progression of NPC, which indicates that vimentin may be an effective predictive indicator for NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a highly malignant head-neck epithelial cancer that most commonly occurs around the Eustachian tube of the pharyngeal wall in the nasopharynx [1,2]. The results of epidemiological investigations suggest that the key factors affecting the distribution of NPC are regionalism and ethnicity. Much of the existing literature mentions that NPC is mainly distributed in South China, Southeast Asia, and Africa. Several reports have shown an incidence rate for nasopharyngeal cancer of over 20 per 100,000 in Southeast Asia and over 15 per 100,000 in South China. The incidence of NPC is lower in Western countries, but Challapalli et al. found differences in NPC survival rate among various ethnic groups in the United States [3–8]. In recent years, the local control rate of NPC has been improved; however, the incidence of NPC in South China is still high compared to most other areas.

In the past, NPC accounted for 3.5% of all new carcinoma cases in the world [9,10]. Furthermore, its higher rate of recurrence and metastasis compared to other tumors seriously reduces the survival time of patients, and metastasis to the lymph nodes in the neck occurs quite readily, even at the early stage of the disease [11,12]. Radiation therapy has always been the most effective treatment modality because of the anatomical restrictions and radio-sensitivity of the nasopharyngeal region. However, the resistance of NPC to radiotherapy and chemotherapy is one of the main reasons for the failure of NPC treatment [13–15]. For instance, 20–30% of NPC patients without initial

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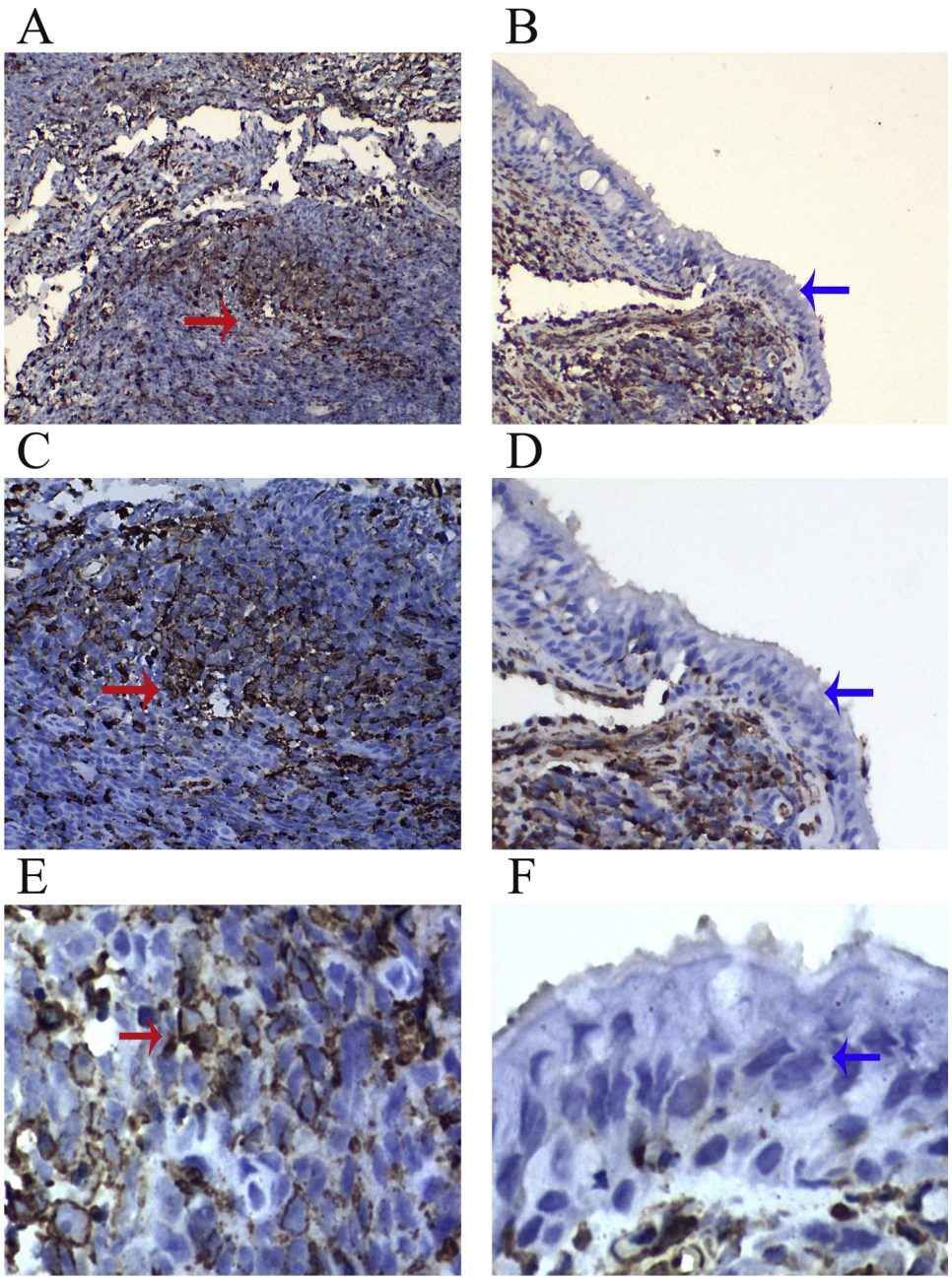

Fig. 1. Immunohistochemical staining of vimentin in nasopharyngeal carcinoma (NPC) and nasopharyngitis (NPG) specimens. (A) NPC (100×). (B) NPG (100×). (C) NPC (200×). (D) NPG (200×). (E) NPC (400×). (F) NPG (400×). Red arrows indicated the expression signals of vimentin in NPC tissues (A, C, E). Blue arrows depicted the negative staining of vimentin in non-cancerous squamous cells in NPG (B, D, F).

metastases will still develop tumor recurrence or distant metastases after radiotherapy [16]. Distant metastasis is also the main cause of treatment failure in patients with advanced NPC [17]. Therefore, a better understanding is needed regarding the factors that modulate the occurrence and molecular mechanism of NPC, as this knowledge may provide new therapeutic directions and targets.

Several factors at both the macroscopic and microscopic levels have different promoting or inhibiting effects in NPC. Many researchers have shown that the risk factors for NPC include environmental factors, genetic susceptibility, Epstein Barr virus infection, occupational exposure, and unhealthy lifestyle habits [18–20]. The gradual deepening and expansion of gene research has also revealed polymorphisms of certain genes, such as COX-2, Pinx1, microRNA-184, microRNA-24, and microRNA-663b, that are associated with the risk of developing NPC [21–25]. However, even considering these previous studies, the level of in-depth research on diverse therapeutic targets for NPC is far from adequate.

One tumor-related gene of considerable interest codes for vimentin, a type-III IF protein involved in cell migration, motility, and adhesion. Vimentin can exert its regulatory effects partially by linking to desmosomes on the cell membrane, and much research has suggested a possible role for vimentin in the epithelial-mesenchymal transition (EMT) [26,27]. The EMT is a physiological process that plays an important role in normal embryonic development and wound healing. During the process of EMT, epithelial cells significantly change their morphological and biochemical behavior; however, recent evidence now supports an abnormal reactivation of the EMT during tumor progression. The EMT is recognized as one of the important forces driving the metastatic dissemination of head and neck squamous cancer, breast cancer, and hepatic cancer by increasing the ability of cells to invade

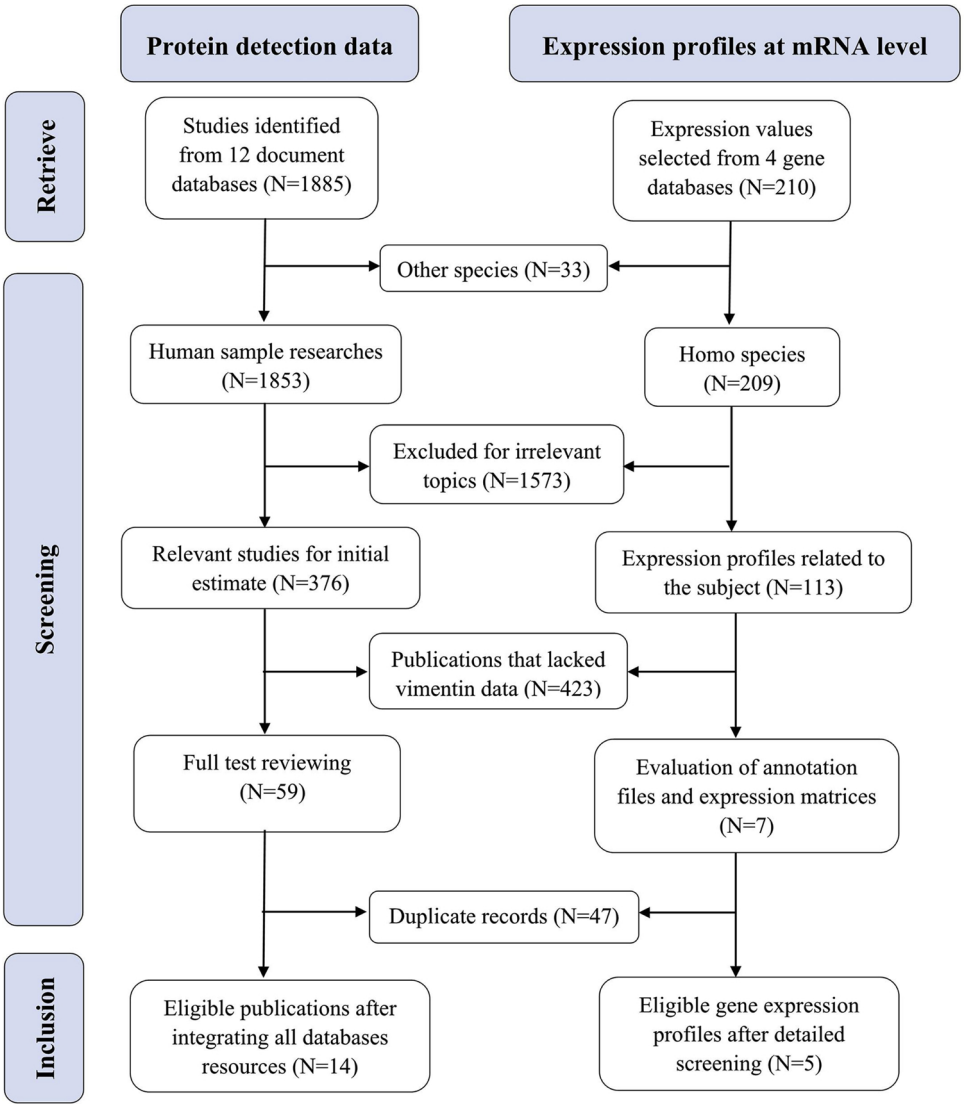

Fig. 2. The flow diagram of the comprehensive search strategy. All the retrieval and screening processes were shown in the figure. The protein detection data and chip data were both included.

and proliferate at distant sites [28,29]. One biochemical hallmark for the EMT is the over-expression of vimentin, which also shows a close relationship with accelerated tumor growth and cancer cell adhesion, migration, and invasion [30,31]. Moreover, accumulating evidence also shows an association between vimentin over-expression, tumor progression, and poor prognosis in several epithelial cancers, including colorectal, gastric, breast, non-small cell lung, cervical, and prostate cancers [28,32–36].

These findings have suggested a relationship between vimentin in cells and various cancers and have prompted studies on the potential diagnostic and therapeutic value of vimentin in NPC. In the past few years, research has proven that the high expression of vimentin predicts an unfavorable prognosis for patients with NPC [37,38]. However, most of these studies have only analyzed cell lines or a few clinical samples by immunohistochemistry, so the molecular mechanisms involved in vimentin interactions in NPC are not yet clear. The aim of the present study was to explore the clinicopathological significance of vimentin in NPC in a large cohort by examining vimentin expression at both the mRNA and protein levels in patients with NPC and by statistically analyzing the available literature.

2. Materials and methods

2.1. Expression analysis of clinical tissues

A total of 77 paraffin-embedded samples, including 57 NPC and 20 nasopharyngitis (NPG) tissues, were obtained from the Third Affiliated Hospital of Guangxi Medical University (Nanning, China). None of the patients who provided samples had received radiotherapy or chemotherapy before the biopsy. The present study was approved by the Third Affiliated Hospital of Guangxi Medical University. In addition, each patient signed an informed consent before the experiment.

2.2. Immunohistochemical analysis of acquired tissues

The expression of vimentin in samples was determined by immunohistochemistry. Briefly, all the paraffin-embedded samples were cut into 4-μm serial slices, deparaffinized, and rehydrated in a graded alcohol series. Subsequently, the sections were subjected to heat-induced antigen retrieval in sodium citrate buffer (10 mM, pH 6.0) in a pressure cooker. Endogenous peroxidase activity was blocked by incubating all sections in 3% hydrogen peroxide. A standard detection system based on an avidin-biotin immunoperoxidase complex was used,

Table 1
Sample information of the included studies.

First author	Publication year	Sample country	Sample size (NPC/NPG)	Vimentin Expression in tissue (P/N)	Gender (M/F)	Age (< 50/≥ 50)	Clinical stage (III-IV/I-II)	T stage (T3-T4/T1-T2)	N stage (N2-N3/N0-N1)	M stage (yes/no)	Lymph node metastasis (yes/no)
Wei-ren Luo [38]	2012	China	122/29	/	92/30	/	89/33	64/58	52/70	15/107	/
Han-guo Jiang [41]	2014	China	130/20	53/97	/	/	/	/	/	/	53/77
Yan-xia Wu [42]	2017	China	119/29	82/66	81/38	66/53	102/17	70/49	77/42	27/92	/
Lei-lei Zhou [43]	2017	China	30/10	16/24	20/10	13/17	18/12	16/14	17/23	12/18	/
You-you Xia [44]	2015	China	124/20	78/66	90/34	69/55	80/44	56/68	58/66	/	/
Wei Wang [37]	2017	China	127/76	76/127	95/32	58/69	/	25/16	/	17/52	62/6
Li-hua Yue [45]	2011	China	88/0	/	/	/	/	/	/	/	18/30
Rong Li [46]	2011	China	28/18	26/20	/	/	/	/	/	/	11/2
Yun-ji Peng [47]	2012	China	60/30	57/33	/	/	/	/	/	/	/
Wei Lu [48]	2010	China	36/20	6/50	/	/	/	/	/	/	/
Qing-ping Jiang [49]	2008	China	58/29	8/79	/	/	/	/	/	/	/
Ai-hua Luo [50]	2013	China	130/0	/	/	/	/	/	/	/	53/77
Wei-ren Luo [51]	2014	China	98/33	92/39	/	/	/	/	/	/	/
Ji Li [52]	2016	China	82/20	23/79	/	/	/	/	/	/	32/50

NPC: nasopharyngeal carcinoma; NPG: nasopharyngitis; P: positive; N: negative; M: male; F: female; /: no corresponding data.

Table 2
Prognostic parameters of the included studies.

First author	Publication year	Sample country	Vimentin Expression in NPC (P/N)	Follow-up (months)	Analysis types for survival	Statistic method	HR (95%CI)	P value
Wei-ren Luo [38]	2012	China	65/57	60	Overall survival	Univariate analysis	3.756 (1.91-7.385)	< 0.001
You-you Xia [44]	2015	China	78/66	36	Overall survival	Univariate analysis	3.364 (1.287-8.793)	0.013
Wei Wang [37]	2017	China	39/31	90*	Overall survival	Survival curves	7.07 (1.14-43.95)	0.0064

NPC: nasopharyngeal carcinoma; NPG: nasopharyngitis; P: positive; N: negative; HR: hazard ratios; 95%CI: 95% confidential interval; *: Follow-up time was obtained from the survival curve.

Table 3
Sample information from the gene expression series.

Author	Publication year	Country	Sample source	Data source	Sample size (NPC/non-cancer)	Platform
Paul Ahlquist	2008	USA	Tissue	GSE12452	31/10	GPL570
Wen-bin Wei	2009	United Kingdom	Tissue	GSE13597	25/3	GPL96
Wen-bin Wei	2012	United Kingdom	Tissue	GSE34573	16/4	GPL570
Chao-nan Qian	2014	China	Tissue	GSE53819	46/45	GPL6480
Wei Xiong	2017	China	Tissue	GSE64634	12/4	GPL570

NPC: nasopharyngeal carcinoma; USA: United States of America.

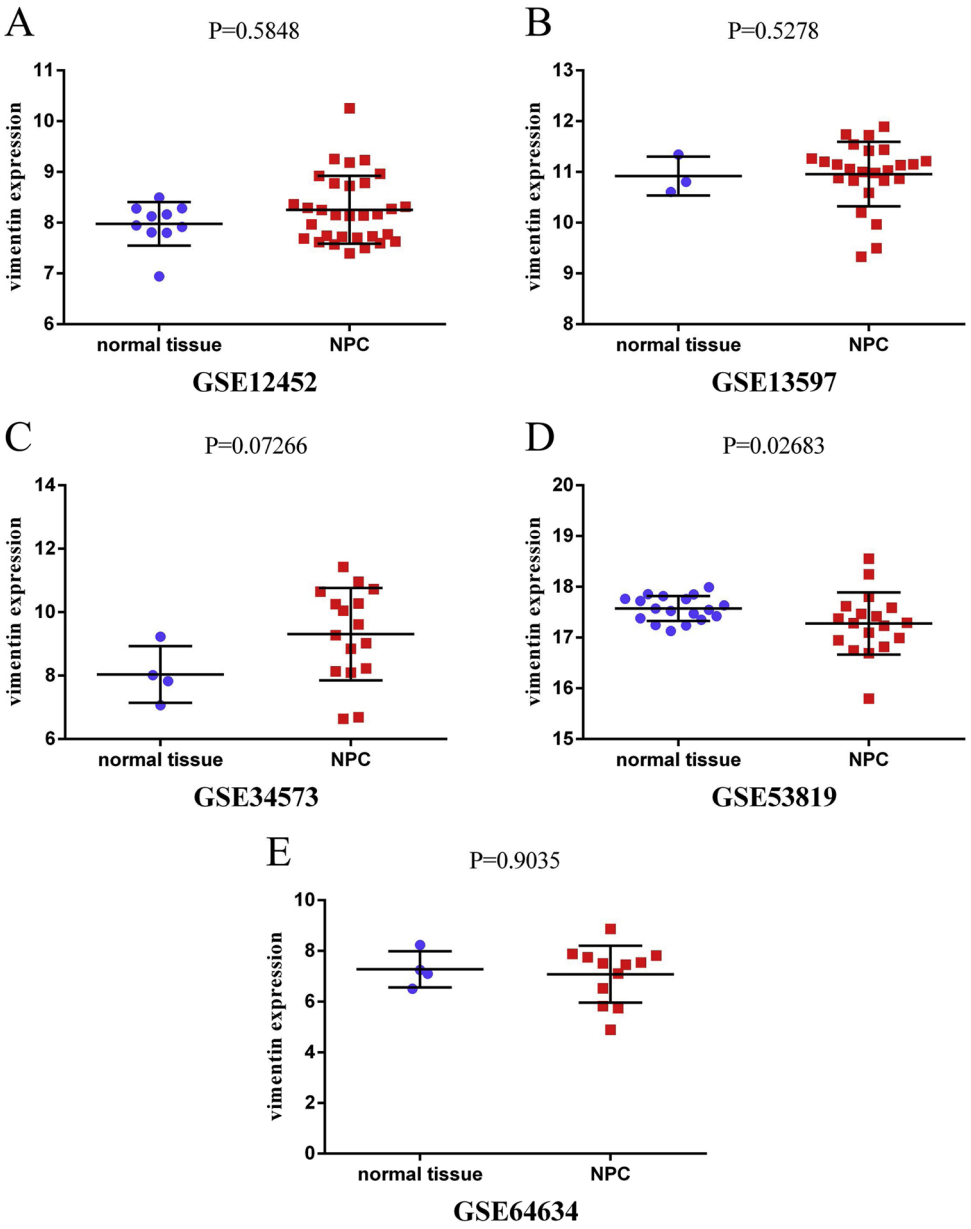

Fig. 3. The scatter plots of vimentin expression levels from different datasets. (A) GSE12452 (P = 0.5848); (B) GSE13597 (P = 0.5278); (C) GSE34573 (P = 0.07266); (D) GSE53819 (P = 0.02683); (E) GSE64634 (P = 0.9035).

according to the instructions of manufacturer, to determine the localization of vimentin expression. The sections were scored by two pathologists (Wei Lu and Mei-hua Wu), who were blinded to the identity of the tissue samples. Any divergence in scores was resolved through discussion. The immunoreactive scores (IRS) of vimentin were calculated as follows: (1) The staining intensity was scored 0 (negative), 1 (weak), 2 (moderate), or 3 (strong), and (2) the proportion of vimentin-positive cells was scored as 1 (0–9% positive), 2 (10–50%), or 3 (> 50%). Ultimately, after multiplying these two scores, samples with a sum IRS ≥ 1 were judged positive for vimentin.

2.3. Search strategy for literature, microarray, and RNA-sequencing data

A comprehensive search strategy, aimed at improving the ability to

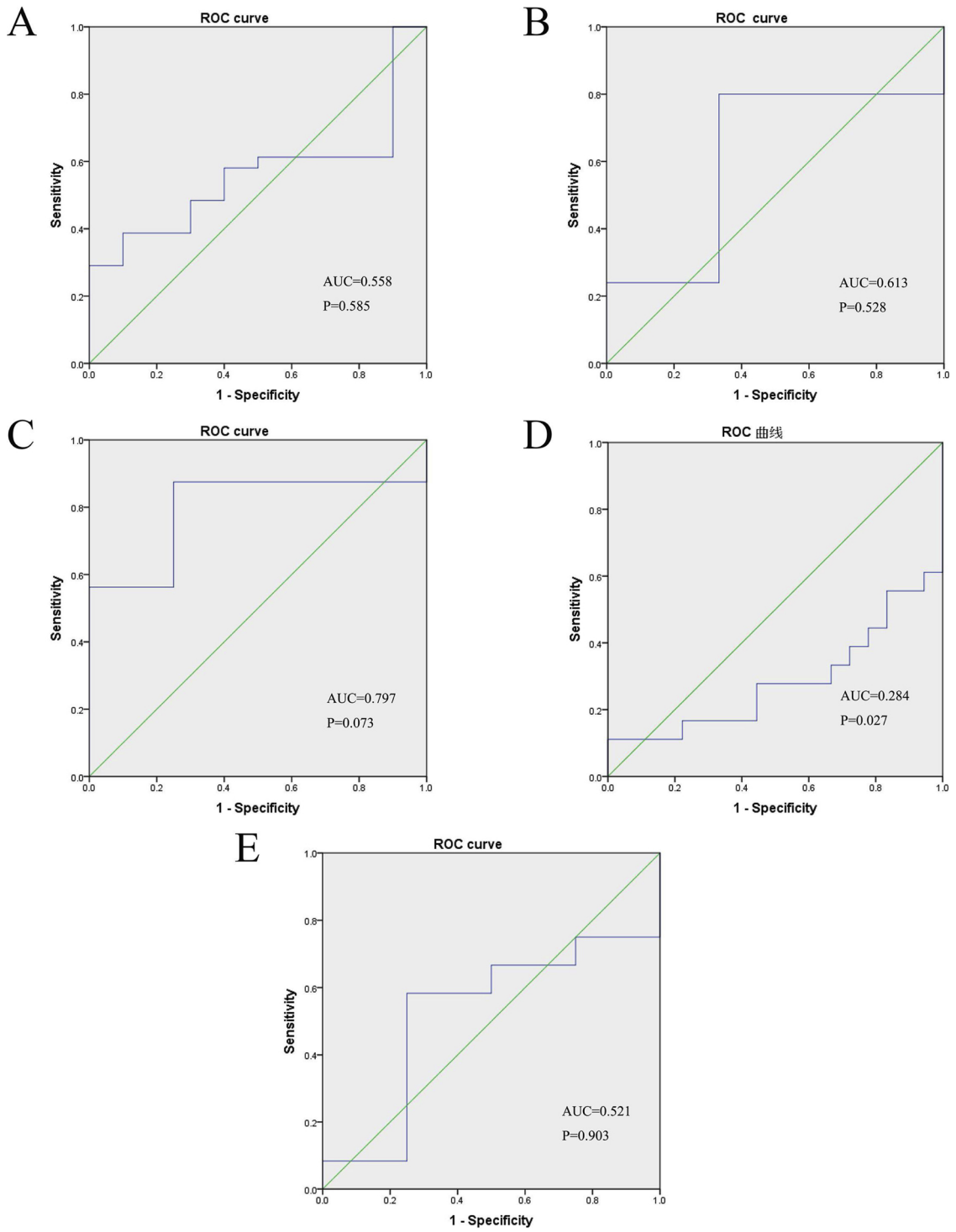

Fig. 4. Receiver operating characteristic (ROC) curves of vimentin expression levels from distinct datasets. (A) GSE12452; (B) GSE13597; (C) GSE34573; (D) GSE53819; (E) GSE64634.

search and view original literature, was used for systematic literature retrieval on vimentin related to NPC. Gene databases were also searched to obtain the corresponding expression values. The following databases were searched: the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), ArrayExpress, Oncomine, PubMed, Web of Science, EMBASE, Ovid, Science Direct, Wiley Online Library, the Cochrane library, Google Scholar, Chinese CNKI, Wan Fang, Chong Qing VIP, and China Biology Medicine. Considering the different database search requirements, the search string contained the following medical subject headings (Mesh) and keywords: (vimentin) AND (nasopharyngeal OR nasopharynx) AND (neoplasm OR cancer OR tumor OR carcinoma OR malignancy). The reference materials in the retrieved publications were examined to uncover further eligible publications. This comprehensive and iterative search ensured the reliability of the search strategy. The search strategy was conducted independently by three individuals (Jia-yuan Luo, Jia-yin Hou, and Xia Yang), and the final screening result was checked by two superiors (Gang Chen and Zhen-bo Feng). The last search date was January 24, 2019.

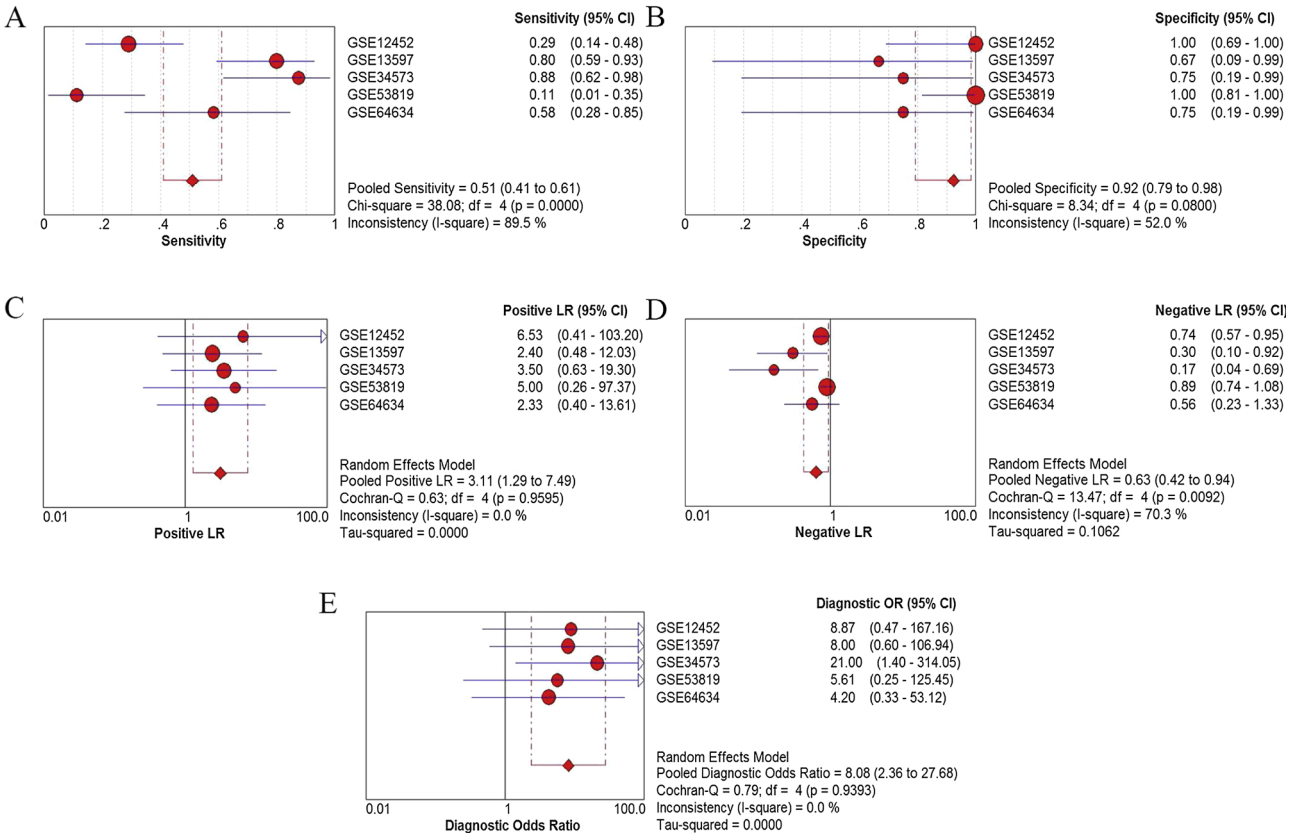

Fig. 5. The distinguishing capacity of vimentin between nasopharyngeal carcinoma (NPC) and nasopharyngitis (NPG) tissues. (A) The pooled sensitivity plot; (B) The pooled specificity plot; (C) The positive LR plot; (D) The negative LR plot; (E) The diagnostic odds ratio plot.

2.4. Publication selection criteria in the search strategy

Three steps of screening were conducted. The first step was publication selection, in which the titles and abstracts of the retrieved articles were carefully screened. The inclusion criteria for preliminary screening of the retrieved articles were as follows: (1) Homo species; (2) data related to vimentin; (3) patients with NPC; and (4) samples from serum, plasma, blood, or tumor tissue. After the preliminary screening, the full text of the remaining articles was read carefully and compared to the following exclusion criteria: (1) data series unrelated to vimentin; (2) publications that lacked clinical and prognostic parameters of vimentin; and (3) reviews, letters, or case reports. Ultimately, after this further examination of the contents of each item, we excluded articles containing the same gene spectrum and clinical parameters.

2.5. Literature data extraction

The included studies were assessed carefully, and their data were extracted. The following relevant information was collected: first author, publication year, sampling country, sample size, incidence of cancer, gender (male/female), age (< 50 / $\geq$ 50), clinical stage (III–IV/I–II), T stage (T3–T4/T1–T2),N stage (N2–N3/N0–N1), lymph node metastasis (yes/no), M stage (yes/no), and prognostic parameters. The expression values of vimentin measured by different probes were averaged for the next calculation. According to the series annotation information, all data not marked as normalized in the annotation file were processed by a log2 scale. The clinical information of the data series from the GEO database was obtained through the GEO Convenience Converter. All relevant data that could be obtained were used for this study.

2.6. Statistical analyses

Based on the data obtained, statistical analyses were used to explore the pivotal role of vimentin in NPC. The scatter plots of vimentin expression from various data series were analyzed using GraphPad Prism, STATA 12.0, SPSS 22.0, and MetaDisc software were used to reveal the receiver operating characteristic (ROC), standardized mean difference (SMD), and summarized receiver operating characteristic (SROC) of vimentin expression. A more comprehensive analysis was obtained by measuring the sensitivity, specificity, positive and negative likelihood ratios (LR + and LR -), and diagnostic odds ratio (DOR). The literature data were also used to attest to the role of vimentin in the development and progression of NPC. A synthesis of all the literature studies and IHC data, odds ratios (OR), and 95% confidence intervals was used to assess the role of vimentin in the development and progression of NPC, while hazard ratios (HR) were used to explore prognostic data. The HR values for survival curves obtained in the literature were extracted for investigation using Engauge and HR calculation software. After integrating all data, the analysis was selected when the information number was greater than or equal to 3. The significance of all the above consequences were explored in statistical analyses.

In all analyses, we used the following criteria for the division of heterogeneity. The fixed effect model was used for initial calculations. The random effects model was used only if heterogeneity occurred. Using I^2 tests and Cochran's Q-test, heterogeneity was defined as $P < 0.1$.

2.7. Enrichment analysis and the protein–protein interaction (PPI) network of vimentin-related genes

We further explored the molecular mechanism of vimentin in NPC by performing a sequence of gene analyses. The PPI network was

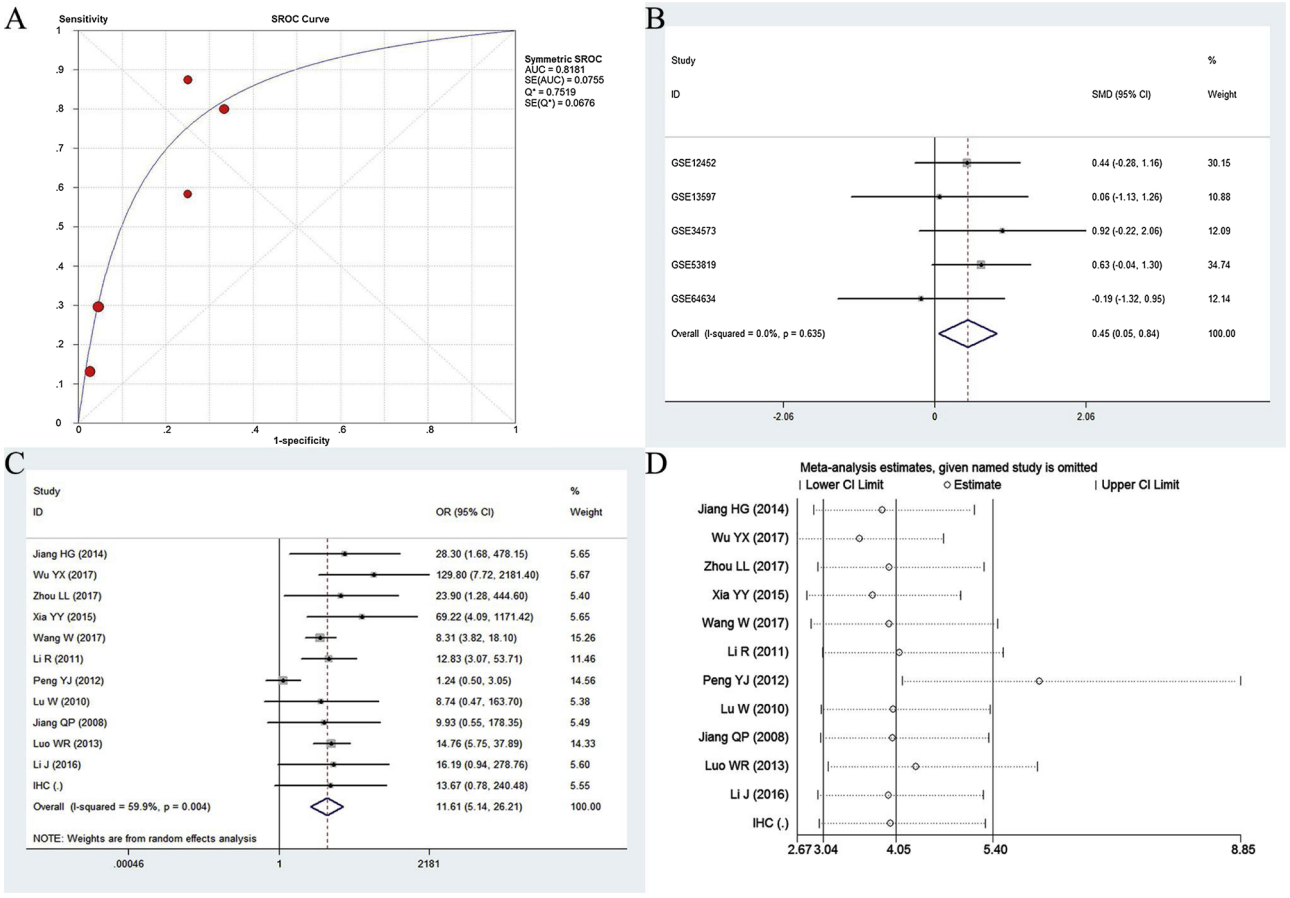

Fig. 6. The role of vimentin expression levels in nasopharyngeal carcinoma (NPC). (A) Summarized receiver operating characteristic (SROC) curves of expression profile data. (B) The forest plot of standardized mean difference (SMD) of expression profiles data. (C) The forest plot showing the significant relationship between vimentin and the occurrence of NPC. (D) The sensitivity test based on the data of vimentin expression in NPC. IHC: the data we obtained from clinical samples by in-house immunohistochemistry.

Table 4
The relationships between the expression of vimentin and clinicopathological covariates in NPC.

Type	Samples	Model	OR(95%CI)	P	I^2	P(I^2)	P(Begg's)	P(Egger's)
Gender	522	Fixed effect model	0.951(0.641–1.412)	0.805	8.1%	0.36	0.462	0.946
Age	400	Fixed effect model	0.982(0.654–1.475)	0.932	0	0.608	0.734	0.897
Clinical stage	395	Fixed effect model	3.763(2.324–6.091)	< 0.001	0	0.737	1	0.797
T stage	436	Fixed effect model	1.819(1.231–2.688)	0.003	48.1%	0.103	0.806	0.912
N stage	405	Fixed effect model	2.496(1.641–3.796)	< 0.001	50.8%	0.107	0.308	0.407
M stage	340	Random effects model	1.741(0.646–4.696)	0.273	63.6%	0.041	0.089	0.037
Lymph node metastasis	471	Fixed effect model	3.968(2.593–6.073)	< 0.001	0	0.475	0.573	0.587
Overall metastasis rate	674	Random effects model	3.475(1.860-6.493)	< 0.001	57.3%	0.022	0.138	0.016

constructed with the STRING online common database (http://www.string-db.org). The minimum required interaction score > 0.4 was selected. Based on this interaction network, we obtained a series of co-expressed genes associated with vimentin. In addition, gene ontology (GO) and the kyoto encyclopedia of genes and genomes (KEGG) analyses were performed using the STRING online common database to identify the target path and function annotation of vimentin. The terms of these three analyses with $P < 0.05$ were considered significant. The GO analysis included the following three items: biological process, cell components, and molecular function. Upon completion of all analyses, we proposed a mechanism for vimentin activity in human cells and predicted its position in NPC tumor cells [39,40].

3. Results

3.1. Protein expression of vimentin in clinical tissues

In this research, we tested the expression of vimentin in 57 NPC and 20 NPG samples. Immunohistochemical results were shown in Fig. 1. Vimentin immunoreactivity was mainly detected in the cytoplasm. The clinical immunohistochemistry detection showed the positive expression ratio of vimentin was 24.6% (14/57) of the NPC specimens, whereas vimentin expression was negative in NPG tissues (0/20, $P = 0.016$). These findings indicated a possible association between elevated vimentin protein expression and the development of NPC.

3.2. Data selection at mRNA and protein levels

The detailed review process of all databases was shown in Fig. 2. A

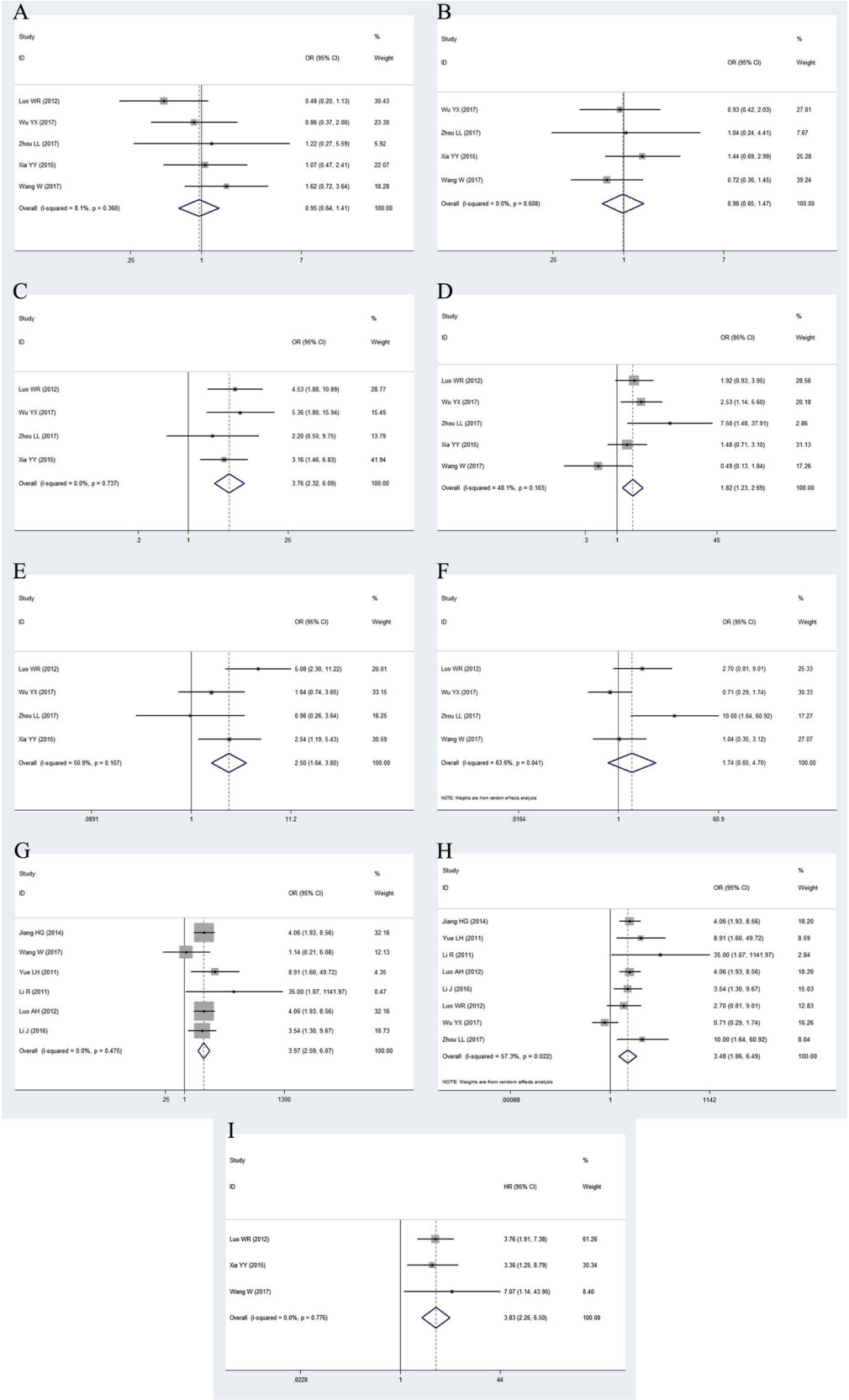

Fig. 7. The clinical value of vimentin in nasopharyngeal carcinoma (NPC).
(A) No significant associations between vimentin and gender; (B) No significant associations between vimentin and age; (C) Significant associations between vimentin and the clinical stage; (D) Significant associations between vimentin and the T stage;
(E) Significant associations between vimentin and the N stage; (F) No significant associations between vimentin and the M stage; (G) Significant associations between vimentin and lymph node metastasis; (H) Significant associations between vimentin and the overall metastasis rate; (I) Significant associations between vimentin and the prognosis of NPC.

total of 1885 articles and 210 data series were retrieved from all the searched databases. After selecting the homo species as the research object and excluding irrelevant data, 376 studies and 113 data series were included by screening titles and abstracts. Careful reading of the full-text contents of these documents and evaluations of the annotation file of gene expression profiles prompted the exclusion of 423 studies because of a lack of vimentin data. Ultimately, 14 qualified publications and five eligible data series were included after integrating all the

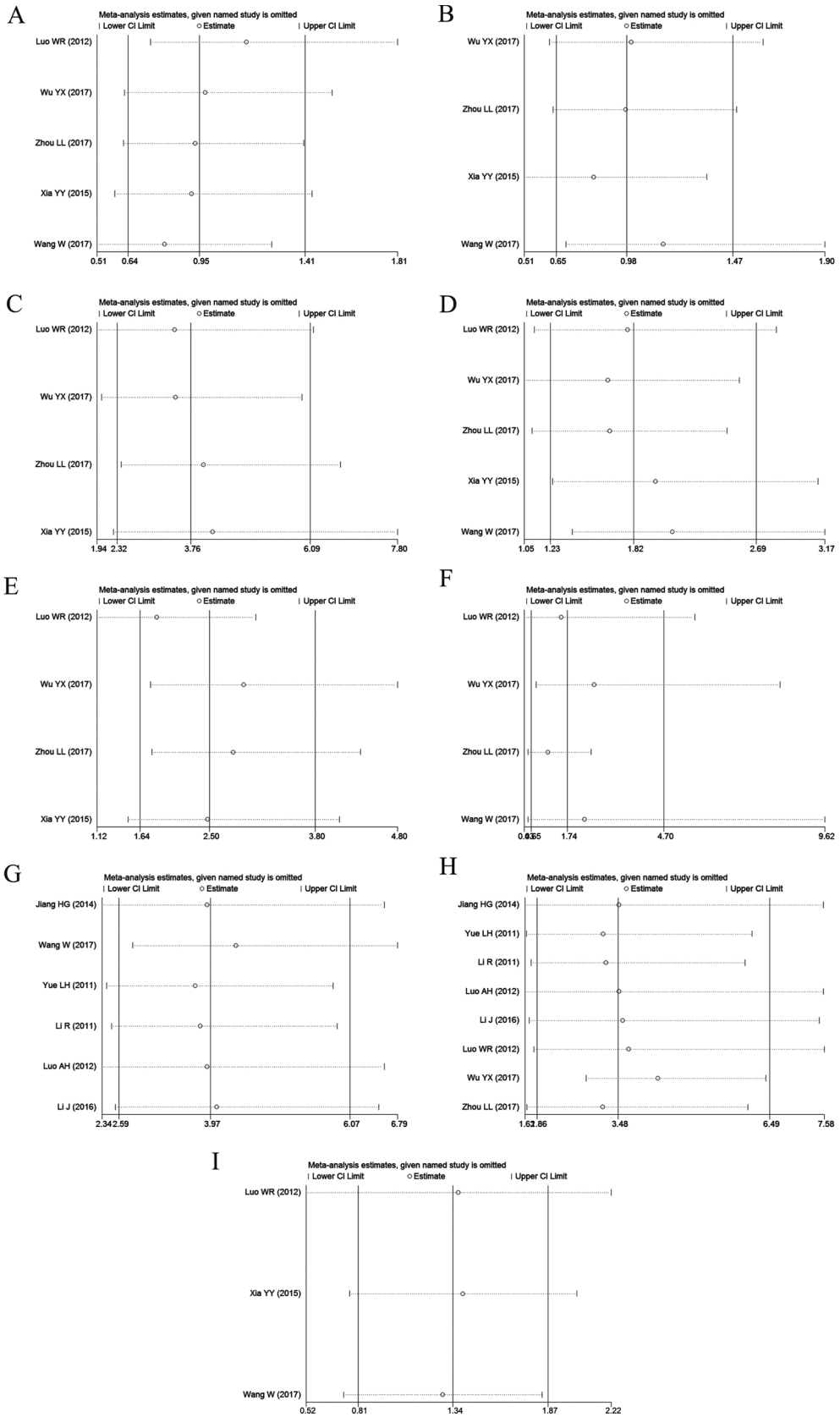

Fig. 8. The sensitivity test of the vimentin expression level related to pathological parameters or prognosis of nasopharyngeal carcinoma (NPC). (A) Gender group; (B) Age group; (C) Clinical stage group; (D) T stage group; (E) N stage group; (F) M stage group; (G) Lymph node metastasis group; (H) Overall metastasis rate group; (I) The sensitivity test based on the studies that included NPC prognostic data.

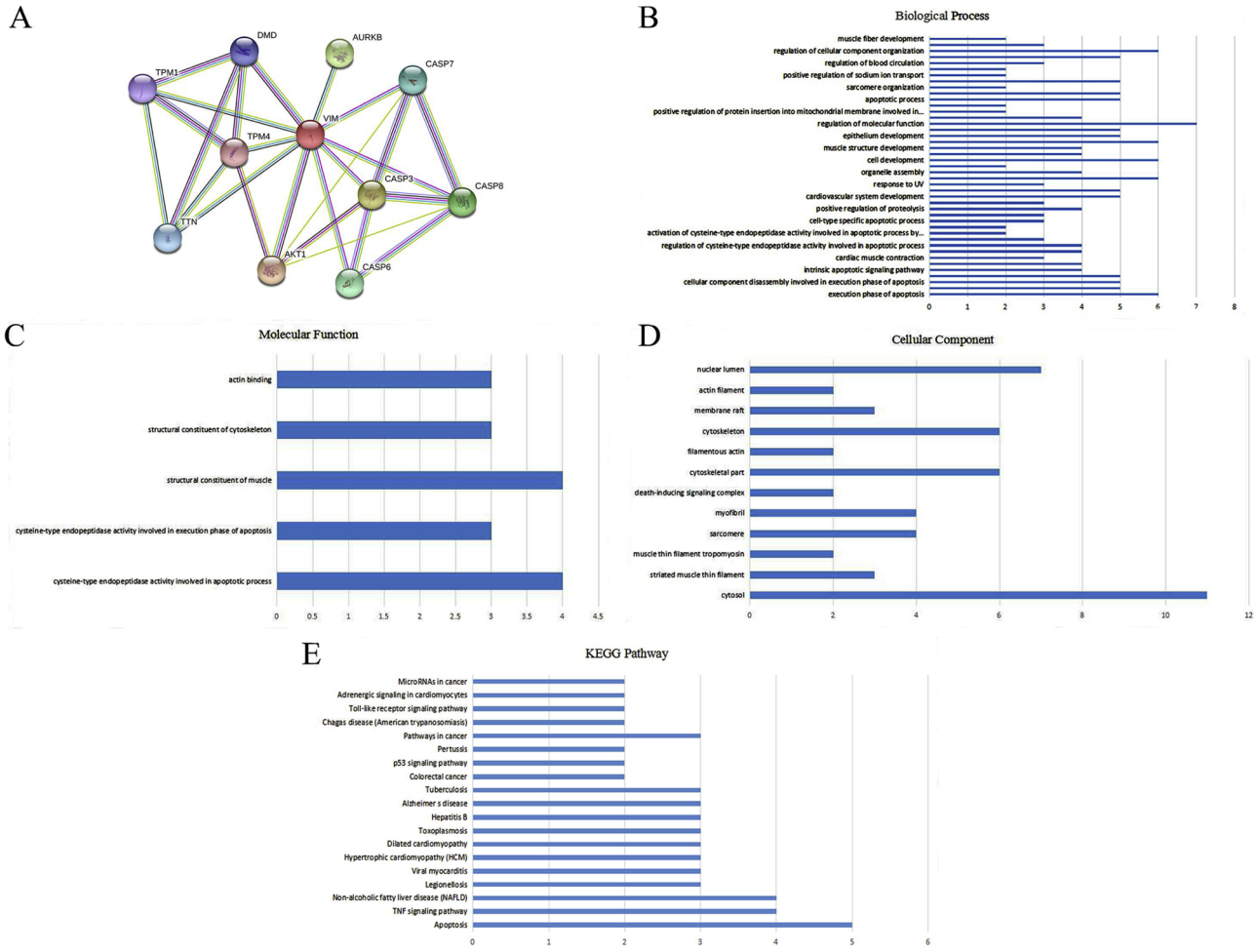

Fig. 9. Enrichment and pathway analysis of genes related to vimentin in nasopharyngeal carcinoma (NPC). (A) Protein-protein interaction (PPI) networks with 11 nodes and 25 edges were analyzed using STRING. (B) The biological processes in which vimentin is involved. (C) The molecular functions that vimentin can perform. (D) The cellular components in which vimentin is involved. (E) Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis based on the PPI network.

database resources. The relevant and prognostic parameters of these publications were displayed in Tables 1 and 2. The information extracted from the ultimately obtained gene expression datasets was shown in Table 3.

3.3. The expression level and potential value of vimentin

Our search of the gene databases identified the following five data series from GEO for inclusion in the study: GSE12452, GSE13597, GSE34573, GSE53819, and GSE64634 with 196 cases. The gene expression profile was standardized before performing the subsequent analysis. Scatter plots represented the expression of vimentin in various datasets (Fig. 3), and a P value < 0.05 was considered meaningful. The receiver operating characteristic (ROC) of vimentin showed the area under the curve (AUC) of these datasets (Fig. 4). A series of results from SROC of vimentin expression revealed that pooled sensitivity, specificity, positive LR (LR +), negative LR (LR -), and diagnostic odds ratio (DOR) were 0.51 (95% CI: 0.41 to 0.61), 0.92 (95% CI: 0.79 to 0.98), 3.11 (95% CI: 1.29 to 7.49), 0.63 (95% CI: 0.42 to 0.94), and 8.08 (95% CI: 2.36 to 27.68), respectively (Fig. 5). The area under the curve (AUC) for vimentin, based on the SROC curve, was 0.8181 ($Q^* = 0.7519$) (Fig. 6A).

The collected information was from several countries and generated by assorted methods. Therefore, we performed a standardized mean difference (SMD) analysis on the expression value of vimentin in NPC. The result of the SMD was 0.448 (95% CI: 0.053–0.843). The I^2 was less than 50%. The result of the forest plot was also displayed in Fig. 6B.

3.4. Statistical analyses of binary categorical variables

Besides the assessment of continuity variables, binary categorical variables extracted from the literature were used for statistical analyses. Based on the data for NPC and non-cancer groups, the odds ratio (OR) values with 95% confidence interval were calculated. The result was 11.608 (95%CI: 5.141–26.214, $P < 0.01$, random effects model) based on 1197 cases. This result indicated an association between the high expression of vimentin and the occurrence of NPC. The forest plot (Fig. 6C) showed that the I^2 was more than 50%. We further assessed the reasons for heterogeneity by conducting Begg's and Egger's tests. The results showed that heterogeneity did not stem from publication bias ($P_{Begg} > 0.537$, $P_{Egger} > 0.095$). We did further sensitivity analysis, as shown in Fig. 6D. The clinical parameters of all studies calculated OR values to reveal the role of vimentin in tumor development. Because of the diverse grouping conditions of clinical parameters in the studies, the relationship between vimentin and lymph node metastasis was divided into two groups (N stage group and lymph node metastasis group). In addition, we combined the data from articles that provided the relationships between vimentin and lymph node metastasis or distant metastasis subgroups to examine the relationship between vimentin expression level and overall metastasis rate. The combined results were summarized in Table 4, and the forest plots were depicted in Fig. 7A–H. The subgroup study showed significant associations between

vimentin and clinical stage, T stage, N stage, lymph node metastasis, and overall metastasis rate. However, no significant associations were noted between vimentin and gender or age.

Analyses of the impact of vimentin expression on the prognosis of NPC patients were also probed. A hazard ratio analysis revealed a relationship between the high expression of vimentin and overall poorer survival compared with low vimentin expression (HR: 3.831; 95%CI: 2.256–6.503; $P < 0.001$; $I^2 = 0$) based on 336 cases. Fig. 7I demonstrated the results more intuitively with forest plots. The results of a sensitivity test of clinical parameters and prognostic data were shown in Fig. 8.

3.5. Protein–protein interaction (PPI) network construction of vimentin-related genes

Protein–protein interaction (PPI) is defined as the correlation between protein molecules and is used to investigate this correlation concerning biochemistry, signal transduction, and genetic networks. PPI network construction could help, for example, in studying molecular mechanisms of disease and in the discovery of drug targets. In the present study, the PPI network was also employed to find other molecules related to vimentin (Fig. 9A). The related genes included AURKB, AKT1, TPM4, DMD, TTN, TPM1, CASP7, CASP3, CASP6, and CASP8.

3.6. Enrichment and pathway analysis

Gene ontology (GO) analysis could be categorized into three parts: biological processes (Fig. 9B), molecular functions (Fig. 9C), and cellular components (Fig. 9D). The results of KEGG analysis were depicted in Fig. 9E. The P value for all results was less than 0.05.

4. Discussion

In recent years, the aberrant expression level of vimentin has been reported in NPC. However, these studies were based on immunohistochemical analyses of samples from small or in vitro experiments with a limited number of cell lines, rather than a comprehensive in silico interpretation taking advantage of big data. More importantly, vimentin-related signaling pathways have not been investigated via in silico approaches. Therefore, this work synthesized all the available data sources, including the literature, microarray and RNA sequencing databases, and the protein expression of clinical samples. This researcher also conducted multiple meta-analyses to confirm that vimentin can promote the unfavorable biological behavior of NPC. Further GO enrichment, KEGG analysis, and the PPI network of vimentin-related genes were employed to examine the possible pathways of vimentin in NPC. The results revealed that vimentin plays a central role in the process of EMT and participates in *TNF signaling pathways* and *P53 signaling pathways*.

In our study, we performed a more comprehensive investigation of the clinical role of vimentin in NPC via mining multiple data sources, including gene-chip and RNA-sequencing data from GEO, ArrayExpress, TCGA, and Oncomine, as well as the information collected from the literature. Altogether, 1762 cases were involved in the current study to improve its reliability compared to the previous studies of a single institute with smaller sample sizes. Since a single study has the disadvantage of using a single detecting method with limited samples, we performed different meta-analyses to show the clinical value of vimentin expression in NPC. For the mRNA data, SMD and SROC were calculated based on five studies (n = 196), and for the protein level of vimentin, OR was calculated based on 14 studies (n = 1566). To validate our results from gene-chip, RNA-sequencing, and the literature, we collected in-house clinical NPC samples to discern vimentin protein level. Like previous reports, [42,43] the current findings based on both public big data and in-house protein detection demonstrated the distinguishing capacity of vimentin to up-regulate vimentin between NPC and NPG tissues, which further supported the oncogenic role of vimentin in the tumorigenesis process of NPC. We attempted to collect vimentin expression data in peripheral blood or other body fluids because of their high praise for non-invasive examinations in clinics. Unfortunately, no such data have been reported so far.

Besides the aforementioned work, we conducted a more comprehensive evaluation of the impact of vimentin on the development of NPC. Because chip and sequencing data lacked clinical-pathology information, the data in this section were derived only from published literature. Many researchers have found that vimentin is one of the mesenchymal markers in EMT, which may enhance the ability of tumor cells for cell proliferation and distant invasion. [53,54] We found that there was no significant relationship between vimentin and distant metastases when analyzing the M stage group separately. We speculated the reason may be the small sample size (n = 340). Therefore, we further explored the significance of vimentin in the overall metastasis rate of NPC (n = 674), including lymph node metastasis and distant metastasis. Our results showed that the up-regulation of vimentin can lead to the metastasis of NPC. In agreement with previous reports [38,41,42,44], the up-regulation of vimentin accelerates the progression of NPC, including its clinical stage, tumor size, and lymph node metastasis, but our findings contradict Wang et al. [37], who reported vimentin expression was not associated with the TMN stage and lymph node metastasis. The reasons may be the different sample size. The sample number of Wang et al.'s study was only 203. Our study has a larger sample size and more reliable information, so we believe that highly expressed vimentin plays a vital role in promoting the progress of NPC. In the present study, we also evaluated the prognostic role of vimentin in NPC based on 498 cases. The result revealed the over-expression of vimentin may lead to a poor prognosis of NPC, suggesting that vimentin can be used as an important prognostic indicator of NPC.

To further investigate the possible mechanism of vimentin in NPC, genes related to vimentin in NPC were analyzed by GO and KEGG analysis to explore the pathway in which vimentin exerts its biological effects. Vimentin is a member of the EMT family. The development of malignant tumors is associated with EMT, which is mediated by the induction of cytoskeleton and a change in the expression of cell surface proteins [55,56]. The current GO analysis showed that vimentin participated in the structural constituent of the cytoskeleton and the regulation of cellular component organization, which further confirmed that vimentin can affect the occurrence and progression of NPC through the EMT process. Moreover, KEGG analysis revealed that vimentin may influence the biological behavior of NPC by participating in *TNF signaling pathways* and *P53 signaling pathways*. These mechanisms merit further verification.

However, the current study had several limitations. First, the present study did not incorporate animal experiments to verify and explore the biological functions of vimentin in NPC. Second, there was no body fluid-related data in our study, which could reflect the diagnostic value of vimentin non-invasively for NPC. Therefore, a larger number of prospective studies is necessary to identify the diagnostic value of vimentin and the specific molecular mechanisms by which vimentin affects the progression and prognosis of NPC in the future.

5. Conclusion

In summary, our study confirms the capacity of vimentin to distinguish NPC from NPG tissues. In addition, this research showed that vimentin promotes the negative biological behaviors of NPC, including its occurrence, malignancy, and poor prognosis. Therefore, vimentin may be useful as a prognostic indicator of NPC. We also found that vimentin and its related genes have important effects on the biological processes, molecular functions, and cellular components associated with NPC.

Disclosure

None.

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