

RESEARCH ARTICLE

Prognostic Value of Overexpressed p16^{INK4a} in Vulvar Cancer: A Meta-Analysis

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Abstract

Objective

This study aimed to examine the prognostic value of overexpressed p16^{INK4a} in vulvar cancer. Although the tumor suppressor p16^{INK4a} has been shown to be of prognostic value in a wide variety of cancers and precancerous lesions, its role in the vulvar cancer is still unclear.

Methods

All publications in English language on the association between p16^{INK4a} and clinicopathological features of vulvar cancer were searched from Pubmed, Embase, and Web of Science, and those in Chinese language were identified manually and online from the China National Knowledge Infrastructure. Strict inclusion and exclusion criteria were followed. Odds ratios(ORs) or risk ratios(RRs) with 95% confidence intervals(CIs) were pooled to assess the strength of association. Publication bias was estimated using funnel plots and the Egger's regression test.

Results

A total of 17 studies with 2309 patients were included. The p16^{INK4a} overexpression was found to correlate significantly with the lower International Federation of Gynecology and Obstetrics stage(I+II vs III+IV; OR = 0.60,95%CI:0.41–0.86,*P* = 0.006),negative lymph node metastasis(negative vs positive; OR = 0.61,95%CI:0.39–0.95,*P* = 0.029),patient's age<55 (OR = 0.54,95%CI:0.31–0.96,*P* = 0.034),human papillomavirus–positive status(OR = 0.01,95%CI:0.00–0.11,*P*<0.001),and higher overall survival(RR = 0.53,95%CI = 0.35–0.80, *P* = 0.003).

Conclusion

The p16^{INK4a} might be associated with a higher survival and indicates better prognosis of vulvar cancer.

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Introduction

Vulvar cancer is the fourth most common malignancy in the female genital tract, accounting for 5% of all gynecological cancers [1]. With an estimate of over 5000 new cases and over 1000 deaths in the United States each year, the incidence of vulvar cancer keeps on rising, particularly in young women [1,2]. The most frequent histological type of vulvar cancer is squamous cell carcinoma (SCC), which represents 80%–90% of the cases and can be divided into two groups based on whether they are related to human papillomavirus (HPV) infection [3,4]. One is the HPV-associated type including the warty or basaloid SCC, and the other is the HPV-independent keratinizing SCC [5,6]. Radical surgery is the main modality of treatment for vulvar cancer, sometimes in combination with presurgical or postsurgical radiotherapy or chemotherapy depending on the tumor size and invasion, but often causes overtreatment in early stage cases when patients present nonmetastasis of lymph node [7]. For this reason, tumor biological markers are needed to predict clinical behavior and metastatic potential of vulvar cancer.

The p16^{INK4a} protein is a cyclin-dependent kinase inhibitor that can act on cyclin-dependent kinases (CDKs) and thus inactivate the cell cycle [8]. The loss of p16^{INK4a} expression seems to be an early event in carcinogenesis. Over the past decades, the prognostic value of p16^{INK4a} protein has been evaluated in a wide variety of cancers [9–14]. Among these, the study of Hellman et al [15] concluded that p16^{INK4A} expression might be used as a marker for HPV positivity in vaginal carcinoma, which shares a very similar etiology with vulvar cancer. However, its role and the etiological effect of its combination with the HPV status in vulvar cancer remain somehow unclear. Tringler and Dong reported a significant association between p16^{INK4a} and longer survival of vulvar cancer patients, while Trietsch denied its independent prognostic role [16–18]. In this regard, the present study performed a meta-analysis to explore the prognostic value of the overexpressed p16^{INK4a} in vulvar cancer.

Methods

Search strategy

To identify all articles that investigated the association of p16^{INK4a} expression and vulvar cancer, a literature search of PubMed database, Embase, Web of Science, and China National Knowledge Infrastructure was conducted between January 1991 and August 2015. All relevant articles were retrieved using the following search terms: “p16 or p16^{INK4a}” and “vulvar neoplasm or vulvar neoplasms”. References of the retrieved publications were also screened for other relevant studies. For multiple publications from the same population, only the largest-scale study was included. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria, by screening the title, abstract, and full-text. Any dispute was solved by discussion. The language of publication was restricted to English and Chinese. Only research articles were included. If an article reported results including different studies, each study was treated as a separate comparison in the present meta-analysis.

Inclusion criteria

The following inclusion criteria were followed in selecting studies for the current meta-analysis: Studies in which the diagnosis of vulvar cancer was proven by histopathological methods; studies of p16^{INK4a} expression that were based on vulvar cancer tissue (after either surgical excision or biopsy sampling); specimens examined by immunohistochemistry; and all studies on the correlation of p16^{INK4a} expression with clinicopathological markers or with the HPV status and the association of p16^{INK4a} overexpression on the overall survival (OS) or disease-free

survival (DFS) or recurrence of vulvar cancer patients. For inclusion into the analysis, no limitation was set on the minimum number of patients of each study. In the case of multiple articles by the same group based on similar patients and using same detection methods, only the largest or the most recent article was included.

Exclusion criteria

The exclusion criteria were defined as follows: Studies based on serum or any other kinds of specimen, and studies using methods other than immunohistochemistry to examine specimens.

Data extraction

Three investigators (Hanyu Cao, Si Wang, and Zhenyu Zhang) extracted independently all articles complying with the aforementioned inclusion criteria. Any discrepancy was resolved by discussion until an agreement was reached between the investigators. The following information was collected from each publication: the first author's name, publication year, patient's country, tumor stage, technique, percentage of p16^{INK4a} positive cells, number of patients, cut-off value of overexpression of p16^{INK4a} in cases and controls.

Statistical analysis

Odds ratios (OR) and 95% confidence intervals (CIs) were pooled to evaluate the association between p16^{INK4a} expression and clinical or histopathological features of vulvar cancer including histological grade, International Federation of Gynecology and Obstetrics (FIGO) stage, lymph node metastasis, age, and HPV status. Risk ratios (RRs) and 95% CIs were synthesized to determine the correlation between p16^{INK4a} expression and 5-year OS. Heterogeneity was examined with I^2 statistics interpreted as the proportion of total variation contributed by between-study variations. Heterogeneity was investigated by the Cochran's chi-square Q test with a significance level of $P < 0.10$ and $I^2 > 50\%$. In this case, the random-effects model was used to estimate the pooled ORs [19]. Otherwise, the pooled ORs were estimated by the fixed-effects model. RRs were pooled to evaluate the association of p16^{INK4a} expression and survival outcome data. The Engauge Digitizer Version 4.1 software (free software downloaded from <http://sourceforge.net>) was used when the survival data could not be extracted directly according to previous studies [20]. Begg's funnel plots and the Egger's linear regression test were performed to investigate publication bias [21]. All statistical tests were performed with the Stata 12.0 software (Stata Corp, TX, USA).

Results

Study inclusion and characteristics

As shown in Fig 1, a total of 17 eligible studies comprising 2309 patients from different countries were included in this meta-analysis, with the number of patients ranging from 4 to 1287 per study [16–17, 22–36]. Among them, eight studies dealt with the FIGO stage, and six studies presented data on lymph node metastasis. Three studies presented data classified according to patients' age (55 years), and seven studies reported histological grade. In addition, four studies reported the association between p16^{INK4a} and the HPV status, whereas only one study [34] included the DFS and OS data concerning the HPV status. Moreover, only three studies provided sufficient data on OS related to p16^{INK4a} expression. All of them used the immunohistochemistry method with different cut-off values. The main characteristics of these studies are summarized in Table 1.

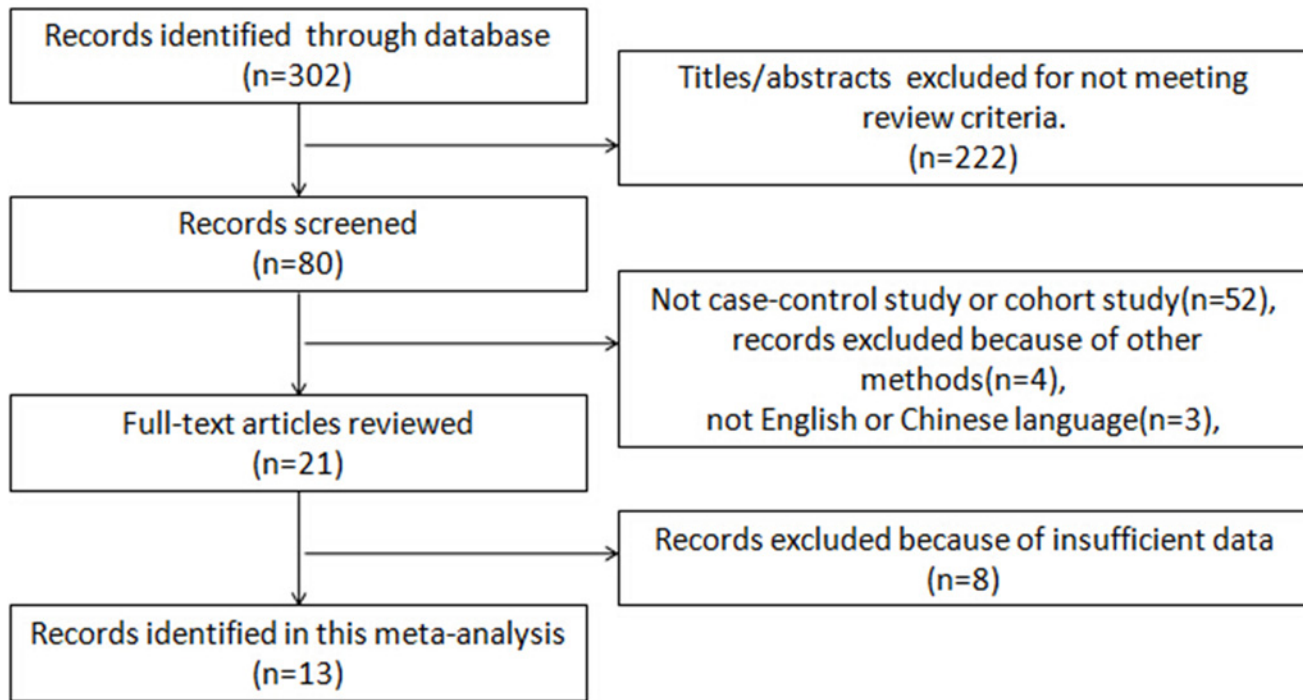


Fig 1. Flow chart demonstrating studies processed for inclusion in the meta-analysis.

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Table 1. Main Characteristics of all Eligible Studies.

Study	Year	Patient's country	Tumor stage	Technique	Percentage of p16INK4a positive cells (%)	Number of patients	Cutoff(IHC)
Chan MK	1998	China	FIGO	IHC	72	30	ND
Zheng	2000	China	FIGO	IHC	40.38	52	ND
Ma	2002	China	FIGO	IHC	64	25	Staining grade>2
Hao	2004	China	ND	IHC	40	30	ND
Knopp S	2004	Norway	FIGO	IHC	31	224	5%
Mauricio	2006	Brazil	ND	IHC	24.3	37	5%
Santos M	2006	Spain	ND	IHC	18	92	25%
van der Avoort	2006	The Netherlands	ND	IHC	75	16	25%
Tringler B	2007	Austria	FIGO	IHC	43	80	ND
Wei	2008	China	FIGO	IHC	29	21	ND
Alonso I	2011	Spain	FIGO	IHC	20	98	Diffuse staining
Guerrero D	2011	Spain	TNM	IHC	70	30	1%
de Sanjosé S	2013	39 Countries	ND	IHC	29	1287	ND
Ma HM	2013	China	FIGO	IHC	90.3	36	Staining grade>2
Lavorato-Rocha	2013	Brazil	FIGO	IHC	29	139	5%
Missaoui N	2014	Tunisia	FIGO	IHC	73	15	ND
Dong	2015	America	ND	IHC	52	97	70%

FIGO, International Federation of Gynecology and Obstetrics; IHC, immunohistochemistry; ND, no data; TNM, tumor node metastases.

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Correlation of p16^{INK4a} expression with clinicopathological parameters

As shown in Fig 2, the overexpression of p16^{INK4a} was significantly associated with the lower FIGO stage of I-II(OR = 0.60,95%CI:0.41–0.86,*P* = 0.006, fixed effect; Fig 2A), no lymph node metastasis(OR = 0.61,95%CI:0.39–0.95,*P* = 0.029, fixed effect; Fig 2B), patient’s age<55 (OR = 0.54,95%CI:0.31–0.96,*P* = 0.034, fixed effect; Fig 2C), and HPV-positive status (OR = 0.01,95%CI:0.00–0.11,*P*<0.001, random effect; Fig 2D). However, the overexpression of p16^{INK4a} was not correlated with the histological grade(OR = 0.99,95%CI:0.71–1.39,*P* = 0.953, fixed effect; Fig 2E).

Correlation of p16^{INK4a} expression with OS

Five-year OS rates were extracted from three studies and were pooled in this meta-analysis using the method mentioned earlier. The overexpression of p16^{INK4a} was significantly correlated with a higher OS(RR = 0.53,95%CI = 0.35,0.80,*P* = 0.003, fixed effect; Fig 3) with no heterogeneity(*I*² = 0.0%,*P* = 0.636).

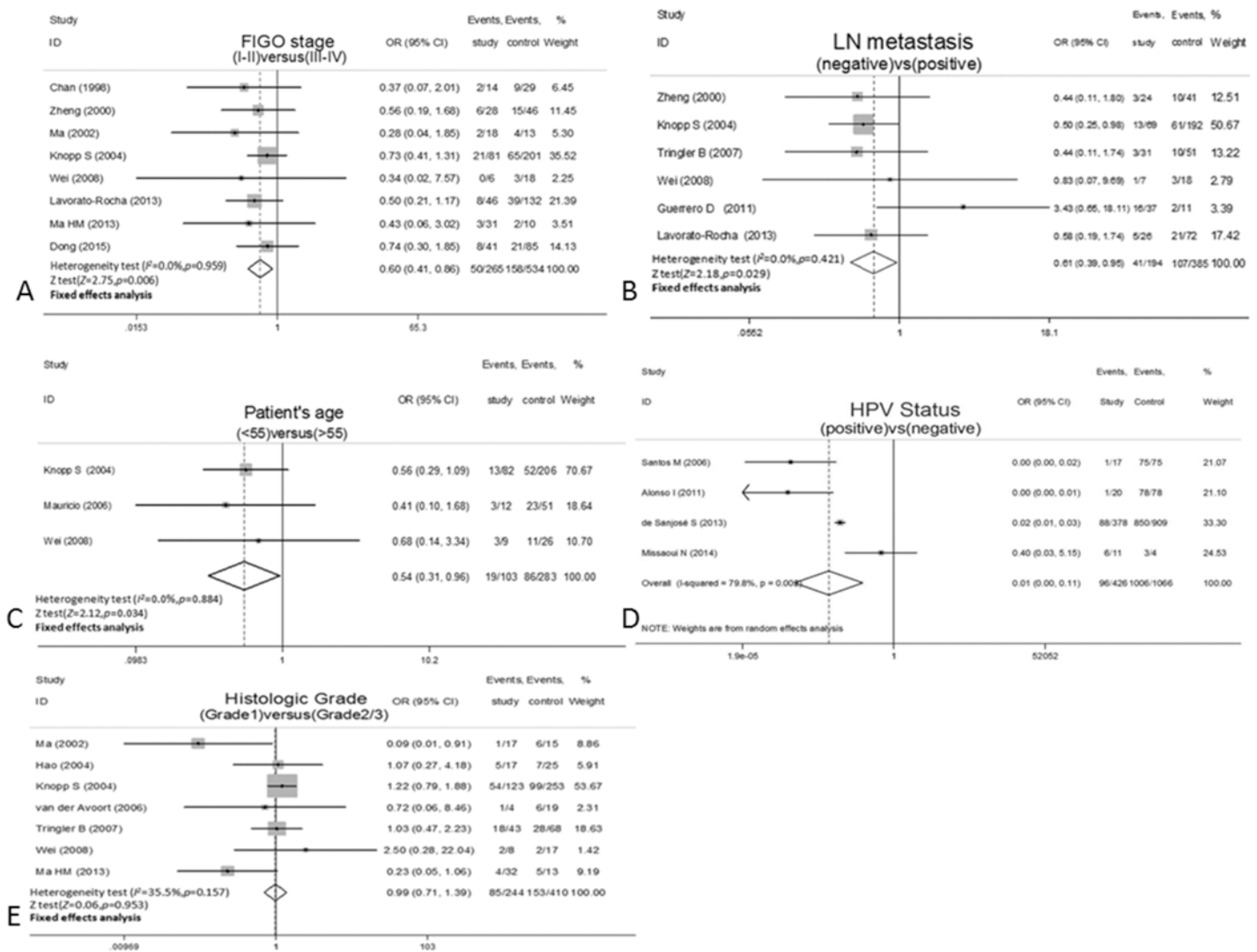


Fig 2. Forest plot for the association between p16INK4a expression and clinicopathological markers(A) FIGO stage.(B) LN metastasis. (C) Patient's age. (D) HPV status. (E) Histological grade. FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; HPV, human papillomavirus.

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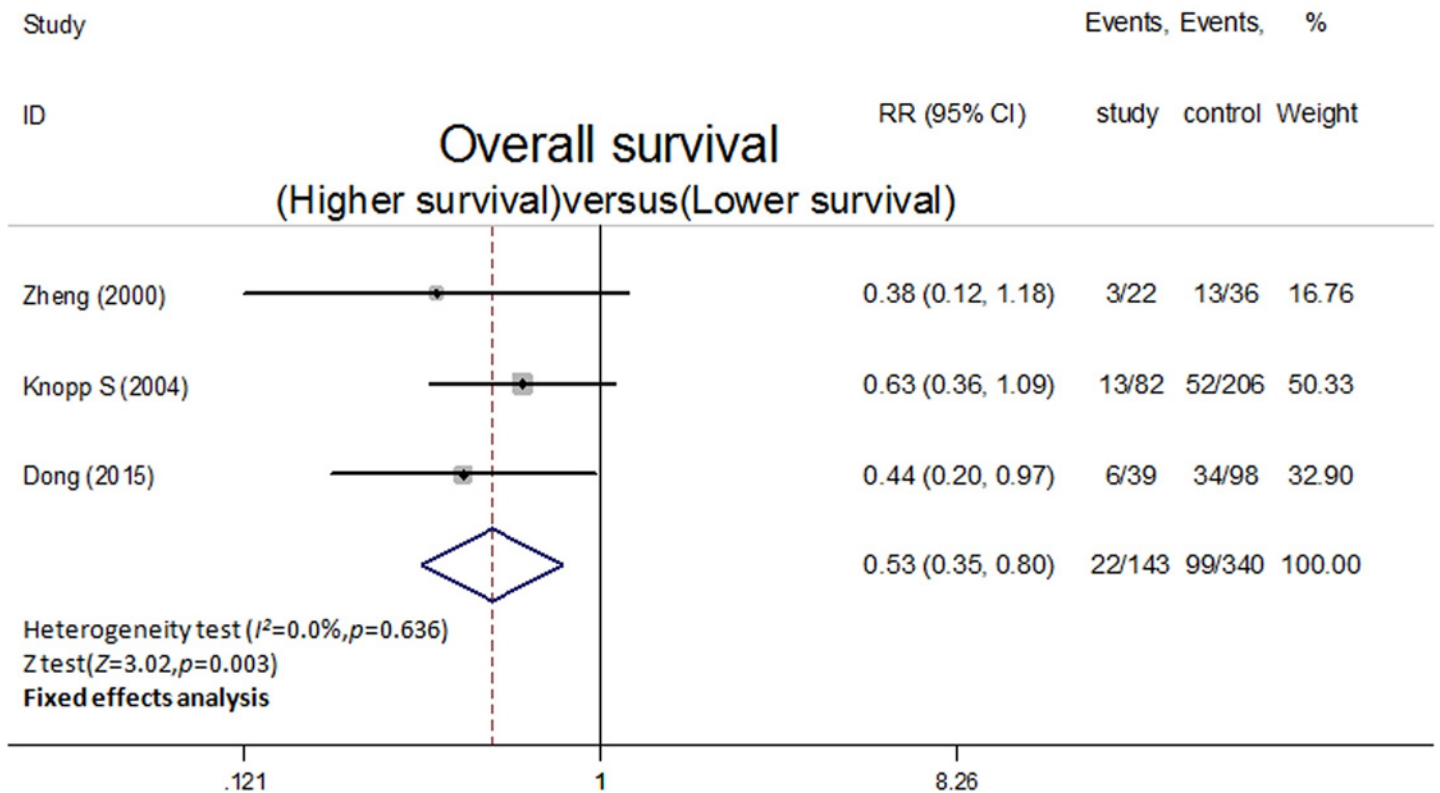


Fig 3. Forest plot for the association between p16INK4a expression and OS.

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Publication Bias

Begg’s funnel plots and Egger’s test were used to assess whether publication bias existed in the 5-year OS studies. No obvious evidence of asymmetry was found in the funnel plot of OS ($P = 0.217$; Fig 4).

Discussion

P16^{INK4a}, as a member of the INK4a family, is known to inactivate cell cycle progression and differentiation by interfering with cyclin D–CDK4/6 assembly and thus block the G1 to S transition [37]. The functional loss of INK4a family has been found in variable human cancers. Some previous studies have provided evidences indicating that p16^{INK4a} is a potential tumor suppressor whose overexpression can inhibit abnormal cell growth, whereas downregulation may lead to tumorigenesis [38]. Although p16^{INK4a} has been shown to be of prognostic value in a wide variety of cancers and precancerous lesions such as gastrointestinal stromal tumor, non-small lung cancer, osteosarcoma, cervical cancer, and cervical intraepithelial neoplasia, evidence of correlation with vulvar cancer is lacking [12–13, 39–41].

Vulvar cancer is uncommon but represents a certain group in the carcinoma of lower genital tract in female. As the treatment for vulvar cancer tends to be more conservative and individualized over the past decade, acquaintance with the prognostic factors seems more essential and is of great value for early diagnosis, but is still a challenge because of scanty evidence compared with other gynecological malignancies [42]. It was found that FIGO stage, lymph node metastasis, and histological grade are the most important prognostic factors in vulvar cancer [43–45]. The age of patients is also a predominant and independent factor in the 5-year

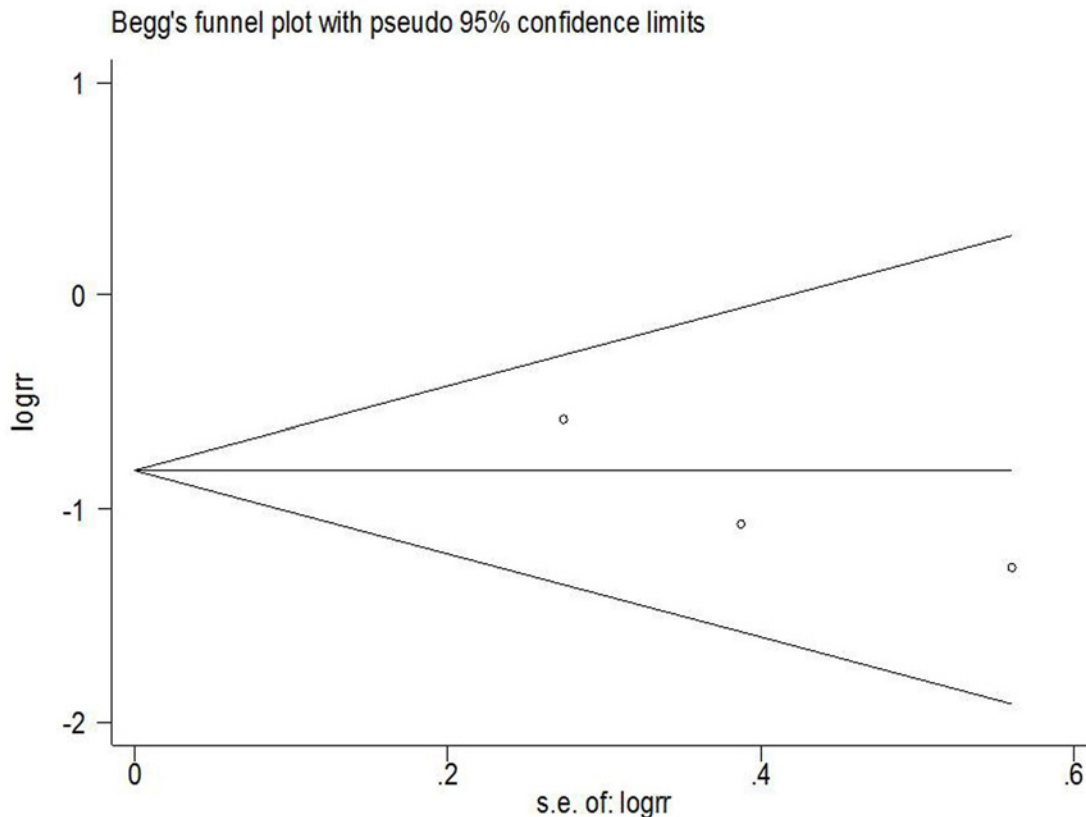


Fig 4. A funnel plot analysis on the detection of publication bias in the meta-analysis of the prognostic value of p16ink4a in the OS of vulvar cancer.

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survival of vulvar cancer[46]. This study is perhaps the first meta-analysis that evaluated the prognostic value of p16^{INK4a} in vulvar cancer.

The present meta-analysis using pooled ORs demonstrated that p16^{INK4a} overexpression correlates significantly with the lower FIGO stage, negative lymph node metastasis, and younger age, consistent with its well-known function of suppressing tumor invasion[47–49]. The pooled RR of OS reveals that p16^{INK4a} overexpression indicates a better prognosis for patients diagnosed with vulvar cancer. The results indicate that p16^{INK4a} may be a potential biomarker for the diagnosis and prognosis of vulvar cancer. Although evidence shows improved survival of HPV-induced and p16-positive carcinoma associated with head and neck cancer[50], no detailed studies are found presenting the survival data including both HPV status and p16^{INK4a}. A very few studies presented recurrence rate related to p16 concerning vulvar squamous cell carcinoma (VSCC) after repeated searches for several times, which may be due to the low frequency of VSCC. Among the included studies, HPV-positive rate independent of p16^{INK4a} ranges between 0% and 41% [23,25,27,34], which is in accordance with the fact that vulvar cancer presents an overall HPV infection rate of only 30%–40%[51–52]. Moreover, the analysis outcome shows that p16^{ink4a} overexpression correlates significantly with the HPV-positive status, strongly supporting the etiological role of HPV infection in the development of vulvar cancer. The E7 and E6 oncoproteins encoded by HPV can be integrated into the host genome, binding to the tumor suppressor proteins pRB and p53, respectively, and leading to their deregulation, which results in the overexpression of p16^{INK4A} as a means of genetic

instability control[53]. All these may reasonably lead to the speculation that p16^{INK4a} is only associated with HPV-induced vulvar carcinoma.

However, this study has some limitations that should be taken into consideration. First, this study included a small sample size. A total of 817 patients may not be able to provide statistical evidence convincing enough to prove the role of p16^{INK4a} in vulvar cancer prognosis. In addition, studies concerning the survival rate of vulvar cancer are even less. Second, the arbitrarily selected cutoff value of positive p16^{INK4a} expression varies in different studies. This may be caused by the lack of a standardized immunostaining point decided by a combination of intensity and the variable methodological factors in immunohistochemistry such as storage time, fixation method, and different antibodies[54], which may result in an inaccurate outcome. All studies were evaluated based on a review of the entire histological section, while some declared the evaluation of the immunostaining results by one or two pathologists at the same time according to previous experience and established criteria in vulvar neoplasia or for uterine cervix[55,56], which ensure their reliability. Third, treatment methods for each patient differed to a certain extent for their multiple kinds of clinical and histological features, which should not be ignored. For these reasons, further research with a larger sample size and more evidence are needed to confirm the findings of this study.

In conclusion, despite the limitations, the results of the present meta-analysis suggested that p16^{INK4a} overexpression was associated with a favorable prognosis in patients with vulvar cancer. The assessment of p16^{INK4a} expression is capable of providing better prognostic information for patients with vulvar cancer.

Supporting Information

S1 PRISMA Checklist. PRISMA 2009 Checklist.
(DOC)

Acknowledgments

All the authors were directly involved in the whole process, therefore it is disclosed that all the authors contributed equally towards the research. Thanks to the editors and anonymous reviewers. The software used in the analysis: stata 12.0 software (Stata Corp, College Station, TX)

Author Contributions

Conceived and designed the experiments: HYC. Performed the experiments: HYC SW ZYZ. Analyzed the data: HYC SW ZYZ. Contributed reagents/materials/analysis tools: HYC SW ZYZ. Wrote the paper: HYC SW ZYZ JYL.

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