Systematic Review and Meta-Analysis

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Meta-analysis of CDKN2A methylation to find its role in prostate cancer development and progression, and also to find the effect of CDKN2A expression on disease-free survival (PRISMA)

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Abstract

Background: Reduction of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) (*p16* and *p14*) expression through DNA methylation has been reported in prostate cancer (PCa). This meta-analysis was conducted to assess the difference of *p16* and *p14* methylation between PCa and different histological types of nonmalignant controls and the correlation of *p16* or *p14* methylation with clinicopathological features of PCa.

Methods: According to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement criteria, articles were searched in PubMed, Embase, EBSCO, Wanfang, and CNKI databases. The strength of correlation was calculated by the pooled odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs). Trial sequential analysis (TSA) was used to estimate the required population information for significant results.

Results: A total of 20 studies published from 1997 to 2017 were identified in this meta-analysis, including 1140 PCa patients and 530 cases without cancer. Only *p16* methylation in PCa was significantly higher than in benign prostatic lesions (OR = 4.72, $P = .011$), but had a similar level in PCa and adjacent tissues or high-grade prostatic intraepithelial neoplasias (HGPIN). TSA revealed that this analysis on *p16* methylation is a false positive result in cancer versus benign prostatic lesions (the estimated required information size of 5116 participants). *p16* methylation was not correlated with PCa in the urine and blood. Besides, *p16* methylation was not linked to clinical stage, prostate-specific antigen (PSA) level, and Gleason score (GS) of patients with PCa. *p14* methylation was not correlated with PCa in tissue and urine samples. No correlation was observed between *p14* methylation and clinical stage or GS. *CDKN2A* mutation and copy number alteration were not associated with prognosis of PCa in overall survival and disease-free survival. *CDKN2A* expression was not correlated with the prognosis of PCa in overall survival (492 cases) ($P > .1$), while *CDKN2A* expression was significantly associated with a poor disease-free survival ($P < .01$).

Conclusion: *CDKN2A* methylation may not be significantly associated with the development, progression of PCa. Although *CDKN2A* expression had an unfavorable prognosis in disease-free survival. More studies are needed to confirm our results.

Abbreviations: CI = confidence interval, *CDKN2A* = cyclin-dependent kinase inhibitor 2A, GS = Gleason score, HGPIN = high-grade prostatic intraepithelial neoplasias, MSP = methylation-specific polymerase chain reaction, OR = odds ratio, PCa = prostate cancer, TSA = trial sequential analysis, TSG = tumor suppressor gene.

Keywords: *CDKN2A*, clinical features, expression, methylation, PCa, prognosis

1. Introduction

According to GLOBOCAN estimates, prostate cancer (PCa) is the most frequent human malignancy and the 3rd-leading cause of cancer-related deaths among men in developed countries. And this disease is the 4th most common cancer and the 6th-leading cause of cancer-related deaths among men in developing countries.[1] Although therapeutic strategies have marked advances in recent years for PCa patients, patients with advanced stage easily develop metastasis, with a poor survival rate.[2–4]

Increasing evidence suggests that epigenetic modifications are an early biotic regulation mechanism in human cancers.[5–7] As a frequent mechanism of epigenetic alterations, DNA methylation is closely linked to cancer initiation and progression.[8,9] Aberrantly methylated tumor suppressor genes (TSGs) have been reported in PCa, such as RAS association domain family protein 1 A,[10] *miR-23b*,[11] and glutathione S-transferase P1.[12] Mapped to human chromosome 9p21, the *p16* and *p14* genes, are encoded by the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and play a key role in cell cycle regulation.[13,14] As a common TSG, downregulation or loss of *CDKN2A* (*p16* and *p14*) expression through DNA methylation frequently occurs

Editor: Giovanni Tarantino.

Authorship: XY and ZC contributed to the conception and design of this research. ZC, LW, and WZ contributed to the drafting of the article and final approval of the submitted version. ZC, LW, WZ, and XY contributed to data analyses and interpretation and completion of the figures and table. All authors read and approved the final manuscript.

The authors have no funding and conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2018) 97:12(e0182)

Received: 20 September 2017 / Received in final form: 17 January 2018 / Accepted: 27 February 2018

http://dx.doi.org/10.1097/MD.0000000000010182

in malignant tumors.[15–17] The *p16* or *p14* gene has been reported to be methylated in PCa.[18–21]

A previous meta-analysis only reported the correlation of *p16* gene methylation between PCa and controls.[22] However, there are some inconsistent and conflicting results regarding *p16* or *p14* methylation in PCa. For example, Yaqinuddin 2013 et al[23] reported that the *p16* gene was not methylated in tissue samples of PCa patients. Although Ameri 2011 et al[24] reported that the *p16* gene was frequently methylated in tissue samples of patients with PCa. Hence, we systematically gathered all available publications to evaluate the correlation of *p16* or *p14* methylation between PCa and different pathological types of nonmalignant tissue samples (adjacent tissues, benign prostatic lesions, or high-grade prostatic intraepithelial neoplasias [HGPIN]). In addition, we also evaluated whether *p16* or *p14* methylation was associated with PCa in blood or urine samples. Finally, we assessed the relationship of *p16* or *p14* methylation with the clinicopathological features of patients with PCa.

2. Materials and methods

2.1. Literature search strategy

A comprehensive search of PubMed, Embase, EBSCO, Wanfang, and CNKI databases was performed to get the eligible studies before March 7, 2017. The following search terms and key words were used: "P16 OR CDKN2A OR INK4A OR cyclin-dependent kinase inhibitor 2A," "p14 OR p14ARF," "methylation OR methylated OR epigene*," "prostate cancer OR PCa OR prostate adenocarcinoma OR prostate tumor OR prostate carcinoma OR prostate neoplasm." The eligible studies were retrieved to identify other potential publications.

2.2. Inclusion criteria

All eligible studies had to meet the following selection criteria in this meta-analysis: all patients were confirmed with primary PCa; studies reported the information of *p16* or *p14* methylation in PCa, with no restriction of sample types (tissue, urine, or blood); studies provided sufficient data to evaluate the association between *p16* or *p14* methylation and PCa in cancer versus different control types (adjacent tissues, benign prostatic lesions, or HGPIN, etc); and studies provided sufficient data to assess the relationship of *p16* or *p14* methylation with clinicopathological features of patients with PCa, including clinical stage, prostate-specific antigen (PSA) level, and Gleason score (GS). The most recent or complete paper was included when duplicate publications were reported using the same study population.

2.3. Ethical statement

This meta-analysis was not primary research involving human samples, but rather a secondary analysis of human subject data published in the public domain.

2.4. Data extraction

Data extraction was performed by 2 independent authors (ZC and LW). Disagreements were discussed to resolve the dispute by all authors. The relevant information was carefully scanned and extracted from all eligible publications: first author's name, year of publication, country, ethnicity, detection method, sample types, age, the number of the study population, control groups, methylation frequency, and the clinicopathological features. Control groups included adjacent tissues, benign prostatic lesions, HGPIN, and blood or urine samples without cancer. The clinicopathological features consisted of tumor stage (stage 3–4 vs stage 1–2), PSA level (PSA >8 vs PSA ≤ 8), and GS (GS ≥ 7 vs GS ≤ 6).

2.5. Statistical analysis

The statistical analyses were conducted by Stata 12.0 software (Stata Corporation, College Station, TX). The strength of association between *p16* or *p14* methylation and PCa risk was estimated by calculating combined odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). The heterogeneity among the included publications was investigated with the Q-test statistic.[25,26] This meta-analysis was performed using a random-effects model. A P value of less than .10 was considered as substantial heterogeneity. We performed a sensitivity analysis to analyze the influence of an individual study on the pooled result and heterogeneity by omitting one study.[27,28] For the analyses with more than 8 studies, the potential publication bias was measured with Egger line regression test.[29,30] For significant results, to decrease the type I and II error rates, the trial sequential meta-analysis (trial sequential analysis [TSA]) was conducted to estimate the needed sample information.[31,32] The type I error rate was defined as 5%, and the type II error rate was set as 20% (a statistical power of 80%). The relative risk reduction (RRR) rate was considered as 20%. When the cumulative Z-curve crossed the trial sequential monitoring boundary or the required information size, which suggested that the evidence is conclusive. Otherwise, a false positive result is achieved.[33,34]

3. Results

3.1. Study characteristics

After searching a range of online databases, 285 articles were initially searched, as shown in Fig. 1, according to the above-mentioned selection criteria, final 20 articles published from 1997 to 2017[18–21,23,24,35–48] were included in the present meta-analysis, including 1140 patients with PCa and 530 cases without cancer. Of the eligible studies, 17 studies evaluated the relationship between *p16* methylation and PCa in cancer versus different control groups.[18–21,23,24,35,39–48] 12 studies including 748 patients with PCa assessed the correlation of *p16* methylation with the clinicopathological characteristics of PCa.[18,19,21,24,36–38,41–43,45,46] Seven publications analyzed the correlation between *p14* methylation and PCa.[20,21,37,41,42,44,45] The basic characteristics of the eligible publications are listed in Table S1, http://links.lww.com/MD/C169.

3.2. Correlation between p16 methylation and PCa in cancer versus different control types

When cancerous tissues were compared to noncancerous tissue samples, the data included 9 studies with 637 PCa patients and 228 benign prostatic lesions, 5 studies with 219 PCa patients and 78 adjacent tissue samples, and 3 studies with 167 PCa patients and 55 patients with HGPIN (Fig. 2). The results of *p16* methylation demonstrated that a significant relationship was found in PCa versus benign prostatic lesions (OR = 4.72, 95% CI = 1.42–15.70, $P = .011$), but no association was observed in PCa versus adjacent tissues or HGPIN (OR = 1.68, 95% CI = 0.24–11.85, $P = .602$; OR = 1.30, 95% CI = 0.41–4.17, $P = .657$, respectively).

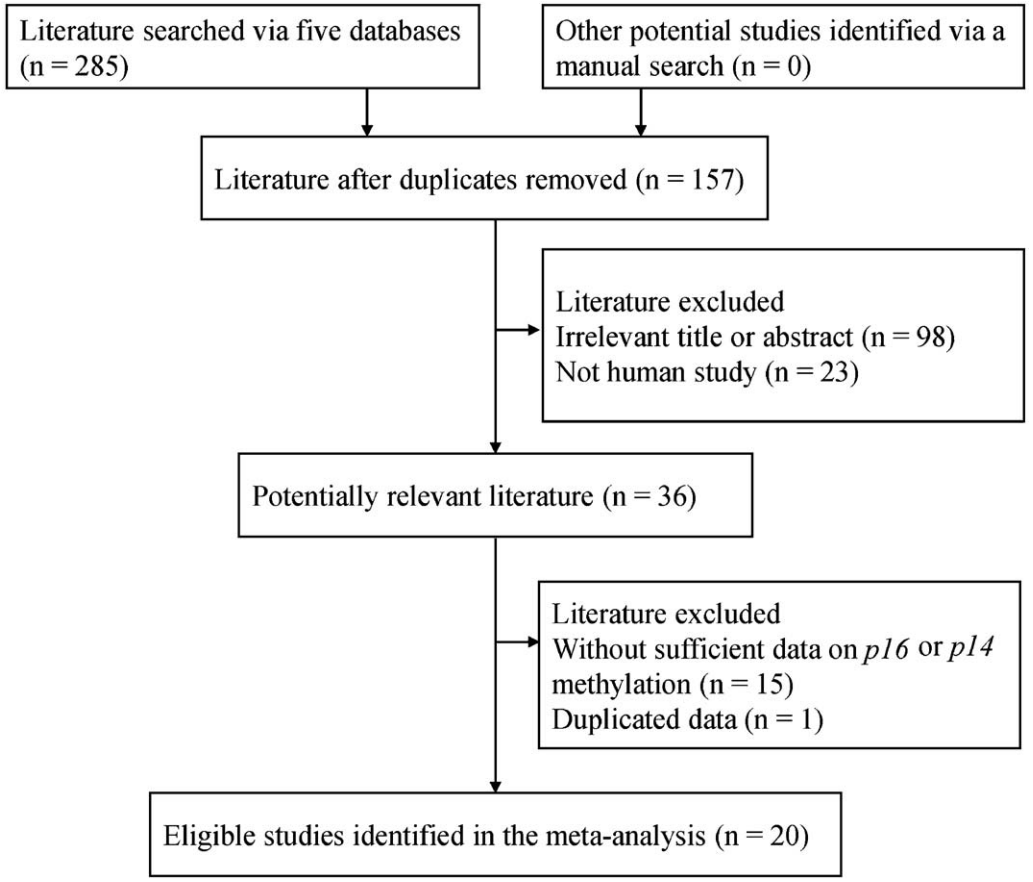

Figure 1. Flow diagram of the procedure for selecting literature.

We analyzed whether *p16* methylation was associated with PCa in the urine and blood (Fig. 2). The result from 2 studies with 97 PCa and 40 cases without cancer showed that *p16* methylation was not correlated with PCa in the blood (OR = 1.10, 95% CI = 0.04–27.63, $P = .956$). The result from 2 studies involving 147 PCa and 129 cases without cancer demonstrated that *p16* methylation was not associated with PCa in the urine (OR = 13.45, 95% CI = 0.24–754.62, $P = .206$).

3.3. Subgroup analysis of p16 methylation in PCa versus benign prostatic lesions

According to ethnicity (Caucasians and Asians) and testing method (methylation-specific polymerase chain reaction [MSP] and non-MSP), subgroup analysis of ethnicity showed a trend toward the correlation between *p16* methylation and Caucasians with PCa (OR = 4.16, 95% CI = 0.91–19.16, $P = .067$) (Fig. 3). A significant correlation was found between *p16* methylation and Asians with PCa (OR = 7.95, 95% CI = 1.49–42.54, $P = .015$) (Fig. 3).

Subgroup analysis of detection method demonstrated that *p16* methylation was correlated with PCa in the MSP method (OR = 7.40, 95% CI = 2.01–27.26, $P = .003$), but not in the non-MSP subgroup (OR = 3.63, 95% CI = 0.53–24.83, $P = .189$) (Fig. 4).

3.4. Sensitivity analysis of p16 methylation in PCa versus benign prostatic lesions

A substantial heterogeneity was detected in the comparison of PCa and benign prostatic lesions ($P = .020 < .1$). One study (Jerónimo 2004 et al)[20] was deleted, the pooled OR was recalculated (OR = 7.80, 95% CI = 3.13–19.45, $P < .001$), with no evidence of heterogeneity ($P = .693$).

3.5. Correlation of p16 methylation with clinicopathological features of patients with PCa

The result demonstrated that *p16* methylation was not associated with clinical stage, PSA level or GS of patients with PCa (all $Ps > .1$) (Fig. 5). These data included 5 studies with 547 PCa patients on clinical stage, 4 studies with 211 PCa patients on PSA level and 12 studies with 747 PCa patients on GS.

3.6. Publication bias

Egger test was used to estimate the potential publication bias in PCa versus benign prostatic lesions and GS (Fig. 6). No obvious evidence of publication bias was found in PCa versus benign prostatic lesions and GS ($P > .5$).

3.7. Correlation between p14 methylation and PCa in cancer versus different control types

No correlation was found between *p14* methylation and different control types in cancer versus adjacent, benign tissue samples, or urine controls without cancer among 2 studies, respectively (all $Ps > .5$) (Fig. 7). Because the sample sizes were small in the current meta-analysis, additional studies with a large population are needed.

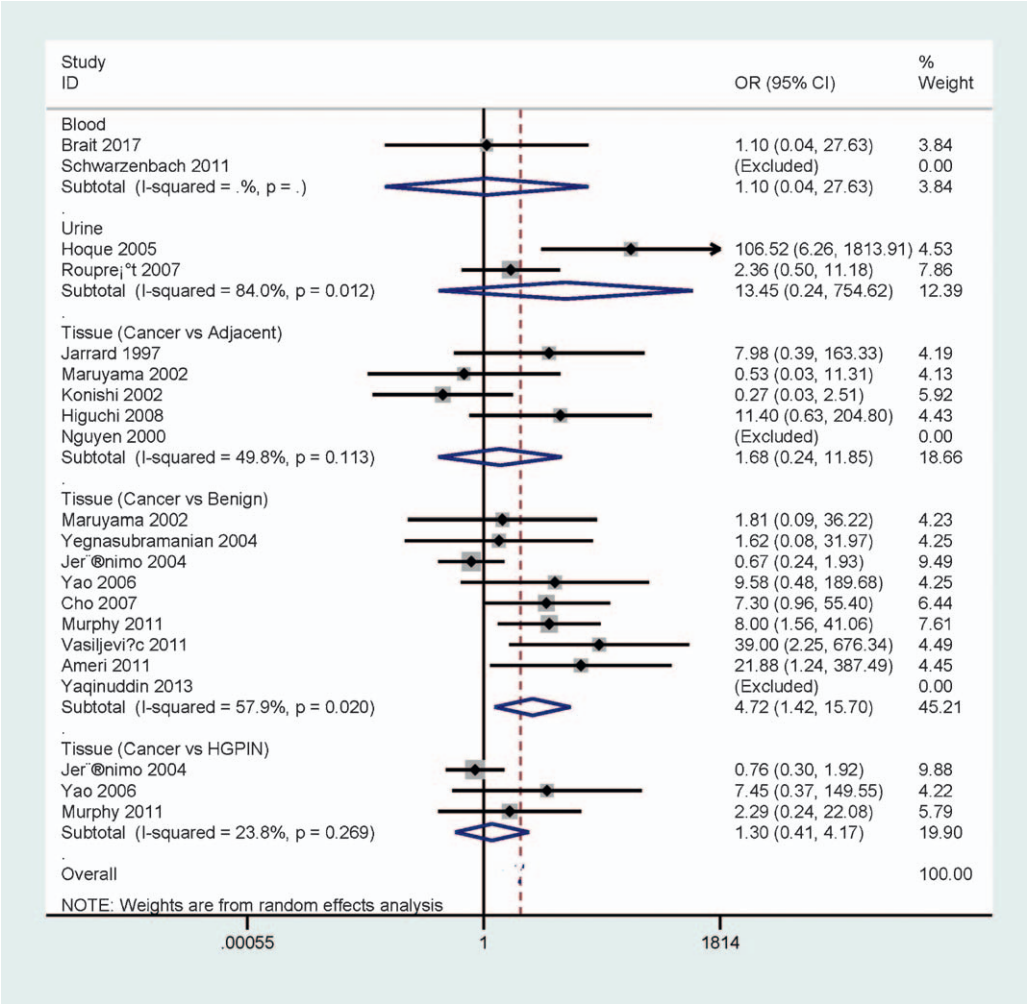

Figure 2. Forest plot of the pooled OR between *p16* methylation and PCa in cancer versus different types of noncancerous tissues, urine, and blood samples. OR = odds ratio, PCa = prostate cancer.

3.8. Correlation of p14 methylation with clinicopathological features of patients with PCa

The result from 3 studies involving 238 PCa patients shown no correlation between *p14* methylation and tumor stage ($P = .666$) (Fig. 8). No relationship was found between *p14* methylation and GS among 5 studies ($P = .269$), including 323 PCa patients (Fig. 8).

3.9. TSA

TSA was performed using the priori anticipated information size (APIS) method in PCa versus benign lesions, as shown in Fig. 9, the required information size was 5116 participants. The result showed that the cumulative Z-curve was not more than the trial sequential monitoring boundary, suggesting a false positive result.

3.10. Prognostic role of CDKN2A

No survival information or data on *CDKN2A* (*p16* and *p14*) methylation was reported in PCa. The data from GEPIA database[49] demonstrated that no association was found between *CDKN2A* expression and the prognosis of PCa in overall survival (492 cases) ($P > .1$) (Fig. 10), while *CDKN2A* expression was significantly associated with a poor disease-free survival ($P < .01$) (Fig. 11).

4. Discussion

DNA methylation of TSGs leads to loss of gene expression and plays an essential role in the development and progression of human cancers.[50–52] Some studies suggest that promoter methylation of TSGs is closely associated with PCa.[53,54] As an important TSG, *p16* methylation has been reported in PCa, the relationship between *p16* methylation and its expression was observed in PCa, with loss of *p16* expression.[18,48] *p16* methylation may play a crucial role in the tumorigenesis of PCa.[24,43] *p14* methylation was significantly higher in PCa than in urine samples without cancer by Hoque et al.[21] However, the exact association between *p16* or *p14* methylation and PCa is still controversial. Therefore, we conducted this meta-analysis to resolve these inconsistent results on the role of *p16* or *p14* methylation in PCa.

PCa develops from normal epithelium into benign lesions (benign prostatic hyperplasias), then progresses from premalignant (HGPIN) to malignant (prostate carcinoma) lesions via

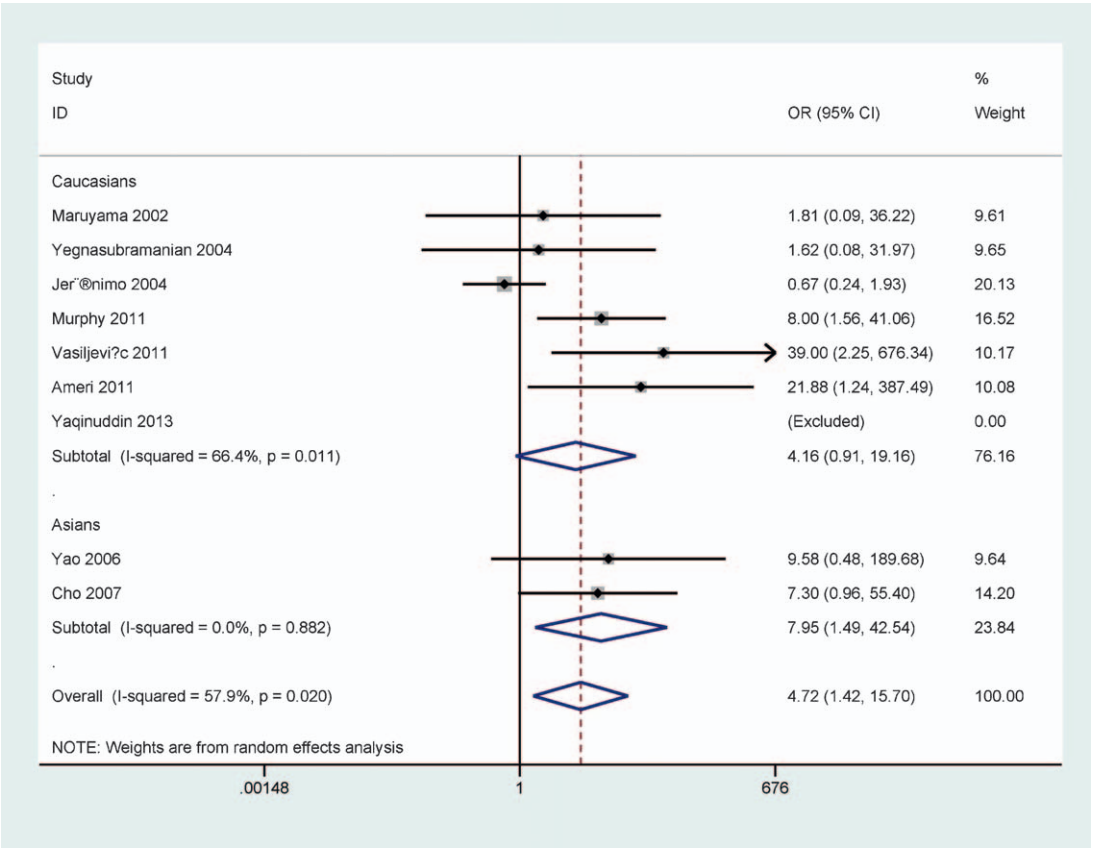

Figure 3. Forest plot of subgroup analysis by ethnicity in prostate cancer (PCa) versus benign prostatic lesions.

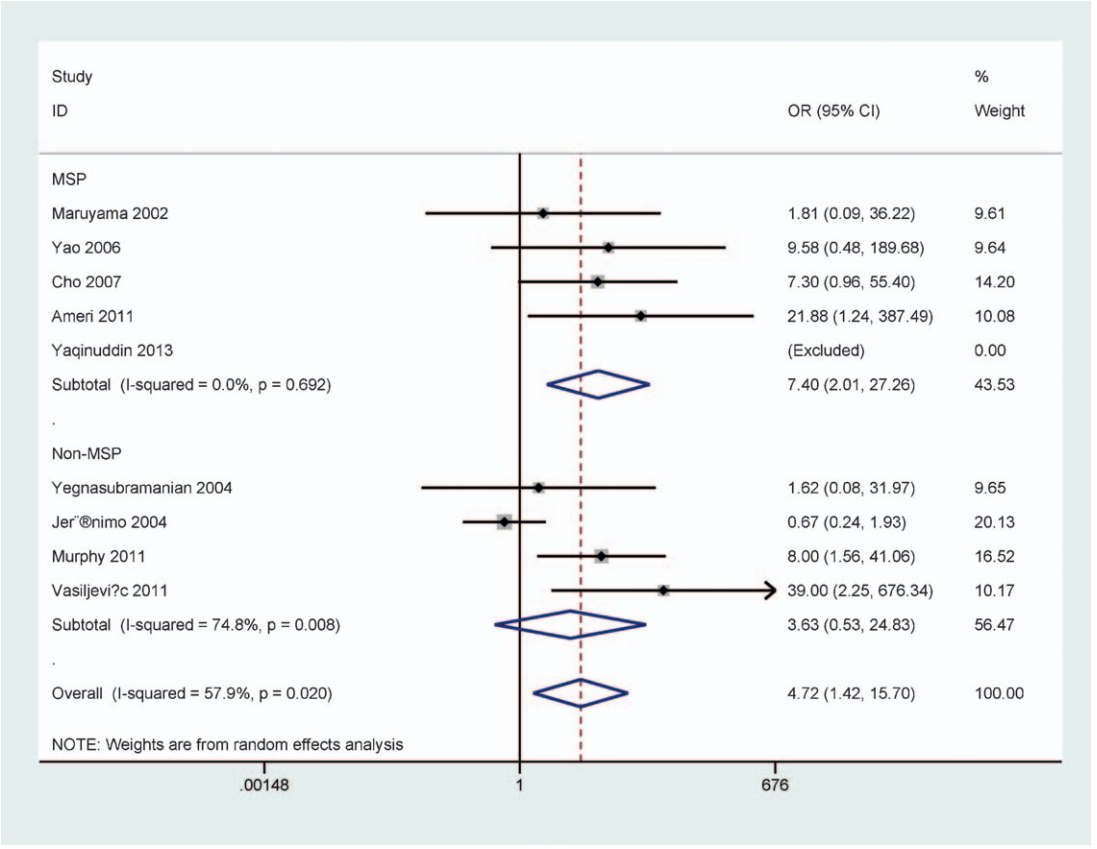

Figure 4. Forest plot of subgroup analysis by the testing method in prostate cancer (PCa) versus benign prostatic lesions.

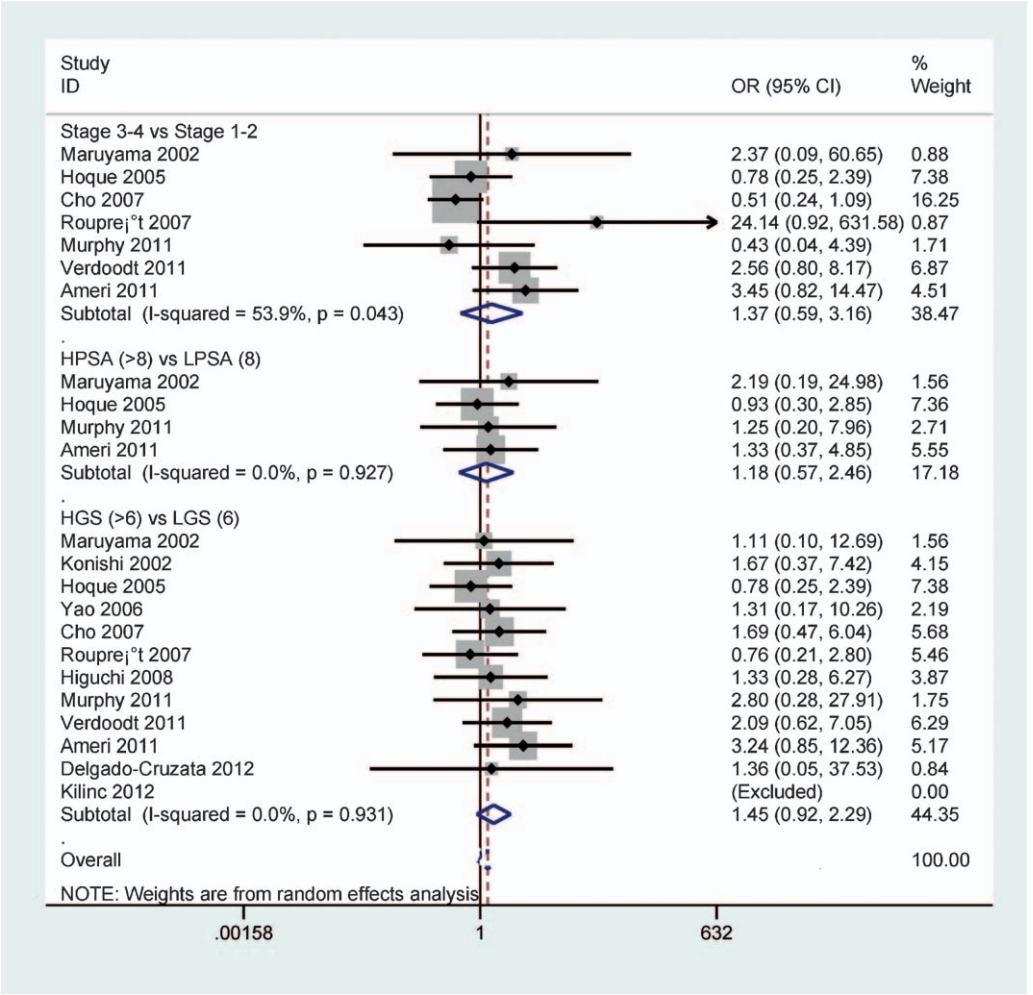

Figure 5. Forest plot of the association between *p16* methylation and clinical stage, PSA level, or GS of patients with PCa (all $P > .1$). GS = Gleason score, PCa = prostate cancer, PSA = prostate-specific antigen.

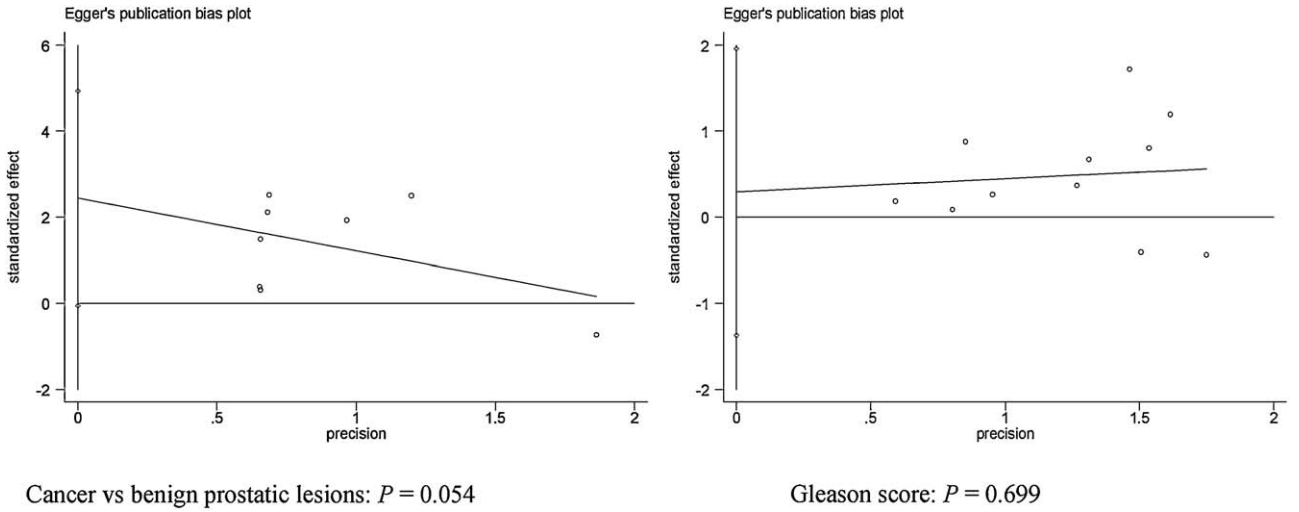

Figure 6. Forest plot of publication bias using Egger test in PCa versus benign prostatic lesions and GS ($P > .05$). GS = Gleason score, PCa = prostate cancer.

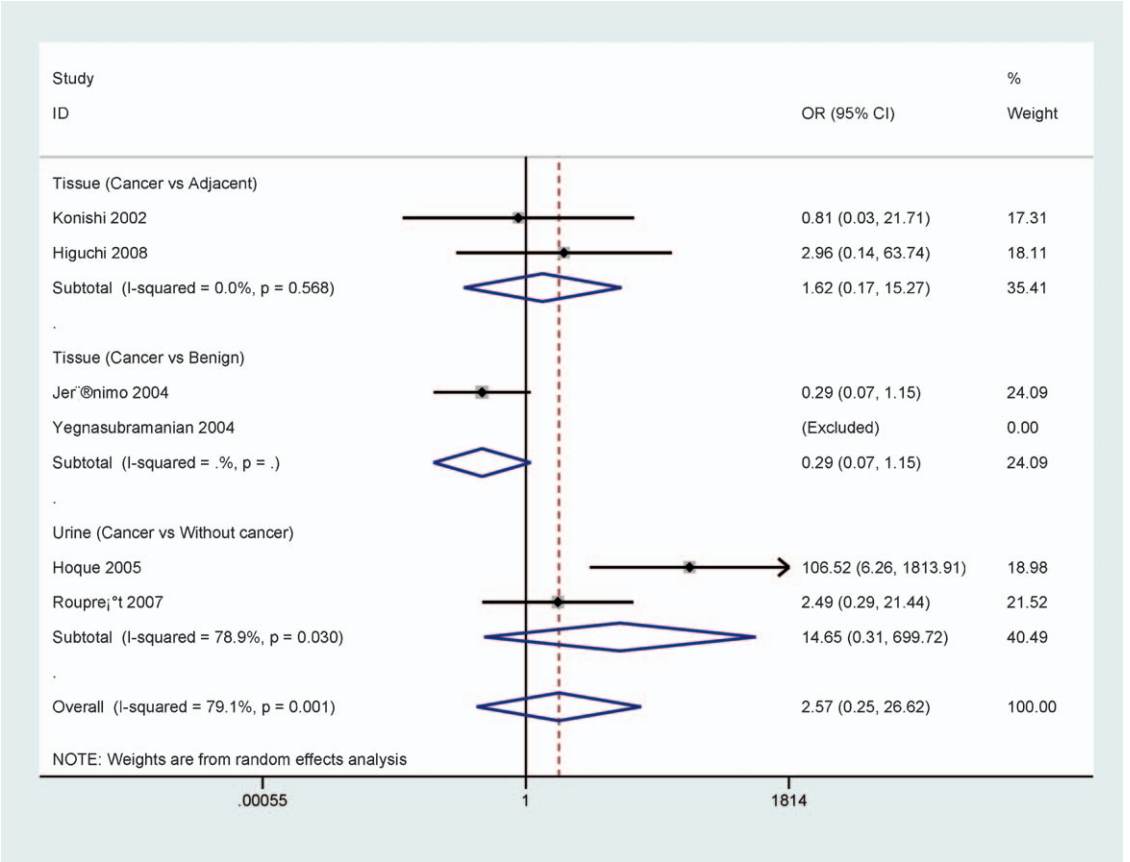

Figure 7. Forest plot of the association between *p14* methylation and prostate cancer (PCa) in cancer versus different control types (all $P > .05$).

multiple sequences.[55–57] When cancerous tissue samples were compared to different types of noncancerous tissue samples, no association between *p16* methylation and PCa was found in cancer versus adjacent tissue samples among all studies.[41,45–48] Moreover, no relationship between *p16* methylation and PCa was observed in cancer versus and HGPIN among all studies.[18–20] Four studies reported that significant correlation between *p16* methylation and PCa was found in cancer versus benign prostatic lesions,[18,24,39,43] but the other studies showed no correlation.[19,20,23,44,46] Our meta-analysis involving more eligible articles showed that *p16* methylation was notably higher in PCa than in benign prostatic lesions (OR = 4.72, $P = .011$), but *p16* methylation had a similar OR value in PCa and adjacent normal tissue samples or HGPIN. In addition, further TSA revealed that the result in the comparison of PCa and benign lesions was false positive, additional studies are necessary to confirm it. Our results suggested that *p16* methylation may not be significantly correlated with PCa development. Because the sample sizes were small, more studies are needed in PCa versus adjacent normal tissue samples and HGPIN.

We also determined whether *p16* methylation was correlated with PCa in urine and blood samples, *p16* methylation was not associated with PCa in the blood.[35,40] One study reported the correlation between *p16* methylation and PCa in urine samples,[21] but another study showed no association between *p16* methylation and PCa in urine samples.[42] Our results suggested that *p16* methylation was not correlated with PCa in the blood and urine. Additional studies with large sample sizes are needed to confirm whether *p16* methylation may be a noninvasive biomarker using blood or urine samples in the future.

No correlation of *p14* methylation was found in the tissue (cancer vs adjacent tissues)[41,45] and (cancer vs benign tissues).[20,44] The current results indicated no relationship between *p14* methylation and PCa in tissue samples, which were consistent with an individual study. *p14* methylation showed a higher frequency in PCa than in urine samples without cancer,[21] but another study demonstrated a similar frequency in PCa and control urine samples without cancer.[42] Our results showed that *p14* methylation was not associated with PCa in the urine. The results of *p14* methylation should be carefully considered because small sample sizes were included in this meta-analysis.

We analyzed whether *p16* or *p14* methylation was correlated with the clinicopathological features, including tumor stage, prostate-specific antigen level, and GS. For eligible studies, no significant relationship was observed between *p16* methylation and tumor stage,[18,21,24,37,42,43,46] prostate-specific antigen level,[18,21,24,46] or GS.[18,19,21,24,36–38,41–43,45,46] Our results comprising more studies with a large population showed that *p16* methylation was not correlated with tumor stage, prostate-specific antigen level, and GS ($P > .1$). Our results were consistent with an individual study regarding *p16* methylation. One study reported the relationship between *p14* methylation and clinical stage,[42] but the other studies showed no association.[21,37] *p14* methylation was negatively associated with GS by Verdoodt 2011 et al,[37] while no correlation was reported among the remaining 4 studies.[21,41,42,45] The results demonstrated that *p14*

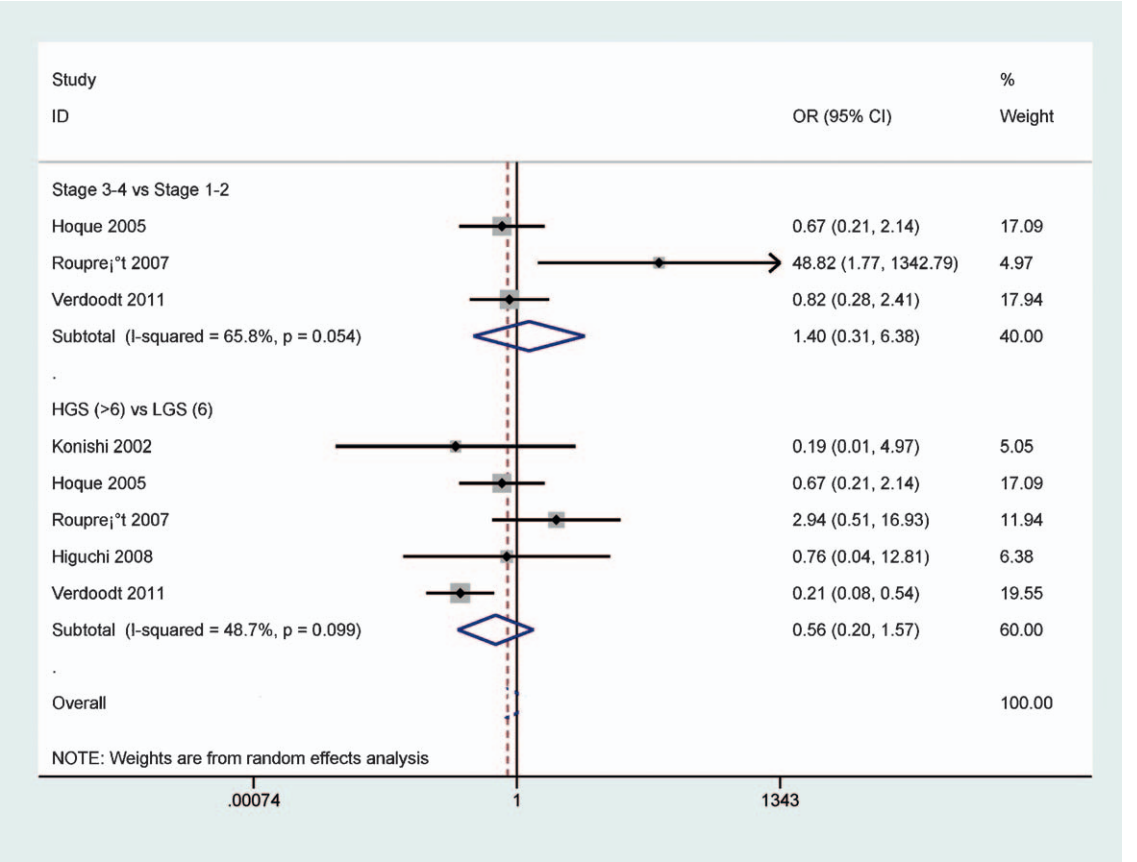

Figure 8. Forest plot of the association of *p14* methylation with clinicopathological features of patients with prostate cancer (PCa) (all $P > .1$).

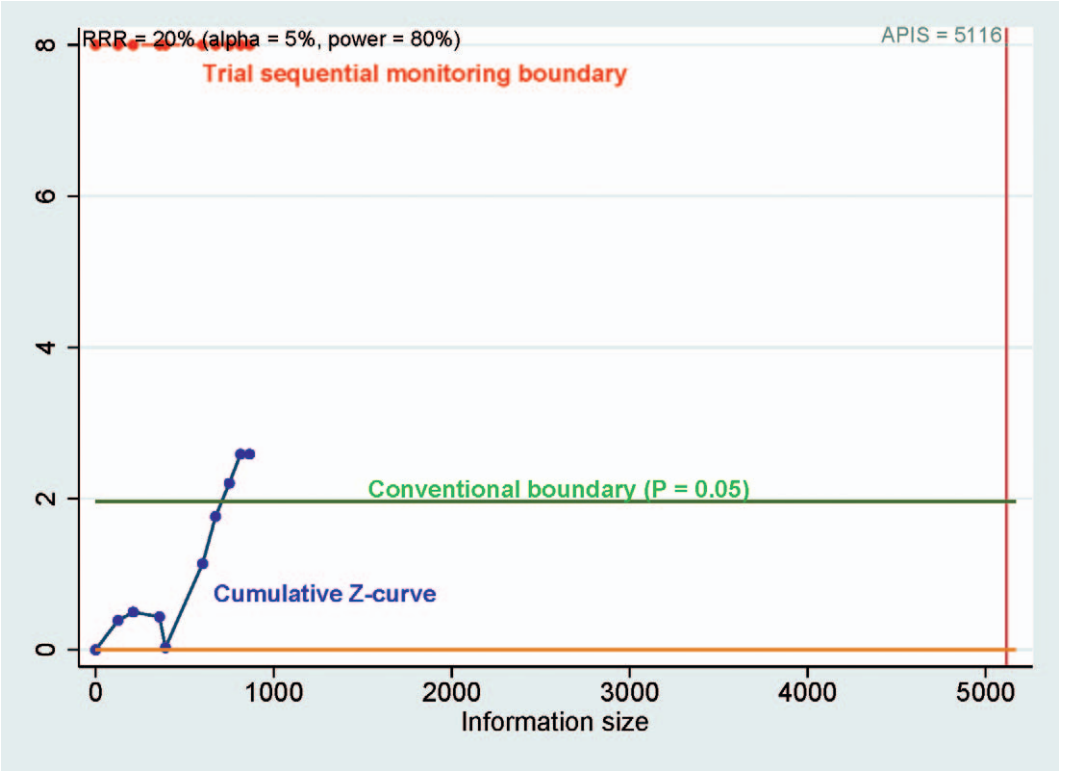

Figure 9. Trial sequential analysis assessing the required sample information in prostate cancer (PCa) versus benign lesions, the cumulative Z-curve crossed the conventional boundary ($Z = 1.96$, $P = .05$), did not cross the trial sequential monitoring boundary, suggesting that the cumulative evidence is inconclusive.

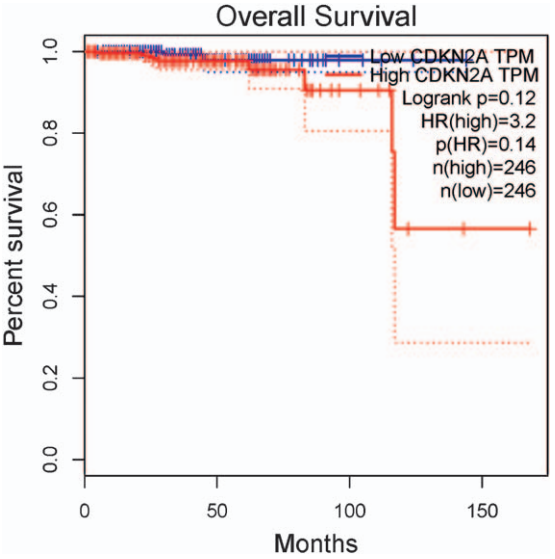

Figure 10. The correlation between *CDKN2A* expression and the prognosis of PCa in overall survival ($P > .1$). *CDKN2A* = cyclin-dependent kinase inhibitor 2A, PCa = prostate cancer.

methylation was not linked to clinical stage and GS of patients with PCa. The above analyses suggested that *p16* and *p14* methylation may not be linked to the clinicopathological features of PCa patients.

Interestingly, we found that *CDKN2A* expression was significantly associated with a reduced prognosis of PCa in disease-free survival. The cBioportal database[58,59] (TCGA, Provisional, 499 samples) was also used to explore the possible prognostic impact of *CDKN2A* mutation and copy number alteration in PCa. *CDKN2A* mutation was shown in 1 (0.2%) of 498 samples and was not associated with the prognosis in overall survival and disease-free survival (data not shown). *CDKN2A* copy number alteration (deep deletion) was shown in 8 (1.6%) of 498 samples and was not correlated with the prognosis in overall survival and disease-free survival (data not shown). The other mechanisms such as allelic loss or amplification may lead to *CDKN2A* expression. And therefore *CDKN2A* expression may influence the prognosis of PCa in disease-free survival. Additional studies are necessary in the future.

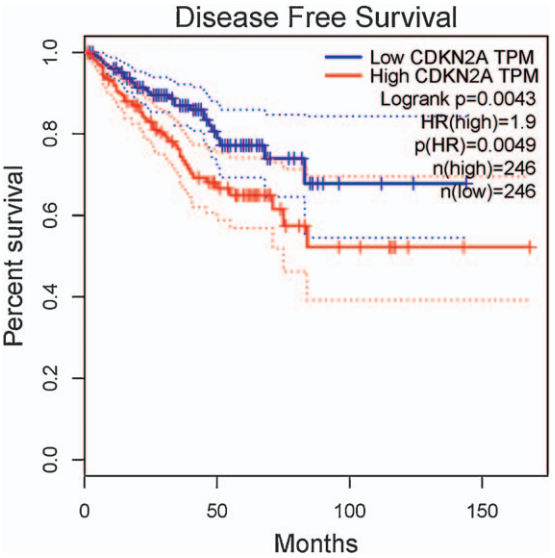

Figure 11. The correlation between *CDKN2A* expression and the prognosis of PCa in disease-free survival ($P < .01$). *CDKN2A* = cyclin-dependent kinase inhibitor 2A, PCa = prostate cancer.

The current meta-analysis compared favorably with this previous meta-analysis by Feng et al,[22] the previous study only reported that *p16* methylation was correlated with PCa in cancer versus controls. The number of study population included in our meta-analysis (n = 1670 samples) was larger than in the previous study (n = 1296 samples). A further TSA was conducted in our study, and we found this false positive result in PCa versus benign lesions. Additionally, this previous meta-analysis did not evaluate the exact correlation between *p16* methylation and the sequence of histological changes of the prostate ([adjacent normal tissues-benign lesions (benign prostatic hyperplasias)-premalignant lesions (HGPIN)-malignant prostate]). Finally, this previous meta-analysis did not analyze the association between *p16* methylation and clinicopathological features of PCa, the association between *p14* methylation and PCa, and the potential prognostic effect of *CDKN2A* methylation, expression, mutation, or copy number alteration in PCa.

A slight heterogeneity was measured in PCa versus benign prostatic lesions (*p16* gene: $P = .020 < .1$). When we removed this study (Jerónimo 2004 et al)[20] and recalculated the overall OR from the remaining studies. The pooled OR did not significantly change (OR = 7.80, $P < .001$), with no heterogeneity ($P = .693$). The possible reasons for substantial heterogeneity were not very clear, possibly because of the use of the inappropriate conditions in the detection of *p16* methylation. The present meta-analysis had several limitations. First, only articles published in English or Chinese were selected in this study. Publications in other languages were excluded based on insufficient information, which may cause a selection bias. Second, only 2 studies of blood or urine samples were analyzed. Third, sample sizes were small among different subgroups and in the comparison of PCa and different control groups. More studies with large populations are necessary to validate these results.

5. Conclusions

Our findings suggest that only *p16* methylation may have a higher frequency in PCa than in benign prostatic lesions. *p16* methylation was not associated with PCa risk in PCa versus adjacent tissues or HGPIN. No correlation between *p16* methylation and PCa was found in blood or urine samples. In addition, *p16* methylation was not correlated with clinical stage, PSA level, and GS of patients with PCa. *p14* methylation was not correlated with PCa in the tissue and urine, and no relationship was observed between *p14* methylation and clinical stage and GS. No correlation was found between *CDKN2A* mutation and copy number alteration and the prognosis of PCa in overall survival and disease-free survival. No association was observed between *CDKN2A* expression and the prognosis of PCa in overall survival, while *CDKN2A* expression was significantly associated with a poor prognosis in disease-free survival. Further large-scale and well-designed trials are essential to confirm our findings in the future.

Author contributions

Conceptualization: Z. Cao, X. Yao.
Formal analysis: Z. Cao, L. Wei, W. Zhu, X. Yao.

Methodology: Z. Cao, X. Yao.
Software: Z. Cao, L. Wei, W. Zhu, X. Yao.
Supervision: X. Yao.
Validation: Z. Cao, X. Yao.
Writing – original draft: Z. Cao, L. Wei, W. Zhu, X. Yao.
Writing – review & editing: Z. Cao, L. Wei, X. Yao.

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