Original Article
APC methylation predicts biochemical recurrence of patients with prostate cancer: a meta-analysis

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Abstract: The promoter region of adenomatous polyposis coli (APC) has been found to be frequently methylated in prostate cancer. However, the prognostic role of APC methylation in prostate cancer was still debated. We performed a meta-analysis by searching PubMed and EMBASE databases. Pooled hazard ratios (HRs) and corresponding 95% confidence intervals (CI) were calculated. Seven studies (1227 patients) were included in this study. After calculation, the overall HR was 1.74 (95% CI: 1.31-2.31), implicating that APC methylation has an unfavorable impact on biochemical recurrence of prostate cancer. A subgroup analysis was performed with detection method, combined HR was 1.53 (95% CI: 1.19-1.96) for Methylation-Specific PCR (MSP), and 2.08 (95% CI: 1.18-3.64) for quantitative Methylation-Specific PCR (qMSP). Another subgroup analysis was conducted according to regions of the patients, combined HR was 2.02 (95% CI: 1.18-3.49) for North America, and 1.64 (95% CI: 1.14-2.36) for European. In conclusion, APC methylation is associated with biochemical recurrence of patients with prostate cancer.

Keywords: APC, methylation, biochemical recurrence, meta-analysis

Introduction

Prostate cancer (PC) is the second leading cause of cancer-related death amongst male patients in the Western world [1, 2], and its incidence is still increasing. Prostate cancer is a complex, multifactorial disease with heterogeneous tumor types. It is still a major challenge to distinct between aggressive and indolent disease [3]. Small et al reported several prognostic markers, such as Gleason grade, clinical stage, and pretreatment prostate-specific antigen (PSA) levels, but had limited prognostic value for individual patient [4]. Therefore, it is necessary to explore effective biomarkers for biochemical recurrence of patients with prostate cancer.

The molecular biomarkers for prostate cancer have been widely investigated, especially in methylation markers, such as ABHD9 [5], GSTP1 [6], HOXD3 [7], adenomatous polyposis coli (APC) [8], PTGS2 [9], RARB [10] and RASSF1A [11]. APC is a tumor suppressor gene that acts as an antagonist of Wnt signaling pathway [12]. It is also involved in other processes including cell migration [13] and apoptosis [14]. In addition, APC has also been reported to correlate to the biochemical recurrence for patients with prostate cancer [15]. The relationship between APC methylation and the biochemical recurrence for patients with prostate cancer is still debated. In this study, a meta-analysis was conducted to evaluate the association of APC methylation with biochemical recurrence in prostate cancer patients.

Material and methods

Study selection

We collected literatures by searching PubMed and EMBASE databases updated to January, 2015. Studies were searched by using the following keywords or text words: "adenomatous polyposis coli (APC)" in combination with "methylation OR hypermethylation", "prostate cancer OR prostate neoplasms" and "recurrence OR relapse OR biochemical recurrence". For each paper, additional studies were selected from its

Table 1. General parameters of the seven studies included

Author	Year	Country	Sample size	Method
Rosenbaum	2005	USA	74	qMSP
Henrique	2007	Portugal	83	qMSP
Alumkal	2008	USA	151	MSP
Richiardi	2009	Italy	459	MSP
Liu	2011	Canada	219	qMSP
Richiardi	2013	Italy	157	MSP
Moritz	2013	Germany	84	qMSP

out these elements to ensure the quality of this meta-analysis.

Data extraction

According to the selection criteria, the data was extracted from each identified paper. The name of the First author, year for publication and total number of cases (N) were demonstrated in **Table 1**. Additional data were reviewed as the following: hazard ratio (HR), 95% confidence intervals (CI) and *P* value.

Statistical analysis

In this study, the random effect model was used for meta-analysis, according to the heterogeneity between studies. Heterogeneity was tested by the Q test (P<0.10 was considered as statistically significant heterogeneity) and the I^2 statistic (values of 25%, 50% and 75% were considered to represent low, medium and high heterogeneity, respectively. The fixed effect model was used when there was no significant heterogeneity ($I^2 < 50\%$); otherwise the random effect model was used. *P* values were calculated by I^2 tests. All the reported *P* values were analyzed by two-tailed student *t* test and *P* values <0.05 were regarded as statistical significance for all included studies.

Analyses were performed using STATA statistical software (Version 12.0). Calculation of dichotomous variables was carried out using the HR with the 95% confidence interval (CI) as the summary statistic. The Mantel-Haenszel method was used to combine HRs for the outcome parameters. Begg's test and Egger's test were used to evaluate the publication bias.
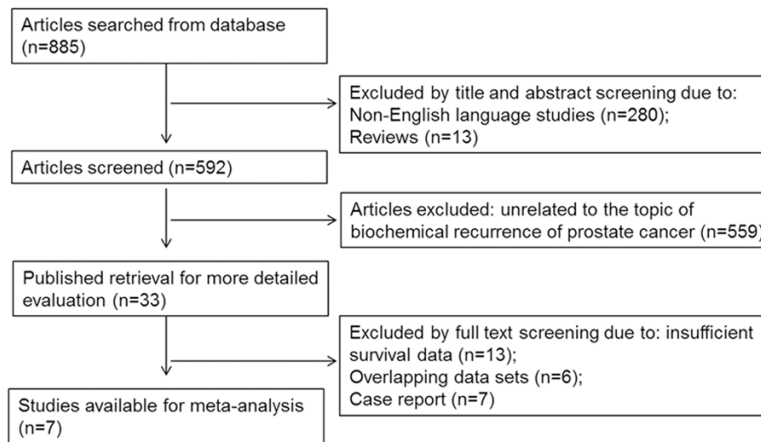

Figure 1. Flow chart of study selection in this meta-analysis.

references, citations and from the PubMed option "Related Articles".

Criteria for inclusion and exclusion

The following criteria were used to select studies: (1) Discussing the patients with the prostate cancer; (2) Investigating the biochemical recurrence or the correlation between APC methylation expression and the clinical variables; (3) Using a Cox proportional hazards modeling, (4) sufficient published data for estimating a hazard ratio (HR) with 95% confidence interval (CI), and (5) article written in English. The following criteria were used to exclude published studies: (1) letters and reviews, (2) lack of data information, (3) non-English language literatures, (4) studies of genetic variation, (5) no tissue samples in the studies, (6) overlapping data sets.

Quality assessment

The following factors were noted: study population, origin of country, method used to detect APC methylation. We excluded the studies with-

Results

Study selection and characteristics

Based on the including and excluding criteria, seven studies were collected in the meta-analysis (**Figure 1**). The methods used to detect
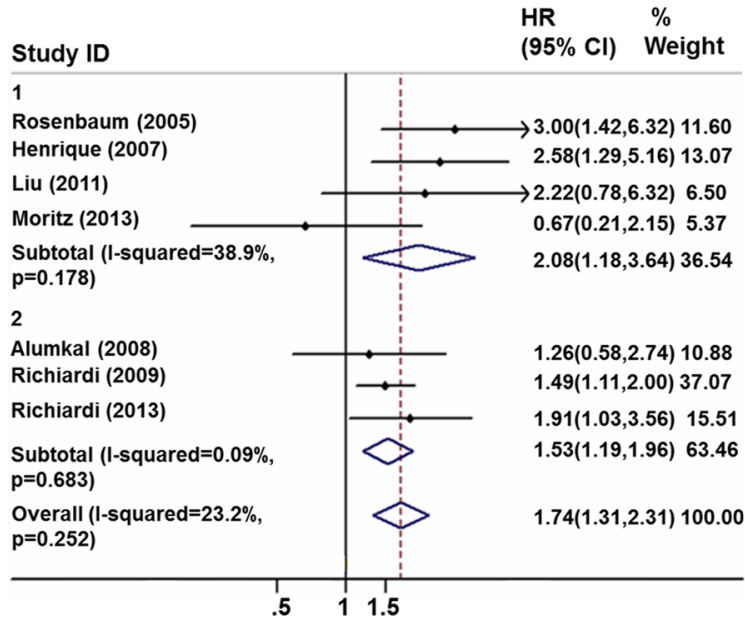
Figure 2. Meta-analysis (Forest plot) showing hazard ratios of the APC methylation. Subgroup analysis of detection method: qMSP (up)/MSP (down) Hazard ratios for each trial are represented by the squares, the size of the square represents the weight of the trial in the meta-analysis, and the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect (labeled total) using the mantel-Haenszel random-effect model. All *P* values are two-sided.
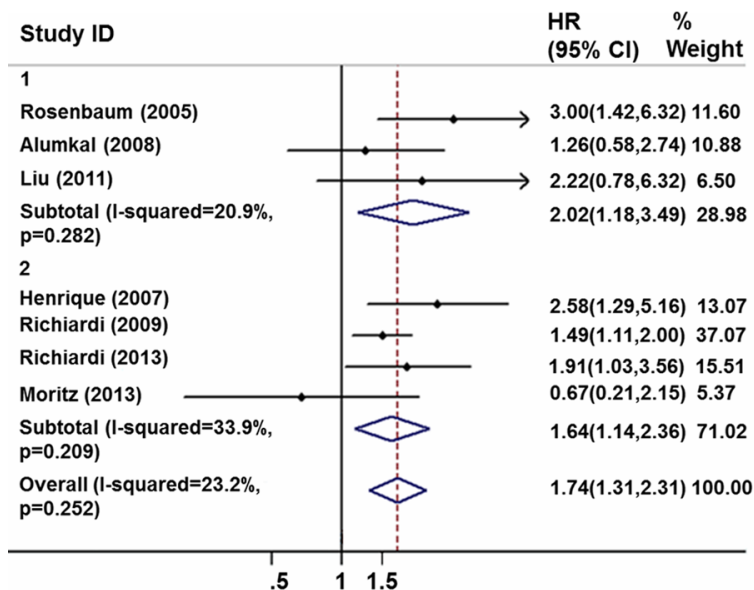
Figure 3. Meta-analysis (Forest plot) showing hazard ratios of the APC methylation. Subgroup analysis of region: North (up)/Europe (down). Hazard ratios for each trial are represented by the squares, the size of the square represents the weight of the trial in the meta-analysis, and the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect (labeled total) using the mantel-Haenszel random-effect model. All *P* values are two-sided.

APC methylation included quantitative Methylation-Specific PCR (qMSP) and Methylation-Specific PCR (MSP): four studies for qMSP [16-19], three studies for MSP [8, 20, 21]. The selected patients were from North America and Europe: three studies from North America [16, 18, 20], four studies from Europe [8, 17, 19, 21]. A total of 1227 cases were collected in this meta-analysis. The general information of those studies was shown in **Table 1**.

Association of APC methylation and biochemical recurrence

The risk factor for biochemical recurrence was analyzed by random effect model pooled OR and 95% CI. The combined analysis of the seven studies showed that APC methylation was associated with biochemical recurrence (HR = 1.74, 95% CI: 1.31-2.31), with the heterogeneity (I^2 statistic) of 23.2% (**Figures 2**, **3**). A subgroup analysis was performed with detection method, combined HR was 1.53 (95% CI: 1.19-1.96) for MSP, and 2.08 (95% CI: 1.18-3.64) for qMSP (**Figure 2**). This result suggested that qMSP might be more sensitive to detect APC methylation in prostate patients. Another subgroup analysis was conducted according to patient region, combined HR was 2.02 (95% CI: 1.18-3.49) for North America, and 1.64 (95% CI: 1.14-2.36) for European (**Figure 3**). These data indicated that APC methylation in North America patients predicted higher biochemical recurrence rate than that in European. Taken together, APC methylation is associated with biochemical recurrence of patients with prostate cancer.
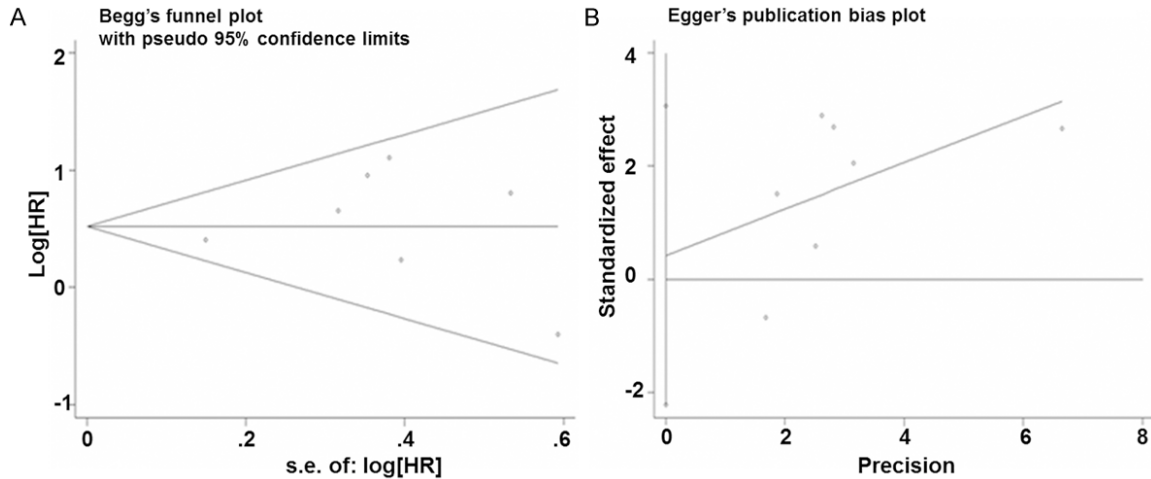
Figure 4. Begg's and Egger's funnel plot with 95% confidence intervals for publication bias testing.

Furthermore, funnel plot analysis excluded the publication bias (Begg's test z = 0.00, P = 1.000, Egger's test t = 0.41, P = 0.698) (**Figure 4A**, **4B**).

Discussion

Aberrant methylation affected the biochemical recurrence of cancers [22-25]. Here, we focused on the APC methylation in prostate cancers to collect complete articles and infer potential prognostic value. The HR of APC methylation in prostate cancer was 1.74, suggesting strong discriminatory ability. In this study, we firstly discussed that APC methylation was associated with a bad prognostic, which suggested that APC methylation might be a potential therapeutic target. Secondly, we conducted a subgroup analysis for PC. Thirdly, it highlighted the importance of an accurate biomarker for its assessment. Finally, the analyses emphasized the value of identifying surrogate markers for APC methylation.

In this study, we suggested that APC methylation was significantly associated with the bad outcome of PC. Despite of significant findings, our research also had several limitations. Firstly, the controls selected varied between studies. The studies that lacked the survival data (e.g. HR, CI or survival curve) were excluded. Secondly, there was statistical heterogeneity which might origin from the differences in the characteristics of patients, technical platforms, normalization controls, the cut-off values or any other technical issues. Finally, the present meta-analysis was limited to the paper published and listed in PubMed and EMBASE up to January 2015. It is possible that some relevant published or unpublished studies, which may have met the inclusion criteria, were missing. In addition, larger-scale and more standard investigations are required to contribute to the role of APC methylation in tumors biochemical recurrence and clinical application.

In conclusion, this meta-analysis is not somewhat perfect due to heterogeneity, biases and other limitations; however, we paid attention to the candidate role of APC methylation as a prognostic biomarker. We demonstrated that aberrant expression of APC methylation was related to biochemical recurrence for patients with prostate cancer.

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Disclosure of conflict of interest

None.

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