Clinical significance of CDH13 promoter methylation as a biomarker for bladder cancer: a meta-analysis

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Abstract

Background: Methylation of the tumor suppressor gene H-cadherin (CDH13) has been reported in many cancers. However, the clinical effect of the CDH13 methylation status of patients with bladder cancer remains to be clarified.

Methods: A systematic literature search was performed to identify eligible studies in the PubMed, Embase, EBSCO, CKNI and Wanfang databases. The pooled odds ratio (OR) and the corresponding 95 % confidence interval (95 % CI) was calculated and summarized.

Results: Nine eligible studies were included in the present meta-analysis consisting of a total of 1017 bladder cancer patients and 265 non-tumor controls. A significant association was found between CDH13 methylation levels and bladder cancer (OR = 21.71, P < 0.001). The results of subgroup analyses based on sample type suggested that CDH13 methylation was significantly associated with bladder cancer risk in both the tissue and the urine (OR = 53.94, P < 0.001; OR = 7.71, P < 0.001; respectively). A subgroup analysis based on ethnic population showed that the OR value of methylated CDH13 was higher in Asians than in Caucasians (OR = 35.18, P < 0.001; OR = 8.86, P < 0.001; respectively). The relationships between CDH13 methylation and clinicopathological features were also analyzed. A significant association was not observed between CDH13 methylation status and gender (P = 0.053). Our results revealed that CDH13 methylation was significantly associated with high-grade bladder cancer, multiple bladder cancer and muscle invasive bladder cancer (OR = 2.22, P < 0.001; OR = 1.45, P = 0.032; OR = 3.42, P < 0.001; respectively).

Conclusion: Our study indicates that CDH13 methylation may play an important role in the carcinogenesis, development and progression of bladder cancer. In addition, CDH13 methylation has the potential to be a useful biomarker for bladder cancer screening in urine samples and to be a prognostic biomarker in the clinic.

Keywords: CDH13 methylation, Bladder cancer, Screening, Biomarker

Abbreviations: CDH13, H-cadherin; CI, Confidence interval; MIBC, Muscle invasive bladder cancer; OR, Odds ratio; TCC, Transitional cell carcinoma; TNM, Tumor/node/metastasis.

Background

Human bladder cancer is the most common urinary system malignancy in the world. According to global cancer statistics, approximately 74,000 cases of bladder cancer will be diagnosed in the USA in 2015, leading to an estimated 16,000 deaths [1]. Bladder cancer consists of three histological and pathological types: urothelial carcinoma, squamous cell carcinoma and adenocarcinoma. Urothelial carcinoma, also known as transitional cell carcinoma (TCC), is the most common type, accounting for 90 % of all bladder cancer cases [2, 3]. Clinically, studies have shown that non-muscle invasive bladder cancer (stages Ta – T1) accounts for approximately 70–80 % of all cases, with the remainder being characterized as muscle invasive bladder cancer (stages T2–T4). Furthermore, 10–30 % of non-muscle invasive bladder cancer (NMIBC) will progress to muscle invasive bladder cancer (MIBC) [4, 5]. MIBC patients...
have a much worse outcome with regards to tumor recurrence and progression, with a 5-year survival rate of 25–60 % [6–8]. Thus, additional noninvasive biomarkers for the prediction and diagnosis of bladder cancer are needed in the clinic.

Epigenetic changes are early and frequent events in cancer that play an important role in carcinogenesis. DNA methylation is the most common epigenetic alteration in human cancers [9–11]. The detection of aberrantly methylated genes can be used as a diagnostic or prognostic biomarker for human cancers, especially when the aberrant methylation silences tumor suppressor genes [12–14]. The CDH13 gene, located on 16q24, encodes a protein that belongs to the cadherin family [15]. CDH13, a tumor suppressor gene (TSG), is also called H-cadherin or T-cadherin and plays a pivotal role in cell–cell adhesion [16]. The expression of CDH13 in human tumor cells can inhibit their invasive potential and markedly reduce their proliferation [17–19]. CDH13 promoter methylation has been reported in some human cancers including bladder cancer [16].

However, the association between CDH13 promoter methylation and bladder cancer remains to be clarified. In this study, a meta-analysis was conducted to evaluate the effect of CDH13 methylation on the clinicopathological features of patients with bladder cancer.

Methods

Search strategy
A systemic literature search for studies published prior to November 16, 2015 was conducted in the PubMed, Embase, EBSCO, CKNI and Wanfang databases without any language restrictions. The following keywords and search terms were used: (CDH13 OR cadherin 13 OR H-cadherin OR T-cadherin) AND (bladder cancer OR bladder tumor OR bladder carcinoma OR bladder neoplasm) AND (methylation OR epigenetic silencing). The reference lists of the retrieved articles and reviews were then manually searched to identify potentially relevant studies.

Inclusion criteria
The eligible studies met the following criteria: 1) the patients were diagnosed with bladder cancer based on histopathology; 2) CDH13 methylation was evaluated in different types of samples, such as tissue, serum, plasma and urine; 3) regarding control samples by cystoscopy and histopathological confirmation, tissue samples belonged to normal tissues, while fluid samples such as serum, plasma or urine were from healthy individuals or patients with benign urological diseases; 4) the studies showed the associations between CDH13 methylation and clinicopathological parameters, including gender (male vs female), cancer tumor/node/metastasis (TNM) stage (T2/T4 vs Ta/T1), grade (grade 3 vs grade1/2) or tumor number (multiple vs single); 5) the methylation frequency of the CDH13 gene was sufficient for the case-control or cohort studies; 6) the studies were published in English or Chinese. The studies that were excluded did not meet our inclusion criteria. When the authors published more than one paper using the same sample data, either the most recent study or the study using the largest sample size was selected. The current meta-analysis was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.

Data extraction
The following pieces of information from the eligible studies were collected: first author surname, year of publication, ethnicity, histological type, types of samples, detection method, number of samples, clinicopathological parameters, gender, stage, grade, tumor number, frequency of CDH13 methylation, etc. As a control group, our meta-analysis used non-cancerous samples including non-cancerous diseases of the bladder and normal healthy tissue, according to each individual study in the original literature. Of these studies, a tumor stage of ≤ 1 was defined as early stage, a tumor stage of ≥ 2 was defined as advanced stage, a tumor grade of ≤ 2 was defined as low-grade, and a tumor grade of 3 was defined as high-grade. The final eligible studies were independently assessed by two reviewers for the current meta-analysis.

Data analysis
The analysis was conducted using STATA 12.0 (Stata Corporation) to evaluate the relationships between CDH13 methylation and bladder cancer via the pooled odds ratio (OR) and the corresponding 95 % confidence interval (95 % CI). The frequency of CDH13 methylation was analyzed according to various cancer characteristics. A statistical test for heterogeneity was performed based on the chi-square test and Q statistic [20]. If substantial heterogeneity (I^2 ≥ 50 % or p < 0.1) was observed, a random-effects model was used to calculate the parameters. Otherwise, a fixed effects model assuming a lack of heterogeneity was used [21, 22]. Egger’s test was used to evaluate for possible publication bias [23]. A p value of less than 0.05 was considered statistically significant.

Results

Study characteristics
The search method described above obtained 49 potentially relevant articles. We carefully reviewed the titles, abstracts and full-texts of the articles. In total, 9 published studies (English, 7; Chinese, 2) met the inclusion criteria of the present meta-analysis [24–32] and
included 1017 bladder cancer patients and 265 controls, as shown in Fig. 1. Of these studies, 8 studies assessed the association between CDH13 methylation and bladder cancer risk, 5 studies evaluated the relationship between CDH13 methylation and gender, 5 studies explored the association between CDH13 methylation and tumor number, 4 studies reported the tumor grade (grade 3 vs grade 1-2), and 5 studies evaluated the effect of clinical stage (T2-T4 vs Ta-T1). The main characteristics of the included studies were presented in Table 1.

CDH13 methylation and the risk of bladder cancer
The heterogeneity among the studies was not significant ($p = 0.495$ and $I^2 = 0.0\%$), and therefore, the fixed effects model was used. The OR value of CDH13 methylation in bladder cancer patients compared with non-tumor controls was 21.71 (95 % CI: 9.83–47.94, $P < 0.001$) (Fig. 2); this analysis included 920 bladder cancer patients and 265 controls. Subgroup analyses were performed to investigate the difference in CDH13 methylation according to sample type (tissue and urine) and ethnicity (Caucasians and Asians) (Table 2). The results showed that the pooled OR value for the tissue group was higher than that of the urine group (OR = 53.94, 95 % CI = 12.83–226.87, $P < 0.001$; OR = 7.71, 95 % CI = 2.65–22.39, $P < 0.001$; respectively). The subgroup analysis according to ethnic populations revealed that the OR value of methylated CDH13 in Asians was higher than in Caucasians.

Table 1 Basic characteristics of the studies included in the current meta-analysis

| First author | Year | Ethnicity | Method | Sample | Methylation % | Number | Gender | Grade | Stage | Tumor number |
|--------------|------|-----------|--------|--------|--------------|--------|--------|-------|-------|--------------|
| Maruyama et al. | 2001 | Caucasians | MSP | Tissue | 29.9 % | 97 | - | - | - | - |
| Meng et al. | 2007 | Asians | MSP | Urine | 15.2 % | 92 | 0 % | - | - | - |
| Yu et al. | 2007 | Asians | MSP | Urine | 16.7 % | 132 | 0 % | - | - | - |
| Cabello et al. | 2011 | Caucasians * | Urine | 27.1 % | 96 | 6 % | - | - | - | 36 60 18 78 |
| Agundez et al. | 2011 | Caucasians | MSP | Urine | 62.6 % | 91 | 0 % | - | - | - |
| Lin et al. | 2011 | Asians | MSP | Serum | 30.7 % | 127 | 0 % | - | 9 | - |
| Lin et al. | 2012 | Asians | MSP | Tissue | 35.3 % | 133 | 0 % | 43 | 39 | 37 96 48 85 82 |
| Lin et al. | 2013 | Asians | MSP | Tissue | 60.6 % | 71 | 0 % | 23 | 49 | 49 23 49 78 74 |
| Lin et al. | 2014 | Asians | MSP | Tissue | 44.9 % | 178 | 0 % | 38 | 124 | 38 124 76 102 |

MSP methylation-specific polymerase chain reaction, *-* indicates data not available
*indicates MS-MLPA (Methylation-Specific Multiplex Ligation-Dependent Probe Amplification)
The relationships between CDH13 methylation and clinicopathological features

The associations between CDH13 methylation and gender, tumor grade and tumor number used the random effects model, while a fixed effects model was used for tumor stage. A significant association was not found between CDH13 methylation and gender in the 5 studies analyzed (OR = 1.46, 95 % CI = 0.99–2.15, P = 0.053), which included 437 male patients and 163 female patients (Fig. 3). The pooled OR from 5 studies including 174 advanced bladder cancer patients and 345 early stage bladder cancer patients indicated that CDH13 methylation was significantly higher in advanced stage tumors than in early stage tumors (OR = 3.42, 95 % CI = 1.72–6.80, P < 0.001) (Fig. 4). Results from 4 studies comparing a total of 169 high-grade patients and 284 low-grade patients showed that CDH13 methylation was significantly associated with high-grade bladder cancer (OR = 2.22, 95 % CI = 1.72–6.80, P < 0.001) (Fig. 5). Results from 5 studies analyzing a total of 319 bladder cancer patients with multiple tumors and 281 bladder cancer patients with single tumors demonstrated that methylated CDH13 was significantly associated with patients harboring multiple tumors (OR = 1.45, 95 % CI = 1.03–2.04, P = 0.032) (Fig. 6).

Table 2: Summary of the relationship between CDH13 methylation and bladder cancer

| Material  | OR (95 % CI) | I², p   | P value | Cases | Controls | p (Egger’s test) |
|-----------|-------------|---------|---------|-------|----------|-----------------|
| Total     | 21.71 (9.83–47.94) | 0.0 %, 0.495 | <0.001 | 920   | 265      | 0.008           |
| Urine     | 7.71 (2.65–22.39)   | 0.0 %, 0.831 | <0.001 | 320   | 110      |                 |
| Tissue    | 53.94 (12.83–226.87) | 0.0 %, 0.986 | <0.001 | 473   | 114      |                 |
| Caucasians| 8.86 (2.91–27.03)   | 24.0 %, 0.251 | <0.001 | 187   | 60       |                 |
| Asians    | 35.18 (11.20–110.55) | 0.0 %, 0.903 | <0.001 | 733   | 205      |                 |

CDH13: H-cadherin, OR: odds ratio, CI: confidence interval
Significant heterogeneity was found in relation to tumor stage in cancer ($I^2 = 59.0\%$, $p = 0.045$). Thus, a sensitivity analysis by omitting a single study was carried out to assess the stability of the pooled OR. When we removed this study by Lin 2011 et al. [25], the heterogeneity was significantly decreased, with the absence of heterogeneity ($I^2 = 14.7\%$, $p = 0.318$). However, the pooled OR of CDH13 promoter methylation was not significantly changed (OR = 2.75, 95 % CI = 1.71–4.44, $p < 0.001$), suggesting the stability of our analyses.

### Publication bias

Egger’s test was performed to assess for publication bias of the included studies (Tables 2 and 3). Egger’s test

#### Table 3

|               | Overall OR (95 % CI) | $I^2$, p    | $P$ value | $p$ (Egger’s test) | Patients |
|---------------|----------------------|-------------|-----------|-------------------|----------|
| Gender        |                      |             |           |                   |          |
| Male          | 1.46 (0.99–2.15)     | 0.0 %, 0.496| 0.053     | 0.085             | 437      |
| Female        |                      |             |           |                   | 163      |
| Grade         |                      |             |           |                   |          |
| High-grade    | 2.22 (1.43–3.43)     | 0.0 %, 0.641| <0.001    | 0.613             | 169      |
| Low-grade     |                      |             |           |                   | 284      |
| Stage         |                      |             |           |                   |          |
| MIBC          | 3.42 (1.72–6.80)     | 59.0 %, 0.045| <0.001    | 0.279             | 174      |
| NMIBC         |                      |             |           |                   | 345      |
| Number        |                      |             |           |                   |          |
| Multiple      | 1.45 (1.03–2.04)     | 0.0 %, 0.779| 0.032     | 0.038             | 319      |
| Single        |                      |             |           |                   | 281      |

*MIBC* muscle invasive bladder cancer (stages T2–T4), *NMIBC* non-muscle invasive bladder cancer (stages Ta – T1), low-grade tumor grade ≤ 2, high-grade tumor grade of 3
test provided strong statistical evidence for a publication bias in the comparison between CDH13 methylation of bladder cancer patients and non-tumor controls \((p = 0.008)\). The relatively small number of control samples (265 controls versus 920 bladder cancer patients) may cause publication bias. Egger’s test indicated a lack of publication bias in the current analysis of CDH13 methylation status and clinicopathological features \((p > 0.05)\). Egger’s funnel plot of the publication bias test for CDH13 methylation was shown in Additional file 1: Table S1.

**Discussion**

DNA methylation in the blood, sputum, urine, feces, and other bodily fluids can be used as a non-invasive biomarker for the early detection of various cancers [12, 33, 34]. Aberrant methylation of the CDH13 gene has been reported in many cancers, including non-small cell lung cancer [35], breast cancer [36], gastric cancer [37], and colorectal carcinoma [38]. However, the potential of CDH13 gene methylation to be a biomarker for bladder cancer has not yet been evaluated.

The methylation rate of CDH13 gene was relatively lower in non-tumor control samples, with a mean methylation frequency of 1.1 % in this study. The findings of the current study showed that CDH13 promoter methylation was significantly higher in bladder cancer patients than in non-tumor control samples \((OR = 21.71, P < 0.001)\), suggesting that the methylation of CDH13 may be involved in the development of bladder cancer. No significant heterogeneity was observed in cancer vs. controls \((p = 0.495\) and \(I^2 = 0.0\) %), indicating the reliability of our results. In addition, the result of a subgroup analysis based on sample type suggested that the CDH13 methylation status was significant in both tissue and urine samples \((OR = 53.94, P < 0.001; OR = 7.71, P < 0.001; respectively)\), indicating that the detection of CDH13 methylation has the potential to be a non-invasive biomarker in the urine, which may aid in the early screening for and the diagnosis of bladder cancer. The OR value in Asians \((OR = 35.18, P < 0.001)\) was significantly higher than in Caucasians \((OR = 8.86, P < 0.001)\), which revealed that CDH13 methylation may be a relatively more important risk factor among Asian populations.
In addition, we conducted meta-analyses to determine the correlations between CDH13 methylation and clinicopathological characteristics. The results showed that the CDH13 methylation status was not associated with gender (OR = 1.46, 95 % CI = 0.99–2.15, P = 0.053). The levels of methylated CDH13 were significantly higher in muscle invasive bladder cancer (stages T2–T4) than in non-muscle invasive bladder cancer (stages Ta – T1) (OR = 3.42, P < 0.001). CDH13 methylation status was significantly associated with high-grade (grade 3) bladder cancer (OR = 2.22, P < 0.001). The levels of methylated CDH13 were significantly higher in bladder cancer consisting of multiple tumors than in bladder cancer consisting of a single tumor (OR = 1.45, P = 0.032). Patients with multiple tumors, high-grade bladder cancer, or muscle invasive bladder cancer are characterized by a high incidence of recurrence and progression and a poorer outcome [39, 40]. Our findings indicated that CDH13 promoter methylation was a very useful biomarker that can predict the recurrence of bladder cancer.

Some limitations of the current meta-analysis should be considered. First, the inclusion of articles published only in English and Chinese might lead to a selection bias. Second, the primary ethnic population of the patients in the current study was Asian, while only two studies involving Caucasians were involved in this meta-analysis. Other ethnicities, such as Africans, were limited. Third, due to the limitation of eligible studies in fluid samples, we did not further evaluate the diagnostic capacity of CDH13 promoter methylation for patients with non-muscle invasive bladder cancer. Thus, more studies based on urine and blood samples are very essential to evaluate whether CDH13 promoter methylation can become a noninvasive biomarker for the detection and diagnosis of non-muscle invasive bladder cancer in the future. Therefore, additional studies incorporating larger sample sizes are required to confirm our results in the future.

**Conclusion**

Our study indicates that CDH13 methylation may play a key role in the initiation and progression of bladder cancer, especially among Asian populations. In addition, CDH13 methylation has the potential to become a useful
biomarker for the clinical screening of bladder cancer in the urine. CDH13 methylation may also be a prognostic biomarker for patients with tumor progression.

Additional file

Additional file 1: Table S1. Egger’s funnel plot of the publication bias test for CDH13 methylation. (DOCX 21 kb)

Acknowledgements

This research was supported by grants from the Ningbo Natural Science Foundation “2009A610115 and 2009A610143”.

Availability of data and materials

Data come from published literature.

Authors’ contributions

FC and XY contributed to the conception, design and final approval of the submitted manuscript. FC, TH, YR, JW, ZL, XW, XF, YC and GW contributed to the completion of article analysis the data extraction and the calculation and design of the figures and tables. All the authors approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was not primary research involving humans or animals but was a secondary analysis of human subject data available in the public domain.

Received: 2 June 2016 Accepted: 23 August 2016 Published online: 30 August 2016

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Invasiveness and tumor formation are significant factors in bladder cancer. The study of in vitro and in vivo models has shown that DNA methylation markers play a crucial role in the detection and diagnosis of bladder cancer. Andreeva et al., 2014 investigated the promoter methylation of h-cadherin gene in bladder cancer. Their findings revealed that aberrant methylation of cdh13 could be a potential biomarker in patients with bladder transitional cell carcinoma. Methylated DNA for monitoring bladder cancer was reported by Kristiansen et al. 2011. These findings highlight the importance of DNA methylation markers in bladder cancer research. The use of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer was reported by Qureshi et al. 2014. This method provides a non-invasive approach for the early detection of bladder cancer.

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