RESEARCH ARTICLE

Quantitative Assessment of the Diagnostic Role of CDH13 Promoter Methylation in Lung Cancer

Yun-Hua Zhong1,2, Hao Peng3, Hong-Zhong Cheng3, Ping Wang3*

Abstract

In order to explore the association between cadherin 13 (CDH13) gene promoter methylation and lung carcinoma (LC) risk, we carried out a meta-analysis with searching of PubMed, Web of Science. Ultimately, 17 articles were identified and analysed by STATA 12.0 software. Overall, we found a significant relationship between CDH13 promoter methylation and LC risk (odds ratio=6.98, 95% confidence interval: 4.21-11.56, p<0.001). Subgroup analyses further revealed that LC risk was increased for individuals carrying the methylated CDH13 compared with those with unmethylated CDH13. Hence, our study identified a strong association between CDH13 gene promoter methylation and LC and highlighted a promising potential for CDH13 methylation in LC risk prediction.

Keywords: CDH13 - lung carcinoma - methylation

Introduction

Lung cancer (LC) is the most frequent cancer worldwide still. There were more than 1.8 million new cases (13% of total cancer incidence) and almost 1.6 million deaths (20% of total cancer mortality), as estimated in 2012 (Bernard W. Stewart, 2014). Moreover, LC is the leading cause of cancer death in men in 87 countries and in women in 26 countries. Despite the advent of new diagnostic techniques, most LCs are detected at a late stage, and the 5-year survival rate of LC is less than 15% in the US (Jemal et al., 2011). Once tumor cells have spread, the long-term prognosis is poor since no curative treatments are available. However, the bottleneck in improving survival is early detection (Zhang et al., 2011). As an important mechanism for tumor suppressor gene inactivation in cancer, DNA hypermethylation could yield powerful biomarkers for early detection of LC, owning in comparable advantages over other traditional markers due to its stable chemical property, detection ability in remote patient media, quantitative signal, convenient low cost in detection, and so on (Li et al., 2012; Mikeska et al., 2012). Therefore, we believe that DNA methylation could become a powerful tool for LC diagnosis.

The cadherin 13 (CDH13) gene, a new member of the cadherin superfamily, was isolated recently and has been mapped to 16q24 (Takeuchi and Ohtsuki, 2001). The introduction of CDH13 in human breast carcinoma cells reduced their invasive potential and markedly decreased their growth rate; in addition, it induced the reversion of morphology from an invasive type to a normal cell-like type (Lee, 1996). Abnormalities in the CDH13 gene have been identified in human malignancies, including lung carcinomas (Sato et al., 1998). Moreover, CDH13 expression is associated with tumorigenicity in non-small cell lung carcinoma (NSCLC) and frequently is silenced by promoter methylation of the CDH13 gene in NSCLC (Toyooka et al., 2001; Hanabata et al., 2004; Kim et al., 2004).

In this article, we conducted a meta-analysis of the CDH13 methylation on LC diagnosis. At the end, we found a strong association between CDH13 gene promoter methylation and LC.

Materials and Methods

Search strategy, data extraction and statistical analysis

This pooled study involved searching a range of computerized databases, including PubMed, Web of Science for articles published in English by September 2014. The study used a subject and text word strategy with ‘lung cancer or lung neoplasm or lung carcinoma’, ‘CDH13 or T-cadherin or cadherin 13 or H-cadherin’, ‘methylation or hypermethylation or epigenetic’, as the primary search terms. Wildcard character of star, dollar or some other truncations were applied according to the rules of the databases to allow effective article collection.

Two independent reviewers screened the titles and abstracts derived from the literature search to identify relevant studies. The following types of studies were...
excluded: animal experiments, case reports, reviews or meta-analyses and studies of non-case-control studies or studies with insufficient data or those proving inaccessible after making contact with the authors. The remaining articles were further examined to see if they met the inclusion criteria: 1) the patients had to be diagnosed with LC; 2) the studies had to contain CDH13 gene promoter methylation data from tissue, blood or serum; 3) the studies had to be case-control studies which included tissue-tissue, blood-blood or serum-serum in case and controls respectively. The reference sections of all retrieved articles were searched to identify further relevant articles. Potentially relevant papers were obtained and the full text articles were screened for inclusion by two independent reviewers. Decisions were made and disagreements about study selection were resolved by consensus or by involving a third reviewer. The following information was extracted from the studies: the first author’s last name, publication year, original country of patients in the subjects, and the number of CDH13 methylation of cases and controls in individuals, etc.

The strength of the association between the CDH13 methylation and LC risk was measured by pooled odds ratio (OR) with its 95% confidence interval (CI). The significance of the pooled OR was determined by the Z test and \( p < 0.05 \) was considered as statistically significant. Subgroup analysis was performed stratified by the study character of tissue sample and blood sample. The heterogeneity assumption was checked by chi-test based on Q-test (significance level of \( p < 0.10 \)) (Dickersin and Berlin, 1992). With a lack of heterogeneity among included studies, the pooled odds ratio estimates were calculated using the fixed-effects model (Mantel-Haenszel) (Mantel and Haenszel, 1959). Otherwise, the random-effects model (DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). Sensitivity analyses were performed to assess the contributions of single studies to the final results. Begg’s funnel plots were used to examine whether the results of a meta-analysis may have been affected by publication bias. Egger’s test was implemented to testing for funnel plot asymmetry (M. Egger, 1997). All statistical analyses were performed using Stata statistical software.

Results

Study characteristics

After being selected in accordance with the inclusive criteria, and finally, 17 studies with data on the relationship between CDH13 gene promoter methylation and LC were pooled for analysis (Table 1) (Toyooka et al., 2001; Zhong et al., 2001; Toyooka et al., 2003; Kim et al., 2004; Kim et al., 2006; Suzuki et al., 2006; Ulivi et al., 2006; Hsu et al., 2007; Kim et al., 2007; Tsou et al., 2007; Yanagawa et al., 2007; Brock et al., 2008; Feng et al., 2008; Jin et al., 2009; Zhang et al., 2011; Kontic et al., 2012; Zhai and Li, 2014). All these articles were written in English and were original study. The 17 studies published between 2001 and 2014.

In total, 1,786 LC tissues/serum and 1,134 normal counterpart tissues/serum were collected. There was 35.78% of LC patients had the methylated CDH13 allele with a frequency ranging from 18.64% to 98.46% in individual trials. However, there was 10.67% of normal had the methylated CDH13 allele with a frequency ranging from 0.00% to 25.49% in individual trials. For histologic type, 15 studies focus on NSCLC, two studies focus on LC; for sample source, 14 and 1 literatures based on the investigation of tissue and plasma, two articles both on tissue and plasma, respectively; for ethnic group, there was 6 articles of the Asian, 3 of the Caucasus and 8 of Mixed-race.

Meta-analysis and subgroup analysis

The ORs for CDH13 methylation in cancer tissues compared with that in normal controls were 6.98 (95%CI: 2.83-17.13) and 0.79 (95%CI: 0.55-1.17) for LC and NSCLC, respectively. There were significant differences in terms of gender and age, with the results showing a higher risk of CDH13 methylation in male patients and older patients. For histological type, there were significant differences in the risk of CDH13 methylation between lung adenocarcinoma and squamous cell carcinoma, with the risk being higher in adenocarcinoma (OR: 2.49; 95%CI: 1.50-4.05) than in squamous cell carcinoma (OR: 0.86; 95%CI: 0.55-1.33). The risk of CDH13 methylation was significantly higher in samples from patients with advanced clinical stages (OR: 1.67; 95%CI: 1.06-2.63) compared with those from patients with early clinical stages. The risk of CDH13 methylation was also significantly higher in patients with positive lymph node involvement (OR: 1.72; 95%CI: 1.14-2.59) compared with those with negative lymph node involvement.

Table 1. Main Characteristics of the Studies Included in the Meta-Analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Population</th>
<th>Method</th>
<th>Sample</th>
<th>Histologic type</th>
<th>Control</th>
<th>Case</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>Zhai et al.</td>
<td>2014</td>
<td>China</td>
<td>Asian</td>
<td>MSP</td>
<td>Blood</td>
<td>NSCLC</td>
<td>0</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>Kontic et al.</td>
<td>2012</td>
<td>Serbia</td>
<td>Caucasian</td>
<td>BSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>15</td>
<td>65</td>
<td>64</td>
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<tr>
<td>Zhang et al.</td>
<td>2011</td>
<td>Germany</td>
<td>Caucasian</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>8</td>
<td>78</td>
<td>38</td>
</tr>
<tr>
<td>Jin et al.</td>
<td>2009</td>
<td>Japan</td>
<td>Asian</td>
<td>QMSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>2</td>
<td>63</td>
<td>52</td>
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<tr>
<td>Feng et al.</td>
<td>2008</td>
<td>USA</td>
<td>Mixed</td>
<td>chip</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>0</td>
<td>49</td>
<td>11</td>
</tr>
<tr>
<td>Brock et al.</td>
<td>2008</td>
<td>USA</td>
<td>Mixed</td>
<td>MSP</td>
<td>Tissue</td>
<td>LC</td>
<td>24</td>
<td>104</td>
<td>74</td>
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<tr>
<td>Yanagawa et al.</td>
<td>2007</td>
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<td>Asian</td>
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<td>Tissue</td>
<td>NSCLC</td>
<td>7</td>
<td>101</td>
<td>26</td>
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<td>Tsou et al.</td>
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<td>USA</td>
<td>Mixed</td>
<td>MSP</td>
<td>Tissue</td>
<td>LC</td>
<td>0</td>
<td>11</td>
<td>37</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>2007</td>
<td>Korea</td>
<td>Asian</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>7</td>
<td>88</td>
<td>26</td>
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<tr>
<td>Hsu et al.</td>
<td>2007</td>
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<td>Asian</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>6</td>
<td>36</td>
<td>28</td>
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<td>China</td>
<td>Asian</td>
<td>MSP</td>
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<td>NSCLC</td>
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<td>Italy</td>
<td>Caucasian</td>
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<td>Tissue</td>
<td>NSCLC</td>
<td>0</td>
<td>15</td>
<td>40</td>
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<td>Ulivi et al.</td>
<td>2006</td>
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<td>Caucasian</td>
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<td>NSCLC</td>
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<td>Kim et al.</td>
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<td>Korea</td>
<td>Asian</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>26</td>
<td>102</td>
<td>35</td>
</tr>
<tr>
<td>Kim et al.</td>
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<td>Korea</td>
<td>Asian</td>
<td>MSP</td>
<td>Tissue</td>
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<td>4</td>
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<td>29</td>
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<tr>
<td>Toyooka et al.</td>
<td>2003</td>
<td>Mixed</td>
<td>Mixed</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>5</td>
<td>84</td>
<td>5</td>
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<tr>
<td>Zhong et al.</td>
<td>2001</td>
<td>USA</td>
<td>Mixed</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>6</td>
<td>35</td>
<td>15</td>
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<tr>
<td>Toyooka et al.</td>
<td>2001</td>
<td>USA</td>
<td>Mixed</td>
<td>MSP</td>
<td>Tissue</td>
<td>NSCLC</td>
<td>2</td>
<td>25</td>
<td>18</td>
</tr>
</tbody>
</table>
CDH13 Promoter Methylation as a Risk Factor for Lung Cancer

4.21-11.56, \( p<0.001 \)) in random effects model pooled, demonstrating a statistically significant increasing in likelihood of methylation in LC tissues comparing to controls (Figures and Table 2). In other words, compared with healthy person, LC patients had a 6.98-fold higher risk for CDH13 methylation.

Subgroup analyses were conducted for different subtypes, which included histologic type (NSCLC and LC), sample source (tissue or blood), ethnic group (Asian, Caucasian and Mixed-race). Histological type ORs showed (Figure 1) that LC risk was increased for individuals carrying the methylated CDH13 compared with those with unmethylated CDH13 in NSCLC group (OR=7.33; 95%CI, 4.33-12.41) and LC group (OR=7.35; 95%CI, 0.29-183.86). Testing materials subgroup analyses showed (Figure 2) that the incidence of CDH13 methylation in LC tissues was higher than that in normal tissues (OR=6.93; 95%CI, 4.07-11.80), and similar results was found in plasma sample (OR=10.75; 95%CI, 0.89-129.16). When stratifying for ethnic-specific (Figure 3), the increased risk of CDH13 methylation in cases than controls was found in Asian populations (OR=5.51; 95%CI, 2.87-10.57), Caucasian populations (OR=28.78; 95%CI, 4.61-179.78) and mixed-race (OR=4.91; 95%CI, 2.34-10.31).

### Table 2. Summary of CDH13 Methylation and Lung Cancer Risk

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>OR</th>
<th>[95% Conf. Interval]</th>
<th>Significance Test(S) of OR=1</th>
</tr>
</thead>
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<td>7.33</td>
<td>4.32-12.41</td>
<td>0.000</td>
</tr>
<tr>
<td>LC</td>
<td>7.34</td>
<td>0.29-183.86</td>
<td>0.000</td>
</tr>
<tr>
<td>Blood</td>
<td>10.74</td>
<td>0.89-129.159</td>
<td>0.000</td>
</tr>
<tr>
<td>Tissue</td>
<td>6.92</td>
<td>0.67-11.80</td>
<td>0.000</td>
</tr>
<tr>
<td>Asian</td>
<td>5.50</td>
<td>2.87-10.572</td>
<td>0.000</td>
</tr>
<tr>
<td>Caucasian</td>
<td>28.78</td>
<td>4.60-179.777</td>
<td>0.000</td>
</tr>
<tr>
<td>Mixed</td>
<td>4.91</td>
<td>2.34-10.31</td>
<td>0.000</td>
</tr>
</tbody>
</table>

#### Figure 1. Forest Plot of Meta-Analysis for Association between CDH13 Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

#### Figure 2. Forest Plot of Meta-Analysis for Association between CDH13 Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

#### Figure 3. Forest Plot of Meta-Analysis for Association between CDH13 Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

#### Figure 4. Sensitivity Analysis of the Summary Odds Ratio Coefficients on the Relationships between CDH13 Promoter Methylation and the Lung Cancer Patients

Sensitivity analyses were conducted to determine whether modification of the inclusive criteria of the meta-
analysis affected the final results. When we excluded the studies of (Kim et al., 2006), there was almost no difference between the remaining 16 studies (Figure 4). Therefore, our results were statistically robust.

Bias diagnosis

Begg’s funnel plot and Egger’s test were carried out to assess the publication bias of the studies. Here, we only used the Begg’s funnel plot and for overall, the shapes of Begg’s funnel plot revealed obvious symmetry (Figure 5). Although, our results showed significant publication bias in testing materials and histological type group, the ethnicity group did not provide any evidence of publication bias (Table 2). Hence, we made a conclusion that Begg’s funnel plot detected any publication bias of our studies.

Discussion

The CDH13 gene has been reported as an important tumor suppressor in colorectal cancer (Dong et al., 2005), and the aberrant of CDH13 methylation had been reported in numerics for cancers, such as hepatocellular carcinoma (Riou et al., 2006), cervical neoplasia (Feng et al., 2007), breast cancer and LC (Sato et al., 1998). However, the diagnostic role of the methylation status of the CDH13 gene in LC lacks quantitative assessment. We therefore performed an integrated analysis to quantify the ability for the CDH13 gene promoter methylation test in LC diagnosis, and a significant association was identified between CDH13 methylation and LC (OR = 6.98, p<0.001), suggesting a strong association of the methylation of CDH13 gene promoter with LC. Three subgroup studies were filled when trim and fill tests were performed to eliminate the influence of publication bias on the random effects model, and the overall OR was still significant, although it was slightly smaller than that in the crude meta-analysis (Table 2), which revealed that CDH13 methylation status is a good biomarker in LC diagnosis.

Meta-analysis has been widely applied in SNP-disease risk association studies because SNPs have specific genome location. Meta-analysis is also gradually starting to boom in the realm of DNA methylation. Since the late 1980s, various studies have shown that the same genetic/epigenetic alterations, such as DNA methylation, in the primitive tumors were also found in the circulating DNA of the patients with tumors (Esteller et al., 1999). Interestingly, in our study, the OR of the serum subgroup was greater than that of the tissue group of the CDH13 methylation test for serum was greater than that for tissue in our meta-analysis, which indicated that the CDH13 methylation test should be a promising serum biomarker for LC diagnosis.

It must be pointed that some limitations may affect the objectivity of our meta-analysis, such as smoking, gender, age and the TNM stage which are differences in CDH13 methylation between cases and controls. Therefore, a meta-analysis including more high-quality designed epidemiology studies and a stratified analysis targeting different status are necessary in the future in this field.

In conclusion, this integrated analysis of the pooled data provides strong evidence that the methylation status of the CDH13 promoter is strongly associated with LC risk. Therefore, the CDH13 methylation test could be a promising diagnostic biomarker which could be applied in the clinical diagnosis of lung adenocarcinoma with remote non-invasive media detection.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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