Clinicopathological significance and potential drug targeting of CDH1 in lung cancer: a meta-analysis and literature review

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Background: CDH1 is a protein encoded by the CDH1 gene in humans. Mutations in this gene are linked with several types of cancer. Loss of CDH1 function contributes to the progression of cancer by increasing proliferation, invasion, and/or metastasis. However, the association between and clinicopathological significance of CDH1 promoter methylation and lung cancer remains unclear. In this study, we systematically reviewed the studies of CDH1 promoter methylation and lung cancer, and evaluated the association between CDH1 promoter methylation and lung cancer using meta-analysis methods.

Methods: A comprehensive search of the PubMed and Embase databases was performed up to July 2014. The methodological quality of the studies was also evaluated. The data were extracted and assessed by two reviewers independently. Analyses of pooled data were performed. Odds ratios (ORs) were calculated and summarized.

Results: Finally, an analysis of 866 patients with non-small cell lung cancer from 13 eligible studies was performed. The CDH1 methylation level in the cancer group was significantly higher than in the controls (OR 3.89, 95% confidence interval [CI] 2.87–5.27, \(P<0.00001\)). However, there were no correlations between CDH1 promoter methylation and clinicopathological characteristics (sex status, OR 0.78, 95% CI 0.41–1.50, \(P=0.46\); smoking history, OR 0.97, 95% CI 0.53–1.79, \(P=0.93\); pathological type, OR 0.97, 95% CI 0.59–1.60, \(P=0.91\); clinical staging, OR 1.48, 95% CI 0.81–2.68, \(P=0.2\); lymph node metastasis, OR 0.68, 95% CI 0.13–3.63, \(P=0.65\); or differentiation degree, OR 1.01, 95% CI 0.34–3.02, \(P=0.99\)).

Conclusion: The results of this meta-analysis suggest that CDH1 methylation is associated with an increased risk of lung cancer. CDH1 hypermethylation, which induces inactivation of the CDH1 gene, plays an important role in carcinogenesis and may serve as a potential drug target in lung cancer. However, CDH1 methylation does not correlate with other factors, such as smoking history, clinical stage, pathological type, sex status, lymph node metastasis, or degree of differentiation.

Keywords: CDH1, methylation, lung cancer, meta-analysis, tumor suppressor gene, odds ratio

Introduction

Lung cancer is a leading cause of cancer mortality in the USA and other developed countries. The two major forms of the disease are non-small cell lung cancer and small cell lung cancer, which account for 85% and 15% of all lung cancers, respectively. Despite advances in preliminary detection and standard treatment with combination chemotherapy and radiotherapy, the prognosis for patients with lung cancer has not improved significantly in the last 20 years, and survival rates have changed little over the past 2 decades. Currently, an enormous amount of research is aimed at understanding the molecular and cellular biology of lung cancer; however, much more
work is needed to better understand the correlation between gene regulation and lung cancer.

DNA methylation is an important epigenetic mechanism for gene silencing. Growing evidence shows that aberrant hypermethylation in 5′-CpG islands in the promoter regions is a major mechanism for silencing tumor suppressor or other cancer-associated genes in many kinds of human cancer.54 Loss of function in cancer suppressor genes may hinder inhibition of growth of cancer cells, which leads to malignant transcription and translation during replication of DNA. A number of genes, including the cyclin-dependent kinase inhibitor (p16), the tumor suppressor gene Ras association domain family protein 1A, Kelch-like ECH-associating protein 1, the DNA repair gene MGMT, and the cystic fibrosis transmembrane conductance regulator are demonstratively methylated in lung cancer.9–12 CDH1, a member of the transmembrane glycoprotein family, also known as cadherin-1, CAM 120/80, epithelial cadherin (E-cadherin), or uvomorulin, is encoded by the CDH1 gene (16q22.1).13 CDH1 is a calcium-dependent cell-cell adhesion glycoprotein containing three domains, ie, five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail.14 CDH1 as a tumor suppressor gene plays an essential role in maintaining cell adhesion and adherent junctions in normal tissues. CDH1 expression is frequently absent in a variety of epithelial tumors, and loss of normal intercellular junctions results in promotion of cancer invasion and metastasis and is correlated with several types of cancers.15–17 However, the association between and clinicopathological significance of CDH1 promoter hypermethylation and lung cancer remains unclear. In this study, we systematically investigate studies of CDH1 promoter hypermethylation and lung cancer, and validate the association between CDH1 promoter hypermethylation and lung cancer using meta-analysis methods. In addition, we summarize these findings and discuss tumor suppressor function, as well as the clinicopathological significance of CDH1 in lung cancer.

Materials and methods
Publication selection
A systematic literature searching was performed using PubMed, Embase, and the Web of Science up to August 13, 2014 without any language restrictions. The following keywords and terms were used: [methylation or DNA methylation or hypermethylation or de-methylation] and [CDH1 or cadherin-1 or CAM 120/80 or epithelial cadherin (E-cadherin) or uvomorulin] and [lung cancer or lung carcinoma or lung tumor]. References from these publications were manually searched to identify additional studies. The published scientific articles were restricted to English language, and conference abstracts were excluded due to lack of sufficient data. Titles, abstracts, and key words in the articles were initially evaluated for inclusion criteria. Details and additional information were identified and collected from the full text of these articles.

Inclusion and exclusion criteria
A study included for meta-analysis needed to have: evaluated the correlation between CDH1 methylation and lung cancer; included a clinical cohort and controls; included at least three patients and controls; used methylation-specific polymerase chain reaction or quantitative methylation-specific polymerase chain reaction to examine CDH1 methylation and expression; and used tissue data rather than blood data. Studies that did not meet our inclusion criteria were excluded. When the same groups of patients were reported in multiple papers, only the most recent and complete paper was selected to avoid overlap.

Data extraction and quality assessment
Two researchers independently collected the information and extracted the data regarding authorship, year, source of publication, inclusion criteria, CDH1 methylation frequency, sex status, smoking history, pathological type, clinical staging, degree of differentiation, lymph node metastasis, epidermal growth factor receptor status, and prognosis in patients and controls. Any discrepancy was resolved by discussion until agreement was reached. The data are summarized in Table 1 according to the criteria mentioned above. Methodological evaluation was assessed by the researchers according to the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines and ELCWP (European Lung Cancer Working Party) score.18,19

Data analysis
The meta-analysis was performed using Reviewer Manager 5 (Cochrane Collaboration, Oxford, UK). Pooled odds ratios (ORs) and confidence intervals (CIs) were calculated to assess the correlation between CDH1 methylation and lung cancer. Cochran’s $Q$ test and $I^2$ were used to assess heterogeneity among the studies.20 A $Q$ test showing $P<0.05$ or an $I^2$ test >50% indicated significant heterogeneity and a fixed-effects model was used to calculate the parameters. Otherwise, a random-effects model was used to pool data and
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Table 1 Characteristics of the included studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Years</th>
<th>Samples</th>
<th>Sex status</th>
<th>Smoking history</th>
<th>Pathological types</th>
<th>Clinical staging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
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<td>Female</td>
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<tr>
<td>Zochbauer-Muller et al</td>
<td>2001</td>
<td>107</td>
<td>104</td>
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<tr>
<td>Yanagawa et al</td>
<td>2003</td>
<td>75</td>
<td>75</td>
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<tr>
<td>Russo et al</td>
<td>2005</td>
<td>49</td>
<td>49</td>
<td>–</td>
<td>–</td>
<td>22</td>
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<tr>
<td>Tsou et al</td>
<td>2005</td>
<td>7</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Kim et al</td>
<td>2007</td>
<td>88</td>
<td>88</td>
<td>70</td>
<td>18</td>
<td>72</td>
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<tr>
<td>Tan et al</td>
<td>2007</td>
<td>20</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Feng et al</td>
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<td>49</td>
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<td>Wang et al</td>
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<td>Wang et al</td>
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<tr>
<td>Vaisiere et al</td>
<td>2009</td>
<td>209</td>
<td>164</td>
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<td>Begum et al</td>
<td>2011</td>
<td>76</td>
<td>30</td>
<td>40</td>
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<td>Guzman et al</td>
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<td>Zheng et al</td>
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<td>37</td>
<td>37</td>
<td>26</td>
<td>11</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: “–” means data not available.

Results

The process followed to select the papers used in this report is shown in Figure 1. Ninety papers were identified by electronic database searching and 20 further papers by manual searching. Eighty-five papers were excluded for being duplicate publications, being an irrelevant title or abstract, or being a relevant title and abstract but not published in the English language. After retrieval of the full-text articles, eleven papers were excluded for not having a large enough study population or for being reviews. Thirteen further studies were screened out (involving 866 cases and 757 controls) based on the inclusion and exclusion criteria in the pooled analysis.

![Flow diagram of the literature search strategy and assessment of studies identified for meta-analysis](image-url)

**Figure 1** Flow diagram of the literature search strategy and assessment of studies identified for meta-analysis.

**Abbreviation:** EMBASE, Excerpta Medica dataBASE.
The characteristics of the studies are shown in Table 1.\textsuperscript{24–36} Six papers were used to study the correlation between CDH1 methylation and sex status. Five papers provided smoking history data, allowing an investigation of the influence of smoking on CDH1 methylation. Six studies included pathological typing (squamous small carcinoma or adenocarcinoma). Three studies investigated the effect of clinical stage, ie, stage I, II, III, or IV. One paper discussed lymph node metastasis and another discussed degree of differentiation.

Analyzing tissue samples from 657 patients and 593 controls, the mean frequency of CDH1 methylation was 32\% (range 8.33\% to 66.32\%) in tumor tissue and 9\% (range 0.00\%–27.78\%) in tissue from controls. This result indicates that the occurrence of CDH1 methylation is higher in tumor tissue than in normal tissue. Using the fixed model, meta-analysis showed that 657 cases and 593 controls from 12 studies were pooled OR as shown in Figure 2 (OR 3.89, 95\% CI 2.87–5.27, $P=0.00001$). These findings indicate that CDH1 methylation is a key molecular event in tumor tissue but not in normal tissue.

The results also show heterogeneity across the included studies ($I^2$ is 61\%, ie, more than 50\%). Given this significant heterogeneity, a subgroup analysis was performed to investigate sex status, smoking history, pathological type, clinical stage, differentiation degree, and lymph node metastasis to observe the relationship between CDH1 methylation and clinical characteristics (see Figure 3). However, there was no correlation between CDH1 promoter methylation and any of these factors (sex status, OR 0.78, 95\% CI 0.41–1.50, $P=0.46$; smoking history, OR 0.97, 95\% CI 0.53–1.79, $P=0.93$; pathological type, OR 0.97, 95\% CI 0.59–1.60, $P=0.91$; clinical stage, OR 1.48, 95\% CI 0.81–2.68, $P=0.2$; lymph node metastasis, OR 0.68, 95\% CI 0.13–3.63, $P=0.65$; and differentiation degree, OR 1.01, 95\% CI 0.34–3.02, $P=0.99$).

The stability of the results was tested by sensitivity analysis. The OR ranged from 0.78 to 3.89, which was not a significant change, suggesting that the results of our meta-analysis were not significantly unstable. The funnel plot shown in Figure 4 is partially symmetric, indicating low publication bias regarding CDH1 methylation in lung cancer.

\textbf{Discussion}

DNA methylation is an important epigenetic mechanism for regulation of gene expression. An imbalance of gene methylation can cause a variety of human diseases. Hypermethylation of tumor suppressor genes and hypomethylation of oncogenes are two essential components of the molecular mechanism involved in the epigenomic regulation of initiation and progression of cancer. As a tumor suppressor gene, CDH1 maintains cell-cell adhesion and keeps epithelial cells arranged in normal arrangement and layer. In vitro studies demonstrate that loss of expression or function of CDH1 can activate transcription factors associated with epithelial-mesenchymal transition, leading to metastasis of cancer cells.\textsuperscript{37} CDH1 methylation has been detected in several types of carcinoma, including breast cancer, gastric cancer, and lung cancer.\textsuperscript{38–40} A comprehensive evaluation of markers of methylation in lung cancer is needed to better understand the relationship between CDH1 methylation and lung cancer. Although a large number of studies have demonstrated a possible relationship between

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Study or subgroup & Cases & Total & Control & Weight & OR M–H, fixed, & OR M–H, fixed, \\
 & & & & & 95\% CI & 95\% CI \\
\hline
Begum et al\textsuperscript{a4} & 47 & 76 & 9 & 30 & 10.8\% & 3.78 (1.53, 9.37) & \\
Feng et al\textsuperscript{a2} & 4 & 49 & 7 & 49 & 14.1\% & 0.53 (0.15, 1.95) & \\
Guzman et al\textsuperscript{a3} & 9 & 26 & 10 & 33 & 12.6\% & 1.22 (0.41, 3.65) & \\
Kim et al\textsuperscript{a2} & 30 & 88 & 5 & 88 & 7.2\% & 8.59 (3.14, 23.44) & \\
Russo et al\textsuperscript{a2} & 18 & 49 & 11 & 49 & 15.2\% & 2.01 (0.83, 4.87) & \\
Tan et al\textsuperscript{a2} & 4 & 20 & 0 & 10 & 1.1\% & 5.73 (0.28, 117.65) & \\
Tsou et al\textsuperscript{a2} & 2 & 7 & 0 & 11 & 0.6\% & 10.45 (0.43, 256.96) & \\
Wang et al\textsuperscript{a2} & 3 & 28 & 1 & 12 & 2.7\% & 1.32 (0.12, 14.14) & \\
Wang et al\textsuperscript{a2} & 63 & 95 & 23 & 95 & 17.0\% & 6.16 (3.27, 11.61) & \\
Yangawara et al\textsuperscript{a2} & 22 & 75 & 11 & 75 & 17.0\% & 2.42 (1.07, 5.43) & \\
Zheng et al\textsuperscript{a2} & 12 & 37 & 0 & 37 & 0.7\% & 36.76 (2.08, 649.15) & \\
Zochbauer-Muller et al\textsuperscript{a2} & 20 & 107 & 0 & 104 & 0.9\% & 48.97 (2.92, 821.28) & \\
\hline
Total (95\% CI) & 657 & 593 & 100.0\% & 3.89 (2.87, 5.27) & \\
\hline
Heterogeneity: $\chi^2$=27.87, df=11 ($P=0.003$); $I^2$=61\%
Test for overall effect: $Z=8.77$ ($P=0.00001$)
\end{tabular}
\caption{Estimates for CDH1 methylation frequency associated with lung cancer in the meta-analysis.}
\end{table}
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Figure 3 (Continued)
CDH1 methylation and lung cancer, a meta-analysis can summarize the relevant studies and compare different subgroup characteristics.

In this meta-analysis, we mainly focus on the correlation between CDH1 methylation and lung cancer. We analyzed data from 13 studies that included 657 tumor tissue samples and 593 control samples. The results show that the CDH1 methylation level in the cancer group was significantly higher than in the control group. The pooled OR using the fixed-effect model was 3.89 (95% CI 2.87–5.27 versus the control group). CDH1 methylation plays a key role in the induction of lung cancer due to silencing of the tumor suppressor gene CDH1. This conclusion is consistent with that of a previous study. Since changes in CDH1 promoter hypermethylation are reversible, drug treatment promoting demethylation may be useful for delaying carcinogenesis and progression. Treatment with 5-aza-2′-deoxycytidine showed that migration of A549 cells decreased markedly upon restoration of CDH1.41 These preclinical studies show the therapeutic potential of restoration of tumor suppressor expression via...
epigenetic modulation. This approach may bring hope for patients with cancer through gene-targeted therapy.

We further determined the clinicopathological significance of CDH1 promoter hypermethylation in patients with lung cancer. For smoking history, the summary OR was 0.97 (95% CI 0.53–1.79) in the 65 cases and 27 controls. The results show that methylation level of CDH1 is not associated with smoking history. Lung cancer is a complicated disease with different genetic and epigenetic profiling. Smoking is not the only risk factor for lung cancer. Smoking may target other specific genes for methylation or mutation, for example, methylenetetrahydrofolate reductase.\(^\text{33}\)

Other subgroup meta-analysis was performed, including for sex status (OR 0.78, 95% CI 0.41–1.50), pathological type (OR 0.97, 95% CI 0.59–1.60), clinical stage (OR 1.48, 95% CI 0.81–2.68), lymph node metastasis (OR 0.68, 95% CI 0.13–3.63), and degree of differentiation (OR 1.01, 95% CI 0.34–3.02). CDH1 methylation determined by clinical staging shows a slightly more significant association than the other subgroups. Interestingly, CDH1 methylation was detected much more frequently in stages III and IV than in stages I and II. However, CDH1 methylation itself does not correlate with pathological type, sex status, lymph node metastasis, or degree of differentiation. Possible reasons for this finding might be the widely heterogeneous results for the subgroups or the lack of cases and controls in the subgroups. Other potentially significant factors may need to be investigated, such as patient age, tumor size, and biopsy sample control.\(^\text{42}\)

**Conclusion**

CDH1 promoter methylation is associated with lung cancer based on this meta-analysis. CDH1 methylation might be a biomarker of lung cancer, with potential value in predicting the prognosis of the disease, and warrants further studies involving more clinical cases for meta-analysis in the future. In addition, the potential variables on CDH1 methylation from different group database are still not clear due to the limitation of the statistical power of meta-analysis.

**Disclosure**

The authors have no financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in this paper.

**References**


