Does hypermethylation of CpG island in the promoter region of the *E-cadherin* gene increase the risk of lung cancer? A meta-analysis

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Abstract

**Background:** Hypermethylation of the CpG island in the promoter regions of tumor suppressor genes is common in the cancer tissue of non-small cell lung cancer (NSCLC) patients. *Epithelial cadherin (E-cadherin)* is a classic tumor suppressor gene of the cadherin superfamily and its promoter region is usually hypermethylated in malignant carcinomas. However, whether hypermethylation of the CpG island in the promoter region of *E-cadherin* increases the risk of lung cancer is unknown. We conducted a meta-analysis of *E-cadherin* gene promoter methylation status in cancer tissue (CT) and autologous controls (AC).

**Methods:** Electronic databases were searched for *E-cadherin* gene promoter methylation and NSCLC. The hypermethylation status between CT and AC of NSCLC patients were compared and pooled by random or fixed effect models according to statistical heterogeneity across the included studies.

**Results:** Eleven publications relevant to *E-cadherin* gene promoter hypermethylation and lung cancer risk were identified and included. *E-cadherin* gene promoter hypermethylation frequency in CT and AC was 0.32 (95% confidence interval [CI] 0.22–0.41) and 0.12 (95% CI 0.04–0.20), respectively, with statistical difference (*P* < 0.05). Significant statistical heterogeneity was found across the 11 studies (I² = 54.5, *P* < 0.05). The data was pooled through a random effect model with an odds ratio of 4.21 (95% CI 2.33–7.58) in CT compared to AC.

**Conclusion:** The frequency of *E-cadherin* promoter hypermethylation in CT is significantly higher than in AC, indicating that promoter hypermethylation of *E-cadherin* plays an important role in NSCLC carcinogenesis.

Introduction

Lung cancer, the leading cause of cancer-related death, is the most commonly diagnosed malignant carcinoma in men and the second most common in women worldwide.1,2 More than one million deaths are attributed to lung cancer each year.3 However, the exact cause and molecular mechanisms of lung cancer are not entirely clear. Lung cancer carcinogenesis is a complex biological process involving a variety of genetic and epigenetic changes.4,5 Tumor suppressor gene promoter methylation is considered an important mechanism that inactivates lung cancer progression.6,7

Methylation of the CpG island is a common DNA modification and can change the activity of a DNA segment without changing the sequence. DNA methylation in the promoter region of certain genes typically acts to repress gene transcription.8,9 DNA methylation has been proven essential for normal development and is associated with a number of key processes, including genomic imprinting, X-chromosome inactivation, repression of transposable elements, aging, and carcinogenesis.8 DNA hypermethylation of CpG in the promoter regions causes inactivation of the tumor-suppressor genes, which are involved in mechanisms such as apoptosis and the cell cycle.8
Epithelial cadherin (E-cadherin), also known as cadherin-1 (CDH1) gene is a classic tumor suppressor gene. Studies have indicated that loss of E-cadherin function or expression is implicated in cancer progression and metastasis. One of the key mechanisms for loss of E-cadherin expression is hypermethylation of the promoter region. Several studies have shown that the promoter region of the E-cadherin gene is hypermethylated and is associated with a loss of expression. In the present study, we included all of the open published studies relevant to E-cadherin promoter hypermethylation and non-small cell lung cancer (NSCLC) to examine any correlation.

Methods

Database search

The electronic PubMed, Embase, Web of Science, Google scholar, China National Knowledge Infrastructure, and Wanfang databases were systematically searched by two reviewers. Studies relevant to E-cadherin gene promoter methylation and NSCLC were identified using the following keywords: “lung cancer,” “carcinoma of the lung,” “non-small cell lung cancer,” “NSCLC,” “methylation,” “hypermethylation,” “E-cadherin,” “epithelial cadherin,” “Cadherin-1” and “CDH1.” The title and abstract of the identified publications were reviewed to exclude unrelated studies. The full text of all potentially relevant publications was then reviewed to identify the suitable publications and extract the data.

Publication inclusion and data extraction

The inclusion criteria were: pathologically or cytologically confirmed NSCLC; and promoter hypermethylation of the E-cadherin gene was detected via methylation-specific PCR (MSP), real-time MSP (RT-MSP), quantitative MSP (q-MSP), and MethyLight. Non-malignant lung tissue or blood from the same patient was used as control material. All included studies were published in English or Chinese. General information, such as first author, study publication year, mean age of subjects included in each individual study, ethnicity, and hypermethylation detection methods, were extracted from each individual study. The hypermethylation status of the cancer tissue (CT) and autologous control (AC) were extracted by two reviewers and checked by a third reviewer, as recommended by the Cochrane Handbook for systematic reviews.

Statistical method

Hypermethylation frequency in CT and AC was calculated as the hypermethylation rate. E-cadherin gene promoter hypermethylation in CT compared to AC was expressed by odds ratio (OR) and 95% confidence intervals (CIs). Before pooling the data, statistical heterogeneity across the 11 included publications was assessed by I² test. Fixed or random effect methods were used to pool the ORs, according to the I² test. Correlation of hypermethylation status between CT and AC of NSCLC patients was evaluated by line regression test. Funnel plot and Egger’s line regression test were used to assess publication bias. All data analysis was performed using STATA/SE 11.0 (StataCorp LP, http://www.stata.com).

Results

General information of the included publications

Seventy-six relevant studies were initially identified. After reviewing the title and abstract, 53 publications were excluded for the following reasons: (i) subjects of the original studies had other malignant carcinomas; (ii) hypermethylation was detected in genes other than E-cadherin; (iii) duplicated publications or data; (iv) control samples were from healthy subjects; and (v) studies were about cell lines. The full text of 32 studies was reviewed. Twelve publications were excluded as they did not include sufficient data to calculate the hypermethylation frequency of CT or AC. Eleven studies were finally included in the meta-analysis (Fig 1). The general characteristics of the included 11 studies are shown in Table 1.

Hypermethylation frequency in cancer tissue (CT) and autologous controls (AC)

The frequency of E-cadherin gene promoter hypermethylation ranged from 0.11 to 0.66 in the CT and 0 to 0.40 in the AC of the included publications. The mean E-cadherin gene promoter hypermethylation frequencies were 0.32 (95% CI 0.22–0.41) and 0.12 (95% CI 0.04–0.20) for CT and AC, respectively, with statistical difference (P < 0.05) (Fig 2).

Meta-analysis

Statistical heterogeneity was evaluated by I² test. Significant statistical heterogeneity was found across the included 11 publications (I² = 54.5, P < 0.05). The data was pooled through random effect model with an OR of 4.21 (95% CI 2.33–7.58) in CT compared to AC (Fig 3).
Correlation of *E-cadherin* gene promoter methylation between CT and AC

Correlation of *E-cadherin* gene promoter hypermethylation between CT and AC was evaluated by line regression test. Hypermethylation correlation between CT and AC can be demonstrated by $Y = 0.3431X + 0.01231$ (Fig 4).

Publication bias

A Begg’s funnel plot and Egger’s line regression test were used to investigate possible publication bias. Slight asymmetry in the bottom of the funnel plot indicated potential publication bias; however, no publication bias was identified by Egger’s line regression test ($t = 0.58, P > 0.05$) (Fig 5).
Figure 2 Scatter plot of E-cadherin gene promoter hypermethylation frequency in cancer tissue and autologous controls of non-small cell lung cancer patients.

Figure 3 Forest plot of E-cadherin promoter hypermethylation in cancer tissue (CT) versus autologous controls (AC). The squares and horizontal lines represent the study-specific odds ratio (OR) and 95% confidence interval (CI). The area of the squares reflects the weight (inverse of the variance). The diamond represents the pooled OR and 95% CI through random effect method.
Tumor suppressor gene promoter methylation is considered an important mechanism to inactivate lung cancer progression.\(^1,2\) E-cadherin is a classic member of the cadherin superfamily. E-cadherin mutation or loss of expression is correlated with the progression of several carcinomas, including lung, gastric, breast, colorectal, thyroid, and ovarian cancers. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis. Promoter hypermethylation is an important mechanism that inactivates the E-cadherin gene.\(^3,13,19,20\) Several studies have investigated the hypermethylation status in CT and AC of NSCLC patients; however, the hypermethylation frequency differs between studies.\(^22,25\) In addition, the statistical power of each individual study was limited by small sample sizes. Therefore, the correlation between hypermethylation of the CpG island in the promoter region of the E-cadherin gene and lung cancer risk is inconclusive.

In the present work, we investigated the correlation between E-cadherin gene promoter hypermethylation and lung cancer risk by meta-analysis. Eleven relevant publications were included and analysis showed that the frequency of E-cadherin promoter hypermethylation in CT is significantly higher than in AC, indicating that promoter hypermethylation of E-cadherin plays an important role in NSCLC carcinogenesis. Hypermethylation of the E-cadherin gene may increase the risk of lung cancer at an epidemiological level. However, the molecular mechanisms are yet to be elucidated.

In conclusion, E-cadherin promoter hypermethylation is common in the CT of NSCLC patients and may play an important role in progression. However, the molecular mechanisms relevant to E-cadherin promoter hypermethylation and lung cancer progression are not yet clear. Therefore, studies of the molecular mechanisms of E-cadherin promoter hypermethylation and lung cancer are needed.

**Disclosure**

No authors report any conflict of interest.

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