Thoracic Cancer

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Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

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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Keywords
Carcinoma of the lung; hypermethylation; meta-analysis; non-small cell lung cancer.

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Received: 28 August 2018;
Accepted: 27 September 2018.

doi: 10.1111/1759-7714.12900

Thoracic Cancer **10** (2019) 54–59

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 ($I^2 = 54.5$, $P < 0.05$). 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.[9]

Epithelial cadherin (E-cadherin), also known as the *cadherin-1* (*CDH1*) gene is a classic tumor suppressor gene.[10,11] Studies have indicated that loss of *E-cadherin* function or expression is implicated in cancer progression and metastasis.[12] 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.[13–15] 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 systematic 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.[16]

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 I^2 test. Fixed or random effect methods were used to pool the ORs, according to the I^2 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[17] 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).[13–15,18–25] 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 I^2 test. Significant statistical heterogeneity was found across the included 11 publications ($I^2 = 54.5$, $P < 0.05$). 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).

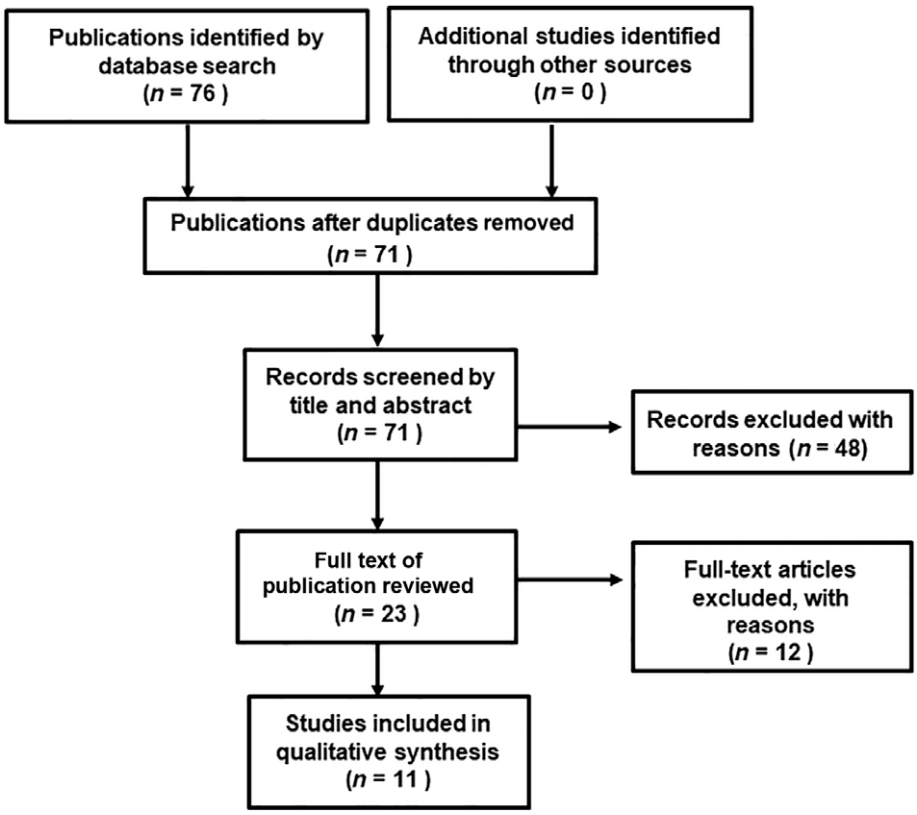

Figure 1 PRISMA flowchart of database search.

Table 1 General characteristics of the included publications

Author	Year publication	Location	Age (years)	Gender (M/F)	Ethnic	CT		AC		Method	Control type
						Me	uMe	Me	uMe		
Zochbauer-Muller *et al.*[18]	2001	Australia	NA	76/31	Caucasian	19	88	0	104	MSP	NMLT
Yanagawa *et al.*[14]	2003	Japan	67.3 (mean)	54/21	East Asian	22	53	11	64	MSP	NMLT
Russo *et al.*[15]	2005	US	NA	NA	Caucasian	18	31	11	38	MSP	Blood
Kim *et al.*[12]	2007	Korea	NA	90/28	East Asian	30	58	5	83	MSP	NMLT
Wang *et al.*[23]	2007	China	57 (median)	14/8	East Asian	9	13	2	20	MSP	NMLT
Gu *et al.*[24]	2007	China	NA	23/18		8	33	0	41	MSP	NMLT
Feng *et al.*[13]	2008	US	64.3 (mean)	26/23	Caucasian	15	34	4	45	MethyLight	NMLT
Wang G *et al.*[20]	2008	China	58 (median)	19/76	East Asian	63	32	23	72	MSP	NMLT
Wang Y *et al.*[21]	2008	China	NA	NA	East Asian	3	25	1	11	MSP	NMLT
Liu *et al.*[22]	2009	US	NA	27/8	Mixed	10	25	4	6	MSP	NMLT
Zheng *et al.*[25]	2012	China	NA	26/11	East Asian	12	25	0	25	MSP	NMLT

AC, autologous control; CT, cancer tissue; F, female; M, male; MSP, methylation-specific PCR; NA, not available; NMLT, non-malignant lung tissue.

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 $Y = 0.3431*X + 0.01231$ (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).

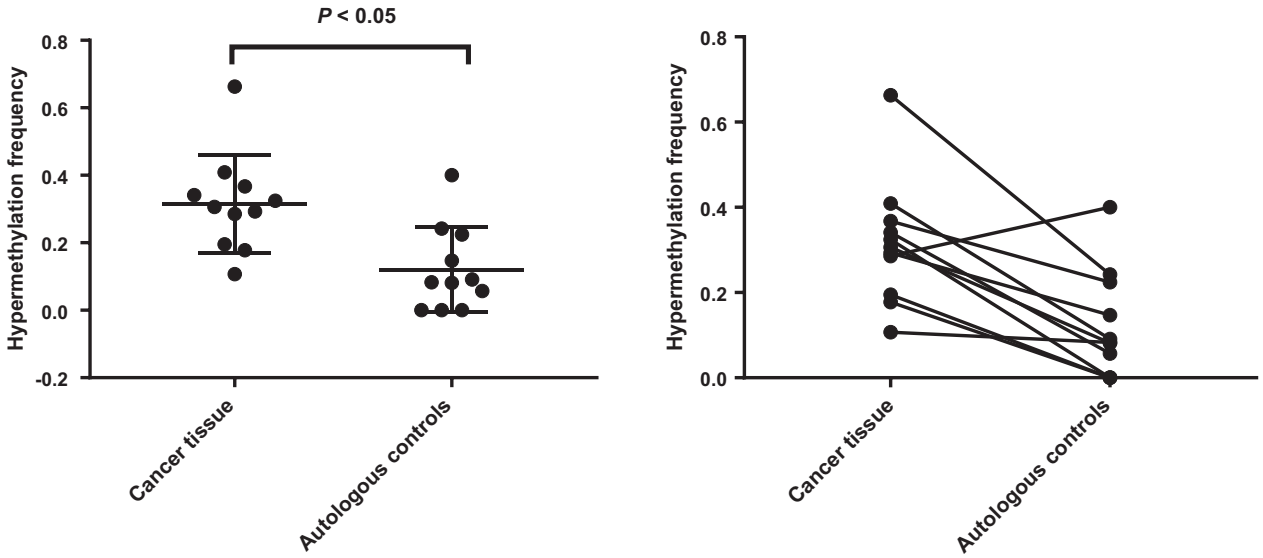

Figure 2 Scatter plot of *E-cadherin* gene promoter hypermethylation frequency in cancer tissue and autologous controls of non-small cell lung cancer patients.

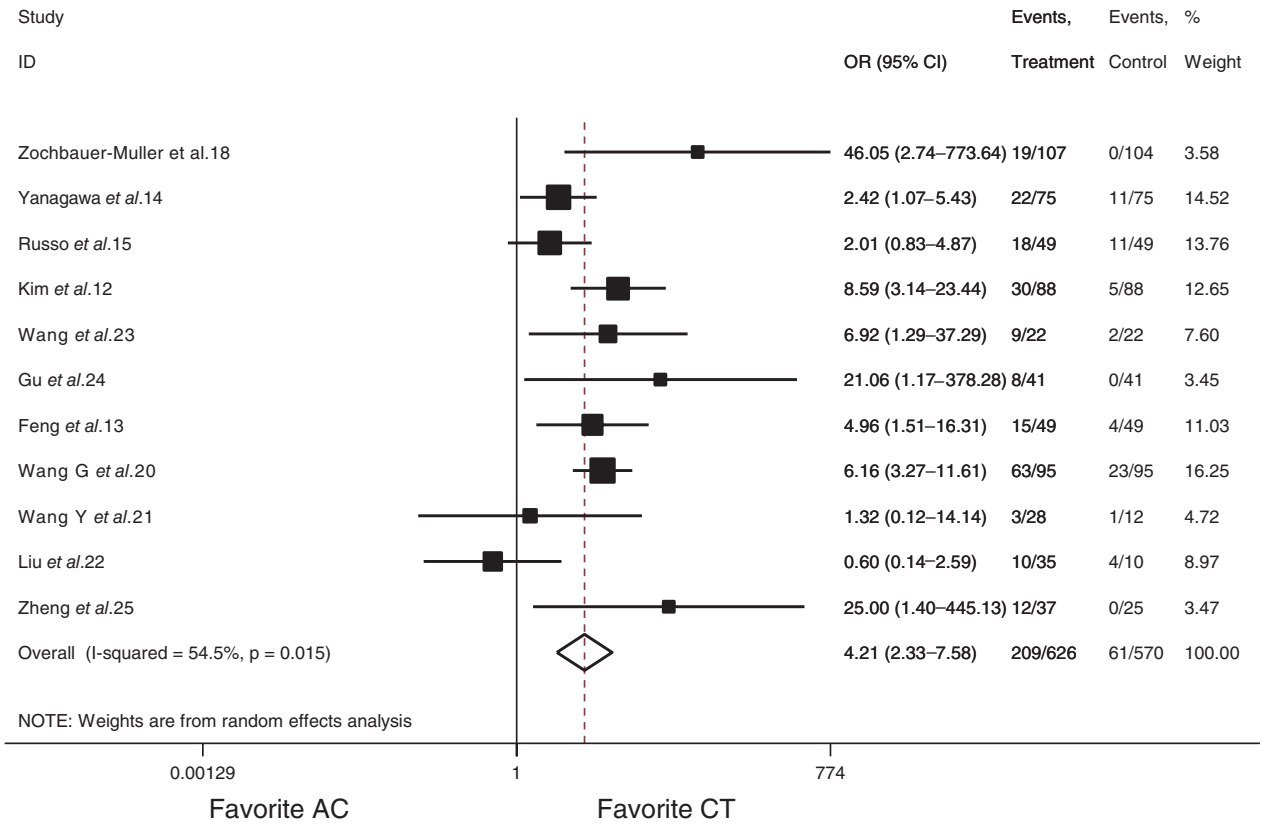

Figure 3 Forest plot of *E-cadherin* promoter hypermethylation in cancer tissue (CT) versus autologous controls (AC). The squares and horizontal lines represent the study-specific odds ratio (OR) and 95% confidence interval (CI). The area of the squares reflects the weight (inverse of the variance). The diamond represents the pooled OR and 95% CI through random effect method.

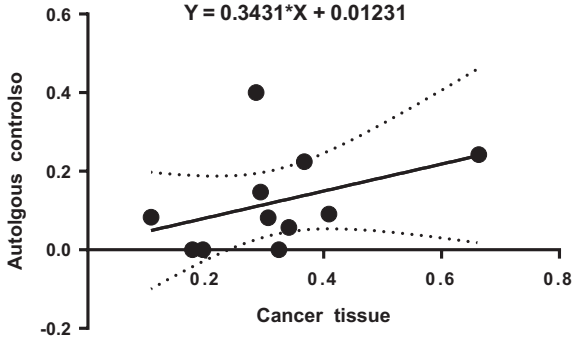

Figure 4 Scatter plot of the correlation of *E-cadherin* gene promoter methylation between cancer tissue (CT) and autologous controls (AC).

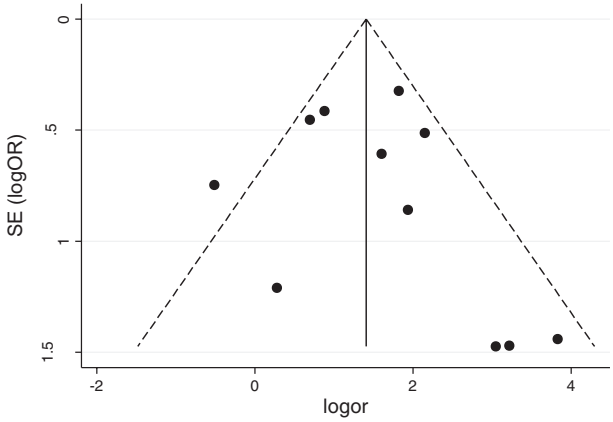

Figure 5 Funnel plot with pseudo 95% confidence limits (each circle represents a separate publication for the indicated association). The area of the hollow circle reflects the weight (inverse of the variance). SE, standard error.

Discussion

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.[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* the 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.

References

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7–30.
2 Torre LA, Siegel RL, Jemal A. Lung cancer statistics. *Adv Exp Med Biol* 2016; **893**: 1–19.
3 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87–108.
4 Inamura K, Ishikawa Y. Lung cancer progression and metastasis from the prognostic point of view. *Clin Exp Metastasis* 2010; **27**: 389–97.
5 Popper HH. Progression and metastasis of lung cancer. *Cancer Metastasis Rev* 2016; **35**: 75–91.
6 Qian X, Ruan L. APC gene promoter aberrant methylation in serum as a biomarker for breast cancer diagnosis: A meta-analysis. *Thorac Cancer* 2018; **9**: 284–90.
7 Li S, Shen L, Chen KN. Association between H3K4 methylation and cancer prognosis: A meta-analysis. *Thorac Cancer* 2018; **9**: 794–9.
8 Bartels A, Han Q, Nair P *et al.* Dynamic DNA methylation in plant growth and development. *Int J Mol Sci* 2018; **19**: E2144.
9 Pfeifer GP. Defining driver DNA methylation changes in human cancer. *Int J Mol Sci* 2018; **19**: E1166.
10 Semb H, Christofori G. The tumor-suppressor function of E-cadherin. *Am J Hum Genet* 1998; **63**: 1588–93.
11 Wong AS, Gumbiner BM. Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol* 2003; **161**: 1191–203.

12 Beavon IR. The E-cadherin-catenin complex in tumour metastasis: Structure, function and regulation. *Eur J Cancer* 2000; **36**: 1607–20.
13 Feng Q, Hawes SE, Stern JE *et al*. DNA methylation in tumor and matched normal tissues from non-small cell lung cancer patients. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 645–54.
14 Yanagawa N, Tamura G, Oizumi H, Takahashi N, Shimazaki Y, Motoyama T. Promoter hypermethylation of tumor suppressor and tumor-related genes in non-small cell lung cancers. *Cancer Sci* 2003; **94**: 589–92.
15 Russo AL, Thiagalingam A, Pan H *et al*. Differential DNA hypermethylation of critical genes mediates the stage-specific tobacco smoke-induced neoplastic progression of lung cancer. *Clin Cancer Res* 2005; **11**: 2466–70.
16 Zafarmand MH, van der Schouw YT, Grobbee DE, de Leeuw PW, Bots ML. The M235T polymorphism in the AGT gene and CHD risk: Evidence of a Hardy-Weinbergequilibrium violation and publication bias in a meta-analysis. *PLoS One* 2008; **3**: e2533.
17 Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629–34.
18 Zochbauer-Muller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD. Aberrant promoter methylation of multiple genes in non-small cell lung cancers. *Cancer Res* 2001; **61**: 249–55.
19 Kim DS, Kim MJ, Lee JY, Kim YZ, Kim EJ, Park JY. Aberrant methylation of E-cadherin and H-cadherin genes in nonsmall cell lung cancer and its relation to clinicopathologic features. *Cancer* 2007; **110**: 2785–92.
20 Wang G, Hu X, Lu C, Su C, Luo S, Luo ZW. Promoter-hypermethylation associated defective expression of E-cadherin in primary non-small cell lung cancer. *Lung Cancer* 2008; **62**: 162–72.
21 Wang Y, Zhang D, Zheng W, Luo J, Bai Y, Lu Z. Multiple gene methylation of nonsmall cell lung cancers evaluated with 3-dimensional microarray. *Cancer* 2008; **112**: 1325–36.
22 Liu RY, Lei Z, Li W *et al*. Infrequently methylated event at sites -181 to -9 within the 5' CpG Island of E-cadherin in non-small cell lung cancer. *Exp Lung Res* 2009; **35**: 541–53.
23 Wang HB, Miao H, Zhang JC *et al*. The relation between E-cadherin promoter CpG Island methylation and E-cadher in protein expression in lung cancer. *China Oncol* 2007; **17**: 603–6.
24 Gu Q, Wang D, Wang XH, Tao S, Zhao LS, Wang YC. A relationship between the methylation of epithelial cadher in gene and biological behaviour of lung cancer. *J Tianjin Med Univ* 2007; **13**: 10–2.
25 Zheng SY, Hou JY, Zhao J, Jiang D, Ge JF, Chen S. Clinical outcomes of downregulation of E-cadherin gene expression in non-small cell lung cancer. *Asian Pac J Cancer Prev* 2012; **13**: 1557–61.

