

Clinicopathological and prognostic significance of *CDH1* hypermethylation in hepatocellular carcinoma: a meta-analysis

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Background: The patients with hepatocellular carcinoma (HCC) have poor prognosis due to being diagnosed at late stage or recurrence following surgery. It's critical to identify effective biomarkers that can improve overall diagnosis and treatment of HCC.

Methods: We performed a meta-analysis of all relative studies reporting the clinicopathological significance of *CDH1* hypermethylation in HCC by using Review Manager 5.2. A comprehensive literature search was performed in EMBASE, PubMed, Web of Science and Google Scholar databases. Kaplan Meier Plotter online database was used for the determination of correlation between *CDH1* mRNA expression and overall survival in patients with HCC. Odds Ratios (OR) with 95% corresponding confidence intervals (CIs) were calculated. A total of 12 relevant studies were included in the meta-analysis with 981 patients.

Results: The positive rate of *CDH1* hypermethylation was significantly higher in HCC than in normal liver tissue; and the pooled OR was 4.34 with 95% CI 2.50–7.56, $P < 0.00001$. *CDH1* promoter in HCC was more frequently hypermethylated compared to the group of chronic liver disease (CLD); OR was 4.83 with 95% CI 2.67–8.72, $P < 0.00001$. However, the rate of *CDH1* promoter hypermethylation was not correlated with different grades as well as stages. High *CDH1* mRNA expression was significantly correlated to better overall survival in all 231 HCC patients compared to 133 HCC patients with low level *CDH1* mRNA expression; HR was 0.6 with 95% CI 0.42–0.85, $P = 0.0034$.

Conclusion: In summary, *CDH1* promoter hypermethylation is a risk factor and promising biomarker for HCC carcinogenesis and diagnosis, as well as a predictor of poor prognosis.

Keywords: hepatocellular carcinoma, *CDH1*, methylation, prognosis, diagnosis, E-cadherin

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the fourth leading cause of cancer mortality globally.¹ The patients with HCC have a poor prognosis due to being diagnosed at late stage or recurrence following surgery.² The only curative therapies are liver transplantation and resection. In order to qualify these two treatments, patients with HCC need to be diagnosed at early stage.³ On the other hand, there is no good prognostic marker to predict survival outcome apart from tumor staging classification system. Therefore, it is very critical to find effective biomarkers for early diagnosis and biomarkers to predict prognosis.

Cadherins were defined as cell surface glycoproteins responsible for the Ca^{2+} -dependent cell–cell adhesion mechanism,^{4,5} including E-cadherin, neural (N-) cadherin, and the placental (P-) cadherin.⁵ E-cadherin functions as a mediator of cell–cell adhesion and the principal organizer of epithelial phenotype.^{6,7} Its adhesive

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function at cell surface holds cells together, facilitates other cell–cell interactions, and physically blocks the movement of cells. Additionally, E-cadherin homophilic binding leads to contact-mediated inhibition of growth via modulation of growth inhibitory signals including the Hippo, growth factor receptor tyrosine kinase (RTK), and Src family kinase signaling pathways. On the other hand, the cytoplasmic tail of E-cadherin is associated with intracellular molecules of alpha-, beta-, and gamma-catenins^{8,9} that link to the cytoskeleton and mediate downstream growth factor signaling pathways such as Wnt, transforming growth factor β , and nuclear factor kappa-light-chain-enhancer of activated B cells.^{10–13} Loss of E-cadherin expression leads to induction of the epithelial to mesenchymal transition (EMT), causing cells to lose cell–cell contacts and acquire increased motility to spread into surrounding or distant tissues, also upregulates growth factor pathways and promoting proliferation. Therefore, loss of E-cadherin plays a crucial role in hepatocellular carcinogenesis and metastasis.¹⁴ *Cadherin-1 (CDH1)* is the gene for E-cadherin, and its downregulation with hypermethylation of CpG islands of region promoter is found in various tumors originating from epithelial cells including HCC.^{15,16} Aberrant DNA methylation has been demonstrated in common HCC,^{17,18} and recent studies showed that CpG methylation around the promoter is very important in the transcriptional inactivation of *CDH1* in HCC.¹⁵ However, the positive rate of *CDH1* promoter hypermethylation in HCC is inconsistent due to small size of samples in individual study.

In the present study, we conducted a meta-analysis study using the qualified studies and determined the association between *CDH1* hypermethylation in HCC and its progression as well as prognosis.

Methods

Selection criteria and study search

We searched the three electronic databases EMBASE, PubMed, and Web of Science from the earliest date up to June 2018 for appropriated articles addressing the focused question. Electronic database searches were conducted by using the following terms: “liver,” “hepatocellular” and “cancer or tumor or neoplasm or carcinoma,” “methylation,” and “*CDH1* or E-cadherin.” Additional studies were searched manually from the reference list of included articles. There were 37 articles from EMBASE, 73 articles found from PubMed, as well as 36 articles from Web of Science.

A total of 146 articles were reviewed and screened by article titles and abstracts. Studies were eligible for inclusion in the current analysis if they met the following criteria: 1)

studies that evaluated the association between *CDH1* hypermethylation and clinicopathological parameters of HCC; 2) *CDH1* hypermethylation detected in the primary HCC tissues; and 3) studies published in English or Chinese. The exclusion criteria were as follows: 1) expert opinion, case reports, reviews, conference abstracts, editorials, letters; 2) all publications regarding cell lines, in vitro/ex vivo studies, and human xenografts; 3) studies in which same population was involved; and 4) studies in which *CDH1* protein expression was evaluated.

After exclusion of non-relevant and/or redundant publications from different databases, the 17 remaining papers were selected and reviewed in the full-text version for inclusion and exclusion criteria. Five articles were excluded due to lack of sufficient data for the present study.

Data extraction and study assessment

Two independent authors (XW and XY) extracted the data from the included studies. Disagreement regarding selection was resolved through discussion. If they could not reach an agreement, a third reviewer (LS) was consulted. The following information was collected from each study: year of publication, the first author name, sample source, number of cases, cancer TNM (tumor-node-metastasis) stage, methylation detection method, and *CDH1* methylation status. Data for study characteristics and clinical responses were collected and organized into a standard table format. Heterogeneity of the included studies was assessed to determine whether or not the data of the various studies could be analyzed for a meta-analysis.

The assessment of methodological quality of included studies was done by two assessors based on a grading system developed by the Newcastle Ottawa Quality Assessment Scale (NOQAS).¹⁹ The three reviewers graded the quality and compared them, and then they reached an agreement for each item. Those scales use a grading system to evaluate on comparability, the selection of quality, exposure, and outcomes for study participants. The NOQAS scores ranged between 0 and 9, and a study with a score of 7 or more indicated a good quality.

Statistical analysis

The meta-analysis was performed using Review Manager 5.2 (Cochrane Collaboration, Update Software, Oxford, UK). ORs with its 95% CIs were calculated. All the events represent the number of HCC with *CDH1* hypermethylation. The evaluation of statistical heterogeneity was finished by using the Cochran's Q statistic and I^2 tests. When the I^2 value was below 50%, fixed-effect model was generated, and when the I^2 value was 50% or greater, a random-effect model was

generated. Sensitivity analysis was used for exploring the reasons of statistical heterogeneity. The pooled frequency of *CDH1* hypermethylation and 95% CIs were estimated. The rate of *CDH1* hypermethylation was compared between different groups by tumor features. The pooled OR was estimated for the correlation between *CDH1* hypermethylation and clinicopathological features. Overall survival was analyzed by using an online database Kaplan premier plotter that was established by using gene expression and the survival information of 364 liver cancer patients (http://kmpplot.com/analysis/index.php?p=service&cancer=liver_rnaseq).²⁰ *P*-value less than 0.05 was regarded statistically significant. Publication bias was evaluated by using funnel plots reported by Egger et al.²¹

Results

Identification of relevant studies and study quality

Twelve studies were included in the meta-analysis after screening 146 studies (Figure 1). The following items were recorded from each study: first author, published year, country, tumor histology, *CDH1* methylation status, differentiation and stages of tumors, as well as methylation detection methods (Table 1).

The correlation of CDH1 promoter hypermethylation with clinicopathological features

The frequency of *CDH1* promoter hypermethylation was significantly higher in HCC than in normal liver tissue;

the pooled OR was 4.34 with 95% CI 2.50–7.56, $z=5.19$, $P<0.00001$, $I^2=0\%$ (Figure 2). *CDH1* promoter in HCC was more frequently hypermethylated compared to the group of chronic liver disease (CLD); OR was 4.83 with 95% CI 2.67–8.72, $z=5.22$, $P<0.00001$, $I^2=0\%$ (Figure 3). However, the rate of *CDH1* promoter hypermethylation was not correlated with different grades; OR was 0.84 with 95% CI 0.40–1.78, $z=0.46$, $P=0.65$, $I^2=0\%$ (Figure 4). The rate of *CDH1* promoter hypermethylation was not significantly different between early and advanced stages; OR was 0.79 with 95% CI 0.42–1.49, $z=0.71$, $P=0.48$, $I^2=0\%$ (Figure 5). High *CDH1* mRNA expression was strongly associated with better overall survival in all 231 HCC patients compared to 133 HCC patients with low-level *CDH1* mRNA expression; HR was 0.6 with 95% CI 0.42–0.85, $P=0.0034$ (Figure 6).

Sensitivity analyses and publication bias

The quality of each study was evaluated using the NOQAS. This scale for non-randomized case-controlled studies and cohort studies was used to evaluate the quality of selection, comparability, exposure, and outcomes for study participants, with the maximum score being 9 points. Among the 12 studies, three of them scored 8 points, seven scored 7 points, and two scored 6 points (data not shown). The score for each study showed a relatively high quality. A sensitivity analysis was performed by removing one study at a time; the result remained stable, indicating the stability of the present analysis. The funnel plots were largely symmetric (Figure 7), indicating there were no publication biases in the meta-analysis of *CDH1* methylation and clinicopathological features.

Discussion

E-cadherin, a Ca^{2+} -dependent cell adhesion molecule, encoded by *CDH1*, regulates cell polarity and tissue morphology.⁸ Disturbance of cell polarity and destruction of normal tissue morphology initiate carcinogenesis. Knocking-down of *CDH1* results in the activation of oncogenic signaling pathways and leads to the transition of epithelial to mesenchymal in the development of epithelial tumor.^{22–24} Cancer invasion is initiated by the dissociation of cells from primary cancer nests due to loosened cell adhesion. Recent studies reported that *CDH1* promoter hypermethylation was observed in HCC. However, the frequency varied from 13.3% to 66.7%.^{25,26} We pooled nine studies including 580 patients, and evaluated the frequency of *CDH1* promoter hypermethylation. The frequency of *CDH1* promoter hypermethylation in HCC was 32% – 4.34 times higher than the one in normal liver tissue. Additionally, the rate of *CDH1* promoter hypermethylation

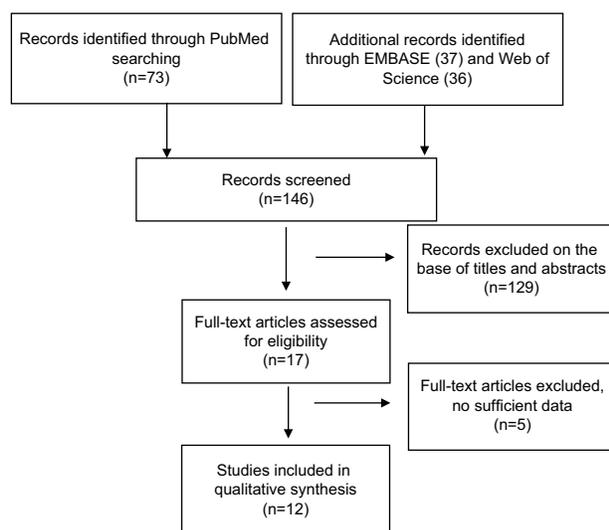


Figure 1 Schematic flow diagram for selection of included studies.

Table 1 Main characteristics of included studies

Authors	Year	Country	Histology (M/T)			Grade (M/T)		Stage (M/T)		Methods
			NLT	CLD	HCC	L	H	I/II	III/IV	
Zekri et al ³⁵	2014	Egypt	2/13	7/38	17/31					MSP
Herath et al ³⁶	2009	Australia	2/20		14/61					MSP
Nishida et al ³⁷	2008	Japan	4/22		21/77					COBRA
Kato et al ³⁸	2006	Japan			26/60	16/32	10/28	20/42	6/18	MSP
Yuan et al ³⁹	2006	China			30/112			20/73	10/39	MSP
Kwon et al ⁴⁰	2005	Korea		1/13	32/64	25/52	7/12	28/56	4/8	MSP
Herath et al ⁴¹	2004	South Africa	2/20		14/50					MSP
Schagdarsurengin et al ²⁶	2003	Germany	0/2		2/15					MSP
Lee et al ⁴²	2003	Korea	4/60	2/30	20/60					MSP
Yang et al ¹⁸	2003	USA	1/14	3/15	25/51					MSP
Matsumura et al ⁴³	2001	Japan	1/30		7/30	7/26	1/4			Southern blot
Kanai et al ²⁵	1997	Japan		11/24	16/24					Southern blot

Abbreviations: CLD, chronic liver disease; COBRA, combined bisulfite restriction analysis; H, high grade; HCC, hepatocellular carcinoma; L, low grade; M, methylation number; MSP, methylation-specific PCR; NLT, normal liver tissue; T, total number.

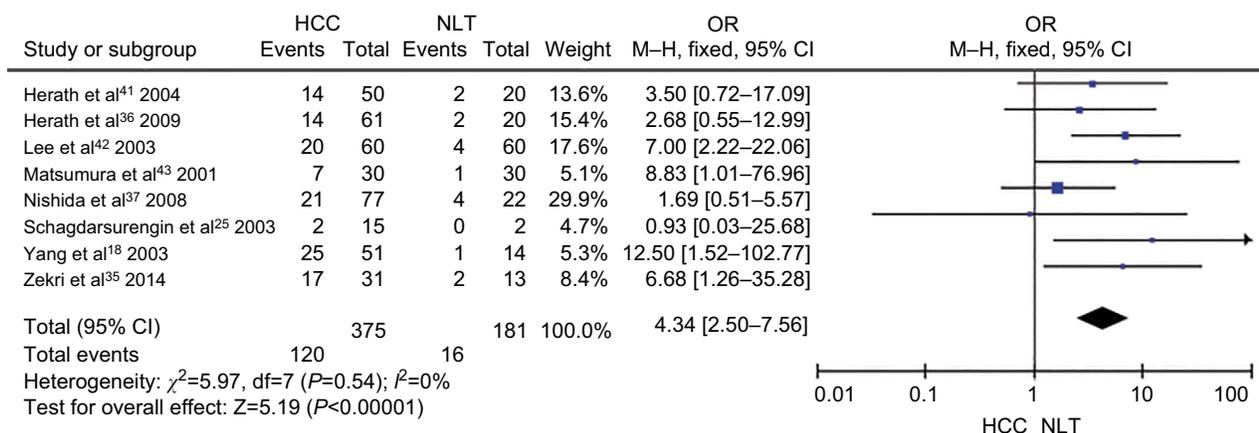


Figure 2 Forest plot for CDHI promoter hypermethylation in HCC and normal liver tissue.

Notes: The squares represent the weight of individual study in the meta-analysis and the line width indicates the corresponding 95% CI. The diamond represents the pooled OR and the width of diamond indicates 95% CI.

Abbreviations: HCC, hepatocellular carcinoma; NLT, normal liver tissue; M-H, Mantel-Haenszel.

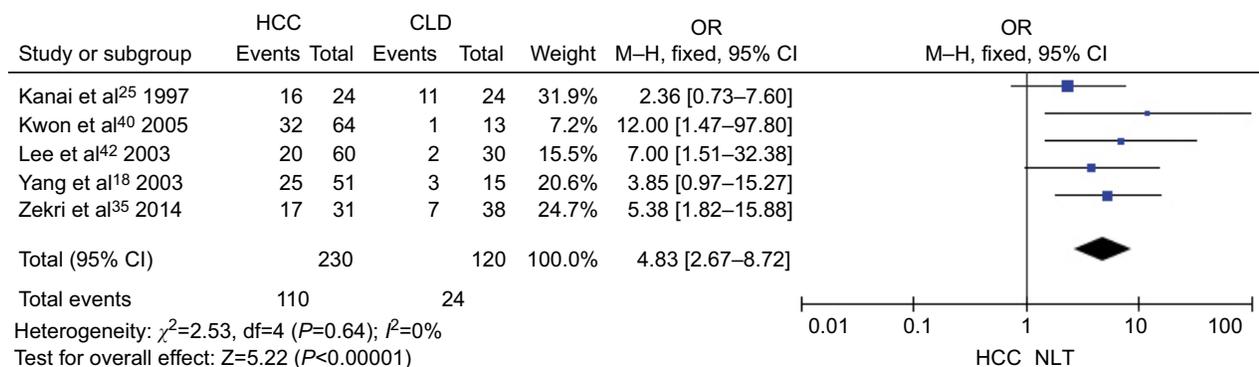


Figure 3 Forest plot for CDHI promoter hypermethylation in HCC and CLD.

Notes: The squares represent the weight of individual study in the meta-analysis and the line width indicates the corresponding 95% CI. The diamond represents the pooled OR and the width of diamond indicates 95% CI.

Abbreviations: CLD, chronic liver disease; HCC, hepatocellular carcinoma; M-H, Mantel-Haenszel.

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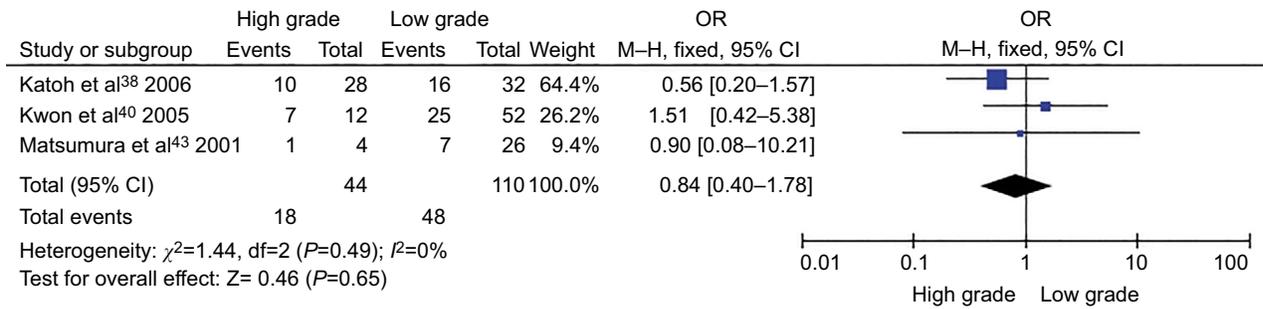


Figure 4 Forest plot for CDHI promoter hypermethylation in high and low grade of histological differentiation.

Notes: The squares represent the weight of individual study in the meta-analysis and the line width indicates the corresponding 95% CI. The diamond represents the pooled OR and the width of diamond indicates 95% CI.

Abbreviation: M-H, Mantel-Haenszel.

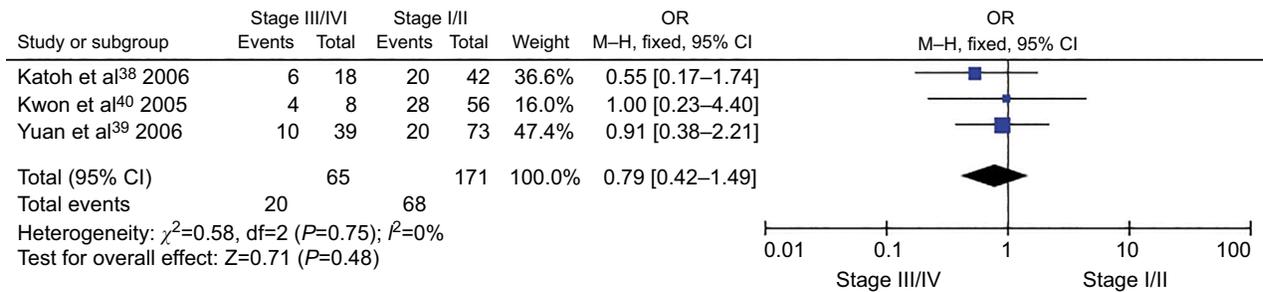


Figure 5 Forest plot for CDHI promoter hypermethylation in different stage of HCC.

Notes: The squares represent the weight of individual study in the meta-analysis and the line width indicates the corresponding 95% CI. The diamond represents the pooled OR and the width of diamond indicates 95% CI.

Abbreviations: HCC, hepatocellular carcinoma; M-H, Mantel-Haenszel.

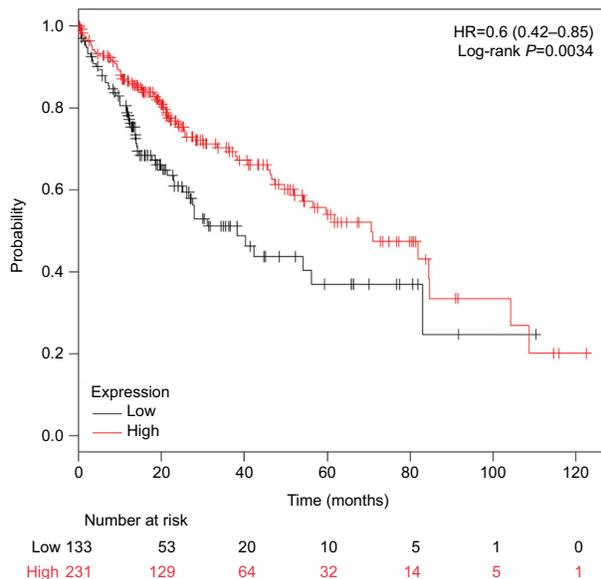


Figure 6 Comparison of overall survival between patients with high and low expression of CDHI mRNA.

Abbreviation: HR, hazard ratio.

in HCC was 4.83 times higher than the one in CLD. Our findings suggest that CDHI promoter hypermethylation could be a biomarker for HCC diagnosis. DNA methyltransferases (DNMTs) are pivotal regulators of the methylation of CpG islands. Previous evidence indicated that increased expression of DNMT1 was correlated with the stages, portal venous invasion, and prognosis of HCC.^{27,28} It has been reported that inhibitors of DNA methyltransferase inhibitors (DNMTis) such as 5-Aza-2'-deoxycytidine (5-Aza-CdR) and 5-fluoro-2-deoxycytidine (FdCyd) are applied to human breast and lung cancer cells, and currently FdCyd is in clinical trials for the treatment of breast cancer and other solid tumors.²⁹⁻³¹ Therefore, inhibitors for DNA methylation could be a new therapy strategy for HCC patients with CDHI promoter hypermethylation.

Overall survival was evaluated by using an online database Kaplan-Meier Plotter that was generated by using mRNA expression and the survival data of 364

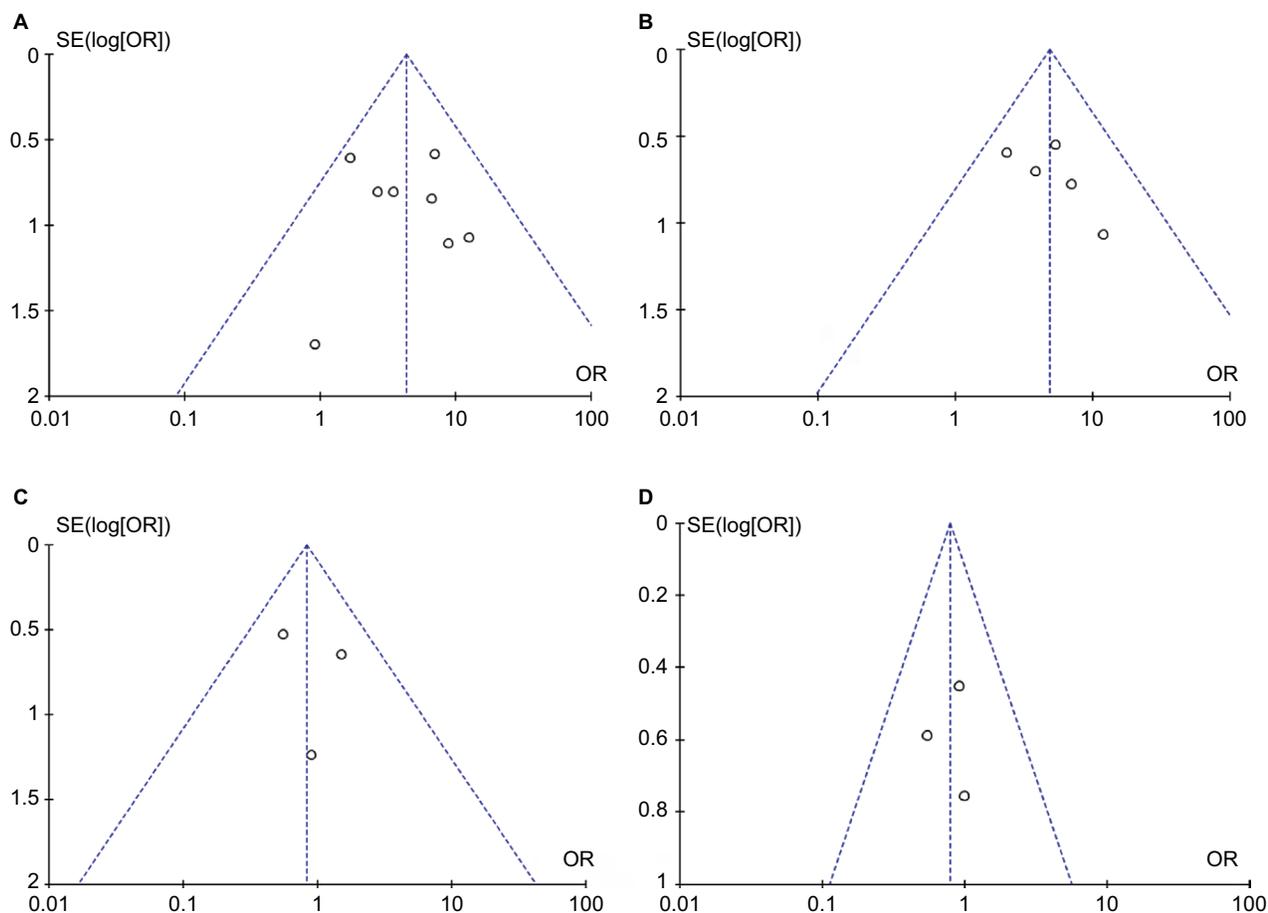


Figure 7 Funnel plot for publication bias.

Notes: (A) *CDH1* promoter hypermethylation in HCC and normal liver tissue; (B) *CDH1* promoter hypermethylation in HCC and CLD; (C) *CDH1* promoter hypermethylation in high and low grade of histological differentiation; (D) *CDH1* promoter hypermethylation in different stage of HCC. Area of the circle represents the weight of individual study.

Abbreviations: CLD, chronic liver disease; HCC, hepatocellular carcinoma; SE, standard error.

liver cancer patients (http://kmpplot.com/analysis/index.php?p=service&caner=liver_rnaseq). Our findings showed that low expression of E-cadherin mRNA was significantly correlated with poor overall survival in HCC patients. Gene methylation is a major mechanism to inactivate gene in HCC.¹⁸ Thus, *CDH1* promoter hypermethylation could be a predictor of poor overall survival. Previous evidence revealed that EMT is an important process correlated with the propensity for invasion and metastases of many types of tumors including HCC.^{32–34} The process of EMT is initiated by suppression of the epithelial markers such as E-cadherin. Loss of E-cadherin function initiates the dissociation of cells from primary cancer nests because of loosened intercellular adhesion, thus resulting in invasion and metastasis. Therefore, *CDH1* promoter hypermethylation in HCC leads to a poor overall survival in patients with HCC. However, our find-

ings showed that *CDH1* promoter hypermethylation was not significantly associated with HCC differentiation and stages. The evaluation needs to be performed when more relative studies are available in future.

Limitations

There were a few limitations of the present study. First, the selection bias was unavoidable due to the selection restricted to the articles published in English and Chinese. Second, the heterogeneity existed in the frequency of *CDH1* promoter hypermethylation between HCC and normal liver tissue. This phenomenon may be caused by the moderate number of samples in the involved studies and inconsistent criteria in selection of controls. Further evaluation with a larger size of samples will be carried out in future for a more reliable conclusion.

Conclusion

In summary, *CDH1* promoter hypermethylation is a promising biomarker for HCC diagnosis and a predictor of poor prognosis.

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Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. The corresponding author had full access to all data and the final responsibility for the decision to submit the article for publication.

Disclosure

The authors report no conflicts of interest in this work.

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