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Hypermethylation of tumor suppressor genes is a risk factor for poor prognosis in ovarian cancer

A meta-analysis

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Abstract

Objective: DNA methylation is the earliest and most studied epigenetic modification in cancer. The literature reported that the abnormal methylation level of multiple genes was associated with poor prognosis in ovarian cancer. However, due to a small sample size, the results reported in the literature vary widely. In this study, the correlation between aberrant methylation level of genes and poor prognosis of ovarian cancer was reviewed in order to clarify the role of DNA methylation in the prognosis of ovarian cancer.

Methods: A systematic research of PubMed, EMbase, Cochrane Library, China Biology Medicine disc (CBMdisc), China National Knowledge Infrastructure (CNKI), Wanfang databases, and EMBASE was performed, and calculated the hazard ratio (HR) of overall survival (OS) and progression-free survival (PFS) and its 95% confidence interval.

Results: HR of the OS obtained of target genes was 2.32 (95% CI: 1.54–3.48, $P = .000$); HR of the PFS obtained of target genes was 1.318 (95% CI: 0.848–2.050, $P = .220$). HR of OS achieved by tumor suppressor genes was 3.09 (95% CI 1.80 − 5.30, $P = .000$).

Conclusion: Hypermethylation of tumor suppressor genes indicate poor prognosis of ovarian cancer.

Abbreviations: CBMdisc = China biology medicine disc, CNKI = China National Knowledge Infrastructure, MSP = methylation-specific polymerase chain reaction, OS = overall survival, PFS = progression-free survival, TSGs = tumor suppressor genes.

Keywords: methylation, ovarian cancer, prognosis, tumor suppressor genes

1. Introduction

Ovarian cancer is the most lethal gynecological malignancy due to the lack of biomarkers for early detection and treatment options.[1] Although there has been a lot of progress in surgery and adjuvant therapy, the survival rate of ovarian cancer has barely changed since the platinum treatment began 30 years ago.[2] The poor overall survival is caused by late presentation, poor surgical outcomes and the development of chemotherapy resistance.[3] It is widely accepted that size of residual disease following surgery, stage, pathological type, peritoneal metastasis, lymph node status, and morphological characteristics are prognostic factors in ovarian cancer.[4]

DNA methylation is the primary and most studied epigenetic modification.[5] Gene hypermethylation in cancer can silence gene expression and regulate biological processes, especially the tumor suppressor genes.[6,7] Aberrant DNA methylation is a common phenomenon in malignancy and the methylation profiles are altered in various tumors which might be associated with clinical outcomes.[8] Epigenetic modifications at specific CpG sites correlate with PFS and OS in ovarian cancer patients treated with conventional chemotherapeutics.[9–12] However, due to a small sample size, the results indicated in the literature vary greatly.[13] In this study, the correlation between abnormal methylation level of genes and poor prognosis of ovarian cancer was reviewed in order to elucidate the role of DNA methylation in the prognosis of ovarian cancer.

2. Materials and methods

2.1. Research strategy and selection criteria

Literature on target genes methylation level as a prognostic factor of ovarian cancer was researched from the PubMed, EMbase, Cochrane Library, CBM, CNKI, Wanfang databases, and EMBASE databases, and the search time was up to July 31, 2018. Search keywords such as "ovarian cancer or ovarian carcinoma or ovarian neoplasm or ovary cancer", "prognosis or prognostic factor" and "DNA methylation or methylation" were combined search (shown in Table 5).

The articles included in this study should meet the following standards:

1. the study is written in English or Chinese;
2. The study reported specific data on ovarian cancer OS and PFS; and
3. the study detects gene methylation level in tissue, serum, or plasma.

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Our study is a meta-analysis and ethical approval is not necessary.

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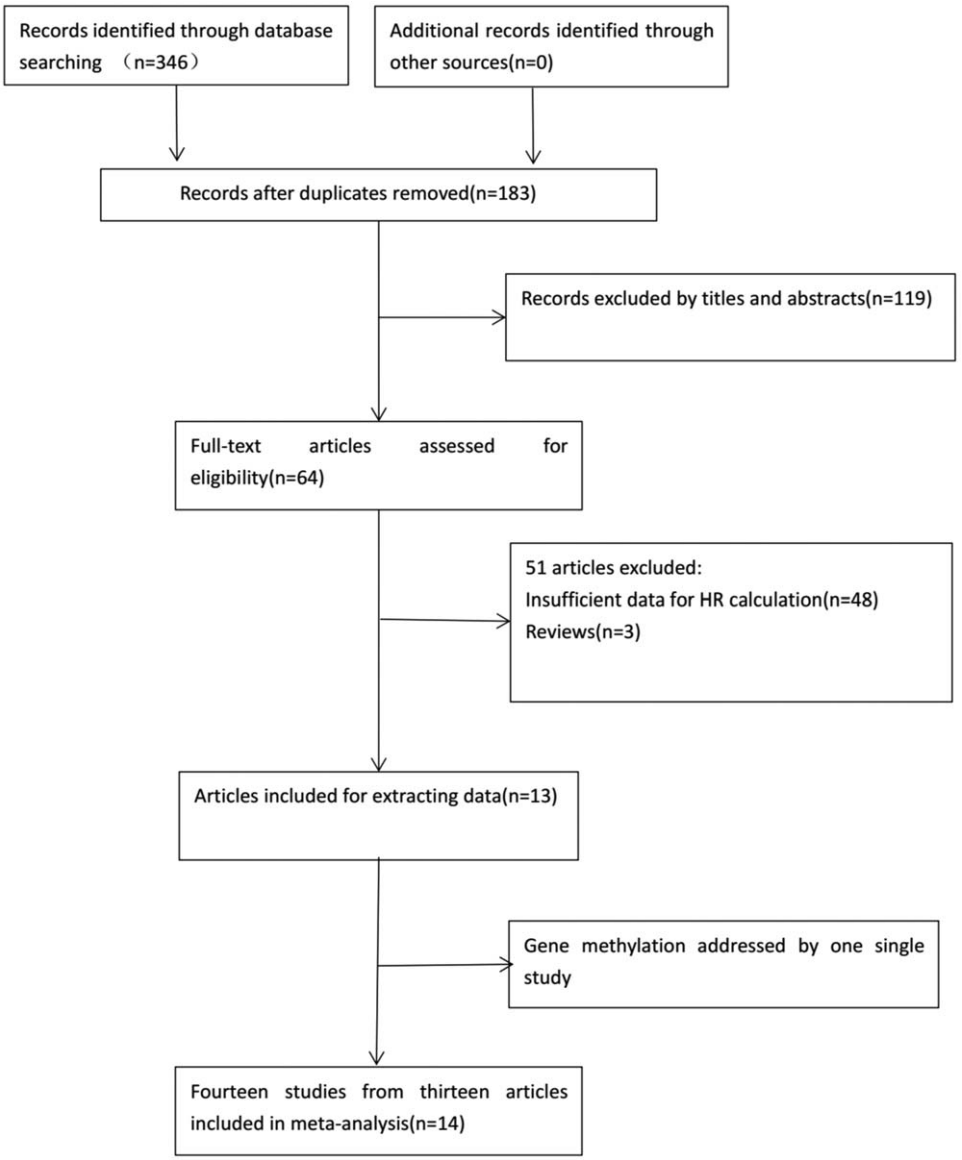


Figure 1. Flow diagram of the study selection process.

Studies that meet the following criteria will be excluded:

1. the article is a review or comment;
2. the study lacks usable data, such as HR of OS and PFS; and
3. the study data were repeated with previous articles.

2.2. Data extraction and quality assessment

The included articles were extracted from the following data by 2 readers: first author, year of publication, country, sample size, methylation detection technology, target gene, cutoff value, follow-up time, and HRs for OS and PFS. Since meta-analysis of prognostic studies have not received a broad consensus on the quality of the literature, the necessity along with the credibility of the score are controversial and we have not been able to grade the literature obtained.[14]

2.3. Statistical analysis

The pooled HRs for OS and PFS were used to evaluate the association between methylation of the target genes and prognosis of ovarian cancer. Sensitivity analysis was used to eliminate a large difference of the study. Q test and I^2 statistics were utilized to detect the heterogeneity of the included studies. $I^2 > 50\%$ or $P < .05$ for the Q test were considered to be statistically heterogeneous, the random-effects model was utilized. Meta-regression and subset analysis was used to analyze sources of heterogeneity. Otherwise, a fixed-effects model was utilized. Publication bias was evaluated using the funnel plot and Begg test.[15] All of the analyses were performed using STATA (version 12.0). P values were 2 sides $< .05$ was regarded as statistically significant.

3. Results

3.1. Characteristic of study

Our study included 2174 ovarian cancer patients in 13 studies published between 2004 and 2017.[12,16–27] Twelve studies reported data on methylation and ovarian cancer OS,

Table 1

The main features of enrolled studies.

Author	Year	Sample size	Population	Sample	Method	Gene	Type	Cut-off	Follow-up (month)
Strathdee	2005	41	England	tissue	Bisulfite sequencing	MCJ	Unknow	>90%	100
Liao	2014	168	China	tissue	QMSP	HIST1H2BN	Unknow	M-index>618	84
Ho	2012	47	China	tissue	MS-MLPA	HIN-1 CACNA1A	tumor suppressor gene tumor suppressor gene	>30%	median 56
Chiang	2013	136	China	tissue	MSP	BLU	tumor suppressor gene	—	34 (1 -193)
Iramaneerat	2011	29	Thailand	tissue	COBRA	HERV-K	Unknow	mean (>51.1%)	60
Zhou	2014	102	China	tissue	QMSP	OPCML	tumor suppressor gene	Unreported	47 (6–60)
Montavon	2012	80	Australia	tissue	MSP	DLEC1	tumor suppressor gene	—	150
Ding	2015	112	China	tissue	MSP	FANCF	Unknow	—	60
Gifford	2004	138	England	plasma	MSP	hMLH1	tumor suppressor gene	—	36
Beeghly	2007	215	America	tissue	MSP	IGF-II P2	Unknow	—	median 31.1 (0.6–114.1)
Phelps	2017	47	England	tissue	Bisulfite sequencing	MYLK3	Unknow	>20%	median 52.4
Ignatov	2014	179	Germany	tissue	MSP	BRCA1	tumor suppressor gene	—	median 21.6 (1.3–90.5) for group I and 14.5 (2.5–62.8) for group II
Flanagan	2013	880	England	plasma	Bisulfite sequencing	SFN	oncogene	mean	mean 18

'—' = Gene methylation when the sample showed positive result in the primers used for methylated gene promoter and negative in the primers used for unmethylated gene, COBRA = quantitative combined bisulfite restriction analysis, MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification; MSP = methylation-specific polymerase chain reaction, QMSP = quantitative methylation-specific PCR.

while 5 studies reported data on methylation and ovarian cancer PFS. The literature retrieval flow chart was shown in Figure 1.

The study of gene methylation involves many genes with different detection methods and cut-off values. Six studies used methylation-specific polymerase chain reaction (MSP) to detect gene methylation levels, and 3 studies used Bisulfite pyrosequencing. Eleven studies reported methylation levels in ovarian cancer tissues, and 2 studies reported methylation levels in plasma. Seven studies reported the genes were tumor suppressor gene, 1 study reported the gene was oncogene. The main features of studies are shown in Tables 1 and 2.

3.2. Meta-analysis of target genes methylation and OS/PFS

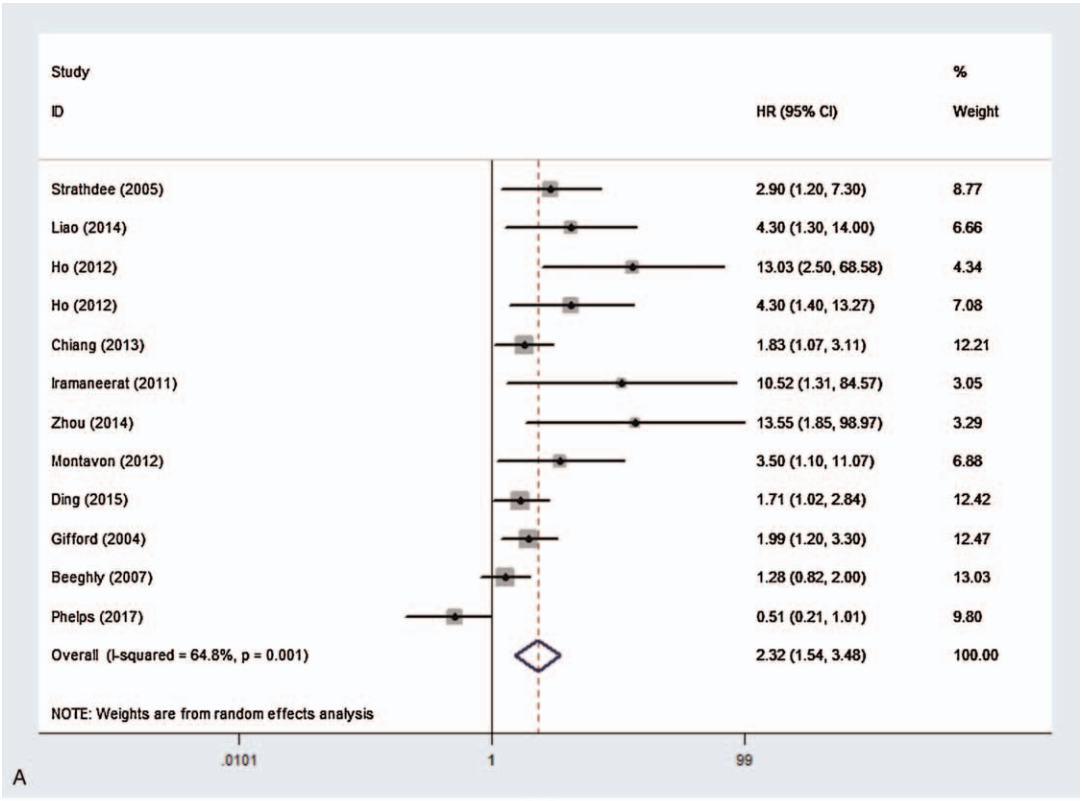
Due to heterogeneity (OS: $I^2 = 64.8\%$, $P = .001$; PFS: $I^2 = 79.4\%$, $P = .001$), the random model was used in our meta-analysis. Target genes hypermethylation indicates a poor overall survival in ovarian cancer patients (HR = 2.32, 95% CI: 1.54–3.48, $P = .000$), (forest map is shown in Fig. 2A). Target genes of hypermethylation and PFS were not statistically significant (HR = 1.318, 95% CI: 0.848–2.050, $P = .220$), (forest map is shown in Fig. 2B). Due to the different biological functions of oncogenes and tumor suppressor genes (TSGs), we conducted a meta-analysis of tumor suppressor genes alone. The result

Table 2

HRs for target genes methylation.

		Sample size		OS		PFS		
Study	Gene	High leve/ Methylated	Low level/ Unmethylated	HR (95% CI)	*P*	HR (95% CI)	*P*	Mthylation level associates with poor prognosis
Strathdee, 2005	MCJ	7	34	2.9 (1.2–7.3)	.023	—	—	Hypermethylation
Liao, 2014	HIST1H2BN	27	141	4.3 (1.3–14)	$P < .05$	4.5 (1.4–14.8)	$P < .05$	Hypomethylation
Ho, 2012	HIN-1	19	28	13.03 (2.5–68.58)	.002	—	—	Hypermethylation
Ho, 2012	CACNA1A	20	27	4.3 (1.4–13.27)	.02	—	——	Hypermethylation
Chiang, 2013	BLU	38	98	1.83 (1.07–3.11)	$P < .001$	1.48 (1.01–2.56)	$P < .001$	Hypermethylation
Iramaneerat, 2011	HERV-K	14	15	10.525 (1.31–84.57)	.027	—	—	Hypomethylation
Zhou, 2014	OPCML	80	22	13.55 (1.85–98.97)	.01	—	—	Hypermethylation
Montavon, 2012	DLEC1	6	73	3.5 (1.1–11.07)	.033	—	—	Hypermethylation
Ding, 2015	FANCF	36	76	1.706 (1.02–2.838)	.04	—	—	Hypermethylation
Gifford, 2004	hMLH1	34	104	1.99 (1.2–3.3)	.007	—	—	Hypermethylation
Beeghly, 2007	IGF-II P2	136	79	1.28 (0.82–2)	.116	1.73 (1.09–2.74)	.008	Hypermethylation
Phelps, 2017	MYLK3	41	6	0.51 (0.21–1.01)	.053	—	—	Hypomethylation
Ignatov, 2014	BRCA1	61	118	—	—	0.52 (0.32–0.85)	.009	Hypomethylation
Flanagan, 2013	SFN	534	346	—	—	1.3 (1.1–1.6)	.04	Hypomethylation

95% CI = 95% confidence interval.



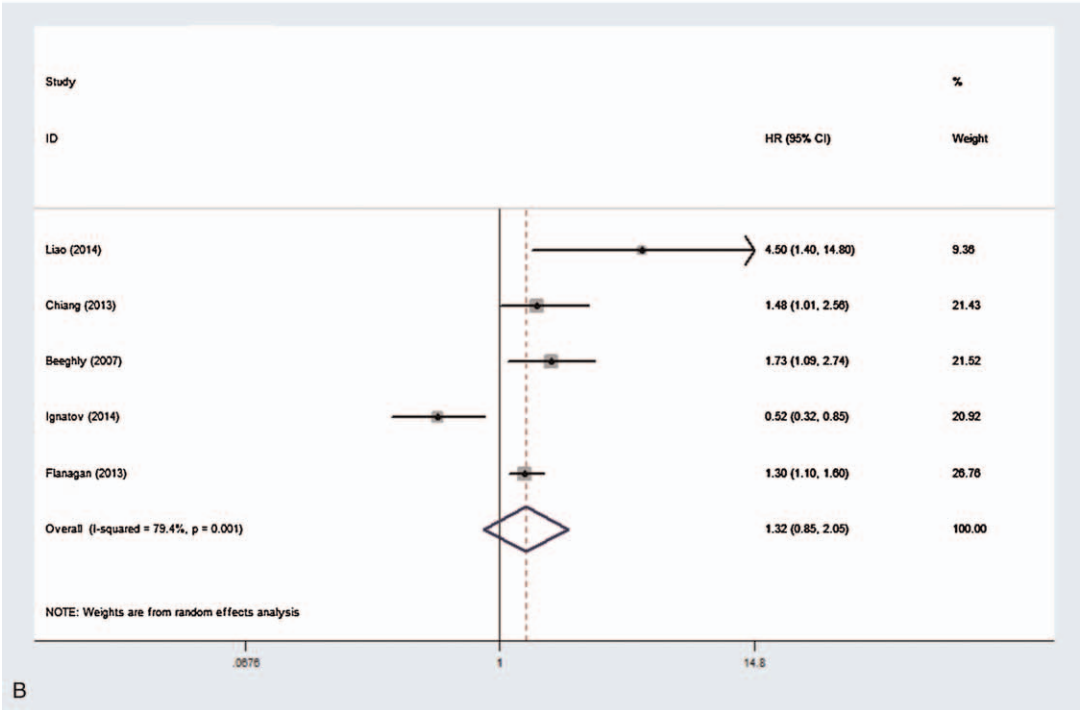


Figure 2. A. Forest plots of the correlation between gene methylation and OS in ovarian cancer patient. B. Forest plot of the correlation between gene methylation and PFS in ovarian cancer patient. C. Forest plot of the correlation between tumor suppressor genes methylation and OS in ovarian cancer patient. D. Subgroup analysis. OS = overall survival, PFS = progression-free survival.

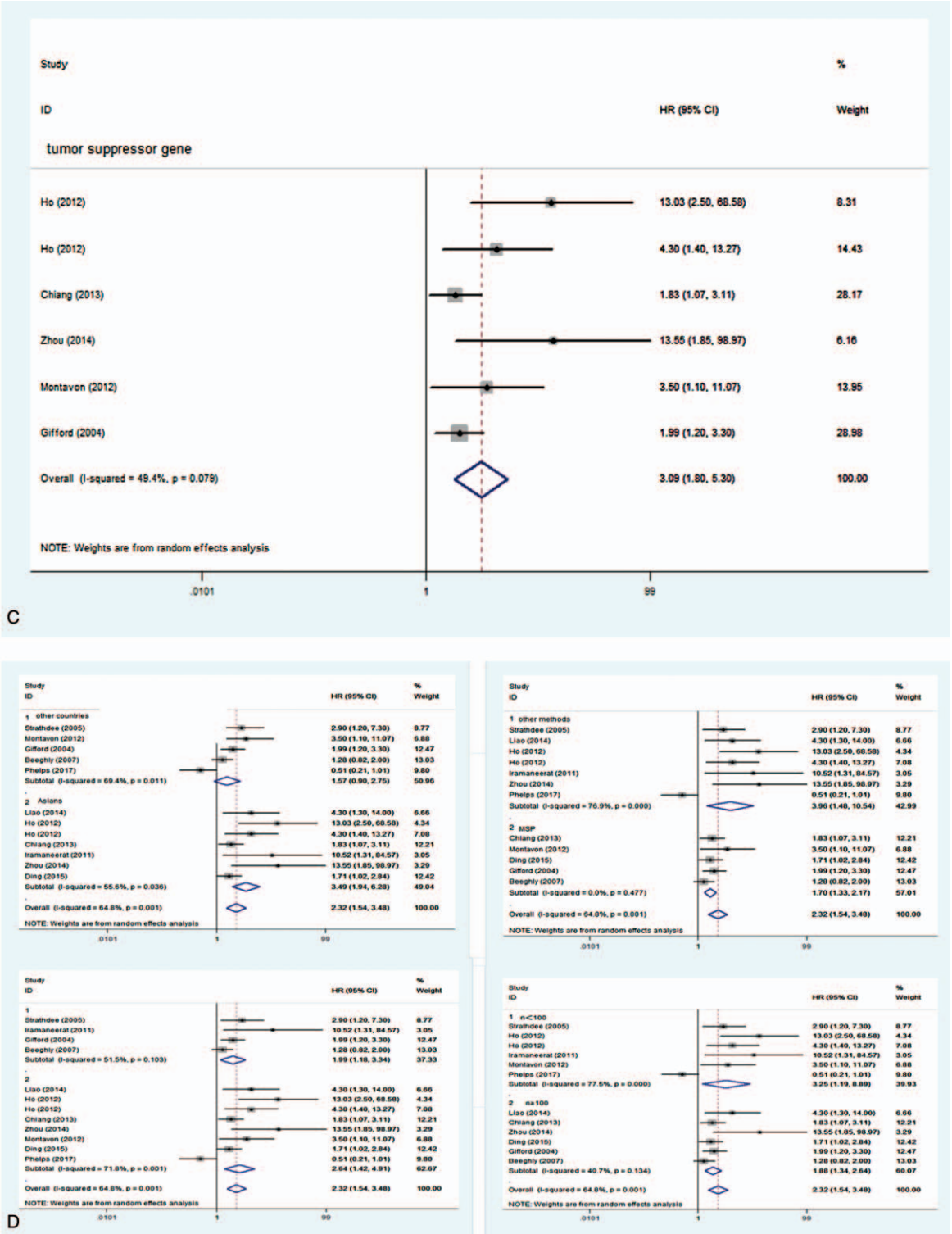


Figure 2. Continued

indicates that tumor suppressor genes hypermethylation indicates a poor overall survival in ovarian cancer patients (HR = 3.09, 95% CI 1.80–5.30, $P = .000$) (forest map is shown in Fig. 2C) and no heterogeneity was found in this meta-analysis (OS: $I^2 = 49.4\%$, $P = .079$). Due to the small size of the studies on oncogenes, this study does not perform the meta-analysis.

3.3. Heterogeneity source analysis

We used meta-regression and subset analysis to explore heterogeneity sources in the study. We conducted a multiple regression model with 7 variables (Country, Sample Type, Method, Methylation level, Gene type, Year, and Sample size) on OS, But the results show that these variables were not the source

Table 3

Results of meta-regression on OS, BS method: REML.

Variables	Coefficient	Standard error	t	*P* value	95%CI
Year	−0.5023824	0.7885683	−0.64	.559	−2.691799, 1.687034
Sample size	−0.5118892	0.8569071	−0.60	.582	−2.891045, 1.867266
Country	1.371694	0.8365332	1.64	.176	−0.950895, 3.694282
Sample type	−0.5483989	1.066723	−0.51	.634	−3.510097, 2.413299
Method	0.6969891	0.9180515	0.76	.490	−1.85193, 3.245909
Mthylation level	−0.5571884	0.9171108	−0.61	.576	−3.103496, 1.989119
Gene type	−0.287298	0.5306622	−0.54	.617	−1.760653, 1.186056

of heterogeneity (shown in Table 3, BS method: REML). Due to the small size of the studies on PFS, this study did not perform the meta-regression analysis. We performed a subset analysis to further analyze the sources of heterogeneity according to country (Asians and other countries), method (MSP and other methods), year (before 2010 and after 2010) and n ($n < 100$ and $n \geqq 100$). No heterogeneity exists in MSP subset in subgroup analysis, all other subgroups had heterogeneity and were calculated using a random-effects model ($I^2 = 0.0\%$, $P = .477$ in MSP subgroup). The HR of the target genes hypermethylation and OS in Asian population was 3.49 (95% CI = 1.94–6.28, $P = .000$) and 1.57 (95% CI = 0.90–2.75, $P = .112$) in people of other countries. The HR of the target genes methylation and OS in MSP subgroup was 1.70 (95% CI = 1.33–2.17, $P = .000$) and 3.96 (95% CI = 1.48–10.54, $P = .006$) in other methods subgroup. The HR of the target genes methylation and OS in before 2010 subgroup was 1.99 (95% CI = 1.18–3.34, $P = .009$) and 2.64 (95% CI = 1.42–4.91, $P = .002$) in after 2010 subgroup. The HR of the target genes methylation and OS in $n < 100$ subgroup was 3.25 (95% CI = 1.19–8.89, $P = .021$) and 1.88 (95% CI = 1.34–2.64, $P = .000$) in $n \geqq 100$ subgroup. Tumor suppressor genes studies did not perform the meta-regression because there was no heterogeneity and insufficient observations.

3.4. Publication bias and sensitivity analysis

The publication bias was detected by funnel plot and Begg test (shown in Fig. 3), the results show that the funnel plot was asymmetrical and the Begg test $P = .003$ ($< .05$), showing that all target genes had publication bias in meta-analysis of OS. But no publication bias was found for the tumor suppressor genes studies used for the meta-analysis for overall survival (Begg test, $P = .133$). Sensitivity analysis was performed on a case-by-case basis for all included studies (shown in Fig. 4). The result indicates that there was no obvious influence of every individual study on the pooled HR. Publication bias and sensitivity analysis were not performed for this study due to the small size of the studies on PFS.

4. Discussion

Since genetic factors cannot be reversed, the potential reversibility of epigenetic mechanisms makes them attractive candidates for the prevention and treatment of ovarian carcinoma. Increasing evidence has shown that epigenetic alterations including DNA methylation play a significant role in cancer, from the silencing of tumor suppressors to the activation of oncogenes and the promotion of metastasis.[16] The majority of studies assessing the

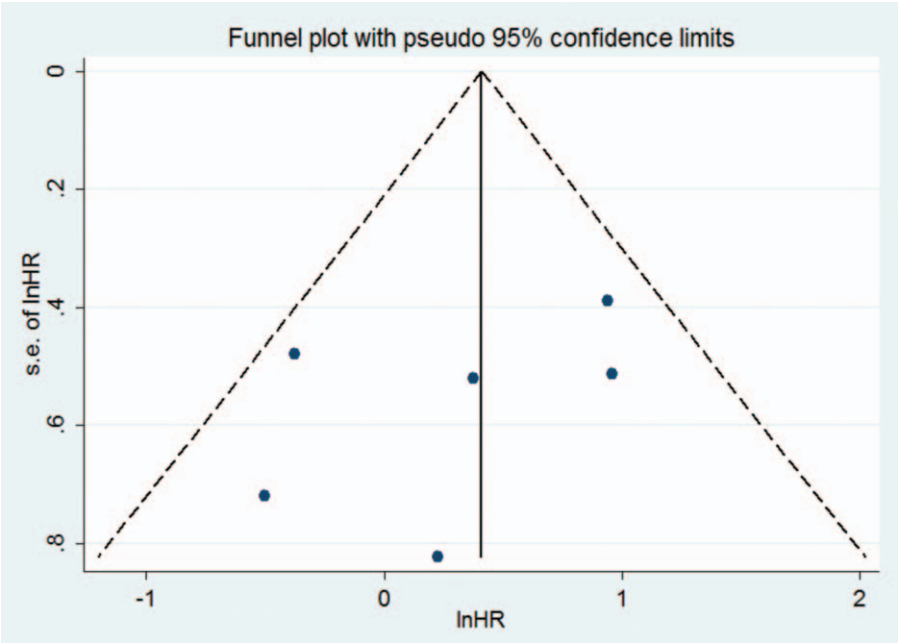


Figure 3. Funnel plot of tumor suppressor genes included in this meta-analysis for OS. OS = overall survival.

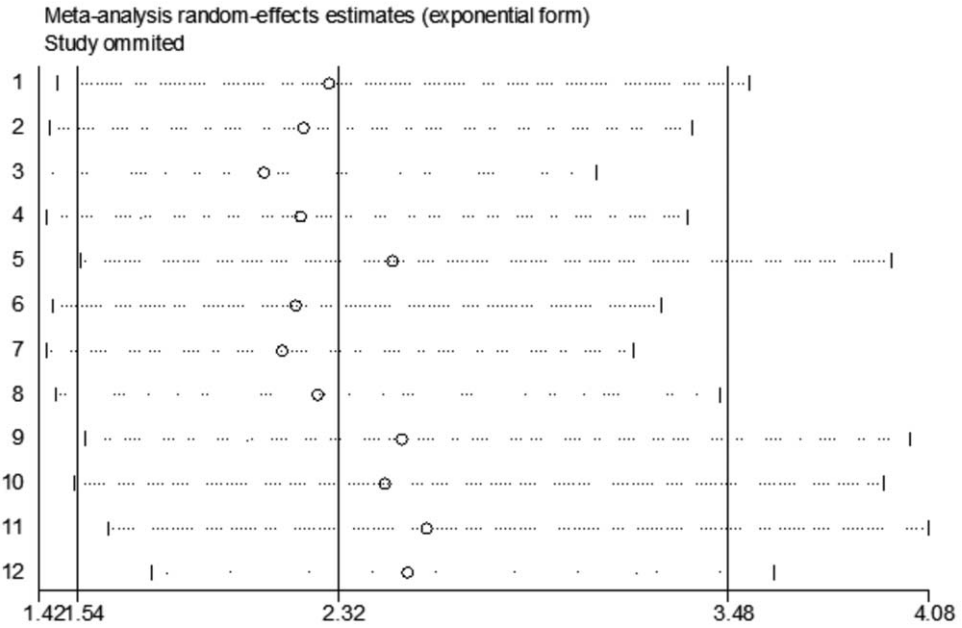


Figure 4. Sensitivity analysis of 12 studies included in this meta-analysis for OS. OS = overall survival.

methylation status of TSGs in ovarian cancer almost focused on a single gene. However, hypermethylation in ovarian cancer has been found to be associated with the inactivation of almost every pathway including DNA repair, cell cycle regulation, apoptosis, cell adherence, and detoxification pathways.[28,29]

Our meta-analysis assessed the role of target genes methylation as a prognostic factor in ovarian cancer. The result indicates that tumor suppressor genes hypermethylation indicates a poor overall survival in ovarian cancer patients (HR = 3.09, 95% CI 1.80–5.30), it suggests that tumor suppressor genes hypermethylation might be promising markers for predicting the survival rate of ovarian cancer. In this meta-analysis, no publication bias was found for the tumor suppressor genes studies on overall survival (Begg test, $P = .133$). This result provides a new idea for finding a combined gene model for prognostic factors in ovarian cancer. For 12 studies which report genes methylation as a prognostic factor of OS, a multiple regression found no source of significant heterogeneity. Subgroup analysis showed that the HR value of Asian population subgroup (HR = 3.49) was higher than that in people of other countries subgroup (HR = 1.57), suggesting that target genes methylation status as prognostic factor in ovarian cancer for Asian population is more valuable. In addition, methylation sequencing results have huge variation even coming from the same sources. Subgroup analysis of methods showed this difference. This has caused that even for the same gene, literature reported different levels of methylation with poor prognosis in ovarian cancer. Different methylation detection methods which in determining a site are high or low methylation have no standardized reference value and repetition rate was low. It needs a further study on how

Table 4

Comprehensive analysis of the correlation between fourteen methylated genes and ovarian cancer multidrug resistance.

Gene	Methylation status in drug-resistant tissue/cell	Expression of gene	Drugs	Regulation manner of drug resistance	Refs.
MCJ	Hypermethylation	Silenced expression	Cisplatin,paclitaxel	Drug delivery system,regulator of mitochondrial respiration	[23,30,31]
HIST1H2BN	Hypomethylation	–	Cisplatin	Structural unit of chromosome	[21]
HIN-1	Hypermethylation	Downregulation	Paclitaxel,cisplatin	Cell growth, apoptosis, AKT signalling pathway	[17]
CACNA1A	Hypermethylation	–	–	–	[17]
BLU	Hypermethylation	Downregulation	Paclitaxel	Apoptosis,colony formation	[16]
HERV-K	Hypomethylation	Upregulation	–	–	[22]
OPCML	Hypermethylation	Downregulation	–	Cell growth,cell adhesion,migration, receptor tyrosine kinases (RTKs)	[19,28,32–33]
DLEC1	Hypermethylation	Downregulation	–	Cell proliferation,colony formation	[12]
FANCF	Hypermethylation	Upregulation	Alkylating agent,cisplatin	Cell cycle,migration,DNA mismatch repair,apoptosis, FA/BRCA pathway	[18,29,34–37]
hMLH1	Hypermethylation	Downregulation	Carboplatin,cisplatin,taxoid	DNA mismatch repair, microsatellite instability , apoptosis	[25]
IGF-II P2	Hypermethylation	–	Fluorouracil and cisplatin	Cell proliferation,apoptosis. AKT signalling pathway	[27,38–39]
MYLK3	Hypomethylation	–	–	Cell migration and invasion,apoptosis,immune response signalling pathway	[26,40–41]
BRCA1	Hypomethylation	Upregulation	Cisplatin	DNA mismatch repair	[20,42–43]
SFN	Hypomethylation	–	–	Cell cycle, DNA damage repair	[24]

Table 5

Search details.

Electronic databases	PubMed, Embase
Search terms # 1	ovarian neoplasm Or ovary neoplasm Or ovary cancer Or ovarian cancer
Search terms # 2	DNA methylation Or methylation
Search terms # 3	prognosis Or prognostic factor
Search terms # 4	Search terms # 1 AND Search terms # 2 AND Search terms # 3

to find stable and reliable markers from these tags. In the future, more standardized standards and testing methods will be needed for the detection of methylation. There were some limitations to our study. Firstly, included studies only included published in English and Chinese, ignoring the published studies in other languages. Secondly, there was some heterogeneity in the included literature. Although meta regression did not find the source of heterogeneity, subset analysis could explain some of the sources of heterogeneity. Thirdly, due to the lack of literature reports, more studies are necessary to confirm the conclusions of PFS in our meta-analysis.

In summary, although there are some defects in this study, the following conclusions can be drawn: tumor suppressor genes promoter hypermethylation indicates a poor overall survival in ovarian cancer patients. Tumor suppressor genes hypermethylation is an effective biomarker for predicting the prognosis of ovarian cancer. At the same time, we consider that gene methylation levels exert biological functions by regulating gene expression.

Chemotherapy resistance is one of the causes of poor prognosis in patients with ovarian cancer. Studies have shown that hypomethylating agents can reverse the sensitivity of ovarian cancer patients to chemotherapy. So what is the mechanism of these genes participates in drug resistance affecting prognosis? To explore this mechanism, we summarized the biological mechanism of the target genes for chemotherapy resistance in our study (Shown in Table 4). Restoration of the function of these methylation genes would be an important step to develop new treatment strategies for ovarian cancer patients with genes hypermethylation.

Author contributions

Data curation: Li-yuan Feng, Chang-xian Chen.
Writing – original draft: Li-yuan Feng.
Writing – review & editing: Li Li.
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