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MGMT gene promoter methylation in humoral tissue as biomarker for lung cancer diagnosis: An update meta-analysis

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Abstract

Objective: To investigate *O*-6-methylguanine-DNA methyltransferase (MGMT) gene promoter methylation in humoral tissue as biomarker for lung cancer diagnosis by pooling relevant open published data.

Methods: Clinical studies relevant to MGMT gene promoter methylation and lung cancer were systematic electronic searched in the databases of Medline, EMBASE, Ovid, Web of Science, and CNKI. Data of true positive (tp), false positive (fp), false negative (fn), and true negative (tn) were extracted from the included studies and made combination. The diagnostic sensitivity, specificity, diagnostic odds ratio (DOR) and summary receiver operating characteristic (SROC) of MGMT gene methylation for lung cancer diagnosis were pooled.

Results: Twelve studies were included in the meta-analysis. The diagnostic sensitivity, specificity, DOR were 0.39 (95% CI = 0.31–0.49) 0.92 (95% CI = 0.77–0.97), and 4.20 (95% CI = 2.09–8.44), respectively under random effect model. The SROC of MGMT gene methylation for lung cancer diagnosis was 0.58 (95% CI = 0.53–0.62).

Conclusion: MGMT methylation rate was higher in plasma and bronchoalveolar lavage fluid (BLAF) of lung cancer cases compared to controls. High diagnostic specificity indicated that MGMT methylation in plasma and BLAF can be applied as lung cancer confirmation test.

KEYWORDS
biomarker, lung cancer diagnosis, meta-analysis, MGMT gene, promoter methylation

INTRODUCTION

Lung cancer including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) is the most common malignant tumor and leading cause of cancer related death.[1] Epidemiology study indicated that 235 760 new cases of lung cancer and 131 880 deaths will be identified in the year 2021 in the United States (US).[2] With the development of radiation technology and target therapy, the general prognosis of lung cancer was improved. However, the overall long-term survival of lung cancer was still unsatisfied because of advanced stages when first diagnosis. How to identify the lung cancer cases at early state is a key for improving the general prognosis of the disease.[3]

In recent years, the research progress of epigenetics has far reaching significance for the early diagnosis and treatment of lung cancer.[4–6] CpG island methylation in the promoter region of tumor suppressor genes is an important mechanism of gene silence.[7–9] Transcriptional inactivation of different genes will have different consequences, such as affecting cell cycle, DNA repair, carcinogen metabolism, cell adhesion, apoptosis, etc.[10] In multiple human carcinomas, the CpG island in the promoter region of tumor suppressor genes showed different degrees of methylation, resulting in transcriptional silencing. It was reported that CpG island methylation in gene promoter region was a common phenomenon in NSCLC patients and an early events.[11]

Bizheng Chen and Xiaozhen Ying contribute equally to this work.

Therefore, DNA methylation may be a potential biomarker for lung cancer diagnosis or even screening.

O-6-methylguanine-DNA methyltransferase (MGMT) gene encodes DNA repair protein that is involved in cellular defense against mutagenesis and toxicity from alkylating agents.[12] The protein catalyzes transfer of methyl groups from *O*(6)-alkylguanine and other methylated moieties of the DNA to its own molecule, which repairs the toxic lesions. Methylation of the genes promoter has been associated with several cancer types, including colorectal cancer,[13] lung cancer, lymphoma, and glioblastoma.[14] MGMT promoter methylation was widely discussed and found methylated in cancer and humoral tissues of the lung cancer patients. However, its value as biomarker for lung cancer diagnosis was not clear yet.

MATERIAL AND METHODS

Studies searching in the electronic databases

Clinical studies relevant to MGMT gene promoter methylation and lung cancer were systematic electronic searched in the databases of Medline, EMBASE, Ovid, Web of Science, and CNKI. The publication electronic searching was limited to English and Chinese with the searching text word of "MGMT", "*O*-6-methylguanine-DNA methyltransferase", "non-small cell lung cancer", "lung cancer", "lung neoplasm. The references of included studies were also reviewed to identify the further suitable publications. The studies electronic searching process was performed by B.C. and X.Y. independently and cross checked.

Inclusion and exclusion criteria

The study inclusion was restricted to study design, patients, methylation detection methods, results, and language. For inclusion criteria, the publication limited to case–control, cohort, diagnostic, or observation study. The patients were restricted to lung cancer with pathology confirmation. The methylation detection methods were methylation specific PCR (MSP) or q-MSP. The result of the included studies should provide the distribution of true positive (tp), false positive (fp), false negative (fn), and true negative (tn). The publication languages were limited to English and Chinese.

For studies exclusion criteria: case report, literature review, or meta-analysis studies were excluded. Lung cancer patients without pathology or cytology validation were excluded. The studies did not provide enough data to calculate tp, fp, fn, and tn were also excluded.

Data extraction

Two reviewers (B.C. and L.B.) reviewed the included original studies and extracted the data independently. The extracted data includes the general information such as first author, country, year of publication, methylation detection methods, and humoral tissue type. The distribution of tp, fp, fn, and tn was directly extracted or calculated.

Statistical methods

All the data was managed by STATA12.0 statistical software. Before combination, the data was tested for statistical heterogeneity by χ^2 test and demonstrated by I^2 ($I^2 > 50\%$ indicated significant heterogeneity, otherwise without statistical heterogeneity). The publication bias was evaluated by Deeks' and Begg's funnel plot.

RESULTS

Studies included in the meta-analysis

A total of 165 clinical studies relevant to MGMT gene promoter methylation and lung cancer were initially identified in the electronic database and other source. After removing the unsuitable studies through reviewing the title, abstract, and full text, 12 studies were finally included for meta-analysis. The studies identification procedure was demonstrated in Figure 1, and the general characteristics of the included 12 studies were shown in Table 1.

Statistical heterogeneity

Significant statistical heterogeneity for MGMT gene promoter methylation in humoral tissue as biomarker for lung cancer diagnosis was identified in effect size of sensitivity ($I^2 = 81.03\%$, $p < 0.05$), specificity ($I^2 = 81.03\%$, $p < 0.05$) and diagnostic odds ratio (DOR) ($I^2 = 66.70\%$, $p < 0.05$). The statistical heterogeneity may originate from the clinical features of the included cases and controls of each included original study.

Meta-analysis

Because of significant statistical heterogeneity across the included 12 studies, the data was pooled in random effect model. The diagnostic sensitivity, specificity, DOR were 0.39 (95% CI = 0.31–0.49), (Figure 2) 0.92 (95% CI = 0.77–0.97), (Figure 2) and 4.20 (95% CI = 2.09–8.44), (Figure 3), respectively, under random effect model. The area under the summary receiver operating characteristic (SROC) of MGMT gene methylation for lung cancer diagnosis was 0.58 (95% CI = 0.53–0.62), (Figure 4).

Subgroup analysis

Subgroup analysis was performed according to the humoral tissue type of plasma and bronchoalveolar

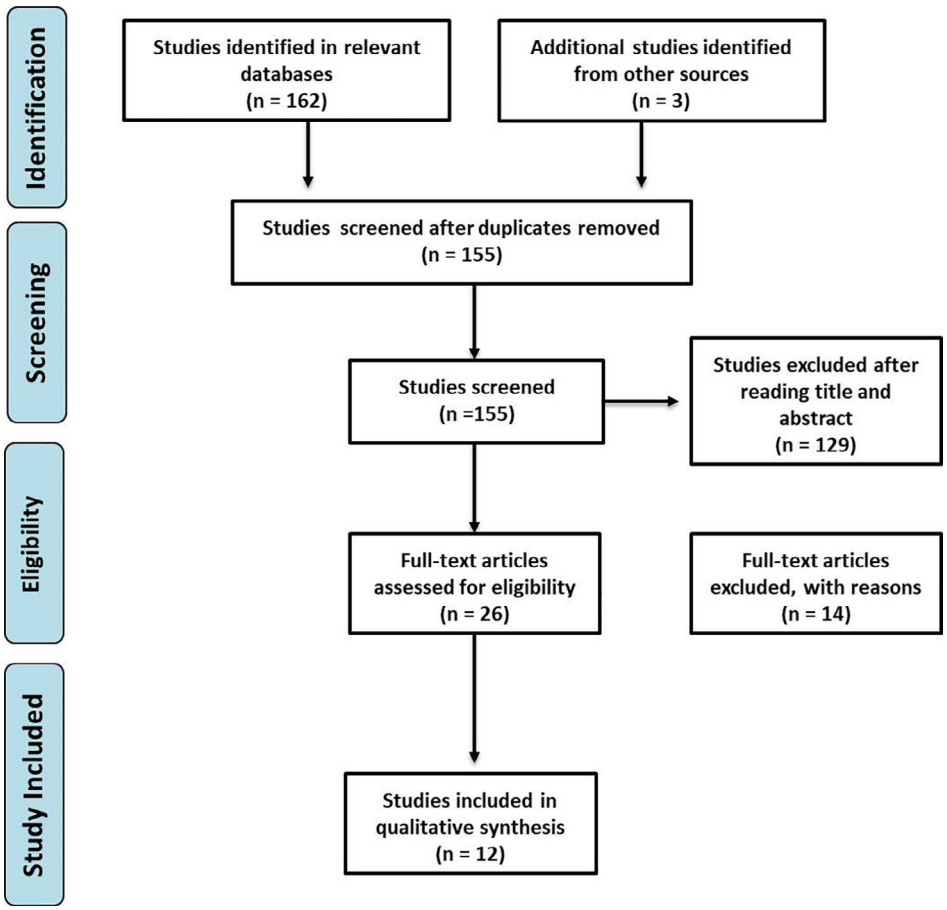


FIGURE 1 Flow chart of studies identification according to inclusion and exclusion criteria

TABLE 1 The general features of the included 12 studies

Author	Year	Sample size	Cancer (M+/M−)	Control (M+/M−)	TNM (I/II/III/IV)	Tissue	Country	Methods
Yao et al.[15]	2005	106	21/32	18/35	NA	Plasma	China	MSP
Kang et al.[16]	2011	106	19/34	15/38	NA	Plasma	China	MSP
Russo et al.[17]	2005	66	18/15	6/27	NA	Plasma	US	MSP
Topaloglu et al.[18]	2004	62	12/19	7/24	17/9/5/−	BLAF	US	MSP
Begum et al.[19]	2011	20	3/7	1/9	NA	Plasma	US	q-PCR
Guo et al.[20]	2004	40	14/6	11/9	NA	Plasma	China	MSP
Belinsky et al.[21]	2007	144	11/61	4/68	NA	Plasma	US	MSP
Wang and Song[22]	2020	100	51/34	1/14	NA	BLAF	China	MSP
Zhong and Fan[23]	2019	200	36/64	0/100	37/34/29/−	Plasma	China	MSP
Zhong and Li[24]	2015	60	15/15	7/23	5/13/9/3	Plasma	China	q-MSP
Zhao et al.[25]	2013	108	17/45	0/46	1/10/24/27	Plasma	China	MSP
Yan and Zhang[26]	2018	180	21/59	0/100	NA	Plasma	China	MSP

Abbreviation: BLAF, bronchoalveolar lavage fluid; M+, methylation positive; M-, methylation negative; MSP, methylation specific PCR; NA, not avaiable.

lavage fluid (BLAF). The diagnostic performance of plasma and BLAF for MGMT promoter methylation as biomarker for lung cancer diagnosis is demonstrated in Table 2.

Publication bias

The Begg's funnel plot was left–right asymmetry especially at bottom. However, the Deeks' funnel plot

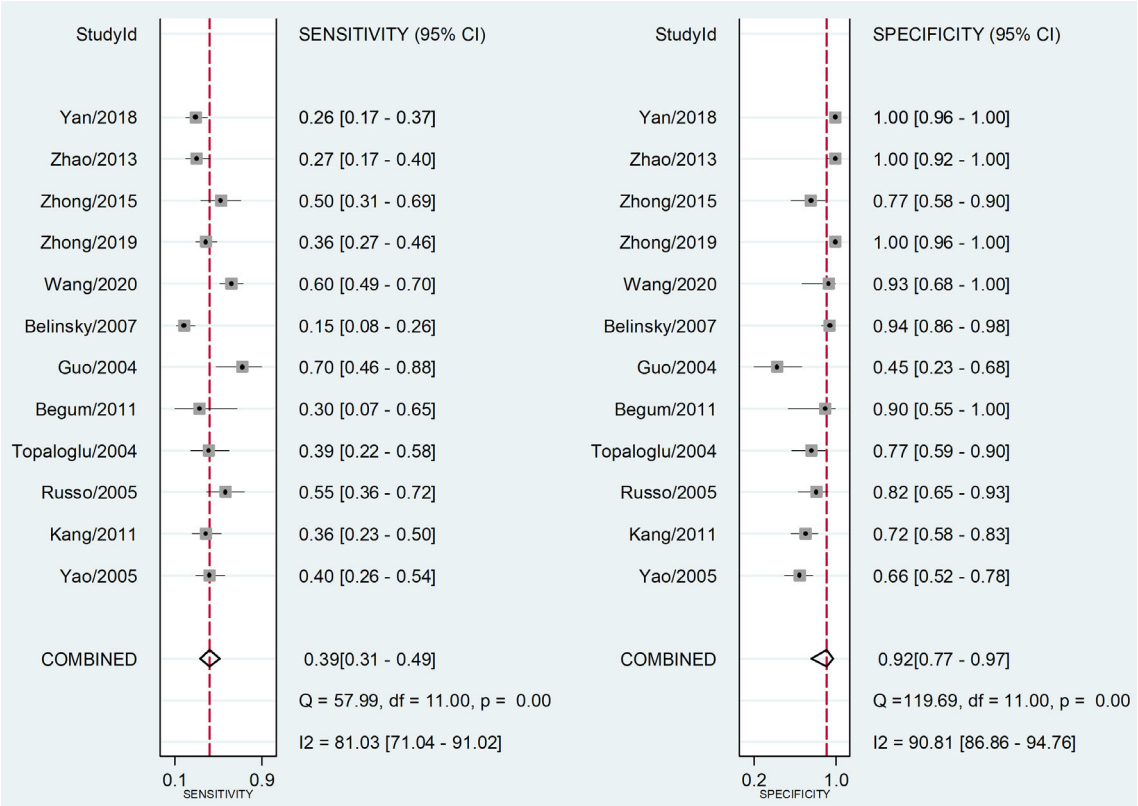
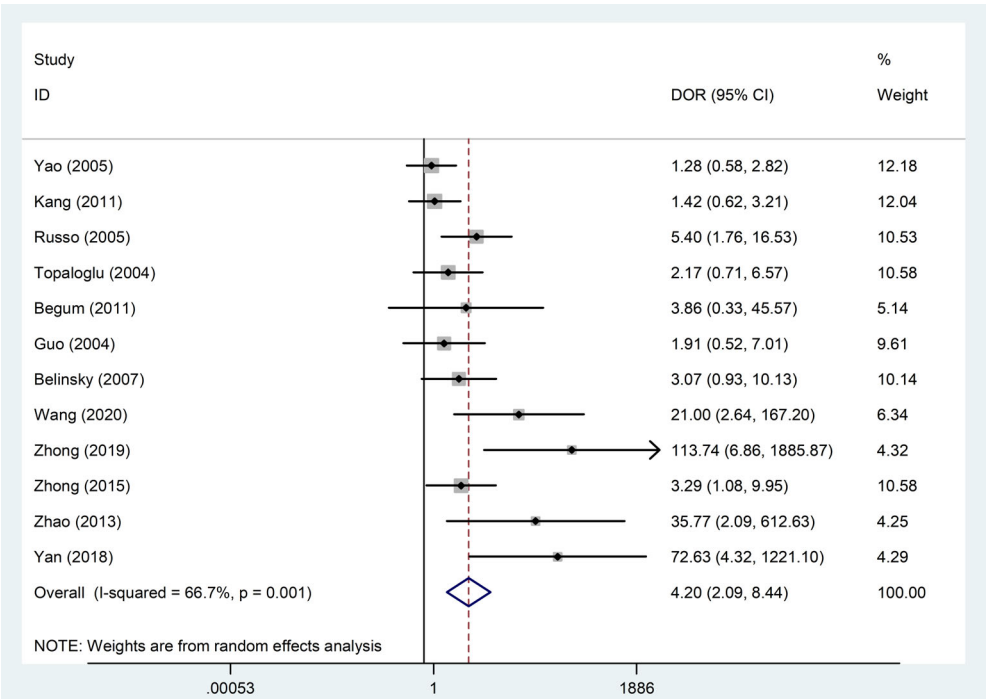


FIGURE 2 Forest plot of diagnostic sensitivity and specificity of MGMT gene methylation for lung cancer diagnosis

FIGURE 3 Forest plot of diagnostic odds ratio (DOR) of MGMT gene methylation for lung cancer diagnosis



did not demonstrated significant publication bias ($p = 0.09$) (Figure 5). We considered that the publication bias was existed in the present meta-analysis.

DISCUSSION

In the present meta-analysis, 12 clinical studies relevant to MGMT gene promoter methylation in plasma and BLAF

were included and made quantitative analysis. The results indicated that the MGMT methylation rate was higher in plasma and BLAF of lung cancer cases compared to controls. High diagnostic specificity indicated that MGMT methylation in plasma and BLAF can be used as lung cancer confirmation test. However, the diagnostic sensitivity of MGMT promoter methylation as reference for lung cancer is extremely low, which indicated it may not be suitable for lung cancer screening. Gu et al.[27] evaluated the association between MGMT promoter methylation and NSCLC by meta-analysis methods. In that study, the authors included 18 studies and compared the MGMT promoter methylation in cancer tissue and normal control tissue. The authors found that the MGMT promoter methylation rate in cancer tissue was significantly higher than that of the controls and a strong association between methylation of MGMT gene and NSCLC was identified. In the present meta-analysis, we also found that the MGMT methylation rate in plasma and BLAF was higher than controls and could be applied as a biomarker for lung cancer confirmations test.

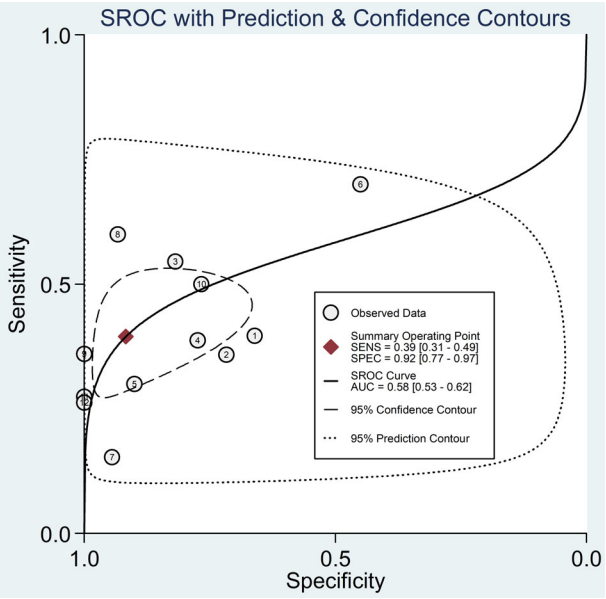


FIGURE 4 Summary receiver operating characteristic (SROC) of MGMT gene methylation for lung cancer diagnosis

Lung cancer is the most common malignant tumor. However, there were few effective and convenient methods for early diagnosis and screening of lung cancer.[28,29] Although histology and cytology are the gold standard for the diagnosis of lung cancer, patients are often of advanced stage at the time of diagnosis by pathology and cytology.[30] Therefore, new diagnostic methods to improve the early diagnosis rate and reduce the mortality are needed. Tumor suppressor gene methylation analysis in plasma and BALF was considered to be a promising diagnostic method with

TABLE 2 Subgroup analysis of MGMT gene methylation for lung cancer diagnosis

Group	SEN	SPE	DOR	AUC
Plasma	0.34(0.50–0.38)	0.88(0.85–0.91)	4.12(1.87–9.04)	0.56
BLAF	0.54(0.45–0.64)	0.83(0.69–0.92)	5.74(0.58–57.06)	–

Abbreviations: AUC, area under roc curve; DOR, diagnostic odds ratio; SEN, sensitivity; SPE, specificity.

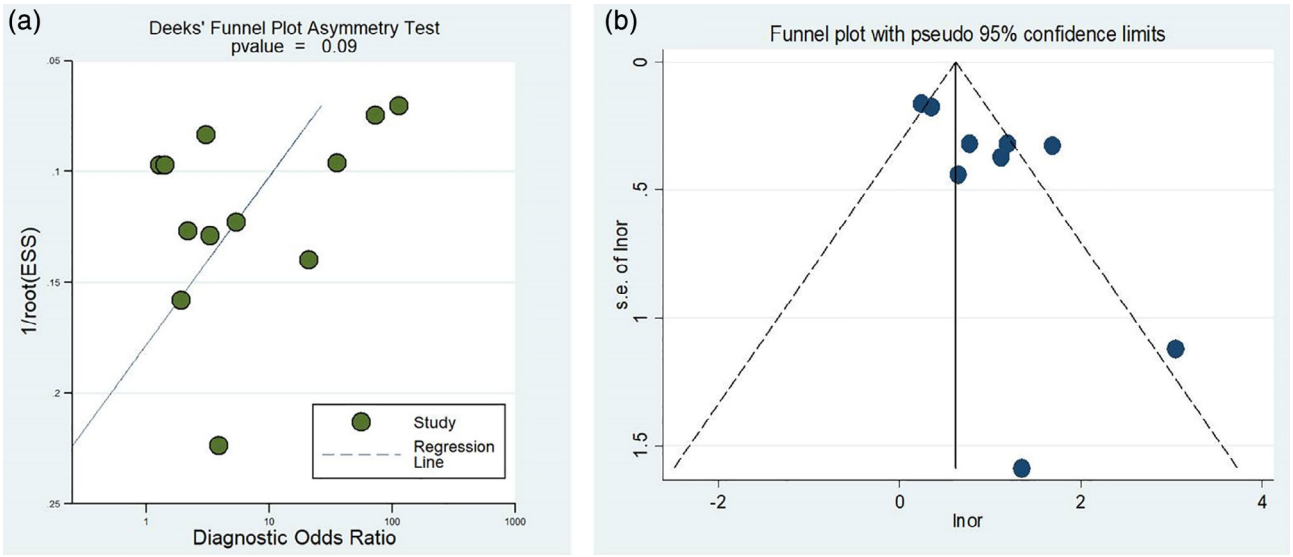


FIGURE 5 Deeks' and Begg's funnel plot in evaluation the publication bias of MGMT gene methylation for lung cancer diagnosis (a) Deeks' funnel plot; (b) Begg's funnel plot)

convenient clinical application and mini invasive.[31,32] Therefore, MGMT gene methylation detection combined with histology, cytology can improve the diagnostic accuracy of lung cancer.

In humans, MGMT gene was located in chromosome 10q26.3 and coded a protein that is involved in the cellular defense against the biological effects of *O*-6-methylguanine (O6-MeG) and *O*-4-methylthymine (O4-MeT) in DNA and repairs the methylated nucleobase in DNA by stoichiometrically transferring the methyl group to a cysteine residue in the enzyme. Previous studies have shown that,[33,34] the expression level of MGMT gene is downregulated in a variety of tumor tissues, suggesting that the inactivation of MGMT gene is related to the occurrence of a variety of tumors. Limitations of the present meta-analysis: (i) small sample size with only 12 original studies included in the meta-analysis; (ii) language restriction with only English and Chinese publications included; (iii) statistical heterogeneity across the included studies were existed; and (iv) potential publications bias was also identified in the preset work.

In conclusion, MGMT methylation rate was higher in plasma and BLAF of lung cancer cases compared to controls. High diagnostic specificity indication MGMT methylation in plasma and BLAF can be used as lung cancer confirmation test. However, because of the statistical heterogeneity and small sample size, the conclusion should be further validated by well-designed prospective diagnostic studies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Yang X, Yang L, Dai W, Ye B. Role of p14ARF and p15INK4B promoter methylation in patients with lung cancer: a systematic meta-analysis. Onco Targets Ther. 2016;9:6977–85.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71:7–33.
3. Senan S, Paul MA, Lagerwaard FJ. Treatment of early-stage lung cancer detected by screening: surgery or stereotactic ablative radiotherapy. Lancet Oncol. 2013;14:e270–4.
4. Chao YL, Pecot CV. Targeting epigenetics in lung cancer. Cold Spring Harb Perspect Med. 2021;11:a038000.
5. Shi YX, Sheng DQ, Cheng L, Song XY. Current landscape of epigenetics in lung cancer: focus on the mechanism and application. J Oncol. 2019;2019:8107318.
6. Quintanal-Villalonga Á, Molina-Pinelo S. Epigenetics of lung cancer: a translational perspective. Cell Oncol (Dordr). 2019;42:739–56.
7. Li D, Hu X, Yu S, Deng S, Yan M, Sun F, et al. Silence of lncRNA MIAT-mediated inhibition of DLG3 promoter methylation suppresses breast cancer progression via the hippo signaling pathway. Cell Signal. 2020;73:109697.
8. Nunna S, Reinhardt R, Ragozin S, Jeltsch A. Targeted methylation of the epithelial cell adhesion molecule (EpCAM) promoter to silence its expression in ovarian cancer cells. PLoS One. 2014;9:e87703.
9. Gong Y, Guo MZ, Ye ZJ, Zhang XL, Zhao YL, Yang YS. Silence of HIN-1 expression through methylation of its gene promoter in gastric cancer. World J Gastroenterol. 2011;17:526–33.
10. Dos Santos P, Machado A, De Grandis RA, Ribeiro DL, Tuttis K, Morselli M, et al. Transcriptome and DNA methylation changes modulated by sulforaphane induce cell cycle arrest, apoptosis, DNA damage, and suppression of proliferation in human liver cancer cells. Food Chem Toxicol. 2020;136:111047.
11. Rauscher GH, Kresovich JK, Poulin M, Yan L, Macias V, Mahmoud AM, et al. Exploring DNA methylation changes in promoter, intragenic, and intergenic regions as early and late events in breast cancer formation. BMC Cancer. 2015;15:816.
12. Ali-Osman F, Srivenugopal K, Sawaya R. The DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med. 2001;344:687 author reply 687-8.
13. Shalaby SM, El-Shal AS, Abdelaziz LA, Abd-Elbary E, Khairy MM. Promoter methylation and expression of DNA repair genes MGMT and ERCC1 in tissue and blood of rectal cancer patients. Gene. 2018;644:66–73.
14. Inoue R, Isono M, Abe M, Abe T, Kobayashi H. A genotype of the polymorphic DNA repair gene MGMT is associated with de novo glioblastoma. Neurol Res. 2003;25:875–9.
15. Yao QF, Zhang LP, Ning Y, Zeng W, Zhou YK. Detection of gene aberrant methylation in patients with lung cancer by nested methylation specific PCR. Chinese J Clin Oncol. 2005;32:894–5.
16. Kang CY, Zhou HC, Tang SP, Xiao H. Values of promoter hypermethylation of FHIT, p16, MGMT and RASSF1A genes in plasma in the diagnosis of lung cancer. Tumor. 2011;31:729–34.
17. Russo AL, Thiagalingam A, Pan H, Califano J, Cheng KH, Ponte JF, 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.
18. Topaloglu O, Hoque MO, Tokumaru Y, Lee J, Ratovitski E, Sidransky D, et al. Detection of promoter hypermethylation of multiple genes in the tumor and bronchoalveolar lavage of patients with lung cancer. Clin Cancer Res. 2004;10:2284–8.
19. Begum S, Brait M, Dasgupta S, Ostrow KL, Zahurak M, Carvalho AL, et al. An epigenetic marker panel for detection of lung cancer using cell-free serum DNA. Clin Cancer Res. 2011;17:4494–503.
20. Guo M, House MG, Hooker C, Han Y, Heath E, Gabrielson E, et al. Promoter hypermethylation of resected bronchial margins: a field defect of changes. Clin Cancer Res. 2004;10:5131–6.
21. Belinsky SA, Grimes MJ, Casas E, Stidley CA, Franklin WA, Bocklage TJ, et al. Predicting gene promoter methylation in non-small-cell lung cancer by evaluating sputum and serum. Br J Cancer. 2007;96:1278–83.
22. Wang YH, Song YC. The value of combined detection p16,MGMT, FHIT and APC gene methylation in alveolar lavage fluid of patients with pulmonary nodules for lung carcinoma early screening. Zhejiang Med Educ. 2020;19:57–60.
23. Zhong YH, Fan MJ. The role of methylation of tumor suppressor genes MGMT, p16, CDH13 and RASSF1 in the diagnosis of lung cancer. J Southeast Univ (Medical Science Edition). 2019;38:333–7.
24. Zhong YH, Li Y. Effect of modified multiplex real-time fluorescent quantitative PCR for determination of methylation of tumor suppressor genes MGMT, p16, CDH13 and RASSF1A in early diagnosis of lung cancer. J Southeast Univ (Medical Science Edition). 2019;38:33–8.
25. Zhao PF, Lu DX, Ji XQ, Zhao Q, Liu ZZ, Sun LH. Joint detection and significance of non-small cell lung cancer serum MGMT, RASSF2, RUNX3 and RASSF1A gene promoter CpG Island methylation. China Med Pharm. 2013;3:20–3. +47.
26. Yan Q, Zhang J. Combined detection of CpG Island methylation of 8 tumor suppressor gene promoters in serum and its significance in

non-small cell lung cancer. Chinese J Cancer Prevent Treat. 2018;25: 8–10.
27. Gu C, Lu J, Cui T, Lu C, Shi H, Xu W, et al. Association between MGMT promoter methylation and non-small cell lung cancer: a meta-analysis. PLoS One. 2013;8:e72633.
28. Yang J, Xu R, Wang C, Qiu J, Ren B, You L. Early screening and diagnosis strategies of pancreatic cancer: a comprehensive review. Cancer Commun (Lond). 2021. https://doi.org/10.1002/cac2.12204
29. Cainap C, Pop LA, Balacescu O, Cainap SS. Early diagnosis and screening in lung cancer. Am J Cancer Res. 2020;10:1993–2009.
30. Rosenthal SA, Curran WJ Jr. The significance of histology in non-small cell lung cancer. Cancer Treat Rev. 1990;17:409–25.
31. Liu B, Ricarte Filho J, Mallisetty A, Villani C, Kottorou A, Rodgers K, et al. Detection of promoter DNA methylation in urine and plasma aids the detection of non-small cell lung cancer. Clin Cancer Res. 2020;26:4339–48.
32. Belinsky SA, Leng S, Wu G, Thomas CL, Picchi MA, Lee SJ, et al. Gene methylation biomarkers in sputum and plasma as predictors for lung cancer recurrence. Cancer Prev Res (Phila). 2017;10:635–40.
33. Michailidi C, Theocharis S, Tsourouflis G, Pletsa V, Kouraklis G, Patsouris E, et al. Expression and promoter methylation status of hMLH1, MGMT, APC, and CDH1 genes in patients with colon adenocarcinoma. Exp Biol Med (Maywood). 2015;240:1599–605.
34. Oliver JA, Ortiz R, Melguizo C, Alvarez PJ, Gómez-Millán J, Prados J. Prognostic impact of MGMT promoter methylation and MGMT and CD133 expression in colorectal adenocarcinoma. BMC Cancer. 2014; 14:511.

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