

REVIEW ARTICLE

The function of DNA methylation in lung cancer: Diagnostic and clinical implications

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Abstract

Currently, the detection of lung cancer depends on chest X-ray, low-dose computed tomography (CT) scans, and tissue biopsy. However, harmful radiation from X-rays and CT scans may increase cancer risk. In contrast, biopsy of the lungs is highly laborious and painful. These diagnostic limitations contribute to delayed diagnosis, and treatment is therefore less effective at advanced stages. DNA methylation is a mechanism in which DNA methyltransferases add a methyl group to the cytosine-phosphate-guanine islands of a gene, which can either enhance or suppress its expression. Previous research has shown that promoter methylation of proto-oncogenes and tumor suppressor genes begins at the early stages of tumorigenesis and can be detected in cell-free DNA derived from blood plasma. With this primary objective, we highlight DNA hypomethylation in proto-oncogenes and hypermethylation in tumor suppressor genes in lung cancer patients, which may serve as therapeutic targets for the development of treatments that may increase the survival rate of lung cancer patients.

Keywords: Methylation; Methyltransferase; Cell-free DNA; Biopsy; Early-stage detection

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Citation: Yadav DR, Kumar S, Chaubey S, Tiwari M, Chipoya PM. The function of DNA methylation in lung cancer: Diagnostic and clinical implications. *Innov Med Omics*. 2026;3(3):025520079. doi: 10.36922/IMO025520079

Received: December 25, 2025

Revised: January 24, 2026

Accepted: February 2, 2026

Published online: March 11, 2026

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1. Introduction

Lung cancer is a leading cause of mortality, with a five-year survival rate of merely 15%. It is primarily categorized into two histological types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is further categorized into three subtypes: adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma. Genetic and epigenetic modifications contribute to the formation of lung cancer, such as SCLC, which is associated with the suppression of tumor protein P53 (*TP53*) and the retinoblastoma (*RB1*).¹ *TP53* and *CDKN2A* are concurrently inactivated in LUSC, whereas *KRAS*, *EGFR*, *TP53*, *KEAP1*, *STK11*, and *NF1* are commonly mutated in LUAD. Epigenetic alterations identified in lung cancer include methylation of DNA, histone modification, and aberrant production of non-coding RNA. DNA methylation,

as an epigenetic factor, modifies gene expression by altering chromatin shape and influencing transcription factor binding. Alterations in DNA methylation levels are characterized by various subtypes of lung cancer, as well as mutational status, smoking history, sex, and race or ethnicity. Consequently, aberrant DNA methylation patterns in cell-free DNA (cfDNA) can serve as diagnostic biomarkers for early-stage lung cancer, and their early detection may improve patient survival rates. Current lung cancer screening relies on noninvasive imaging modalities, including X-ray imaging and computed tomography (CT) scans, and on invasive diagnostic procedures such as biopsy. These procedures are uncomfortable and labor-intensive, resulting in diminished treatment efficacy due to late-stage diagnosis. The development of less invasive and less labor-intensive techniques can enhance survival rates in lung cancer patients by detecting DNA methylation in cfDNA, thereby facilitating early-stage lung cancer detection. Abnormal DNA methylation has produced promising signatures for the diagnosis of early-stage lung carcinomas, which cells utilize to modulate

gene expression. DNA methylation plays a significant role in embryonic development, genomic imprinting, X-chromosome inactivation, and the silencing of repetitive DNA.² DNA methylation involves the addition or removal of a methyl group at the 5'-carbon of cytosine within cytosine-phosphate-guanine (CpG) dinucleotides, a process facilitated by DNA methyltransferase enzymes (DNMTs). Methylation within the promoter regions of tumor suppressor genes (TSGs) results in their silencing, whereas reduced methylation of proto-oncogenes can convert them into oncogenes, ultimately contributing to lung cancer development. Nonetheless, although numerous studies have identified specific methylation-based markers, a comprehensive synthesis encompassing both diagnostic and therapeutic aspects of lung cancer remains lacking. This review aims to highlight that aberrant DNA methylation patterns, including hypermethylation of TSGs and hypomethylation of oncogenes, can serve as early-stage diagnostic indicators and therapeutic targets in lung cancer (Figure 1).

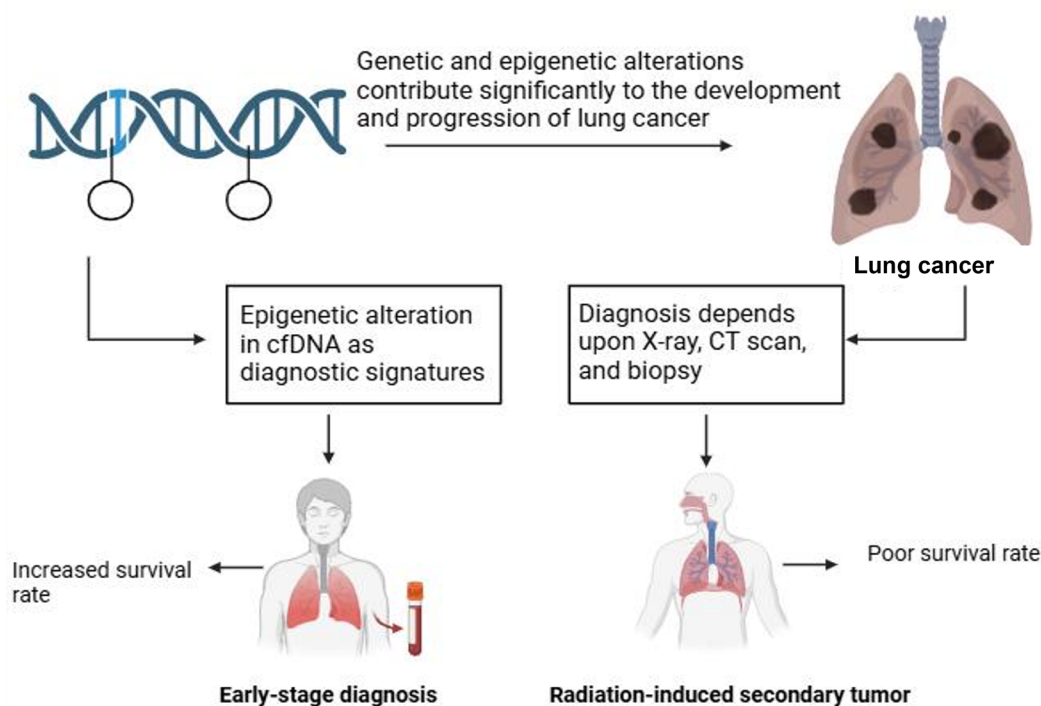


Figure 1. Illustration of the role of genetic and epigenetic alterations in lung cancer progression. Blood-derived cfDNA biomarkers can serve as a noninvasive approach for early-stage diagnosis, potentially leading to improved survival outcomes compared with imaging- and biopsy-based methods. Image created by the authors.

Abbreviations: cfDNA: Cell-free DNA; CT: Computed tomography.

2. Methodology

2.1. Information sources

The literature search was conducted through various databases, including PubMed, Google Scholar, Web of Science, and ResearchGate. The reference lists of selected articles were also reviewed to identify related studies. To ensure complete information, the selected articles were carefully reviewed and read to extract important data.

2.2. Search keywords

Various combinations of search terms were used, including “lung cancer,” “non-small cell lung cancer,” “small cell lung cancer,” “early-stage diagnosis,” “epigenetics,” “methylation,” “hypermethylation,” and “hypomethylation.”

2.3. Inclusion and exclusion criteria

Peer-reviewed articles published in English, studies related to cancer, and articles providing information on methylation were included in this study. Articles published in other languages, editorials, conference papers without full-text versions, and articles not related to cancer were excluded from the study.

2.4. Outcome extraction

Outcomes from each included article were extracted, and the information used in the study included cohort size, techniques used, key findings, study design, inclusion and exclusion criteria, level of methylation, type of methylation, and study type.

3. Cell-free DNA and circulating tumor DNA

Cell-free DNA, released during apoptosis or necrosis, consists of nucleic acids that can be identified in body fluids such as blood, saliva, urine, or feces. The nucleic acid released by tumor cells into body fluids is referred to as circulating tumor DNA (ctDNA). ctDNA is more fragmented and exhibits higher concentrations than non-tumor-derived cfDNA due to aberrant apoptotic control and increased cell proliferation in cancer cells, with elevated levels in lung cancer patients significantly contributing to tumor diagnosis and staging.³ A study by Emmalyn Chen *et al.*⁴ found that the concentration of ctDNA in 112 prostate cancer patients and 122 metastatic castration-resistant prostate cancer patients was elevated compared to control subjects. The size of ctDNA fragments is reported to be less than 167 base pairs.⁵ This reduction in fragment size may result from increased fragmentation or abnormal necrosis in the tumor, which is currently being investigated for its diagnostic potential.

4. Mechanistic understanding of DNA methylation in the progression of lung cancer

DNA methylation is crucial for preserving genomic integrity; hence, irregular and uncontrolled methylation patterns are often observed in various forms of lung cancer. In DNA methylation mechanisms, S-adenosyl-L-methionine (SAM) transfers a methyl group, resulting in the formation of 5-methylcytosine, N6-methyladenine, and 7-methylguanine, predominantly at the cytosine-C5 position within CpG islands and their shorelines. DNA methylation does not modify the DNA sequence; however, it influences gene expression via interactions with methylation-binding proteins. Methylation-binding proteins are classified into the methyl-CpG binding domain family (MBDs), SET and RING-associated (SRA) domain-containing proteins, and methyl-CpG binding zinc fingers.⁶ MBDs adhere to methylated CpG islands and exhibit diverse transcriptional regulatory actions,⁷ and are considered oncogenic. SRA domain-containing proteins comprise two domains: UHRF1 and UHRF2.⁸ UHRF1 regulates the expression of TSGs and modulates aberrant cell proliferation through the UHRF1/SIRT4 axis and other mechanisms.⁹ Methyl-CpG binding zinc fingers interact with methylated CpG islands via C-terminal zinc finger motifs and recruit additional proteins that inhibit gene expression, thereby representing potential therapeutic targets for cancer treatment. The addition of a methyl group to the carbon-5 position of the cytosine residue within the promoter region of TSGs by DNMTs is termed DNA hypermethylation. Aberrant methylation patterns in TSGs lead to transcriptional silencing, whereas widespread DNA hypomethylation diminishes methylation at CpG dinucleotides, resulting in the activation of previously silenced oncogenes and transposable elements, ultimately contributing to cancer development. The extent of DNA methylation at CpG sites is governed by the coordinated activities of DNMTs and DNA demethylases.¹⁰ The presence of CpG islands within promoter regions of TSGs provides evidence for gene silencing mediated by hypermethylation. Kulis *et al.*¹¹ reported that hypomethylation of suppressed oncogenes during the early stages of lung cancer is associated with increased genomic instability, cellular transformation, and progression to advanced metastatic stages of the disease (Figure 2).

4.1. DNA methyltransferase

Humans possess five distinct forms of DNMTs: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1 functions at hemi-methylated loci within CpG islands,

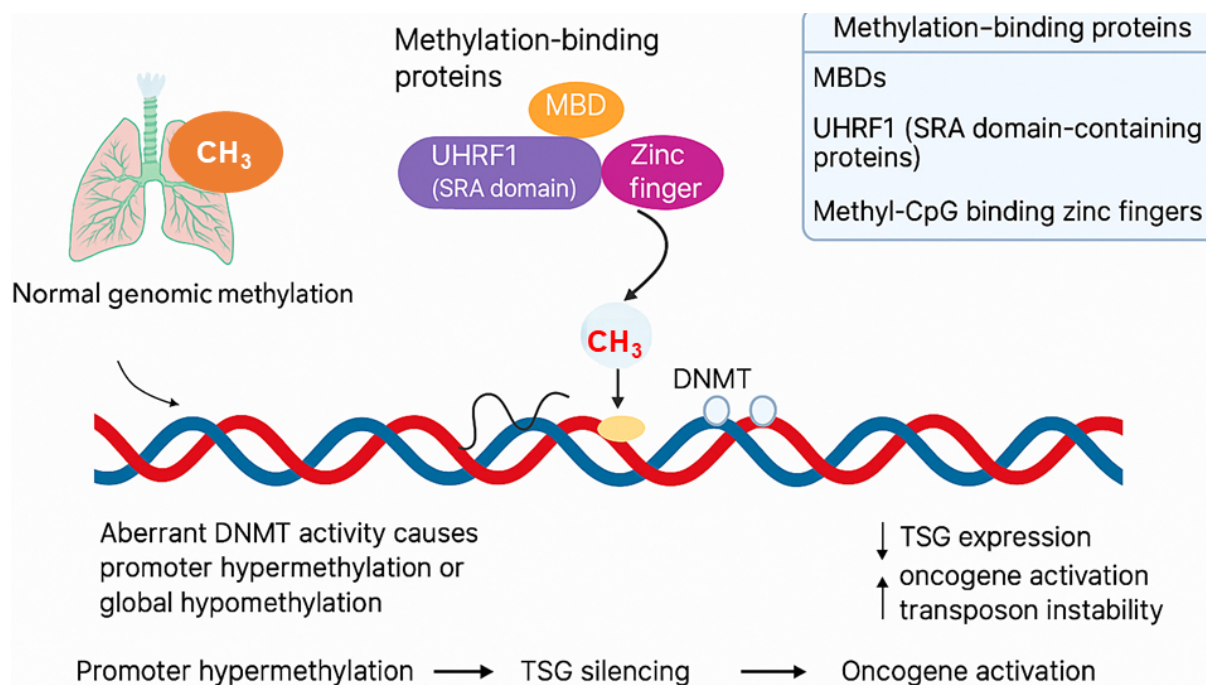


Figure 2. Mechanistic analysis of methylation reveals that DNMTs facilitate the covalent attachment of a methyl group to cytosine residues within CpG islands located in gene promoter regions. Aberrant hypermethylation of promoter regions in TSGs leads to transcriptional suppression, thereby activating oncogenic pathways and promoting lung cancer development. Image created by the authors.

Abbreviations: CH₃: Methyl group; CpG: Cytosine-phosphate-Guanine island; DNMTs: DNA methyltransferases; MBD: Methyl-CpG-binding domain; SRA domain: SET and RING-associated domain; TSG: Tumor suppressor gene; UHRF1: Ubiquitin-like with PHD and RING finger domains.

preserving methylation patterns on newly synthesized DNA after replication.¹² DNMT3A and DNMT3B are crucial for establishing methylation patterns during germ cell development and the early stages of embryogenesis. DNMT2, also known as tRNA aspartic acid methyltransferase, plays an essential role in the methylation of transfer RNAs. DNMT3L lacks a catalytic domain; yet, it enhances the methylation activity of DNMT3A and DNMT3B, indicating that elevated expression of the DNMTs is associated with the occurrence of lung cancer. Belinsky *et al.*¹³ showed that a reduction in DNMT1 levels decreases the incidence of lung cancer in a tobacco carcinogen-induced mouse model, as dysregulation of DNMTs leads to growth arrest, increased apoptosis, and inhibition of TSGs in lung cancer.¹⁴ Several mechanisms that adversely regulate the dysregulation of DNMTs include transcription factors such as specificity protein 1 and the downregulation of microRNA (miRNA)

expression, such as miR-10.¹⁵ Husni *et al.*¹⁶ reported that downregulation of DNMT3A facilitates the progression of lung LUAD via hypomethylation of oncogenes. In contrast, other studies have shown that DNMT3A is upregulated in SCLC but downregulated in NSCLC cell lines, which may be attributed to differences in histological subtypes of lung malignancies. Aberrant DNMT expression contributes to lung carcinogenesis via various mechanisms, including upregulation associated with increased cellular proliferation and cell cycle dysregulation. This is supported by findings from Sato *et al.*,¹⁷ which demonstrated that overexpression of DNMTs enhances the cell proliferation rate in human lung cancer. DNMTs are also involved in ribosome biogenesis, facilitating proliferation by participating in ribosomal RNA maturation and maintaining the nucleolar compartment required for ribosome assembly. Wu *et al.*¹⁸ revealed that DNMT1 methylates promoter regions and enhances cell proliferation by downregulating the

expression of *MLH1* and *MSH2*, which are involved in cell cycle suppression. Silencing of TSGs by DNA methylation, mediated by the upregulation of DNMTs, occurs through inhibition of methylation-sensitive transcription factors and the requirement of histone deacetylases for the MBD–CpG complex, ultimately leading to the formation of repressive chromatin structures.^{19,20}

4.2. DNA hypermethylation

DNA hypermethylation is an epigenetic phenomenon that reduces gene expression. When hypermethylation occurs in the promoter regions of TSGs, it leads to their inactivation, contributing to abnormal cell proliferation, dysregulated apoptosis, and cancer development. The *CDKN2A* gene, located on chromosome 9p21, inhibits phosphorylation of the RB1 protein by binding to cyclin-dependent kinases 4. Loss of *CDKN2A* function due to promoter hypermethylation results in RB1 phosphorylation and may lead to uncontrolled progression of the cell cycle from the G1 to the S phase.²¹ Singhal *et al.*²² proved that epigenetically mediated silencing of TSGs in lung cancer occurs through hypermethylation of promoter regions, particularly within the CpG island of the *CDKN2A* gene.^{23,24} A study by Liu *et al.* reported promoter hypermethylation of p16INK4a in NSCLC patients, indicating that methylation of *CDKN2A* is a common molecular event that may contribute to early tumor development.²⁵ Non-adrenergic imidazoline1 receptor protein, encoded by the *NISCH* gene and located on the short arm locus of chromosome 3, is a tumor suppressor involved in cytoskeleton organization of actin, cell–cell communication, apoptosis, intracellular signaling, and negative regulation of cell migration. According to a study by Ostrow *et al.*,²⁶ methylation of the *NISCH* promoter region was significantly higher in lung cancer patients and tobacco smokers compared with non-smoker controls. Balgouranidou *et al.*²⁷ investigated the methylation of *BRMS1* in plasma-derived ctDNA and reported promoter hypermethylation, suggesting that this hypermethylation

suppresses *BRMS1* expression and contributes to the development of a more advanced metastatic state in lung cancer. *SOX17*, a member of the high-mobility group box transcription factor superfamily and a homolog of the *SRY* gene, plays a key role in tumor angiogenesis. *SOX17* promotes the degradation of beta-catenin/T-cell factor complex via the Wntless-related integration site signaling pathway, and hypermethylation of the *SOX17* gene has been associated with the progression of lung cancer.²⁸

4.3. DNA hypomethylation

DNA hypomethylation contrasts with hypermethylation, resulting in a diminished quantity of methylated cytosine bases relative to normal levels. This phenomenon leads to aberrant activation of oncogenes and transposons through various mechanisms, including reduced availability of SAM, a universal methyl donor, compromised DNA integrity, and decreased expression of methyltransferase enzymes. Hypomethylation occurs at short interspersed nuclear elements, long interspersed nuclear elements (LINEs), subtelomeric repeats, and duplicated segments²⁹ in lung cancer patients, with LINE-1 hypomethylation being directly associated with prognosis and significantly contributing to the suppression of retrotransposon elements and chromosomal rearrangements. A study by Vachtenheim *et al.*³⁰ indicated that hypomethylation in the 3' tandem repeat region of Harvey rat sarcoma virus oncogene in NSCLC patients contributes to gene loss, whereas elevated hypomethylation of retrotransposable elements such as LINE-1 increases transcription levels, which is strongly correlated with enhanced genomic instability in NSCLC. DNA hypomethylation has also been observed in coding regions and introns, leading to the activation of oncogenes such as the *MYC* and *RAS*.³¹ Consequently, hypomethylation reactivates genes previously repressed by promoter methylation, suggesting that hypomethylation of oncogenes may result in their activation (Table 1).

Table 1. DNA methylation changes in selected genes in lung cancer. For each gene, the table shows whether it is aberrantly hypermethylated or hypomethylated in lung tumors, the lung cancer subtype studied, study details, assay and sample type, cohort size and stage, and reported sensitivity

Gene (alias)	Methylation Change	Cancer Type	Study	Assay	Sample	Cohort (n)	Stage	Sensitivity (%)	Specificity (%)	Key Findings
<i>CDKN2A</i> (p16)	Hypermethylation	NSCLC (primarily)	Belinsky <i>et al.</i> ³²	MSP	Tumor tissue, sputum	72 NSCLC patients (stage III)	Stage III (all cases)	62 (sputum vs tumor)	79 (sputum vs tumor)	p16 promoter hypermethylation was found in 47% of NSCLC tumors; it was detected in 40% of paired sputum samples. Sputum MSP testing predicted tumor methylation with 62% sensitivity and 79% specificity, indicating p16 methylation as a potential lung cancer biomarker.
<i>NISCH</i>	Hypermethylation (promoter)	Lung cancer (NSCLC/SCLC)	Krishnamurthy <i>et al.</i> ³³	MSP	Plasma cfDNA	40 lung cancer cases; 60 controls (30 smokers, 30 non-smokers)	No stage correlation	-	-	<i>NISCH</i> (tumor suppressor gene) promoter methylation was significantly higher in lung cancer patients' plasma cfDNA than in non-cancer controls ($P < 0.05$). Methylation was common even in high-risk (smoker) controls. There was no association of <i>NISCH</i> methylation with tumor stage or grade. Authors suggest that <i>NISCH</i> methylation in cfDNA could serve as a marker for lung cancer across different etiologies.
<i>EGFR</i>	Hypermethylation (promoter CpG island)	NSCLC (lung adenocarcinoma)	Sadri <i>et al.</i> ³⁴	MSP	Tumor tissue	50 NSCLC (tissue pairs)	Higher-stage tumors	-	-	<i>EGFR</i> promoter region II showed significantly higher methylation in NSCLC tumors compared to adjacent normal tissue ($P = 0.02$). Importantly, patients with <i>EGFR</i> methylation had significantly more advanced stage disease ($p = 0.03$). This suggests that <i>EGFR</i> epigenetic silencing may contribute to NSCLC pathogenesis (no sensitivity or specificity was reported).

Abbreviations: cfDNA: Cell-free DNA; EGFR: Epidermal growth factor receptor; MSP: Methylation-specific polymerase chain reaction; NISCH: Nischarin; NSCLC: Non-small cell lung cancer; p16: Cyclin-dependent kinase inhibitor 2A.

4.4. Association between smoking and methylation of DNA

Tobacco smoking is significantly linked to the occurrence of lung cancer compared with non-smoking status. Previous studies have demonstrated a strong association between tobacco smoking and the onset of SCLC, with only approximately 2.5% of SCLC cases occurring in non-smokers, highlighting a connection between smoking-induced DNA methylation alterations and lung cancer development. Gao *et al.*³⁵ reported that smoking induces aberrant gene methylation, contributing to lung cancer initiation. Similarly, Zhang *et al.*³⁶ proposed that hypomethylation of the *AHRR* and *F2RL3* genes is associated with smoking and increases lung cancer risk. Baglietto *et al.*³⁷ identified abnormal hypomethylation at CpG sites cg21566642, cg05951221, and cg23387569 in smokers, indicating potential susceptibility to lung cancer development. Therefore, tobacco smoking demonstrates a strong association with elevated DNA methylation levels in the *CDKN2A*, *APC*, *MGMT*, *ANK1*, and *MTHFR* genes, which are somatically associated with lung cancer progression. Pulling *et al.*³⁸ reported that abnormal *MGMT* promoter methylation was more prevalent in non-smokers than in smokers. Although the reasons for these discrepancies remain unclear, Liu *et al.*³⁹ suggested that the use of paraffin-embedded tissue instead of fresh tumor tissue may account for these variations. In a multi-gene methylation analysis of advanced NSCLC, Pesek *et al.*⁴⁰ reported variability in *CDH13* promoter methylation, indicating that smoking is an etiological factor; however, methylation of *CDH13* alone does not reflect a smoking-dependent pattern. Overall, tobacco smoking is strongly associated with oncogenic mutations that promote tumor development; however, there is no direct and consistent correlation between DNA methylation patterns and smoking, as various exogenous and endogenous factors influence the DNA methylation process.

5. DNA methylation as a diagnostic biomarker for the diagnosis of early-stage lung cancer

Mitsuhiro *et al.*⁴¹ examined 28 samples of NSCLC associated with chromate exposure and found hypermethylation of the *MLH1* gene in 25 patients, a finding similarly described by Ali *et al.*⁴² Hu *et al.*⁴³ reported hypermethylation in DNA repair genes among industrial workers exposed to chromate. Hung *et al.*⁴⁴ studied 69 cases of NSCLC and observed hypermethylation of *CCND2* in 22 patients. The aberrant DNA hypermethylation of *CCND2* was further corroborated by studies conducted by Castro *et al.*⁴⁵ and Oshimo *et al.*⁴⁶ Wang *et al.*⁴⁷ analyzed *NID2* gene methylation

levels in 38 cancer tissue specimens and reported elevated methylation across all samples. This finding was confirmed by Geng *et al.*,⁴⁸ who established a significant association between the *NID2* gene and cancer, noting methylation in 29% of colon cancer cases and 95% of gastric cancer cases. Su *et al.*⁴⁹ documented hypermethylation of the *CLEC14A* gene in 40 of 45 clinical samples, corroborated by other studies indicating that *CLEC14A* is upregulated in lung cancer.⁵⁰ Most studies have focused on gene-specific DNA hypermethylation as an early-stage diagnostic marker in lung cancer, while comparatively few have explored the clinical utility of hypomethylation, despite its critical role in genomic instability and tumor progression.

6. Methods of methylation analysis

Methylation-specific polymerase chain reaction (PCR) (MSP) and quantitative methylation-specific PCR (q-MSP) require two sets of primers—one for the methylated allele and another for the unmethylated allele—necessitating two PCR reactions per sample. Primers must be designed for identical DNA sites, containing up to three CpGs and thymine residues from bisulfite-converted cytosines to ensure selective amplification of converted DNA. In addition, primers should have similar melting temperatures, differing by only a few degrees Celsius. A detailed description of methylation-specific PCR has been published previously.⁵¹ In MSP, PCR products are analyzed by gel electrophoresis, whereas q-MSP allows quantification using cycle threshold values. Primer design for methylated sequences can be challenging, which is a limitation of these methods.⁵² DNA methylation arrays based on hybridization allow analysis of targeted loci with minimal DNA inputs. Methylation EPIC BeadChip quantifies over 850,000 CpG island sites, including enhancers, open chromatin, transcription factor binding sites, and promoter regions. Agilent's human DNA methylation microarrays are based on methylated DNA immunoprecipitation.⁵³ Next-generation sequencing enables comprehensive analysis of methylation patterns across genes, while pyrosequencing allows precise quantification of DNA methylation levels, and it is used for analyzing the methylation status of CpG sites in a particular gene.

7. Pre-analytical confounders in cell-free DNA methylation

Ethylenediaminetetraacetic acid vials are cost-effective for regulatory-approved plasma separation; however, plasma must be separated within four hours of blood collection to prevent genomic DNA contamination from lysed cells. Plasma separation should be carried out by centrifugation at 16,000 × g for 15 min, allowing blood cells and platelets to settle at the bottom while plasma forms the top layer,

which is then transferred to a fresh microcentrifuge tube. For short-term storage (up to one year), plasma should be maintained at -20°C , whereas long-term storage requires -80°C to preserve cfDNA quality. The efficiency of bisulfite conversion is also an important factor: insufficient conversion can result in false-positive methylation signals, while over-conversion can degrade DNA and reduce its quality. Furthermore, fragment size selection is also important; cfDNA fragments are typically around 167 bp in healthy individuals but are shorter, approximately 90 bp in cancer patients, due to increased fragmentation.

8. DNA methylation as a therapeutic target for lung cancer treatments

Aberrant DNA methylation alters gene expression levels, and research by Kwon *et al.*⁵⁴ demonstrated that reversing hypermethylation restores expression levels, highlighting a critical distinction between mutations in TSGs and the phenomenon of epigenetic gene silencing. DNA methylation thus represents a potential epigenetic therapeutic target to restore gene expression, as demonstrated by Laird *et al.*⁵⁵ and others, who provided evidence that reducing methylation levels through treatment with 5-aza-2'-deoxycytidine in tumor cell lines reactivated gene expression.⁵⁶ Methylation levels in tumor and proliferative cells are directly dependent on DNMT

expression, so reducing DNMT activity is a viable strategy to restore DNA methylation levels. n-Butylidenephthalide, an organic compound, inhibits DNMT and induces cell cycle arrest in the G0/G1 phase.⁵⁷ Azacytidine and decitabine (clinical trials NCT01845805, NCT02959164) are cytidine analogues developed by Sorm and his colleagues in 1964.⁵⁸ They are transported into cells via concentrative nucleoside transporter-1 and converted to 5-azacytidine 5'-tris and 5-aza-2'-deoxycytidine-5'-triphosphate, and are then degraded by cytidine deaminase. Once incorporated into DNA, they disrupt the interaction between DNMTs and DNA, ultimately recruiting proteins that initiate DNA damage signaling and promote the destruction of sequestered DNMTs.⁵⁹ Although DNMT inhibitors have shown promising results in preclinical and early clinical studies, their use in lung cancer therapy remains limited. Further *in vivo* validation and clinical trials are required. Nevertheless, these inhibitors represent a promising avenue for future therapeutic strategies in the treatment of lung cancer (Figure 3).

9. Discussion

This review article highlights the importance of blood-derived cfDNA methylation biomarkers for the early-stage diagnosis of lung cancer. It also summarizes the therapeutic potential of targeting DNMTs to reverse abnormal

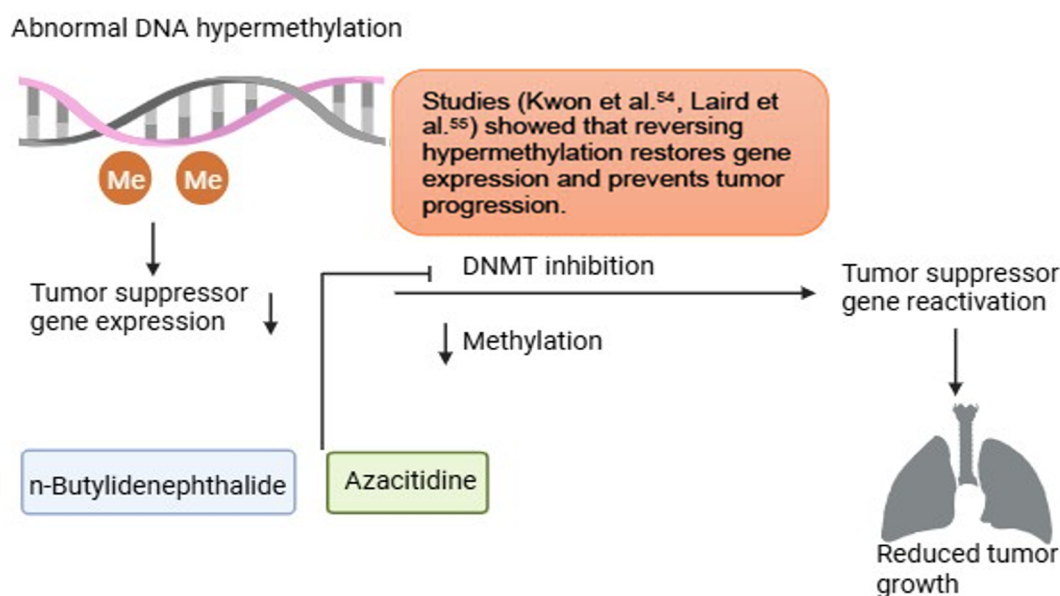


Figure 3. Inhibition of DNA methyltransferase. DNMT expression positively influences the methylation of tumor suppressor gene promoters, leading to their transcriptional silencing. Conversely, inhibition of DNMTs by azacytidine and n-Butylidenephthalide reinstates their expression and may contribute to the prevention of tumor development. Image created by the authors.

Abbreviations: DNMT: DNA methyltransferase; Me: Methyl group.

methylation patterns. In addition, the relationship between smoking and DNA methylation changes in lung cancer progression is discussed. By integrating diagnostic and therapeutic aspects, this review provides a comprehensive overview of the clinical relevance of DNA methylation in lung cancer. Currently, the detection of lung carcinomas relies on chest X-rays, low-dose CT scans, and biopsies, which are limited by false-positive rates, harmful radiation effects, and associated costs. Indeed, biopsy, accompanied by immunohistochemistry, serves as an invasive supplementary diagnostic method; however, both procedures are uncomfortable and labor-intensive. Prior research has elucidated that promoter methylation in proto-oncogenes and TSGs begins at the initial stages of carcinogenesis and can be detected in DNA released by tumor cells into the bloodstream.^{60,61} Furthermore, methylation-based blood tests offer high specificity and sensitivity, notably with the methylation of the short *SHOX2* and *RASSF1A* genes, which can be detected at early stages of cancer.^{62,63} Gaga *et al.*⁶⁴ reported a specificity/sensitivity of 78.4%/62.2% for the blood-based methylation tests in stage I NSCLC and 100.0%/88.9% in extensive-stage SCLC. Shahnaz *et al.*⁶⁵ performed quantitative methylation-specific PCR on serum or plasma from lung cancer patients, achieving 100% specificity for the *DCC* gene. Additionally, Christoph *et al.*⁶⁶ reported sensitivity rates for blood-based methylation tests in the *SHOX2* gene, with 80% for SCLC, 63% for LUSC, and 39% for LUADs. The cfDNA-methylation assay exhibits a low false-positive rate of approximately 0.5% in published cohorts,⁶⁷ whereas the low-dose CT scan demonstrates a significantly higher false-positive rate of 24–27%, largely due to the difficulty in distinguishing the lung cancer subtypes from pneumonia on CT imaging.⁶⁸ The use of liquid biopsies is gaining popularity in clinical practice due to their noninvasiveness and high specificity for early-stage diagnosis.^{69,70} This article highlights the extent of aberrant methylation in oncogenic and TSGs among lung cancer patients, while also examining the role of DNMTs in methylation mechanisms. Elevated expression of DNMTs has been associated with poor prognosis in lung cancer, suggesting that targeting abnormal DNMT expression may restore normal methylation patterns and serve as a potential epigenetic therapeutic target for lung cancer treatment. Hypermethylation of TSGs is a primary driver in lung cancer initiation, often occurring at an early stage. This phenomenon can serve as a diagnostic signature, potentially improving treatment regimens and augmenting patient survival rates. DNA hypomethylation has been comparatively less investigated as a clinically actionable biomarker, due to technical challenges in distinguishing it from normal epigenetic variation. Although DNA

methylation significantly influences cancer development, only a limited number of methylated genes have been established as clinical biomarkers. The advent of epigenetic analyses has enabled the identification of a considerable number of hypomethylated or hypermethylated tumor-related genes in cfDNA derived from blood samples. Plasma has emerged as the most advantageous testing matrix for the assessment of biological markers in early-stage lung cancer diagnosis, with numerous studies validating its utility over serum. Research can be undertaken to examine the methylation patterns in DNA using blood plasma. Smoking exposure remains a significant source of oncogenic mutations that initiate tumorigenesis, while other exogenous and endogenous factors, such as contaminants, inflammation, aging, and chronic cellular stress, contribute to epigenetic modifications that facilitate lung cancer development even in the absence of tobacco carcinogens.

10. Conclusion

This review illustrates the potential for developing methylation-based biomarkers for early detection and therapeutic intervention in lung cancer. Future studies should include appropriate biological and technical controls, validation cohorts, and standardized cfDNA processing protocols to minimize false-positive methylation signals and improve reproducibility.

Acknowledgments

The authors sincerely thank the Department of Respiratory Medicine and Department of Biochemistry, King George's Medical University, Lucknow, for their support and contributions to this study.

Funding

The research was financially supported by the Uttar Pradesh Council of Science and Technology (UPCST), Government of Uttar Pradesh (Grant No. CST-D/1850).

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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