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Epigenetic Silencing in Lung Cancer: The Role of DNA Hypermethylation

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ABSTRACT

Lung cancer, a primary global cause of cancer mortality, arises from complex genetic and epigenetic changes. This review examines the role of key epigenetic mechanisms—specifically DNA hypermethylation, Histone deacetylases (HDACs), and microRNAs (miRNAs)—in lung cancer pathogenesis. DNA hypermethylation frequently leads to the silencing of TSGs, causing unregulated cell proliferation and progression of tumor. By modulating chromatin structure, HDACs influence gene expression patterns critical to cancer development, making them promising therapeutic targets. miRNAs, acting as oncogenes or tumor suppressors, control gene expression at the post-transcriptional stage, further impacting lung cancer biology. Understanding these mechanisms provides insight into novel diagnostic approaches that aim to enhance cancer management and clinical outcomes.

Keywords: Epigenetics, DNA Hypermethylation, Lung Cancer, Histone Deacetylases (HDACs), microRNAs (miRNAs).

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INTRODUCTION

Cancer arises when cells proliferate uncontrollably because of transformative changes which occurs genetically as well as epigenetically. These alterations disrupt cellular homeostasis, enabling autonomous cell growth independent of organismal regulatory mechanisms. The oncogenes triggering and inactivation of TSGs drive significant changes in cellular pathways, facilitating cancer progression. Cellular metabolism functions as a dynamic network, allowing tissues to sustain both homeostasis and growth. In cancer, tumor cells undergo metabolic adaptations driven by various internal and external stimuli [1]. This adaptability, referred to as metabolic plasticity, results in metabolic reprogramming that disrupts cellular homeostasis. Consequently, biochemical changes has been considered as a hallmark of cancer, encompassing persistent alterations in glucose, glutamine, and mitochondrial metabolism [1].

There are two types of lung cancer such as Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) comprising roughly 85% and 15% of cases respectively [2]. Adenocarcinoma and Squamous Cell Carcinoma are the primary subtypes of Non-Small Cell Lung Cancer. This classification is based on the fact that these subtypes arise from different types of cells within the lungs [3]. Further molecular classification is facilitated by the identification of specific DNA mutations [2]. Early diagnosed in NSCLC presents a favourable prognosis when removed surgically boasting survival rates over a 5-year period for localized small tumors extending from 70% to 90% [4]. However, majority of patients, around 75% are analyzed as advanced-stage (classified as stage III/IV) at the time of diagnosis [2]. Small Cell Lung Cancer (SCLC) has different DNA methylation patterns than other types of lung cancer, particularly in genes related to neuroendocrine cell development [3].

2.2 million individuals were diagnosed with the lung cancer leading to 1.8 million deaths in 2020. Globally, lung cancer is the second most common cancer accounting for 11.4% of all cancer cases and 18.0% of all cancer related deaths. It ranks third after breast and colorectal cancers in women whereas in men it is the most frequent and deadliest. The ratio of incidence and mortality varies across different geographical region, while in general it is significantly higher in men than in women. In 2020 Lung cancer accounted for 5.9% of all cancer cases and 8.1% of all cancer-related deaths in India presenting a notable public health challenge. Factors such as use of tobacco, smoking and air pollution are the leading cause of lung cancer incidence and mortality in the country (NCDIR, India). The rising prevalence is partially attributed to declining mortality rates from stroke and heart disease in various regions [5].

Lung cancer has specific genetic changes that cause it to grow along with changes in epigenetic markers on genes [3]. According to World Health Organization, cancer is considered as a leading cause of death in 2020 (6). Epigenetics refers to heritable changes in gene expression or chromosomal stability driven by mechanisms like DNA methylation, histone modifications, or non-coding RNAs, occurring without alterations to the DNA sequence [7]. Epigenetic alterations refer to inheritable modifications in function of genes that occur independently of any Modifications in the DNA sequence [8].

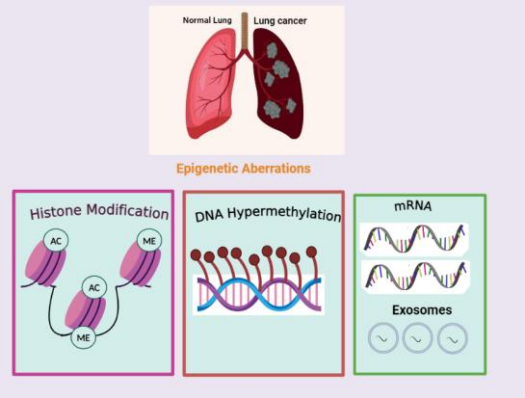

Figure 1: This illustrates key epigenetic alterations in lung cancer, including histone modifications that impact gene expression, DNA hypermethylation drive silencing of TSG, and changes within the mRNA and exosome profiles that influence tumor growth and communication.

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Epigenetic factors play an important function in precisely regulation of gene expressions, that are essential for fundamental processes in biology like cellular differentiation and embryogenesis and exists a compelling proof suggesting that epigenetic reprogramming significantly contributes to the dynamic variability seen in cancer at the transcriptomic level [9]. Epigenetic changes cause differences in how genes are expressed, even when there are no mutations in the genes themselves [10]. While epigenetic changes occur more frequently than somatic mutations, there exists a notableinteractions between these two processes. Epigenetic silencing can contribute to genetic mutations, which, in turn leads to disturbance in epigenetic [11] [12]. Glucose-regulated protein 78 (GRP78), Src, Toll-like receptor 7 (TLR7), caveolin-1, and dopamine receptor D2 (DRD2) play pivotal roles in the binding and entry of Japanese encephalitis virus (JEV) into neurons. Interestingly, these receptors are also implicated in cancer progression, where their expression and activity are often modulated by epigenetic mechanisms, including DNA methylation. Aberrant methylation of genes encoding these receptors may influence their expression, contributing to oncogenic signaling pathways and tumorigenesis [13]. Dietary compounds are emerging as promising agents for cancer prevention due to their lower toxicity compared to conventional drugs. Phytochemicals, in particular, exhibit chemopreventive properties by targeting multiple stages of carcinogenesis. Notably, many of these compounds can modulate epigenetic mechanisms, including the reversal of DNA hypermethylation, which plays a critical role in the silencing of tumor suppressor genes in lung cancer [14]. By restoring normal gene expression, these natural agents hold potential for mitigating epigenetic silencing associated with lung tumorigenesis [15].

HDAC (Histone deacetylases)

HDACs belong to a group of enzymes essential for controlling gene expression by deacetylating histone proteins, leading to chromatin condensation and the suppression of transcription. Their activities are counterbalanced by histone acetyltransferases (HATs), which attach acetyl groups, resulting in a relaxed chromatin configuration and facilitating active gene transcription. HDACs have been linked to various biological processes and diseases, including cancer, neurological disorders, and inflammatory diseases, which makes them promising targets for therapeutic intervention [16]. HDACs are grouped into four categories "due to their resemblance to yeast" proteins and their cellular localization [17] (Table 1).

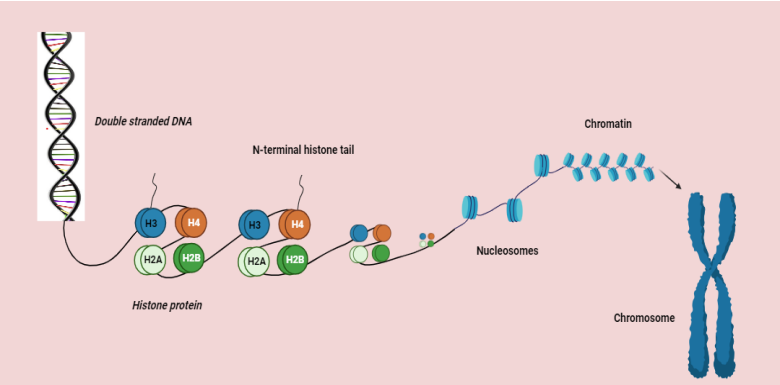

Figure 2: Illustrations of the hierarchical organization of DNA, beginning with the dsDNA winding around Histone proteins' octamer combine to create nucleosomes, which are the basic building blocks of chromatin. The nucleosomes further compact into higher-order structures, ultimately forming chromatin fibers that are organized into chromosomes. This structural arrangement is essential for efficient DNA packaging, gene expression regulation, and genomic integrity maintenance.

Table 1: Classifications of Histones deacetylases (HDACs) and their functions.

Class	Name	Location	Function	References
Class 1 HDAC	HDAC 1,2,3,8	Predominantly Nucleus	regulation of cell cycle and differentiation.	[16]
Class IIa HDACs	HDAC 4,5,7,9	Nucleus & Cytoplasm	muscle differentiation and heart development.	[18]

Class IIb HDACs	HDAC 6, 10	Mainly Nucleus	HDAC 6 - cytoskeletal dynamics and stress responses. HDAC 10- involved in DNA damage response.	[18]
Class III HDACs (Sirtuins)	HDAC 1,2,3,4,5,6,7	Nucleus Cytoplasm	involved in aging, metabolism, and stress resistance.	[19]
Class IV HDACs	HDAC 11	Nucleus	implicated in immune response regulation.	[16]

miRNA

Small, non-coding RNA molecules called microRNAs are crucial for controlling the expression of genes. The RNA polymerase II enzyme in the cell nucleus initiates their production by converting miRNA genes into primary miRNAs, or pri-miRNAs [20]. The RNase III enzyme Drosha and its cofactor DGCR8 forms precursor miRNAs (pre-miRNAs) to process primary RNAs inside the nucleus. Exportin 5 then carries these pre-miRNAs to the cytoplasm. The pre-miRNAs are converted into miRNA duplexes, which are roughly 22 nucleotides long, in the cytoplasm with the help of enzyme Dicer. One strand of this duplex is integrated inside the miRNA-induced silencing complex (miRISC), that guides the complex to target mRNAs, which in turn results in silencing of genes via degradation of mRNA and translational repression [7].

Biological Roles of miRNAs

miRNAs are considered as an essential regulator of many biological processes, including development and cell growth, apoptosis (programmed cell death), metabolism, and neural development. Their capacity to optimize gene expression positions them as essential players in maintaining cellular homeostasis. The dysregulation of miRNAs has been associated with many pathological state. In cancer, miRNAs can function as tumor suppressor or oncogenes (oncomiRs). OncomiRs are typically overexpressed in tumors and facilitate cancer progression by inhibiting tumor suppressor genes. Conversely, tumor-suppressive miRNAs are frequently downregulated in cancers, resulting in the unchecked expression of oncogenes [7].

Oncogenic miRNAs in Lung Cancer

Oncogenic role of several microRNAs promotes tumorigenesis through various mechanisms such as miR-221/miR-222, miR-155, and miR-21. These miRNAs target TSGs like PTEN and p53, block Ras pathway inhibitors, promote TRAIL-mediated resistance to apoptosis inhibition, facilitate cell migration via AKT activation, and reduce tissue inhibitors that modulate Metal-dependent proteinases [3]

miRNAs in Lung Cancer Pathogenesis

Cluster of miR-17-92

The group of miR-17-92, which referred as oncomiR-1, comprises miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a and their overexpression affects lung cancer development. For instance, the synergistic interaction between miR-18a and miR-92a offers important information on how NSCLC develops. Moreover, in xenografted mouse models, antagomiR-18a treatment has been demonstrated to suppress tumour growth [21].

miR-21: It is the most thoroughly researched miRNAs in cancer, miR-21 mechanisms as an oncogene in NSCLC. It reduces the expression of tumor suppressor genes such as PTEN at the post-transcriptional level, thereby inducing invasion and progression of cells in cancer [21].

miR-34b/c: For NSCLC at stage I, hypermethylation of miR-34b/c DNA has predictive value. The hypermethylation leads to the downregulation of these miRNAs, contributing to lung cancer pathogenesis [21].

miRNAs and Epigenetic Regulation

The interaction between miRNAs and epigenetic mechanisms is dynamic and reciprocal. Histone modifications and DNA methylation are examples of epigenetic alterations that regulate miRNAs.

Epigenetic changes can alter miRNA expression, affecting their role in gene regulation. On the other hand, miRNAs can modulate DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) as an epigenetic regulators. This interaction forms a regulatory circuit wherein epigenetic changes and miRNAs mutually regulate each other, significantly impacting gene expression and cancer development. For instance, treatment with HDAC inhibitors (HDACi) can restore miR-373 expression and reverse an epithelial-mesenchymal transition (EMT) phenotype. The miR-29 family, which is often down-regulated in lung cancer, causes increased expression of DNMT3A and DNMT3B, both of that inhibit different TSGs [3].

DNA Methylation

The process of a methyl group becoming covalently bonded to the 5'carbon of cytosine ring, leading to the production of 5-methylcytosine is usually occurring within CpG dinucleotide regions is known as DNA methylation (22) Epigenetic modification has an important role in regulating the stable gene expression in mammals. The distribution of DNA methylation in the genome is bimodal, with most CpG sites being either highly methylated (ranging from 85% to 100%) or largely unmethylated (ranging from 0% to 5%). This methylation pattern is crucial for proper development, the suppression of transposable elements, genomic imprinting, X-chromosome inactivation [7]. The DNA methyltransferase (DNMTs) facilitates this process in which DNMT3A and DNMT3B playing key role in establishing de novo methylation and DNMT1 supporting methylations during DNA replication (23). The TET (ten-eleven translocation) enzyme family demethylates 5-methylcytosine by oxidizing it to 5-hydroxymethylcytosine and other oxidation products. Aberrant DNA methylation patterns, including promoter global hypomethylation and hypermethylation are hallmark features of lung cancer and contribute significantly to tumor progression and metastasis by altering gene expression [8]

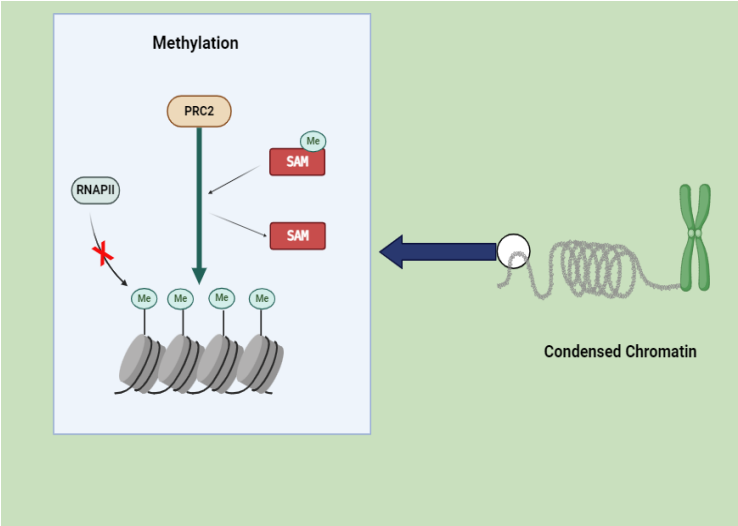

Figure 3: PRC2-mediated histone methylation, which uses SAM as a methyl donor to add methyl groups to histones, leading to chromatin condensation. This process blocks RNA Polymerase II binding, silencing gene expression. The result is a more compact and transcriptionally inactive chromatin structure.

DNA methylation and oncogenesis

Methylation of DNA plays a significant role in tumorigenesis which is linked to normal human physiological processes. The TSG p16 has been found to play a crucial role in cancer development within mouse embryonic stem cells. The study revealed that 27% of mice with p16 methylation developed malignancies such as lung cancer, leukemia, and sarcoma, while no tumor formation was observed in the wild-type control mice. These observations suggest a potential causative role of DNA methylation in cancer onset [24].

Under normal physiological conditions, the human genome consist of CpG sites which are always remains methylated while a small subset remaining unmethylated, forming CpG islands (CGIs). These CpG islands are predominantly located in promoter regions of genes and, to a lesser extent, in exonic regions. They are enriched with tumor suppressor genes, housekeeping genes, and DNA repair genes [25]. Typically, CpG islands are maintained in an unmethylated state, but the exact mechanisms that preserve this state are not yet fully understood. Ginno et al. identified that CpG islands might form R-loop structures, which could prevent methylation by blocking the binding of methyltransferases [26]. It has been demonstrated that the demethylation of CpG islands by ten-eleven translocation protein 1 (TET1) is promoted bt growth arrest and DNA damage protein (GADD45A) through its interaction with the R-loop structure [27]. Disruption of these protective mechanisms may leads to tumor suppressor genes and DNA repair genes silencing, and the proto-oncogenes gets activated, resulting in the expression tumor-related proteins and subsequent tumorigenesis [28].

Mechanisms of DNA Methylation

The process includes the addition of a methyl group to the cytosine base in DNA, primarily at CpG dinucleotides. This process is regulated by several key components [29]:

Methyltransferases

DNMT1: During DNA replication the primarily functions of DNMT1 is to maintain DNA methylation which exhibits a significant preference for DNA which is hemimethylated, ensuring that methylation patterns are accurately transferred to the daughter strand. This maintenance function is critical for the preservation of the epigenetic landscape through successive cell divisions [29].

DNMT3A and DNMT3B: The key factors such as DNMT3A and DNMT3B in *de novo* methylation are found to be responsible for establishing novel methylation patterns during embryogenesis and in response to environmental stimuli. These enzymes target unmethylated CpG dinucleotides, thereby setting up the initial methylation landscape [29].

DNMT3L: Although DNMT3L lacks intrinsic catalytic activity, it serves as a regulatory cofactor for DNMT3A and DNMT3B. DNMT3L enhances their catalytic efficiency as the affinity for the methyl group donor S-adenosylmethionine (SAM) gets imcreased, which helps in promoting the establishment of new methylation [29].

Demethylation

TET Enzymes: The Ten-Eleven Translocation (TET) enzymes, including TET1, TET2, and TET3, have a crucial function in the demethylation process. These enzymes like oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) can undergo further processing to achieve both active and passive demethylation. Passive demethylation takes place during DNA replication when 5hmC fails to recognized by DNMT1, this leads to its progressive dilution. Active demethylation involves subsequent enzymatic steps that ultimately substitute the modified cytosine with an unmodified cytosine [29].

DNMT and Lung Cancer Therapy

Scientists have suggested the strategy of demethylating TSG and DNA repair genes to restore their normal expression and inhibit tumor progression. Among the therapeutic approaches, DNMTis have been the most extensively studied. Research has identified DNMT as an important target for epigenetic therapies in cancer treatment [30]. Notably, Combining DNMT inhibitors (DNMTis) with histone deacetylase inhibitors (HDACis) has been shown to markedly improve treatment outcomes in NSCLC [31]. This combination therapy has proven effective in suppressing growth in both human and mouse models cancer cells. Clinically, DNMT inhibitors like 5-Azacytidine and its analog 5-Aza-2'-deoxycytidine have been shown to demethylate tumor suppressor genes, effectively suppressing tumor growth. However, 5-Aza-CdR has notable limitations, including low specificity and the potential for tumor metastasis with improper dosing, which restrict its clinical utility [32]. Additionally, the impact of DNMTs on the methylation status of other genes is still unclear and requires further exploration.

DNA methylation serves as an early indicator of lung cancer

Statistical data indicates that five-year survival for individuals diagnosed with early cancer of the lung is 92%. In contrast, the survival rate at an advanced stage is only 5%. This underscores the critical importance of early diagnosis for improving patient outcomes. In the last 2 decades, early detection and timely treatments have significantly lowered mortality rates in both males and females in the United States [33]. This advancement is primarily credited to the widespread adoption of LDCT (low-dose computed tomography) scans, which assess the risk of lung cancer by analyzing the imaging features of pulmonary nodules [34]. Furthermore, LDCT screening presents several Drawbacks, such as heightened radiation exposure and less-than-ideal diagnostic precision, and reduced efficiency [35]. In contrast, DNA methylation analysis offers a more sensitive and stable alternative, effectively addressing the shortcomings of imaging techniques and improving the prompt detection of lung cancer.

Regulation of Gene Expression

Transcription factors (TFs) play a crucial role in regulating expression of gene by interacting with DNA to facilitate the transcription process. Moreover, the work has been demonstrated that methylated DNA in transfected cells was transcriptionally inactive, which could be reactivated following DNA demethylation [36]. This results in the methylation of DNA which could have a significant function in regulating gene transcription in eukaryotes. Further studies, have supported this notion, suggesting that DNA methylation is integral to regulatory networks involved in gene transcription [37]. Specifically, DNA methylation modulates expressions in gene by obstructing TF binding to methylated DNA sequences, altering chromatin structure, and facilitating the interaction of methyl-binding proteins (MBPs) with transcriptional repressors. Additionally, [38] discovered that DNA methylation can induce a conformational change from B-DNA to Z-DNA, although the underlying mechanism remains unclear. Further studies identified that differences in gene body methylation compared to promoter methylation (MeGDP) can act as an indicator for expressions in gene [37].

Hypermethylation in Lung Cancer

Hypermethylation involves an excessive increase in DNA methylation, particularly in CpG islands within gene promoter regions. This abnormal methylation is a characteristic feature of various cancers, including lung cancer, where it silences tumor suppressor genes, aiding in tumorigenesis. For example, the hallmark characteristics of cancer cells are uncontrolled growth and survival, which are caused by promoter hypermethylation, which silences genes involved in DNA repair, cell cycle regulation and programmed cell death. This abnormal methylation pattern affects multiple genes, disrupting several cellular pathways simultaneously [8].

In lung cancer, DNA methylation patterns are often altered, leading to gene silencing or activation that contributes to tumorigenesis. The aberrant hypermethylation of CpG islands in the 5'regions of genes associated with cancer is one such alteration. About 5% to 10% of CpG island genes are affected by this promoter hypermethylation, which is commonly seen in lung cancer. This epigenetic modification locks the affected genes into an inactive state, directly contributing to carcinogenesis by silencing tumor suppressor genes and other regulatory elements which involved in controlling the cell growth and differentiation. CpG islands are found in around 60% of all gene promoters [39]. In healthy lung tissue, these islands are left unmethylated to permit active or prospective gene expression. However, in lung cancer, hypermethylation of these CpG island promoters disrupt normal gene regulation, leads to unchecked proliferation of cell and tumor growth [40]. The frequency and significance of CpG island hypermethylation in lung cancer highlight its potential as a target for epigenetic therapy, aiming to reverse these changes and restore normal gene function [7].

Hypomethylation in Lung Cancer

Hypomethylation in lung cancer leads to chromosomal instability, disrupted genomic imprinting, and abnormal tumor metabolism and differentiation. The process of gene body hypomethylation observed in tumors is not fully understood but may involve active demethylation by TET family proteins or passive loss during tumor replication [41].

Mutations in Methylation-Related Genes

Mutations in genes associated with DNA methylation regulation, such as TP53, are common in lung cancer. These mutations often occur at CpG sites, where methylation can predispose the sites to mutations [42]. Epigenetic therapies targeting DNA methylation are being explored to reactivate silenced TSGs and inhibit oncogenes in lung cancer. Inhibitors of DNMTs and drugs targeting other epigenetic modifiers are under investigation to reverse abnormal methylation patterns and restore normal gene function.

Hypermethylated Genes in Lung Cancer

ADAMTS18

ADAMTS18 is hypermethylated in different forms of cancer, such as gastric, breast, renal, and reproductive cancers. Although it is hypermethylated in India, this hypermethylation does not occur in lung cancer. However, in other regions like China, the inactivation of ADAMTS18 due to aberrant promoter hypermethylation is implicated in lung cancer progression. This suggests a geographical variation in the epigenetic regulation of ADAMTS18 and highlights the importance of considering regional differences in cancer epigenetics.

FHIT (Fragile Histidine Triad)

In many cancers, FHIT is hypermethylated, such as cervical, colorectal and oral squamous cell carcinoma in India, but not in lung cancer. Similar patterns are observed in China, where FHIT promoter methylation shows diagnostic role in NSCLC. This indicates that while FHIT methylation is a useful biomarker in certain cancers, its role in lung cancer remains limited or inconsistent.

RASSF1A

Across different geographical regions, including India, the RASSF1A gene undergoes hypermethylation in lung cancer. Previous studies have shown associations between methylation in promoter regions of RASSF1A and mutations of KRAS in NSCLC and SCLC, particularly in smokers. Other research has highlighted RASSF1A's potential as a tumor biomarker and its correlation with cancer prognosis based on cell-free DNA methylation. This gene's consistent hypermethylation in lung cancer makes it a valuable target for diagnostic and prognostic purposes.

p16

The p16 gene shows hypermethylation in lung cancer both in India and other countries like China and Italy. This methylation leads to resistance to the chemotherapy drug paclitaxel and affects transcriptional activity in lung cancer cell lines. In India, the p16 gene, along with GSTp1, p14, and RASSF1A, exhibits promoter hypermethylation in smokers, underscoring the gene's involvement in cancer of the lung development and its effective role as a target for treatment.

MGMT

MGMT is also hypermethylated in numerous cancers such as, ovarian, head and neck squamous cell carcinoma, colorectal and cervical cancer but not in lung cancer in India. In China, MGMT methylation of promoter region have been extensively studied for lung cancer diagnostic biomarker, with varying associations to the clinicopathological characteristics of NSCLC. This discrepancy points towards need for region-specific studies to validate biomarker role of MGMT in lung cancer.

LATS1 and LATS2

LATS1 and LATS2 genes, in India, are not hypermethylated in lung cancer but previously shown hypermethylation in other regions like Israel, China, and Korea. These genes are part of the Hippo pathway and their expression levels influence chemotherapy response and prognosis in advanced NSCLC. The lack of hypermethylation in India suggests potential differences in pathway regulation or environmental factors affecting gene methylation. However, in OSCC, these genes have been shown to undergo hypermethylation, suggesting cancer-specific and population-specific variations in methylation patterns, potentially driven by differences in pathway regulation or environmental factors [43].

DAPK

The DAPK gene is frequently inactivated through hypermethylation in various cancers. While it is hypermethylated in childhood ALL, where it contributes to leukemogenesis and serves as a potential biomarker for prognosis[44]. DAPK gene promoter hypermethylation is observed in lung cancer in both India and other countries such as China, Dubai, and Egypt. Research shows that in NSCLC, DAPK promoter methylation in linked to a variety of clinicopathological and predictive traits. In India, studies concentrate on identifying DAPK methylation in tissue samples from patients with advanced-stage lung cancer and employing plant extracts. DAPK's function as a universal biomarker for lung cancer is supported by it's constant methylation across geographical areas.

Impact of Hypermethylation on Lung Cancer Development

In lung cancer, DNA hypermethylation facilitates malignant transformation by disrupting essential cellular processes critical for regulating cell proliferation, maintaining genomic integrity and apoptosis, and DNA repair mechanisms [45]. Critical physiological processes involved in regulating the cell cycle, DNA repair and apoptotic signaling mechanisms are disrupted when hypermethylation-mediated TSGs silencing occurs [46]. For instance, the RASSF1A gene's function in controling progression of cell cycle and apoptotic pathways is disrupted by hypermethylation, which is commonly seen in NSCLC [47]. Inhibiting this tumor suppressor gene promotes unregulated cellular proliferation and enables evasion of apoptosis. Reactivation of tumor suppressor genes silenced by promoter hypermethylation represents a crucial approach in cancer therapy. In this context, the ethanolic extract of *Withania somnifera* has shown promising epigenetic-modulating properties. Treatment of HeLa cells, a cervical adenocarcinoma cell line, with 20 μg/ml of this extract for six days resulted in the demethylation of the *RARβ2* gene promoter, restoring its expression. Such findings emphasize the potential of natural compounds to reverse DNA hypermethylation and reactivate critical tumor suppressor genes, offering therapeutic prospects for addressing epigenetic silencing in lung cancer [48].

DAPK is a key gene involved in pro-apoptotic signaling pathways and is frequently affected in lung cancer. Cancer cells are more likely to survive when DAPK is silenced by methylation, which inhibits apoptotic processes [49]. Research has demonstrated that the agressiveness of small cell lung cancer, which is characterized by a marked resistance to apoptotic cell death, is significantly influenced by hypermethylation of apoptosis-regulating genes [50].

Molecular Pathways Affected by Hypermethylation in Lung Cancer

Abnormal hypermethylation of tumor suppressor genes disrupts several biochemical pathways critical for maintaining cellular homeostasis[51]. Genes associated with DNA repair mechanisms, including MGMT, are often hypermethylated in lung cancer, compromising the DNA repair capacity of the cell and contributing to genomic instability [52]. Additionally, hypermethylation of apoptosis-related genes, such as DAPK, enables cancer cells to evade programmed cell death, thereby promoting tumor survival and progression [53]. This dysregulation also impacts cell cycle regulatory pathways; for instance, hypermethylation of the p16 gene, a key regulator of the cell cycle checkpoints—removes an essential control on cell division, thereby facilitating malignant transformation [54].

DNA Hypermethylation as a Biomarker in Lung Cancer

DNA hypermethylation patterns may serve as indicators for the lung cancer diagnosis and early detection [55]. Hypermethylated genes, including p16, RASSF1A, and DAPK, are commonly seen in the plasma and tissue of lung cancer patients and serve as non-invasive markers for early detection [56]. Additionally, evidence suggests that the hypermethylation status of these genes correlates with clinical outcomes, underscoring their potential prognostic significance [57]. Targeting DNA hypermethylation represents a promising therapeutic approach in lung cancer treatment. Demethylating agents, such as azacitidine and decitabine, inhibit DNA methyltransferases (DNMTs), thereby reversing hypermethylation and restoring tumor suppressor gene expression[58]. These agents are currently undergoing clinical investigation for solid tumors, including lung cancer, and have shown effectiveness in treating hematologic cancers. Demethylation could improve the effectiveness of conventional therapies and decrease resistance

by reactivating the key genes expression[59]. Despite the potential benefits, challenges remain, including toxicity, off-target effects, and the complex dynamics of the tumor microenvironment [60]. Comprehending methylation patterns in DNA among lung cancer has significant clinical implications. Hypermethylation of certain genes serve as indicators of cancer diagnosis, prognosis, and treatment choice. Methylation-based assays are used to detect cancer early and track the course of the disease. The reversibility of DNA methylation also makes it an attractive therapeutic target. Epigenetic drugs inhibiting DNMTs, such as azacitidine and decitabine, have shown promise in treating certain cancers by reactivating silenced tumor suppressor genes [61].

CONCLUSION

Epigenetic mechanisms, including DNA hypermethylation, HDAC activity, and miRNA regulation, play significant roles in lung cancer development by silencing tumor suppressor genes and altering cellular pathways. These changes are valuable as diagnostic and prognostic biomarkers, with genes like RASSF1A, p16, and DAPK highlighted for their potential in early detection. Epigenetic therapies, like HDAC and DNMT inhibitors, show promise in reversing these changes and enhancing conventional treatments. But problems including toxicity, side effects and methylation pattern variability still exist. Continued research is essential to overcome these barriers and develop more targeted, personalized treatments. Understanding and targeting these epigenetic mechanisms could greatly improve lung cancer management and patient outcomes.

Abbreviations

TSG- Tumor Suppressor Genes,
SCLC- Small Cell Lung Cancer,
NSCLC-Non-small Cell Lung Cancer

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