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EPIGENETICS (ATY LAU, SECTION EDITOR)

The Diagnostic and Therapeutic Potential of the Epigenetic Modifications of Lung Cancer–Related Genes

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Abstract
Lung cancer is characterized as a series of genetic mutations and epigenetic modifications, resulting in the silencing of tumor suppressor genes and activating of tumorigenic genes. In this review, we will explore those lung cancer–related genes that undergo significant epigenetic modifications. These genes include CDKN2A, DAPK, RASSF1A, FHIT, CHD13, DAL1, APC, RUNX3, CDH1, TSLC1, and PTEN. We will discuss the role of epigenetic modifications used as diagnostic biomarkers and therapeutic predictive biomarkers in lung cancer. The epigenetics of lung cancer have been widely studied in recent years. It will improve our understanding about lung cancer early detection and personalized treatment.

Keywords Lung cancer · Epigenetics · Methylation · Diagnostic biomarker · Predictive biomarker

Introduction

An Overview of Lung Cancer

Lung cancer is a predominant form of cancer and a leading cause of both cancer- and non-cancer-related mortality worldwide. Although lung cancer rates have declined over recent years, there were approximately 234,000 new lung cancer cases and 154,000 lung cancer deaths in the USA alone in 2018 [1]. Despite significant advances in cancer research, diagnosis, and treatment, lung cancer remains highly fatal regardless of the stage of diagnosis and retains a poor prognostic outcome, with the 5-year survival rate of only 5% [1]. While smoking remains the primary risk factor for the development of lung cancer, other risk factors include exposure to a variety of chemicals, including asbestos and radon [2].

The most predominant form of lung cancer is non–small cell carcinoma (NSCLC), which accounts for approximately 85% of all lung cancer cases. The other major form of lung cancer is small cell carcinoma, which accounts for the remaining 15% of lung cancer cases [3]. However, these two major classes of lung cancer also encompass a number of subtypes, including squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [2, 4].

The hallmarks of lung cancer include cellular, genetic, and epigenetic changes of the respiratory epithelium that lead to modifications of cell functions such as cell proliferation and apoptosis, as well as DNA repair [2]. Like other forms of cancer, somatic genetic mutations occur in lung cancer patients. However, epigenetic modifications account for the vast majority of the dysregulation which affects oncogenes and tumor suppressor genes. These epigenetic modifications play a significant role in lung cancer pathogenesis [3, 5].

An Overview of the Epigenetics of Lung Cancer

One of the earliest carcinogenic events in lung cancer pathogenesis is the silencing of tumor suppressor genes via DNA methylation, in a process known as hypermethylation [6]. Hypermethylation occurs as a result of the methylation of the promotor sequence of tumor suppressor genes, leading to its inactivation [3, 7]. The silencing of tumor suppressor genes is associated with the dysregulation of important cellular processes, including cell cycle and DNA repair. Through this dysregulation, pre-malignant and ultimately cancerous cells are formed.

DNA methyltransferases (DNMT) are enzymes which play a significant role in the early stages of lung cancer

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pathogenesis. These enzymes are responsible for the facilitation of promoter silencing, through the attachment of a methyl group, often to unmethylated CpG sites [8, 9]. Among the earliest tumor suppressor genes to be silenced in lung cancer is *CDKN2A* [10]. This gene is responsible for encoding tumor-suppressing proteins such as p16INK4a, which are important for cell cycle regulation [11]. The suppression of the *CDKN2A* gene can be seen in pre-malignant cells in lung cancer patients and this effect is known to progress as lung cancer stage increases, highlighting the importance of this gene in lung cancer pathogenesis [12]. Interestingly, the disruption of p16INK is a prevalent epigenetic modification in NSCLC but is considerably less common in SCLC [2, 3].

Many of the epigenetic modifications in lung cancer predominantly occur in genes responsible for the regulation of many cellular functions, which when dysregulated give rise to a carcinogenic phenotype. These genes include those associated with the regulation of cell proliferation and apoptosis, viz. *CDKN2A*, *DAPK*, *RASSF1A*, *FHIT*, *CDH13*, and *DAL-1* [13–15]; many of these genes also function as tumor suppressor genes along with *APC*, *RUNX3*, *CDH1*, *TSLC1*, and *PTEN* [14, 16, 17] which are also affected. *MGMT* which is associated with DNA repair and *hTERT* which is associated with cellular immortalization also undergo epigenetic silencing in lung cancer pathogenesis [13, 18].

As the understanding of the epigenetic modifications that occur in lung cancer continues to expand, attention is turning to many of these genes not only as potential therapeutic targets but also as diagnostic biomarkers. In this review, we will discuss how these genes have been and are continuing to be explored in both lung cancer treatment and diagnosis.

Epigenetic Targets for the Treatment and Diagnosis of Lung Cancer

CDKN2A

Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) is a member of the cyclin-dependent kinase (CDK) family. These proteins are responsible for the regulation of progression of the cell cycle. CDKs are regulated by their associated CDK inhibitor proteins, which act as tumor suppressor genes. *CDKN2A* encodes the protein p16INK4a, which functions as a cell cycle inhibitor [19]. *CDKN2A* is frequently deleted in a number of cancers, including lung cancer. More specifically, in work conducted by Tam et al. [20], *CDKN2A* was found to be inactivated in as many as 75% of NSCLC samples tested. These inactivations included mutations, deletions, and methylations of the protein [21]. The inactivation or deletion of *CDKN2A* has also been observed in SCLC-derived samples [22]. The prevalence of *CDKN2A* deletion, which frequently results in the subsequent loss of p16INK4a [19], makes *CDKN2A* an ideal candidate for both the treatment of and diagnosis of lung cancer.

Jeong et al. studied the CDK inhibitors flavopiridol and dinaciclib. These inhibitors were tested in vitro utilizing a *CDKN2A*-defective squamous cell lung cancer (SqCLC) cell line. When applied, the CDK inhibitors were found to exhibit anti-tumor effects and had the capacity to induce apoptosis, demonstrating the potential for CDK inhibition to normalize cell function in the absence of *CDKN2A* [23]. The use of CDK inhibitors has also been tested in human clinical trials. The CDK4 and CDK6 inhibitor palbociclib was recently tested in NSCLC patients in a phase II basket trial. In this trial, Ahn et al. [24] demonstrated that in NSCLC patients exhibiting *CDKN2A* mutation or loss, treatment with palbociclib was capable of inducing an anti-tumor response. These studies demonstrate that the effect of targeting CDKs in lung cancer has potential as a therapeutic strategy and the anti-tumor effects of this approach can be demonstrated in vitro, in vivo, and in human subjects with *CDKN2A* deletion or mutation. Although unsuccessful, phase II clinical trials targeting CDK inhibition in SCLC patients with *CDKN2A* mutations have also been conducted in recent years (ClinicalTrials.gov; NCT02688907).

DAPK

Death-associated protein kinase (*DAPK*) is a tumor suppressor gene associated with a number of crucial cellular processes including apoptosis [25]. Like *CDKN2A*, its function is commonly dysregulated in a number of cancers, usually as a result of the hypermethylation of the *DAPK* promoter [25, 26] and has been heavily implicated of having a significant role in lung cancer.

In a meta-analysis, *DAPK* hypermethylation was found to be evident in more than 40% of lung cancer cases [27]. The study revealed not only that *DAPK* methylation was higher in lung cancer samples than in normal lung tissue but also that *DAPK* methylation correlated with prognosis. Based on the prevalence of *DAPK* hypermethylation in lung cancer cases, DAPK presents a potential target for the diagnosis of lung cancer patients and further research is being carried out in this area.

RASSF1A

Ras-associated domain family 1A gene (*RASSF1A*) is another gene widely associated with lung cancer. *RASSF1A* is a tumor suppressor gene, the loss or modification of which has been associated with a number of cancers, including lung cancer [28]. *RASSF1A* dysfunction in lung cancer is the result of methylation in the promoter region of the gene, leading to loss of expression [29]. This loss of *RASSF1A* expression in lung cancer is seen in both NSCLC and SCLC cases, with both major forms exhibiting *RASSF1A* loss in 80–90% of cases [30, 31]. Hence, *RASSF1A* has potential as a treatment and/

or diagnostic biomarker for all major types of lung cancer. In a work conducted by Mengxi et al., the methylation of *RASSF1A* was analyzed in vitro using the A549 NSCLC cell line and the anti-cisplatin cell line. This work demonstrated that methylation of RASSF1A was significantly different in the NSCLC-derived cell line [32]. This highlighted the potential of RASSF1A as a potential biomarker in NSCLC diagnosis.

FHIT

The protein fragile histidine triad (FHIT) is a member of the histidine triad protein family and is encoded by the *FHIT* gene [33, 34]. FHIT is an important tumor suppressor protein which is believed to have a role in the regulation of cell cycle processes including apoptosis and the control of cell proliferation [34–37]. The silencing of *FHIT* is believed to be an early event associated with the tumorigenesis of a number of cancers, including lung cancer. This silencing is believed to be primarily caused by the hypermethylation of *FHIT*.

With respect to lung cancer, *FHIT* inactivation is more associated with NSCLC than with SCLC. In a meta-analysis of NSCLC patients, it was shown that *FHIT* hypermethylation was higher in NSCLC patient samples than in samples from normal lung tissues. Furthermore, although the hypermethylation of *FHIT* did not correlate with cancer stage, hypermethylation was associated with a poorer prognosis and survival outcome [38]. These results confirmed a significant role for *FHIT* in NSCLC cases, and identifying it as a potential diagnostic and/or therapeutic target.

The epigenetic silencing of *FHIT* has also been associated with the efficacy of cancer therapeutics. In a study by Andriani et al. [39] in vitro, the transfection of *FHIT* was able to improve the sensitivity of NCI-H460 NSCLC cells towards a number of cancer therapeutics including cisplatin. The work by this group highlighted the necessity of incorporating *FHIT* as part of any therapeutic approach for the treatment of lung cancer. In an in vitro approach to explore ways to normalize *FHIT* expression, Wu et al. demonstrated that the high expression of cMyc in lung cancer cells led to the reduction of microRNA-29, which in turn was responsible for the promoter methylation of *FHIT*. The group proposed that the application of cMyc inhibitors may be beneficial for preventing *FHIT* loss and improving outcomes in NSCLC patients, thus presenting a potential therapeutic approach for normalizing *FHIT* function in lung cancer [40].

CDH13

It is well-established that cadherin 13 (*CDH13*) acts as an anti-oncogene and that its polymorphisms are associated with cancer development [41]. *CDH13* is a member of the cadherin gene superfamily and its expression is reduced or absent in a variety of tumors. The hypermethylation of the gene promoter region is the chief cause of its downregulation. The downregulation of *CDH13* was correlated to a poor prognosis in lung cancer patients [42]. Consequently, *CDH13* re-expression can reduce tumor growth by inhibiting cell proliferation and invasiveness. Moreover, *CDH13* has been suggested as a potential early detection marker for lung cancer [43]. A meta-analysis by Pu et al. [44] strongly demonstrated *CDH13* promoter methylation status as a promising diagnostic biomarker for the diagnosis of non-invasive lung adenocarcinoma.

Wang et al. [45] established that *CDH13* promoter methylation regulates cisplatin resistance of A549/DDP NSCLC cells. Inhibition of *CDH13* methylation restored its expression and altered drug resistance of NSCLC cells. Therefore, this study clarified that CDH13 could serve as a molecular marker for predicting the efficacy of chemotherapy for NSCLC.

DAL-1

DAL-1 (differentially expressed in adenocarcinoma of the lung) inhibits metastasis of NSCLC cells by attenuating epithelial-mesenchymal transition. *HSPA5* (heat shock protein 5) gene, which promotes tumor progression, directly binds to *DAL-1* [46]. *HSPA5* induces epithelial-mesenchymal transition through activation of the phosphatidylinositol 3-kinase/Akt pathway in NSCLC cells [47]. Qiu et al. [48] demonstrated that *DAL-1* is downregulated and *HSPA5* is upregulated in NSCLC. They demonstrated that *DAL-1* inhibits metastasis by downregulating *HSPA5*. Furthermore, they discovered that *DAL-1* inhibits the phosphatidylinositol 3-kinase/Akt/Mdm2 signaling pathway by suppressing *HSPA5*. Interestingly, Cai et al. [49] investigated the possible microRNAs of *DAL-1* using bioinformatics and concluded that microRNA-26a regulates the expression levels of annexin A1 (a *DAL-1* associated protein) but not DAL-1.

APC

Adenomatous polyposis coli (*APC*) gene encodes a tumor suppressor protein which is involved in cell cycle regulation, adhesion, and apoptosis [50]. It is mutated in numerous cancers and is a well-known regulator of β-catenin and WNT signaling [51–53]. In SCLC cells, activation of WNT signaling via *APC* knockdown induces chemoresistance. Wagner et al. [54] demonstrated that SCLC cells could be re-sensitized to chemotherapy through overexpression of wild-type *APC* devoid of untranslated regions (UTRs). In addition, they induced etoposide resistance and activation of WNT signaling through CRISPR/Cas9-mediated deletion of *APC* in the H82 SCLC cell line. *APC* promoter 1A hypermethylation has been reported in a range of cancers, including NSCLC [53]. A meta-analysis by Hu et al. [55] revealed that *APC* promoter 1A methylation plays a vital role in NSCLC carcinogenesis.

RUNX3

Runt-related transcription factor 3 (*RUNX3*) was established as a target of microRNA-301a and a regulator of epithelial-mesenchymal transition [56]. *RUNX3* overexpression in epithelial cells inhibits epithelial-mesenchymal transition, while the loss of *RUNX3* has the opposite effect [57, 58]. Furthermore, *RUNX3* activates the $p14^{ARF}$-p53 pathway to inhibit *Kras*-induced lung adenoma formation [59]. Li et al. [60] demonstrated that microRNA-301a promotes lung tumorigenesis via suppressing *RUNX3*. Deficiency of microRNA-301a in lung tumor cells reduces tumor metastasis by elevating *RUNX3* expression, while microRNA-301a knockdown reduces tumorigenesis. *RUNX3* promoter hypermethylation has been found to be associated with the regulation of alveolar differentiation and epithelial tumorigenesis [61, 62]. A meta-analysis by Xu et al. [63] revealed that *RUNX3* hypermethylation is correlated with a higher risk and lower survival rate in NSCLC and hence plays a significant role in carcinogenesis and clinical outcome. Chen et al. [64] established that loss of expression of *RUNX3* predicted worse outcomes in NSCLC and could be utilized as a prognostic biomarker in predicting in NSCLC patients.

CDH1

CDH1 (cadherin-1), a cell-cell adhesion glycoprotein, is encoded by the *CDH1* gene [65]. *CDH1* as a tumor suppressor gene is involved in the maintenance of cell adhesion and adherent junctions. Its expression is usually absent in various epithelial tumors and leads to metastasis [66]. A meta-analysis by Yu et al. [67] validated the association between *CDH1* promoter hypermethylation and NSCLC. *CDH1* hypermethylation plays a key role in carcinogenesis and could be exploited as a potential prognostic biomarker in lung cancer [68]. Gao et al. [69] demonstrated that silencing of long-chain non-coding RNA H19 inhibits epithelial-

Table 1 Current clinical trials on combination of epigenetic therapies with other therapeutic agents for lung cancer

ClinicalTrials.gov identifier	Phase	Cancer type	Epigenetic drugs	Other drugs
NCT00602030	II	Advanced NSCLC	Entinostat	Erlotinib
NCT00635791	I	NSCLC	Vorinostat	Sorafenib
NCT00251589	I/II	Relapsed/refractory NSCLC with *EGFR* mutation	Vorinostat	Erlotinib
NCT00481078	II	Advanced/metastatic NSCLC	Vorinostat	Carboplatin + paclitaxel
NCT01628471	II	Advanced NSCLC	5-Fluoro-2′-deoxycytidine	Tetrahydrouridine
NCT00473889	I/II	Advanced NSCLC (stage IV), not priorly treated with chemotherapy	Belinostat	Carboplatin, paclitaxel, and bevacizumab
NCT00978250	I	Metastatic or locally advanced NSCLC	Vorinostat	Gemcitabine + platinum
NCT01090830	I/II	NSCLC	Belinostat	Erlotinib
NCT01928576	II	Recurrent metastatic NSCLC	Azacitidine	Nivolumab
NCT02959437	I/II	Advanced solid tumors	Azacitidine INCB059872 INCB057643	Pebrolizumab Epacadostat
NCT03220477	I	Advanced NSCLC	Guadecitabine Mocetinostat	Pembrolizumab
NCT03085849	I	Extensive-stage SCLC	SGI-110	Durvalumab Tremelimumab
NCT03233724	I/II	Inoperable locally Advanced/metastatic NSCLC	Decitabine Tetrahydrouridine	Pembrolizumab
NCT02998567	I	Refractory solid tumors	Guadecitabine	Pembrolizumab
NCT02635061	Ib	Unresectable NSCLC	Citarinostat	Nivolumab
NCT02546986	II	Advanced/metastatic NSCLC	Oral azacitidine	Pembrolizumab
NCT02805660	I/II	Advanced/metastatic solid tumors	Mocetinostat	Durvalumab
NCT02664181	II	Metastatic NSCLC	Oral decitabine Tetrahydrouridine	Nivolumab
NCT02638090	I/II	Advanced NSCLC	Vorinostat	Pembrolizumab
NCT02909452	I	Advanced solid tumors	Entinostat	Pembrolizumab
NCT02954991	II	Advanced/metastatic NSCLC	Mocetinostat	Nivolumab Glesatinib Sitravatinib

NSCLC, non–small cell lung cancer; *SCLC*, small cell lung cancer

mesenchymal transition and proliferation while augmenting apoptosis of lung adenocarcinoma cells via inhibition of *CDH1* promoter methylation. In another study, Liu et al. [70] showed that *CDH1* acts as a microRNA-25 mediator in NSCLC progression and cell migration.

TSLC1

Tumor suppressor in lung cancer 1 (*TSLC1*) has been associated with cancer progression and its expression is reduced or absent in most NSCLC cases [71, 72]. Interestingly, re-expression of *TSLC1* occurs after treatment of NSCLC cells with 5-aza-2′-deoxycytidine. Elevated expression of *TSLC1* inhibits NSCLC cell proliferation, migration, and invasiveness [73]. Reduction or loss of *TSLC1* expression is also observed in SCLC cell lines [15]. In a significant number of NSCLC cases, *TSLC1* expression is reduced or lost by promoter hypermethylation [73, 74]. Thus, methylation of *TSLC1*, leading to loss of its expression, is a key event in the pathogenesis of NSCLC.

PTEN

PTEN (phosphatase and tensin homologue), a tumor suppressor gene, negatively regulates the phosphatidylinositol 3-kinase/Akt signaling pathway. Phosphorylated Akt is overexpressed and is associated with the loss of *PTEN* in NSCLC cells, which results in poor prognosis [75]. The *PTEN*/phosphatidylinositol 3-kinase/Akt pathway is an oncogenic pathway which modulates cell proliferation, motility, invasiveness, and intracellular trafficking [76]. microRNA-21 downregulates the expression of *PTEN* and stimulates the proliferation and invasion in NSCLC [77]. Also, *PTEN* hypermethylation is not a strong predictor of PTEN protein expression. Using small molecule inhibitors which inhibit *PTEN* phosphorylation is a potential strategy to activate *PTEN* function in lung cancer [78]. *PTEN* inactivation has been correlated with resistance to treatment with EGFR-tyrosine kinase inhibitors as well as with lower survival in NSCLC patients [79]. Hence, *PTEN* status could be exploited to tailor combination therapies for lung cancer patients with *PTEN*-deficient tumors or with high levels of phosphorylated *PTEN*.

Translation of Epigenetic Knowledge Into Clinical Practice

Along with being major events during lung cancer development and progression, epigenetic alterations may also

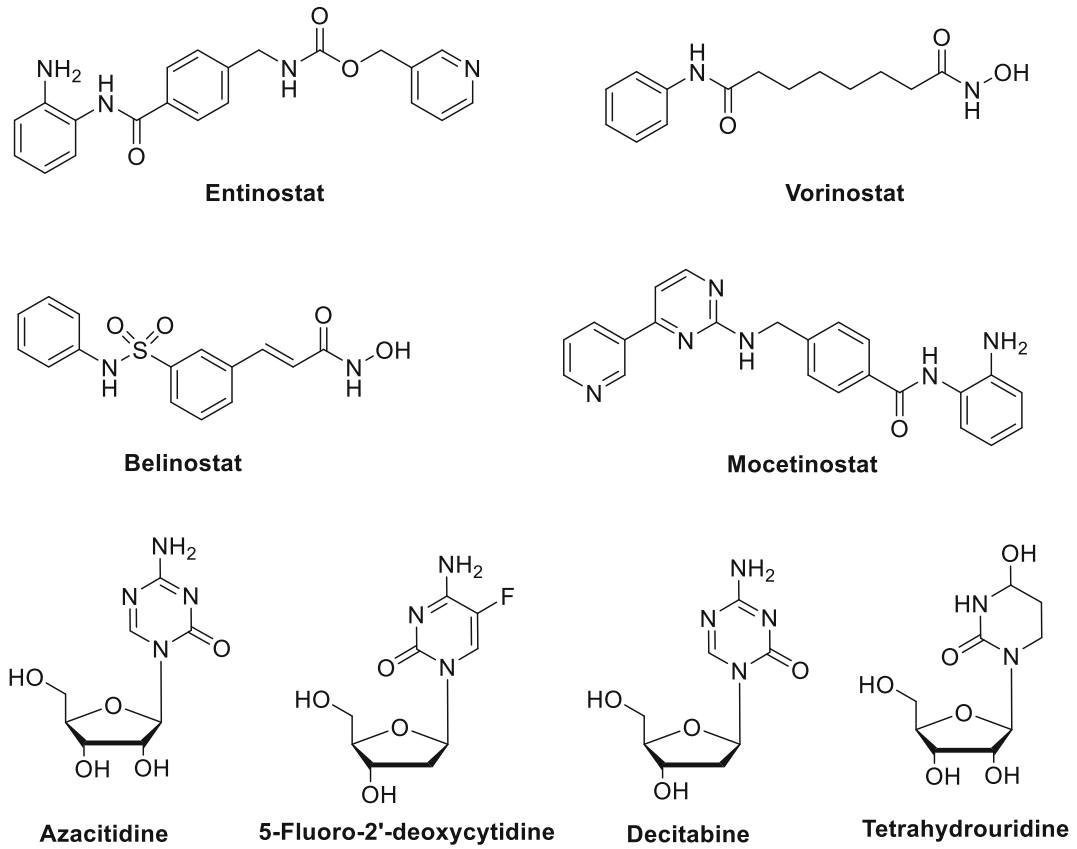

Fig. 1 Examples of epigenetic drugs for targeting lung cancer

play a crucial role in the development of therapeutic approaches. Numerous epigenetic biomarkers have been reported as predictors of chemoresistance. In addition, epigenetic inactivations of tumor suppressor genes have been projected to be responsible for chemoresistance. For instance, *GSTP1* and *RARβ2* are downregulated through methylation in lung squamous cell carcinoma and adenocarcinoma, while the *IGFB3* promoter is hypermethylated in cisplatin-resistant lung cancer cells [80, 81]. The unmethylated status of *OCT4* and *SLUG* has been negatively correlated with their expressions in chemoresistant NSCLC cells in vitro [80]. Epigenetic therapies can be classified into epigenetic regulators, targeting writers, erasers, or readers. In spite of their specificity and low toxicity, these agents display limited efficacy when used as monotherapy. Hence, recent efforts are aimed at the evaluation of the efficacy of combination therapies (Table 1). Some of the epigenetic drugs currently under investigation for lung cancer are presented in Fig. 1.

Conclusions and Future Perspectives

Despite considerable progress with respect to prognosis and mortality for many other forms of cancer, lung cancer remains a leading cause of cancer-related deaths. The reason for this is partially related to the late-stage diagnosis for many patients, rendering many treatment options limited in their efficacy. The importance therefore of cutting-edge developments in the area of lung cancer–related epigenetic modifications cannot be understated. By furthering advances in this field, hopefully more reliable and expedient diagnosis can be made and treatments developed with efficacy at a wide range of cancer stages. Additionally, application of high-throughput epigenetic screening methods could facilitate the discovery of prognostic epigenomic biomarkers. Combinations of epigenetic drugs with chemotherapy or immunotherapy are currently being investigated in clinical trials. In the future, combining data from genomics, transcriptomics, and epigenomics may aid the discovery of epigenetic therapeutic targets and offer novel avenues for improving survival outcomes in lung cancer patients.

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Compliance with Ethical Standards

Conflict of Interest No conflict interests declared.

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- • Of importance
- •• Of major importance

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