



Epigenetics of Lung Cancer

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Abstract

Lung cancer is the biggest cause of cancer-related death, with a 5-year survival rate of just 18%. Thanks to recent developments in targeted pharmacological medications and immunotherapies, the survival rates of some patients have increased considerably. However, patients are usually only provided standard chemotherapy, which has a history of providing mediocre outcomes in the clinic. Accordingly, innovative methods of therapy are urgently required. Recent advances in epigenetic assessments and their use in research of cancer have shown the significance in the regulation of epigenetic lung cancer's progression, development, initiation, and treatment. There are several epigenetic modifications that occur in lung cancer at different phases of progression, and some of them are essential for tumour growth. Improved consideration of the natural science behind lung cancer growth and the facilitation of the creation of novel treatment options are both dependent on the continued development of state-of-the-art technologies like single-cell epigenomics. As a consequence of this strategy, treatments that use either a single medication or a combination of medicines to target epigenetic modifiers have been created and are now being explored in clinical trials. In this chapter, we discuss the function of epigenetics in lung cancer at different stages of the disease and how this knowledge is being used in clinical practice.

Keywords

Lung cancer · Oxidative stress · Epigenetics · Inflammation · Therapy

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7.1 Introduction

Lung cancer is the foremost cause of death due to cancer worldwide, with only around an 18% 5-year survival rate. In the majority of instances (80%), lung cancer is fatal if not caught early [1]. There have been only slight improvements in survival rates as a consequence of the therapy. About 220,000 instances of lung and bronchus cancer are diagnosed each year in the United States, and use of tobacco is the major cause. The two most frequent types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (non-SCLC) (NSCLC). NSCLC accounts for around 85% of all lung cancer diagnoses; its subtypes include squamous cell carcinoma (40%), adenocarcinoma (40%), and giant cell carcinoma (10%). SCLC accounts for around 15% of lung cancer diagnoses [2, 3]. The histologic classification of LC has been revised in light of the development of novel targeted therapies and chemotherapy regimens. Variations in kinases like HER2, BRAF, EGFR, ALK, RET, ROS1, and NTRK have been associated with therapeutically relevant adenocarcinomas. When it comes to treating SCLC, PI3K, FGFR1, and DDR2 inhibitors have shown very modest efficacies in clinical trials [4]. Immune frontier drugs have newly also shown groundbreaking results in the treatment of lung cancer, with some patients seeing durable responses. However, the wide variety of the condition means that even with these advancements, a single, effective treatment method cannot be applied to everyone. For this reason, specialised analyses of molecules are essential to decipher the complex network of relevant clinical phenotypes that regulate lung cancer growth, to define effectual methods of diagnostic for initial lung cancer recognition, and to advance novel approaches to increase clinically beneficial efficiencies for lung cancer [5]. Developments in high-throughput and high-resolution molecular technologies, our considerate mechanisms of molecular study of causal lung cancer progression, especially the complexity of its driving variables, have increased. Epigenetic alterations, which regulate gene expression and preserve genomic integrity, have been linked to lung cancer and may play a significant role in the disease's progress. In this chapter, we concentrate on lung cancer and address the potential therapeutic and diagnostic applications of epigenetic (de)regulation in the clinic [6, 7] (Fig. 7.1).

7.2 Cancer Epigenetics

Without changing the DNA sequence itself, epigenetic mechanisms allow for the guideline of gene expression and genome steadiness. Examples of alterations that may work together include modification of histone, nucleic acid methylation, chromatin remodelling, and changes in the production of non-coding RNAs. Many illnesses and disorders have been connected to epigenetic changes that are passed down via families [8, 9]. In a typical environment, DNA methylation is a strictly regulated process. This process, which occurs at cytosines and especially at CpG nucleotides and is often seen in gene regulatory elements, is mediated by the enzymes DNA methyltransferases (DNMTs) and demethylases. However, a

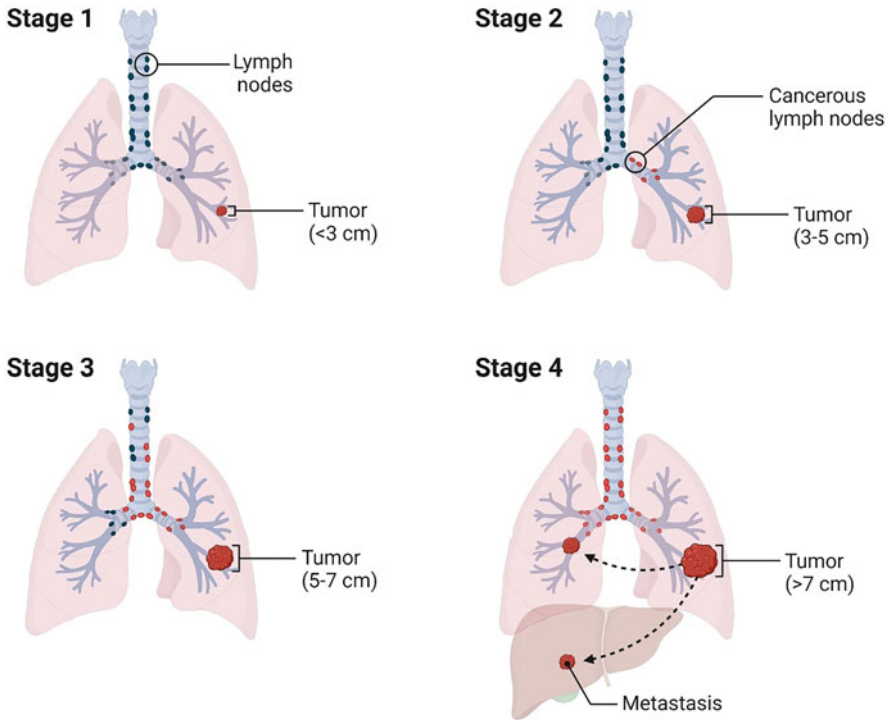


Fig. 7.1 Lung cancer stages

disturbance in DNA methylation equilibrium in tumour tissue may lead to modification of epigenetic key regulatory sections linked with transforming gene or tumour suppressor genes [10, 11]. Malignancies may have a more prominent hypo- to hypermethylation spectrum than noncancerous tissues. Hypomethylation has been related to a wide variety of pathologies, including chromosomal instability and aneuploidy, imprinting loss, transposon reactivation, and most importantly, oncogene activation. In contrast, DNA hypermethylation, the opposite process, may have dual roles depending on its position in the human genome [12]. Numerous tumour suppressor genes have been shown to be silenced by hypermethylation in CpG-rich regions inside gene promoters. Indirectly or directly, DNA hypermethylation may bring in complexes of protein affinity which is high for DNA methylation, which can decrease transcription factor binding. This might lead to the recruitment of chromatin remodellers like histone deacetylases and methylases that may result in the genes silencing like tumour suppressor genes or RNAs non-coding that have been linked to malignant transformation [13]. Increased expression of a gene could be correlated with high levels of methylation not only in the promoter region but also in the gene body. The fascinating potential is raised by our findings that hypermethylation of gene bodies may increase oncogene production [14]. Potentially, the state of chromatin is vigorously changed by a system of proteins with a wide variety of modes of

action. Eukaryotic chromatin is composed mostly of nucleosomes, which communicate with histone binding proteins. Histones may be covalently changed to adjust their interface with DNA and so recruit histone modifiers to alter chromatin structure, mute or activate genes, or do both. The glycosylation, phosphorylation, and ubiquitination of serine, arginine, and threonine remains are a few more examples. Other modifications include histone methylation and lysine acetylation. While chromatin remodelling involves a large number of proteins and chemical complexes, cancer researchers have only studied a small portion of those factors [15]. Histone deacetylases (HDACs) and polycomb repressive complexes (PRCs) may both inhibit gene expression in ways unrelated to their enzymatic activity by altering histones or by directly compacting chromatin. Two other examples of chromatin remodelling with a greater relevance for cancer are the variants of histone, which can replace the canonical core of histones in nucleosomes and confer diverse functional and structural possessions that may distress chromatin compaction, and Switch/Sucrose non-fermentable developments, which can modify expression of gene patterns by repositioning nucleosomes and chromatin remodelling [16, 17]. Cancer also has a strong epigenetic component, which is noncoding RNAs. These RNAs do not get translated into proteins because they serve other functions. There is rising sign that non-coding RNAs (ncRNAs) play an important role in pathological situations like cancer, where a number of ncRNAs have been identified as oncogenic drivers or tumour suppressors. Both the transcriptional and post-transcriptional stages of gene expression are susceptible to regulation by ncRNAs. MicroRNAs (miRNAs) are small RNAs that target mRNAs for destruction, hence lowering gene target expression, while non-coding long RNAs interrelate with modifying chromatin enzymes and remodelling of chromatin factors, altering their activity [18]. In addition, scientists' focus on modifications of epigenetic RNA molecules is growing. Changes like cytosine or adenine methylation are only two of the numerous known variations that may influence the stability, translation, localization, splicing, and/or targeting of RNA. These changes may play a role in carcinogenesis by amplifying or dampening tumour characteristics like invasion and proliferation [19, 20] (Fig. 7.2).

7.3 Epigenetics of Lung Cancer

Genome-wide investigations of huge number of patient associates have shown the relevance of epigenetic changes in many cancer types, most notably lung cancer. Lung cancers are among those most often affected by the high mutation frequency in SWI/SNF and HDAC gene components (about 20%). Tobacco use is the primary cause of lung cancer deaths, and these changes might be related to this behaviour [21]. Further evidence suggests that smoking causes far-reaching impacts on DNA methylation, which seem to be long-lasting even after stopping. Changes of methylation in disorder-related genes has been seen in complete DNA blood samples from lung cancer patients. Several genes, including the aryl hydrocarbon repressor receptor and F2R like trypsin receptor 3 or thrombin, have had their methylation levels reduced in people who smoke and are at a higher risk of growing lung cancer, as

The Lung Tumor Immune Microenvironment

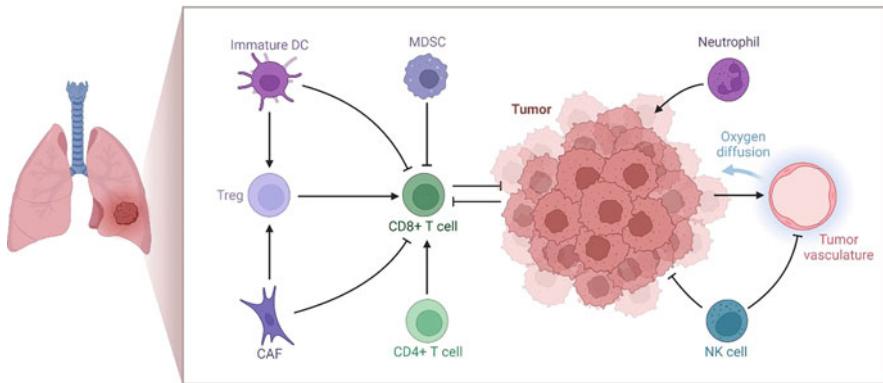


Fig. 7.2 The lung tumour immune microenvironment

shown by blood testing (F2RL3) [22]. Bossé et al. found a significant association between alterations in the expression of AHRR in non-tumour tissues of lung and cigarette smoking. Research by others has shown that BECs derived from lung cancer patients exhibit hypermethylation of the p16 and, to a lower extent, the death-associated protein (DAP) kinase promoters. This abnormal methylation occurred in BECs from present and previous smokers who did not develop lung cancer, suggesting a connection between tobacco use and cancer [23]. Certain expression of miRNA patterns in BECs have also been accompanying with cigarette smoking, perhaps because to the presence of intrinsic toxins in cigarettes. DNA methylation at individual CpG sites may have a dose- and cell-type-specific association with smoking; therefore, these results should be interpreted cautiously [24].

7.4 Epigenetics of NSCLC

Many studies have looked at how epigenetic events and interactions with tumour microenvironment (TME) affect the development and progression of NSCLC [25].

7.5 Epigenetics of NSCLC Tumour Initiation

The downstream fate-specifying of cell and other factors of transcription may contribute to tumour initiation, as shown by multi-omics investigations. The most common cause of lower gene expression is hypermethylation. The homeobox genes HOXA4, HOXA2, and NKX2-1, along with other factor of transcription genes, including GATA2 and ZNF132, were demonstrated to have a role in the discovery of possible tumour gene suppressor for SCLC [26]. The hypermethylation of genes

including ZEB2, KCNIP4, and FOXF1 was found in lung cancer. Also, transcription factor genes hypermethylation like HOXA5, TAL1, FOXJ1, FOXA2, and HLF has been seen in both types of lung tumours COPD is a risk factor for lung cancer, and the hypermethylation of certain of these genes has been associated to COPD as well. Multiple additional epigenetic mechanisms besides hypermethylation have also been associated with lung cancer [27]. Overexpression of DNMT3A, DNMT1, and DNMT3B, for instance, has been associated to the onset of lung cancer. These enzymes have been demonstrated to contribute to NSCLC risk by inducing methylation errors in DNA and chromatin remodelling at an early stage in the disease's progression. When DNMT1 is upregulated during the onset of lung squamous cell carcinoma, tumour suppressor genes including RASSF1A, which is intricate in RAS signalling, and CDKN2A, a cyclin-dependent kinase blocker intricate in arrest of cell cycle at the G1/S phase, are silenced by hypermethylation (LUSC) [28, 29]. Also, it has been found that in lesions of preneoplastic squamous histology, aberrant promoter methylation affects the expression of multiple genes, including FHIT, whose degradation is associated with invasiveness and proliferation, and miR47b, a tumour suppressor whose downregulation is linked with tumour growth via the stemness-related Wnt pathway. The involvement of chromatin-remodelling complexes in LUSC development has also been identified. Aberrant chromatin remodellers reduce levels of H4K20me3 and elevate EZH2 expression leading to Silencing of tumour suppressor miRNAs [30]. Lung cancer has been connected to the downregulation of genes involved in apoptosis (DAPK), immortalization (hTERT), insulin metabolism (PTPRN2), and DNA repair (MGMT). The preneoplastic lesions of this histologic category have been reported to express CDKN2A, p741, RASSF1A, and many Wnt pathway antagonists [31, 32].

7.6 Epigenetic Determinants of NSCLC Progression and Metastasis

Aggressive NSCLC has been associated to changes in DNA methylation throughout the whole genome. Teschendorff et al. discovered a novel methylation of DNA pattern in cells of buccal that correlates with smoking and has the potential to be used to differentiate between advanced and lung in situ cancers. In lung cancer, hypermethylation was shown to downregulate 164 genes involved in differentiation, EMT, and cell cycle progression, whereas hypomethylation upregulated 57 genes. An epigenomic signature indicative of invasive lesions was identified by Teixeira et al. among 12,064 differentially methylation sites associated with 2695 genes in squamous cell carcinoma. In pro-metastatic phenotypic samples, many members of the homeobox family, including NKX2-1, were shown to exhibit hypermethylation and, as a result, to have reduced expression among the identified impacted genes [33, 34]. Others have recognized potential targets of key methylation associated with the acquisition of an invasive phenotype in NSCLC, including PTGDR, MAGE family members, FBP1, CDO1, and AJAP1, and alterations in

the methylation pattern of PGC-related coactivator (PRC) members of family involved in regulation of cell cycle, invasion, and proliferation [35].

The epigenetic regulation of miRNA expression has been implicated as a cause of NSCLC. Highly aggressive NSCLC has been related to oncogenic miRNAs like miR-135b, which target components of the Hippo pathway and de-methylate their promoters. For instance, the miR-200 family controls EMT in NSCLC by targeting various effectors of this process such as miR-132, GATA3, ZEB1, and miR-149, which control FOXM1 and ZEB2. Thus, these miRNAs suppress invasion, migration, and metastasis in NSCLC by inhibiting the mesenchymal conversion process [36, 37].

NSCLC invasion and metastasis have been linked to the overexpression of certain epigenetic “writers” and “erasers” involved in chromatin remodelling. Proteins like H3K36 demethylase activating mitogen-activated protein kinase (KDM2A), H3K9 methyltransferase inducing Wnt (SETDB1), and H3.3 histone variant encoding (H3F3A) are all examples [38]. Mutations in BAF and PBAF, which are also components of SWI/SNF chromatin-remodelling complex of the human, have also been associated with NSCLC. There is evidence that mutations in the SMARCA4 gene contribute to tumour growth through altering the expression of other genes in the body [39].

Numerous lncRNAs have been identified to have tissue-specific expression patterns; several of them are downregulated in lung tumours. Genes like RCC2 and LCAL1 and KPNA2 have been connected to the growth and metastasis of lung tumours, while ENST00000439577 and LOC146880 have been correlated with the expression of these genes [40]. Other lncRNAs have been discovered to have a role in metastasis of lung cancer and/or EMT, in addition to HOTAIR and MALAT1, which produce a pro-metastatic gene expression profile. EZH2 downregulates a number of genes that play a role in modulating EMT, including BANC1 and, most significantly, SPRY4-IT1, which does so via activating E-cadherin and repressing vimentin. Metastasis of lung cancer and EGFR-based therapy resistance has been related to the BC087858, lncRNAs, UCA1, and GAS5, perhaps via the activation of Akt signalling. Both EMT and treatment resistance may be influenced by these lncRNAs [41, 42]. Several epigenetic mechanisms have been associated with treatment resistance in NSCLC. It has been demonstrated, for example, that a drug-tolerant subset of NSCLC cells dependent on anti-EGFR treatment requires a KDM5A demethylase-induced altered chromatin state for survival of histone [43].

7.7 Epigenetics of Interactions Between NSCLC Cells and the TME

Numerous researches have been conducted to determine what role epigenetics plays in the interaction between cancer cells and the tumour microenvironment (TME). Researchers have discovered that connections between tumours and TMEs, which may be produced epigenetically, contribute to the development of lung tumours [44]. For instance, in NSCLC, miRs targeting TIMP3, a protein involved in the

regulation of cytokines and growth hormones, are considered to contribute to tumour development by downregulating their expression. It is possible that TME, such as prostaglandin, will be found to have a role in the body. The overexpression of c-Myc in stromal cells is controlled by prostaglandin, which in turn affects the synthesis of the miR-17-92 cluster, which inhibits apoptosis in NSCLC tumour cells by targeting the tumour suppressor PTEN [45]. Other connections between tumours and TMEs have been identified to promote angiogenesis as well. There is an uptick in the expression of let-7b and miR-126 in cancer cells and their surrounding tissue. Similar to this, cancers may have the capacity to change the TME in ways that promote spread [46]. The interaction between a tumour and its TME may have a tumour-suppressing impact in certain situations. Exosomes released from lung tumours inhibited invasion and metastasis by decreasing ICAM, TME IL-8, and CXCL1 production, according to one research [47].

Multiple lines of evidence suggest that epigenetics plays a role in the control of antitumour immune responses in lung cancer. It has been shown that downregulating the DAP12 expression, a crucial transduction of signal receptor in NK cells, is one way in which TGF induces the miR-183 release from lung cancer cells, hence reducing the antitumour cytotoxic activity of NK cells. A second miRNA, miR-9, is upregulated in lung cancer and inhibits the immune system from recognising downregulating tumour cells by major histocompatibility complex (MHC) class I gene [48, 49].

7.8 Epigenetics of SCLC

Epigenetic changes have been hypothesised to have a role in the growth of SCLC. Neuroendocrine markers like NEUROD1 have been shown to be present in 75% of instances of SCLCs, which distinguishes SCLCs from other kinds of lung cancer. Samples of SCLC with very similar histological and genetic features have been shown to cluster with varied DNA methylation and gene expression, indicating that these biomarkers may be helpful for discriminating among SCLC subtypes [50]. The tumour-suppressor genes methylation is common in small cell lung cancer. Hypermethylation of the RASSF1A promoter was seen in almost all SCLC tumours, suggesting a function for this mechanism in tumour development. The DAPK tumour suppressor gene is methylated in 30% of patients with SCLC. RASSF1A promoter and DAPK tumour suppressor are only two examples of genes that play a role in signalling pathways linked with death receptor-mediated apoptosis [51].

Besides methylation-level anomalies, changes in chromatin remodelling enzymes have been detected in SCLC, signifying that they may play a key role in the tumour's progression. Lung neuroendocrine tumours like SCLCs have been shown to lose H4 methylation, which has been associated to increased proliferation. It has also been shown that several mutations exist in the genes that code for remodelling enzymes [52]. The MLL2 gene has the most frequent somatic mutations (8% of SCLC tumours). These modifications are associated with dysfunctional enhancers, which in turn cause genes to become dormant. EP300, CREBBP, and KAT6B are histone

acetyltransferases; ARID1A, PBRM1, and ARID1B are chromatin remodelling factors; and inactivating mutations in these genes have been observed less often. Researchers have also shown that chromatin remodellers are expressed abnormally in these malignancies, in addition to the mutations already known to be present [53, 54]. In a subset of SCLC tumours, overexpression of the histone methyltransferase EZH2 accelerates E2F-driven carcinogenesis, and PCR2-related protein is increased. ASXL3, has been found in main SCLC tumours and is linked with enhanced cell lines growth of SCLC [55].

In the latter phases of small cell lung cancer growth, epigenetic changes may potentially play a role. Nfib, discovered in a recent research to have a role in increasing chromatin accessibility in several intergenic areas and in inducing neuronal gene expression programmes that drive the metastatic capacity of SCLC cells, is a transcription factor. Overexpression of MYCL, a member of the EMT-involved MYC family, is common in SCLC, and has been shown to be regulated by great enhancers and dependent on acetylation of histone [56, 57].

Although chemotherapy is the backbone of care for treating advanced SCLC tumours, drug resistance may develop quickly in certain patients. It has been suggested that one epigenetic modification causing treatment resistance in this collection of cells is the overexpression of EZH2, a DNA damage repair protein that is connected to the silencing of SLFN11 chemoresistance [58].

7.9 Translation of Epigenetic Knowledge to Clinical Practice

The development and course of lung cancer may be influenced significantly by epigenetic alterations, which might potentially be vital in the progress of successful treatments. It has been hypothesised that the epigenetic inactivation of specific tumour suppressor genes lies at the heart of chemoresistance, and many biomarkers of epigenetic have been found as possible predictors of chemo-resistance [59]. Through methylation, GSTP1 and RAR2 expression is silenced in SCLC and adenocarcinoma cells. Not only that, but the IGFB3 promoter in cisplatin-resistant lung cancer cell lines is hypermethylated. One such cell is the cancer stem cell (CSC), which, if it survives therapy, may repopulate the tumour and cause resistance. Among stem-related genes, unmethylated expression is inversely correlated with treatment resistance in NSCLC cells in vitro for OCT4 and SLUG [60, 61].

It is possible to classify therapeutic strategies for epigenetic regulation as either focusing on writers, focusing on erasers, or focusing on readers. To reactivate silenced tumour suppressor genes or revive production of tumour suppressor proteins, researchers have created DNMTi and histone-modifying enzyme inhibitors for the first category [62]. Tumour cells are more effectively arrested in their growth cycle, induced to undergo apoptosis, and differentiated when treated with either kind of inhibitor. Two types of DNMTis are used in research that are nucleoside analogues and non-nucleoside analogues. AZA and Decitabine, two cytidine nucleoside analogues, belong to the first group and work by inducing DNA hypomethylation and inhibiting DNMTs [63]. Both the Food and Drug

Administration and the European Medicines Agency have approved these drugs for the treatment of myelodysplastic syndrome (MDS). AZA was the first treatment to prolong life expectancy in people with MDS. The effectiveness of decitabine in treating acute myeloid leukaemia is also acknowledged by the European Union [64]. Downregulating the epigenetic miR-200/Z1EB axis with decitabine prevented TGF-1 from inducing epithelial-mesenchymal transition and mortality in lung cancer preclinical trials, as described by Zhang et al. In NSCLC, however, both decitabine and AZA have been demonstrated to be somewhat ineffective when administered alone. Promising results have been shown with the use of zebularine and other DNMTis since they provide a less risky option to conventional therapy. Highly selective for cancer cells yet harmless to healthy tissue, zebularine is a potent cytidine deaminase inhibitor [65]. In studies employing adenocarcinoma-derived cell lines, zebularine was shown to induce cell death by lowering intracellular reactive oxygen species (ROS) and increasing glutathione levels, and to halt cell development by causing a cell cycle arrest. Several current clinical trials are evaluating the finished chemical for its effectiveness against various cancers [66]. NCT01696032 is for patients with advanced SCLC, while NCT02131597 is for patients with leukaemia (NCT03085849). There have been five studies (NCT01534598, NCT01479348, NCT00359606, NCT01041443, and NCT00978250) looking at the effects of 5-fluoro-2'-deoxycytidine (FdCyd) on patients with solid tumours, acute myeloid leukaemia, and myelodysplastic syndromes [67].

These drugs do not deliver the best therapeutic outcomes when taken alone, despite their targeted nature and lower toxicity. That's why researchers are now interested in gauging the cumulative benefits of several therapies. Inhibitors of poly (ADP-ribose) polymerase (DNMTis) and PARP1 have been demonstrated to enhance chromatin PARP1 binding in myeloid leukaemia and breast cancer triple-negative. Adding the Bcl-2 inhibitor venetoclax to decitabine or AZA has been shown to increase patients response with relapsed/refractory and formerly untreated severe myeloid leukaemias [68]. The combination of azacitidine with entinostat, on the other hand, has not been found to enhance the efficacy of chemotherapy in any clinical trials. In the beginning, irinotecan showed promising results in xenograft models taken from patients with lung cancer. NSCLC patients in stages IIIb and IV have a new therapeutic option according to the publication of research data on the combination of decitabine and genistein (NCT01628471). It has been postulated that genistein's anticancer activity may be attributable, in part, to its ability to decrease DNMT expression. The combination of AZA and erlotinib showed encouraging effectiveness in treating erlotinib-resistant lung cancer [69, 70].

HDAC are a family of drugs that have been shown to be effective against both solid tumours and blood malignancies. There are several different kinds of HDACis, including benzamides, cyclic peptides, hydroxamic acids, fatty acids, and electrophilic ketones. In contrast, trials with single HDACis have shown either poor replies or transient effects, and these drugs may be rather dangerous. In an in vitro model of lung tumour growth, the cyclin tetrapeptide romidepsin was able to restore wild-type gene expression [71]. It has been postulated that romidepsin acts by inhibiting the

overexpressing p21Waf1/Cip1, PI3K/Akt pathway, and lowering Rb phosphorylation. The findings of animal research on romidepsin for SCLC were encouraging, but the drug has not been effective in aiding humans (SCLC). It has been shown by Peifer et al. that mutations of histone are amongst the most prevalent changes in SCLC. The possibility of using them as therapeutic targets has stoked renewed interest in their investigation and advancement [72]. Together, cisplatin and a hydroxamate-based HDACi (vorinostat) had a stronger anticancer effect in vitro. The anti-cancer effects of valproic acid on NSCLC cells have been shown as well. Recent research suggests that panobinostat (a pan-HDACi) may greatly decrease NSCLC and render tumours more susceptible to carboplatin. Meanwhile, TAZ is inhibited by panobinostat. The compensatory of EGFR mechanism is reduced, making NSCLC more sensitive to gefitinib. These results demonstrate that the combination of gefitinib and panobinostat inhibits the growth of tumours with both KRAS mutations and EGFR wild-type sensitivity [73]. It has been demonstrated that the benzamide-based HDACi entinostat may slow the development of cancers that express the stem cell factor SALL4. Patients with high E-cadherin expression had a long median overall survival when linked to those treated with erlotinib plus placebo, despite the fact that a phase II study of entinostat in combination with erlotinib for progressive NSCLC showed no improvement in clinical outcome. The progress and division of SCLC cells have also been inhibited in vitro by the thricostatin valpromide, HDAC, and valproic acid, with thricostatin [74]. Researchers have shown that A thricostatin, like valproic acid, may boost Notch signalling. Dibenzazepine, an inhibitor of Notch signalling, combined with either HDACi, improved efficacy above the use of either drug alone. For this reason, numerous different histone deacetylase inhibitors (HDACis) may be useful in treating this disease. From this knowledge, studies involving HDAC inhibitors in lung cancer, such as NCT02728492, NCT01935947, NCT00738751, and NCT01059552, have greatly benefited [75]. Among the latest epigenetic medications in development are those that target the enzymes enhancer of disruptor of telomeric silencing1-like (DOT1L), zeste homolog 2 (EZH2), protein arginine nine N-methyltransferase, lysine-specific demethylase 1 (LSD1), and bromodomain and extraterminal motif. Evanno et al. discovered that epithelial-to-mesenchymal transition in NSCLC cells may be partially restored using a combination deacetylase inhibitor of histone and an inhibitor of BET. Studies using the LSD1 Inhibitor T-3775440 have showed encouraging results in slowing the development and spreading of SCLC cells [76, 77].

The microenvironment inside tumours has been hypothesised to have a significant role in tumour development. Besides tumour cells, the tumour microenvironment consists of vascular endothelial cells, adipocytes, pericytes, and immune system cells. Tumour cells may primarily generate immunological checkpoint dysregulation by dampening the body's natural defences against them. Learning more about these procedure has re-energized the lung cancer immunotherapeutic treatment [78]. The potential for immune-based therapies has so expanded. Furthermore, epigenetics has been proven to play a role in modulating immunological signals. There is evidence that combining epigenetic therapy with immunological

checkpoint therapy may reawaken the immune system and effectively combat several forms of cancer, like lung cancer. Topper et al. discovered that mocetinostat, givinostat, and entinostat all had a potent effect of anti-tumour, and that givinostat displayed a considerable antiproliferative action in NSCLC when paired with AZA [79]. AZA was hypothesised to make tumour cells more vulnerable to HDACis, to cause a downregulation of MYC expression, and to alter the phenotype of T cells such that they become memory and effector cells. Based on these findings, researchers designed a clinical study in which patients with advanced NSCLC received escalating dosages of mocetinostat, guadecitabine, and an anti-PD1 antibody (NCT03220477; pembrolizumab). Numerous recent medical trials are looking at the effectiveness of combining epigenetic medicine with immune inhibitors checkpoint for the therapeutic effect on lung cancer [80]. The effects of decitabine in combination with immunological check point inhibitors and tetrahyrouridine are being studied in two-stage I/II clinical trials (NCT03233724 and NCT02664181, respectively) including patients with NSCLC. Moreover, two clinical trials are investigating the efficacy and safety of entinostat coupled with pembrolizumab in patients with advanced NSCLC (NCT02909452 and NCT02437136). The common goal of all of these studies is to find a way to increase immune activation via epigenetic change and so improve the efficiency of treatment [81, 82].

7.10 Conclusions and Future Perspectives

Epigenetic study has been a huge help in understanding the molecular processes underlying cancer genesis, which has huge therapeutic ramifications. Epigenetic regulation is highly organised in lung cancer and plays a role at every step of the disease's course, from the earliest stages of formation through treatment resistance in the later stages. Evidence suggests that epigenetic therapies may be useful for treating cancer. To combat haematological cancers, researchers first studied these medicines and then altered them. Learning more about the proteins that play a role as epigenetic regulators has led to the development of novel drugs and treatment methods. In addition, a patient's distinctive characteristics may be employed to enhance the beneficial effects of a certain therapy. Tyrosine kinase inhibitors, chemotherapy, and/or immunotherapy in combination with epigenetic drugs have demonstrated beneficial effects in the prevention of lung cancer. The potential for reviving latent immune responses via the use of epigenetic medications in combination with immunotherapy has far-reaching consequences for the current state of immunotherapy. To identify successful strategies for preventing lung cancer, more research, both preclinical and clinical, is required. Because methylation of DNA might be specific in type of a cell, heterogeneity of cancer cell is an important concern during sample analysis that may serve as a confounding influence when doing epigenomics in bulk. In recent years, single-cell transcriptomics has demonstrated its capacity to distinguish between the molecular structures of various intratumor cell populations. Since this technology is becoming more important in precision cancer therapy, our present knowledge of cancer biology is evolving. We

expect that the newly advanced and refined methylation of single-cell technique will play a crucial role in intra-tumoral dissection of heterogeneity and deliver responses to questions concerning tumour initiation, progression, and therapy response. Less-intrusive techniques, together with developments in molecular technology, have showed promise for precision oncology. Lung cancer epigenetics research may therefore make use of a wide range of human bodily fluids, not only resection materials and biopsies, but also serum, sputum, plasma, saliva, and bronchoalveolar lavage. Accessibility isn't the only perk of these sources; they also let you gather data in a logical order. Cell-free DNA in the blood has recently been revealed to include methylation patterns that may be indicative of the origin of tumours and/or the development of diseases.

In conclusion, the development of cutting-edge technology and the expansion of the types and numbers of clinical trials that may be used for epigenomic research will be vital in furthering our understanding of the biology of lung cancer. The increasing clarity of this knowledge at the single-cell level bodes well for the progress of novel analytical and therapeutic approaches to this particularly tenacious kind of cancer.

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