Chapter 6

Epigenetic Modifications and Potential Treatment Approaches in Lung Cancers

Metin Budak and Mustafa Yildiz

Additional information is available at the end of the chapter

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Abstract

Alteration of methylation is a process seen across a wide variety of species ranging from bacterial microorganisms to mammals, defined as the adaptation method the organism develops against environmental or intrinsic effects, or employs to switch off the genome regions which are no longer required through the evolutionary process. Scientific advancements have allowed detecting the regions that undergo different patterns of methylation. It has been demonstrated that the control on changes in gene expression is not guided by transcription factors alone and that epigenetic alterations are also involved in this process. Furthermore, epigenetic modifications have been shown to be considerably important in cancer development. This section focuses on epigenetic changes and potential treatment options in lung cancer.

Keywords: lung cancer, epigenetic, acetylation, methylation, treatment

1. Introduction

Lung cancer is one of the leading causes of death worldwide. The five-year survival rate is approximately 15% as the condition is often diagnosed at an advanced stage. Smoking is the underlying cause of about 90% of all lung cancers, and smokers constitute the major risk group for developing lung cancer. World Health Organisation (WHO) stratifies lung cancers into two histological groups: non-small cell lung cancer (NSCLC), accounting for 85% of the cases, and small cell lung cancer (SCLC), which constitutes the remaining 15%. The most valid hypothesis for the development of these cancers suggests that multi-phase genetic alterations and a series of epigenetic events result in lung cancer [1].

Owing to the technological advances of the twenty-first century, current practices offer personalised treatment alternatives by means of detailed diagnostics and a range of treatment approaches in lung cancer. While World Health Organisation classified lung cancers almost entirely under the category of adenomas until 1960s, the utility of advanced molecular tests has allowed convenient and thorough identification of subtypes. In addition with these developments, new molecular targets have emerged, leading to novel therapies [2]. During the last 20 years, studies on molecular mechanisms seeking to elucidate the development of cancers and the underlying mechanisms have shown the importance of epigenetic changes in these processes, leading to increasing interest and studies in this field.

Like all cancers, lung cancer develops with the deviation from a normal cell structure due to a number of problems that arise during cell cycle and differentiation. Deviations from normal state to tumour formation result from alterations in cell growth-signalling pathways and apoptosis mechanisms, including a series of epigenetic modifications, all of which are critical for the cell [3].

Epigenetic modifications usually occur in two forms: (1) acetylation, which occurs mainly in histone proteins at protein level and (2) methylation, which is often seen at DNA level.

2. Mutations in lung cancer

Small cell lung cancer (SCLC) accounts for approximately 16–20% of all lung cancers. Owing to the rapidly spreading pattern, SCLC is accepted as an aggressive and widespread disease; therefore, chemotherapy (CT) remains an important modality for the treatment of SCLC.

Non-small cell lung cancer (NSCLC) is responsible for about 85% of lung cancers. Postoperative adjuvant treatment approaches have become the standard of care in early stage tumours, and various systemic treatments are utilised in a metastatic setting. Although the systemic treatment approach in NSCLC according to the subtype has not changed profoundly through the years, the selection of a systemic treatment in metastatic cases has recently begun to differ based on molecular alterations and different histological subtypes of NSCLC. In addition to molecular changes that provide predictions for targeted therapies, separating NSCLC into two groups, that is, squamous and non-squamous, has been suggested to aid in selecting a more effective chemotherapy agent [4–6].

Lung cancer can be histologically divided into two main groups as NSCLC and SCLC. Mutations may be seen in genes such as EGFR, RET, PIK3CA, ALK, HER2, KRAS, BRAF, MET, NRS, MEK1 and ROS1, which are often referred to as signalling pathways and considered as drivers since they cause cancer development in NSCLCs. These mutations can be seen in current smokers, ex-smokers and non-smokers, whereas mutations in EGFR, ALK, HER2, ROS1 and RET genes are seen only in people who have never smoked. Such mutations may be observed in all histological subgroups of NSCLCs including adenocarcinomas, squamous cell carcinomas (SCCs) and large cell carcinomas [7–10].

Smoking causes chronic inflammatory stress on biological systems, thereby interfering with the cell cycle, cell development and differentiation. Long-term inflammation is associated with DNA methylation and contributes to lung cancer development via methylation mechanisms.

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For example, genes such as APC, FHIT, RASSF1A and CCND2 are inactivated only in smokers due to promoter hypermethylation. Increased promoter hypermethylation of P161NK4A, MGMT, RASSF1A, FHIT and MTHFR, depending on the intensity of smoking, shows a strong correlation with NSCLCs compared to non-smokers [11, 12]. Promoter methylation of RARβ, FHIT, P161NK4A and RASSF1A increases as smoking intensifies.

Moreover, mutations in epigenetic regulator genes create a complicated situation owing to the fact that they prevent these genes from functioning properly, which is expected to impact cell cycle and cell development, thereby resulting in the development of cancer. However, these mechanisms may also offer certain advantages in favour of treatment as they may open new ways for cancer therapies (such as DNTM inhibitors) [13].

3. Acetylation of histones

Human genome, as that of any eukaryotic organism, is organised in a highly complicated manner. Except for the alterations in the genes effective in human development, the histone proteins that pack the genome serve to control the genome by undergoing various modifications. This is accomplished through various changes that occur during events such as DNA replication, repair and expression [14].

For instance, the amino terminal of the histone core is quite important as it contains a flexible and fairly simple tail domain, constituting a target region for several post-translational modifications. Histone modifications primarily occur in the form of addition of acetyl and methyl groups to lysine amino acids, addition of phosphorus to serine amino acids and methyl groups to arginines. These modifications play a critical role in the control of biological processes such as transcription [15].

Modifications in histone proteins develop by means of enzymatic pathways. Histone deacetylase inhibitors (HDACIs) also act as antagonists in cell differentiation by acetylating histones and non-histone proteins. Proteins in this group inhibit DNA repair, apoptosis and gene expression mechanisms. In addition, such proteins contain a zinc-binding group (ZBG) and a chain linking two proteins from these two groups referred to as the surface recognition polypeptide [16]. Therefore, molecules able to inhibit such proteins may be potential anti-cancer agents. For example, peptide-containing cyclic hydroxamic acids (CHAPs) may be suitable for therapeutic use as a group of potential anti-cancer agents. The molecule CHAP31, which acts on HDACIs, has been shown to have a highly effective anti-tumour effect on certain types of cancer such as lung, breast and gastric cancer as well as melanoma *in vivo* [17].

Fibrosis is one of the important factors contributing to cancer invasion and metastasis. Fibrosis results from fibroblast activation, which degrades and alters the physical structure of extracellular matrix (ECM). The fibrosis-induced increase in ECM fragility leads to pathological conditions such as epithelial-mesenchymal transition (EMT) with the transformation of normal cells into cancer. The change in the structure of collagen, one of the most important proteins in tissue structure, is another contributing factor. For this reason, collagen receptors are of particular importance regarding the progression of cancer. The discoidin domain receptors

(DDRs), DDR1 and DDR2, are overexpressed receptor proteins. DDRs mechanically increase the acetylation of c-Myb, the transcription factor of histone acetyltransferase (HAT) on rigid ECM, thereby leading to c-Myb binding to DDR2 promoter together with LEF1, and result in DDR2 upregulation in a rigid environment. Silenced c-Myb may cause DDR2 inhibition and invasion of lung cancer cells, and recovery of physical characteristics of the tissue has been shown when external interventions allow DDR2 expression [18].

Proteins in the Snail group, a zinc-binding superfamily of transcription factors, are responsible for cell migration and invasion during both the embryonic period and cancer process [19]. Snail proteins bind to the E-box sequence located in the promoter region of E-cadherin gene, which encodes the protein that is responsible for cell–cell adhesion. As a result, since E-cadherin synthesis is no longer possible, cell–cell adhesion cannot be achieved, and cancer cells gain metastatic properties [20]. Snail proteins are overexpressed in several types of cancer as they are strategically important for cancer cells. Rui et al. have emphasised that Snail, acetylated by P300, may be of value in terms of developing personalised treatments for lung cancer [21].

Furthermore, HDACs allow E-cadherin expression through non-coding RNAs. In addition, miRNAs serve to control EMT in lung cancer via TGF-β-, EGF- and HGF-signalling pathways. MiR200b and miR200c are effective on H3 acetylation in E-cadherin promoter (**Figure 1)** [22].

Epigenetic readers recognise modified histones by means of a group of polypeptides referred to as 'reader', and these polypeptides are involved both in normal cell growth and in cancer development by controlling several processes conducted together with chromatin [23].

Recently, Wenyi mi et al. identified the YEATS domain, defined as a novel acetyl-lysine-binding module. With regard to human cancers, the functional importance of proteins containing this domain remains unknown; however, the overexpression of the YEATS2 gene has been shown in non-small cell lung cancers. This domain is thought to decrease histone acetylation, thereby inactivating key genes [24, 25].

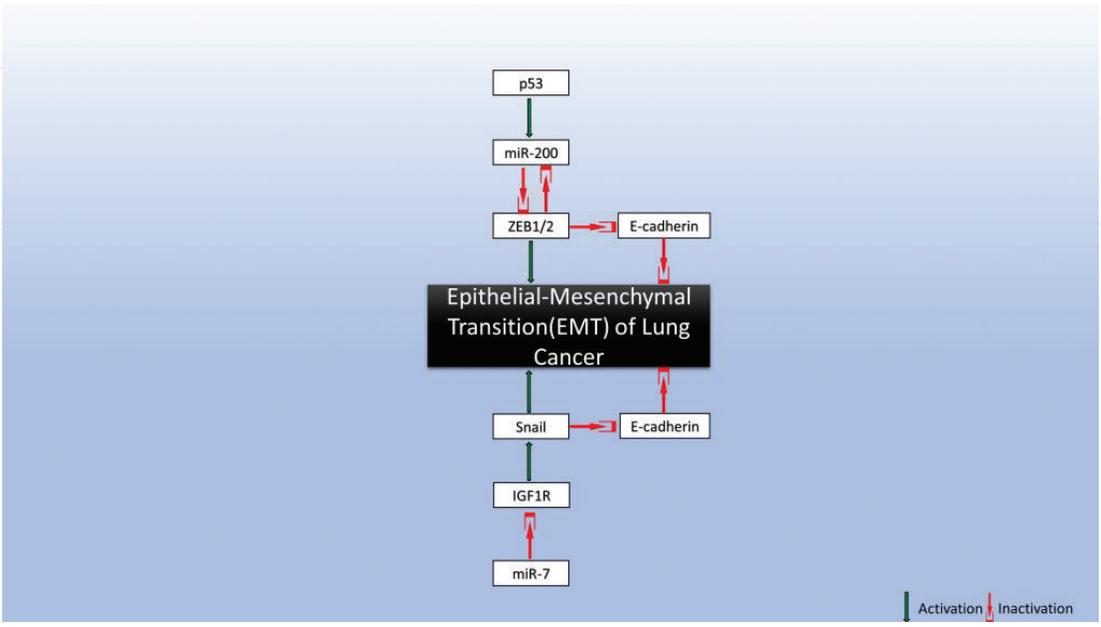

Figure 1. IGF and p53 pathways in lung cancer.

'Lunasin' is a soy protein consisting of 43 amino acids known to protect mammals against chemical carcinogens. In addition, it has been shown to be potentially beneficial in several conditions [26, 27]. A derivative of this protein originating from wheat has been reported to be effective even when taken orally and potentially effective in cancer prevention, regardless of the suppressed acetylation of core H3-H4 histone proteins [28].

As is the case in any intracellular event, the acetylation process occurs by means of enzymatic pathways. The removal of acetyl groups from histones is conducted by enzymes called histone deacetylases (HDACs). Cell viability continues normally as this is carried out in a balance of acetylation-deacetylation processes [29, 30]. Sometimes, hypoacetylation may occur when the process shifts towards deacetylation, and this is accompanied by cancer development. HDAC inhibitors are thought to possess the potential to reverse this process, leading to epigenetic reactivation of the suppressed anti-tumour genes [30]. This may help the suppression of certain tumours depending on which genes are expressed and which proteins are suitable.

Long Chen et al. believe that lysine acetyltransferase accelerates tumour formation due to the acetylation of histones and non-histone proteins despite the anti-tumour effect provided by acetylation-related HDAC inhibitors, and suggest that acetylation may shift to both sides (acetylation-deacetylation) depending on which genes are active at the time [30]. They highlighted the necessity to elucidate the relationship between lysine acetyltransferases (KAT) or HDAC and other proteins such as transcription factors in order to enhance the specificity.

Human MOF (hMOF and MYST1) is a member of the histone acetyltransferase (HAT) protein family. These proteins convert histone H4 acetylation to H4K16Ac, an epigenetic marker of active genes, in particular by adding an acetyl group to the lysine-16 amino acid. Irregularities in these epigenetic markers affect cell biology, potentially leading to cancer development. In correlation with H4K16Ac, hMOF has been shown to be overexpressed in non-small cell lung cancers. Investigators have indicated that this group of proteins may have potential oncological tasks and this may be a potential therapeutic target [31].

Although the interferon regulatory factor 3 (IRF-3), an important transcription factor for interferon genes, is often functional in viral infections, the regulation mechanism of IRF-3 expression in cancers has not been fully understood. The concurrent use of histone deacetylase inhibitors and Trichostatin A (TSA) has been shown to increase IRF-3 expression in lung adenocarcinoma A549 cells by altering GATA-1 acetylation, and targeting IRF-3 is therefore thought to be a novel therapeutic approach [32].

In light of all this information, one may conclude that some genes or proteins contribute to carcinogenesis when they function towards acetylation, while others contribute to carcinogenesis when they function towards deacetylation [22].

4. Methylation

In living organisms, all molecular structures and events are generated by specific sequences called genes that are found in the DNA molecule. However, these genes need to be governed and controlled so that they can participate in biological processes at the optimal time. Genes are actively controlled by specific genes and proteins referred to as transcription factors. However, there is a

different mechanism that also determines gene expression, which can be transmitted from generation to generation and from cell to cell. This is called the epigenetic code [12]. DNA sequence does not undergo any changes during the formation of this code, but the relevant part of the DNA fragment becomes no longer meaningful. The most common epigenetic modifications are the changes in histone proteins and DNA methylation. The most widely studied and the most well-established epigenetic mechanism is DNA methylation. It is an enzymatic change where cytosines are converted to 5'-methylcytosine. The cytosine-end methylation seen in mammalian genome often occurs at the nucleotide pairs which are also called the CpG dinucleotides. Detection of these methylated gene segments on the genome is highly informative in terms of the effects of genes in several biological processes from carcinogenesis to ageing [33, 34].

Methylation-related changes may occur anywhere in the genetic material of a eukaryotic cell. In eukaryotic cells, genetic material is found in two organelles: the nucleus, which contains almost all of the genetic material, and mitochondrion, which has a very small genome compared to the nucleus. Certain methylations are specific to the genomes of these two organelles [1].

For several years since being discovered, the pattern of DNA hypomethylation in CpG dinucleotides has been shown to be highly important in cancer cells [35, 36].

A methylated gene becomes inactivated and therefore cannot synthesise the product, that is, the RNA or protein. Gene methylation occurs more commonly in promoter regions. Methylation in the promoters of tumour suppressor genes is mostly associated with carcinogenesis and often occurs in the form of hypermethylation [12].

Apart from global DNA hypomethylation reported in earlier stages, hypomethylation may also occur in the CpG islands in promoter regions of several specific genes or in the nucleotide pairs of genes that are activated by hypomethylation and silenced by hypermethylation. Methylation of a promoter region does not necessarily produce any protein or RNA [37]. The SHOX2 gene, a member of the Homeobox gene family, has been shown to be a specific biomarker with 60% sensitivity and 90% specificity when investigated by bisulphite modification-PCR in blood plasma samples during a case–control study of approximately 400 subjects with lung cancer. Also, in the same study, comparison against the results from another study conducted with bronchial aspiration samples revealed a higher level of sensitivity compared to results from blood plasma samples [38]. In bronchoalveolar lavage samples obtained from NSCLC patients, 24% methylation was observed in the promoter region of the CDKN2A gene, also known as P16INK4A, in addition to microsatellite instabilities and p53 mutations [39, 40].

The lungs are highly exposed to external factors owing to the nature of their functions. This constitutes a major risk factor in terms of epigenetic modifications as well as being associated with pulmonary diseases caused by environmental factors and smoking in particular. DNA methylation, histone modification and non-coding RNA have been shown to be increased in smokers [11].

Lung cancer develops upon the accumulation of numerous genetic and epigenetic alterations in the respiratory epithelium. Early promoter methylation and tumour suppressor gene inactivation are considered as signs of pulmonary carcinogenesis [12].

Defects in the apoptotic pathway are among the main reasons contributing to the high fatality of lung cancers. Apoptosis, also known as programmed cell death, has a wide range of physiological

effects from embryonic stages to tumour formation. Inhibition of apoptosis is particularly detrimental in cancer treatments. The main reason of this is the fact that most treatments exert their effects by activating apoptotic mechanisms. Targeting the apoptotic pathway ensures the effectiveness of anti-cancer treatments [41, 42].

Survivin, one of the apoptosis inhibitor proteins, plays a critical role in cell division and in the continuation of cell survival [43, 44]. Since increased expression of survivin in human tumours leads to aggressive tumour development and resistance to main cancer treatments such as chemotherapy and radiotherapy, survivin gene and protein have been found to be important markers regarding the outcome of treatment [45, 46].

Computer analyses have shown a potential methylation region in exon 1 of the survivin gene; however, no methylation was found in lung cancer patients, and it has been shown that survivin gene expression in any cell may be effective not only with methylation but also through other transcription factors [47].

5. Importance of apoptosis

Apoptosis occurs during the normal development of multicellular organisms and continues throughout life. This mechanism is responsible for embryonic development and organ formation through cell differentiation. For example, toes are separated from one another by means of apoptosis.

Apoptosis also controls the immune system. T-lymphocytes are involved in the destruction of infected or damaged cells during cellular immunity. The T-lymphocytes produced in the thymus gland need to be active against foreign antigens before being released into bloodstream and should not show any activity against normal cells. Any inactive or semi-active T-cell is bound to be destroyed by apoptosis before they may begin their task.

Inhibitors of apoptosis proteins from the anti-apoptotic protein family have been identified in vertebrate and invertebrate species, and they are known to be negative regulators of programmed cell death. Some homologues identified in mammals include XIAP, cIAP1, cIAP2, NAIP, Bruce, Survivin and IAP. Most of these block cell death by directly binding to and inhibiting caspase-3, caspase-7 and caspase-9 [32].

Various diseases may arise in the event of any defect within the regulation of apoptosis. Among these, cancer is a condition characterised by little or no apoptosis. The mutations in cancer cells result in different cell-signalling and cell growth processes compared to normal cells. Under normal circumstances, when cells become damaged, they become apoptotic while cancer cells do not undergo apoptosis as a result of the cancerous mutations, which leads to uncontrolled cell differentiation and tumour formation. It is often difficult to eliminate such tumours with cell-damaging treatments such as chemotherapy and radiotherapy. In addition, some cancer cells develop resistance to treatments that target tumours with mutations in apoptotic pathways. Further understanding of the regulation of apoptosis in cancer cells is expected which allow developing novel therapies. While apoptosis is reduced in cancer, conditions with increased apoptosis lead to different problems. For example, neurodegenerative diseases such as Alzheimer's and Parkinson may be the result of increased cell death [43–46, 48].

6. Survivin and apoptosis

In early studies, caspase-3 suppression was suggested to be directly responsible for the anti-apoptotic mechanism of action of survivin. However, three-dimensional structural studies have shown that BIR (baculovirus IAP repeat domain) of survivin is not long enough to block the enzymatically active region of caspase-3. Survivin is thought to directly bind to caspase-9 as three-dimensional studies have revealed the similarity between the BIR domain of survivin and BIR domain of another IAP, XIAP, which directly binds to and inhibits caspase-9 *in vitro*. Another relevant mechanism is the inactivation of a pro-apoptotic molecule called SMAC/Diablo released together with cytochrome-c during mitochondrial apoptosis. SMAC/Diablo binds to IAPs and prevents their caspase-suppressing effect. Theoretically, survivin binds to SMAC/Diablo. Survivin bound to SMAC/Diablo protects other IAPs from the inhibitory effect of this protein. Thus, caspase suppression continues, leading to apoptosis blockade. There is evidence indicating that survivin plays an important role in p53-associated apoptosis. p53 blocks survivin transcription both through direct and indirect pathways. Conversely, overexpression of survivin inhibits p53-dependent apoptosis.

Phosphorylation of threonine-34 residues is necessary for survivin to bind to caspase-9. This is conducted by a kinase called p34cdc2-cyclin B1. Survivin-caspase interaction is shown in **Figure 2** [38].

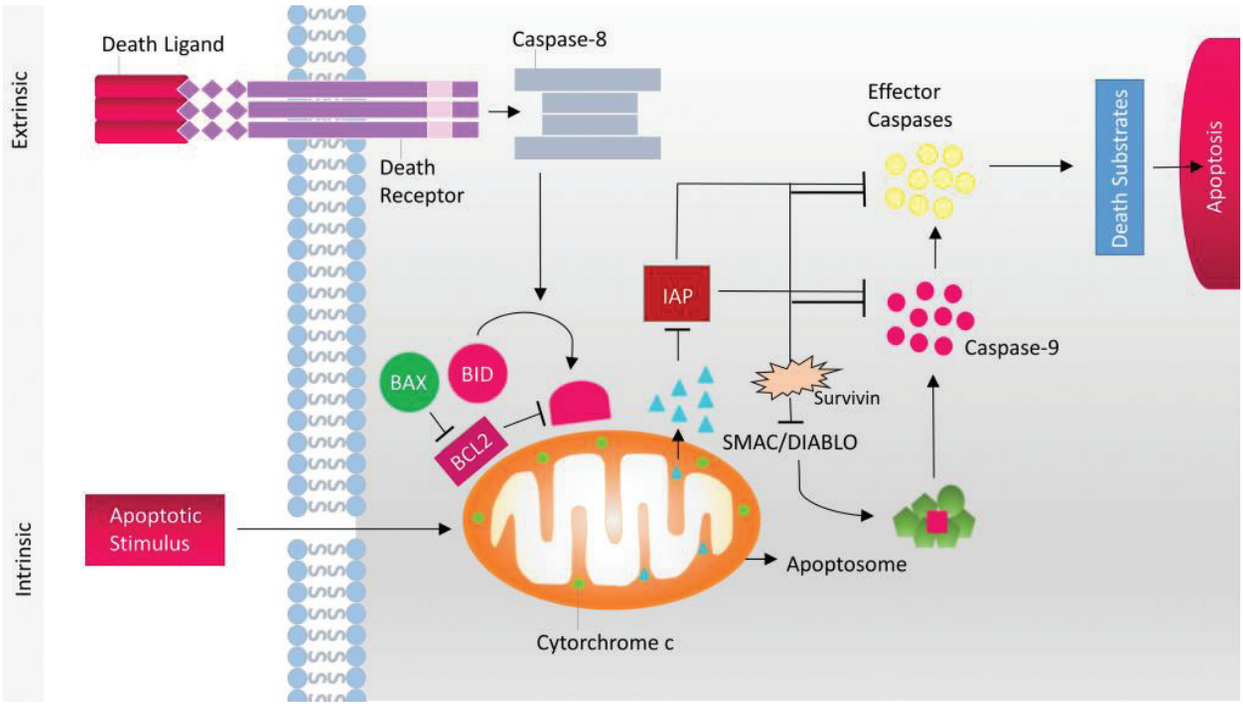

Figure 2. Apoptotic pathways.

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7. Survivin gene and protein

Survivin molecule is expressed in special tissues and cells during certain phases of cell cycle. In humans, survivin expression occurs in the heart, liver, gastrointestinal tract and other foetal tissues from embryonic development until the end of foetal period, and in stem cells, epithelial cells and pancreatic endocrine cells during adulthood. Increased survivin expression has been shown in solid tumour tissues of adults, for example, lung, breast, brain, stomach, oesophagus, pancreas, liver, uterus and ovarian tumours. Increased survivin expression has also been reported in certain tumour tissues such as neuroblastoma, colorectal cancer and gastric cancer and has been associated with poor prognosis in these patients [49, 50]. Some studies have indicated the promotion of tumour development via survivin overexpression. There are numerous studies showing that survivin plays an important role not only in cell division and inhibition of apoptosis but also in cancer development [50, 51].

The human survivin gene is located on the telomeric position of chromosome 17 with a size of 14.7 kb. Survivin gene consists of four exons and three introns and encodes the survivin protein of 16.5 kD. While other IAPs contain more than one BIR in their structure, the survivin gene contains only one BIR region at the N-terminal domain and also contains an alpha-helix structure at the C-terminal domain. Survivin protein interacts with caspase-7 and caspase-9 via the BIR region while its alpha-helix structure interacts with tubulin subunits during mitosis [52, 53].

Survivin expression is suppressed during the G1 and S phases of the cell cycle but increases during G2/M. This control mechanism mostly functions at transcription level, occurring through cell cycle-dependent elements (CDEs) and cell cycle homology regions (CHRs). The CDE/CHR suppressor protein binds to this region, thereby suppressing gene expression. The CHR region is located at the proximal end of the survivin gene promoter [52, 53].

Cell-culture studies have demonstrated the association between SurKex methylation region and histone acetylation in the promoter region of the survivin gene. These studies have also reported a decreased mRNA expression in the survivin gene with the methylation of survivin promoter [54].

The methylated survivin promoter region has also been shown to inhibit p53 binding, which may render p53 ineffective in cell cycle [55].

For this reason, the studies on survivin gene region and protein have been intensified, with a steady increase in the number of studies investigating the expression analysis of survivin as well as survivin promoter-related polymorphisms and mutations.

8. Mitochondrial methylation

Mitochondrial functions provide several protein components synthesised from mitochondrial DNA (mtDNA) for the oxidative phosphorylation mechanism. In mammals, 12S and 16S

rRNAs are encoded with 13 proteins from mtDNA. These genes are effective on cell homeostasis and apoptotic pathways [56].

Since mitochondria are dominant organelles regarding intracellular energy and because they have their own independent DNA genome, it is important to look at the genomic alterations of this organelle in certain diseases. These alterations are associated particularly with various pulmonary diseases and lung cancers [57, 58].

As mentioned earlier, mitochondrial methylation may also be affected in lung cells, which are considerably exposed to environmental effects. In their study dated 2013, Byun et al. have shown that mitochondrial RNA may undergo methylation [59].

9. Mechanisms of current therapies in lung cancer

Several treatment modalities are utilised in different subtypes and stages of lung cancer. Surgery is the first-choice treatment if tumour margins are well defined while adjuvant or neoadjuvant chemotherapy and hormone therapies are also applied with or after surgery. Treatment often continues in the form of chemotherapy, with practices that differ from country to country. The major chemotherapeutic agents approved for lung cancer include cyclophosphamide, doxorubicin, vincristine, cisplatin and mitomycin-C [60–62]. Almost all of these agents share the common feature that they induce apoptosis in the cell by triggering DNA damage in various ways.

Cyclophosphamide (Cytoxan, Neosar) is an immunosuppressant used for the treatment of lung cancer, and its metabolite, phosphoramide mustard, is the molecular structure form which exerts the actual effect. This agent alters DNA structure by forming irreversible cross-links between N-7 atoms of the guanine base in the DNA strand. This altered DNA structure stimulates intracellular apoptotic pathways, allowing the cell to undergo apoptosis [63].

Doxorubicin, sold under the commercial name Adriamycin, is another chemical agent that can be administered via intravascular route and interacts with DNA by intercalation. This agent prevents the biosynthesis of DNA macromolecules, inhibits DNA replication by stabilising topoisomerases and thereby shows the anti-cancer effect [64, 65].

Cisplatin is a platinum molecule with two chloride ions. This agent interferes with DNA, prevents replication and induces apoptosis in cells that are rapidly proliferating. Because of the different chloride concentration in intracellular and extracellular environment, cisplatin readily enters the cell and interacts with the water molecule to form a complex. This complex replaces the N-heterocyclic bases in DNA and has a particularly strong binding effect on guanine. This new structure forms the cis-[PtCl(NH3)2(N7-ACV)]$^{+}$ structure. In this situation, DNA repair mechanisms cannot work, and degradation of DNA is initiated in apoptotic cells [66, 67].

Mitomycin-C is another bactericidal chemotherapeutic agent. The mechanism of action of this agent is to generate DNA damage by alkylating the guanine nucleotide in 5′-CpG-3′ sequence via cross-links. Mitomycin-C exerts the anti-cancer effect by activating apoptotic pathways [68].

Another treatment modality employed for the treatment of lung cancers is radiation therapy. Radiotherapy is a radiation-based application that utilises ionised radiation, such as high-energy

X or gamma radiation. Radiotherapy can be applied before or after surgery and can also be combined with chemotherapy, depending on the localisation and stage of the tumours that are being treated. In this therapy, high-energy radiation beams cause DNA damage in the cell and result in apoptosis [69, 70].

The abovementioned therapeutic approaches share a common mechanism of action, in that almost all chemotherapeutic agents and radiotherapies induce DNA damage by activating apoptotic pathways and destroy the tumour with the help of apoptosis [71]. However, this requires the presence of intact apoptotic pathways; in other words, apoptotic pathways should not have been inhibited in order for these treatments to be effective. If anti-apoptotic mechanisms are activated, these treatments often fail, and drug resistance may develop. Specific targets of certain chemotherapeutic agents may be located in base sequences that undergo methylation, and motif changes may occur in the relevant DNA sequence due to methylation. In this case, chemotherapeutic agents may prove to be ineffective.

DNA methylations, chromosome acetylations and inhibited apoptotic pathways are among the reasons of resistance to therapeutic agents and radiotherapy. Studies have shown that epigenetic agents are highly promising in terms of overcoming the resistance to chemotherapy in various tumours [72]. A good understanding of acetylation, methylation and apoptosis mechanisms will allow developing more effective and targeted novel molecules.

10. Potential novel treatment approaches

Excision of the tumour tissue and the surrounding lymph nodes with the most recent surgical approach remains the optimal treatment in lung cancers. However, this may not always be possible owing to the anatomic location, spread pattern and metastasis status of the cancer. Chemotherapy or radiotherapy or both may be used in such cases. Response to treatment, however, is often not promising [73]. It is at this point where epigenetic alterations during cancer development emerge as therapeutic options. Due to their reversible characteristics, epigenetic modifications are therapeutic targets which may prove to have very good anticancer effects. For this reason, the US Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMEA) have started granting approval for certain drugs such as histone deacetylation inhibitors and DNA methyltransferase inhibitors. Among these, inhibitors of DNA methylation are the most effective treatment options and they appear to be effective in lung cancer as well [36].

DNA DNMTs are molecules that transfer methyl groups to cytosines via S-adenosyl methionine (SAM). Hypo- and hyper-methylation of DNA may occur in any cancer cell and silence tumour suppressor genes or inactivate T-cell recognition genes, which provide immune response, or affect the genes that trigger metastasis, angiogenesis and invasion [74]. The most important investigational DNA methyltransferase inhibitors and their analogues are presented in **Table 1** [74].

CI-994 is one of the candidate therapeutics in clinical testing phase. CI-994 is an orally bioavailable histone deacetylase (HDAC) inhibitor that causes histone hyperacetylation in viable cells. CI-994 shows inhibitory effects based on the concentration of HDAC1 and HDAC2.

DNA Methyltransferase Inhibitors Substance Group	Substance Name
Nucleoside analogs	Decitabine (5-aza-2′-deoxycytidine, Dacogen®, DAC)[1,2]
	Azacitidine (5-azacytidine, Vidaza®)[1,2]
	5-aza-fluoro-2′-deoxycytidine (FCdR)
	CC486 (oral azacitidine)
	Guadecitabine (SGI-110)
	Sinefungin
	Zebularine
Antisense oligonucleotide	DNMT1 ASO
(ASO) inhibitors	MG98
Others	1-Hydrazinophthalazine
	CBC12
	Epigallocatechin gallate (EGCG)
	Procainamide
	Psammaplin A
	RG-108
	SGI-1027
	Thioguanine

[1]Food and Drug Administration (FDA)-approve drugs, [2]European Medicine Agency (EMA)-approved drugs

Table 1. DNA methyltransferase inhibitors and their analogues [74].

Specifically, it mediates the arrest of cell cycle at G1, inhibits proliferation and induces apoptosis both *in vitro* and *in vivo* [75, 76].

FDA-approved epigenetic therapy agents are shown in **Table 2** together with their indications and year of approval [77].

Acetylation-based drug study is an early phase 2 trial of vorinostat (Zolinza®) [78]. Vorinostat is an HDAC inhibitor from the hydroxamate group. In this phase 2 study, vorinostat was

FDA-approved epigenetic therapy

Agent	Class	Approval Date	Indication
Azacitidine	DNMT Inhibitor	2004	Myelodysplastic syndrome
Decitabine	DNMT Inhibitor	2006	Myelodysplastic syndrome
Vorinostat	Pan-HDAC Inhibitor	2006	Cutaneous T-cell lymphoma
Romidepsin	Class I HDAC Inhibitor	2009	Cutaneous T-cell lymphoma
Bellnostat	Pan-HDAC Inhibitor	2014	Multiple myeloma
Panobinostat	Pan-HDAC Inhibitor	2015	Peripheral T-cell lymphoma

HDAC, Histone Deacetylase: DNMT, DNA methyltransferases.

Table 2. FDA-approved epigenetic therapy agents [77].

evaluated in breast, colorectal, and non-small lung cell cancers; however, adequate clinical response was not obtained, and authors reported that studies are to continue to determine appropriate doses [78]. While having a wide spectrum of tolerable side effects, it is a promising molecule in terms of chemotherapeutic utility [79].

In addition to survivin gene vaccines, a study has been conducted to target the methylated oligonucleotides of the survivin gene in non-small cell lung cancer. The study in question aimed to break down the apoptosis resistance of NSCLC by interfering with the survivin gene expression via oligonucleotides called SurKex1, which are specific to the promoter region of the methylated survivin gene. Data from this study were the first to show the utility of SurKex1 owing to its downregulating effects on survivin expression by means of DNMT1 activation [80, 81]. This study, although conducted in a cell-culture setting, is promising with regard to targeted survivin gene therapy in the near future.

Chemotherapy combined with immunotherapy may also be effective in treatment. High rates of response to treatment were demonstrated through PD-1 blockade via activated IFN signals by hypomethylation in treatments combined with IRF1/7 following treatment with decitabine (5-aza-2′-deoxycytidine or 5-Aza-Cdr or DAC), which was the first cytosine analogue synthesised by Pliml and Sorm in 1960. DAC, known to inhibit DNMT during cell division, is also a candidate for use in cancer therapy as an FDA-approved promoter hypomethylation inhibitor [82, 83].

Cystatin A (CSTA), a member of the type 1 cystatin superfamily, is essential to protect cells from cytoplasmic proteolysis and is mainly expressed in epithelial and lymphoid tissue. Furthermore, while cathepsins B, H and L, CSTA and cytoskeleton are known to be involved as tumour suppressors in oesophageal cancers, they have been found to exert such effects also in lung cancers. Histone methylation and acetylation play an important role in CSTA gene silencing in lung cancers. DAC treatments have an inhibitory effect on DNMT1, which is responsible for replicating DNA in a methylated form during replication. While limited CSTA expression is associated with high grades in squamous cell carcinoma (SCC), silencing in CSTA promoter region has also been demonstrated in the absence of CpG islands through epigenetic mechanisms such as partial methylation [84]. This renders DNMT1s a good target for novel treatment approaches.

In lung cancers, miR-9-3 hypermethylation occurs and the resulting downregulation of miR-9-3 expression leads to poor prognosis. Sulforaphane (SFN), a natural plant-derived molecule with anti-cancer properties, has been reported to decrease miR-9-3 methylation by attenuating DNMT activity in lung cancers and has a potential effect in improving the cancer prognosis [85].

It has been shown that Runx transcription factors (Runx1, Runx2 and Runx3), which play a critical role in organogenesis and cell differentiation pathways, are involved in lung cancers as they cause epigenetic silencing of a tumour growth inhibitor called BMP-3B. In this respect, downregulation of BMP-3B and lung cancers are closely related. Therefore, Runx transcription factors now appear to be a potential epigenetic target in lung cancers [86].

MARVELD1, a recently identified nuclear factor, is known to be extensively expressed in all human tissues and downregulated via promoter methylation in multiple cancer tissues. By working in combination with DNA methylation and histone acetylations, the epigenetic silencing of MARVELD1 leads to a decreased expression, causing unfavourable effects on

histopathology and malignancy in lung cancers. This decreased MARVELD1 status in lung cancers eliminates the NMD complex-forming activity with UPF1/SMG1, resulting in premature termination codons and non-functional RNA. Epigenetic MARVELD1 silencing, which may serve as a diagnostic biomarker in lung cancers, appears to be a good target for anti-tumourigenesis [87].

Highly tumourigenic stem-like cells, which are thought to be tumour-initiating cells, cause the initiation, recurrence and drug resistance of cancers. Ca + 2/calmodulin-dependent protein kinase IIy (CaMKIIy), which is abnormally overexpressed in highly tumourigenic stem-like cells, is also associated with poor prognosis in lung cancers. Oct4 is one of the mRNA expression factors for pluripotent stem cells and regulates the differentiation of these cells by inhibiting CaMKIIy through epigenetic regulation. Therefore, Oct4 may be considered as a novel target approach in lung cancers [88].

The long non-coding RNAs are now also thought to offer a potential biomarker in non-small cell lung cancers. Studies have shown that they are particularly increased in non-small cell lung cancers. Because such RNAs occur mainly through methylation at DNA-gene level, long non-coding RNAs appear to be good markers and targets for novel treatment approaches in non-small cell lung cancers with regard to epigenetic mechanisms [89].

Among lung cancers, the metastatic risk is high in adenocarcinomas. In a study conducted to reveal possible biomarkers and therapeutic agent targets in these carcinomas, DNA methylation profile was downloaded from Gene Expression Omnibus (GEO) database, and DNA methylation profile of lung adenocarcinoma was investigated. This study concluded that methylated PTPRF, HOXD3, HOXD13 and CACNA1A genes may be potential biomarkers for the diagnosis and treatment of lung adenomas [90].

11. Conclusions

In light of all the information described earlier, one may conclude that acetylation at histone level and DNA methylations may be potential biomarkers and also good target molecules for treatment, particularly in lung cancers. Especially, the promoter regions of several tumour suppressor genes, and regions such as exon 1, although to a smaller extent, are inactivated through methylation. While the DNMT1 enzyme is in the position of a general target for inhibition to prevent these methylations, approaches such as inactivation of certain specific genes, oncogenes or apoptosis-inhibiting genes by means of methylated DNA oligo-primers appear to be a considerably good option. In the future, a combination of all these possibilities may allow treatments with significantly reduced side effects compared to current treatments as well as improved targeted approaches which destruct or prevent the progression of tumours.

Conflict of interest

There is no conflict of interest.

Author details

Metin Budak* and Mustafa Yildiz

*Address all correspondence to: genomicdna2@yahoo.com

Biophysics Department, Faculty of Medicine, Trakya University, Edirne, Turkey

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