Chapter 11
Epigenetics and Its Role in Human Cancer

Utkarsh Raj and Pritish Kumar Varadwaj

Abstract Cancer is often associated with heritable epigenetic changes, which are characterized by the change in gene expression profile without changing the underlying DNA sequence. The most prominent epigenetic modification is methylation of DNA, which to a large extent is connected to modifications of histone proteins. Epigenetic modifications resulting in a normal gene are reversible, thus endow functional flexibility and diversity to the genome, and these modifications can be cured with selective epigenetic target inhibitors. The role of epigenetics in human cancer has been vastly studied and reported in recent decade with emerging evidences about the significance of epigenetic alterations to comprehend various cellular mechanisms. The cellular mechanisms which are crucial for controlling the growth and progression were seen to be impaired by epigenetic changes, which result into development of various human cancer diseases. Although several targets for cancer epigenetics have been identified and annotated in recent past, the development of novel anticancer treatments for these targets is still in nascent stage. By recognizing the spectrum of cancer epigenetics, an array of new drug discoveries has been possible these days. In this chapter, we presented an overview of such epigenetic modifications which occurs and resulted into human cancer and the relationship between those epigenetic enzyme classes and cancer types, with a note on preclinical utilizations of inhibitors for the treatment of such cancer types. This chapter focuses on the practical understanding of human cancer epigenetics and its perspective use for drug designing.

Keywords Cancer epigenetics • DNA methylation • Histone modifications • CpG methylation • miRNAs

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11.1 Introduction

The essential role of epigenetics in various cellular processes of normal and cancerous cells has drawn considerable attentions in recent years. It has been reported that the selective expression of gene resulted due to epigenetic modifications was instrumental in deciding the fate of proteins involved in binding of chromatin and the related machinery of transcription. These findings had revealed critical infection-related epigenetic components and pathways which are crucial for discovery of novel therapeutics. A major fraction of such reported cases comprised of epigenetic misregulation related to human cancer. Cancer epigenetics is the investigation of epigenetic alterations to the genome of tumor cells that don't essentially include a change or variation in the nucleotide succession.

The earliest indications of an epigenetic connection to cancer were resultant of various studies on gene expression and methylation of DNA. The quantum of such studies was well discussed elsewhere in a survey article by Feinberg enumerating the historical backdrop of growth of epigenetics (Feinberg and Tycko 2004). The International Cancer Genome Consortium (ICGC) has significantly strengthened these early observations. The whole genome sequencing in an immeasurable cluster of cancers has given an index of recurrent somatic mutations in several epigenetic controllers (Forbes et al. 2011; Stratton et al. 2009). Epigenetic data is contained in the cell in various forms that incorporate methylation of DNA, modification of histones (methylation, phosphorylation, acetylation, and so forth), positioning of nucleosome, and microRNA expression; these data together constitute the epigenome (Campbell and Tummino 2014). All these modifications in the chromatin structure lead to the activation or silencing of the expression of genes (Herceg and Ushijima 2010; Baylin 2008; Jones and Baylin 2007; Kouzarides 2007a; Kelly et al. 2010). Although an exhaustive understanding of epigenomic dysregulation in specific type of cancer has not been clarified yet, there exists a comprehension of tumor-specific types of modification which occurs in human cancer (Baylin and Jones 2011a; Croce 2009). The remodeling of chromatin is carried out with the help of two important mechanisms: the cytosine residue methylation in DNA and an array of posttranslational modifications (PTMs) occurring at the N-terminal ends of histone proteins. These PTMs comprise of methylation, acetylation, ubiquitylation, phosphorylation, glycosylation, sumoylation, ADP-ribosylation, citrullination, biotinylation, and carbonylation (Sidoli et al. 2012; Gardner et al. 2011). Among all such PTMs, the lysine amino acid residues of histone tails are reported to be methylated, acetylated, or ubiquitylated; also the arginine amino acid residues are found to be methylated, whereas threonine and serine amino acid residues were seen to undergo phosphorylation (Cosgrove et al. 2004; Cruickshank et al. 2010; Imhof 2006; Weake and Workman 2008; De Koning et al. 2007). These covalent alterations have the propensity to bring cross talk, which is known as the histone code that can be positively or negatively associated with specific states of transcription or chromatin organization (Cruickshank et al. 2010; Sippl and Jung 2010; Chi et al. 2010). Human tumors are considered fundamentally to be a disease of

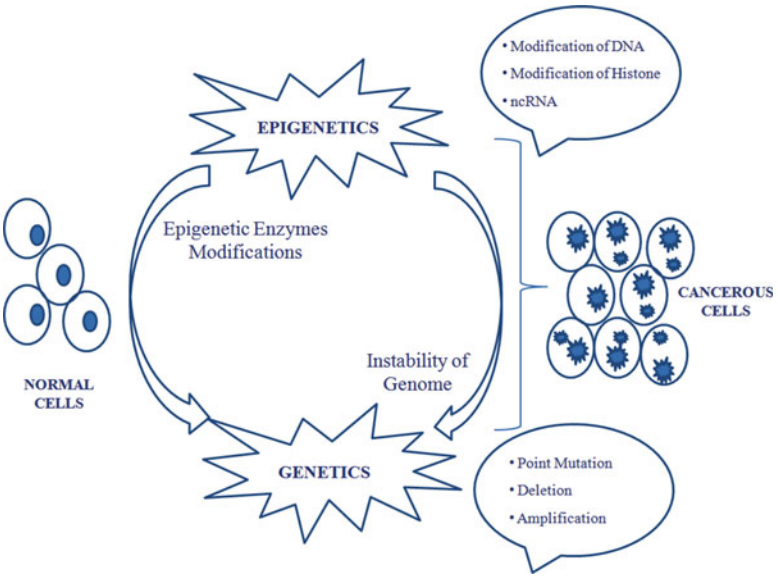

Fig. 11.1 Oncogenic mutation in normal cells due to epigenetic modifications

genetic level, where several genes get mutated or abnormally proliferated during the formation of cancer (Martin 2004; Vogelstein and Kinzler 2004). In the meantime, epigenetic modifications such as methylation of DNA, histone modifications, and microRNAs (miRNAs) lead to abnormal expression of genes (Chen et al. 2014), which induce instability of genome as explained in Fig. 11.1. Hence, an epigenetic can be safely characterized as a steadily inherited phenotype resulting out of progressions in a chromosome without apparent modifications in the DNA arrangement. In fact, all the various cellular pathways contributing to the neoplastic phenotype are affected by epigenetic genes in cancer (Jiang et al. 2015; Fornaro et al. 2016; Delpu et al. 2013). They are being investigated as biomarkers in clinical use for early detection of disease, tumor classification, and response to treatment with classical chemotherapy agents, target compounds, and epigenetic drugs (Mack et al. 2015; Andreol et al. 2013). These sorts of subtle adjustments are fundamental for ordinary cell physiology and function, aiding in the initiation or restraint of essential qualities in different phases of advancement. There are occurrences, however, in which the changes can be modified to actuate sporadic transcription of gene. In these cases, the results can incite different types of tumors in humans, with two key zones of modification, viz., methylation of DNA sequences and changes on the histones encompassing DNA. Since the disclosure of their association in the change of expression of the gene, modification of histones and methylation of DNA have been involved in sicknesses other than malignancy. One paramount part of epigenetic methylation is its reversibility; this key property has made a guaranteeing field of epigenetic treatment, which has prompted the

improvement of a few FDA sanction drugs for treatment of tumors. It has likewise produced a few new and energizing thoughts for future ways of treatment.

11.2 Epigenetics and Cancer Types

In this segment, we portray the present understanding about different types of cancer with their associated epigenetic enzyme classes, taking into account that established cause-consequence might not so much specific that these receptor targets can be accepted for anticancer drug discovery. In Table 11.1, we enumerated the associations between the major types of cancer and diverse epigenetic targets classes, which can be so much informative to fetch relevant drug discovery information (Andreol et al. 2013).

11.2.1 Breast Cancer

Epigenetic modifications including methylation of DNA and remodeling of chromatin play an important role in the development of breast cancer. In the similar manner, altered expression of microRNAs has also been reported to control important genes in the breast cancer development and progression (Veeck and Esteller 2010). Besides, various synthetic drugs based on epigenetic therapy which can decrease hypermethylation of DNA and deacetylation of histones are currently in preclinical and clinical trials (Lustberg and Ramaswamy 2010).

Table 11.1 Cancer types with their associated epigenetic enzyme classes

Epigenetic enzymatic classes	Cancer type
Methyltransferases	Breast cancer, colorectal cancer, leukemia, ovarian cancer, liver cancer, prostate cancer
Deacetylases	Breast cancer
Deacetylases (classes I, II, and IV)	Colorectal cancer, leukemia, ovarian cancer, gastric cancer, prostate cancer, liver cancer
K and R methyltransferases	Breast cancer, leukemia, myeloma, ovarian cancer, prostate cancer
Acetyltransferases	Leukemia, prostate cancer
Demethylases	Kidney cancer
miRNAs regulating proteins	Breast cancer, colorectal cancer, leukemia, myeloma, lung cancer, ovarian cancer, liver cancer
Kinases/phosphatases	Liver cancer

Epigenetic enzyme classes shown as bold characters are validated targets for the associated cancer types, whereas epigenetic enzyme classes shown as normal characters are partially validated targets

11.2.2 Ovarian Cancer

Epigenetic alterations, viz., aberrant methylation of DNA and unregulated distinct microRNAs expression, have resulted in altered expression of gene favoring survival of cells (Asadollahi et al. 2010). With reference to other cancerous diseases, the therapeutic improvement went for turning around oncogenic chromatin abnormalities which have been principally examined with DNA methyltransferase and histone deacetylase inhibitors. Moreover, the examination of various epigenetic events in which there is posttranscriptional gene regulation by small noncoding microRNAs has also been done (Ahluwalia et al. 2001).

11.2.3 Colorectal Cancer

Modification or extensive loss of DNA methylation patterns at several steps involved in the progression of colorectal cancer contributes fundamentally to epigenetic dysregulation (Kim and Deng 2007). In addition to this, epigenetically miRNAs modification has also been established to perform an important part in colorectal cancer (Grady and Markowitz 2002). Since major pathways of colorectal carcinogenesis are closely linked with changes in epigenetics, emerging evidence demonstrates that the risk of colorectal cancer can be impacted by lifestyle and factors affecting environment (Nyström 2009).

11.2.4 Prostate Cancer

In this cancer, epigenetic modifications come into view earlier and more frequently than the mutations occurring at genetic level. The identification of the silencing of multiple genes due to epigenetic alterations has been done (Chin et al. 2011). Preclinical confirmation including the epigenome as a key go-between in this cancer type involved preliminary clinical tests with epigenetic drugs, viz., histone deacetylase inhibitors (Kim and Deng 2007).

11.2.5 Leukemia

DNA and histone posttranslational modifications have been exhibited to be connected with a few changes in epigenetic targets for distinctive hematologic malignancies (Bishton et al. 2007). Biological players that are being used for clinical applications comprise deacetylases (Altucci and Minucci 2009), histone and DNA methyltransferases (Rodríguez-Paredes and Esteller 2011), and miRNA

(Florean et al. 2011). In this type of cancer, the function of different epigenetic enzyme classes is being studied primarily for acute promyelocytic leukemia (Petrie et al. 2009) and acute myeloid leukemia (Florean et al. 2011).

11.2.6 Gastric Cancer

The abnormal changes that occurred due to acetylation of histones which is regulated by histone acetyltransferases and histone deacetylases have been associated with gastric cancer (W-jian et al. 2012). Despite the fact that different connections between gastrointestinal malignancy and histone acetyltransferases and histone deacetylases have been distinguished, contrasting with other cancers, fewer advances have been accounted for to treat gastrointestinal carcinogenesis with epigenetic drugs.

11.2.7 Myeloma and Lymphomas

The importance in the modulation of epigenetic enzymes has been significantly raised for the treatment of myelomas and lymphomas, mainly as combination therapies (Mahadevan and Fisher 2011). For example, histone deacetylase inhibitors and DNA methyltransferase inhibitors are being already investigated for the cure of non-Hodgkin's lymphomas (Cotto et al. 2010; Yoshimi and Kurokawa 2011).

11.2.8 Liver Cancer

Methylation of DNA and RNA interference, as well as several modifications in histones, has been established as epigenetic events which contribute to the progression of hepatocellular carcinoma (Herceg and Paliwal 2011; Tischoff 2008). At present only histone deacetylase inhibitors have been studied for the treatment of such type of carcinoma (Lachenmayer et al. 2010).

11.2.9 Lung Cancer

Epigenetic changes, viz., methylation of DNA and covalent modifications of histone and chromatin with the help of epigenetic enzymes and miRNAs, are involved in the silencing of tumor suppressor genes and in enhancing the oncogene expression level (Yang 2011; Heller et al. 2010; Herman 2004). The restoration in

the expression level of silenced genes involved in epigenetics with novel targeted strategies and combined therapy with entinostat and azacitidine, as well as DNA methyltransferase inhibitors and histone deacetylase inhibitors, was examined in phase I/II clinical trials for the treatment of non-small-cell lung carcinoma (Heller et al. 2010).

11.2.10 Kidney Cancer

The modifications occurring due to methylation of DNA at an early stage of cancer may expose renal tissue to various changes taking place both at the epigenetic and genetic level, producing more cancerous growth (Dressler 2008). Currently, there are some clinical trials of phase I/II for testing inhibitors involved in deacetylation of histones which can lead to advanced renal cell carcinomas (Gan et al. 2009).

Most of the structural information about the enzyme classes involved in cancer epigenetics is well known and is used in the application of targetable molecules as mentioned in Table 11.2. First-generation epigenetic inhibitors such as histone deacetylase inhibitors and DNA methyltransferase inhibitors have as of now been affirmed for treatment of cancer. Extensive efforts have been made in current drug development that mainly focused to investigate more selective inhibitors which can be useful in multi-targeted approach therapy for the treatment of cancer.

11.2.11 Mechanisms of Epigenetic Regulation of Cancer

There are numerous chemical alterations that influence DNA, as well as RNA and proteins, and make diverse epigenetic layers. Out of these alterations, DNA methylation is the most well-studied epigenetic modification; in any case, it turns out to be progressively acknowledged that DNA methylation does not work alone yet rather is connected to different alterations, for example, histone modifications. As evident from Fig. 11.2, studies focusing on the methylation of the DNA cover almost half of the cancer epigenetic research (Razvi 2013). miRNA studies also become an integral part of the epigenetic research as nearly a quarter of the research community working on cancer epigenetics focuses on it.

11.2.11.1 DNA Methylation

Methylation of DNA is a prevalent alteration in bacteria, plants, and mammals. DNA methylation which occurs during the replication of DNA is a stable gene-silencing mechanism. It involves the addition of a –CH3 group to 5′ end of the CpG dinucleotide of the cytosine ring. Catalyzation of this reaction is being carried out by the DNA methyltransferase (DNMT) family, which comprises of DNMT1,

Table 11.2 Information about epigenetic enzyme classes and their connections with drug discovery for the cancer treatment

Epigenetic enzyme classes	Structural data	Clinical trials	Approved drugs	Known ligands
DNA methyltransferases	✓	✓	✓	✓
Histone acetylation				
Deacetylases class I, II, IV	✓	✓	✓	✓
Deacetylases class III	✓			✓
Acetyltransferases	✓	✓		✓
Histone ADP-ribosylation				
Mono-ADP-ribosyltransferases	✓			✓
Poly-ADP-ribosyltransferases	✓	✓		✓
Histone biotinylation				
Biotin ligase	✓			
Histone citrullination				
Deiminases	✓			✓
Histone glycosylation				
Glycosyltransferases/glycosidase	✓			✓
Histone methylation				
K and R methyltransferases	✓	✓		✓
Demethylases	✓	✓		✓
Histone phosphorylation				
Kinases/phosphatases	✓			
Histone ubiquitination and sumoylation				
E1, E2, and E3 enzymes	✓			✓
microRNA expression				
miRNA-regulating proteins	✓			✓

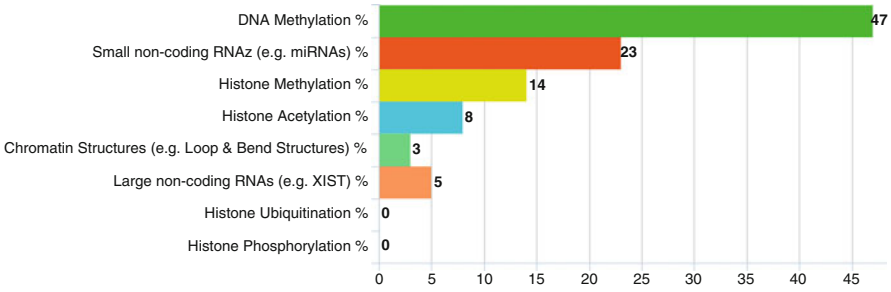

Fig. 11.2 Breakout of epigenetic research on the basis of epigenetic modifications

DNMT3A, and DNMT3B. Methylation of DNA is performed by DNMT3A and DNMT3B, which further results in the formation of 5-methylcytosine from cytosine residues of CpG dinucleotides during the formation of embryo, while DNMT1 is involved in maintaining the status of methylation during the process of embryo

Table 11.3 Aberrant DNA-methylated genes with associated cancer types

Gene	Cancer type
DNA methyltransferase	
DNMT 1	Ovarian and colorectal cancer
DNMT3b	Colon, colorectal, breast, ovarian, squamous cell carcinoma, esophageal cancers
Methyl-CpG-binding proteins	
MBD1	Prostate, lung, and colon cancer
MBD2	Prostate, lung, and colon cancer
MBD3	Colon and lung cancer
MBD4	Stomach, colon, endometrium cancers
MeCP2	Rett syndrome and prostate cancer
Kaiso	Lung, intestinal, and colon cancer

These genes can be overexpressed or silenced and cause cancer when their methylation activity is affected

formation. Conversion of 5-hydroxymethyl-2′-deoxycytidine from 5-methylcytosine is further carried out by the ten-eleven translocation (TET) family enzymes (Tahiliani et al. 2009). CpG islands are referred to the regions of DNA in the genome of human normally ranging from 0.5 to 5 kb in size and frequently occur in the promoter region of genes. Although the process of methylation of DNA in 5′ promoter region has been thoroughly investigated in various studies and has exhibited suppression of gene expression, the significance of 5-hydroxymethylation is still unclear and under investigation. Recent studies reported that the methylation of DNA occurs downstream in the promoter region (both intra- and intergenic) of genes as well as in regions with low CpG density neighboring CpG islands (Maunakea et al. 2010; Hansen et al. 2011). The following is the list of some DNA methylation genes that get altered in different human cancer types as mentioned in Table 11.3.

11.2.11.2 Histone Modification

The fundamental structure of nucleosome is comprised of histones, namely, H2A, H2B, H3, and H4, which together form the histone octamer around it (Luger et al. 1997). The N-terminals of histones protrude outward from the core of the nucleosome, whereas an array of covalent modifications, viz., methylation, acetylation, phosphorylation, sumoylation, ubiquitination, etc., occurs in the amino acid residues of this terminal. These covalent modifications can change the structure of chromatin from an open to a closed, condensed form and vice versa. The mono-, di-, or trimethylation of histones occurs at the ϵ-NH2 group of lysine amino acid residues, followed by mono- or dimethylation at arginine amino acid residues. In addition to other abovesaid covalent modifications, histone protein methylations are thought to represent an epigenetic code by the creation of binding interfaces for

Table 11.4 Aberrant histone-modified genes and their cancer-causing diseases (these histone-modifying genes can also be silenced or overexpressed by aberrant activity to cause cancer)

Gene	Cancer
SIRT1	Colon cancer
SIRT2	Glioma and gastric cancer
SIRT3	Breast cancer
SIRT4	Acute myeloid cancer
SIRT5	Breast cancer
SIRT6	Prostate, breast cancer
SIRT7	Thyroid carcinoma, breast cancer
HDAC1	Colorectal, cervical dysplasia, gastric, colon, stromal sarcomas, and prostate cancer
HDAC2	Colon and multiple gastric carcinomas
HDAC3	Prostate, colon cancer
HDAC4	Breast, prostate, and colon cancer
HDAC5	Acute myeloid cancer, colon cancer
HDAC6	Breast and acute myeloid cancer
HDAC7	Colon cancer
HDAC8	Colon cancer
HDAC9	Breast, lung cancer
HDAC10	Gastric cancer
P300	Ovarian, breast, oral, colorectal, hepatocellular cancers
CBP	Breast, colon, acute myeloid cancer, ovarian cancer
MOZ	Neurogenic progenitors, hematopoietic, leukemia cancer
PCAF	Colon cancer
MORF	Uterine, leiomyomata
Tip60	Prostate, colorectal cancer
DOT1L	Mixed lineage leukemia
MLL1	Cervical tumor
EHMT1, EHMT2	Esophageal squamous cell carcinoma

proteins involved in the regulation of chromatin (Sharma et al. 2010; Suzuki and Bird 2008).

The modifications of histones have a great impact on several biological processes, viz., transcriptional repression, activation of genes, and repair of DNA, with the exception of the packaging of chromatin. Based on the function, there are three classes of histone interacting proteins: (i) the writers which place the modification of histones, (ii) the erasers which remove these histone alterations, (iii) and the readers that recognize these alterations and may provide histone, nucleosome, or DNA-modifying enzymes (Kouzarides 2007b). The following is the list of a few histone-modified genes which get altered in different types of human cancers as mentioned in Table 11.4.

Some other posttranslational modifications are as follows:

Phosphorylation: Phosphorylation of histone plays an important role in DNA repair, gene silencing, cell cycle control, signal transduction pathway, cellular differentiation, and chromatin structure. It basically occurs on threonine, serine, and tyrosine amino acid residues.

ADP-ribosylation: ADP-ribosylation is the process in which one or more ADP-ribose molecules are added to a protein. It has been observed that histone protein is described to be mono-and poly-ADP ribosylated; thus they have a connection between codes. It is involved in different processes like cell signaling, gene regulation, and DNA repair; thus improper functioning causes disease like cancer.

Biotinylation: It is the process of attachment of biotin to a protein, nucleic acid, or other kind of molecule. This process is described in various histone variants and involved in various biological processes like cellular response to damage DNA, gene silencing, and cell proliferation.

Acetylation and Deacetylation: Acetylation and deacetylation of histone are the processes in which lysine residues of N-terminal tail of the nucleosome are acetylated or deacetylated, and it takes part in gene regulation. These processes are essential for gene regulation and catalyzed by enzymes like HDACs and HATs. Increased activity or overexpression of these enzymes can lead to formation of metastasis and tumor (cancer).

Citrullination/Deimination: This is the process of conversion of arginine residue in a protein into citrulline. In this process primary ketimine group (=NH) is replaced by a ketone group (=O) by the activity of enzymes like PADs (petideylarginine deiminases).

Carbonylation: It is the process in which RCS (reactive carbonyl species) covalently modifies cysteine residues in histone. RCS are produced with the help of enzymes like tyrosine kinases/phosphatases, transcription factors like p53, Nrf2, NFkB, and peroxiredoxins by redox signaling process. There is also a worse condition that some abnormal changes in redox signaling process can lead to the formation of malignant cells or cancerous cells.

Ubiquitination/Sumoylation: Ubiquitination and sumoylation are two very important PTM processes that play their roles in protein trafficking, cell survival, DNA damage response, signaling regulation, and cancer. Deregulation of these two processes causes abnormal activity of proteins; thus it contributes to disease like cancer.

Other PTMs: There are also kinds of posttranslational modifications such as histone proline isomerization and histone tail clipping. Histone tail clipping process removes N-terminal tail of histone molecule during transcription process and in other process which involve process like chromatin remodeling. Proline isomerization is a specific posttranslational modification that does not include covalent modifications, but it does include isomerization of proline residue.

11.2.11.3 microRNAs

microRNAs are short, endogenous, and noncoding RNAs normally 19–25 nucleotides in length and are conserved throughout evolution. These miRNAs mainly belong to the 3′ untranslated regions (3′ UTR) of target mRNA to control the expression of genes in two ways: (i) silencing of posttranscriptional process (ii) and target mRNA degradation (Rouhi et al. 2008). The relationship between several epigenetic mechanisms and these miRNAs is a quite convoluted and complex regulatory network (Iorio et al. 2010). miRNA expression is tissue specific and is controlled by various epigenetic changes, viz., methylation of DNA and alterations of histones (Friedman et al. 2009). miRNAs can also have an effect on epigenetic mechanisms which control the transcription of genes and the capability to target posttranscriptional silencing of genes (Kasinski and Slack 2011). Convincing proof now demonstrates that miRNAs are liable to both hypo- and hypermethylation in a tumor as well as tissue-specific manner (Wee et al. 2014). Recent studies also suggest that hypermethylation can mimic small chromosomal deletions or loss of heterozygosity with the help of long-range epigenetic silencing (Malkhosyan et al. 1996; Duval et al. 2001). The concept of long-range epigenetic silencing is no longer "one methylated CpG island – one silent gene" but rather involves large regions which may include several genes (Perucho 1996).

11.2.12 Identification Methods for Epigenetic Modifications Involved in Cancer

Earlier, the profiles associated with epigenetics were restricted to individual genes only, but these days, the researchers have adopted a whole genomic approach in order to find out an entire genomic profile for cancer cells versus normal cells.

Prominent methodologies for measuring CpG methylation in cells consist of:

- Bisulfite sequencing: This type of sequencing involves the use of bisulfite treatment of DNA to determine its methylation patterns. Treatment of DNA with bisulfite converts cytosine residues to uracil but leaves 5-methylcytosine residues unaffected. Subsequently, bisulfite treatment brings about specific changes in the sequence of DNA that rely on upon the methylation status of individual cytosine residues, yielding single-nucleotide determination data about the methylation status of a DNA segment.
  Different investigations can be performed on the altered sequence to retrieve this information. The target of this examination is consequently reduced to differentiating between single-nucleotide polymorphisms (cytosines and thymidine) coming about because of bisulfite conversion.
- MethyLight: It is a highly sensitive assay, equipped for recognizing methylated alleles within the sight of a 10,000-fold excess of unmethylated alleles. The test is likewise exceedingly quantitative and can very precisely decide the relative

prevalence of a particular pattern of methylation of the DNA. The most striking point of interest of MethyLight, when contrasted with existing procedures, is its capability to permit the fast screening of hundreds to thousands of samples.

- Pyrosequencing: It is a strategy for DNA sequencing (determining the order of nucleotides in DNA) based on the "sequencing by synthesis" principle. It contrasts from Sanger sequencing, in that it depends on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides.
- Arbitrary primed PCR: A deoxyribonucleic acid (DNA) fingerprinting technique in which one short arbitrary primer is used to amplify multiple DNA fragments of different length, which yield a fingerprint after separation in gel electrophoresis. It is also known as random amplification.
- Combined bisulfite restriction analysis (COBRA): A molecular biology technique that takes into account the sensitive quantification of levels of DNA methylation at a specific genomic locus on a DNA sequence in a small sample of genomic DNA. This method is a modification of bisulfite sequencing, which combines bisulfite conversion-based polymerase chain reaction with restriction digestion.
- Restriction landmark genomic scanning: It is a genome investigation technique that takes into consideration fast concurrent visualization of thousands of landmarks or restriction sites. By utilizing a combination of restriction enzymes, some of which are specific to modifications of DNA, the method can be used to visualize differences in the levels of methylation across the genome of a given organism.
- Chromatin immunoprecipitation (ChIP): An immunoprecipitation experimental technique used to investigate the interaction between proteins and DNA in the cell. It intends to figure out whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters or other DNA-binding sites and potentially characterizing cistromes. It also aims to determine the specific location in the genome where various histone modifications are linked with, demonstrating the target of the histone modifiers.
- HELP assay (Hpall tiny fragment enrichment by ligation-mediated PCR): This is one of a few procedures utilized for figuring out if DNA has been methylated. The system can be adjusted to look at DNA methylation within and around individual genes, or it can be extended to inspect methylation in a whole genome.
- Methylated DNA immunoprecipitation: MeDIP or mDIP is a large-scale (chromosome- or genome-wide) purification technique which is used to enrich for methylated DNA sequences. It consists of isolating methylated DNA fragments via an antibody raised against 5-methylcytosine (5mC). In any case, comprehension of the methylome stays simple; its study is entangled by the way that, as other epigenetic properties, patterns vary from cell type to cell type.
- Profiling of the expression of genes using DNA microarray: Comparing mRNA levels from diseased cell lines prior and then afterward treatment with a demethylating agent.

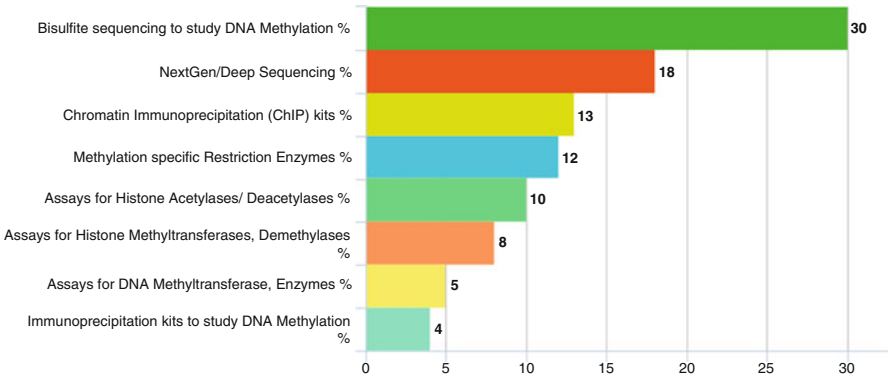

Fig. 11.3 Segmentation of the epigenetic research on the basis of deployment of assay classes

In view of the fact that bisulfite sequencing is an important marker strategy to measure CpG methylation, when one of alternate methods is utilized, results are normally affirmed utilizing this technique. The bisulfite conversion kits to study the methylation of DNA cover around 30% of the epigenetic research market as clear from the Fig. 11.3. The ChIP utilizing antibodies specific for methyl-CpG-binding domain proteins also occupies around 13% of the research based on the cancer epigenetics (Razvi 2013). The well-known methodologies for determining profiles of histone modification in healthy versus cancerous cells comprise of the two techniques, i.e., mass spectrometry and chromatin immunoprecipitation assay.

11.2.13 Clinical Use of Epigenetics

Currently, there are two noteworthy zones of interest for the clinical use of epigenetics, to be specific, biomarkers and therapeutics.

1. *Cancer Biomarkers:* The methylated genomic DNA has a wide range of properties, which makes it an alluring molecule for biomarker utility. Initially, it is steady in biofluids, for example, blood, saliva, and urine. Secondly, in most of the cases, methylation in CpG is obtained amid malignant transformation and hence specific to neoplasia. Lastly, the systems utilized for detection of methylated DNA are promptly manageable to automation.
2. *Cancer Therapeutics:* Both epigenetic proteins and protein markers are great focuses for the improvement of new anticancer medications. The verification of idea for epigenetic treatments is the FDA and EMEA approval of demethylating agents and histone acetylase (HDAC) inhibitors for the treatment of MDS, AML, and certain types of lymphomas, respectively. In any case, we ought not to overlook that these agents are nonselective without having their side effects clearly known.

11.2.14 Future Perspective for Cancer Epigenetics Therapy

Various reported studies on genome-wide mapping suggested the information about how normal genomes are developed significantly illuminating our perspective of epigenetic variations in cancer. From a period that started with recognizing cancer-specific abnormalities in the methylation of DNA, both gains and losses, we now comprehend that these must not just be connected to characterize key-related changes in chromatin but also viewed in the perspective that all genomic regions are not equal for susceptibility to these modifications (Baylin and Jones 2011b; Berman et al. 2012). An important example is the disclosure that both the gains and losses of methylation of DNA in cancer can be biased to different genomic regions associated with nuclear lamin, late-replicating DNA, i.e., enriched for low-transcription developmental genes with bivalent chromatin in the promoter region (Hegi et al. 2009). In both the embryonic and adult stem cells, such chromatin is necessary for maintenance of the state of stem cells and appears susceptible to evolve epigenetic variations during progression of tumor. This susceptibility may seriously include stresses, viz., increased ROS that intensely shifts a complex of proteins, comprising DNA methyltransferases and polycomb proteins into CpG islands. The confinement of such proteins may lead to aberrant methylation of DNA. Several stimulating examples of the effectiveness of such methodologies have emerged, and these will without a doubt increment significantly in the near future.

The use of epigenetic drugs with an intention to reestablish sensitivity to hormonal as well as cytotoxic drugs is a big challenge in cancer therapy. The restoration of the hormonal sensitivity in breast cancer is of highest medical significance and has come under serious consideration in various reported studies of the most recent decades (Baylin and Jones 2011b; Berman et al. 2012). Altogether 25% of breast cancers have the repressed estrogen receptor alpha (ERalpha) because of hypermethylation of the ER promoter and don't react to endocrine treatment. Recent reported studies established that decitabine and histone deacetylase inhibitors, viz., trichostatin A, entinostat, and scriptaid, can restore ER mRNA expression (Raha et al. 2011).

Striking advancement has been made in the last few years on the methylation of DNA and modifications of histones involved in the transcription of genes; however, the significance of these phenomena in epigenetic regulation of cancer has not been fully clarified. On the other hand, a lot of research advancement has been made in context to improve drugs associated with cancer epigenetics which can target chromatin and enzymes taking part in the modification of histones. Numerous epigenetic medications, comprising a histone deacetylase inhibitor and two DNA methyltransferase enzyme inhibitors, have been sanctioned by the FDA as viable medications for the treatment of cancer. In the meantime, different inhibitor drugs, for example, SAHA (Marks 2007), MS-275 (Bracker et al. 2009), and FK228 (Saijo et al. 2012), have as of now been the prime focus and are in step III clinical tests.

Therefore, more specific and potent inhibitors should be developed to diminish undesirable side effects. Studies on understanding of the impact of epigenetic changes occurring in cancer and tumor pathology are likely to improve the capability to detect and treat cancer (Mack et al. 2015; Wu et al. 2016).

11.3 Conclusion

The utmost challenge for researchers working on cancer therapy is to integrate the available data to understand the translational prospective of specific expression profile. However, various studies on epidemiology have acknowledged both the environmental and dietary factors as also related with cancer; animal models are capable to recognize the mechanisms as well as correlation between these environmental factors and carcinogenesis. In spite of the fact that the viability of epigenetic treatment for cancer therapy remains unproven till now, there is a strong urge to consider the use of epigenetic agents, perhaps informed by epigenetic profiling of individual patient, which may facilitate the therapeutic window for personalized medication. In addition, such studies will also facilitate the identification of specific subtypes of cancer which are more prone to chemotherapy (Lv et al. 2015; Cho 2011). This will also help in effective use of various epigenetic target inhibitors, comprising DNA-demethylating agents, histone deacetylase inhibitors, or several other promising therapies which are undergoing preclinical and clinical tests. The plethora of genetic modifications in epigenetic regulators offers numerous conceivable focuses for drug discovery and will probably draw in the consideration of the pharmaceutical business. Therefore, the characterization of the progression of tumor at the molecular level, involving both genetic and epigenetic profiles, is considered to be an important step in assessment of the progress of individualized treatment modalities as well as personalized therapies available for such cancers.

References

Ahluwalia A, Hurteau JA, Bigsby RM, Nephew KP. DNA methylation in ovarian cancer. II. Expression of DNA methyltransferases in ovarian cancer cell lines and normal ovarian epithelial cells. Gynecol Oncol. 2001;82(2):299–304.

Altucci L, Minucci S. Epigenetic therapies in haematological malignancies: searching for true targets. Eur J Cancer. 2009;45(7):1137–45.

Andreol F, Barbosa AJM, Daniele Parenti M, Rio AD. Modulation of epigenetic targets for anticancer therapy: clinicopathological relevance, structural data and drug discovery perspectives. Curr Pharm Des. 2013;19(4):578–613.

Asadollahi R, CAC H, Zhong XY. Epigenetics of ovarian cancer: from the lab to the clinic. Gynecol Oncol. 2010;118(1):81–7.

Baylin SB. Epigenetics and cancer. Mol Basis Cancer. 2008:57–65.

Baylin SB, Jones PA. A decade of exploring the cancer epigenome – biological and translational implications. Nat Rev Cancer. 2011a;11(10):726–34.

Baylin SB, Jones PA. A decade of exploring the cancer epigenome – biological and translational implications. Nat Rev Cancer. 2011b;11:726–34.
Berman BP, Weisenberger DJ, Aman JF, Hinoue T, Ramjan Z, Liu Y, Noushmehr H, Lange CPE, Dijk CMV, Tollenaar RAEM, Berg DVD, Laird PW. Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. Nat Genet. 2012;44(1):40–6.
Bishton M, Kenealy M, Johnstone R, Rasheed W, Prince HM. Epigenetic targets in hematological malignancies: combination therapies with HDAC is and demethylating agents. Expert Rev Anticancer Ther. 2007;7(10):1439–49.
Bracker TU, Sommer A, Fichtner I, Faus H, Haendler B, Hess-Stumpp H. Efficacy of MS-275, a selective inhibitor of class I histone deacetylases, in human colon cancer models. Int J Oncol. 2009;35(4):909–20.
Campbell RM, Tummino PJ. Cancer epigenetics drug discovery and development: the challenge of hitting the mark. J Clin Invest. 2014;124(1):64–9.
Chen QW, Zhu XY, Li YY, Meng ZQ. Epigenetic regulation and cancer (Review). Oncol Rep. 2014;31(2):523–32.
Chi P, Allis CD, Wang GG. Covalent histone modifications–miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer. 2010;10(7):457–69.
Chin SP, Dickinson JL, Holloway AF. Epigenetic regulation of prostate cancer. Clin Epigenet. 2011;2(2):151–69.
Cho WC. Epigenetic alteration of microRNAs in feces of colorectal cancer and its clinical significance. Expert Rev Mol Diagn. 2011;11(7):691–4.
Cosgrove MS, Boeke JD, Wolberger C. Regulated nucleosome mobility and the histone code. Nat Struct Mol Biol. 2004;11(11):1037–43.
Cotto M, Cabanillas F, Tirado M, García MV, Pacheco E. Epigenetic therapy of lymphoma using histone deacetylase inhibitors. Clin Transl Oncol. 2010;12(6):401–9.
Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009;10(10):704–14.
Cruickshank MN, Besant P, Ulgiati D. The impact of histone posttranslational modifications on developmental gene regulation. Amino Acids. 2010;39(5):1087–105.
De Koning L, Corpet A, Haber JE, Almouzni G. Histone chaperones: an escort network regulating histone traffic. Nat Struct Mol Biol. 2007;14(11):997–1007.
Delpu Y, Cordelier P, Cho WC, Torrisani J. DNA methylation and cancer diagnosis. Int J Mol Sci. 2013;14(7):15029–58.
Dressler GR. Epigenetics, development, and the kidney. J Am Soc Nephrol JASN. 2008;19(11):2060–7.
Duval A, Rolland S, Compoint A, Tubacher E, Iacopetta B, Thomas G, Hamelin R. Evolution of instability at coding and non-coding repeat sequences in human MSI-H colorectal cancers. Hum Mol Genet. 2001;10:513–8.
Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004;4:143–53.
Florean C, Schnekenburger M, Grandjenette C, Dicato M, Diederich M. Epigenomics of leukemia: from mechanisms to therapeutic applications. Epigenomics. 2011;3(5):581–609.
Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, Jia M, Shepherd R, Leung K, Menzies A, et al. COSMIC: mining complete cancer genomes in the catalogue of somatic mutations in cancer. Nucleic Acids Res. 2011;39(Database issue):D945–50.
Fornaro L, Vivaldi C, Caparello C, Musettini G, Baldini E, Masi G, Falcone A. Pharmacoepigenetics in gastrointestinal tumors: MGMT methylation and beyond. Front Biosci (Elite edition). 2016;8:170–80.
Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, Zhou X, Jones PA. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res. 2009;69(6):2623–9.
Gan HK, Seruga B, Knox JJ. Targeted therapies for renal cell carcinoma – more gains from using them again. Curr Oncol. 2009;16(S1):45–51.

Gardner KE, Allis CD, Strahl BD. Operating on chromatin, a colorful language where context matters. J Mol Biol. 2011;409(1):36–46.
Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. Annu Rev Genomics Hum Genet. 2002;3(37):101–28.
Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, Wen B, Wu H, Liu Y, Diep D, Briem E, Zhang K, Irizarry RA, Feinberg AP. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43(8):768–75.
Hegi ME, Sciuscio D, Murat A, Levivier M, Stupp R. Epigenetic deregulation of DNA repair and its potential for therapy. Clin Cancer Res. 2009;15(16):5026–31.
Heller G, Zielinski CC, Zöchbauer-Müller S. Lung cancer: from single-gene methylation to methylome profiling. Cancer Metastasis Rev. 2010;29(1):95–107.
Herceg Z, Paliwal A. Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. Mutat Res/Rev Mutat Res. 2011;727(3):55–61.
Herceg Z, Ushijima T. Introduction: epigenetics and cancer. Adv Genet. 2010;70:1–23.
Herman JG. Epigenetics in lung cancer: focus on progression and early lesions. Chest. 2004;125(5 Suppl):119S–22S.
Imhof A. Epigenetic regulators and histone modification. Brief Funct Genom Proteom. 2006;5(3):222–7.
Iorio MV, Piovan C, Croce CM. Interplay between microRNAs and the epigenetic machinery: an intricate network. Biochimica et Biophysica Acta (BBA)-Gene Regul Mech. 2010;1799:694–701.
Jiang W, Liu N, Chen XZ, Sun Y, Li B, Ren XY, Qin WF, Jiang N, Xu YF, Li YQ, Ren J, Cho WC, Yun JP, Zeng J, Liu LZ, Li L, Guo Y, Mai HQ, Zeng MS, Kang TB, Jia WH, Shao JY, Ma J. Genome-wide identification of a methylation gene panel as a prognostic biomarker in nasopharyngeal carcinoma. Mol Cancer Ther. 2015;14(12):2864–73.
Jones PA, Baylin SB. The epigenomics cancer. Cell. 2007;128(4):683–92.
Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat Rev Cancer. 2011;11(12):849–64.
Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nat Biotechnol. 2010;28(10):1069–78.
Kim YS, Deng G. Epigenetic changes (aberrant DNA methylation) in colorectal neoplasia. Gut Liver. 2007;1(1):1–11.
Kouzarides T. Chromatin modifications and their function. Cell. 2007a;128(4):693–705.
Kouzarides T. Chromatin modifications and their function. Cell. 2007b;128:693–705.
Lachenmayer A, Alsinet C, Chang CY, Llovet JM. Molecular approaches to treatment of hepatocellular carcinoma. Dig Liver Dis. 2010;42(Suppl 3):S264–72.
Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature. 1997;389:251–60.
Lustberg MB, Ramaswamy B. Epigenetic therapy in breast cancer. Curr Breast Cancer Rep. 2010;3(1):34–43.
Lv JF, Hu L, Zhuo W, Zhang CM, Zhou HH, Fan L. Epigenetic alternations and cancer chemotherapy response. Cancer Chemother Pharmacol. 2015;77(4):673–84.
Mack SC, Hubert CG, Miller TE, Taylor MD, Rich JN. An epigenetic gateway to brain tumor cell identity. Nat Neurosci. 2015;19(1):10–9
Mahadevan D, Fisher RI. Novel therapeutics for aggressive non- Hodgkin's lymphoma. J Clin Oncol. 2011;29(14):1876–84.
Malkhosyan S, Rampino N, Yamamoto H, Perucho M. Frameshift mutator mutations. Nature. 1996;382(6591):499–500.
Marks PA. Discovery and development of SAHA as an anticancer agent. Oncogene. 2007;26(9):1351–6.
Martin GS. The road to Src. Oncogene. 2004;23:7910–7.
Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine

VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJM, Haussler D, Marra MA, Wang T, Costello JF. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010;466(7303):253–7.
Nyström M. Diet and epigenetics in colon cancer. World J Gastroenterol. 2009;15(3):257.
Perucho M. Microsatellite instability: the mutator that mutates the other mutator. Nat Med. 1996;2:630–1.
Petrie K, Zelent A, Waxman S. Differentiation therapy of acute myeloid leukemia: past, present and future. Curr Opin Hematol. 2009;16(2):84–91.
Raha P, Thomas S, Munster PN. Epigenetic modulation: a novel therapeutic target for overcoming hormonal therapy resistance. Epigenomics. 2011;3(4):451–70.
Razvi E. Epigenetic research classes and assay trends. GEN Reports: Market & Tech Analysis. 2013; 1–10.
Rodríguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nat Med. 2011;17(3):330–9.
Rouhi A, Mager DL, Humphries RK, Kuchenbauer F. MiRNAs, epigenetics, and cancer. Mamm Genome. 2008;19:517–25.
Saijo K, Katoh T, Shimodaira H, Oda A, Takahashi O, Ishioka C. Romidepsin (FK228) and its analogs directly inhibit phosphatidylinositol 3-kinase activity and potently induce apoptosis as histone deacetylase/phosphatidylinositol 3-kinase dual inhibitors. Cancer Sci. 2012;103(11):1994–2001.
Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31:27–36.
Sidoli S, Cheng L, Jensen ON. Proteomics in chromatin biology and epigenetics: elucidation of post-translational modifications of histone proteins by mass spectrometry. J Proteome. 2012;75(12):3419–33.
Sippl W, Jung M. Epigenetic targets in drug discovery. Chem List. 2010;104:131–2.
Stratton MR, Campbel PJ, Futreal PA. The cancer genome. Nature. 2009;458:719–24.
Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet. 2008;9:465–76.
Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930–5.
Tischoff I. DNA methylation in hepatocellular carcinoma. World J Gastroenterol. 2008;14(11):1741.
Veeck J, Esteller M. Breast cancer epigenetics: from DNA methylation to microRNAs. J Mammary Gland Biol Neoplasia. 2010;15(1):5–17.
Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004;10:789–99.
Weake VM, Workman JL. Histone ubiquitination: triggering gene activity. Mol Cell. 2008;29(6):653–63.
Wee S, Dhanak D, Li H, Armstrong SA, Copeland RA, Sims R, Baylin SB, Liu XS, Schweizer L. Targeting epigenetic regulators for cancer therapy. Ann N Y Acad Sci. 2014;1309(1):30–6.
W-jian S, Zhou X, Zheng J-hang LMD, Nie JY, Yang XJ, Zheng ZQ. Histone acetyltransferases and deacetylases: molecular and clinical implications to gastrointestinal carcinogenesis. Acta Biochim Biophys Sin. 2012;44(1):80–91.
Wu K, Sharma S, Venkat S, Liu K, Zhou X, Watabe K. Non-coding RNAs in cancer brain metastasis. Front Biosci (Scholar edition). 2016;8:187–202.
Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. Am J Respir Crit Care Med. 2011;183(10):1295–301.
Yoshimi A, Kurokawa M. Key roles of histone methyltransferase and demethylase in leukemogenesis. J Cell Biochem. 2011;112(2):415–24.