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Chapter

Evolution of Epigenome as the Blueprint for Carcinogenesis

Zeenat Farooq, Ambreen Shah, Mohammad Tauseef, Riyaz Ahmad Rather and Mumtaz Anwar

Abstract

Epigenetics "above or over genetics" is the term used for processes that result in modifications which are stably inherited through cell generations, without changing the underlying DNA sequence of the cell. These include DNA methylation, Post-translational histone modification and non-coding RNAs. Over the last two decades, interest in the field of epigenetics has grown manifold because of the realization of its involvement in key cellular and pathological processes beyond what was initially anticipated. Epigenetics and chromatin biology have been underscored to play key roles in diseases like cancer. The landscape of different epigenetic signatures can vary considerably from one cancer type to another, and even from one ethnic group to another in the case of same cancer. This chapter discusses the emerging role of epigenetics and chromatin biology in the field of cancer research. It discusses about the different forms of epigenetic mechanisms and their respective role in carcinogenesis in the light of emerging research.

Keywords: Epigenetics, DNA Methylation, Histone Modifications, Cancer

1. Introduction

Transmission of characters in a stable, inheritable manner is governed by the genetic make-up of a cell. This information for vertical transmission of characters is carried by the macromolecule deoxyribonucleic acid (DNA). The linear sequence of nucleotides in the DNA dictates the sequence of amino acids in the proteins and hence controls all the vital processes occurring within the cell. However, the linear length of DNA molecules is very long. For example, a typical human cell contains about 2 meters long DNA. Therefore, in order to accommodate DNA into nucleus, this genetic information is contained in the form of a nucleoprotein complex called chromatin [1]. This is particularly true about eukaryotic cells. Though prokaryotic cells also contain a nucleoid, it, however, is not well-organized.

The organization of DNA into chromatin is particularly important for two main reasons.

1. To bring about compaction of the large DNA molecule into a small nuclear space in an ordered manner.

2. To facilitate regulated gene expression.

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Alongside DNA, chromatin mainly consists of small, basic positively charged group of proteins called histones. The positively charged histones bind with the negatively charged DNA in an energetically favorable manner inside chromatin [2]. These proteins have remained the focus of intensive research for many years now. Apart from DNA and histones, chromatin also contains a huge array of non-histone proteins, most of which are not as well characterized and well-studied as histones.

Earlier it was thought that compaction of DNA into chromatin solely occurs to accommodate DNA. But later it was realized that this compaction plays a paramount role in orderly organization of DNA and thereby helps in differential gene expression. The fundamental repeating unit of chromatin is the nucleosome which consists of two copies each of histones H2A, H2B, H3 and H4 wrapped around 146 bp of DNA in a left-handed helical manner [1]. The histone proteins are named in the order in which they were discovered. Because of being associated with the nucleosome core, these histone proteins are known as the core histones. Another class of histones binds DNA at the entry and exit sites into nucleosomes. This is known as the linker histone H1 and paves way for further compaction of nucleosomes into higher order chromatin structures (**Figure 1**).

Upon observation under a microscope, chromatin appears as two distinct entities within the nucleus. These are termed as euchromatin and heterochromatin. Euchromatin is the lightly stained part of chromatin which mostly lies towards the interior regions of nucleus and contains actively transcribed genomic regions. Heterochromatin is the darkly stained fraction which mostly lies towards the periphery of nucleus [3]. It contains regions which are transcriptionally silent and mostly contains repetitive DNA sequences. This spatial organization of chromatin is maintained through various mechanisms. These mechanisms serve as the "epigenetic carriers of nuclear information" within the cell and include covalent histone modifications, non-coding RNAs and chromatin remodeling complexes and lately also included DNA methylation (**Figure 2**).

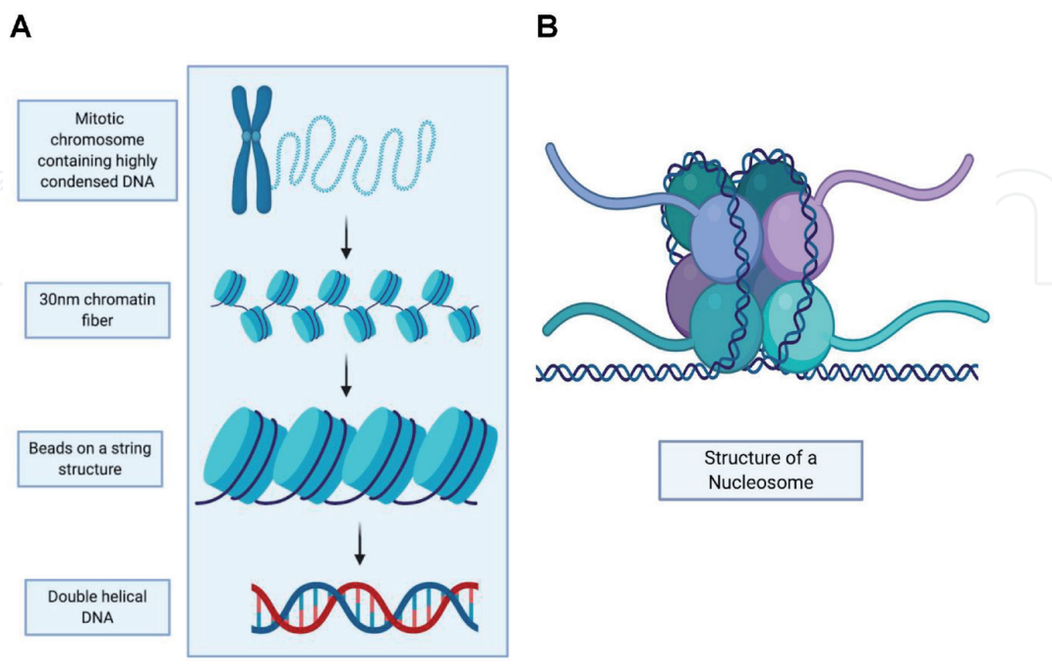

Figure 1.
Representation of different levels of hierarchical chromatin organization. (A) Inside a compact chromosome, DNA and proteins are organized at different levels. (B) Ultrastructure of a nucleosome containing two copies of H2A,H2B,H3 and H4 inside 147 bp of DNA.

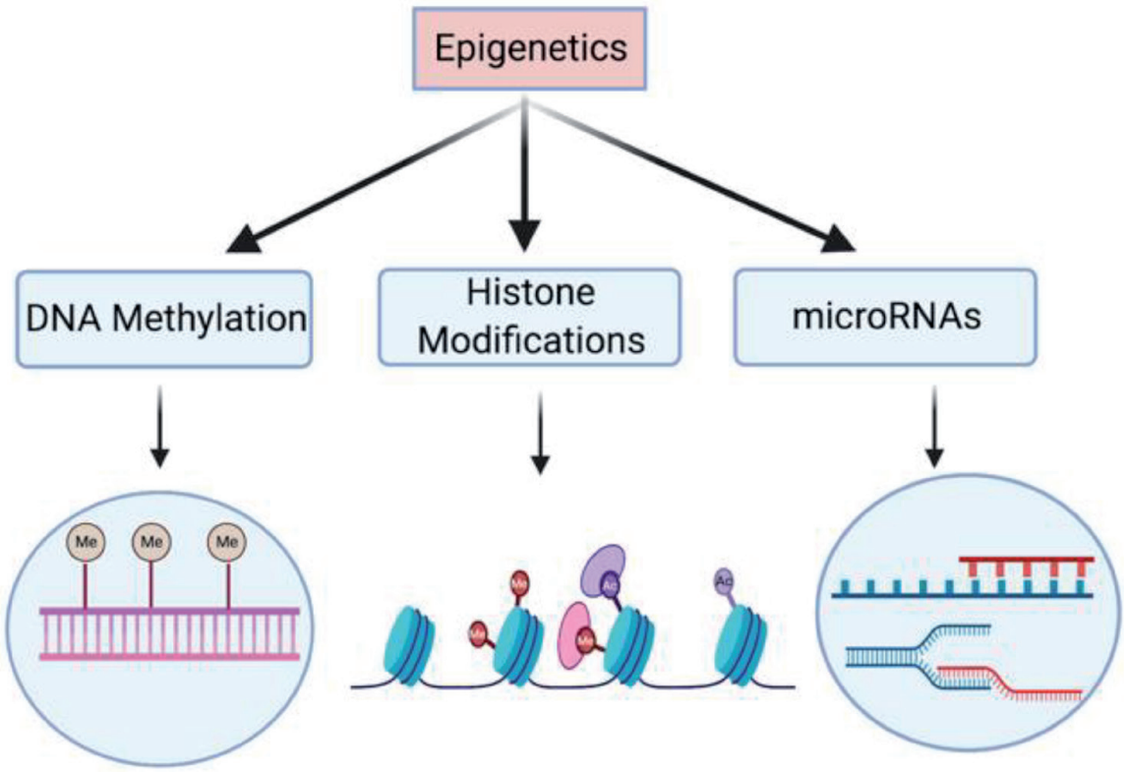

Figure 2.
Major players involved in the propagation of epigenetic mechanisms in cells. DNA methylation and micro RNAs are involved in gene silencing, histone modifications are involved in both silencing and expression of genes.

2. Epigenetics and chromatin biology: unifying themes and differences

At its heart, epigenetics refers to the study of heritable changes in gene expression without changes in the DNA sequence. This term was coined by Waddington and as the name indicates, epi (above or over genetics) is any moiety that can be stably inherited by cells across many generations without altering the sequence of nucleotides in the DNA. The study of epigenetics previously involved study of covalent histone modifications and non-coding RNAs. However, DNA methylation has also been increasingly recognized as an epigenetic phenomenon owing to its non-sequence based heritable nature and its importance in maintaining cellular homeostasis and association of its perturbations with various diseases. Therefore, the definition and scope of epigenetics has changed dynamically since the inception of the field.

Quite often, epigenetics and chromatin biology are very loosely stated terms. However, to be more precise, epigenetics refers to the study of "epigenetic marks or signatures" which play a prominent role in maintenance of cellular homeostasis whereas chromatin biology refers to the study of "chromatin structure and function". This encompasses nuclear dynamics, topology, localisation, organisation and three-dimensional (3D) structure [3]. There is a huge overlap between the two terms, and these are often used interchangeably. For example, epigenetic signatures and modifications play a paramount role in the maintenance of nuclear topology, overall chromatin organization and chromatin states.

Field of epigenetics is very interesting because of the reversible nature of epigenetic changes. This means that although these changes can be stably inherited, however, unlike DNA sequence, these changes can also be reversed under particular

conditions. In fact, mechanisms are well in place within the cells which lead to the reversal of these modifications [4]. Interestingly, these changes can also be targeted for the reversal externally, using specific enzymes, under desired conditions. This may include the reversal of epigenetic modifications involved in disease progression with the help of enzymes [5]. For example, reversal of an epigenetic modification that is involved in carcinogenesis by an enzyme specific for the reversal to alleviate some of the symptoms.

Epigenetic modifications play a very prominent role in almost all the cellular processes like growth, cell division, maintenance of cellular identity etc. Therefore, any changes in these modifications can lead to serious outcomes. Perturbations in epigenetic modifications have been observed to be involved in various deleterious conditions including cancer [6].

In this chapter, we shall discuss about the various epigenetic mechanisms, their importance, major functions that they carry out in the cells and changes to these marks and their implications in cancer.

3. DNA methylation

DNA methylation involves transfer of a methyl group from S-adenosylmethionine to the 5'position of cytosine residues in DNA. DNA methylation is one of the most prominent epigenetic events that take place within the cells and has been shown to play important roles in various cellular processes like genome integrity, genome imprinting, X chromosome inactivation and development [7–9].

DNA methylation at 5 methyl cytosine is catalyzed by two groups of methyltransferases.

1. DNMT1 which catalyzes methylation on the newly synthesized hemi-methylated DNA strand, utilizing the parental strand as template for copying of methylation pattern. This class of enzymes are known as the maintenance methyltransferases as they play role in maintaining the methylation status following replication. These are critically important enzymes for mammals as mice deficient in DMNT1 display embryonic lethality [10].

2. DNMT3a and 3b. These are the enzymes which play role in methylating DNA at 5′ methyl cytosine without utilization of a methylated template. These enzymes are therefore known as *de novo* methyltransferases and these have been known to catalyze methylation events during various important cellular phases like development. These enzymes are therefore highly expressed during embryogenesis and display reduction in expression pattern in adult tissues [11]. DNMT 3a and 3b are also extremely important for mammals since DNMT 3b deficient mice, similar to DNMT 1, are embryonic lethal whereas those deficient in DNMT 3a die by the age of 4 weeks [10].

Another member of the DNMT family of enzymes is DNMT 3 L. It was discovered in 2000. DNMT 3 L lacks an intrinsic methyltransferase activity but assists DNMNT3a and 3b in methylating retrotransposons [12].

In eukaryotes, DNA methylation occurs predominantly within repetitive sequences in order to maintain genomic integrity [13]. Methylation on cytosine

residues usually takes place in the context of CG dinucleotides (Known as CpG) and around 75% of CpG dinucleotides in humans remain methylated. These CpG dinucleotides are unevenly distributed but are concentrated in stretches of high frequency known as CpG islands. These islands remain mostly unmethylated and can be found in the promoters of constitutively expressed genes like housekeeping genes [14]. In humans, almost half of the estimated 29,000 CpG islands remains unmethylated under normal conditions [15–17].

Methycytosine residues often co-operate with other effectors to bring about a silenced chromatin state. Methyl binding domain (MBD) proteins recognize and bind to methylated cytosines. These MBD proteins act as a signal/binding platform for histone modifying and chromatin remodeling enzymes to bring about further compaction of chromatin [18]. Apart from binding methylated DNA, MBD 2 (a member of MBD family of proteins) has also been shown to promote the DNA methyltransferase activity of NuRD (chromatin remodeling complex) by interacting with NuRD [19, 20]. This interaction brings NuRD complex in close proximity of cytosine residues which are later methylated by NuRD. Till date, six members of methyl binding domain proteins have been identified that include MBD1, MBD2, MBD3, MBD4, methylcytosine binding protein 2 (MECP2) and Kaiso [21]. All of these proteins are under intense investigation and efforts are being made to identify more members of the family.

Various genes contain regions of CpG dinucleotides in their promoters with variable degrees of methylation levels [14]. These levels are crucial for normal functioning of the cells and any mis-regulation in this level is associated with a number of physiological outcomes. Methylated DNA elements often co-operate with other epigenetic elements to ensure proper silencing of chromatin and any increase in levels of DNA methylation are often involved in silencing of cognate genes which can lead to carcinogenesis [15, 22]. For example, it has been observed that increase in the levels of promoter DNA methylation in tumor suppressor genes leads to a decrease in their expression and hence a steady decline in their cellular activity is observed [15, 23–25]. Hypermethylated promoters can also serve as targets for transition mutations due to spontaneous deamination of 5'methyl cytosine into thymine [7, 26]. This leads to transmission of DNA with errors during replication to new cells. These cells are genomically unstable and with time, accumulate more and more mutations which in the absence of proper surveillance, eventually lead to cancer initiation [7, 16, 27]. Decrease in the DNA methylation of tumor suppressor genes has been observed in a number of primary tissues from cancer patients at various geographical locations.

Global hypomethylation can also ensue which can lead to loss of repression from the repetitive DNA sequences (like transposons) and imprinted genomic sequences. This can be accompanied by loss of methylation from genomic regions involved in maintaining chromosome stability like peri centromere. This can cause gross genomic instability which is a characteristic of many forms of cancer. Though the relationship between global loss of DNA methylation and cancer has not been very well studied and needs more research (**Figure 3**) [16, 28, 29].

Alternatively, certain genes undergo hypomethylation and therefore experience increase in expression that has been associated with carcinogenesis. Genes predominantly affected by hypomethylation include developmentally critical genes, enzymes, growth regulatory genes and tissue-specific genes such as germ cell-specific tumour antigen genes [30]. Various other genes which have been shown to be involved in carcinogenesis as a result of aberrant DNA methylation are listed in **Table 1**.

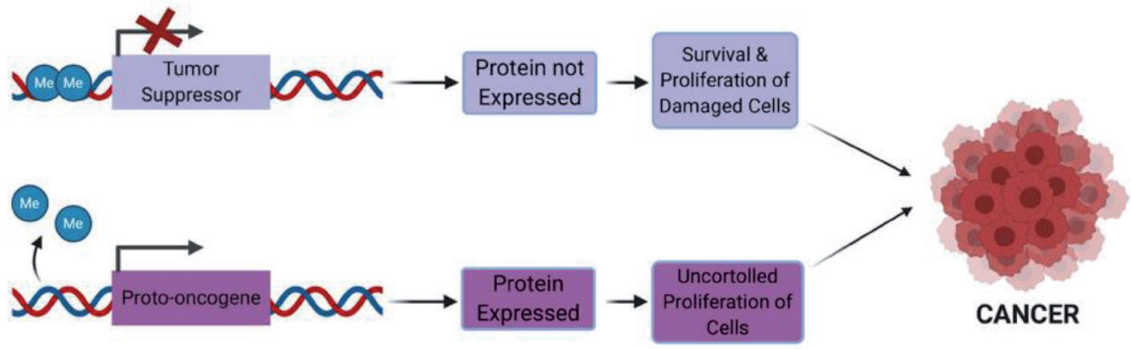

Figure 3.
Schematic of two broad mechanisms involved in cancer progression through DNA methylation. Hypermethylation and silencing of tumor suppressor gene promoters to allow unchecked growth of damaged cells to accumulate more damage and generate cancer phenotype. Hypomethylation of proto-oncogenes to favor uncontrolled proliferation of cells to generate cancer mass.

S.No.	Name of gene	DNA methylation change	Change in gene expression	Type of cancer	References
1.	P16	Increase	Decrease	Colorectal, Renal Lung, Oral, Head and neck, Hepatic	[23, 29, 31–39]
2.	Hmlh1 and hMSH2	Increase	Decrease	Colorectal, Renal	[40–42]
3.	P Cadherin	Increase	Decrease	Breast, Hepatic, Pancreatic, Lung, Salivary gland	[26, 37, 43–45]
4.	Cyclin D2	Decrease		Gastric	[46]
5.	MAGE	Decrease		Melanoma	[47]
6.	P15	Increase	Decrease	Oral carcinoma	[32]
7.	RASSF1	Increase	Decrease	Nasopharyngeal Hepatic, Bladder	[37, 48–50]
8.	MGMT	Increase	Decrease	Oral, Head and neck Bladder, Lung	[33, 35, 38, 39, 50, 51]
9.	FHIT	Increase	Decrease	Lung	[23, 43]
10.	DAP-K	Increase	Decrease	Oral, Nasopharyngeal Head and neck, Lung Pancreatic, Renal	[32, 33, 35, 48, 38, 39, 51–53]
11.	APC	Increase	Decrease	Colorectal, Lung	[40, 51, 54]
12.	RAR (retinoic acid receptor)			Nasopharyngeal Head and Neck Lung	[23, 38, 39, 43]

Table 1.
Changes in DNA methylation of different genes in different forms of cancer.

4. Epigenetic modifications in context of chromatin

The organization of DNA into chromatin, although very necessary, imposes constraints on all the nuclear processes which require DNA as a template like replication, transcription and repair. Therefore, in order to gain access to the underlying DNA, chromatin structure is dynamically regulated through various mechanisms. This flexibility is permitted by mechanisms like histone modifications, incorporation of histone variants and chromatin remodeling [2].

Histone modifications act as binding platforms for various effectors for appropriate downstream signaling. Histone variants are incorporated by replacing canonical histones under specified conditions into nucleosomes. The variants possess different bio-physical properties compared to their canonical counterparts and hence play crucial roles in cellular processes like DNA repair. Chromatin remodeling leads to sliding of nucleosomes along chromatin, exposing regions of genome which could be acted upon by trans-acting factors for specified outcomes.

4.1 Histone modifications

Histone proteins undergo a variety of covalent modifications which can either lead to compaction or relaxation of the underlying DNA within chromatin. The outcome of these modifications is dictated by the type of modification, degree of modification as well as stage of the cell cycle. Histone proteins consist of a highly structured C-terminal globular domain and an unstructured N-terminal tail. Globular domains are generally involved in mediating histone-histone and histone-DNA interactions while as N-terminal tails act as sites for covalent modifications. Among the different classes of histone proteins, histone H3 and H4 generally undergo covalent modifications in their tails. Though recently, H2A and H2B have also been observed to undergo certain modifications [55, 56]. Similarly, many modifications have been observed in the globular domain of histone H3 as well [1]. Histone modifications play role in numerous biological processes like gene regulation, DNA repair, chromosome condensation and spermatogenesis [57]. Some of the well-recognized histone modifications include acetylation and ubiquitination of lysine (K) residues, phosphorylation of serine (S) and threonine (T) residues, methylation of arginine (R) and lysine (K) residues as well as other less known modifications [58, 59]. These modifications are largely postulated to affect chromatin function through two distinct mechanisms: By altering the electrostatic charge of histones, these could alter the structural properties or the binding of histones to DNA. As against the first mechanism, some of the modifications create binding surfaces for the recruitment of specific functional complexes to their sites of action e.g., proteins containing bromodomains recognize acetylated residues while those containing chromodomains recognize methylated residues [60, 61]. It was, In fact, the potential specificity of these interactions which prompted Struhl and Allis to propose the *'histone code hypothesis'* according to which "*specific combinatorial sets of histone modification signals dictate the recruitment of particular trans-acting factors to accomplish specific functions*" [62]. Initially, it was thought that histone proteins undergo covalent modifications after translation (post translational modifications) in a manner dictated by nucleosomal context. But recently, it has been observed that histones can undergo co-translational modifications as well, depending upon the cellular context. This observation has added an additional layer into the role of histones in regulation of cellular homeostasis and clearly calls for more research in the field. Perturbations in histone modifications is associated with many physiological disturbances, including carcinogenesis [5].

4.1.1 Histone acetylation and deacetylation

Acetylation is the most widely studied post translational modification in histones. This modification involves transfer of an acetyl group from N-acetyl-Co-A to the € amino group of lysine with the help of histone acetyltransferases (HATs). Histone acetylation is associated with loosing of chromatin structure due to neutralization of the positive charge on histones with the negative charge on acetyl group which is responsible for increase in transcription. In fact, various transcription activator or co-activator complexes contain HAT activity such as CBP 300, TAF II 250. Reversal of acetylation is carried out by another class of enzymes known as histone deactylases (HDACs). Both HATs and HDACs have been studied extensively in relation to various diseases, including neurodegeneration and cancer [4]. Depending upon the gene/s being involved (oncogenes or tumor suppressor genes), HATs and HDACs can have different effects on the cancer outcomes.

4.1.2 HATs, HDACs and cancer

Relationship between histone acetylation status and cancer has been demonstrated in various studies. For example, a loss of acetylation on lysine 16 of histone H4 (H4K16) has been observed in cancer cell lines and primary human tissues by Fraga et al. [63]. Decrease in promoter acetylation and consequent decline in expression of P21 gene has been observed in some forms of cancer with subsequent rescue of expression upon treatment of cells with HDAC inhibitors under similar conditions [64]. Another study has linked decrease in histone acetylation with tumor invasiveness and metastasis [65]. Accumulating data also shows that HDACs are involved in hematological malignancies like acute promyelocytic leukemia (APL) due to aberrant recruitment to non-target promoters, as a result of interaction with translocation-induced fusion proteins like RAR-PML [66]. Downregulation of E-cadherin due to decrease in promoter acetylation levels has been implicated in the invasive potential by carcinomas [67, 68]. A number of studies have also linked levels of specific classes of HDAC enzymes with different forms of cancer like increase in HDAC1 expression in gastric [69], prostate [70], colon [71], breast carcinoma [72], increase in HDAC2 expression in cervical [73], gastric [74] and colorectal carcinoma [75]; increase in HDAC3 expression in colon carcinoma [76] and increase in HDAC 6 in breast carcinoma [71]. Mutations in HDAC2 gene has also been reported in sporadic colorectal carcinomas [77].

Various mechanisms are responsible for the role of specific forms of enzymes in specific cancer types, largely depending upon their interaction partners and the pathways involved. For example, HDAC1 has been shown to play a role in transcriptional repression of various oncogenic targets of retinoblastoma gene (Rb). Therefore, loss of HDAC1 activity leads to compromise in efficiency of Rb in downregulation of target oncogenes [78]. HDAC3 has also been seen to interact with retinoblastoma protein (Prb) in cancer, Perhaps the most important HDAC III enzyme in cancer is SIRT1 due to its role in regulation of protein factors like P53 [79], androgen receptor [80], p300 [81], E2F1 [82], DNA repair factor ku70 [83] and most importantly, NF-KB [84].

4.1.3 Histone methylation

Histone methylation involves transfer of methyl group(s) from S-adenosyl-methionine to lysine or arginine residues on histones. The enzymes catalyzing histone methylation are known as histone methyltransferases (HMTs). Depending on the

target residue, histone methyltransferases are of two kinds 1. Histone lysine methyltransferases (HKMTs) and histone arginine methyltransferases (HRMTs). Also, lysine residues have three replaceable amino groups on the β-carbon. Therefore, lysine can undergo mono, di or tri-methylation whereas arginine can undergo only mon and di methylation.

Histone methylation is most commonly observed on lysine residues of H3 and H4 tails [85]. It is the most diverse histone modification in terms of complexity and is involved in various functions, depending on the physiological context. Histone methylations commonly associated with gene activation include H3K4, H3K36 and H3K79 and those associated with gene inactivation include H3K9, H3K27 and H4K20 [86]. Furthermore, variations in the degree of methylation on a single residue can also amplify the histone code further. For example, monomethylated H4K20 (H4K20me1) is involved in the compaction of chromatin and therefore transcriptional repression. However, H4K20me2 is associated with repair of DNA damage [63].

Histone methylation is involved in several cellular functions like maintenance of chromatin structure, DNA repair, gene silencing, prevention of hyper-recombination, maintenance of genome integrity et cetera. It is also involved in maintenance of X-chromosome integrity and silencing through excessively methylation of H3K9 on the second copy of human X chromosome in female cells. This provides a binding surface for methyl domain binding (MDB) protein and heterochromatin protein (HP1) to heterochromatinize and silence the second copy of X-chromosome [87, 88]. Since histone methylation plays a paramount role in regulation of gene expression and represents the most stable and complex histone modification, even slight changes to the methylation pattern can have deleterious effects on the organism. In *Saccharomyces cerevisiae*, a lethal mutation that leads to H3K4, H3K36 and H3K79 methylation inactivates many genes required for cell cycle progression and hence causes a delay in mitosis. It has been discovered that deletion of the methyltransferase genes which play role in the above-mentioned methylations allows this organism to live since the lysine residues in question are not methylated [89].

4.1.4 HMTs and cancer

Cancer cells use a diverse range of molecular mechanisms to alter histone methylation landscape. These include mis-regulation of histone methyltransferases and/or demethylases, mistargeting of histone methyltransferases and mutations in methyltransferases. For example, if areas around oncogenes become unmethylated, these genes will attain the potential of being transcribed at an alarming rate. On the contrary, if areas around tumor suppressor genes become highly methylated, these genes will lose their activity and therefore cancer will be more likely to occur [90]. Accumulating data suggests that histone methylation is mis-regulated in various forms of cancer [91, 92]. Fraga et al. [63] have observed that loss of H4K20 trimethylation that leads to hypomethylation of repetitive sequences is a common event in human cancers which occurs at a early stage during tumorigenesis. Mutations on the genes encoding histone proteins are also linked with cancers. 30% of paediatric glioblastomas have mutations at key post translational modification sites in histone genes [93]. Recently, mutations in metabolic enzymes have also been observed to have a role in histone methylation status alteration. The mutated metabolic enzymes produce altered metabolites (popularly known as oncometabolites) which jeopardize the function of methylase enzymes. For instance, inhibition of histone demethylation Jumonji C enzymes by the oncometabolite d-2-hydroxyglutarate [94–97].

4.1.5 Histone phosphorylation

Phosphorylation of histones takes place on serine, threonine, tyrosine and histidine residues, predominantly in the N-terminal tails of all nucleosomal histones by histone kinase enzymes which transfer a phosphate group from ATP to the hydroxyl group of the target amino-acid side chain. Phosphate group contains significant negative charge and therefore phosphorylation is generally associated with transcriptional upregulation. Various proteins have been identified which contain phosphor-binding domains [98, 99]. Histone phosphorylation changes dynamically with the transcriptional profile of the cell [100]. For example, H3Ser10 phosphorylation correlates with gene activation in mammalian cells and heat shock response induced transcription in *Drosophila* [101]. However, the same phosphorylation is associated with chromosome condensation and segregation during mitosis and meiosis [102]. Histone phosphorylations also play a pivotal role in response to DNA damage e.g., phosphorylation of H2A(X) on serine 139 in mammalian cells (referred to as γ H2AX) and S129 of H2A in yeast [103].

4.1.6 Histone phosphorylation and cancer

Regulation of the level of histone H3 phosphorylation by an interplay of the activities of kinases and phosphatases serves as a means of promoting chromosomal condensation and segregation in mitosis [104]. Phosphorylation of H3S10 has also been linked to the expression of proto-oncogenes like *c-fos* [105–107]. It has been detected with the aid of ChIP assay that phosphoacetylation of H3 tails exist at the promoters of several MAP- kinase activated genes as well as the promoters of *c-fos* and *c-jun* [108]. H2A(X) phosphorylation is involved breast cancer [109] and colon cancer [110]. Histidine phosphorylation on histone H4 has been shown to be involved in liver regeneration and cancer [111]. Phosphoacetylation of histones, involving phosphorylation of histone H3 on residue serine 10 and acetylation of histone H4 on lysine 12 has been shown to have a role prognosis of oral squamous cell carcinoma [112].

4.1.7 Histone ubiquitination

It is a process in which ubiquitin molecules are added to lysine residues of histones. Monoubiquitination is the major form of ubiquitination in histones. However, histones H2A and H2B can also be modified by polyubiquitination. The first ubiquitinated histone to be identified was H2A [113]. H2A and H2B also hold the distinction of being the most abundantly ubiquitinated proteins in the nucleus [113, 114]. In addition, H3, H4 as well as H1 have been reported to be modified by ubiquitin but the biological function of these ubiquitinations has not been well characterised [115, 116]. Histone ubiquitinations perform a number of important nucleosomal functions. Chromatin immunoprecipitation (ChIP) experiments have revealed enrichment of monoubiquitinated H2A (H2Aub) in the satellite regions of genome and of H2Bub in transcriptionally active genes [117, 118].

4.1.8 Histone ubiquitination and cancer

Several recent studies have linked ubiquitination, especially H2Bub with inflammation and cancer [119–121]. Histone H2Bub1 predominantly resides downstream to transcription start sites (TSS), a position which allows association with highly

transcribed genes and therefore makes this protein a likely target in cancer [117]. RNF20/RNF40 has been shown to negatively regulate cancer- related inflammation in mice and humans through increased recruitment of repressive NF-κB subunit p50 to various gene targets to downregulate their transcription [121]. RNF40 is also known to modulate NF-κB activity in colorectal cancer in mice [122] while as RNF20 and H2B ubiquitylation have also been shown to be involved in breast cancer [123]. Loss of H2B monoubiquitination has also been shown to activate immune pathways by alteration of chromatin accessibility in ovarian cancer [124–126].

5. Conclusion

Epigenome in a typical eukaryotic cell is packaged as an entity containing nucleoproteins-DNA and histones. This epigenome is compartmentalized into euchromatin and heterochromatin and contain various marks which are transmitted from one cell generation to another [127]. Covalent DNA and histone modifications are the carriers of epigenetic inheritance which are required for the maintenance of a stable epigenome [128]. Any disturbance in the propagation and maintenance of a stable epigenome is associated with diseases like transformation and cancer. The process of cellular transformation is associated with changes in the epigenetic landscape of DNA methylation and histone post-translational modifications. In recent past, genome wide studies have identified various genes related to diseases like cancer and neurodegeneration [4]. Many of these genes have been observed to code for key epigenetic enzymes like HDACs, which raises the possibility of their involvement in far reaching pathological problems. In recent years, non-coding RNA has also been increasingly investigated in relation to carcinogenesis and various types of non-coding RNAs have been associated with different forms of cancer [129, 130].

A stable epigenome also requires proper chromatin conformation. It has been observed that upon transformation, the 3D organization and nuclear topology also undergoes certain changes. These topological changes can be both cause and consequence of alterations in histone and DNA modifications. Topological changes in chromatin structure are associated with increased expression of repetitive DNA elements, which leads to hyper-recombination and gross genomic instability which can further lead a cell on the path of transformation.

Studies performed on chromatin structure and covalent modifications have paved way for better understanding as well as therapeutic intervention of various forms of cancer. Epigenetic approach of therapeutic intervention in cancer is definitely a better approach for cancer treatment since it aims at reversal of inheritable changes without changing the DNA or without affecting normal physiological processes. Also, tumor forms have recently been discovered with anatomical restrictions which contain mutations in histone variant genes. For example, H3.3, a variant of histone H3, contains a point mutation at residue 34 in which glycine changes to valine or arginine (H3.3G34V or H3.3G34R). These tumors are found almost exclusively in the cerebral hemispheres [131, 132]. Tumors with point mutations in histone variant H3.1 (H3.1K27M) are restricted to pons of brainstem while as H3.3K27M tumors are found along the midline of the brain [133]. This "anatomical restriction" in tumor types and the corresponding mutations in histone variants are indicative of an exciting new dimension of the role of epigenetics in tumor biology [134, 135]. This also provides cues about the role of epigenetics in defining tumor micro-environment. Alternatively, many more tumor types can be screened for mutations in genes coding

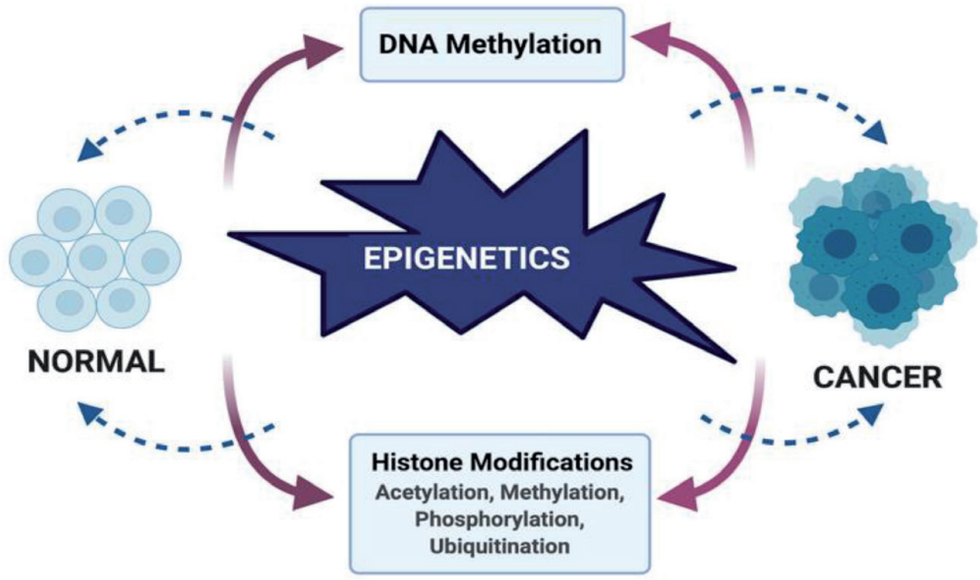

Figure 4.
Schematic depicting two major pillars of epigenetic mechanisms that is, DNA methylation and histone modifications, their importance in maintaining normal cellular morphology and function and their mis-regulation leading to cancer.

for epigenetic factors to have better insights into the role of epigenetics in tumor progression. These findings also encourage the possibility of exploration of epigenetic therapy in resetting the balance in tumor micro-environment for therapeutic targeting. However, the field of epigenetic studies and epigenetic cancer therapy is still in its infancy and intense investigations are required for further exploration of the possibility of epigenetic targeting and treatment of cancer (**Figure 4**).

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