EPIGENETIC MODIFICATION IN CANCER THERAPY: A REVIEW

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ABSTRACT

The mechanism of epigenetic are essential in the normal development and maintenance of gene expression patterns in mammals. Disruption of epigenetic processes results in malignant cellular transformation and altered gene function. Epigenetic aberrations has led to the emergence in the promising field of epigenetic therapy, with the recent FDA approval of epigenetic drugs for cancer treatment. The aim of this review work is to explain the current progression in the alterations of epigenetics in cancer and the changes in cancer initiation, progression and the potential use of this knowledge in designing most effective treatment methods.

KEYWORDS: Epigenetics, DNA Methylation, Histone Modification, Chromatin Remodelling.

INTRODUCTION

Cancer occurs due to the accumulation of genetic and epigenetic alterations.[5] The appearance and behavior of the cells are identified by genes that are present in the cell.[1] The abnormal activity of these cancer cells can be explained by invoking both the quantitative and qualitative changes in this activated gene set.[1] Epigenetic modifications are classified into three interacting processes: methylation of DNA, modification of histone (acetylation and methylation) and chromatin remodeling.[2] DNA methylation is mediated by a enzymes termed DNA methyltransferases(DNMTs), while histone modification patterns are established and maintained by a various set of enzymes by adding or subtracting the acetyl, methyl and other modifications to various amino acids of histone proteins.[3] The complex of various proteins and DNA that composes chromosomes. Chromatin packages and DNA fits into the nucleus and controls gene expression by allows mitosis and meiosis.
structure changes may affect the DNA methylation and histone modifications.\cite{4} Both regulatory mechanisms cooperate to determine the expression potential of individual genes.\cite{3}

**DNA METHYLATION**

DNA methylation is an adding a methyl group to 5 carbon cytosine within the dinucleotide CpG. The maintenance of DNA methylation in dinucleotide CpG islands plays an important role in regulation of a wide variety of molecular processes including the stability of chromosomal structure and controlling the gene expression.\cite{6} The oldest approach shows that the methylation-sensitive restriction enzymes (MSREs) will distinguish the methylated and nonmethylated sites. These MSREs were mostly used in conjunction with Southernblotting to analyze methylation status at candidate genes. This method is labor-intensive, needs large quantities of high-quality DNA not readily obtained from tumors, and depends on enzyme’s specific recognition sites.\cite{2} DNA methyltransferases (DNMTs) is a group of enzymes responsible for DNA methylation. DNMTs shift a methyl group from the methyl donor S-adenosyl-L-methionine to the 5 carbon cytosine.\cite{7} DNMT1, DNMT3A and DNMT3B. DNMT3A/B are the three mammalian DNA methyltransferases that act as de novo methyltransferases. There are most important for establishing DNA methylation patterns, whereas, DNMT1 acts as a maintenance enzyme, preserving methylation patterns after cell replication and deletion cause apoptosis.\cite{8,9} Silencing of genes can be initiated by DNA methylation through various mechanisms. If CpG dinucleotide islands are methylated, the corresponding gene were repressed directly due to a poor transcription factors and recruitment of proteins involved in chromatin remodeling, such as methylated binding domain proteins (MBDs). Though these enzymes having same structure of C-terminal catalytic domain and large N-terminal regulatory domain and also they have equal functions and expression patterns.\cite{11,12} Dnmt1 preferentially methylates the hemimethylated DNA. During replication of DNA, Dnmt1 localizes on replication fork and synthesized new hemimethylated DNA.\cite{13} Dnmt1 binds on newly formed DNA that undergo methylates and blocks the original methylation pattern present before DNA replication.\cite{14} Additionally, Dnmt1 also has the ability to repair DNA methylation.\cite{15} Dnmt1 maintains the original DNA pattern methylation in a cell linkage so it is called as the maintenance. When Knockout of DNA methyltransferases in mice will conclude in embryonic lethality between E 8.0 and E 10.5\cite{16}, and also this knockout embryos shows a two-thirds loss in DNA methylation, to various apoptotic cells.\cite{17} However, in vitro differentiation results in massive cell death, recapitulating the phenotype observed in knockout embryos.\cite{18} These results shows that
Dnmt1 plays an important role in cellular differentiation. Dnmt3a and Dnmt3b are having similar structure and function. Unlike Dnmt1 these Dnmt3a and Dnmt3b are more expressed in methylating both native and synthetic DNA without preferring hemimethylated DNA. For this conversion of methylation into naked DNA Dnmt3a and Dnmt3b are referred to as de novo. Although Dnmt3a is expressed relatively ubiquitously, Dnmt3b is less expressed in majority of differentiated tissues but there will exception in testes, thyroid and bone marrow. Similar to Dnmt1, the knockout of Dnmt3b in mice is embryonic lethal. Or Dnmt3a knockout mice are runted but survive to B4 weeks after birth. From the results it shows that Dnmt3b is needed in early development for normal cellular differentiation. The final member is Dnmt3L in Dnmt family, a protein that suffers from less number of catalytic domain. Dnmt3L is expressed in first stage of development and it is restricted by the thymus and germ cells. Although there is no own catalytic function in Dnmt3L, where Dnmt3a and Dnmt3b will increases the methyltransferase activity. In early development of germ cells in mice, Dnmt3L is needed in establishing both paternal and maternal genomic imprinting, for methylating retrotransposons, for compaction of the chromosome X. Though this Dnmt3L is expressed on developing brain it is downregulated the neuronal differentiation and is not observed in the brain postnatally.

Besides, other than methylation ability, DNMTs have certain other roles in gene silencing, by acting on a transcriptional repressors, or by transferring as binding scaffolds for transcriptional repressors and histone deacetylases methyltransferases.

HISTONE MODIFICATIONS

Modifications of core histones play a significant roles in epigenetic regulation. Histones are the basic element of nucleosomes that has basic of nuclear DNA. Every nucleosome comprises of histones H2A, H2B, H3, and H4 variants forms a globular core. Around base pairs of DNA 146 are wrapped. Internucleosomal interactions are further packed as nuclear DNA as chromatin fiber. The chromatin functions is to organize the DNA and to provide mechanisms for regulating transcription factors accessibility and expressions of the gene. In condensed chromatin the nucleosomes are compacted tightly and not easily accessible on transcriptional machinery as chromatin were histones and DNA are not as tightly packed.

Histone Acetylation/Deacetylation

It occurs by an addition of a acetyl group (COCH₃) from acetyl coenzyme A. The step of acetylation is involved in regulation the various cellular processes including chromatin
transcription and dynamics, DNA replication, cell cycle progression, apoptosis, differentiation, gene silencing, DNA repair neuronal repression and nuclear import and neuronal repression. The modifying enzymes are also needed in acetylation of histone are called histone acetyltransferases (HATs) which plays an major role in controlling both histone H3 and H4 acetylation. More than 20 HATs identified and differentiated into five families: MYST, GNAT1, P300/CBP, TAFII250, P300/CB and nuclear receptor coactivators such as ACTR. By inhibition of histone deacetylases (HDACs) Histone H3 acetylation are increased and decreased by HAT inhibition.

The Histone acetyltransferases (HATs) and catalyze the hydrolytic removal of acetyl groups in histone lysine residues. An imbalancing of histone acetylation are associated with cancer progression and tumorigenesis. Detecting whether histone H3 is acetylated to lysine residues will give an useful information for further characterization of acetylation patterns thereby provides a path for better understanding of epigenetics and gene activation in the preparing of newly targeted HAT-drugs. And also HATs, HDACs provides a major role in different cellular processes involving Histone 3 and Histone 4. Class I HDACs comprises of 1, 2, 3, and 8. Class II HDACs consists of 4, 5, 6, 7, 9, and 10. Class III enzymes (sirtuins) require NAD+ cofactors and also consist of SIRTs 1-7. The Class IV enzyme contains only HDAC11. HDAC inhibition resulting on effects on differentiation in cancer cells, apoptosis, and cell cycle arrest. HDAC inhibitors used in developing as anticancer agents now a days.\[33\]

**Histone Methylation/Demethylation**

Histone methylation is defined as the transfer of methyl groups from S-adenosyl-L-methionine to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs). HMTs regulate methylation of DNA through chromatin-dependent transcriptional repression or activation. In the cell nucleus, when histone methylation occurs, specific genes within the DNA complex were the histone may be activated or silenced.\[32\] Several different histone methyltransferases exist that are specific for the arginine or lysine which they modify. On histone H3 for example SET7/9, Ash1, ALL-1, SET1, MLL, ALR, SMYD3 and Trx. This histone methyltransferases catalyzes the methylation of histone H3 at lysine 4 (H3-K4). ESET, SUV39-h1, G9a, SUV39-h2, SETDB1, Dim-5, and Eu-HMTase are the histone methyltransferases and catalyzes the methylation of histone H3 at lysine 9 (H3-K9) in mammalian cells. G9a and polycomb group enzymes such as EZH2 are histone
methyltransferases used in catalyze methylation of histone H3 at lysine 27 (H3-K27) in mammal cells.\[33\] Both H3-K9 and H3-K27 methylation mediates heterochromatin formation that also participates on silencing various gene expression. The Increased H3-K27 methylation involved in cancer progression.

And also arginine methylation of histones H3 and H4 promotes transcriptional activation and is mediated by a protein arginine methyltransferases (PRMTs). In humans nine types of PRMTs are there but only 7 are methylate histones. They can initiate dimethylation or mono of arginine residues. Based on adding methyl group, PRMTs are classified into type I (CARM1, PRMT1, PRMT2, PRMT3, PRMT6, and PRMT8) and type II (PRMT5 and PRMT7).\[34\] Type II PRMTs are strongly implicated in diseases like cancer. For example, PRMT5 plays an major position in representing suppressing tumor genes like RB tumor suppressors while PRMT7 overexpression is seen on breast cancer. Activity detecting and inhibiting in type II PRMTs and also HMTs is important in mechanisms of silencing, as well as benefiting cancer diagnostics and therapeutics and epigenetic regulation of gene activation. Histone demethylation is the subtracting of methyl groups in modified histone proteins via histone demethylases. These demethylases have potential oncogenic functions and involvement in other pathological processes. The identification of histone demethylases shows that histone methylation is more dynamic process and not permanent. Two major families of demethylases have been discovered: Lysine specific demethylase 1 (LSD1) and Jumonji domain containing (JmjC domain) histone demethylases (JMJD2, JMJD3/UTX and JARIDs). The specific amino acid and amount of methylation determines the demethylation enzyme. For example, on histone H3, mono, di methylated lysine 4 are demethylated by LSD1 (BHC110, KDM1) and tri-methylated lysine 4 by JARID (1A-1D); di- and tri-methylated lysine 27 are demethylated by JMJD3 and UTX (KDM6A) and mono and dimethylated lysine 9 are demethylated by JMJD1 and tri-methylated lysine 9 is demethylated by JMJD2\[35\]. Histone demethylases inhibition may lead to histone re-methylation at specific residues important for chromatin gene expression and dynamics. Furthermore, detection and inhibition of enzymes are needed in preparing the mechanisms for silencing and epigenetic regulation in gene activation and may benefit cancer therapeutics.

**Chromatin remodeling** is the rearrangement from a condensed state to transcriptionally accessible state that allows other DNA binding proteins or transcription factors to access control gene expression. Chromatin composed of various proteins and DNAAt packed in
nucleus. DNA is condensed tightly and wrapped on nuclear proteins called histones. DNA-histone complex, comprises of base pairs 146 of double-stranded DNA surrounded by eight histone proteins called a nucleosome.

Generally, high condensed chromatin is harder for transcription factors and binding DNA proteins is to access DNA and perform their duties. When chromatin is packed tightly it is not actively transcribed called heterochromatin. If chromatin is more loosely packed, and accessible for transcription called euchromatin. Remodeling of chromatin is highly implicated in epigenetics. Epigenetic modifications to histone proteins such as demethylation/acetylation can change the structure of chromatin resulting in activating transcriptional or repression. [36]

Tools of the Trade

Chromatin Immunoprecipitation (ChIP). The interaction between the DNA and proteins shows result in chromosome segregation, gene transcription signal transduction, DNA replication, epigenetic silencing and recombination.

Chromatin immunoprecipitation (ChIP) used for studying DNA-protein interactions. It also used in determining a particular protein attaches to a particular gene sequence, like target sequence of a transcription to compare the histone methylation level associated with a specific promoter gene region between normal and abnormal tissue. Identifying the DNA binding proteins genetic targets to revealing the mechanism of DNA -protein interaction is difficult for understanding cellular processes.

In a Chromatin immunoprecipitation assay, chromatin is sheared, extracted and then added into a microwell where protein-DNA complexes are identified by affinity antibodies. The identified DNA is released and purified. Eluted DNA is needed in different downstream applications including ChIP-on-chip, ChIP-PCR, and ChIP-seq.

A nucleosome comprises of core histone proteins like H2A, H2B, H3 and H4. These histone proteins are mostly responsible in structural organization of chromatin in eukaryotic cells that moves to several posttranslational modifications that will changes the interaction between DNA and nuclear proteins. Modifications of the histone tails include methylation, acetylation, ubiquitination, citrullination, phosphorylation, sumoylation and ADP-ribosylation occurring in the N-terminal domain tail, which result in restructuring of the
nucleosome as open conformation more accessible to transcription complexes (H2A can also be modified).

Combinations of these changes constitute and so-called “histone code” which can be studied and interpreted by different cellular factors leading to transcriptional activation or repression.\cite{36} For example, a general characteristic of euchromatin is tri-methylation at lysine residues of histone H3 – lysine 4 (K4), lysine 36 (K36), and lysine 79 (K79) and a high level of histone acetylation, while heterochromatin is characteristically enriched in trimethylation of other histone H3 lysine residues K9, K20, and K27.

CONCLUSIONS

The importance of epigenetics in cancer has been identified and interest in the field has grown vast over the last few decades. Recent advanced technique in epigenomic approaches allow mapping of the acetylation/methylation state and miRNA levels in the genome with high accuracy, which helps in the identification of biomarkers in various diseases. An understanding between cancer and epigenetic deregulation will help in designing better treatment strategies.

REFERENCE


