Review Article

Epigenetic Therapy: Emerging Role in Treatment of Human Diseases

Ashwani Sharma[1], Seema Jain[1*], Nidhi B Agarwal[2]

[1]Department of Pharmacology, University College of Medical Sciences & GTB Hospital, Delhi 110095, India.

[2]Assistant Professor, Centre for Translational and Clinical Research,Jamia Hamdard, New Delhi 110062, India.

Abstract

Epigenetics refer to heritable deoxyribonucleic acid (DNA) and chromatin modifications occurring in gene expression without any change in the underlying DNA sequence. Epigenetic modifications contribute to the pathogeneses of various diseases such as various cancers, neurological diseases such as psychosis and bipolar disorder and other diseases. Epigenetic changes can occur due to DNA methylation, histone modifications, microRNAs (miRNAs)–monitored gene activity and nucleosome remodeling. A new therapeutic strategy in the form of epigenetic therapy has emerged in the recent years. The basis of this therapy is the underlying epigenetic etiology rather than that of genetic origin. Epi-drugs are mainly classified as DNA methyltransferase (DNMT) inhibitors or DNA demethylating drugs and Histone deacetylase inhibitors (HDAC) inhibitor drugs which either induces changes in the methylation pattern of DNA or causes modification of histone proteins. This paper discusses the current information about epi-drugs and their potential role as emerging therpautics for various diseases.

Keywords: Epigenetic, DNA methyltransferase inhibitors, Histone deacetylase inhibitors, Cancer

INTRODUCTION

Epigenetics is the study of heritable deoxyribonucleic acid (DNA) and chromatin modifications occurring in gene expression without any change in the underlying DNA sequence. Directly adjoining the prefix epi to genetics describes the numerous mechanisms by which gene expression and phenotypes are influenced, free from of any changes to the primary DNA sequence. In other words, DNA and epigenetic modifications work similar to hard drive and software, respectively where DNA carries the information required to conduct every cellular functions and epigenetic modifications controls the packaging of DNA and regulate the patterns of gene expressions. Thus, epigentic changes affect only the phenotype of an organism without causing any alteration in genotype.[1] It is well established that not only genetic defects but epigenetic modifications also contribute to the pathogeneses of certain diseases such as various cancers, virtually all areas of science and medicine:

**Corresponding Author: Dr. Seema Jain, Professor, Department of Pharmacology, University College of Medical Sciences, Delhi, India. Email- dr.seemajain@gmail.com*

chemistry and genetics, development and differentiation, immunology, aging, and neuroscience. This new mechanism paved the way for the discovery of newer drugs with a specific target in various diseases specifically cancer and evoked the development of a diagnostic tool that might help in detection of the underlying cause of epigenetic alterations of other conditions as well.[2] Feinberg and Vogelstein in 1983, first hypothesized the involvement of epigenetic factors in human carcinogenesis. Later, epigenetic changes were also found as the underlying cause in a plethora of diseases like neurological diseases such as psychosis and bipolar disorder and another disease state like α- thalassemia, fragile-x syndrome, lymphomas and aging.[3,4,5]

MECHANISMS OF EPIGENETICS

The epigenetics refers to meiotically and mitotically inherited changes in gene expression without alteration of DNA structure.[2,6] These heritable changes in gene expression termed as “epimutations” which are potentially reversible and regulated by environmental factors. There are four mechanisms through which epigenetic changes can occur:

1. DNA methylation
2. Histone modifications
3. MicroRNAs (miRNAs)–monitored gene activity
4. Nucleosome remodeling

These mechanisms have essential roles in the interpretation of various genetic information. Various epigenetic diseases occur due to disruption in any of the arrangement as mentioned earlier, which in turn results in altered gene expression or silencing.[6,8]

DNA methylation

It is the most frequent covalent chemical modification of DNA. DNA methylation refers to the addition of a methyl group at the C-5 position of cytosine, resulting in the conversion of cytosine into 5-methyl cytosine.[9] S-adenosyl-methionine acts as a source of the methyl group for methylation.[2,4] In human genome cytosine and guanine bases, fertile regions (CpG islands) are the region that mostly remain unmethylated. The DNA methylation process can affect the expression of a gene by targeting these regions.[10] The methylation process is carried out in the presence of DNA methyltransferases (DNMTase) enzymes. Mainly four DNA methyltransferases, namely DNMT 1, DNMT 2, DNMT 3a, DNMT 3b have been found responsible for methylation of CpG site in the human genome.11 These enzymes not only methylate DNA but also play an essential role in defense against viral sequences, transposable elements-silencing and transcriptional silencing of specific genes.[2,4,12] DNMT 1 referred to as maintenance methyltransferase. During replication of DNA, it copies a DNA methylation pattern from parent strand to the newly synthesized strand.[13]

On the other hand, DNMT 3A and DNMT 3B are being considered as de novo methyltransferases that regulate DNA methylation pattern early in the development. The role of DNMT-2 is still not established.[14] The aberrant methylation of cytosine residue in DNA or increase expression of DNMTs enzymes act as

precipitating factors for human cancers and occurs at an early stage in carcinogenesis.[14]

Histone modification

The post-translational modifications of amino acids that make histone protein play a significant role in epigenetics.[15] The human genomic DNA is complexed with histones in the nucleus to form DNA-protein complex called chromatin.[16] Histones are necessary proteins rich in lysine and arginine, which may undergo post-translational modifications by acetylation, deacetylation, methylation, phosphorylation, and ubiquitination.[11,15] Acetylation of histone protein relaxes the tightly supercoiled chromatin. This process enhances the accessibility of DNA-binding transcriptional regulatory proteins to the promoter region. The promoter region thus becomes accessible to DNA binding transcriptional regulatory proteins and activates gene transcription. Deacetylation of the histone protein is associated with gene transcription silencing.[16,17] The acetylation and deacetylation of histone proteins are carried out by histone acetyltransferase (HAT) and histone deacetylases (HDAC) enzymes, respectively. In mammalian cells, 18 histone deacetylases enzymes have been identified and are classified into four classes. Class I (HDAC1-3, HDAC8), Class II (HDAC4-7, HDAC9, HDAC10) and Class IV (HDAC11) are NAD+-independent enzymes while Class III (SIRT1-7) belongs to sirtuin family and are NAD+- dependent enzymes.[18,19]

Dysregulation of histone acetyltransferase and histone deacetylases has been suggested to play an essential role in the etiology of cancer by altering the expression pattern of various genes.[20,21]

It is now well established that DNA methylation and histone modification are interconnected and the interaction of HDACs, histone methyltransferases, and methylcytosine binding proteins leads to DNMTs recruitment. Thus, these interconnected mechanisms are involved in the pathogenesis of epigenetic diseases like cancer, metabolic syndrome, type-2 diabetes mellitus and others.[22]

MicroRNAs (miRNAs)–monitored gene activity

Recently, the RNA-associated post-transcriptional silencing of a gene has gained much interest among the researchers. RNA in three forms, antisense transcripts, non-coding RNAs, and RNA interference (RNAi) might accentuate the transcriptional silencing.[2] These forms of RNA facilitate histone modification and DNA methylation thereby mediating heritable and stable silencing. A single case report of α- thalassemia demonstrated the role of antisense transcripts in DNA methylation and silencing of the globin gene.[23] These abnormally silenced genes require certain drugs that could modify the altered gene expression.

EPIGENETIC THERAPEUTICS

A new therapeutic strategy in the form of epigenetic therapy has emerged in recent years and gained the attention of many researchers. The basis of this therapy is the underlying epigenetic etiology rather than that of genetic origin. To date, several epigenetic drugs are in various stages

of clinical trials for their assessments in certain tumors like solid tumors and hematological malignancies.[24] To correct epigenetic defects, these agents either induces changes in the methylation pattern of DNA or causes modification of histone proteins.

Currently available epigenetic drugs or epi-drugs are classified into two groups: -

1. DNA methyltransferase (DNMT) inhibitors or DNA demethylating drugs
2. HDAC inhibitor drugs[4,25]

DNMT INHIBITORS

These drugs act by inhibiting DNA methyl-transferases (DNMTs), which carries out DNA methylation. In turn, a rapid reactivation of expression of genes occurs which have undergone silencing due to epigenetic changes. Most of these drugs lack specificity for DNMTs.[24] Based on the structure, these drugs are classified as:

a. Nucleoside analogs
b. Non-nucleoside analogs
c. Anti-sense oligonucleotides

a) Nucleoside analogs

DNMTs cause methylation of a cytosine nucleotide base at CpG site. These drugs are analogs of cytosine. 5-azacytidine (5-aza-CR) and decitabine (5-aza'-deoxycytidine, 5-aza-cdr) are prototype drugs of this subgroup. Initially, these agents were developed as cytotoxic agents but now considered as DNMT inhibitors based on their potent DNA methylation inhibitor property.[2,13] These drugs first get phosphorylated to active deoxynucleotide triphosphates which get incorporated into replicating DNA instead of cytosine and form irreversible covalent bonding with DNA methyltransferase enzymes. These drugs are often termed S-phase specific drugs as they cause degradation of DNMT during S-phase of cell division.[2]

In several clinical trials, both drugs are effective against acute leukemias and myeloid dysplastic syndrome in low doses.[26,27,28] However, these drugs also hold certain demerits viz., poor oral bioavailability, instability, and myelotoxicity. These cytotoxic effects occur as a result of the binding of these drugs to DNA through covalent bonding.[13,17] Besides, 5-azacytidine also binds to RNA, thereby interfering with the synthesis of proteins.[29]

Zebularine is a newer cytosine analog. It is an effective inhibitor of DNA methylation. As compared to 5-azacytidine and decitabine, it is more stable, less toxic, and can be given through oral route.30 However, it has to be delivered in high doses.[31]

b) Nonnucleoside analogs:

Structurally, these substances are different from cytosine nucleotide, thereby developed to exclude the cytotoxic effects of nucleoside analogs inhibitors. Some of these compounds directly block the active site of DNMT enzyme without any covalent trapping of the protein.

Several drugs and natural substances which act as nonnucleoside DNMT inhibitors are:

Epigallocatechin-3-gallate (EGCG)

EGCG is a polyphenolic compound derived from green tea. It has been found

to have antiproliferative. antiangiogenic, proapoptotic and cell cycle are inhibiting properties in various in vitro and in vivo models of cancers. These growth inhibitory effects have been observed more in the cancer cell than in healthy cells. The specific mechanism of EGCG is still unknown. However, in human cancer cell lines it has been found to bind and block the active site of human DNMT-132, Yet some researchers have proposed other mechanisms like inhibition of DNA topoisomerase and modification of transduction pathways.

Procaine: This drug belongs to the class of local anesthetic drugs and has demonstrated a demethylating effect in various cellular assays. It binds to CpG site and blocks the binding of DNMTs to DNA. However, this effect is observed only at higher doses of procaine.[33,34]

Procainamide : It is an established drug for the treatment of arrhythmias. Like procaine, it binds with CpG abundant sequences and therefore blocks the DNA methylation process. Additionally, it also acts by decreasing the affinity of DNMT1 for both DNA and S- adenosyl L- methionine.[35]

Psammaplin (PSA): It is a naturally occurring biphenolic compound isolated from the Psammaplysella sponge. It has been found to possess significant antibacterial and anti-tumor properties. It showed significant cytotoxicity against human lung, ovary, spine, and colon cancer cell lines. These effects are attributed due to inhibition of certain enzymes like topoisomerase II, DNA methyltransferase, and histone deacetylase activity.[36]

RG 108: RG108 and its maleimide derivatives RG108-1 and RG119-1 act as direct inhibitors of human DNMTs and are found to reactivate the tumor suppressor genes. They appear to have very low toxicity in human cancer cell lines.

In addition, polyphenols and flavonoids compounds,curcumin, genistein, quercetin, laccaic acid, lycopene silibinin and luteolin also show DNMT inhibiting properties and can be used as demethylating agents.[13,37]

c) Antisense oligonucleotides (AS-ONs):

These are a short-defined segments of about 15-25 nucleotides, hybridize with mRNA, and block translation process. Antisense oligonucleotides act as competitive inhibitors of DNMTs enzyme. Several AS-ONs (e.g., MG98, MTC- 423, 427 and 433, miR29b, miR-155-5p asCEBPα-2) are in various phases of trials which target human DNA methyltransferase one and result in demethylation.[38]

HDAC INHIBITORS

Changes in the acetylation pattern of histone proteins play an essential role in transcriptional regulation of genes. Deacetylation of the histone proteins through HDAC enzymes leads to transcriptional repression of genes. HDAC inhibitor drugs act by blocking this deacetylation of histones. Thus, resulted accumulation of acetylated histone proteins modulates various cellular functions like cell cycle arrest, cellular differentiation, and apoptosis in many tumor cells. These drugs upregulate epigenetically silenced genes without modifying the DNA sequence. Currently, various HDAC inhibitors have been approved and many are different

phases of clinical trials for human cancer.[39] HDAC inhibitors can be classified in to various types:

Hydroxamic acids (hydroxamates): include various compounds like trichostatin A, Givinostat, Abexinostat, Resminostat, Rocilinostat, Quisinostat, Practinostat, CHR-3996, Vorinostat (SAHA) belinostat and Panabiostat. These HDAc inhibitors are in preclinical, phase I and phase II clinical trials for various carcinoma except vorinostat and belinostat (approved for cutaneous T-cell lymphoma) and panabiostat (approved for multiple myoma).[40,41]

Cyclic peptides have epoxide (epoxyketones) moiety which reacts with zinc cation or amino acid in the binding pocket to trap HDAC. Trapoxins (TPX) A and B, apicidin and romidepsin belong to this class. The antiproliferative activity of TPX has been reported to occur as a result of irreversible inhibition of HDACs at nanomolar concentration. Apicidin is isolated from fungus Fusarium species. It has been reported to be effective against Plasmodium berglhoi malaria in mice at nanomolar concentration. Its antiparasitic effect is considered due to inhibition of histone deacetylase enzyme in parasites leading to hyperacetylation of histones in treated parasites. Apicidin has potent broad-spectrum antiproliferative, antiangiogenic and anti-invasive activity against various cancer cell lines.[39] It appears to be a potential therapeutic agent for human acute promyelocytic leukemia. Romidepsin (depsipeptide) is a natural product obtained from Chromobacterium violaceum and has been approved for the treatment of cutaneous T - cell lymphoma.[42]

Benzamides: These are less potent than hydroxamates and cyclic tetrapeptides. Benzamides include tacedinaline, entinostat, mocetinostat and 4SC202. Tacedinaline, entinostat and 4SC202 are selective inhibitors of class I HDACs while Mocetinostat inhibit both class I and class IV HDACs. Benzamides are in different phases of clinical trials for various carcinomas including Hodgkin's lymphoma, non -small cell lung cancer, breast cancer etc.[40,43]

Short chain fatty acids: Valproic acid which is used in treating epilepsy and bipolar disorder, also inhibits HDAC at physiological concentrations used for treating these neurological conditions. Being as an inhibitor of HDAC, valproic acid acts as a competitive inhibitor to acetyl groups on histone N-terminal tails by binding to the catalytic site of the enzyme. Butyric acid and phenylbutyric acid are other short chain fatty acids HDAC inhibitors that are in clinical trials for various diseases.[40,44]

Sirutin inhibitors: Nicotinamide, sirtinol and cambinol act by inhibiting the HDAC enzyme that belongs to sirtuin family. Nicotinamide acts by inhibiting all Class III (SIRT1-7) enzymes whereas sirtinol and cambinol act on SIRT 1 and 2 only. These drugs are in preclinical and clinical trials for carcinomas.[40,45]

CONCLUSION

Role of epigentic modifications has been identified in various diseases specially in cancer. As epigenetic changes are reversible and are associated with change in only phenotypic characterisitics so various agents or drugs that target these epigenetic

modification might act as therpautic options in the treatment of several diseases. DNA methyltransferase and HDAC inhibitors are the two main classes of drugs that act by targeting on these epigenetic changes and termed as epi-drugs.

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