Epigenetic mechanisms as a new approach in cancer treatment: An updated review

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Abstract  Epigenetic, along with genetic mechanisms, is essential for natural evolution and maintenance of specific patterns of gene expression in mammalians. Global epigenetic variation is inherited somatically and unlike genetic variation, it is dynamic and reversible. They are somatically associated with known genetic variations.

Recent studies indicate the broad role of epigenetic mechanisms in the initiation and development of cancers, that they are including DNA methylation, histone modifications, nucleosomes changes, non-coding RNAs. The reversible nature of epigenetic changes has led to the emergence of novel epigenetic therapeutic approaches, so that several types of these medications have been approved by the FDA so far.

In this review, we discuss the concept of epigenetic changes in diseases, especially cancers, the role of these changes in the onset and progression of cancers and the potential of using this knowledge in designing novel therapeutic strategies.

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Introduction

Epigenetic mechanisms as a new therapeutic approach has generated considerable recent research interest in the last decades. The biological nature of living organisms is determined by genetic features such as DNA sequencing and epigenetics. They are essential for the survival of cells
and tissues of living organisms. Epigenetics is defined by inherited somatic changes that are not associated with changes in the DNA sequence. So, the epigenetic outlook of a cell is determined by status of DNA methylation, histones covalent modifications, chromatin structure, and non-coding RNAs and networking with each other. The importance of this regulatory system will become more apparent when it comes to some diseases that are caused by defects in the epigenetic system. For example, a mutation in the DNA methyltransferase 3-A (DNMT3A) enzyme that plays a role in the histone methylation can lead to hematological malignancies such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), and increased chance of progression to AML. DNA methylation occurs when a methyl group is added to the position of the 5'-cytosine ring of the CpG dinucleotides. Histones can be modified covalently by adding methyl, acetyl, phospho, ubiquitol, or SUMO moieties.

Whether this modification plays a facilitating or inhibiting role in the expression of a gene depends on the moderating residual and the type of modification. A nucleosome is made up of DNA that spins one and two-thirds around the protein core containing 2 copies of each H2A, H2B, H3 and H4 histones. X-ray crystallography has shown that a histone octamer, the eight protein complexes found at the center of a nucleosome core particle, has a disc-like structure and is composed of histone subunits that are locked together. The continuous and interconnected structure of DNA and histones makes coordination between DNA methylation and histone modifications. The precise combination of modified amino acids in the histone tail assists in controlling the density and compressibility of the chromatin and its stability or instability for transcription, replication and repair. This can be seen with the use of dye-bound DNA by an electron microscope. Finally, long non-coding RNAs (lncRNAs), that contain microRNAs on top, can have the tumor suppressor or oncogenic roles according to their complementary DNA sequences. Despite the large amount of research on epigenetic mechanisms, it seems that there remains a need for wrapping up the recent data.

In the following article we discuss epigenetic mechanisms that are involved in diseases, combined epigenetic therapy and relationship between epigenetics and cytotoxic treatments. The purpose of this article is to review recent advances in the treatment of diseases, especially cancers, using epigenetics mechanisms. We also emphasize the importance of further investigations in elucidating the underlying mechanisms of epigenetic in cancer progression.

Epigenetic mechanisms involved in cancers

Epigenetic mechanisms are widely involved in human diseases (Fig. 1). The majority of the cells in the body have the same genome, and it is the function of the epigenetic system of the cells to regulate the distinctive features of each cell morphologically and functionally so that each cell can perform its specific function. Diseases that have been raised with impaired epigenetic systems are including cancers, diabetes, lupus, asthma, and neurological diseases. In cancers, global hypomethylation occurs in the DNA, which is accompanied by hypermethylation at other sites. Aberrant hypomethylation causes the expression of certain genes, such as oncogenes, while hypermethylation causes inhibition of tumor suppressor genes.

Epigenetic modifications can initiate the disease and may also be predictive of the clinical outcomes. For example, lower levels of H3K4me2 are associated with a poor prognosis in prostate, lung and kidney cancers. Lower levels of H3K18ac and H3K9me predict a worse prognosis in kidney and lung cancer. While the expression of higher levels of H3K9ac in patients with lung cancer is associated with a lower survival. Certain patterns of H3K9me are associated with certain clinical outcomes in acute myeloid leukemia.

H3 acetylation and H3K9 demethylation can be a diagnostic difference between prostate cancer (PSA) and non-malignant prostate tissue. Also, H3K4 trimethylation can be used as an important predictor of PSA recurrence. The expression of EZH2 is an independent diagnostic marker that is a sign of malignancy in the prostate, breast and endometrial cancers. The expression of DNA repair gene, O-6-methylguanine-DNA methyltransferase (MGMT), neutralizes chemotherapy and radiotherapy. As a result, MGMT depletion by hypomethylation is associated with a positive response to treatment; in addition, epigenetic changes may form tumors and, as a result, are potential sources of diagnosis to determine the risk of disease. For example, bacterial infection with Helicobacter pylori is accompanied by hypermethylation of DNA in certain genes, which are mainly methylated in cancers. So the reversal of epigenetic changes that occur as a result of an acute disease can prevent the progression of the disease to the chronic level. With the advent of analytical technologies and the study of the epigenome, a new epigenetic branch named Pharmacoeigenomic is emerging that can be used to study epigenetic profiles to investigate molecular pathways for assessment of drug sensitivity in cancers and determine the best available therapeutic approaches.

In non-small cell lung cancer (NSCLC), an unmethylated promoter called IGFBP3 is an indication of the response to chemotherapy by Cisplatin. A polymorphism in the variant of CYP2C19*17 for cytochrome P450 requires higher dosages of valproic acid (VPA) to reach target plasma levels. Additionally, monitoring epigenetic changes can be used to evaluate the efficacy of treatment and disease progression. PITX2 methylation can be used to predict the primary outcome of breast cancer patients following treatment with adjuvant Tamoxifen. Patients with bladder cancer with p16 hypermethylation were less likely to relapse after receiving IL2 treatment compared to those who did not have hypermethylated p16 and received the same treatment.

Since the epigenetic mechanisms determine which genes and signaling pathways be activated, they can play a significant role in determining the best treatment and monitoring approach for the disease.

Epigenetic modifications are inherited somatic and reversible. This important feature has made them to be considered as potential therapeutic targets. Several epigenetic defects are commonly found in the patient’s tissues, leading to a change in epigenetic status. Cancer
cells may become abnormally addicted to epigenetics status like the process of cancer cell addiction to the oncogenic pathway; this will make them more susceptible to epigenetic treatments than healthy cells. An example of this is addiction to the MET oncogene, which is a tyrosine kinase, and acts as a receptor for hepatic growth factor (HGF) and tissue homeostasis regulator in normal cells.\(^{28}\) Interestingly, despite the role of MET in the healthy cells as well as in cancer cells, the latter is more sensitive to MET suppressors as a result of being more dependent on the MET signaling pathway.\(^{19}\) As a result, cancer cells will become addicted to the increased activity of a number of important oncogenes. These cells may also be dependent on the extinction of some important tumor suppressor genes. Since it has been shown that a number of tumor suppressor genes are extinguished by means of epigenetic mechanisms in cancers,\(^{6}\) it can be said that cancerous cells may be addicted to a disrupted epigenetic condition and therefore become more susceptible to epigenetic treatment than normal cells.

**DNA methylation**

Although there are various mechanisms for changing the expression of a gene epigenetically, DNA methylation is a common procedure used to silence genes expression in eukaryotic cells. DNA methylation is different from histone methylation. In recent decades, the importance of DNA methylation in cell biology has been identified, such as embryonic development, inactivation of chromosome X, silencing of genes at different stages of evolution and their expression at appropriate times. DNA methylation is accompanied by the addition of a methyl group to the C5 position of the cytosine ring by DNA methyltransferase (DNMTs).\(^{29}\)

The highest amount of DNA methylation (98%) occurs in CpG islands in the promoter of certain genes in somatic cells.\(^{30}\) Methylation is regulated by the family of DNA methyltransferases (DNMTs) including DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L.\(^ {31}\) The mechanisms of silencing gene expression by DNA methylation in the promoters is that specific transcription factors and other transcriptional regulator components cannot access to the promoter, thus DNA expression will be suppressed. By contrast, hypomethylation in this regions will turn on the expression.\(^{32}\) Disturbance in DNA methylation is associated with many diseases, such as cancers, lupus, muscular dystrophy and a series of congenital abnormalities.\(^{29,33}\) Hypomethylation of DNA is common in cancers.\(^ {13,34,35}\) Hypomethylation causes tumorigenesis and cancer by triggering transcription of oncogenes. Also, since methylation causes genome stability, hypomethylation can increase genetic instability by causing genetic mutations in DNA sequence.\(^ {36}\)

On the other hand, many cancers are caused by the aberrant methylation of TSG genes, such as P53 and P16 (which are required to regulate normal growth and cellular differentiation), and stop their expression. In addition, the study of the methylation pattern of some genes can determine the response or lack of response to treatment. For example, a specific pattern of hypermethylation in MGMT gene (a type of TSG tumor suppressor gene), may indicate a good response to chemotherapy (alkaloid therapy). Hypermethylation in some cancers, such as colon cancer, can be used as an early diagnosis biomarker.\(^ {29}\)

There are two important drugs with a DNMT inhibitory effect for the treatment of some diseases caused by
hypermethylation of the DNA: azacitidine and decitabine (5-aza-2’-deoxycytidine). They are mainly used in the treatment of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and chronic myelomonocytic leukemia (CMML). 37 The therapeutic utility of these drugs is not limited to hematological malignancies and is also used in the treatment of other tumors. 38

The third group of DNMT inhibitors, which are analogues of cytidine, are from the Zebularine family. The low dose DNMT inhibitors of aza-nucleosides can inhibit the methyl transferase property of DNMTs and thereby cause the expression of the desired gene expression. The reason for the use of these drugs at low dosages is that their high concentrations have cytotoxic effects, and at high dosages they can interfere with DNA synthesis and cause damage to it. 39 DNMT inhibitors interfere with DNA in the S-phase of mitotic cell cycle and blocks cancer cells proliferation. 40 The potential of these drugs in inhibiting DNMTs are not the same, so that azacitidine only has 10% of the ability of decitabine to inhibit DNA methylation. 41 In spite of the efficacy against tumors caused by hypermethylation, azacitidine and decitabine have limitations too, including low biological sustainability and high toxicity. For this reason, the new generation of Zebularine family of drugs with a higher sustainability and minimal toxicity seems to be a potential antitumor agent. 42, 43 The lower toxicity of Zebularine compared to the two other drugs can be due to the difference in the expression of uridine cytidine kinase in normal cells and cancer cells. Expression of this enzyme in cancer cells increases the substitution of these aza-nucleosides among nucleic acids. 44

**Histone modifications**

Histones are highly alkaline proteins packaged in DNA packets called nucleosome. 6 Histone modifications include lysine methylation, arginine methylation, arginine citrullination, lysine acetylation and Serine/Threonine/Tyrosine phosphorylation. Most histone modifications regulate DNA transcription.

For the first time in 1964, Allfrey et al. Discovered histone acetylation. 45 The regulation of histone acetylation is very dynamic and is controlled by two families of enzymes with reverse function: histone acetyltransferase (HAT) and histone deacetylase (HDAC). 46 HAT adds acetyl to histone in the position of the Lys and causes the positive charge of the Lys to be neutralized. As a result, the link between the histone and the DNA is weakened and the DNA is removed from the packaging. HDACs with an anti-HAT function remove the Lys group and maintain histones with positive charge that result in chromatin stability in the respective position.

Phosphorylation of histones, like histones itself, is very dynamic, and is often carried out on serine, threonine and tyrosine residues by the reversible performance of kinases and phosphatases, which respectively add and remove the phosphate moiety. 46 With the addition of phosphate, the negativity of the histones adds up, which has an effect on the chromatin structure. Due to the rapid turnover of phosphorylation in histones, there is relatively little information about the role of this modulation. Histone methylation is performed on lysine (K) and arginine (R) residues by lysine methyl transferase (KMT) and proline arginine methyl transferase (PRMT). Lysine can be mono, di, and tri-methylated, while Arg can be mono or dimethylated (symmetrical/non-symmetric). Histone methylation modifications, in contrast to the mentioned histone modifications (histone acetylation and phosphorylation), do not change the charge of histone protein, but affect the affinity of the genome transcription factors. 47 In 2004, the first enzyme Lysine-demethylase, the lysine-1 specific demethylase enzyme (LSD1), was identified. 48

The histone methylation can activate or restrict transcription. For example, H3K4, H3K36, and H3K79 are the most important places where their methylation results in transcription of genes, and vice versa, methylation of H3K9, H3K27, and H4K20 is accompanied by silence of transcription. 49 Even the number of methylation is also involved in the regulation of transcription, for example, the mono-methylation of lysine in H3K9 activate transcription and its trimethylation causes transcriptional inhibition.

Disruption in the function of methyltransferase enzymes can lead to changes in the site and the number of methylation and, as a result, has an important role in the development of malignancies. One of the most obvious examples of hematologic malignancies in this field is the disruption of Enhancer Zeste Homolog 2 (EZH2).

The Polycomb Protein Group (PcG) is a part of proteins that plays a role in transcription inhibition and determines the fate of the cell. Polycomb proteins include PRC1 and PRC2, which with coordinated activity and post-translation changes in histones. The core of the PRC2 is EZH1/2, EED and SUZ12. The main enzyme of PRC2 is EZH2, which has H3K27 methyltransferase activity. Then H3K27 methylation is detected by PRC1. 50 PRC1 compresses DNA and suppresses transcription by ubiquitination the H2K119 51, 52 (Fig. 2). Increased enzymatic activity of EZH2 in many cancers leads to inappropriate inhibition of tumor suppressor genes, including B cell lymphoma, in germinal centers. 53 In such diseases, EZH2 malignant activity may be inhibited by using specific small molecules. 54, 55 Also, in 25% of patients with follicular lymphoma (FL), mutations with increased EZH2 activity leads to the aberrant methylation of H3K27. 55

**Figure 2 Collaboration of PRC1 and PRC2 in suppressing transcription:** The PRC2 with its main subunit (EZH2) triggers H3K27 trimethylation. PRC1 via chromobox (CBX) interacts with H3K27me3 and catalyzes the ubiquitination of H2AK119. So the expression of genes such as the tumor suppresor genes (TSGs) is inhibited and one of the hits for tumor initiation is performed.
MicroRNAs (miRNAs)

MiRNAs are a class of non-coding RNAs (ncRNAs). These small RNAs are about 19–24 nucleotides long, and only by using about 3–4 of their nucleotides, called seed site, bound to the 3’UTR region of target mRNA, in this way it prevents the translation of mRNA and protein production. As miRNAs play critical roles in regulating functions of the cells, disruption in their structure and turnover can causes diseases. CLL is the first human disease that is associated with miRNA disorders. Such miRNAs that somehow have an oncogenic role are called Onco-miRs.

The miR-101 reduces EZH2 expression. In several types of cancer, the amount of miR-101 decrease, resulting in an increase in the expression of EZH2 and an increase in such methyllations like H3K27me3 in the tumor suppressor genes that will reduce TSG products and will increase incidence of cancers. In case of increase in the expression of miR-101, the process of disease will be reversed and the growth of cancerous cells will stop.

Another application of miRNAs is as cancer diagnosis biomarker and also determinant of cancer prognosis and patient overall survival.

For example, in a meta-analysis performed by Zhu H and Leung sw in 2015, it was found that 40 types of miRNAs were deregulated in type 2 diabetes. These include miR-29a, miR-34a, miR-375, miR-107, miR-132, miR-142-3P and miR-144, which are potential biomarkers for this disease. In addition, miR-199-3P and miR-223 are potential tissue biomarkers for type 2 diabetes.

In another study to detect miRNAs as non-invasive biomarkers for the diagnosis of endometriosis by Wang WT et al. In 2013 it was shown miR-199a, miR-145* and miR-542-3P could be investigated as serum endometriosis biomarkers. It was also found that miR-199a plays a significant role in detecting disease progression.

MiRNAs can be used to classify myeloid malignancies. For example, 12 different miRNAs have different levels of expression in patients with different stages of MDS. In addition to the mentioned therapeutic applications of miRNAs, induction of miRNAs into malignant stem cells inhibits malignancy by suppressing production of specific proteins. But due to the lack of a suitable in vivo carrier for an accurate transmission of miRNA to the cells and presence of various barriers in the body, this type of treatment needs more evolution.

Combined epigenetic therapies

Due to the presence of several epigenetic disorders in some diseases, combined epigenetic drugs seem to be potential therapies for this type of disorders. The use of combined epigenetic drugs has been successfully developed over the last 25 years. Many studies have proven the usefulness of this kind of therapies.

New findings suggest the role of various epigenetic mechanisms including DNA methylation, histone modifica-
tions, and non-coding RNAs in the evolution and progression in the development of different cardiovascular diseases (CVDs). For this reason, combinations of epigenetic drugs that treat such diseases with a variety of different strategies are more suitable. It should be noted that the optimal performance of such treatments is based on knowing the specific genetic and epigenetic profiles of each patient.

In bladder cancer, epigenetic changes occur in two pathways. Genes that are expressed in natural cells, such as tumor suppressor genes have open chromatin, unmethylated promotor, acetylated and active histones, as well as free nucleosomes in their upstream region.

During tumorigenesis, the genes are turned off by two mechanisms: through the polycomb repressive complex (PRC) or through de novo methylation of the DNA.

Suppression of the expression induced by PRC can be treated with EZH2 inhibitors such as Dzep, and the de novo methylation function can be reversed through DNA...
methylation inhibitors (DNMTi) such as 5-Aza-CdR, 5-Aza-CR, Zebularine and S110.

The therapeutic value of each of mentioned drugs increases when combined with histone deacetylination inhibitors (HDACi) such as SAHA, PBA, and TSA. In a study conducted by Juergens et al. in 2011, it was found that the use of low dose and a combination of azacitidine and entinostat, which are DNA inhibitors and DNA deacetylination inhibitors respectively, have a lasting and complete response in most patients with Non-small cell lung cancer. Another study that examined the efficacy of these two drugs in metastatic colorectal cancer (mCRC) confirmed their synergistic effects.

A study by Pera et al. in 2016 it was found that the use of traditional chemotherapy treatments for patients with Relapsed-Refractory Diffuse Large B Cell Lymphoma (RR-DLBCL) due to its aggressiveness and resistance is not effective. However, chemotherapy with epigenetic treatments such as DNA methyltransferase inhibitors (DNMTi) has a negative effect on the growth of RR-DLBCL cells and increases the sensitivity of malignant cells to chemotherapy.

Another study also found that the combination of two drugs, DNMTi and HDI, is an effective therapeutic strategy for the treatment of RR-DLBCL. The two drugs act as a combination therapy to boost the anti-lymphoma effect in a synergistic manner against RR-DLBCL in vitro and in vivo without any toxicity.

Despite all the positive aspects of combined epigenetic therapy, it is possible that these drugs also treat illness with goals other than epigenetic goals.

Epigenetics and cytotoxic treatments

One of the problems with traditional chemotherapy is that sometime after treatment, cancerous cells undergo acute epigenetic changes due to the cytotoxicity of chemotherapy that promotes resistance to therapy, ineffectiveness of this type of treatment and as a result, proliferation of malignant cells, and if the cytotoxic therapy is stopped, the cancerous cells will begin to grow and repopulate. Two major epigenetic changes due to the cytotoxicity of high dosages of chemotherapy drugs are the methylation of CpG islands and histone acetylation, which can be modulated and reversed by DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), respectively. In addition, it has now been shown that the use of chemotherapy drugs such as low dosages of azacitidine by improving epigenetic modifications and having non-toxicity for bone marrow, is a more appropriate treatment than high dosages of drugs.

Studies have shown that the combination of epigenetic therapy and chemotherapy will rein induce the response to chemotherapy and resolve the resistance to cytotoxic agents. Data on many types of tumors including chronic myeloid leukemia (CML), lung, stomach, intestine, breast, bladder, and ovarian cancers indicate that treatment with DNMT inhibitors (azacitidine or decitabine) resulted in a response in 13–18% of patients with the myelodysplastic syndrome (MDS) with survival rates of 280–330 days.

No response to chemotherapy accompanied by epigenetic therapy such as DNMTs and cisplatin in some patients, can be a result from the specific epigenetic profile of patients that is unique for each individual, and this necessitates the identification of molecular biomarkers (like the methylation profile) in patients for choosing a suitable and unique epigenetic treatment.

Conclusion

Epigenetics therapy has been proven to be a successful approach for the treatment of different malignancies. The inhibition of DNMTs and HDACs are two FDA approved treatments for cancers, while the underlying mechanisms of DNMT and HDAC inhibition are not fully recognized. Future studies may be able to identify methods for recognizing response mechanisms by combining genomic sequencing and gene expression profiles. In addition, histones may be phosphorylated, ubiquitinated, sumoylated, methylated and acetylated. However, these modifications have been less studied in diseases and may also be able to demonstrate other therapeutic targets.

An important challenge in epigenetic therapy is to know which genes are the driver and which genes are stimulated. Recent developments in Genome-wide sequencing, along with RNA data profiles, chromatin immunoprecipitation (ChiP), or bisulfate conversion have led to a massive amount of information that can be used to accurately identify epigenetic changes. Comparing and reconciling this massive amount of information will help identify epigenetic changes that occur as a cause and effect or are completely dependent on each other. As a result, in the future, patients may be screened using precise techniques or classified by genetic modifications in driver genes. That way, it has made it possible to implement a personal and unique therapeutic approach to the treatment of each patient.

Currently, epigenetic therapy is successfully applied in clinics for the treatment of hematological malignancies, but little success has been achieved in the treatment of solid tumors.

Until recently, they used therapeutic regimen in early clinical trials, but later found that they were less than optimal and therefore, have fewer positive clinical responses. Recently, the implementation of therapeutic programs and the use of more advanced dosages, with the grouping of tumors based on their molecular properties, have been able to increase the efficacy of epigenetic therapy for solid tumors. In addition, solid tumors are a heterogeneous cell population, often made up of cells with different stages of differentiation. Consequently, determining which of the cells has undergone epigenetic changes, ensuring that the therapeutic agents maintain their sustainability, their capacity to penetrate the tumor mass and target malignant cells will increase the clinical success of the treatment.

The use of epigenetics as a major contributing factor in the development of normal and abnormal cells will open new sights for the advent of new therapeutic approaches. Epigenetic therapy can be combined with the traditional therapies to provide certain treatments for reversal of the drug-resistant tumors. Also with this therapeutic approach,
the drug dosages can be reduced to eliminate the side effects of treatment and, consequently, the patient’s healing problems and increase the patients’ quality of life.

Conflict of interest

The authors declare no competing financial interests.

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