The Role of Epigenetics in Cancer Drug Resistance

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Abstract

Cancer is caused by aberrant genetic and epigenetic changes in genes expression. DNA methylation, histone modification, and microRNAs gene deregulation are the most known epigenetic changes in different stages of cancer. Since every tumor has its own specific epigenome, any abnormal pattern is a potential biomarker for classification of different types of tumors. Despite, tumorigenesis, abnormal epigenetic changes are highly correlated with drug resistance in various stages of cancer. But, reversible nature of these abnormalities is the basis of epigenetic cancer treatment. Drugs affecting the epigenome are the new hopes in cancer treatment. The aim of this study was to investigate the role of epigenetics in tumorigenesis and also drug resistance in cancers.

Introduction

Epigenetics are heritable factors that change gene expression, but do not change the nucleotide sequences (1). Epigenetic mechanisms are involved in many biological processes including genomic imprinting, chromosome x inactivation, gene silencing, differentiation, embryogenesis and neoplasia (2). Since epigenetic changes induce changes in the natural pattern of gene expression, these changes can cause treatment initiation, progression and resistance in some cardiovascular diseases, metabolic syndrome, neurological disorders, pulmonary disease, and cancer (3). The aim of this review was to investigate the role of epigenetics in cancer drug resistance.

Epigenetic Mechanisms of Cancer

DNA methylation, histone modifications and changes in the gene expression levels of microRNAs (miRNAs) are the most known epigenetic changes in different stages of cancer (4-6). DNA Methylation has been studied more than other changes because CH_3 is enzymatically transferred from S-adenosyl methionine (SAMe) to cytosine nucleotides in CpG dinucleotides; this change is induced by a variety of DNA-methyltransferase enzymes in mammals (7). Although there are other epigenetic changes such as phosphorylation, ubiquitination, histones, acetylation and methylation, which are caused by a variety of histone acetyltransferases (HATs) and histone deacetylases (HDACs), have been more studied

(8). MicroRNAs are 17-25 nucleotide RNA sequences that bind to the end of the 3-UTR of target mRNA to set its expression (6). The expression of some miRNAs in cancer cells compared with that of normal cells has been increased or decreased, and dysregulation of gene expression, can dysregulate the expression of oncogenes or target tumor inhibitors (5, 9).

Mechanisms of Resistance to Anticancer Drugs

Cancer drug therapy or chemotherapy is the basic treatment in cancer patients. Although many types of cancers initially respond to chemotherapy, but after a while, resistance to the treatment and recurrence or metastasis may occur (10). Despite advances in treatment, a definite treatment for cancer has not been found (3). There are two main theories about lack of a definite treatment for cancer: genetic changes (mutations) and epigenetic (10-12). These two important factors with their different mechanisms influence the development of drug resistance (13).

Changes in the chromatin level can control drug sensitivities, and this feature has opened new horizons for more effective strategies in the treatment of cancer by combination therapies. The main mechanisms of drug resistance are drug inactivation, alteration of drug targets and expulsion of drugs from the cells, DNA repairs, cell death prevention and epithelial-mesenchymal transition (EMT). EMT is a process by which epithelial cells lose their cell-cell adhesion and become able to migrate and invade to become mesenchymal stem cells. In the case of cancer cells, this feature leads to metastasis (14).

In these mechanisms, gene expression can be also controlled by epigenetic (10, 15). The well-known mechanism of drug resistance is multi-drug resistance (MDR) phenotype, which is observed in many cancers (16). Phenotype of MDR is caused by increased expression of ABC transporters, including ABCC1 (MRP1), ABCB1 (MDR1), ABCG2 (BCRP) and ABCC2 (MRP2) that accordingly leads to the expulsion of hydrophobic anticancer drugs from cancer cells. Histone modifications and ABCB1 promoter methylations are involved in the increased expression of ABCB1 and formation of MDR phenotype. The ABCG2 methylation is also associated with MDR (17- 19). Another example is DNA repair enzyme or MGMT that prevent tumor cells death by chemotherapy alkylating agents. MGMT methylation leads to gene silencing and lack of gene expression, which ultimately reduces MGMT product. Some relationships between change in chromatin conformation and epigenetic changes in MGMT expression have been reported (10).

Cancer Epigenetics and Environmental Factors

Hypermethylation in the CpG islands (CGIs) of promoter is called as CpG island methylator phenotype (CIMP). CIMP, first, was detected in colorectal cancer and has been widely studied (20, 21). In the studies performed during the last ten years, CIMP has been reported for a variety of tumor types, including tumors of bladder, breast, endometrium, stomach, glioblastoma (glioma), hepatocellular, lung, ovarian, pancreas, renal cell and prostate as well as leukemia, melanoma, adenocarcinoma, and many different carcinomas (22). Thus, CpG island hypermethylation in human cancers is considered as a biomarker for the prediction of and response to the treatment (23). The results of studies have shown that CIMP is related to the environmental factors and lifestyle, although its main involved factors have not been identified yet (24). One of the first events leading to the CIMP, is V600E mutation of BRAF gene. However, despite identifying this mutation, its functional evidence has not well known (25). Mutations in the isocitrate dehydrogenase (IDH) are also known as involved

factors in the development of CIMP (26). According to the results of studies performed on some types of cancer, different cancers can be classified in different panels: CIMP+ and CIMP- (27). For example, colorectal cancer can be classified in CIMP+. In this cancer, CpG islands of all genes were hypermethylated, while similar genes in some types of cancer were hypomethylated. CIMP has important role in prediction, prognosis, and response to the treatment of a variety of tumors (27, 28). Today, heterogeneity of cells in a tumor mass is known as one of the causes of failure of cancer treatment (29). On the other hand, genetic and epigenetic differences causing individual variations in patients as well as in tumor cells, which makes them different from tissues of the same origin, are involved in the development of drug resistance. In addition, heterogeneity of tumor cells caused by genetic and epigenetic differentiates cancer cells from precursor cells and this reduces the effect of treatment (30). Cancer stem cells (CSCs), due to factors such as mutations and epigenetic changes, are involved in the management and development of cancer. Epigenetic changes are key features in the formation of cancer-initiating cells and Sub-cell populations in tumor and can be considered as the main factors of treatment resistance that in association with gene mutations, directly influence therapeutic strategies. Several abnormal epigenetic changes in this field have been known (31). Also, the mechanisms of resistance to various chemical agents in CSCs have been identified. CSCs are a subgroup of cancer cells that are found in blood cancers and also in solid tumors and are characterized with stem cell-like features such as self-renewal and the ability to give rise to all kinds of cells found in a particular cancer sample. CSCs are tumorigenic cells with special mechanisms and the main factors of cancer therapeutic resistance, recurrence and metastasis (32-35). For example, abnormal epigenetic changes in these cells lead to aberrant

expression and inappropriate activity of the ABC transporter (36), which its role was described previously. Some drug combinations target epigenetic enzymes in order to make therapeutic sensitivity in the cancer stem cells (37). As mentioned previously, the main problem in chemotherapy is drug resistance in cancer cells. The basic cause of this resistance is individual variations. Lack of response to drug therapy is the main cause of cancer mortality all over the world. Evidences have shown that epigenetic changes are the most important factors in the development of drug resistance. The most important environmental factors that may influence a person's epigenetic status include: nutrition, place of residence or work environment, drug treatment, and unhealthy habits (38). For example, a high fat diet can increase DNA hypermethylation of tumor suppressor genes (antioncogenes) (4, 38, 39). Environmental factors can also alter the epigenetic status of a tissue. For example, hypermethylation of antioncogenes that occurs in lung tissue of smokers, does not occur in that of non-smokers (38). Considering the important role of epigenetic changes in drug resistance, new cancer treatments are rapidly developing (40). Since epigenome changes can help to decide about the clinical interventions on the basis of the methylation patterns in cancers and consider new therapeutic goals, targeting epigenetic abnormalities has been considered as a new and effective cancer treatment strategy (41, 42). High level of epigenetic change in tumors leads to variations in gene expression patterns, which accordingly can be used for drug selection during the treatment period to decrease drug resistance (43). This can extensively create ambiguity in classification of chemotherapy that is based on the mutations in biomarkers. Epigenetic changes have been proven by studying the mechanism of steroid hormones and antihormone activities of nuclear receptors in the breast cancer (44).

The Importance of Epigenetic Drugs in Chemotherapy

Epigenetic drugs that target histone-modifying enzymes or DNA methylation, have shown remarkable results in clinical studies; for example, Genistein has high potential for cancer treatment through inhibiting DNA methylation. Therefore, this drug can enhance the effect of chemotherapy drugs (45). Studies have shown that in patients treated with epigenetic drugs, more stem cells or precursor cells are destroyed in tumor and the rate of recurrence will be reduced. These drugs make cancer cells more sensitive to the other treatments. With identification of this mechanism, researchers have proposed sensitization of cancer cells before treatment with standard chemotherapy, using epigenetic drugs, as much as possible, rather than other cytotoxic (anti-cancer) drugs (19).

Epigenetic and Anti-cancer Drugs

Hypomethylation of DNA, modified histones, gene mutation in histone-modifying enzymes and conformational changes in double-stranded DNA can change the access of transcription factors to gene promoter regions (46). Changes in the normal rate of miRs can also alter the normal expression of numerous genes. Despite insufficient knowledge of the role of abnormal epigenetic changes in cancer, some molecular events of cellular epigenetic associated with drug resistance in cancer cells, have been known. Drugs affecting epigenomes, are new hope for the cancer treatment. Today, many new and effective drugs in cancer treatment play their role through different epigenetic mechanisms (Table 1). In different stages of clinical trials, some of these drugs have shown their significant effect (47). On the other hand, nutrition or environmental factors influence gene expression through methylation. These factors, through various metabolism cycles by methyl or acetyl, can also change histone modifications. DNA hypermethylation in CpG-rich promoters of some genes

are observed in transformation and metastasis of various cancers (48, 49). It is thought that treatments inducing epigenome reconstruction can be effective in cancer patients (48, 49). By identifying epigenetic changes, as well as new molecular biomarkers, mechanisms of carcinogenesis has been indicated. In cancers, the highest levels of hypermethylation occur in the promoter of antioncogenes involved in the message pathways, DNA repair, cell adhesion, cell cycle control, and apoptosis. There are relationships between metabolic disorders, epigenetic changes and cancer (50). Recent studies obviously have shown that drug resistance in cancer cells is a multi-factor phenomenon caused by mutations and epigenetic changes (51). Unlike genetic changes, epigenetic changes are reversible and this feature allows resetting the abnormal cancer epigenome, through the use of drug combinations (52). Therefore, epigenetics is the most promising field in the biomedical research. Today more effort is made to use epigenome- regulator drugs. By determining the exact epigenetic changes in cancer, targeted therapies can lead to successful treatment of cancer patients. By knowledge about the relationship between epigenetics and its role in the cancer treatment, the future of cancer treatment seems bright because cancer treatment by drug-induced sensitivity is more promising than other treatments (53). Understanding the mechanisms of drug resistance and its causes is more important for new therapeutic strategies in the treatment of cancer patients, and recent studies have shown that drug resistance is the main obstacle to the successful treatment of cancer (54). Although drug resistance can be stopped using epigenetic therapies in the experimental models, clinical studies have shown great challenges and identifying methods for better targeting is required. Today it is well established that some epigenetic aberrations occur in the early stages of malignant transformation. Therefore, reliable and

valid diagnostic screenings will be allowed for evaluating the resistance to chemical compounds using these epigenetic biomarkers, hence, futile chemotherapies will be prevented (55). In some cases, these changes are clinically valuable for the diagnosis and prognosis of cancer. Some miRNAs have the ability to expulse outside of the cells floating in the body fluids. Therefore, they can be also considered as biomarkers for cancer detection. Moreover, some strategies can be considered to regulate their expression (56). It seems that each of the algorithms, alone or in combination, can play an important role in cancer control.

Table 1. Chemotherapy drugs with epigenetic mechanisms used as effective anticancer drugs in different stages of clinical experiments (47).

Epigenetic Mechanism of Drug	Chemotherapy Drugs	Cancer Type	Stages of Clinical Trials
DNA Methylation Inhibition	5-Azacytidine	haematological malignancies	III
	5-Aza-2′-deoxycytidine	haematological malignancies; cervical, non-small-cell lung cancer	III,II
	5-Fluoro-2′-deoxycytidine		I
	5,6-Dihydro-5-azacytidine	ovarian cancer and lymphomas	I,II
	Hydralazine	cervical cancer	I
	MG98	advanced/ metastatic solid tumors	I
Histone deacetylase inhibition	Butyrate	colorectal	I,II
	Valproic acid	AML, leukaemias	I
	Suberoylanilide hydroxamic acid (SAHA)	haematological and solid tumors	I,II
	Depsipeptide (FK-228, FR901228)	CLL, AML, T-cell lymphoma	I,II
	CI-994 (N-acetyl dinaline)	solid tumors	I,II
	MS-275	solid tumors and lymphoma	I,II

Conclusion

Combination epigenetic therapies in cancer, allows the re-expression of inhibitors to create drug-induced sensitivity; thus, instead of conventional chemical treatments, new and more effective methods can be provided to treat cancer in the future. Also, using this method, CSCs, which are basically involved in the resistance to treatment and also suitable options for management of solid tumors, can be eliminated. In the future, anti-cancer drug regimens, including epigenetic therapy, particularly in combination with pathway inhibitors in fundamental message pathways, will effectively reduce chemical drugs resistance and prevent recurrence in cancer patients (57). Targeted epigenetic therapy is still in the early stages of its development. But, the most important reason to continue the research in this field is its role and function in cell regulation and clinical therapies, which makes combination epigenetic therapy, eventually, the best choice.

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