Epigenetic Therapy in Malignant and Chronic Diseases

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Abstract

The role of epigenetics in cancer development establishes enzymes that regulate epigenetic modifications as vital targets for cancer therapy. Inhibition of DNA Methyltransferase (DNMT) and Histone Deacetylase (HDAC) enzymes proved to be a successful strategy in the treatment of some types of cancer. There is currently growing interest in studying the effect of inhibition of enzymes affecting other histone modifications, like histone methylation, and how they can affect cancer development and progression. A major limitation of epigenetic therapy is the lack of specificity with consequent global induction of epigenetic changes. Additionally, optimal dosing, single or combined therapy and the sequence of delivery of combined therapy are clinical issues associated with the use of these drugs. Herein, we will summarize the impact of using the different classes of epigenetic drugs in cancer and other chronic diseases.

Keywords: DNA methylation; Histone acetylation; miRNAs; Decitabine; 5-Azacytidine

Introduction

Cancer is a complex disease that involves genetic and epigenetic changes. The World Health Organization (WHO) has identified several approaches to fight cancer including prevention, early detection and comprehensive treatment plans for patients with advanced disease [1]. Epigenetic therapy is a novel therapeutic approach that modulates gene expression by targeting the DNA methylation machinery, histone covalent modifications or microRNAs (miRNAs). Drugs targeting DNA methylation (5-azacytidine and decitabine) and histone acetylation (vorinostat, romidepsin) are currently FDA approved for the treatment of myelodysplastic syndromes (MDS) and Cutaneous T-Cell Lymphoma (CTCL), respectively. On the other hand, drugs targeting miRNAs and other histone covalent modifications are still under development. Several epigenetic agents demonstrated efficacy as chemo preventive agents, adding another dimension for their future use in medicine. This review will discuss the recent advances in epigenetic therapy and a future perspective for the use of epigenetic modifiers in the treatment of other diseases.

DNA Methyltransferase (DNMT) inhibitors

The methylation of cytosine bases in CpG dinucleotides was the first described covalent DNA modification. This modification was the focus of extensive research studies after recognizing the inverse relation between promoter DNA methylation and gene expression [2-4]. In normal mammalian cells genome, CpG islands exist in the proximal promoter regions of almost half of the genes and are usually unmethylated [5]. However, DNA repeat sequences, centromeres, telomeres and inactive X-chromosomes are methylated in normal cells [6]. On the contrary, tumor cells show an opposite pattern with increased gene promoter hypermethylation and decreased global methylation. Recent advances in DNA sequencing technology facilitated a more detailed genome wide comparisons of the DNA methylene in normal and tumor cells and discovered additional methylation changes in other genomic regions like CpG shores within the gene body and in gene promoters of non coding RNA [7].

Other than the spontaneous deamination of 5-methylcytosine into uracil, DNA methylation was considered an irreversible modification for a long time. Earlier reports claimed the existence of a mammalian demethylase specific for methylated CpGs [8,9]. Recently, the discovery of the 5-hydroxymethylcytosine (5hmC) modification altered this concept and proved that 5-methylcytosine is metabolized by hydroxylation into 5hmC by a family of enzymes known as ten-eleven translocation (TET1-3) [10,11]. Further oxidation of 5hmC by the TET enzymes results in the formation of 5-formylcytosine and 5-carboxycytosine. It’s speculated that decarboxylation of 5-carboxycytosine is the final process of reversing DNA methylation and the generation of unmethylated cytosine [12]. The physiological function of 5hmC and the downstream intermediates is not clear yet.

Inhibition of DNA methyltransferase (DNMT) enzymes either directly or indirectly re-express epigenetically silenced genes by reversing DNA methylation. Direct inhibition of DNMT involves binding of an inhibitor to one of the DNMT isotypes, while indirect inhibition involves the trapping of DNMT isotypes by cytidine analogs after their incorporation into DNA [13]. The chemically synthesized compound 2-(1,3-dioxo-1,2-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl) propanoic acid or RG108 is a direct DNMT1 inhibitor with demethylating activity both in vitro and in vivo. On the other hand, 5-aza-2’-deoxycytidine (decitabine, DAC) and 5-azacytidine (5AC) are nucleoside analogs that indirectly inhibit DNMT. The incorporation of 5AC into RNA with consequent inhibition of protein synthesis is the major difference from DAC, which fully incorporates into DNA. Although 10-20% of 5AC is incorporated into DNA versus full incorporation of DAC into DNA; 5AC has been shown to be more clinically effective than DAC [14]. The question of which strategy (direct versus indirect) is more effective in inhibiting DNA methylation is intriguing. A comparison of the demethylating activity of the direct, indirect inhibitors and the natural compound (−)-epigallocatechin-3-gallate (EGCG) revealed that the indirect inhibitors are the most potent demethylating agents [15]. However, direct inhibition is not associated

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with the cytotoxicity observed with the use of indirect inhibitors. A major drawback of DNMT inhibition, either directly or indirectly, is the induction of global demethylation with consequent undesired activation of oncopgenes and chromosomal instability.

Non-nucleoside analogues like hydralazine (antihypertensive agent) and procainamide (management of cardiac arrhythmia) demonstrated DNA demethylating activity. The mechanism of their demethylating activity is not clear and is speculated that they bind to CpG-rich regions [16]. Unfortunately, the demethylating activity of these drugs is not reproducible and requires administration of clinically irrelevant high doses [13].

There are several approaches to use DNMT inhibitors in the clinical setting. The combination of indirect DNMT inhibitors with chemotherapy to harness their gene expression modulation with consequent sensitization of cancer cells has been applied in different tumors. 5AC restored the sensitivity of bladder cancer cells to cisplatin [17]. 5AC reversed platinum resistance in patients with platinum-refractory epithelial ovarian cancer [18]. The clinical utility of DNMT inhibitors was challenged by their cost effectiveness. The economic burden of the drug decitabine was compared to the use of best supportive care (red blood cell transfusion, erythropoiesis stimulating agents, colony stimulating factors, deferoxamine, as well as platelet transfusion) in treating intermediate-high risk MDS [19]. Five days dosing regimen of decitabine was shown to be a cost effective option in treating intermediate high risk MDS when compared to best supportive care.

**Histone Deacetylase Inhibitors**

Histone deacetylase (HDAC) inhibitors are another class of epigenetic drugs. Although their name implies inhibition of histone deacetylation, they also inhibit the deacetylation of other proteins like p53 and NF-kB [20]. Most HDAC inhibitors share a common structure that is characterized by three main regions; a surface recognition domain, a linker, and a metal binding domain that binds to Zn at the enzyme core (Figure 1). Romidepsin is an HDAC inhibitor with a cyclic structure that requires reduction of the disulfide bond to expose the sulfur access the active site of the HDAC enzyme and binds to Zn with consequent HDAC deactivation.

A major drawback associated with the use of HDAC inhibitors is the non-selective inhibition of the different classes of HDAC enzymes. Current studies focus on HDAC inhibitors that are highly selective. For instance, the orally active mocetinostat (class I and IV selective HDAC inhibitors) demonstrated no or little hematological toxicity like thrombocytopenia when compared to non-selective HDAC inhibitors [22]. Similar to other non-selective HDAC inhibitors, mocetinostat induced other off-target effects like autophagy, microtubules destabilization and cell death [23-25]. Mocetinostat demonstrated in vitro synergistic effects with the proteasome inhibitor bortezomib, giving the opportunity of using lower doses of both drugs to minimize their toxicity [26].

The pharmacodynamics of HDAC inhibitors is dose-dependent. In lower doses, they act by modulating gene expression. At higher doses, they induce cytotoxicity through different mechanisms [27]. In a phase II clinical trial enrolling patients with relapsed classical Hodgkin lymphoma, lower doses of mocetinostat showed better outcome than higher doses, in favor of non-cytotoxic mechanism of action [28]. Another phase II clinical trial examined the efficacy of the orally active vorinostat in combination with bortezomib, a proteasome inhibitor, for the treatment of recurrent glioblastoma [29]. The study was a follow up on previous pre-clinical studies, which demonstrated synergistic cytotoxicity when combining HDAC inhibitors with proteasome inhibitors in glioblastoma cells. Study results showed no improvement among the 34 subjects included with only one partial response in one patient; further supporting a non-cytotoxic mechanism of action of these drugs [29].

The combination of HDAC inhibitors with chemotherapeutic agents is another treatment strategy that depends on the induction of pro-apoptotic genes and repression of anti-apoptotic genes by HDAC inhibitors. A Phase II randomized, double blinded, placebo controlled study enrolling non-small cell lung cancer (NSCLC) patients combined vorinostat with Carboplatin and paclitaxel. Results showed an enhancement in the efficacy of paclitaxel and carboplatin for NSCLC treatment, predicting vorinostat as a promising future drug in treating NSCLC [30].

The combination with DNMT inhibitors is another widely used treatment strategy. A recent phase II clinical trial investigated the effectiveness and the safety of hydralazine (DNMT inhibitor) and magnesium valproate (HDAC inhibitor) in the treatment of MDS. The results of the study showed less progression to acute myeloid leukemia (AML) and fewer requirements for blood transfusion [31]. Several other studies adopted the sequential combination of DNMT inhibitors and HDAC inhibitors in MDS and AML and showed promising results and are reviewed elsewhere [32,33].

The clinical utility of HDAC inhibitors is not confined to neoplastic diseases. Other metabolic diseases like the Maple SyrupUrine Disease (MSUD) demonstrated improvement after the use of the non-FDA approved HDAC inhibitor, phenyl butyrate. MSUD is a condition that is caused by an error in the metabolism of amino acids due to a deficiency in the mitochondrial branched-chain keto dehydrogenase complex (BCKDC) [34]. Consequentially, branched chain amino acids (BCAA) and the corresponding branched chain alpha keto acids (BCKA) starts accumulating in plasma and tissues which, ends up in a maple syrup odor in urine as well as other symptoms that range from neurological deterioration to weight loss due to feeding problems [34]. Phenyl butyrate treatment resulted in lowering the neurotoxic BCKA
miRNAs are 18-24 nucleotides non-coding RNA that down regulate the expression of their target genes via translational repression or cleavage of mRNAs [37]. Recently, it was proposed that miRNAs may also upregulate the translation of their target genes [38]. The number of human miRNAs is estimated to be more than 1100 and they can function as oncogenes or tumor suppressor genes (TSG), depending on their miRNA target [32]. miRNAs-based therapeutics is a rational therapeutic approach in cancer treatment by modulating the expression of oncogenes and TSG. Currently, there are no FDA approved miRNAs modifier drugs available in the market because of their stability. The stability and specificity of miRNAs are among the major hurdles that impact the development of miRNA-based therapeutics. Modifications in the nucleotides structure, such as 2’-O-methyl and 2’-O-methoxymethyl anti-miRs, improved the stability of miRNAs [39].

miRNAs delivery is another challenge that obstructs the development of this type of therapy. Non-viral delivery systems like liposomes and nanoparticles demonstrated promising results in animal studies [40,41]. Exosomes, vesicles of endocytic origin, shuttle different types of RNA between cells and can be utilized to deliver miRNAs [42]. Exosomes would provide a stable environment for miRNAs preventing their degradation and can be modified externally to target miRNAs to specific type of cells. Viral gene delivery is another approach that critically enhances miRNAs delivery. Lentiviral gene delivery promotes stable expression of miRNAs by integrating into the human genome. Although this integration is thought to have a minimal impact on the genome, there is always a risk of disrupting the genomic integrity. The use of the episomal adeno viral vectors could provide an alternative approach to avoid viral genome integration; however, the development of immune response and transduction efficiency are major limiting factors to this approach [43].

Recent studies have been focusing on miRNAs as a diagnostic tool due to the fact that miRNAs are very stable in human plasma. This phenomenon can be utilized in detecting biomarker miRNAs to identify certain diseases and to distinguish between the different stages of a disease. For instance, the fluctuation of serum miRNA-141 levels can be used as a reliable and sensitive method to distinguish prostate cancer patients from healthy individuals [44]. Current studies have been investigating miRNAs as biomarkers in drug induced liver injury [45], type II diabetes [46], coronary artery disease [47,48], Barrett’s Esophagus progression [49], as well as AML [50].

A recent phase II clinical study investigated the levels of circulating miRNA-122 and miRNA-192 among patients with acetoniminophen poisoning [45]. This was a follow up on a previous study that investigated acetoniminophen induced acute liver injury in mice. miRNA-122 and miRNA-192 serum levels were significantly higher in acetoniminophen-induced liver injury patients when compared with healthy individuals. The same observation was also true among chronic kidney disease patients (CKD); albeit lower than acetoniminophen-induced liver injury patients.

The role of miRNAs in other chronic diseases like diabetes was investigated. A phase II clinical trial investigated the hypothesis that miRNAs may contribute to type II diabetes mellitus (DM) progression [46]. Different miRNAs were screened using microarrays followed by quantitative PCR and showed lower plasma levels of different miRNAs, when compared to healthy individuals; with miRNA-126 being the most associated with DM [46]. The main function of miRNA-126 is to control angiogenesis, wound repair as well as maintaining vascular integrity and is known to be highly expressed in endothelial cells as well as endothelial apoptotic bodies [51,52]. miRNA-126 reduction is believed to contribute to the peripheral angiogenic signaling impairment associated with diabetic patients [46]. miRNA-126 down regulation was also associated with impairment of vascular integrity and angiogenesis in mouse embryonic cells. miRNA-126 keeps the vascular integrity and maintain homeostasis of endothelial cells via the down regulation of two VEGF pathway regulators; phosphoinositidol-3 kinase regulatory subunit 2 (PK3R2) and the Sprouty-related protein (SPRED1) [52]. The role of miRNAs in the discrimination of Barrett’s Esophagus (BE) with and without dysplasia was evaluated [49]. A total of 22 BE patients with 11 dysplasia patients were evaluated. The study demonstrated that BE patients with dysplasia can be discriminated by using miRNAs as biomarkers with clinical accuracy [49].

Modulation of different miRNAs has been reported in various leukemias [53,54]. A recent study compared miRNAs expression in normal myeloid early progenitor cells (CD34+) to that of newly diagnosed AML patients [54]. About 26 miRNAs were shown to be down regulated in AML samples when compared to normal CD34+cells [54]. Of note, miRNA-29b (targets DNMT enzymes) was shown to be down regulated in AML patients [50,55]. Accordingly, the role of miRNA-29b as a biomarker for decitabine treatment in AML was studied [50]. miRNA-29b was evaluated in 53 AML patients as a pretreatment determinant for the use of the DNMT inhibitor decitabine [50]. The study demonstrated that the pretreatment levels of miRNA-29b can be used to evaluate the subsequent response of decitabine; the higher its level, the better is the clinical response to decitabine.

In a follow up clinical trial, the expression of miRNA-29b and miRNA-101 was examined to determine whether it can predict the response to the combination of 5-azacytidine, ATRA and valproic acid in AML patients [56]. The study reported the downregulation of miRNA-29b in AML patients. However, there was no significant difference in the expression of miRNA-101 when compared to healthy controls [56]. A follow up among responders and non-responders showed no difference in the expression of both miRNAs, indicating the absence of a relationship between the response to 5-azacitidine, ATRA, and valproic acid therapy and the level of miRNA-29b [56].

Conclusion

Recent advances in the field of epigenetics underlie many promising clinical applications including prediction of patient response to treatment, prediction of prognosis and biomarkers for early detection of cancer and other chronic diseases like DM and CKD. The histone modifications associated with cancer progression and other diseases started to gain focus and provided an explanation of how cancer cells acquire a DNA methylation pattern that is different from their normal counterparts. Indeed, histone modifications can guide DNMT enzymes and consequently DNA methylation [57]. However, the large number of histone modifications associated with cancer development and the sequence of these modifications remains to be discovered. Although four FDA-approved drugs (Table 1) are believed to act through induction of epigenetic modifications, none of them is indicated for the treatment of solid tumors. Fortunately, several HDAC and DNMT inhibitors are currently in preclinical and clinical trials for the
treatment of different types of solid tumors. Combination therapy with romidepsin and decitabine in clear cell renal cell carcinoma induced synergistic re-expression of the TSG sFRP1 and induced apoptosis and cell cycle arrest [58]. DAC was shown to be effective against pancreatic ductal adenocarcinoma and slowed down its progression in vivo without inducing side effects [59]. The development of miRNA-based therapeutics is feasible but curbed by drug delivery issues and it is hard to predict when they will get FDA-approval. Limitations to epigenetic therapy do exist with lack of specificity considered as the major limitation. Although target specificity was achievable (for instance, targeting specific class of HDAC enzymes), the substrate of the targeted enzyme is global leading to global epigenetic changes and not gene specific changes.

References


Table 1: FDA approved epigenetic drugs and their labeled and unlabeled uses.

<table>
<thead>
<tr>
<th>Generic name (brand)</th>
<th>Mechanism of action</th>
<th>Uses</th>
<th>Dosage/route of administration</th>
</tr>
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<tbody>
<tr>
<td>Azacitidine (Vidaza®)</td>
<td>DNMT inhibitor with possible cytotoxic effect</td>
<td>Labeled: myelodysplastic syndrome (MDS)</td>
<td>MDS: 75 mg/m²/day x 7 days (subcutaneous, IV) Repeated every 4 week</td>
</tr>
<tr>
<td>Decitabine (Dacogen®)</td>
<td>DNMT inhibitor with possible cytotoxic effect</td>
<td>Labeled: MDS Unlabeled: AML and Sickle Cell Anemia</td>
<td>MDS: 15 mg/m² every 8hrs (IV) (~45 mg/m²/day x 3 days). It is recommended to administer the drug for at least 4 cycles; continue until patient has no benefit</td>
</tr>
<tr>
<td>Vorinostat (Zolinza®)</td>
<td>Histone deacetylase (HDAC) inhibitor (class 1 and 2)</td>
<td>Labeled: cutaneous T-cell lymphoma (CTCL) [progression, persistent &amp; recurrent] CTCCL: 400 mg orally once daily (until disease progresses or unacceptable toxicity develops)</td>
<td></td>
</tr>
<tr>
<td>Romidepsin (Istodax®)</td>
<td>HDAC inhibitor (potent class I inhibitor)</td>
<td>Labeled: refractory CTCL and refractory Peripheral T-cell Lymphoma (PTCL)</td>
<td>CTCCL: 14 mg/m² (IV) on days: 1, 8 and 15 in a 28-day cycle PTCL: 14 mg/m² on days 1, 8, &amp; 15 in a 28-day cycle. (Repeat cycles as long as there is benefit &amp; patient is tolerated)</td>
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