Chahin et al., J Pharmacogenom Pharmacoproteomics 2013, 4:2
DOI: 10.4172/2153-0645.1000118

Journal of
Pharmacogenomics & Pharmacoproteomics

Review Article Open Access

Epigenetic Therapy in Malignant and Chronic Diseases

Hussein Chahin, Bassey Ekong and Tamer E Fandy*

Department of Pharmaceutical Sciences, Albany College of Pharmacy, Colchester, VT 05446, USA

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 Methylation 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 methylome 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-carboxylcytosine. It's speculated that decarboxylation of 5-carboxylcytosine 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

***Corresponding author:** Tamer E Fandy, Department of Pharmaceutical Sciences, Albany College of Pharmacy, 261 Mountain View Dr, Colchester, VT 05446, USA, Tel: 802-735-2634; Fax: 802-654-0716; E-mail: tamer.fandy@acphs.edu

Received June 25, 2013; **Accepted** July 20, 2013; **Published** July 27, 2013

Citation: Chahin H, Ekong B, Fandy TE (2013) Epigenetic Therapy in Malignant and Chronic Diseases. J Pharmacogenom Pharmacoproteomics 4: 118. doi:10.4172/2153-0645.1000118

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 oncogenes 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, erythropeoiesis 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 deacytelation 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 group [21]. Consequently, the linker attached negatively charged 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

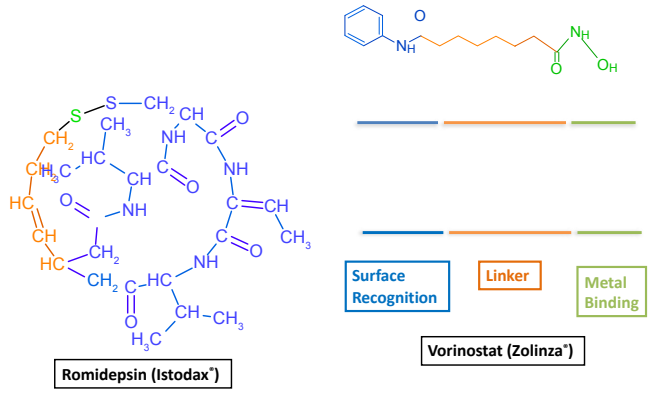

Figure 1: Chemical structures of the FDA approved HDAC inhibitors. The left panel shows the cyclic structure of romidepsin showing the three domains in different colors. The right panel shows the different domains in the structure of vorinostat. The green color indicates the metal binding domain, the orange color indicates the linker domain and the blue color indicates the surface recognition domain.

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]. Consequently, 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

and BCAA levels by increasing the overall activity of the BCKDC enzyme complex. Other HDAC inhibitors are currently tested in liver and kidney fibrosis [35] and neurological disorders like spinal muscular atrophy and myotonic dystrophy [36].

miRNAs-based Therapeutics

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 mRNA 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-methoxyethyl 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 adenoviral 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 acetaminophen poisoning [45]. This was a follow up on a previous study that investigated acetaminophen induced acute liver injury in mice. miRNA-122 and miRNA-192 serum levels were significantly higher in acetaminophen-induced liver injury patients when compared with healthy individuals. The same observation was also true among chronic kidney disease patients (CKD); albeit lower than acetaminophen-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; phosphoinositol-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-azacytidine, 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

Generic name (brand)	Mechanism of action	Uses	Dosage/route of administration
Azacitidine (Vidaza®) FDA approval 2004	DNMT inhibitor with possible cytotoxic effect	Labeled: myelodysplastic syndrome (MDS) Unlabeled: Acute Myelogenous Leukemia (AML)	MDS: 75 mg/m^2/day x 7 days (subcutaneous, IV) Repeated every 4 week
Decitabine (Dacogen®) FDA approval 2006	DNMT inhibitor with possible cytotoxic effect	Labeled: MDS Unlabeled: AML and Sickle Cell Anemia	MDS: 15 mg/m^2 every 8hrs (IV) (~45 mg/m^2/day x 3 days). It is recommended to administer the drug for at least 4 cycles; continue until patient has no benefit
Vorinostat (Zolinza®) FDA approval 2006	Histone deacetylase (HDAC) inhibitor (class 1 and 2)	Labeled: cutaneous T-cell lymphoma (CTCL) [progression, persistent & recurrent]	CTCL: 400 mg orally once daily (until disease progresses or unacceptable toxicity develops)
Romidepsin (Istodax®) FDA approval 2009	HDAC inhibitor (potent class I inhibitor)	Labeled: refractory CTCL and refractory Peripheral T-cell Lymphoma (PTCL)	CTCL: 14 mg/m^2 (IV) on days: 1, 8 and 15 in a 28-day cycle. PTCL: 14 mg/m^2 on days 1, 8, & 15 in a 28-day cycle. (Repeat cycles as long as there is benefit & patient is tolerated)

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

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

1. Cao Y, DePinho RA, Ernst M, Vousden K (2011) Cancer research: past, present and future. Nat Rev Cancer 11: 749-754.
2. Jones PA, Taylor SM (1980) Cellular differentiation, cytidine analogs and DNA methylation. Cell 20: 85-93.
3. Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 187: 226-232.
4. Christman JK, Price P, Pedrinan L, Acs G (1977) Correlation between hypomethylation of DNA and expression of globin genes in Friend erythroleukemia cells. Eur J Biochem 81: 53-61.
5. Bird AP, Wolffe AP (1999) Methylation-induced repression--belts, braces, and chromatin. Cell 99: 451-454.
6. Dawson MA, Kouzarides T (2012) Cancer epigenetics: from mechanism to therapy. Cell 150: 12-27.
7. Baylin SB, Jones PA (2011) A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 11: 726-734.
8. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M (1999) A mammalian protein with specific demethylase activity for mCpG DNA. Nature 397: 579-583.
9. Ramchandani S, Bhattacharya SK, Cervoni N, Szyf M (1999) DNA methylation is a reversible biological signal. Proceedings of the National Academy of Sciences of the United States of America 96: 6107-6112.
10. Kriaucionis S, Heintz N (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324: 929-930.
11. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, et al. (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324: 930-935.
12. Ito S, Shen L, Dai Q, Wu SC, Collins LB, et al. (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science 333: 1300-1303.
13. Fandy TE (2009) Development of DNA methyltransferase inhibitors for the treatment of neoplastic diseases. Curr Med Chem 16: 2075-2085.
14. Gidwani R, Khan ZM, Fenaux P, Beach CL, Pashos CL (2012) A cost-effectiveness analysis of using azacitidine vs. decitabine in treating patients with myelodysplastic syndromes. J Med Econ 15: 145-154.
15. Stresemann C, Brueckner B, Musch T, Stopper H, Lyko F (2006) Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines. Cancer Res 66: 2794-2800.
16. Cornacchia E, Golbus J, Maybaum J, Strahler J, Hanash S, et al. (1988) Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. J Immunol 140: 2197-2200.
17. Ramachandran K, Gordian E, Singal R (2011) 5-azacytidine reverses drug resistance in bladder cancer cells. Anticancer Res 31: 3757-3766.
18. Fu S, Hu W, Iyer R, Kavanagh JJ, Coleman RL, et al. (2011) Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. Cancer 117: 1661-1669.
19. Pan F, Peng S, Fleurence R, Linnehan JE, Knopf K, et al. (2010) Economic analysis of decitabine versus best supportive care in the treatment of intermediate- and high-risk myelodysplastic syndromes from a US payer perspective. Clin Ther 32: 2444-2456.
20. Condorelli F, Gnemmi I, Vallario A, Genazzani AA, Canonico PL (2008) Inhibitors of histone deacetylase (HDAC) restore the p53 pathway in neuroblastoma cells. Br J Pharmacol 153: 657-668.
21. Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, et al. (2002) FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer Res 62: 4916-4921.
22. Boumber Y, Younes A, Garcia-Manero G (2011) Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. Expert Opin Investig Drugs 20: 823-829.
23. El-Khoury V, Moussay E, Janji B, Palissot V, Aouali N, et al. (2010) The histone deacetylase inhibitor MGCD0103 induces apoptosis in B-cell chronic lymphocytic leukemia cells through a mitochondria-mediated caspase activation cascade. Mol Cancer Ther 9: 1349-1360.
24. Chia K, Beamish H, Jafferi K, Gabrielli B (2010) The histone deacetylase inhibitor MGCD0103 has both deacetylase and microtubule inhibitory activity. Mol Pharmacol 78: 436-443.
25. Wei Y, Kadia T, Tong W, Zhang M, Jia Y, et al. (2010) The combination of a histone deacetylase inhibitor with the Bcl-2 homology domain-3 mimetic GX15-070 has synergistic antileukemia activity by activating both apoptosis and autophagy. Clin Cancer Res 16: 3923-3932.
26. Buglio D, Mamidipudi V, Khaskhely NM, Brady H, Heise C, et al. (2010) The class-I HDAC inhibitor MGCD0103 induces apoptosis in Hodgkin lymphoma cell lines and synergizes with proteasome inhibitors by an HDAC6-independent mechanism. Br J Haematol 151: 387-396.
27. Fandy TE, Carraway H, Gore SD (2007) DNA demethylating agents and histone deacetylase inhibitors in hematologic malignancies. Cancer J 13: 40-48.

28. Younes A, Oki Y, Bociek RG, Kuruvilla J, Fanale M (2011) Mocetinostat for relapsed classical Hodgkin's lymphoma: an open-label, single-arm, phase 2 trial. Lancet Oncol 12: 1222-1228.

29. Friday BB, Anderson SK, Buckner J, Yu C, Giannini C, et al. (2012) Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. Neuro Oncol 14: 215-221.

30. Ramalingam SS, Maitland ML, Frankel P, Argiris AE, Koczywas M, et al. (2010) Carboplatin and Paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. J Clin Oncol 28: 56-62.

31. Candelaria M, Herrera A, Labardini J, González-Fierro A, Trejo-Becerril C, et al. (2011) Hydralazine and magnesium valproate as epigenetic treatment for myelodysplastic syndrome. Preliminary results of a phase-II trial. Ann Hematol 90: 379-387.

32. Fandy TE, Gore SD (2010) Epigenetic targets in human neoplasms. Epigenomics 2: 221-232.

33. Loaiza-Bonilla A, Gore SD, Carraway HE (2010) Novel approaches for myelodysplastic syndromes: beyond hypomethylating agents. Curr Opin Hematol 17: 104-109.

34. Brunetti-Pierri N, Lanpher B, Erez A, Ananieva EA, Islam M, et al. (2011) Phenylbutyrate therapy for maple syrup urine disease. Hum Mol Genet 20: 631-640.

35. Van Beneden K, Mannaerts I, Pauwels M, Van den Branden C, van Grunsven LA (2013) HDAC inhibitors in experimental liver and kidney fibrosis. Fibrogenesis Tissue Repair 6: 1.

36. Gottesfeld JM, Pandolfo M (2009) Development of histone deacetylase inhibitors as therapeutics for neurological disease. Future Neurol 4: 775-784.

37. Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75: 855-862.

38. Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science 318: 1931-1934.

39. Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, et al. (2005) Silencing of microRNAs in vivo with 'antagomirs'. Nature 438: 685-659.

40. Wiggins JF, Lynnsie Ruffino, Kevin Kelnar, Michael Omotola, Lubna Patrawala, et al. (2010) Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res 70: 5923-5930.

41. Rai K, Takigawa N, Ito S, Kashihara H, Ichihara E, et al. (2011) Liposomal delivery of MicroRNA-7-expressing plasmid overcomes epidermal growth factor receptor tyrosine kinase inhibitor-resistance in lung cancer cells. Mol Cancer Ther 10: 1720-1727.

42. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, et al. (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9: 654-659.

43. Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, et al. (2009) Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 137: 1005-1017.

44. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105: 10513-10518.

45. Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, et al. (2011) Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 54: 1767-1776.

46. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, et al. (2010) Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 107: 810-817.

47. Tabuchi T, Satoh M, Itoh T, Nakamura M (2012) MicroRNA-34a regulates the longevity-associated protein SIRT1 in coronary artery disease: effect of statins on SIRT1 and microRNA-34a expression. Clin Sci 123: 161-171.

48. Takahashi Y, Satoh M, Minami Y, Tabuchi T, Itoh T, et al. (2010) Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. Clin Sci (Lond) 119: 395-405.

49. Bansal A, Lee IH, Hong X, Anand V, Mathur SC, et al. (2011) Feasibility of mcroRNAs as biomarkers for Barrett's Esophagus progression: a pilot cross-sectional, phase 2 biomarker study. Am J Gastroenterol 106: 1055-1063.

50. Blum W, Garzon R, Klisovic RB, Schwind S, Walker A, et al. (2010) Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. Proc Natl Acad Sci USA 107: 7473-7478.

51. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, et al. (2008) The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 15: 261-271.

52. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, et al. (2008) miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 15: 272-284.

53. Fabbri M, Garzon R, Andreeff M, Kantarjian HM, Garcia-Manero G, et al. (2008) MicroRNAs and noncoding RNAs in hematological malignancies: molecular, clinical and therapeutic implications. Leukemia 22: 1095-1105.

54. Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, et al. (2008) MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood 111: 3183-3189.

55. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, et al. (2009) MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood 113: 6411-6418.

56. Yang H, Fang Z, Wei Y, Hu Y, Calin GA, et al. (2011) Levels of miR-29b do not predict for response in patients with acute myelogenous leukemia treated with the combination of 5-azacytidine, valproic acid, and ATRA. Am J Hematol 86: 237-238.

57. Fuks F (2005) DNA methylation and histone modifications: teaming up to silence genes. Curr Opin Genet Dev 15: 490-495.

58. Cooper SJ, Von Roemeling CA, Kang KH, Marlow LA, Grebe SK, et al. (2012) Reexpression of tumor suppressor, sFRP1, leads to antitumor synergy of combined HDAC and methyltransferase inhibitors in chemoresistant cancers. Mol Cancer Ther 11: 2105-2115.

59. Shakya R, Gonda T, Quante M, Salas M, Kim S, et al. (2013) Hypomethylating therapy in an aggressive stroma-rich model of pancreatic carcinoma. Cancer Res 73: 885-896.