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Chromatin modifying agents – the cutting edge of anticancer therapy

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Chromatin modifying compounds are emerging as the next generation of anticancer therapies. By altering gene expression they could be able to correct uncontrolled proliferation and, in certain cases, aberrant apoptotic pathways, which are hallmarks of malignant cells. The modulation of gene expression is regulated via chromatin remodelling processes that include DNA methylation and chromatin modifications. The identification of aberrant methylation of genes and dysregulated histone acetylation status in cancer cells provides a basis for novel epigenetic therapies. Currently available chromatin modifying agents, a group that includes DNA methyltransferase and histone deacetylase inhibitors, exert anticancer effects by reactivating tumour suppressor genes, inhibiting proliferation and inducing apoptosis. It is anticipated that massive parallel sequencing will identify new epigenetic targets for drug development.

Introduction

DNA methylation and histone acetylation are epigenetic processes that play a major part in the regulation of genes that control vital cellular processes such as proliferation, survival and differentiation [1]. The balance in the activity of DNA methyltransferases (DNMTs), DNA demethylases, histone acetyltransferases (HATs) and histone deacetylases (HDACs) is crucial in mediating these epigenetic processes. An imbalance in the activity of these enzymes could result in hypermethylation of tumour suppressor genes, global genomic hypomethylation and gene silencing via histone deacetylation in cancer cells, suggesting an important role for abnormal chromatin metabolism in tumourigenesis [1,2].

Epigenetic changes, in contrast to genetic modifications, are typically reversible, creating a unique target for therapeutic strategies in cancer. DNMT and HDAC inhibitors can reactivate epigenetically silenced tumour suppressor genes and decrease tumour cell growth *in vitro* and *in vivo* [3]. For example, overexpression of HDAC1, 2, 5, 7 and 8, and an increased expression of DNMT1, 3A and 3B, have been reported in various solid tumours, including lung, gastric and breast cancers, compared with normal tissue [3]. These epigenetic abnormalities provide the basis for the development of chromatin modifying compounds as anticancer therapeutics. However, despite numerous investigational chromatin modifying agents currently in preclinical or clinical trials, only four epigenetic-based drugs have been approved by the FDA to date. These are the DNMT inhibitors (DNMTIs) 5-azacytidine (Vidaza®) and 5-aza-2′-deoxycytidine (Decitabine®); and the HDAC inhibitors (HDACIs) suberoylanilide hydroxamic acid (SAHA; vorinostat; Zolinza®) and romidepsin (depsipeptide; Istodax®) [1]. Here, we provide an overview of the FDA-approved epigenome-targeting compounds and other novel chromatin modifying agents (DNMTIs and HDACIs) as antineoplastic agents (Fig. 1). In addition, the controversies surrounding these types of therapies and potential future directions in the field will be addressed.

DNA methyltransferase inhibitors

The inactivation of DNMT activity reduces DNA methylation and has been shown to correct the abnormal hypermethylation of selected genes, such as tumour suppressor genes including p53, RIZ1 and glutathione-*S*-transferase π, in malignant cells; thereby restoring the activation of the

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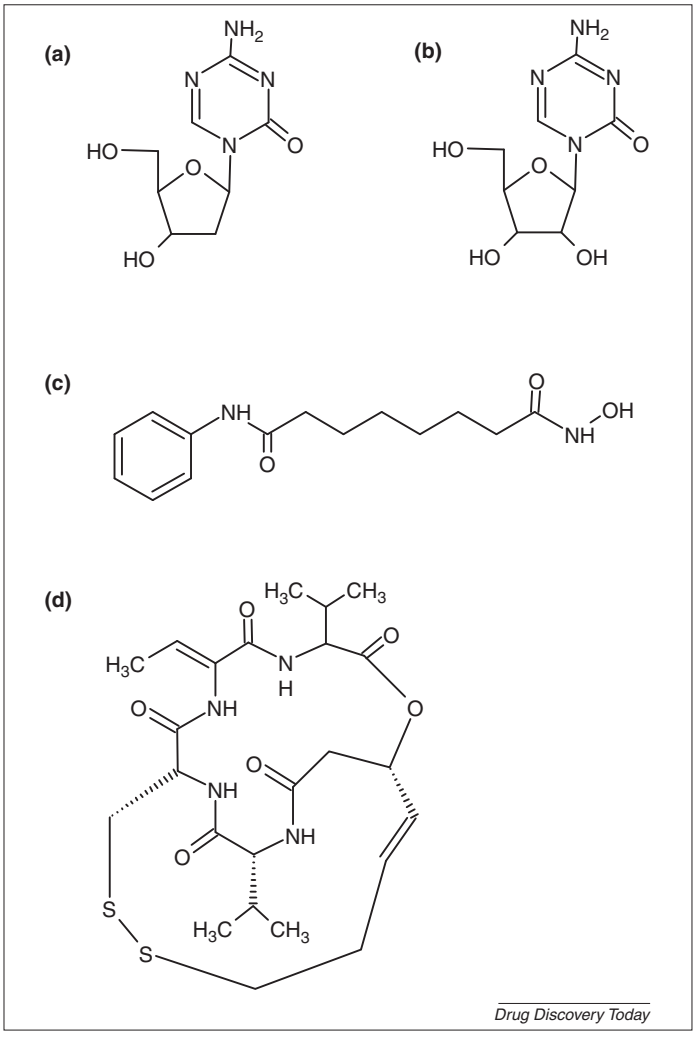

FIGURE 1

Chemical structures of FDA-approved epigenome-targeting compounds. **(a)** Vidaza® (5-azacytidine); **(b)** Decitabine® (5-aza-2′-deoxycytidine); **(c)** Zolinza® (suberoylanilide hydroxamic acid; SAHA); **(d)** Istodax® (romidepsin).

genes [4]. Further, by inhibiting DNMTs, reactivation of genes encoding immunological molecules such as human leukocyte antigen (HLA) class I molecules has also been shown to restore immunorecognition of tumour cells by cytotoxic T lymphocytes, leading to immune-mediated tumour destruction [5]. Given these beneficial effects, the development of DNMTIs has been actively pursued.

In the presence of methyltransferases DNMTIs are incorporated into nucleic acids [6]. Formation of stable complexes between the inhibitors and DNA methyltransferases results in reduction of DNA methylation by the saturation of the cell methylation machinery [1]. Advantages of DNMTIs include effective antitumour activity as single drug therapies in haematological malignancies as well as enhanced cell-killing effects when used in combination with chemotherapeutic agents and HDACIs. Importantly, DNMTIs can cross the blood–brain barrier – allowing treatment of brain malignancies [7]. Additionally, DNMTIs have the capacity to impede metastasis by decreasing blood vessel formation and reactivating growth inhibition genes [8].

FDA-approved DNMT inhibitors

The most well-characterized and clinically relevant DNMTIs are 5-azacytidine (azacytidine) and 5-aza-2′-deoxycytidine (decitabine) (Table 1).

In 2004, azacytidine was approved by the FDA for the treatment of myelodysplastic syndrome. The compound is metabolized intracellularly to 5-azacytidine monophosphate by a reaction catalysed by uridine–cytidine kinase. The compound is further converted to the diphosphate by cytosine monophosphate kinase, and to the triphosphate by diphosphate kinase [9]. The drug is incorporated into RNA and causes disassembly of polyribosomes, disrupting tRNA methylation, an effect that has been implicated in the regulation of tRNA folding and stability [10]. This results in the inhibition of protein synthesis and interrupts the nucleic acid metabolism in the cytoplasm and nucleus. Further, 5-azacytidine can undergo reduction to 5-aza-2′-deoxycytidine diphosphate – a reaction catalysed by ribonucleotide reductase. In this form, the drug is incorporated into DNA, inhibiting synthesis [1]. In one randomized trial azacytidine improved survival, response rates and quality of life while reducing the risk of leukaemia transformation in patients with myelodysplastic syndrome [11]. Several combinations involving azacytidine and conventional chemotherapeutics or HDACIs resulted in improved response (14–82%) and complete remission (3–55%) rates in various types of cancers. However, dose-limiting toxicities such as neutropenia and thrombocytopenia have been observed [12].

The DNMTI 5-aza-2′-deoxycytidine was approved by the FDA for the treatment of myelodysplastic syndrome in 2006, and has been shown to reactivate several hundred genes that are typically downregulated in cisplatin-resistant cells, suggesting that DNMTIs could ameliorate chemoresistance [13]. The long-term stability of demethylation and subsequent reactivation of tumour suppressor genes following exposure to 5-aza-2′-deoxycytidine in human breast cancer cells support its use to treat solid tumours [14]. In addition, this DNMTI has been found to increase the susceptibility of breast cancer cells to anticancer drugs such as paclitaxel, adriamycin and 5-fluorouracil [15]. Similarly, co-administration of 5-aza-2′-deoxycytidine and the patented HDACI MGCD0103 (Methylgene™) led to an enhanced cytotoxic effect in small cell lung cancer cells.

TABLE 1

Pharmacological inhibitors of DNA methylation

DNMT inhibitors
5-Azacytidine[a]
5-Aza-2′-deoxycytidine[a]
DHAC (dihydro-5-azacytidine)
Epigallocate, chi-3-gallate (EGCG)
Fazarabine (1-β-D-arabinofuranosyl-5-azacytidine)
MG98
Procainamide
Procaine
Psammaplins
Zebularine

[a] These DNMT inhibitors have been clinically approved by the FDA.

Interestingly, the loss of small cell lung cancer cell viability was not correlated with the inhibition of DNMT1 caused by 5-aza-2′-deoxycytidine, suggesting that synergistic interaction between 5-aza-2′-deoxycytidine and MGCD0103 might have been caused by possible off-target effects of these inhibitors (i.e. biological effects mediated through the inhibition of these chromatin remodelling enzymes but not attributed to stable chromatin modifications) [16].

Potential DNMT inhibitors

Despite the success of DNMTIs, the use of the current FDA-approved compounds is limited by toxicity caused by non-specific targeting of normal cells. Also because relatively high doses are required for DNMT inhibition; rapid clearance, stability, efficacy and the requirement for intravenous administration are also issues [12]. Furthermore, these DNMTIs induce global DNA hypomethylation which, in certain cases, can trigger the activation of certain pro-metastatic genes, such as urokinase plasminogen activator, in malignant cells [17]. Therefore, the development of other DNMTIs that are not only more specific, stable and effective but also less toxic is necessary.

A recently developed nucleoside analogue, 5-fluoro-2′-deoxycytidine (FdCyd) has been tested in clinical trials. However, the DNMT-inhibitory properties of this analogue were limited by rapid conversion to inactive metabolites by cytidine deaminase *in vivo* [18]. Fortunately, simultaneous administration of FdCyd and the cytidine deaminase inhibitor tetrahydrouridine prevents rapid metabolism of FdCyd leading to fewer side effects and greater efficacy [18].

The most recent addition to the family of DNMTIs is a cytidine without the 4-amino group: 1-(β-D-ribofuranosyl)-2(1H)-pyrimidinone, also known as zebularine [19]. Zebularine inhibits DNA methylation, reactivates silenced genes, potentiates the sensitivity of cancer cells to chemo- and radiotherapy, and enhances antiproliferative effects in combination with HDACIs [20]. Advantages of this DNMTI over FDA-approved DNMTIs include high stability and a long half-life. This allows oral administration and results in prolonged periods of demethylation. A disadvantage of zebularine is that a higher dose is needed to achieve a similar level of demethylation to that induced by 5-aza-2′-deoxycytidine. This is possibly caused by the lack of a permanent covalent bond with DNMTs [19]. Additionally, combination therapies involving zebularine will need to be further scrutinized because it was shown to antagonize the apoptotic effect of 5-fluorouracil in human oral squamous cell carcinoma [21].

Finally, S110, a derivative of 5-aza-2′-deoxycytidine containing deoxyguanosine, has been reported to have DNMT-inhibitory effects and is less susceptible to deamination by cytidine deaminase. In addition to being more stable than 5-aza-2′-deoxycytidine, S110 is well tolerated in mice, adding to the potential of this agent as an anticancer DNMTI [4].

Histone deacetylase inhibitors

Inhibitors of HDAC activity are classified as a group of novel chromatin modifying agents. Common examples of HDACIs include Zolinza®, Istodax®, valproic acid and trichostatin A (Table 2) [22]. HDACIs generally contain a polar terminal moiety, which binds a zinc ion of the HDAC catalytic pocket. The ability of HDACIs to interfere with chromatin remodelling processes results in the accumulation of hyperacetylated histones. This, in turn, inhibits transcriptional activation of many genes; some of which are involved in the cell cycle (e.g. p21), apoptosis (e.g. Bcl-2 survivin), angiogenesis (e.g. fibroblast growth factor) and metastasis (e.g. tissue inhibitor of metalloproteinases-1) [22]. An important aspect with respect to the cytotoxic effects of HDACIs is that they are much more pronounced in malignant cells compared with normal cells – by a factor of 10 or more [23].

FDA-approved HDAC inhibitors

Two HDAC inhibitors (Zolinza® and Istodax®) have received FDA approval for use in the clinic; for the treatment of cutaneous T-cell lymphoma (CTCL), in 2006 and 2009, respectively.

Zolinza® increases expression of death receptor 5, tumour necrosis factor, p21 and p27, and subsequent cell cycle arrest and apoptosis in malignant cells [1]. Additionally, Zolinza® was found to increase the Bax:Bcl-2 ratio in melanoma cells but upregulated Bax and did not change levels of Bcl-2 in CTCL cells, which resulted in less pronounced cell death in these cells [24]. This suggests that a single HDACI might not have universal effects across all malignancies, highlighting the issue of selective antitumour activity by HDACIs.

Istodax®, a natural product obtained from *Chromobacterium violaceum*, has been reported to show antiproliferative and apoptotic effects in various malignancies including small cell lung cancer cells via the activation of caspase 3 and 9 and the downregulation of Bcl-2 and Bcl-xl [25].

TABLE 2

Inhibitors of histone deacetylases

Group	HDAC inhibitors
Hydroxamic acid-derived compounds	Azelaic bis-hydroxyamic acid (ABHA) M-carboxycinnamic acid bis-hydroxymide (CBHA) LBH589 NVPLAQ824 Oxamftlain PXD101 Pyroxamide Scriptaid Suberoylanilide hydroxamic acid (SAHA)[a] Trichostatin A (TSA)
Short-chain fatty acids	AN9 (Pivanex) Phenyl acetate (PA) Phenyl butyrate (PB) Sodium butyrate (SB) Valproic acid (VA)
Cyclic tetrapeptides	Apicidine CHAPS Chlamydocin Depsipeptide (Romidepsin, FK228, FR901228)[a] Depudesin HC-toxin Trapoxin
Ketones	Trifluoromethyl ketone α-Ketomides
Synthetic benzamide derivatives	CI994 (*N*-acetyldinaline)
Synthetic benzamide derivatives	MS275

[a] These HDAC inhibitors have been clinically approved by the FDA.

Istodax® inhibits all known zinc-dependent HDACs.

The capacity to trigger the intrinsic and extrinsic apoptotic pathways enables HDACIs to lower the apoptotic threshold and ultimately render malignant cells more susceptible to cytotoxic agents during combination therapy [22]. This enables either the use of HDACIs alone or in combination with conventional chemotherapeutic drugs for the potential treatment of chemoresistant cancer cells. However, not all concurrent administrations of drug combinations involving HDACIs enhance antitumour activity. For example, Zolinza® and the antileukaemic DNA-damaging drug cytarabine have been reported to act antagonistically [25]. Specifically, the G_1/G_2 cell cycle arrest caused by Zolinza® reduced the availability of S phase cells for cytarabine, thereby limiting the DNA-damaging effect [25]. Interestingly, when Zolinza® was administered before cytarabine (with a Zolinza®-free interval) in an acute leukaemia setting cytotoxic synergism became apparent [25]. The effects of combination therapy of HDACIs with radiotherapy or chemotherapeutics in normal tissues have not been widely investigated. Recent findings have indicated that pre-treatment of cardiomyocytes with trichostatin A potentiates the hypertrophic and toxic effects of the anthracycline doxorubicin in these cells. Therefore, evaluation of the effects of combination therapies in normal tissues is paramount [26].

Potential HDAC inhibitors

Second-generation HDACIs that utilize alternative zinc-binding groups (e.g. sulfamates) have also been designed [1]. One example is benzamide which lacks the metabolic lability, and problems associated with hydroxamic acids as a result of Phase II conjugation reactions during drug metabolism which may produce inactive drug metabolites [1]. The benzamides SNDX275 (Syndax™) and MGCD0103 (Methylgene™) are in Phase I/II clinical trials for non-small-cell lung cancer and Phase I trials for leukaemia and solid tumours [27]. However, MGCD0103 was found to cause pericarditis and pericardial effusion in some patients. Hence, although second-generation benzamide agents with low IC_{50} values might improve antitumour potency, it is imperative that the safety profile of these drugs is well-characterized. Recently, bifunctional HDACIs that are synthesized by the hybridization of a chemotherapeutic drug (e.g. a kinase inhibitor) to a HDACI scaffold are of particular interest. An example is CUDC101, which consists of an erlotinib quinazoline scaffold. This bifunctional HDACI has been shown to have greater tumour-specific inhibitory effects compared with HDACI alone and is currently being tested in Phase I clinical trials in patients with breast, stomach, liver and head and neck cancers [28].

Controversies surrounding DNMT and HDAC inhibitors

Although current FDA-approved and emerging DNMTIs and HDACIs have shown promise as antitumour therapeutics, success in clinical trials has been limited to haematological malignancies with inconsistent efficacy against solid tumours [24,29]. The reduced efficacy of chromatin modifying agents in solid tumours compared with haematological malignancies could be because only actively proliferating cells permit the incorporation of these drugs into the DNA [29]. As mentioned earlier, a concern with the use of DNMTIs is drug-induced global hypomethylation which can promote cancer progression [2]. Similarly, HDACIs have been reported to hyperacetylate non-histone cytoplasmic proteins, including heat shock protein 90 and β-catenin, that are important in cell growth, apoptosis and cell migration [22]. This could lead to the functional inactivation of these proteins which would interfere with HDACI-induced anticancer effects. Generally, chromatin modifying agents are well tolerated and HDACIs show little toxicity to normal cells. However, it is thought that the lack of tumour specificity of epigenetic drugs is the reason for the poor efficacy observed in a majority of cancers [30]. Despite these limitations, the fact that most HDACIs and DNMTIs can trigger various pathways independently of p53 is a major advantage, particularly in cancer patients who have a non-functional p53 and might not be sensitive to conventional chemotherapeutic drugs.

Despite significant evidence supporting the synergistic antitumour effects of various drug combinations, DNMTIs and/or HDACIs have been shown to interact antagonistically with certain types of chemotherapeutic drugs. Hence, it is vital that all drug combinations involving these chromatin modifying agents are scrutinized carefully in preclinical trials to avoid possible antagonistic interactions that would negate the potential benefits of any combinatorial strategy. Further, as discussed above, the effects of combination therapies on normal tissue toxicity should be systematically investigated.

Concluding remarks

Chromatin modifying agents have emerged as a new generation of anticancer therapeutic with clinical success, particularly in haematological malignancies. Inhibitors of DNMT and HDAC enzymes have been the primary focus of epigenetic-based treatments for various malignancies. It is expected that these compounds will be most effective when used in combination with conventional chemotherapy and radiotherapy. In this context, an important issue is the appropriate selection of optimal drug combinations to avoid any suboptimal clinical outcomes and 'off-target' toxicities. Finally, with respect to appropriate targets, it is anticipated that the rapid advances from whole genome massive parallel sequencing will help identify additional aberrant epigenetic processes in malignant cells and aid further drug development.

Conflicts of interest

The authors of this Feature article have no conflicts of interest to declare.

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