

Cancer Epigenetics: Mechanisms and Crosstalk of a HDAC Inhibitor, Vorinostat

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Abstract

In recent years, histone deacetylase inhibitors (HDACis), a novel class of agents that targets mechanistic abnormalities in cancers, have shown promising anti-cancer activity in both hematological and solid cancers. Among them, vorinostat was approved by FDA to treat cutaneous T-cell lymphoma and is being evaluated in other cancer types. Although initially designed to target histone deacetylase, vorinostat were found to have additional effects on other epigenetic machineries, for example acetylation of non-HDAC, methylation and microRNA (miRNA) expression. In this review, we examined all known mechanisms of action for vorinostat. We also summarized the current findings on the 'crosstalk' between different epigenetic machineries. These findings suggest that improved understanding of epigenetic regulatory role of vorinostat and/or other HDACis will provide novel insights in improving utilization of this class of novel agents.

Keywords: Vorinostat; Epigenetic modifications; HDACi; Acetylation; Methylation; MicroRNA expression

Introduction

Epigenetic modifications of DNA-template processes have been a rising field of study for cancer therapy in the past 15 years. Epigenetic modifications refer to the reversible changes in the genome of cells that do not involve any alteration in the DNA sequence [1]. As promising preclinical and clinical results support the potential role of drugs targeting epigenetics in cancer therapy, the field of cancer epigenetics is gaining heightened attention. Increasing evidence suggests the epigenetics is as important as that of genetic mutations, deletions, rearrangements, and gene amplifications in cancer initiation, progression, treatment and prognosis.

To date, there are three major epigenetic machineries that are known to be related to cancers: altered histone acetylations, modified DNA methylations, and misregulated microRNA (miRNA) expression.

Cancer Epigenetics Overview

Histone modifications

Histone acetylation and deacetylation are essential parts of gene regulation. The lysine residues in the N terminus and the surface of the nucleosome core (consists of 4 histone and DNA) are either acetylated or deacetylated during gene transcription [2]. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the important families of catalytic enzymes that regulate transcription [3]. HATs acetylate the ϵ -amino group of lysine (K) residues on histones to neutralize the positive charge and create loosely coiled chromatin conformation to allow transcription. HDACs, on the other hand, remove the acetyl group from the histones, creating a tightly coiled, non-permissive chromatin conformation that in turn represses the transcription of genes [4,5]. Depending on the target gene, chromatin structure alterations by both histone hyperacetylation and hypoacetylation can either propagate or suppress cancer progression. Acetylation of proteins by HATs and HDACs is one of the most widely studied post-translational modifications as it is critical for the dynamic regulation of gene transcription processes including cellular proliferation, differentiation, and apoptosis [6]. To date, many histone and non-histone proteins have been identified as acetylation targets

with direct involvement in tumorigenesis, contributing to the growing effort to control their regulations [6].

DNA methylation

DNA methylation is another important regulator of gene transcription as alterations in DNA methylations are often found in a variety of cancer cells [7]. When compared to normal cells, carcinoma cells tend to have significant disruptions in the DNA methylation patterns. DNA methylation, a covalent chemical modification, refers to an addition of a methyl group at the carbon 5 position of the cytosine ring, involving mostly CpG dinucleotides [8]. The human genome has both methylated and unmethylated segments, and the areas called CpG islands designate small regions rich of CpG sites [8]. Both regionally hypermethylated CpG islands and globally hypomethylated genome can be the cause of oncogenesis, disrupting the genomic landscape of cancer cells [7]. Numerous genes, especially those involved in cell cycle regulation, DNA repair, apoptosis, differentiation, angiogenesis, and metastasis, have been reported to undergo hypermethylation in tumor cells [9]. Hypermethylation prevents transcription of the promoter regions of tumor suppressor genes, leading to gene silencing due to loss-of-function. While hypermethylation of certain genes—*RASSF1A* and *p16*, for example—are commonly observed in a variety of cancers, cancer-specific patterns of gene hypermethylation are also common [8]. DNA methyltransferases (DNMTs) is a group of enzymes that facilitates the regulation of DNA methylation. Global hypomethylation in cancer cells, on the other hand, is commonly found in intergenic regions that normally consist of methyl-cytosine content of a cell. Global hypomethylation can lead to activation of transposable elements, contributing to the genomic instability in cancer cells [10].

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miRNA expression

While the underlying mechanisms of miRNA expression regulation are not fully understood yet, altered miRNA expressions are often found in tumorigenesis and metastasis. miRNAs are short, noncoding RNA molecules that regulate gene expression at the posttranscriptional level [11]. Under normal conditions, miRNAs bind to the 3'-UTRs of the target mRNAs, which result in gene silencing via degradation of the target mRNAs or translation repression [12]. miRNA expressions can be either down-regulated or up-regulated in tumor cells. Upon down-regulation, tumor suppressor miRNAs that target growth-inducing genes are affected [13]. Upon up-regulation, on the other hand, oncogenic miRNAs that target growth-inhibiting genes are affected [14]. Interestingly, it has been reported that miRNAs can regulate genes that encode epigenetic regulators such as DNMTs and HDACs [15]. The implication leads to the possibility of epigenetic modifiers, such as DNMTs and HDACs, to indirectly affect posttranscriptional repression of target genes.

Disruptions in specific miRNA expressions are promising epigenetic markers for cancer diagnosis as miRNAs regulate multiple genes that are involved in modulation of cell proliferation and differentiation. miRNAs play an important role in various biological processes that affect tumorigenesis, including migration, invasion, epithelial to mesenchymal transition (EMT), and metastasis [16]. While the precise mechanism by which miRNAs regulate carcinogenesis remains to be fully elucidated, it is known that approximately 50% of the human miRNAs can be found at fragile genomic regions involved in cancer [17]. This implicates that the aberrant miRNA expressions may be extensively related to tumorigenesis.

Overall, the growing evidence on epigenetic alterations in cancer has instigated the development of novel anti-cancer agents that focus on targeting these cancer abnormalities. We will focus on a promising class of epigenetic anti-cancer therapy agent, HDACs, in this review.

Histone deacetylase inhibitors

HDACi was originally designed to target and inhibit HDACs. They have a similar net effect to that of HATs, facilitating open chromatin structures that enhance transcription levels. During the past 15 years, a number of different HDACs have shown effective antitumor activities *in vitro* [18]. With an implication for neoplastic cells selectivity in preclinical studies, the study of HDACs have spurred the development of additional compounds that have entered phase I trials in various malignancies [19]. There are two FDA approved HDACs, including vorinostat (suberoylanilide hydroxamic acid; SAHA) in 2006 and romidepsin (depsipeptide) in 2009 [20]. These agents have shown efficacy against cutaneous T cell lymphoma (CTCL) with response rates of over 30% in patients [21]. Furthermore, several other HDACs are currently under clinical evaluations [22].

While the main function of HDACs is the restoration of misregulated histone acetylation, HDACs are also known to alter non-histone protein acetylations, DNA methylations, and miRNA expressions. The crosstalk among these different paths of epigenetic regulations is important, as effective and selective strategies for anticancer therapies are pivotal in combating cancer in the most successful manner. A better understanding of the crosstalk among different epigenetic modifications may lead to an advanced approach, entailing further reductions in side effects and the proper dosage of the drugs used. Therefore in this review, we take a concerted effort to examine the pharmacological mechanisms of an HDACi-vorinostat,

in particular-in relation to different epigenetic modifications and their crosstalk.

Vorinostat Mechanism of Action in Cancers

Acetylation

Histone acetylation, highly regulated by HATs and HDACs activities, is one of the major regulation mechanisms for gene transcription. In addition to histone acetylation, class I and II HDACs, in particular, have abundant non-histone protein targets that also aid in epigenetic regulation of cancer [23]. While hyperacetylation on proto-oncogenes can increase their expression and develop into oncogenes, hypoacetylation on tumor suppressor genes can repress their expression levels [24]. HDACi plays a major role in modulating such misregulations as it can induce not only differentiation, cell cycle arrest, and apoptosis in tumor cells, but also re-expression of tumor suppressor genes [20]. Details about vorinostat's effect on epigenetic regulation in various cancer types can be found in Table 1.

Vorinostat has proven its efficacy in restoring misregulated acetylations in various cancers, including both solid and hematological malignancies. Among these, CTCL-as FDA approved indication for vorinostat-is one of the most sensitive cancers for vorinostat [51]. Till date, the exact mechanisms in which vorinostat demonstrates the most effective clinical response against CTCL among other tumors have not been fully understood yet. However, it is known that one of the main causes of CTCL is the aberrant, or lack of acetylated histones, HDAC activity [23]. When CTCL cell lines were treated with vorinostat, an accumulation of acetylated histones (H2B, H3, and H4) was detected [52]. Furthermore, vorinostat treatment at concentrations inducing apoptosis was accompanied by this accumulation of acetylated histones in CTCL cell lines [52]. Treatment with vorinostat at concentrations of 1-5 μ M for 48 hours on CTCL patients demonstrated an increased apoptosis level as well, representing vorinostat as a powerful therapeutic agent for the treatment of CTCL [52]. In addition, the positive correlation between the concentration-dependent growth inhibitions of relapsed lymphoma cells and the level of acetylated H3 and H4 proteins further supports the effective antitumor activities induced by vorinostat [53].

Besides acetylating histones as a therapeutic target, vorinostat also acetylates non-histone proteins to induce chemotherapeutic activities. Vorinostat targets a variety of non-histone proteins that are part of the transcription factor complexes [23]. These include proteins that regulate cell proliferation (e.g., p53), protein stability (e.g., Hsp90), apoptosis (e.g., Bcl-2 family of proteins), cell motility (e.g. α -tubulin), and angiogenesis (e.g., HIF-1 α) [23]. While the mechanisms underlying the anticancer effects of vorinostat has not yet been fully uncovered, the ability of vorinostat targeting non-histone proteins has been proven promising in many different cancers. In a human xenograft mice model of epidermoid squamous cell carcinoma, for example, an increased acetylation of p53 at both lysine 379 and lysine 286 sites upon vorinostat treatment was reported, along with an increase in histone H3 acetylation [46]. Thus, the acetylation of a non-histone tumor suppressor protein, p53, upon vorinostat treatment resulted in the heightened activation of p53, contributing to the blocking of the pathogenesis. By inducing cell cycle arrest, inhibition of proliferation, and apoptosis, vorinostat acts as a potent chemotherapeutic agent against epidermoid squamous cell carcinomas. While the comprehensive machineries of vorinostat still have areas to be unveiled, the multiple targets of the acetylation of vorinostat may account for the efficacy of vorinostat as a potent chemotherapeutic drug.

Regulations	Cancer Type	Target Gene/miRNA	Direction of effect	Cells	Animals	Patients	Reference
Acetylation	CTCL	<i>H2A, H2B, H3, H4</i>	Up-regulation	✓	✓	✓	[25]
	MCL	<i>BIM, BMF, NOXA</i> promoters	Up-regulation	✓	✓	✓	[26]
	DLBCL	<i>Ku70, Ku86</i>	Up-regulation	✓	✓	✓	[27]
	HL	n/a	n/a	✓	✓	✓	[28]
	NHL	n/a	n/a	✓		✓	[29]
	NSCLC	<i>H3, BIM</i>	Up-regulation	✓	✓	✓	[30,31]
	Breast cancer	<i>H3, H4, hsp90, MTRF1L</i>	Up-regulation	✓	✓	✓	[32-36]
	Colorectal cancer	<i>H3, p21</i>	Up-regulation	✓	✓	✓	[37]
	Prostate cancer	<i>H3K9</i>	Up-regulation	✓	✓	✓	[36,38-40]
	Epithelial Ovarian cancer	<i>tublin</i>	Up-regulation	✓			[41]
	GBM	<i>H3, H4, H2B, H3K9</i>	Up-regulation	✓	✓	✓	[42,43]
	Head and Neck Squamous carcinoma	<i>H3, H4</i>	Up-regulation	✓	✓	✓	[44,45]
Epidermoid Squamous carcinoma	<i>H3, p53</i>	Up-regulation	✓	✓		[46]	
Methylation	MM and Leukemia	<i>c-myc</i>	Up-regulation	✓			[47]
	NSCLC	<i>DNMT1, DNMT3b</i> (of hTERT promoter)	Down-regulation	✓			[48]
miRNA expression	Colorectal cancer	hsa-miR-7-1, hsa-miR-9, (28 more miRs)	Up/Down-regulation	✓			[12]
	NSCLC	hsa-miR-345, hsa-miR-593, (62 more miRs)	Up/Down-regulation	✓			[49]
	Pancreatic cancer	miR-34a (p53)	Up-regulation	✓			[16]
	Osteosarcoma	miRNAs at 14q32 locus	Down-regulation	✓			[50]

CTCL=cutaneous T-cell lymphoma, MCL=mantle cell lymphoma, DLBCL=diffuse large B-cell lymphoma, HL=hodgkin's lymphoma, NHL=non-hodgkin's lymphoma, NSCLC=non-small cell lung cancer, GBM=glioblastoma multiforme, MM=multiple myeloma.

Table 1: Vorinostat effect on epigenetic regulations.

Methylation

A disruption in DNA methylation patterns is another epigenetic modification often detected in various types of carcinogenesis, including both hematologic and solid tumors. Some of the target genes for hypermethylation in cancer cells include genes that play a role in DNA repair (e.g., *BRCA1*), cell proliferation (*p15*), apoptosis (*DAPK*), and metastasis (*E-cadherin*) [8]. Although there are far more reports of hypermethylation in cancer than those of hypomethylation in cancer, hypomethylation contributes to tumorigenesis in a wide variety of cancers as well. Global hypomethylation induces oncogenesis by stimulating the activation of oncogenes (e.g., *cMYC*) [8].

Studies that focus on the aberrant methylation patterns in various cancers, ranging from hematologic cancers to solid tumors, have demonstrated the effective anticancer potency of vorinostat. Vorinostat has currently been proven to restore altered methylation patterns as therapeutic means in chronic myeloid leukemia and non-small cell lung cancer (NSCLC) [3,48]. Among the studies conducted on various cancers, the studies on lung cancer demonstrate one of the most effective usages of vorinostat as an anticancer agent controlling the methylation pattern. In the study conducted on NSCLC cells, for example, vorinostat was proven to successfully repress the telomerase activity via epigenetic regulation of methylation patterns [48]. The human telomerase reverse transcriptase (hTERT) is known to provide major contributions to the immortality of cancer cells by protecting the lengths of the ends of chromosomes [48]. Upon treatment with vorinostat, hTERT expression was drastically reduced, as vorinostat induced demethylation of CpGs on the hTERT promoter by down-regulating DNMT1 and DNMT3b [48]. By reducing the binding

activities of DNMTs, vorinostat down-regulated DNMTs, leading to the consequent inhibition of the hTERT transcription controlled by methylation. On a similar note, in prostate cancer, the expression of a tumor suppressor gene named “deleted in liver cancer” (*DLC1*), was efficiently increased upon vorinostat treatment. In primary prostate carcinomas (PCAs), *DLC1* is recurrently down-regulated [54]. However, vorinostat treatment resulted in an increased level of methylation in the promoter region of *DLC1*, inducing the activation of the *DLC1* [54].

These two major studies strongly suggest the efficient anticancer ability of vorinostat to induce both the down-regulation of genes that contribute to oncogenesis and the up-regulation of tumor suppressor genes via methylation regulation.

miRNA Expression

Vorinostat has proven its efficacy in regulating aberrant miRNA expressions in a number of different cancers, including pancreatic cancer stem cells (CSCs), NSCLC, colon carcinoma cells, and osteosarcoma cell lines [17,50,52,55]. A detailed example of the effective usage of vorinostat in regulating miRNA expression for treatment of cancer is found in the study on pancreatic CSCs. In pancreatic cancer, miR-34a, a transcriptional target of *p53*, is often down-regulated [17]. However, upon treatment with vorinostat, miR-34a in human pancreatic CSCs and in human pancreatic cancer cell lines was re-expressed [17]. Consequent, this resulted in the suppressed tumorigenesis via inhibition of the cell proliferation, cell cycle progression, self-renewal, EMT, and invasion [17]. The study further validated vorinostat's anticancer regulation of stem cell characteristics

via miRNA modulations by inhibiting miR-34a using antagomiR [17]. The inhibition led to the subsequent abrogation of the anticancer effects of vorinostat [17]. Therefore, the restoration of the altered miRNA expression via vorinostat provides a novel mechanistic insight for improving existing chemotherapies.

Vorinostat was also observed to take a part in modulating miRNA expressions in NSCLC cells. In the study conducted, vorinostat was found to significantly influence the expression of certain miRNAs, up to 2-fold [50]. These include miRNAs with target genes involved in angiogenesis, apoptosis, chromatin modification, cell proliferation and differentiation [50]. Therefore, the discovery that vorinostat has the ability to regulate miRNAs related to cell cycle regulations suggests the potential of vorinostat in modulating such regulatory factors to treat cancer pathogenesis.

Crosstalk between epigenetic regulations

Aberrant modifications in acetylation, methylation, and miRNA expressions are important epigenetic regulations that provide significant implications on cancer-specific therapeutic targets. While each of these epigenetic misregulations is complex, accumulating evidence indicates that there exist crosstalk links among these epigenetic mechanisms. Although it is not yet clearly understood how each epigenetic regulation may affect one another and which genes are the major players in tumor formation, clarifying the precise mechanism by which they interact has a potential to elucidate the development of the most effective cancer therapies possible.

A crosstalk between acetylation and miRNA upon vorinostat treatment was identified in pancreatic cancer. As mentioned above, in pancreatic cancer, miR-34a, which acts as a tumor suppressor in tumorigenesis, is down-regulated [17]. Upon vorinostat treatment, however, miR-34a is re-expressed, inducing apoptosis by activating caspase-3/7 [17]. Besides, the up-regulation of miR-34a after vorinostat treatment consequently induced the acetylation of *p53*, a tumor suppressor gene, and its transcriptional targets *p21^{WAF1}* and *PUMA* [17]. In this case, the restoration of miRNA expression levels contributes in the stabilization of *p53* via acetylation, leading to increased transcriptional activity of *p53* and the consequent promotion of apoptosis. This result strongly suggests novel prospects for clinical innovations that focus on the restoration of miR-34a by pharmacological intervention. As shown, the two epigenetic regulations of miRNA expression and non-histone protein acetylation are coordinated in an interdependent manner in pancreatic cancer [17]. This study provides possible directions for future experiments that focus on the relationship among different epigenetic regulations in cancers. A further understanding of the relationship will provide an insight on the significance of this crosstalk and lay the groundwork for novel experiments to be conducted.

Recently, the focus on the crosstalk between different epigenetic regulations has unveiled an additional regulatory tier to add to the complexity of anticancer mechanisms. A better understanding of the crosstalk mechanism holds great promise for a further development to induce an effective synergy with standard chemotherapy agents to result in a more enhanced chemosensitivity in various cancers.

Conclusion

The epigenetic machineries have proven roles in a wide variety of cancers. Given the interplay among the machineries, the crosstalk among different epigenetic regulations has been undergoing investigation recently as well. As one of the two FDA approved HDACi, vorinostat can interact with all three epigenetic machineries directly or

indirectly. Improved understanding of the exact mechanism by which vorinostat interacts with different epigenetic systems would allow the better usage of this agent to target correct diseases.

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