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Review

Histone acetyltransferases and deacetylases: molecular and clinical implications to gastrointestinal carcinogenesis

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Histone acetyltransferases and deacetylases are two groups of enzymes whose opposing activities govern the dynamic levels of reversible acetylation on specific lysine residues of histones and many other proteins. Gastrointestinal (GI) carcinogenesis is a major cause of morbidity and mortality worldwide. In addition to genetic and environmental factors, the role of epigenetic abnormalities such as aberrant histone acetylation has been recognized to be pivotal in regulating benign tumorigenesis and eventual malignant transformation. Here we provide an overview of histone acetylation, list the major groups of histone acetyltransferases and deacetylases, and cover in relatively more details the recent studies that suggest the links of these enzymes to GI carcinogenesis. As potential novel therapeutics for GI and other cancers, histone deacetylase inhibitors are also discussed.

Keywords tumorigenesis; carcinogenesis; gastric cancer; colorectal cancer; histone acetylation; histone acetyltransferase; histone deacetylases

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Introduction

A malignant type of tumorigenesis (or neoplastic transformation), carcinogenesis, refers to the process whereby a normal cell tolerates accumulation of chromosomal aberrations and genomic aneuploidies, loses cell-cycle checkpoint control, undergoes uncontrolled proliferation and deregulated differentiation, and forms benign and eventually malignant tumors [1]. Gastrointestinal (GI) carcinogenesis causes some of the most common types of tumors worldwide. Anatomically, the main GI track comprises the esophagus, stomach, bowels, and anus, if excluding the accessory organs such as liver, bile ducts, gallbladder, and pancreas. Colorectal, gastric, and esophageal cancers were, respectively, ranked the third, fourth, and eighth most common ones based on new cases diagnosed around the world in 2008 (http://www.wcrf.org/cancer_statistics; the latest data available). It has been widely considered that the major reason for carcinogenesis is that at least one genetic lesion, such as point mutation, deletion and translocation, either activates an oncogene or inhibits the function of a tumor suppressor gene [1]. Recent research indicates, however, that in addition to genetic lesions, epigenetic changes such as DNA methylation and histone modifications play a pivotal role in tumor initiation and malignant progression along the GI track and at other cancer sites [2,3].

Epigenetics is defined as the study of heritable changes that occur independent of changes in the primary DNA sequence. Among the various epigenetic alterations that lead to altered gene expression, the most important ones are DNA methylation and histone modifications [2,4,5]. In mammals, DNA methylation occurs primarily through covalent addition of the methyl group to cytosine in CpG dinucleotides [2]. Tumor initiation and progression are accompanied by profound changes in DNA methylation patterns, the first epigenetic alterations that were identified in cancer [2]. In addition, nucleosomal histones are targets of a large number of post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation [4–6]. Among these modifications, acetylation has been extensively investigated in the past two decades or so [7]. This modification is dynamically maintained *in vivo* by the opposing histone acetyltransferase (HAT) and deacetylases (HDAC) activities (**Fig. 1**) [8]. Here we provide an overview of histone acetylation and the enzymes controlling this modification and cover

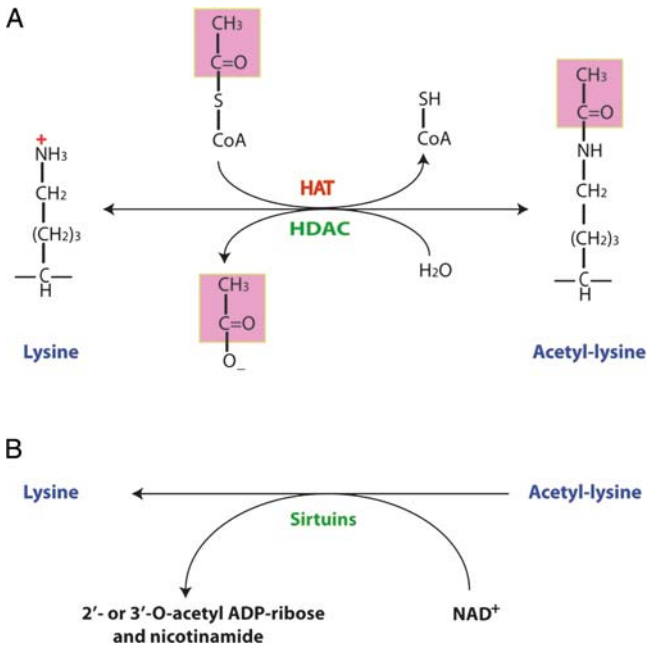

Figure 1 Cartoon illustrating reversible histone acetylation controlled by the opposing actions of acetyltransferases and deacetylases (A) HAT (red) is responsible for transferring the acetyl moiety (in pink) from acetyl-CoA to the ε-group of a lysine residue. The Rpd3/Hda1 (*h*istone *dea*cetylase 1) or classical family of HDAC (green) removes the acetyl group from acetyl-lysine, releasing acetate, and the reaction requires Zn^{2+}. (B) In contrast, in an NAD^{+}-dependent manner, sirtuins (green) utilize a completely different catalytic mechanism to remove the acetyl group from acetyl-lysine residues, releasing products that are different from acetate. In addition to acetylation, some HATs and HDACs may also act on acetylation-like modifications such as malonylation, succinylation, and crotonylation. This figure was adapted from [8] and is published with permission from Elsevier.

the links to cancer initiation and progression, with a special focus on gastric and colorectal carcinogenesis as well as on the potential use of HDAC inhibitors as single-agent therapeutics or in combination with other therapies.

Histone Acetylation as a Key Component of the Epigenetic Language

In normal or cancer cells, the genetic material DNA is not naked but forms a nucleocomplex that is well known as chromatin [9,10]. Chromatin comprises repeated units of nucleosomes, and each nucleosomal core contains 146 bp DNA wrapping around a histone octamer consisting of two copies of each of the four core histones H2A, H2B, H3, and H4. The fifth histone, histone H1, binds to the DNA linking two neighboring nucleosomal cores and is thus known as linker histone. Different from histone H1, each core histone is composed of a C-terminal globular domain (70–90 residues) and an unstructured N-terminal tail (20–30 residues) [11]. The globular domain is well structured and responsible for forming the histone octamer [11], whereas the unstructured tails contain many residues for covalent modifications, including acetylation [5]. Each N-terminal tail possesses multiple lysine residues for specific acetylation although some acetylation sites have also been found in the globular domains of histones H3 and H4. Each core histone contains different variants and acetylation occurs on many of them. To make the matter even more complicated, histone acetylation does not act alone but actively crosstalks with other histone modifications such as methylation, phosphorylation and ubiquitination, as well as with DNA methylation. Thus, histone acetylation has a broad meaning as a simple linguistic term and is an integral part of the sophisticated and newly recognized language of chromatin modifications [4,6,12].

Impact of Histone Acetylation on Transcription and Other Processes

In normal cells, differential acetylation of histones plays an important role in regulating chromatin-templated nuclear processes, such as gene expression, DNA replication, repair and recombination, thereby controlling various cellular and developmental programs [5]. The dynamic state of post-translational protein acetylation is also intimately linked to aging and to several major diseases such as cancer, retroviral pathogenesis, neurodegenerative disorders, and cardiovascular diseases [13–15]. Abnormal histone acetylation leads to cancer development through affecting many nuclear and cellular processes [5,16]. In a word, we only briefly discuss the impact on transcription and cell-cycle progression and describe the interplay of histone acetylation with DNA methylation.

Histone acetylation and transcription

As stated above, acetylation occurs on numerous lysine residues that are mainly located within the N-terminal tails of core histones, e.g. lysine 4, 9, 14, and 18 of histone H3 as well as lysine 5, 8, and 12 of histone H4 [5]. Histone acetylation modulates transcription in multiple ways. Its enzymes, acetyltransferases, and deacetylases (**Fig. 1**) can regulate transcription by modifying the acetylation state of histones or other promoter-bound transcription factors. Histone acetylation effectively reduces the positive charge of histones, and this has the potential to disrupt electrostatic interactions between histones and DNA. This presumably leads to less compact chromatin structure, thereby facilitating access of DNA to molecular machineries involved in transcriptional control. Conversely, histone deacetylation favors transcriptional repression by inducing chromatin compaction [17]. Moreover, numerous chromatin-associated factors have been shown to specifically interact with modified histones via many distinct domains, such as the bromodomain, which is often found in HATs and ATP-dependent chromatin remodeling complexes [18]. For example, the

Swi2/Snf2 chromatin-remodeling complex contains bromodomains that target it to acetylated histones, thereby recruiting the complex to 'open' up the targeted chromatin region for transcription to occur [19]. Specific recruitment of HAT and HDAC containing complexes to selected promoter elements generates localized domains of modified histones that influence transcriptional activity [20,21]. Consistent with the well-established function of HDACs as transcriptional repressors, diminished histone acetylation at promoter regions generally correlates with gene silencing, but there is also evidence that HDACs activate expression of some genes. For example, in yeast, the Hos2 (*H*da *O*ne *s*imilar 2) deacetylase is required for gene activation, and deletion of the Rpd3 (*r*educed *p*otassium *d*ependency 3) deacetylase leads to repression of transcription at telomeric loci [22–24]. The activation by HDACs could be due to reversing repressive rare histone acetylation events (such as acetylation of histone H3 at lysine 4 or histone H4 at lysine 12) or acting on acetylation of non-histone proteins [7]. Related to the latter possibility, direct acetylation and deacetylation of transcription factors and other proteins has also been shown to have positive and negative consequences on gene transcription [8,25].

Histone acetylation in cell-cycle regulation, invasion, and metastasis

Tumor is characterized by loss of cell-cycle check point control and recent studies have identified an important cross-talk between proteins involved in the cell-cycle regulatory apparatus and those regulating histone acetylation. Disruption of HAT or HDAC activity may play an important role in uncontrolled growth and proliferation of tumor cells. The first specific HDAC inhibitor, trichostain A, was in fact discovered as a result of its capacity to induce cellular differentiation and cell-cycle arrest [26]. All HDAC inhibitors studied to date, with the possible exception of tubacin, promote cell-cycle arrest at G1/S [27]. This is often associated with p53-independent induction of $p21^{WAF1/CIP1}$, which acts as a cyclin-dependent kinase inhibitor and stimulates hypophosphorylation of the retinoblastoma tumor suppressor protein [28,29]. Effects on G2/M have also been observed [16,30]. Furthermore, treatment with trichostatin A increased histone acetylation and induced the expression of many genes, which encode suppressors of invasion and metastasis as well as negative cell-cycle regulators and apoptosis-related molecules [31].

Interplay of DNA methylation and histone acetylation

Histone acetylation does not act alone but interplays with other chromatin modifications, including DNA methylation [32]. Global DNA hypomethylation plays a significant role in tumorigenesis and occurs at various genomic sequences including repetitive elements, retrotransposons, CpG poor promoters, introns, and gene deserts [33]. In contrast to hypomethylation, site-specific hypermethylation contributes to tumorigenesis by silencing expression tumor suppressor genes [2]. The link between DNA methylation and histone acetylation is mediated by a group of proteins with methyl DNA-binding activity, including KAISO, MBD1, and MeCP2 [34]. These proteins localize to methylated promoters, as shown in colorectal cancers for genes of the cell-cycle regulator p16 [35] and the multidrug resistance protein MDR1 [36], and recruit protein complexes that contain HDACs [37,38]. In addition, DNA methyltransferases may play a role in direct repression of transcription through cooperation with HDACs in late S-phase [39]. DNA methylation can provide binding sites for methyl-binding domain proteins, which can mediate gene repression through interactions with HDACs, thereby leading to gene silencing and chromatin condensation [37,40]. Interaction between the DNA methylation machinery and HDACs, therefore, enhances further the complexity of epigenetic regulation of gene expression.

Enzymes that Govern Histone Acetylation

As illustrated in **Fig. 1(A)**, HATs utilize acetyl CoA as the coenzyme and catalyze the transfer of an acetyl group to the ε-amino group of lysine side chains. Three major groups of HATs have been identified, including Gcn5 (*g*eneral *c*ontrol *n*on-derepressible 5)-related N-acetyltransferases (GNATs), E1A-associated protein of 300 kDa (p300)/CREB (*c*AMP-*r*esponsive *e*lement *b*inding protein)-binding protein (CBP), and MYST proteins [41,42]. While the GNAT and MYST families have members from yeast to humans, the p300/CBP group is unique to metazoans.

Through removal of acetyl groups from lysine resides of histone tails and non-histone proteins [**Fig. 1(B)**], HDACs oppose the activities of HATs. Four classes of HDACs have been identified. Class I consists of HDAC 1, 2, 3, and 8, which are mainly localized to the nucleus. Class II contains HDAC 4, 5, 6, 7, 9, and 10, some of which present in both the nucleus and cytoplasm for signal-dependent regulation by nucleocytoplasmic trafficking. Class III comprises seven sirtuins [*Sir*2(*tu*) like prote*ins*], SIRT1-7; some of them are mainly nuclear, whereas the others are also found in the cytoplasm. Within class IV, there is only one member, HDAC11, which is mainly nuclear; its sequence displays characteristic features of class I and II members [16].

One emerging concept is that many HATs and HDACs are part of large multisubunit protein complexes [41]. Within these complexes, non-catalytic subunits tend to regulate the specific activity and substrate specificity of catalytic subunits [7]. It is thus important to consider that these complexes as functional units and to recognize that

deregulation of these non-catalytic subunits may also be the pathological reasons for human diseases such as cancer. In the following sections, more details on HATs and HDACs, and their links to cancer are covered.

Links of HATs to General and GI Carcinogenesis

As overviewed in this section, there are various molecular and cellular links of the three families of HATs to GI and other cancers.

The GNAT family

Members of this family share several conserved sequence motifs [43]. In humans, there are two Gcn5-like proteins: PCAF (*p*300/*C*BP-*a*ssociated *f*actor) and GCN5. Both can interact with p300/CBP, form similar HAT complexes, and are involved in transcriptional regulation and cell-cycle control [44–46]. Overexpression of PCAF can lead to growth arrest. This effect may, at least in part, be explained by an unbalanced interaction of PCAF with two important cell-cycle regulators: E2F and p53. PCAF is therefore involved in two opposing scenarios: promote cell-cycle progression by activating E2F or cause cell-cycle arrest by activating p53. Mutations in regions that control the HAT activity or specificity of PCAF (and possibly other HATs of the GNAT family) are thus expected to have significant effects on cellular proliferation and tumor formation [47]. Histone acetylation by yeast Gcn5 has been implicated in displacement of promoter nucleosomes during transcriptional activation [48,49], and in aiding recruitment of RNA polymerase II, the TATA-box binding protein TBP, and other general coactivators to yeast promoter regions [50–52]. Yeast Gcn5 also increases the efficiency of trimethylation of H3 at lysine 4 in transcribed coding sequences [47].

The p300/CBP family

This family consists of two highly homologous transcriptional coactivators, both of which are widely expressed and play critical roles in cell growth, differentiation, transformation, and apoptosis [53]. CBP was originally identified as a coactivator for the transcription factor CREB [54], whereas p300 was isolated as a target of the adenoviral oncogenic protein E1A [55]. Recombinant CBP/p300 acetylates all four histones in their free forms as well as in nucleosomes, demonstrating higher efficiency and less substrate specificity than the other HATs. Apart from histones, CBP/p300 acetylates a wide variety of transcription regulatory proteins, such as the tumor suppressor p53 and others [56–58]. For example, p53 exerts anti-proliferation effects through its ability to function as a sequence-specific DNA-binding transcription factor. p53 can be modified by acetylation both *in vivo* and *in vitro*. Remarkably, the p53 sites that are acetylated by p300 reside within a C-terminal domain known to be critical for regulation of ubiquitination and stability [59].

Colorectal tumors frequently display loss of heterozygosity on chromosome 22q, suggesting that inactivation of a tumor suppressor gene(s) at this chromosomal band participates in cancer development. Neurofibromatosis 2 and p300, whose genes are located on 22q, are thought to be candidates for the tumor suppressor. Mutations of the p300 gene in 27 colorectal and two gastric carcinomas have been analyzed using PCR-SSCP, RT-PCR-SSCP, and direct sequencing methods [60]. Missense mutations of p300 gene were detected in a gastric carcinoma, and in a colorectal carcinoma, no mutation of NF2 gene was detected. The p300 mutations were somatic and coupled to deletion of the second allele of the gene, suggesting inactivation of p300 in these carcinomas. The mutations are located within the cysteine/histidine-rich regions, which are assumed to play important roles in the function of p300. These are the first cases in which the p300 gene has been found to be altered in both alleles, suggesting that inactivation of p300 may be involved in the development of carcinomas, and that this gene may be the target of loss of 22q in carcinomas of the digestive tract.

The MYST family

In humans, there are five members within this family, including MOZ (*mo*nocytic leukemia *z*inc finger protein), MORF (*MOZ*-related *f*actor), HBO1 (*H*AT *b*ound to *O*rc1), Tip60 (*T*at-*i*nteracting *p*rotein of 60 kDa), and MOF (*m*ales absent *o*n the *f*irst) [47]. The link between leukemia and MOZ has been well established [61]. Similarly, the MORF gene is also rearranged in leukemia [62]. HBO1 interacts with the human origin recognition complex [63] and has been reported to function as a transcriptional coregulator for several nuclear hormone receptors [64]. Furthermore, HBO1 is overexpressed in a specific subset of human primary cancers. Immunohistochemistry for HBO1 in 11 primary human tumor types revealed strong HBO1 expression in carcinomas of the testis, ovary, breast, stomach, esophagus, and bladder. The results are consistent with the hypothesis that the HBO1 activity is a key regulator of DNA replication and cell proliferation [65].

Tip60 was initially identified as a cellular protein that interacts with the HIV viral protein Tat [66]. The acetyltransferase activity of Tip60 has been implicated in regulating DNA repair and apoptosis [67]. Tip60 may also play an important role in regulating tumorigenesis, through modulating signaling events involving ATM following DNA damage and regulating the transcriptional activities of p53 and Myc [68]. Dependent on the context and by virtue of being an acetyltransferase and transcriptional co-activator toward other transcription factors, Tip60 may either act as

a tumor suppressor or promote oncogenesis. Further investigation of the link between Tip60 and p53 is likely to elucidate a pathway to tumor progression that may involve inhibition of p53 acetylation by Tip60. Indeed, a recent large-scale screen revealed a significant down-regulation of Tip60 expression in tissues from colon and lung carcinomas [69].

As the fifth member of the MYST family, MOF is the ortholog of *Drosophila* Mof, which is important for controlling dosage compensation in male flies by specifically acetylating Y chromosome-associated histone H4 at lysine 16 [70]. This acetyltransferase is important for DNA damage response in cultured mammalian cells [71] and is frequently repressed in breast carcinoma and medulloblastoma [72]. As with many other HATs mentioned above, very little is known about the role of MOF in GI carcinogenesis.

Links of HDACs to General and GI Carcinogenesis

Class I HDACs are often components of distinct multisubunit corepressor complexes and functional knockdown or deletion of different class I HDACs can result in diverse cellular effects [73]. For example, knockdown of HDAC2 and HDAC1 but not HDAC3 suppressed proliferation of colon carcinoma cells *in vitro* [74]. In contrast, knockdown of HDAC3 was more effective in inhibiting the growth of another set of colon carcinoma cells than knockdown of HDAC1 or HDAC2 [75]. Moreover, knockdown of HDAC3 and HDAC2 induced DNA damage and concomitant apoptosis [76]. Knockdown of HDAC4, a class II member, inhibited cell proliferation and induced apoptosis [77]. Knockdown of HDAC7 (another class II member) in endothelial cells did not affect cell growth or survival, but inhibited cell migration and the capacity to form capillary tube-like structures [78]. A further role for class II HDACs in regulating angiogenesis was suggested by the study of HDAC6 and HDAC10 as their knockdown resulted in depletion of vascular endothelial growth factor receptors [79]. Functional studies done thus far indicate that class I HDACs predominantly regulate cell proliferation and apoptosis whereas class II HDACs are more specifically involved in regulating cell differentiation, migration, and angiogenesis [73].

Individual HDACs have been linked to GI carcinogenesis. HDAC1 is overexpressed in gastric and colon cancers, whereas HDAC2 is overexpressed in colorectal tumors [75,80]. Loss of the adenomatosis polyposis coli (APC) tumor suppressor gene resulted in enhanced expression of HDAC2 via activated β-catenin/c-Myc, and specific knockdown of HDAC2 in APC-deficient colon carcinoma cells overexpressing HDAC2 resulted in robust induction of apoptosis [81]. Moreover, ectopic expression of HDAC2 antagonized APC induced apoptosis in colon carcinoma cells [81]. Truncation mutations in HDAC2 have been detected in a subset of microsatellite unstable colorectal cell lines and primary tumor samples [82]. The mutations generate loss of expression and enzymatic activity of HDAC2, and lead to decreased sensitivity to apoptosis induced by HDAC inhibitors [82]. Finally, studies using the APC^{min} colon cancer mouse model showed that HDAC2 is selectively up-regulated in normal colonic mucosa cells and is further induced in tumors from the mice; importantly, treatment of the mice with the HDAC inhibitor valproic acid significantly reduced the number and size of adenomas. Knockout of *Hdac2* gene APC^{min} mice resulted in decreased intestinal tumor development [83]. HDAC2 is also overexpressed in human gastric cancer [84]. Moderate-to-strong expression of HDAC2 was found in 44 out of a total of 71 tumors that were analyzed. Interestingly, HDAC2 expression appeared to be associated with tumor aggressiveness as elevated expression was observed in advanced gastric cancer and in positive lymph node metastasis [84]. HDAC2 expression was correlated significantly with progression of adenoma to carcinoma when cancer and non-cancer cases were compared. These results suggest that HDAC2 expression is associated with colorectal cancer progression [85].

In addition to HDACs themselves, their regulators are linked to cancer. For example, esophageal squamous cancer patients with a higher level of histone H4 acetylation had a better prognosis, and metastasis-associated protein 1 (MTA1, a regulatory subunit of an HDAC1/2 multiprotein complex) might be involved in the alteration of chromatin structure and transcription repression [86,87]. Immunostaining patterns of MTA1 and acetylated histone H4 were inversely correlated [86,87].

Infection by the bacterium *Helicobacter pylori* is a main cause of gastric cancer and is also associated with an increased risk of gastric mucosa-associated lymphoid tissue lymphoma (http://www.cancer.gov/cancertopics/factsheet/Risk/h-pylori-cancer). Acute infection by this bacterium causes gastritis. Cyclooxygenase 2 (Cox-2) is linked to inflammation, a pre-stage of tumor initiation. A recent study reported that the bacterium-induced Cox-2 expression in gastric epithelial cells decreased HDAC activity in the nucleus and reduced expression of HDAC1, 2, and 3 accordingly [88]. This study thus suggests an interesting link between *H. pylori* infection and HDAC expression. A puzzling issue is that the infection reduced HDAC activity, which would mimic HDAC inhibition by small-molecule inhibitors (this is something expected to be beneficial; see the following section about HDAC inhibitors). It is possible; however, whether HDAC inhibition is detrimental or beneficial is really context dependent.

Like classical HDACs, sirtuins play an important role in tumor onset and progression. They may regulate cellular senescence, DNA repair, chromosomal stability, and cell-cycle progression [89–92]. Consistent with this notion, overexpression of SIRT1, SIRT2, SIRT3, and SIRT7 has been documented in a range of cancers [89,90]. The possible role of SIRT1 in cancer may be context dependent. As a promoter of cell survival, SIRT1 is expected to possess an oncogenic activity. In this regard, SIRT1 deacetylates and down-regulates p53. On the other hand, as a gene promoting organismal survival and delaying aging, a tumor suppressor role is expected for SIRT1. The first suggestion that SIRT1 might be oncogenic was the finding that HIC1 (*h*ypermethylated *i*n *c*ancer 1) binds to the SIRT1 promoter and represses its activity [93]. As HIC1 is silenced in certain tumors, up-regulation of SIRT1 was proposed to stimulate tumorigenesis. However, other studies are consistent with SIRT1 having anti-proliferation and anti-apoptotic effects during cancer development. Eight different cancers (colon, lung, breast, stomach, bladder, liver, skin, and thyroid) exhibited reduced levels of SIRT1 when compared with the corresponding normal controls [94]. In addition, a transgenic mouse strain with gut-specific Sirt1 overexpressing exhibited protection when crossed with a colon cancer mouse model [95].

Therefore, as discussed in the above two sections, there are various links of HATs and HDACs to cancer development, which form the basis for modulating the activities of these enzymes for treating cancer. As detailed below, inhibitors of HDACs have emerged as novel anti-cancer therapeutics.

HDAC Inhibitors for Treating GI and Other Cancers

Action of HDAC inhibitors shifts the balance between the deacetylating activity of HDACs and the acetylating activity of HATs, in favor of increased histone acetylation and up-regulated gene expression. This is based on the assumptions that histone acetylation promotes gene activation and histones are the major substrates. Except for a few rare cases, the first assumption is true. But the second assumption does not always hold and needs to be interpreted with caution, because it is clear now that there are thousands of acetylated proteins and many of them are targets of known HATs and HDACs [8].

Within the HDAC superfamily, there are two types of enzymes, which are either $zinc^{2+}$ [class I, II, and IV; **Fig. 1**(**A**)] or NAD^{+} dependent [class IV, **Fig. 1**(**B**)] [96]. These two types are also known as the classical and sirtuin families, respectively. Most HDAC inhibitors target the classical family and such inhibitors are thus often referred to as classical HDAC inhibitors [97–100]. Here we therefore focus on this family of compounds only. Tumor cells generally show higher sensitivity to such inhibitors than normal cells [101]. Therefore, these inhibitors constitute a new exciting addition to the cancer therapy arena. Numerous such inhibitors have been identified and some of them have recently been used in clinical trials for cancer treatment [97–100]. Known classical HDAC inhibitors are classified into five major groups: short-chain fatty acids, hydroxamic acids, cyclic peptides, benzamides, and hybrid molecules [97,102].

Short-chain fatty acids

Short-chain fatty acids, such as butyrate, phenyl-butyrate, and valproic acid, have become a favorite topic in cancer research because they are thought to be produced from bacterial fermentation of dietary fiber and might protect against colon cancer [103]. An end-product of intestinal microbial fermentation of dietary fiber, butyrate is an important energy source for intestinal epithelial cells and plays a role in the maintenance of colonic homeostasis. It exerts potent effects on a variety of colonic mucosal functions such as inhibition of inflammation and carcinogenesis, reinforcing various components of the colonic defense barrier and decreasing oxidative stress [104]. Butyrate not only plays a role in oxidative stress in the healthy colonic mucosa [105], but also modulates expression of genes in response to oxidative and metabolic stress in primary human colon cells [106] and enhances the responsiveness of colon cancer cells to all-*trans* retinoic acid [107]. Strikingly, down-regulation of a butyrate transporter has been shown in human colon cancer tissue [108–110]. The down-regulation results in reduced uptake and metabolism of butyrate in colonocytes. In addition, the butyrate transporter activity was positively correlated with disease-free survival [111]. Furthermore, a lower butyrate to acetate ratio has been found in luminal samples of patients with adenomatous polyps or colon cancer versus healthy controls [112].

Related to butyrate, phenyl-butyrate is an aromatic short-chain fatty acid able to inhibit HDAC activity [113]. It was evaluated in phase I trial for patients with solid malignant tumors such as colon carcinoma, rectal hemangiopericytoma (a type of soft tissue sarcoma resulting from pericytes), and pancreatic carcinoma [114,115]. This compound was also studied in combination with 5-fluouracil in a phase I trial for patients with advanced colorectal cancer [116].

Also related to butyrate, valproic acid is a known drug used in patients for its anticonvulsant and mood-stabilizing activities. It has been studied in a phase I trial for intravenous administration in patients with advanced colorectal and esophageal cancers [117].

Hydroxamic acids

Hydroxamic acids, such as trichostatin A, suberoyl bishydroxamic acid, suberoylanilide hydroxamic acid (now known as Vorinostat), LBH589, and PXD101, display high efficacy, with nanomolar potency against class I/II HDACs [118,119]. Vorinostat has significant activity in a wide range of cancers [120]. Studies performed in colon and breast cancer cell lines showed that exposure to Vorinostat reactivated expression of a subset of genes silenced in these cells, resulting in cell growth arrest, differentiation, and apoptosis [121]. In a multicenter phase II single-agent study, 16 patients with breast, colon, and lung cancers received Vorinostat at doses of 200, 300, and 400 mg *b.i.d.* (*bis in die*, i.e. twice a day) for 14 days [122]. Disease stabilization was observed in half of the patients [122]. In a phase I trial for 16 Japanese patients with GI cancer, the dose-limiting toxicity of Vorinostat was determined for grade 4 thrombocytopenia. In these Japanese patients, 300 mg *b.i.d.* for 3 consecutive days followed by a 4-day rest each week was found to be the tolerable regimen [123]. Vorinostat 200 mg *b.i.d.* was also evaluated in a single-agent phase II study for patients who had recurrent/metastatic transitional cell carcinoma and had failed in platinum therapy [124].

Vorinostat has also been investigated in combination with other therapies. With capecitabine in a phase I trial for patients with advanced solid tumors, the two drugs were found to be well tolerated and active in several tumor types [125]. Also in a phase I study with GI carcinoma, Vorinostat was combined with pelvic radiotherapy [126,127]. The results indicated that it was safe to combine Vorinostat with radiotherapy.

PCI-24781 and PXD101 are novel hydroxamate-type HDAC inhibitor [128,129]. A phase I study was done in patients with solid tumors [130,131]. Trichostatin A inhibits growth of chemotherapy-resistant hepatoma cell *in vitro* [132]. Its anti-proliferative activity is paralleled by a comparable rate of apoptosis. Thus, trichostatin A may be a promising agent for treatment of hepatocellular carcinoma. Scriptaid, a novel HDAC inhibitor, is effective in cell-cycle arrest and growth suppression and in reversal of repressive chromatin marks at the promoter region of a hypermethylated p16 gene in colorectal cancer [133]. It may also enhance the response of human tumor cells to radiation [134].

The inhibitor panobinostat (or LBH589) achieves potent inhibition of classical HDACs implicated in cancer and displays potent anti-tumor activity in preclinical models and promising clinical efficacy in cancer patients [135]. It significantly induces necrosis, apoptosis, and arrest of tumor cell proliferation. In combination with imatinib, therapeutic effects were enhanced [136]. The therapeutic potential of combining lapatinib with panobinostat in colorectal cancer cell lines was also evaluated. It was found that the combination showed greater antitumor activity than either agent alone [137]. Thus, combinations with established therapies (chemotherapy or other targeted agents) can be expected to increase the therapeutic efficiency of the HDAC inhibitor.

Cyclic tetrapeptide

Depsipeptide (FK228 or FR901228) is a potent bicyclic depsipeptide [138]. FK228 was studied in combination with gemcitabine in a phase I trial for patients with advanced solid tumors [139]. Apicidin is another novel cyclic tetrapeptide whose structure is related to trapoxin [140]. Apicidin displayed marked antiproliferative effects in a wide variety of human cancer cell lines, including those of osteosarcoma, breast and stomach origin, as well as in v-Ras-transformed NIH3T3 cells [141]. The growth inhibitory effects were associated with changes in the expression of $p21^{CIP1/WAF1}$ and gelsolin, two proteins that are involved in regulation of cell-cycle control and cell morphology, respectively [141].

Benzamides

These compounds consist of a structurally diverse group of agents containing the benzamide moiety [140]. This group was postulated to bind zinc at the catalytic site of classical HDACs [142]. Two compounds have been described as members of this group, MS-275 and CI-994. MS-275 is structurally dissimilar from many other HDAC inhibitors [143,144]. As with other compounds of this class, MS-275-associated HDAC inhibition is accompanied by an increase in expression of $p21^{CIP1/WAF1}$ and accumulation of cells at G1 phase [143]. MS-275 displays anti-proliferative activity toward several human cancer cell lines, including breast, colorectal, leukemia, lung, ovary, and pancreas [143]. MS-275 was investigated in patients with solid tumors in a phase I trial [145]. A phase II trial was done in patients with refractory metastatic melanoma [146]. CI-994 is an investigational anticancer drug with a broad spectrum of activity in murine and human tumor xenografts [147]. CI-994 was investigated in patients with refractory metastatic in a phase II trial [146]. CI-994 was also investigated in phase I trial for solid tumors, in combination with gemcitabine [148], capecitabine [130,149], paclitaxel, and carboplatin [150].

Hybrid molecules

MGCD0103 is a hybrid compound evaluated in a phase I/II trial in combination with gemcitabine in patients with solid tumors [151]. Twenty-nine patients were enrolled (25 in phase I and 4 in phase II). Dose levels of MGCD0103 ranged between 50 and 110 mg. The maximum tolerated and recommended phase II dose was determined to be 90 mg. Two of five pancreatic cancer patients achieved

partial response [151]. As a member of hybrid molecules derived from glucosinolates in cruciferous vegetables, such as water cress, cabbage (including the Chinese cabbage bok choi), and broccoli, isothiocyanate has anti-oxidative properties and chemopreventive effects on the development of lung and colon cancers [152,153]. In a cohort of Chinese men at high risk for gastric cancer, isothiocyanates protected against the development of gastric cancer. Glutathione *S*-transferases may modify the chemopreventive effect of isothiocyanates [154].

Therefore, since identification of butyrate and trichostain A as HDAC inhibitors in the late 1970s and early 1990s, respectively, various studies have been performed with classical HDAC inhibitors to evaluate their value as anti-cancer therapeutic agents, individually or in combination with other types of therapy.

Perspective

As discussed above, HATs and HDACs maintain the dynamic equilibrium of reversible lysine acetylation *in vivo* (**Fig. 1**). These two groups of enzymes have been found to participate in the regulation of cellular proliferation and differentiation as cofactors of several mammalian transcriptional complexes. It must be considered that many acetyltransferases and deacetylases act primarily in protein complexes containing multiple cofactors and other enzymes responsible for a variety of post-translational modifications and that cellular processes are driven by the coordinated action of such complexes [41,42,155]. In the past decade or so, it has become very clear that HATs and HDACs also act upon non-histone proteins. While only a few dozens of such enzymes have been identified, recent studies indicate that 5%–10% of human proteins (thus in the order of thousands of proteins) may be acetylated on specific lysine residues [8], thereby raising the intriguing issue how the specificity is achieved and begging the important question whether there are additional such enzymes awaiting identification and characterization. Furthermore, it is noteworthy that the some HATs and HDACs may also be responsible for maintaining acetylation-like modifications [156], as very recently shown for SIRT5-mediated desuccinylation [157]. Answers to these intriguing and important issues shall shed important novel light on carcinogenesis along the GI track and at other cancer sites.

Since their discovery in the mid-1990s, HATs and HDACs have emerged as promising molecular targets for developing anti-cancer agents. In this regard, HDAC inhibitors have been actively evaluated as novel therapeutics for different types of cancer. As a result, two such inhibitors have been approved for treating lymphoma [158]. In comparison, much less progress has been made about HDAC inhibitors in treating cancers resulting from GI carcinogenesis. It should be noted that the outcome from phase I studies about combination of Vorinostat with radiotherapy in GI carcinoma is encouraging [126,127]. These and other studies have laid a solid foundation for additional research to improve the therapeutic potential of HDAC inhibitors for treating GI and other tumors.

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