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Combination Therapies Targeting HDAC and IKK in Solid Tumors

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Abstract

The rationale for developing histone deacetylase (HDAC) inhibitors (HDACi) as anti-cancer agents was based on their ability to induce apoptosis and cell cycle arrest in cancer cells. However, while HDACi have been remarkably effective in treatment of hematological malignancies, clinical studies with HDACi as single agents in solid cancers have been disappointing. Recent studies have shown that in addition to inducing apoptosis in cancer cells, class I HDACi induce I κ B-kinase (IKK)-dependent expression of pro-inflammatory chemokines, such as interleukin-8 (CXCL8), resulting in increased proliferation of tumor cells, and limiting the effectiveness of HDACi in solid tumors. Here, we discuss the mechanisms responsible for the HDACi-induced CXCL8 expression, and opportunities for combination therapies targeting HDACs and IKK in solid tumors.

Keywords

Combination therapies; histone deacetylases (HDACs); histone deacetylase inhibitors (HDACi); interleukin-8 (IL-8; CXCL8); I κ B kinase (IKK); solid cancers

Development of HDACi

Histone deacetylases (HDACs; see Glossary) are epigenetic modulators that remove acetyl groups from histone and non-histone proteins, and play an important role in the regulation of gene expression. In general, increased acetylation is associated with transcriptional induction, while decreased acetylation is associated with transcriptional repression. Based on their function and sequence homology, HDACs are grouped into four classes, class I–IV (Box 1). Increased expression of HDACs, particularly class I HDACs, has been associated with a variety of cancers, including hematological malignancies and solid cancers, and correlates with poor prognosis and development of chemoresistance [1].

Box 1

Classification of HDACs

Class I HDACs are localized mainly in the nucleus; they include HDACs 1, 2, 3, and 8.
Class II HDACs are localized both in the nucleus and in the cytoplasm; they include

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HDACs 4–7, 9, and 10. Class IV contains only HDAC 11, which shares sequence similarity with HDACs class I and II. In contrast to HDACs class I, II, and IV, class III HDACs, or sirtuins, do not contain zinc, but require nicotinamide adenine dinucleotide (NAD⁺) for their catalytic activity.

The rationale for developing HDAC inhibitors (HDACi) as anti-cancer agents was based on their ability to induce hyperacetylation of histones and non-histone proteins, resulting in increased differentiation, apoptosis, and cell cycle arrest of cancer cells [1–3]. HDACi have been used in the treatment of hematological malignancies, since they exhibit excellent differential action on normal and cancer cells at therapeutic dosages [1–3]. In contrast, clinical trials with HDACi as single agents in the treatment of solid tumors have produced disappointing results, but the specific responsible mechanisms are largely unknown. These mechanisms are likely multifactorial, and may include a limited distribution of HDACi in solid tumors, inactivation of specific anti-tumor immune responses, and increased expression of I κ B kinase (IKK)-dependent pro-inflammatory and pro-angiogenic chemokines in solid cancer cells, thus limiting effectiveness of HDACi in solid tumors. This review specifically focuses on the mechanisms of how HDACi induce IKK-dependent transcription of pro-inflammatory and pro-angiogenic chemokines, particularly interleukin-8 (CXCL8), in solid cancer cells, and on the opportunities for combination therapies targeting HDACs and IKK in solid tumors. Since HDACs class I, particularly HDAC1, 2, and 3, are involved in the HDACi-induced pro-inflammatory gene expression in solid tumor cells, this review focuses mainly on these HDACs.

HDACi effects are cell and gene specific

HDACi are structurally diverse compounds that can be divided into several classes, including hydroxamates, cyclic peptides, aliphatic acids, ketones, and benzamides. Four HDACi have been approved by FDA as of 2017: Vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza), which is the first FDA approved HDACi and has been used to treat cutaneous T cell lymphoma (CTCL); romidepsin (depsipeptide, FK228, Istodax), which has been used to treat CTCL and peripheral T-cell lymphoma (PTCL); belinostat (PXD101, Beleodaq), approved for PTCL; and panobinostat (LBH589, Farydak), which has been used to treat patients with multiple myeloma (MM). In addition, many other HDACi have been tested in pre-clinical studies and clinical trials, either as a monotherapy or in combination therapies. Since HDACi have many different, often opposing, cellular effects, combination therapies will likely be more effective than a monotherapy. Some HDACi, such as vorinostat, belinostat, and panobinostat are non-selective (pan-inhibitors), and inhibit all HDACs, while other HDACi are class, or even isozyme specific. For example, romidepsin specifically inhibits class I HDACs, particularly HDAC 1, 2, and 3 [4].

Since HDACi induce hyperacetylation of histones and non-histone proteins, they can reactivate tumor suppressor genes and regulate key oncogenic signaling pathways. In addition to increasing acetylation of histones, HDACi induce acetylation of tumor suppressors, such as p53 and RUNX3, proto-oncogenes, including c-myc, and transcription factors involved in immune regulation and cancer signaling, such as the STAT transcription

factors, hypoxia-inducible factor-1 α (HIF-1 α), Foxp3, and NF κ B [1, 5–9]. By increasing acetylation of histones and different transcription factors, HDACi can both induce and repress gene expression, thus providing a rationale why so many genes are differentially regulated by HDACi. Gene expression profiling studies have shown that HDACi affect expression of approximately 2–10% genes, with approximately equal number of genes being activated and suppressed [10–12]. HDACi exhibit their anti-cancer effect by increasing expression of the cyclin-dependent kinase inhibitor p21, resulting in cell cycle arrest and differentiation; increasing apoptosis by upregulating pro-apoptotic genes and downregulating anti-apoptotic genes; regulating DNA damage, ROS production, proteasome activity and DNA repair; and increasing ER stress and autophagy [2, 13]. In addition, HDACi regulate immune recognition of cancer cells by increasing expression of MHC class I and II molecules [14, 15], inducing expression of PD-L1 [16, 17], and promoting differentiation and function of regulatory T cells [18].

Importantly, the HDACi-mediated effect is cell- and HDAC-specific; different effects and outcomes have been observed in different cell types, and targeting different HDAC isoforms. While HDACi exhibit a strong pro-apoptotic potential in human leukemia cells [19–22], they have a limited ability to induce apoptosis in solid cancer cells [23–25]. While HDAC inhibition by vorinostat inhibits CXCL8 expression in CTCL Hut-78 cells, it increases CXCL8 expression in ovarian cancer (OC) cells [26, 27]. Targeting class I HDACs induces IL-10 expression in macrophages, whereas HDAC6 suppression inhibits IL-10 production in macrophages [28–30]. HDAC6 effectively deacetylates tubulin dimers, but the deacetylation rate of tubulin polymeric forms is much slower [31]. Identification of cellular targets of the individual HDACs and HDACi in different cell types is crucial for the development of targeted HDAC-based anti-cancer strategies.

HDACi have anti-inflammatory effect in stimulated leukocytes, but pro-inflammatory and pro-angiogenic effect in solid cancer cells

Even though HDACi were originally developed as anti-cancer drugs, based on their ability to induce apoptosis in hematopoietic malignancies, they also exhibit anti-inflammatory properties. In most immune cells including monocytic cells, macrophages, and dendritic cells stimulated with lipopolysaccharide or TNF, HDACi suppress expression of NF κ B-dependent pro-inflammatory cytokines including TNF, IL-1, and IL-6 [32–37]. Intriguingly however, in solid cancer cells characterized by constitutively increased NF κ B activity, HDACi actually increase expression of NF κ B-dependent pro-inflammatory genes, and this is associated with increased survival, proliferation, and migration of solid cancer cells (Table 1).

In cervical cancer HeLa cells, inhibition of HDAC class I and II activity by trichostatin A (TSA) increased both basal and TNF-induced NF κ B-dependent CXCL8 expression [38, 39]. HDAC1 and HDAC2 were found to regulate the NF κ B transcriptional activity through a direct association of HDAC1 with the Rel homology domain of p65. While HDAC2 does not seem to interact with p65 directly, it can regulate NF κ B activity through its association with HDAC1 [38]. The CXCL8 expression, as well as expression of the NF κ B-dependent pro-

survival gene cIAP1, was induced in HeLa cells also by apicidin, which preferentially inhibits HDAC class I [40]. The apicidin-induced CXCL8 and cIAP1 expression in HeLa cells was mediated by IKK, and associated with resistance to apicidin-induced apoptosis [40]. Inhibition of HDAC activity by pan-HDACi failed to induce apoptosis also in non-small cell lung cancer (NSCLC) cells, and this was associated with increased transactivation potential of p65, recruitment of the p300 transcriptional co-activator and histone acetyltransferase (HAT) to chromatin, and increased expression of CXCL8, Bcl-xL, and MMP9 [23, 24]. Interestingly, in addition to inducing expression of CXCL8 in NSCLC cells, TSA induced expression of CXCL8 receptors CXCR1 and CXCR2 that are also regulated by NF κ B, while it suppressed expression of CXCL1, CXCL2, and CXCL3 [41]. Inhibition of class I HDACs by romidepsin also induced CXCL9 and CXCL10 expression in human lung cancer cells, mouse lung tumors, and tumor-infiltrating macrophages and T cells [16]. In human breast cancer cells, TSA induced IKK-dependent CXCL8 expression and release that were associated with increased histone H3 promoter acetylation and nuclear p65 accumulation [42]. In human ovarian cancer cells, inhibitors of HDACs 1, 2, and 3, CI994 and romidepsin, but not inhibitors of HDACs class II, specifically induced the IKK-dependent CXCL8 expression that was associated with increased p65 promoter recruitment [26, 27]. Suppression or neutralization of the CXCL8 induced by class I HDACi in OC cells increased their pro-apoptotic and anti-proliferative effect [26, 27].

Interleukin-8 (CXCL8), originally discovered as the neutrophil chemoattractant and inducer of leukocyte-mediated inflammation, is a pro-inflammatory and pro-angiogenic chemokine that contributes to cancer progression through its induction of tumor cell proliferation, survival, and migration [43–46]. Increased production of CXCL8 confers a tremendous growth advantage to malignant cells, and increased CXCL8 levels correlate with poor prognosis in solid tumors, including ovarian, breast, lung, and prostate cancer [46–51]. Interestingly, recent studies have shown that HDACi induce the CXCL8 expression also in cancer associated fibroblasts (CAF) [52, 53]. The HDACi-induced CXCL8 released by solid tumor cells and CAF then activates tumor-associated macrophages and neutrophils to release more CXCL8, which further amplifies the pro-angiogenic and metastatic effect (Figure 1). Since suppression of the HDACi-induced CXCL8 release potentiates the cytotoxic and pro-apoptotic effect of HDACi [26], these data indicate that targeting the HDACi-induced, IKK-dependent expression of CXCL8, and other pro-inflammatory chemokines, may increase effectiveness of HDACi in ovarian cancer and other solid tumors characterized by the HDACi-induced chemokine expression.

HDACi effect on proteasomal regulation of I κ B α and p65 levels

At the transcriptional level, expression of CXCL8, as well as many other pro-inflammatory chemokines, is regulated by the transcription factor NF κ B, which is constitutively activated in solid cancers including ovarian, lung, and breast cancer, and conveys a poor outcome [54–56]. Activation of NF κ B is mediated by the enzymes of IKK complex that consists of two catalytic subunits, IKK α and IKK β , and a regulatory subunit IKK γ (NEMO). IKK phosphorylates the NF κ B inhibitor, I κ B α , resulting in I κ B α proteasomal degradation and nuclear translocation of NF κ B subunits [57, 58]. In addition to phosphorylating I κ B α , IKKs can phosphorylate histones and p65 NF κ B, thus increasing p65 acetylation, promoter

recruitment, and transcriptional activity [57, 58]. While the proteasomal degradation of I κ B α , resulting in the nuclear translocation and accumulation of NF κ B subunits, represents a general step in NF κ B activation, specificity and duration of NF κ B-regulated responses are mediated by the protein composition of NF κ B complexes and their post-translational modifications [59–63]. The NF κ B family includes five structurally related proteins, p65 (RelA), p50, p52, c-Rel, and RelB, which form homo- and heterodimers; p65/p50 heterodimers are the most abundant dimers. In addition to I κ B α , the p65 subunit of NF κ B can also undergo proteasomal degradation [63]. Thus, inhibition of proteasome activity can stabilize both I κ B α and p65 NF κ B, potentially resulting in two diametrically different outcomes in the regulation of NF κ B-transcriptional activity.

Since HDACi can modulate the proteasome activity [5, 64, 65], they can inhibit or activate the NF κ B-dependent transcription, depending on the cellular context. Inhibition of HDAC activity with pan-HDACi reduces expression of the catalytic beta subunit of the proteasome, resulting in the inhibition of the inducible proteasomal degradation of I κ B α [65]. Therefore, in stimulated immune cells, HDAC inhibition is likely to inhibit the inducible cytoplasmic degradation of I κ B α thus preventing the nuclear translocation of NF κ B subunits and NF κ B dependent transcription, as was observed in stimulated leukocytes [32–37]. The importance of cell stimulation for the HDACi-mediated suppression of NF κ B-dependent transcription is evident from the study by Grabiec et al [35]. In the absence of cell stimulation, exposure of human macrophages to TSA had no influence on basal IL-6 or TNF expression, but TSA inhibited IL-6 and TNF expression in TNF- or LPS-stimulated macrophages [35].

However, in most cancer cells, NF κ B is constitutively activated, and the NF κ B proteins are already localized in the nucleus. Thus, the HDACi-mediated stabilization of cytoplasmic I κ B α is likely to have a little effect on the nuclear levels and activity of NF κ B in cancer cells. Instead, HDACi may prevent the proteasomal degradation of nuclear p65, thus increasing its nuclear accumulation and promoter binding (Figure 2). Indeed, several studies have demonstrated that HDACi increase the nuclear p65 levels in cancer cells [24, 26, 39, 40, 66]. For example, in NSCLC and OC cells, pan-HDACi induce the IKK-dependent p65 nuclear accumulation, resulting in the increased CXCL8 expression [24, 26]. Furthermore, several studies have shown that HDACi increase IKK activity and IKK-dependent promoter and p65 acetylation, resulting in the increased p65 transcriptional activity in cancer cells [23, 26, 38, 39, 66, 67].

HDACi-induced CXCL8 expression in solid cancer cells is p65- and IKK-dependent

In contrast to other NF κ B-dependent genes, the CXCL8 transcription is regulated predominantly by NF κ B p65 homodimers [68, 69]. Seven acetylated lysines have been identified within p65 NF κ B: K122, 123, 218, 221, 310, 314, and 315 [62]. Acetylation of K122 and 123 reduces p65 binding to DNA, and promotes its nuclear export [70]. Acetylation of K221 enhances p65 DNA binding and impairs its binding to I κ B α , while acetylation of K310 is required for full transcriptional activity of p65 [60]. Interestingly, acetylation of p65 at K314 and 315 does not affect p65 DNA binding, but facilitates its

promoter specificity in stimulated cells [71, 72]. Acetylation of p65 is regulated by the HATs, p300 and CBP, and HDAC1, 2, and 3 [38, 73–79]. However, the specific regulation of p65 by the individual HATs and HDACs differs in different cells and tissues, and may depend on the cellular status of other transcription factors and co-regulators. For example, while p65 was reported to directly interact with HDAC2 in kidney mesangial cells [80], such interaction was not observed in other cell types [38, 70, 78]. Since different acetylation sites differentially regulate the p65 functions and promoter specificity, modulation of p65 acetylation by targeting specific HATs and HDACs might prove useful in targeting specific NFκB-dependent genes.

However, HDACi can also increase acetylation of other transcription factors that regulate the p65 transcriptional activity. The HDACi-induced activation of p65 can be negated by acetylated STAT1, which can specifically bind to p65 and inhibit its transcriptional activity [81–83]. Interestingly, acetylated STAT1 was found in chemotherapy sensitive but not resistant ovarian cancer cells [84]. Since activated NFκB mediates chemoresistance by inducing expression of pro-survival genes [58], these data suggest that the HDACi-induced STAT1 acetylation may suppress the p65-mediated expression of pro-survival genes, thus modulating chemoresistance. In addition, HDACi can modulate p53 activity and its interaction with p65, thus representing yet another mechanism regulating the p65-dependent transcription [6, 85].

The CXCL8 expression is specifically regulated by class I HDACs, particularly HDAC1, 2, and 3 [27, 77, 86, 87]. However, since acetylation of different lysines can both activate and inhibit the p65 transcriptional activity, HDACs can both induce and repress the CXCL8 expression. Indeed, suppression of HDAC1, 2, and 3 was reported to both inhibit and induce the CXCL8 expression, depending on the cell type, stimulus, and presence of additional transcriptional regulators [27, 77, 86, 87]. In breast cancer cells, knockdown of HDAC1 specifically suppressed the CXCL8 expression, resulting in their reduced proliferation and migration [86]. In contrast, in lung cancer cells infected with respiratory syncytial virus, the CXCL8 transcription was inhibited by Bcl3-mediated recruitment of HDAC1, indicating that HDAC1 attenuates the CXCL8 promoter activity under these conditions [87]. Interestingly, while an individual suppression of HDAC1, HDAC2, or HDAC3 inhibited the CXCL8 expression in ovarian cancer cells, their simultaneous suppression induced the CXCL8 expression, suggesting that HDAC1, 2, and 3 may form complexes with other transcriptional regulators, and suppression of HDAC protein levels in these complexes disrupts their function and reduces the CXCL8 transcription [27]. In this context, previous studies have shown that in addition to p65, HDAC1, 2, and 3 form complexes with IκBα, NuRD, N-CoR/SMRT, and other transcriptional activators and repressors important for cancer cell survival and growth [38, 70, 73, 74]. Thus, the impact of pharmacologic inhibition of HDACs will likely differ from the effect of HDAC protein suppression.

Intriguingly, in ovarian cancer cells, class I HDACi dramatically increase only the CXCL8 expression, while they do not have any substantial effect on the expression of other NFκB-dependent genes, including TNFα, IL-6, CXCL5, TGFβ1, cIAP-1, Bcl-2, p65, p50, IκBα, CXCR-1, and CXCR2 [26, 27]. The HDACi-induced CXCL8 expression in OC cells is mediated by a gene specific, IKKβ-dependent recruitment of p65 homodimers to CXCL8

promoter. In addition, HDAC inhibition increases K314/315 acetylation of p65, and its promoter-specific occupancy at the CXCL8 promoter [26]. The HDACi-induced CXCL8 expression is mediated by IKK β and p65 NF κ B also in breast cancer cells [42]. Why do HDACi induce predominantly the CXCL8 expression in solid cancer cells? Since HDACi increase the nuclear levels of p65 in cancer cells [24, 26, 39, 40, 66], they may specifically increase expression of genes regulated by p65 homodimers. In addition, the HDACi-increased K314/315 acetylation of p65 may favor its specific recruitment to CXCL8 promoter, as was observed in OC cells [26]. Thus, the HDACi-induced CXCL8 expression in solid tumor cells is likely a result of several HDACi-mediated mechanisms that include the HDACi-increased nuclear accumulation of p65, increased IKK activity and IKK-dependent p65 K314/315 acetylation, and promoter-specific recruitment of K314/315 acetylated p65 homodimers to CXCL8 promoter (Figure 2).

Opportunities for combination therapies targeting HDAC and IKK in solid tumors

Inhibition of IKK activity decreases the CXCL8 expression induced by HDAC inhibition in ovarian cancer cells [26]. Since CXCL8 induces proliferation and survival in cancer cells [26, 44–48], these data indicate that therapies targeting the IKK-mediated CXCL8 expression may increase effectiveness of HDACi in OC treatment. This is supported by *in vitro* data demonstrating that suppression of the HDACi-induced CXCL8 by siRNAs, or its neutralization by anti-CXCL8 monoclonal antibodies, increase the HDACi pro-apoptotic and anti-proliferative effect in OC cells [26], and by *in vivo* studies demonstrating that suppression of CXCL8 reduces ovarian tumor growth [47, 48]. In addition, Sonnemann et al. have shown that HDACi and aspirin synergistically induce cell death in OC cells, independently of cyclo-oxygenase [88]. Since aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), in addition to inhibiting cyclooxygenase activity, inhibit IKK activity [89], it seems plausible that the observed synergistic effect in OC cells might have been mediated by IKK inhibition and suppression of the HDACi-induced CXCL8 expression. Disruption of NF κ B-signaling potentiates the HDACi pro-apoptotic effect also in other solid cancer cells, including NSCLC, head and neck squamous cell carcinomas, prostate cancer cells, hepatocellular carcinoma, and thyroid cancer [23, 24, 40, 90–94].

Importantly, our recent *in vivo* results have demonstrated that combining HDAC and IKK inhibitors significantly reduces ovarian tumor growth when compared to either drug alone [26]. The slowest tumor growth in the HDACi/IKK inhibition combination group was associated with the lowest CXCL8 tumor and plasma levels, and with the lowest tumor expression of the murine neutrophil [7/4] antigen, indicating a reduced tumor infiltration with mouse neutrophils. Recent studies have demonstrated a key role of the CXC chemokine receptor, CXCR2, in pancreatic cancer development and progression [95, 96]. Inhibition of the CXCR2 signaling significantly reduced metastases, prolonged survival, and enhanced sensitivity to anti-PD-1 immunotherapy in a mouse model of pancreatic ductal adenocarcinoma [95]. The CXCL8-CXCR1/2 signaling plays a crucial role in the initiation and progression of solid tumors [46]. Thus, targeting the HDACi-induced, IKK-dependent CXCL8 expression may increase effectiveness of HDACi in treating ovarian cancer and

possibly other solid tumors characterized by the increased CXCL8 expression (Figure 3, Key Figure).

Targeting IKK activity and NF κ B-dependent expression of pro-survival genes induced by HDACi has been investigated in the treatment of hematological malignancies [66, 67, 97, 98]. Inhibition of IKK activity and NF κ B signaling by Bay 11-7082 or parthenolide potentiated the HDACi-mediated cell death in leukemia cells [66, 97]. Inhibition of IKK activity by Bay 11-7082 or the selective IKK β inhibitor IKK-2 inhibitor IV also augmented the HDACi-pro-apoptotic effect in multiple myeloma cells [67]. A novel IKK β inhibitor, LY2409881, exhibited a strong cytotoxic synergistic effect with romidepsin in diffuse large B-cell lymphoma (DLBCL) cell lines as well as *in vivo*, in a DLBCL xenograft model [98]. In addition, the HDACi-induced activation of NF κ B in hematological malignancies has served as the basis of many synergistic strategies combining HDACi with proteasome inhibitors that suppress the proteasomal degradation of I κ B α [2].

In contrast to hematological malignancies, combination of IKK and HDAC inhibitors has not been considered in the treatment of solid tumors, perhaps because of the limited effectiveness of HDACi in solid cancers as single agents. Many compounds can inhibit IKK activity, including the IKK inhibitors PS1145, BMS345541, SC514, SPC839, ML120B, BAY 11-7082, and the newly developed IKK β inhibitor LY2409881. In addition, IKK activity can be inhibited by NSAIDs, such as aspirin [89], and by naturally occurring agents, such as curcumin [93, 99], which are pharmacologically safe and have long been used for their chemopreventive and anti-inflammatory properties. Even though IKK inhibitors are not highly potent as single agents, accumulating evidence indicates that they may synergize with HDACi in treating solid cancers [26, 27, 42, 88, 91, 93, 100]. The mechanistic basis of this synergy consists of the HDACi-induced and IKK-dependent expression of CXCL8, which increases survival and proliferation of solid cancer cells. Inhibition of IKK activity suppresses the induced CXCL8 expression, thus potentiating the effectiveness of HDACi in reducing solid tumor growth.

Concluding Remarks and Future Perspectives

Even though HDACi have been remarkably effective in treating hematological malignancies, they have produced poor results in solid cancers. Several mechanisms likely contribute to the limited effectiveness of HDACi in solid tumors; the induced IKK-dependent CXCL8 expression may represent one of these mechanisms. Since different HDACs and mechanisms may regulate the p65 CXCL8 promoter occupancy and transactivation potential in different cell types, it will be important to identify the specific HDACs and mechanisms responsible for the HDACi-induced CXCL8 expression in different types of solid cancers (see Outstanding Questions). In addition, it will be important to determine whether HDACi induce the CXCL8 expression in primary patient samples, particularly in patients with hematological malignancies *vs.* solid tumors. It may be possible that HDACi induce CXCL8 expression only in selected solid tumors, and in these cancers, targeting the induced CXCL8 expression may increase the HDACi effectiveness. Thus, determining in which patient samples HDACi induce CXCL8 expression may identify patients who would respond to the HDACi/IKK inhibition combination therapies.

Outstanding Questions

- What are the specific mechanisms responsible for the HDACi-induced CXCL8 expression in different types of solid cancers?
- Do HDACi induce CXCL8 expression in primary cancer patient samples, and is there a difference between hematological malignancies and solid tumors?
- Could screening of primary solid cancer samples for the HDACi-induced CXCL8 expression identify tumors that would respond to the HDACi/IKK inhibition combination therapies?
- Can anti-inflammatory, IKK-inhibiting drugs increase effectiveness of HDACi in ovarian cancer and other solid cancers characterized by increased CXCL8 expression?

Targeting IKK activity and NF κ B-dependent transcription induced by HDAC inhibition has been investigated in the treatment of multiple myeloma and other hematological malignancies. In solid tumors, combination of IKK and HDAC inhibitors has never been considered, perhaps because of their limited effectiveness as single agents. Recent data indicate that by suppressing the CXCL8 expression, IKK inhibitors may increase effectiveness of HDAC inhibitors in ovarian cancer and other solid cancers characterized by the increased CXCL8 expression (Figure 3). Future studies and clinical trials should examine the effect of IKK inhibiting agents on increasing the effectiveness of HDAC inhibitors in ovarian cancer and other solid cancers characterized by the increased CXCL8 expression.

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GLOSSARY

Interleukin-8 (CXCL8)

Pro-inflammatory and pro-angiogenic chemokine originally discovered as a neutrophil chemoattractant; has an important regulatory role in promoting survival, proliferation, and migration of solid cancer cells.

Histone deacetylases (HDACs)

Epigenetic modulators that remove acetyl groups from histone and non-histone proteins.

Histone deacetylase inhibitors (HDACi)

Chemical compounds that inhibit histone deacetylases and have a significant anti-cancer potential.

I κ B kinase (IKK)

A central enzymatic complex that is a part of the NF κ B signaling; in addition to phosphorylating I κ B α and p65 NF κ B, IKK can phosphorylate other proteins involved in the transcriptional regulation.

Nuclear Factor κ B (NF κ B)

Transcription factor regulating expression of genes involved in a broad range of biological processes including immune responses, inflammation, stress responses, lymphoid organogenesis, and cell survival and proliferation.

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Trends Box

- The rationale for developing histone deacetylase (HDAC) inhibitors as anti-cancer agents was based on their ability to induce apoptosis and cell cycle arrest in cancer cells.
- While HDAC inhibitors are very effective in the treatment of hematological malignancies, they are much less effective as single agents in the treatment of solid tumors, such as ovarian cancer.
- In addition to inducing apoptosis in solid cancer cells, HDAC inhibitors induce I κ B-kinase (IKK)-dependent expression of pro-inflammatory chemokines, such as interleukin-8 (CXCL8).
- Suppression of the IKK-dependent CXCL8 expression induced by HDAC inhibition increases the pro-apoptotic and anti-proliferative effect of HDAC inhibitors in ovarian cancer cells, indicating that anti-inflammatory agents targeting the IKK activity may increase effectiveness of HDAC inhibitors in ovarian cancer and other solid cancers characterized by the increased CXCL8 expression.

Figure 1. Role of HDACi-induced CXCL8 in promoting proliferation, angiogenesis, and metastasis in solid tumors

In solid cancer cells and cancer-associated fibroblasts (CAF), HDACi induce expression of CXCL8, which induces tumor cell proliferation, survival, and migration. In addition, the increased expression of CXCL8 activates tumor-associated macrophages (TAM) and neutrophils to release more CXCL8, which further amplifies the proangiogenic and metastatic effect.

Figure 2. HDACi have anti-inflammatory effect in stimulated immune cells, but pro-inflammatory and pro-angiogenic effect in solid cancer cells

In stimulated immune cells, the HDACi-mediated suppression of proteasome activity inhibits the cytoplasmic degradation of IκBα, thus preventing the inducible nuclear translocation of NFκB subunits and NFκB-dependent transcription. In contrast, in most solid cancer cells, NFκB is constitutively activated, and the NFκB proteins are already localized in the nucleus. Thus, the HDACi-mediated stabilization of cytoplasmic IκBα has a little effect on the nuclear levels of NFκB in solid cancer cells. Instead, HDACi prevent the proteasomal degradation of nuclear p65, causing its increased nuclear accumulation. In addition, HDAC inhibition increases IKK activity, and IKK-dependent p65 acetylation. Since HDAC inhibition in cancer cells increases the nuclear levels of p65, NFκB-dependent genes, such as CXCL8, regulated by p65 homodimers, may be specifically increased by HDAC inhibition in solid cancer cells.

Figure 3. IKK inhibition increases effectiveness of HDACi in solid tumors by suppressing the HDACi-induced, IKK-dependent CXCL8 expression

While HDAC inhibition induces apoptosis in cancer cells, it also increases IKK-dependent expression of CXCL8, which induces tumor growth. Inhibition of IKK activity suppresses the induced CXCL8 expression, thus potentiating the pro-apoptotic effect of HDAC inhibitors, and increasing their effectiveness in reducing tumor growth.

Table 1

HDACi induce NFκB-dependent pro-inflammatory and pro-survival gene expression in solid cancer cells

HDACi	HDAC involved	Effect on NFκB-dependent transcription	Cells	Reference
TSA, apicidin	HDAC 1, 2	Induction of IKK-dependent CXCL8 and cIAP1 expression associated with increased resistance to apoptosis	HeLa cells	[38–40]
Romidepsin, TSA, butyrate, vorinostat		Induction of CXCL8, CXCL9, CXCL10, Bcl-xL, MM 9, CXCR1, CXCR2; increased resistance to apoptosis	Lung cancer cells	[16, 23, 24, 41]
TSA		Induction of IKK-dependent CXCL8 expression	Breast cancer cells	[42]
CI994, romidepsin, vorinostat	HDAC 1, 2, 3	Induction of IKK-dependent CXCL8 expression; increased resistance to apoptosis	Ovarian cancer cells	[26, 27]