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Cytotoxicity Mediated by Histone Deacetylase Inhibitors in Cancer Cells: Mechanisms and Potential Clinical Implications

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Abstract

Aberrant expression of epigenetic regulators of gene expression contributes to initiation and progression of cancer. During recent years, considerable research efforts have focused on the role of histone acetyltransferases (HATs) and histone deacetylases (HDACs) in cancer cells, and the identification of pharmacologic agents that modulate gene expression via inhibition of HDACs. The following review highlights recent studies pertaining to HDAC expression in cancer cells, the plieotropic mechanisms by which HDAC inhibitors (HDACi) mediate antitumor activity, and the potential clinical implications of HDAC inhibition as a strategy for cancer therapy.

Keywords

Epigenetics; cancer; HDAC inhibitor

Introduction

During recent years, considerable research efforts have focused on potentially reversible alterations in chromatin structure, which modulate gene expression during malignant transformation. The basic structure of chromatin is the nucleosome, which is composed of ~146 bp of DNA wrapped twice around an octamer of core histones (H3-H4 tetramer, and two H2A-H2B dimers). Core histone proteins contain a basic N-terminal tail region, a histone fold, and a carboxy-terminal region. All of these regions-particularly the positively charged N terminal tails protruding from the DNA helix, are sites for a variety of covalent modifications such as acetylation, methylation, phosphorylation, ubiquitination, biotinylation, ADP ribosylation, sumoylation, glycosylation, and carbonylation (1). These dynamic alterations modulate interactions between DNA, histones, multiprotein chromatin remodeling complexes and transcription factors, thereby enhancing or repressing gene expression (2;3).

The emerging delineation of histone alterations that coincide with aberrant gene expression and malignant transformation provides impetus for the development of agents that target histone modifiers for cancer therapy. The following discussion will focus on recent insights

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regarding the mechanisms by which histone deacetylase (HDAC) inhibitors mediate cytotoxicity in cancer cells.

Histone Acetyltransferases and Histone Deacetylases

Acetylation of core histones is governed by opposing actions of a variety of histone acetyl transferases (HAT) and histone deacetylases (HDACs). Histone acetylases mediate transfer of an acetyl group from acetyl-co-A to the ε-amino site of lysine, and are divided into two groups. Type A HATs are located in the nucleus, and acetylate nucleosomal histones as well as other chromatin-associated proteins; as such, these HATs directly modulate gene expression. In contrast, Type B HATs are localized in the cytoplasm, and acetylate newly synthesized histones, thus facilitating their transport into the nucleus and subsequent association with newly synthesized DNA (4;5). Type A HATs typically are components of high-molecular complexes and comprise five families; GNAT, P300/CBP, MYST, nuclear receptor coactivators, and general transcription factors (4). Some HATs, notably p300 and CBP, associate with a variety of transcriptional regulators including Rb and p53, and may function as tumor suppressors. In addition, HATs acetylate a variety of non-histone proteins including p53, E2F1, Rb, p73, HDACs, and heat shock protein (Hsp) 90(6;7) (Table 1).

HDACs are currently divided into four classes based on phylogenetic and functional criteria (reviewed in ref (7)). Class I HDACs (1, 2, 3, and 8), which range in size from ˜40–55 Kd, are structurally similar to yeast transcription factor, Rpd-3, and typically associate with multi-protein repressor complexes containing sin3, Co-REST, Mi2/NuRD, N-COR/SMRT and EST1B (8). HDACs 1, 2, and 3 are localized in the nucleus, and target multiple substrates including p53, myo-D, STAT-3, E2F1, Rel-A, and YY1 (9;10). HDAC 8 is localized in the nucleus as well as the cytoplasm; no substrates of this Class I HDAC have been defined to date.

Class II HDACs (4, 5, 6, 7, 9, 10), which range in size from ˜70 – 130 Kd, are structurally similar to yeast HDA1 deacetylase and are subdivided into two classes. Class IIA HDACs (4, 5, 7, and 9) contain large N-terminal domains that regulate DNA binding, and interact in a phosphorylation-dependent manner with 14–3-3 proteins, which mediate movement of these HDACs between cytoplasm and nucleus in response to mitogenic signals (7). Class IIB HDACs (6 and 10) are localized in the cytoplasm. HDAC 6 is unique in that it contains two deacetylase domains and a zinc finger region in the c-terminus. HDAC 10 is similar to HDAC 6, but contains an additional inactive domain (7;10).

In contrast to Class I HDACs, Class II HDACs exhibit family-restricted interactions with a variety of proteins including ANKRA, RFXANK, estrogen receptor (ER), REA, HIF1α, Bcl-6, and Fox3P. These HDACs have a variety of non-histone target substrates including GATA-1, GCMa, HP-1, and SMAD-7, as well as FLAG-1 and FLAG-2 (9;10). Relatively little information is available regarding binding partners for HDAC 6 and HDAC 10 (11;12). Notably, HDAC 6 has emerged as a major deacetylase of α-tubulin as well as Hsp90 ; as such, HDAC 6 mediates cell motility, and stability of oncoproteins such as EGFR, RAF1, and ABL, that are client proteins of Hsp90 (13). Additionally, HDAC 6 can interact via its

zinc finger with ubiquitin to modulate aggressesome function and autophagy (14). Recent studies suggest that HDAC 10 may also function to modulate acetylation of Hsp90 (15).

Class I and Class II HDACs are zinc-dependent enzymes containing catalytic pockets that can be inhibited by zinc chelating compounds such as hydroxamic acid. HDAC 11, the only known Class IV HDAC, exhibits conserved residues within the catalytic domain that are shared with Class I and Class II HDACs; binding partners and target substrates for this HDAC have not been defined to date (10;16). Class I, Class II and Class IV HDACs are referred to as “classical” HDACs, and are targeted by histone deacetylace inhibitors (HDACi) currently in clinical development. In contrast, Class III HDACs (sirtuins) are structurally similar to yeast SirT2, and require NAD+ as a co-factor for enzymatic activity (17). Recent studies suggest that sirtuins are critical regulators of energy-dependent transcription. These latter HDACs, which are rapidly emerging as potential novel targets for cancer therapy will not be further discussed here due to the limited clinical experience with sirtuins inhibitors. Several recent reviews have focused on the biochemistry and potential clinical significance of Class III HDACs (18;19).

HDAC Activity in Normal and Malignant Tissues

Despite structural similarities, HDACs exhibit non-redundant functions during embryogenesis, and aberrant HDAC expression in cancers may be a manifestation of tissue-specific epigenetic reprogramming events (9;10). Knock-out of HDACs 1, 2, 3, or 7 results in embryonic lethality in mice due to aberrant cell cycle regulation or abnormal blood vessel development; in contrast, mice lacking HDACs 4, 5, 6, or 9 are viable, yet exhibit markedly abnormal cardiovascular, bone and muscle development (reviewed in ref (10)). The unique roles of individual HDACs regarding embryonic development and maintenance of organ function may account, in part, for activities and potential systemic toxicities of HDACi currently evaluated in clinical settings.

In light of the complex roles of HDACs during embryogenesis and their expression profiles in normal tissues, it is not surprising that the effects of targeted inhibition of HDACs in cancer cells appear tissue-dependent. For example, knock-down of HDAC 1 inhibits proliferation of cultured colon cancer cells, and induces apoptosis in osteosarcoma and breast cancer cells (20;21). Knock-down of HDAC 2 induces growth arrest in colon cancer cells, but has no such effects in osteosarcoma or breast cancer cells (20;21). However, inhibition of HDAC 2 down-regulates ER/PR expression, and potentiates tamoxifen-induced apoptosis in ER/PR positive breast cancer cells (22). Knock-down of HDAC-2 enhances p53-dependent gene activation/repression, and inhibits proliferation of cultured breast cancer cells (23). Knock-down of HDAC 8, which modulates telomerase function by inhibiting ubiquitin-mediated degradation of hEST1B (24), inhibits proliferation of lung, colon, cervical carcinoma and neuroblastoma cells, and induces apoptosis in cultured lymphoma/leukemia cells (25–27).

To date, targeted inhibition of Class II HDACs has not been systematically examined. However, knock-down of HDAC 6 or HDAC 10 enhances acetylation of Hsp90 in various

cancer cell lines, resulting in destabilization of client oncoproteins such as Bcr-Ab1, and VEGF-R (15;28).

Clinical Manifestation of HDAC Expression in Cancer

A number of studies have been performed recently to examine expression and potential relevance of HDAC expression in cancer tissues. The majority of reports have focused on Class I HDACs, and suggest the clinical manifestations of aberrant HDAC expression may be histology dependent (29). Nakagawa, et al (30) systematically examined expression levels of HDACs 1, 2, 3, and 8 in a variety of cultured cancer lines and a broad panel of primary human lung, esophageal, gastric, colon, pancreas, breast, ovary and thyroid cancers. Seventy-five per cent of esophageal, gastric, colon and prostate cancers, as well as corresponding adjacent “normal” tissues exhibited “high-level” Class I HDAC expression. Although HDAC expression in tumors often was not higher than corresponding normal tissues, 5 – 40% of these cancers exhibited HDAC over-expression; esophageal and prostate cancers tended to exhibit more consistent over-expression of Class I HDACs. Additional studies suggest that high level HDAC 1 expression correlates with advanced stage of disease in lung cancer patients(31), as well as aggressive tumor histology, advanced stage of disease, and poor prognosis in patients with pancreatic carcinoma(32). In contrast, HDAC 1 expression in breast cancer is associated with ER/PR expression, earlier stage of disease (T as well as N classifications), and improved patient survival(33;34).

In a large retrospective study, Weichert, et al (35) observed simultaneous over-expression of HDACs 1, 2, and 3 in approximately 30 % of 150 gastric cancers. An additional 30% of tumors exhibited very low or undetectable expression of these HDACs; global HDAC 1 over-expression in primary cancers correlated significantly with nodal metastases and diminished patient survival. In an additional study, high-level expression of HDACs 1, 2, and 3, was observed in 36%, 58% and 73% of 140 colon carcinomas. HDAC expression correlated significantly with proliferation index, poorly-differentiated histology, and diminished patient survival; HDAC 2 expression was an independent prognosticator of poor outcome. In a related study (36), high level expression of HDAC 1, 2, and 3 was observed in 70%, 74% and 95% of 192 prostate cancers. Over-expression of HDAC 1 and/or HDAC 2 correlated with poorly differentiated tumors, and diminished prostate specific antigen-associated disease free survival. Simultaneous over-expression of all three Class I HDACs coincided with increased proliferation index. HDAC 2 over-expression was an independent prognosticator of poor outcome in prostate cancer patients. Over-expression of HDAC 2 also correlates with advanced stage of disease and diminished survival of oropharyngeal carcinoma patients (37). HDAC 8 expression correlates with aggressive histology and advanced stage of pediatric neuroblastomas, as well as diminished survival of patients with these neoplasms; spontaneous regressions of neuroblastomas coincide with down-regulation of this HDAC (38).

Relatively limited information is available regarding the frequency and clinical relevance of Class II HDAC expression in human cancers. Over-expression of HDAC 4 has been observed in breast cancers, relative to renal, colorectal, or bladder cancers, whereas colon cancers appear to have relatively higher levels of HDACs 5 and 7 (29). Decreased expression

of several Class II HDACs – particularly HDACs 5 and 10, appears to correlate with advanced stage of disease and diminished survival of lung cancer patients (39). HDAC 6 expression correlates with advanced stage of disease in oropharyngeal cancers (40). In contrast, HDAC 6 expression in breast cancers coincides with early stage tumors, ER/PR expression, response to tamoxifen, and in some cases, improved patient survival (41).

Cytotoxic Effects of HDAC Inhibitors

During recent years, intense efforts have focused on the development of HDACi for cancer therapy. These initiatives have been prompted by considerable pre-clinical evidence of plieotropic cytotoxic effects of HDACi of diverse structural classes in cultured cancer cells and various human tumor xenografts (Figure 1), as well as encouraging results of early phase trials in cancer patients.

Effects on Gene Expression:

HDACi mediate complex effects on global gene expression by directly modulating chromatin structure via acetylation of core histones, as well as “marking” chromatin for subsequent recruitment of chromatin remodeling complexes (42). Equally and perhaps more importantly, these agents influence gene expression via acetylation of numerous non-histone proteins involved in signal transduction and transcription (6)(Table 1). In general, acetylation increases the negative charge of core histones, resulting in relaxation of chromatin structure; whereas chromatin de-condensation often enhances gene expression, the net effect of histone acetylation regarding transcriptional activity of different genes is influenced by concomitant alterations in chromatin structure mediated by DNA as well as histone methylation, and the summation of activators and repressors recruited to the respective promoters (43;44). These issues account for the fact that only ~10% of genes are modulated by HDACi, with approximately equal if not more numbers repressed as induced by these agents (reviewed in ref (9). Gene expression profiles in cancer cells mediated by HDACi of diverse structural classes including sodium butyrate, vorinostat, MS-275, TSA, and FK228 are time and dose dependent; while many similarities have been observed regarding effects of various HDACi on gene expression, some profiles appear agent specific (45–49).

The majority of such micro-array studies pertain to analysis of gene expression in cultured cancer lines; limited information is available regarding gene expression alterations in primary tumors from patients receiving HDACi. In a recent clinical trial, long oligo array techniques were used to examine global gene expression profiles in laser-captured tumor cells from pre- and post-treatment biopsies from lung cancer patients receiving FK228 infusions. Pre-treatment RNA was used as the reference for each respective post-treatment array. Considerable heterogeneity was detected in baseline as well as post-treatment gene expression profiles. Only 16 genes were induced 2 fold or more in one or more patients following FK228 treatment. In contrast, more than 1000 genes were repressed 2 fold or more in one or more patients following FK228 infusion (50). Results of these arrays were compared to a large, robust data set pertaining to gene expression profiles in laser captured lung cancer cells and adjacent histologically normal bronchial epithelia from patients undergoing potentially curative resections. Those genes which were induced or repressed

twofold or more by FK228 appeared to be down-regulated or over-expressed, respectively, in the resected primary lung cancers relative to adjacent, histologically normal bronchial epithelial cells (Figure 2).

One of the genes consistently induced by HDACi in vitro and in-vivo is p21, which is up-regulated via p53 dependent as well as p53 independent mechanisms (51–53). Activation of p21 coincides with acetylation of histones H3 and H4, methylation of several histone sites within the p21 promoter, and alterations of multi-protein complexes that regulate p21 transcription. Vorinostat as well as TSA-mediated activation of p21 coincides with dissociation of HDAC 1 and c-myc, and recruitment of RNA polymerase II within the p21 promoter (54;55). Other genes, such as Aurora B, are directly down-regulated via HDACi mediated recruitment of repressor complexes (Figure 3) (44).

It is well established that HDACi enhance activation of aberrantly methylated tumor suppressor gene promoters in cancer cells by DNA demethylating agents such as 5-azacytidine (5-AC) and 5-aza-2'deoxycytidine (DAC) (56;57). In addition, HDACi potentiate de novo induction of germ-cell restricted genes such as NY-ESO-1 and MAGE family members in cancer cells by DNA demethylating agents (58;59). Although these phenomena have been attributed to acetylation of core histones, more recent studies suggest that potentiation of 5-AC or DAC-mediated gene induction by HDACi may be more complex. For example, Xiong et al (60), observed that TSA decreases stability of DNMT3b mRNA, resulting in diminished de novo methylation activity in human endometrial cancer cells. You et al (61), observed that apicidin down-regulates DNMT1 in Hela cells; repression of DNMT1 coincided with localized deacetylation of histones H3 and H4 at the E2F1 binding site with recruitment of Rb and HDAC1, dissociation of RNA pol II, and trimethylation of H3K9 and K3K27 (repressive histone marks) within the DNMT1 promoter. Additional studies have indicated that TSA destabilizes DNMT 1 mRNA in leukemia cells (62). Zhou, et al (63), observed that vorinostat and panobinostat mediate intranuclear acetylation of Hsp90, leading to destabilization of Hsp90-DNMT 1-HDAC 1 complex, with subsequent depletion of HDAC 1, proteosomal degradation of DNMT1, and up-regulation of ER gene expression in cultured breast cancer cells. Knock-down of HDAC 1 - but not HDAC 6, induced depletion of DNMT 1 in these cancer cells. Wu et al (64) observed that FK228 and the structurally related cyclic peptide apicidin mediate demethylation of a variety of tumor suppressor gene promoters including p16, SALL-3, and GATA-4 in lung, colon, and pancreatic cancer lines. FK228 and apicidin- but not TSA, inhibited expression of G9A and SUV39H1 histone methyltransferases, thereby decreasing di- and tri-methylation of H3K9, and diminishing binding of repressive heterochromatin protein (HP) 1α and 1β, as well as DNMT1 to these promoters. Collectively, these recent studies highlight the complexity of mechanisms by which HDACi mediate epigenetic regulation of gene expression in cancer cells.

Modulation of Cell Cycle Progression:

Depending on exposure conditions, HDACi of various structural classes induce G1/S and/or G2/M arrest, and disrupt mitotic progression in normal as well as malignant cells. Cell cycle arrest mediated by HDACi coincides with decreased expression of cyclins A, B, D, and E, as

well as their respective cyclin-dependent kinases, hypophosphorylation of Rb, and induction of p21 and p27 (65).

Presently, the mechanisms contributing to aberrant mitotic progression in cancer cells following exposure to HDACi are less fully defined. HDAC3 is critical for maintaining deacetylated histone tails that become phosphorylated by Aurora B as cells enter mitosis, and inactivation of HDAC 3 induces mitotic delay and apoptosis in murine embryonic fibroblasts(66;67). Ma et al (68), observed that TSA induced prometaphase arrest in Hela cells, characterized by aberrant microtubule-kinetochore attachments, and HP1 localization at pericentromeric heterochromatin, as well as disruption of the chromosome passenger complex. TSA as well as FK228 deplete levels of several kinetochore proteins including HBUB1, CENP-E, and CENP-F, and decrease pre-mitotic phosphorylation of histone H3 in pericentromeric heterochromatin during G2, resulting in deficient assembly of kinetochores(68;69). In addition, TSA disrupts localization of the kinetochore protein BubR1, and decreases phoso-histone H3 after paclitaxel treatment(70). Park et al(71) observed that LAQ824 depletes Aurora-A in gastric cancer cells via inhibition of HDAC 6 mediated de-acetylation of Hsp90. Although Aurora B also associates with Hsp90, LAQ824 did not appear to destabilize this complex. Zhang et al (44) observed that FK228, TSA, and vorinostat inhibit transcription of Aurora A, Aurora B, and survivin in a panel of cultured lung cancer cells. Transcriptional repression mediated by these HDACi was more pronounced in cells expressing wild-type p53. Depletion of Aurora A and survivin protein levels preceded depletion of Aurora B, possibly due to combinatorial effects of these agents on transcription as well as post-translational stabilization of these proteins. Additional experiments revealed that down-regulation of Aurora B expression coincided with increased total acetylation of histone H3, decreased levels of acetylated H3K9, and dimethyl H3K4, and recruitment of MBD1, MBD2, and MBD3 to the Aurora B promoter. Diminished expression of Aurora A, Aurora B, and survivin in lung cancer cells exposed to FK228 or TSA resulted in apparent mitotic catastrophe. More recent studies indicate that panobinostat induces proteosomal degradation of Aurora A and Aurora B in renal cancer cells via inhibition of HDAC 3 and HDAC 6. Degradation of Aurora A and Aurora B coincided with G2/M arrest and apoptosis in these cancer cells (72).

Autophagic/Apoptic Effects of HDAC Inhibitors in Cancer Cells:

Tremendous research efforts have focused on molecular pathways regulating HDACi-mediated cytotoxicity in cancer cells (9;65). Depending on exposure conditions, these agents mediate caspase-independent autophagy as well as caspase-dependent apoptosis in cancer cells of diverse histologies.

Autophagy is complex process by which proteins and organelles are sequestered in autophagosomes, and subsequently degraded following fusion with lysosomes. Autophagy is induced by nuclear (but not cytoplasmic) p53 via upregulation of damage-regulated autophagy modulator (DRAM), as well as p73 in response to cellular stress(73;74). Recent studies indicate that mTOR regulates autophagy by inhibiting p73-mediated activation of a variety of genes including ATG5, ATG7, and UVRAG(75;76), and that p53 can inhibit mTOR via activation of AMPK(75).

Autophagy induced by HDACi appears related to inhibition of HDAC 1 as well as HDAC 6 (77;78). Shao et al(79) observed that sodium butyrate and vorinostat mediated autophagy as well as apoptosis in HeLa cells; constitutive over-expression of Bcl-Xl inhibited caspase activation, but did not appear to diminish cell death mediated by these HDACi. Hrzenjak et al(80) observed that vorinostat diminished mTOR expression, and mediated caspase-independent cytotoxicity in endometrial sarcoma cells via autophagic mechanisms. Furthermore, Watanabe et al(81) observed that FK228-mediated autophagy in rhabdomyosarcoma cells coincided with nuclear translocation of apoptosis-inducing factor (AIF); knock-down of AIF abrogated autophagy following FK228 exposure. Chloroquine, an inhibitor of autophagy, enhanced FK228-mediated apoptosis in these cells. Carew, et al (82) examined the relative contributions of autophagy and apoptosis regarding vorinostat-mediated cytotoxicity in cultured CML cells. Chloroquine exposure dramatically increased reactive oxygen species formation and enhanced vorinostat-mediated apoptosis in these cells. The relative contributions of autophagy and apoptosis with regard to tumor regressions in clinical settings have yet to be fully elucidated. Of particular concern are observations that depending on tissue histology/context, autophagy may be cytoprotective (83). For example, autophagy enhances anti-estrogen resistance in cultured breast cancer cells (84), and protects cancer cells from hypoxia (85).

Cancer cells exhibit a variety of defects in caspase-mediated apoptotic pathways due to upregulation of decoy receptors such as TRAIL-R3 and TRAIL-R4 that inhibit activation of death receptors by ligands such TNF or TRAIL, as well as aberrant expression of anti-apoptotic proteins including Bcl-2, Bcl-xl, and XIAP family members, which inhibit caspase activation (9;65). HDACi including TSA, FK228, vorinostat, and panobinostat, decrease expression of Bcl-2, Bcl-xl, and XIAP, and enhance expression of pro-apoptotic proteins such as BAX and BAK, thereby enhancing TRAIL-mediated cytotoxicity in a variety of cancer cells via amplification of intrinsic as well as extrinsic apoptotic pathways (86;87). Agents such as flavopiridol that potentiate mitochondrial injury enhance apoptosis mediated by HDACi in cancer cells(88). In addition, HDAC inhibitors augment apoptosis mediated by a variety of conventional chemotherapeutic agents by up-regulating death receptor 5 or other components of apoptotic pathways such as AIF, thereby enhancing caspase activation(89;90). Recently, Xu, et al (91) observed that MS275 as well as vorinostat induce TRAIL expression without altering DR4 or DR5 levels in breast cancer cells; HDACi induction of TRAIL by was mediated via SP1, and markedly enhanced adriamycin cytotoxicity in these cells.

Additional studies have examined the effects of HDACi on nuclear receptor signaling in cancer cells; results have varied depending on agents and exposure conditions, and hormone receptor status of cells used for these experiments(92). For example, TSA potentiates de-repression of ER- α mediated by DNA demethylating agents, and enhances response to tamoxifen in ER-negative breast cancer cells(93); TSA, as well as vorinostat, and valproic acid alone induce only modest up-regulation of ER-α in these breast cancer cells. Jang et al(94) observed that TSA markedly induced ER-β but not ER-α expression, and enhanced nuclear transport of ER-β, resulting in activation of ER target genes and increased tamoxifen sensitivity in ER negative cells. Fiskus et al (95) observed that LAQ824, vorinostat, and panobinostat depleted ER-α via acetylation of Hsp90, thereby diminishing response to E2,

and enhancing tamoxifen sensitivity in ER-positive breast cancer cells. Bicaku, et al (22) observed that co-treatment of cultured breast cancer cells with vorinostat or valproic acid depleted ER as well as progesterone receptor (PR), and synergistically enhanced tamoxifen-mediated cytotoxicity in ER+ or ER+/PR+ breast cancer cells. Knock-down experiments revealed that depletion of ER and PR, and potentiation of tamoxifen-mediated cytotoxicity by these HDACi was attributable to inhibition of HDAC 2 (but not HDAC 1 or HDAC 6) activity in these cells.

HDACi of various classes modulate androgen receptor (AR) expression, and enhance activity of AR-mediated blockade in prostate cancer cells(96). TSA, vorinostat and MS-275 inhibit expression of TMPRSS2-ERG fusion transcripts, and enhance apoptosis mediated by flutamide in androgen responsive prostate cancer cells in part by inhibiting translocation of AR from cytoplasm to the nucleus(97); in contrast, HDACi do not potentiate androgen blockade in AR-negative prostate cancer cells(98). Welsbie, et al comprehensively examined the mechanisms underlying the synergistic cytotoxic activity of AR blockade and HDAC inhibition in prostate cancer cells. Vorinostat and panobinostat inhibited AR-mediated activation of downstream target genes including TMPRSS2. Knock-down of HDAC 1 or HDAC 3 suppressed expression of androgen regulated genes, and mimicked the effects of HDACi exposure in these cancer cells. Interestingly, inhibition of AR signaling by HDACi was independent of AR protein depletion. Additional experiments indicated that HDACi prevent assembly of co-activator/ RNA pol II complexes after AR binds to enhancer elements of target genes.

A variety of studies have been performed to examine the effects of HDACi on retinoic acid signaling in cancer cells(100). Elegant functional genetic screening experiments revealed that apoptosis of p53-deficient murine embryonic fibroblasts expressing oncogenic ras exposed to PXD101, vorinostat, MS-275, sodium butyrate and spiruchostatin A could be inhibited by constitutive expression of RARα or the preferentially expressed antigen of melanoma (PRAME), which represses retinoic acid (RA) signaling(101;102). The anti-apoptotic effects of RARα and PRAME did not appear attributable to modulation of histone acetylation. Additional studies have demonstrated that combined valproic acid/RA or MS-275/RA regimens enhance apoptosis and decrease in-vivo growth of neuroblastoma and renal carcinoma cells(103;104).

Effects on Anti-tumor Immunity:

HDACi can potentially modulate anti-tumor immunity via numerous mechanisms including up-regulation of tumor antigens, enhancement of cellular immune recognition and lysis of tumor targets by T cells and NK cells, and alteration of T cell subsets as well as inflammatory cytokine profiles. FK228 as well as TSA enhance deoxyazacytidine-mediated activation of genes encoding cancer testis antigens such as NY-ESO-1 and MAGE family members, and augment NY-ESO-1 and MAGE expression in cancer cells exhibiting de-repression of these germ-cell restricted genes; up-regulation of antigen expression facilitates recognition of cancer cells by cytolyic T cells in vitro(105;106). Whereas HDACi such as TSA and FK228 enhance HLA expression(107), exposure to these agents does not appear to restore antigen presentation in cancer cells deficient in antigen processing(105;106).

Several recent studies indicate that HDACi can modulate TH1/TH2 effector function(108), and enhance the activity of Foxp3-positive T regulatory cells, which contribute to immune tolerance in cancer patients(109). Furthermore, TSA abrogates interferon-gamma (IFN-γ)-mediated inhibition of TNF-α-induced activation of inflammatory cytokine genes such as IL-6 and IL-8, which enhance metastatic potential of cancer cells(110;111).On the other hand, FK228 enhances NK cell- mediated lysis of tumor cells of various histologies by up-regulating DR5 (TRAIL-R2) expression without altering expression of MHC-class I, DR-4 (TRAIL-R1), MIC A/B, or FAS (CD95) on tumor cells(112). Consistent with these findings, Deirmayr et al(113) observed that valproic acid enhances expression of NKG2D ligands on AML cells, thereby enhancing their susceptibility to NK cell mediated lysis.

Sensitivity and Mechanisms of Resistance:

Whereas HDACi induce cell cycle arrest in normal as well as non-transformed cells, the proapoptotic effects of these agents are observed primarily in cancer cells. Preferential tumoricidal activity mediated by HDACi appears related, at least in part, to differential responses of transformed and normal cells to oxidative stress. HDACi such as vorinostat decrease expression of thioreduxin (TRX) in transformed but not normal cells. TRX is a scavenger of reactive oxygen species (ROS), and a hydrogen donor for numerous proteins involved in DNA synthesis and transcription. In addition, TRX inhibits apoptosis signaling regulating kinase-1 (ASK-1). Vorinostat also increases expression of TRX binding protein, an inhibitor of TRX, thereby increasing expression ofASK-1. The net result is an accumulation of ROS, which triggers apoptosis/autophagy in cancer cells(114).

Generation of ROS cannot fully account for sensitivity of cancer cells to HDACi. Increasing evidence indicates that even within given histologies, cancer lines or xenografts exhibit differential sensitivities to these agents. Several studies have been performed recently to define gene expression profiles that correlate with response to HDACi in cancer cells. Susakawa et al(115) identified a 76 gene expression signature that coincided with sensitivity of human tumor xenografts to FK228. Miyanaga et al(116) identified a nine gene expression signature that correlated with response of cultured lung cancer cells to TSA and vorinostat; modulation of three of these genes (NOG1, SEC23A: upregulated; PSNE2: down-regulated) markedly correlated with sensitivity to these HDACi. More recently, Dejligbjerg, et al(117) observed that modulation four genes (ODC1, SKI, STAT1, TYMS) correlated with belinostat sensitivity in a broad panel of cultured cell lines. Dokamanovic et al(117) observed that depletion of HDAC 7 coincided with sensitivity of cultured cancer cells of various cytologies to vorinostat and FK228; interestingly, knock-down of HDAC 7 only modestly inhibited cancer cell proliferation.

Stapnes et al(118), examined responses of cultured AML cells from 59 patients to multiple HDACi, including valproic acid, TSA, PDX101, and sodium butyrate. At high concentrations, all of these HDACi mediated dose-dependent apoptosis. However, exposure to low or intermediate doses of these agents paradoxically increased proliferation in a subset of cell lines. Expression of 25 genes with fold change ≥3.0 discriminated between FLT3-ITD+ AML cells with and without growth enhancement mediated by intermediate doses of HDACi.

An important issue regarding these studies is that HDACi sensitivity often was determined by proliferation rather than cytoxicity assays. Diminished proliferation in response to HDACi appears related, at least in part, to induction of p21, which may be cytoprotective in cancer cells exposed to chemotherapeutic agents and HDACi. Indeed, abrogation of p21 expression by agents such as flavopiridol, markedly enhances apoptosis mediated by HDACi in cultured cancer cells(119). Furthermore, HDACi increase expression of NFκB, which mediates a variety of pro-survival pathways in cancer cells(120); the relevance of NFκB activation regarding sensitivity of cancer cells to these agents is highlighted by the fact that parthenolide which inhibits NFκB function, as well as proteosome inhibitors such as bortezamib, which prevent degradation of IκB markedly enhance cytotoxicity of TSA, vorinostat, valproic acid, and FK228 in cancer cells(121–123).

Recently, Fantin et al(124) observed that activation of signal transducer and activation of transcription (STAT)-1,−3, and −5 correlated with vorinostat resistance in cultured lymphoma cells. Janus-activated kinase inhibition enhanced vorinostat-mediated cytotoxicity in these cells. Subsequent studies revealed that nuclear accumulation of STAT1 and increased levels of nuclear phospho-STAT3 in skin biopsies correlated with lack of response to vorinostat in patients with CTCL.

Observations that HDACi modulate apoptosis thresholds in cancer cells have prompted considerable interest in utilizing these agents to potentiate the effects of standard chemotherapetic regimens or radiation therapy in clinical settings(125–127). However, HDACi may induce resistance that may be clinically relevant. For instance, FK228 is a substrate for P-glycoprotein (Pgp) and multi-drug resistance-associated protein-1 (MRP1); up-regulation of Pgp appears to be a major mechanism of resistance to FK228 as well as apicidin in cultured cancer cells(128;129). Robey et al(130) observed an eight-fold increase in expression of MDR-1, which encodes Pgp, in circulating tumor cells from patients with hematologic malignancies receiving FK228. Additional studies have demonstrated that FK228 induces expression of ABCG2; chromatin alterations within the ABCG2 promoter induced by FK228 are similar to those observed in drug-resistant cells(131).

To date, the mechanisms mediating resistance to other HDAC inhibitors in cancer cells have not been fully defined. Vorinostat and valproic acid-induced resistance appears irreversible and unrelated to MDR-1 expression, and does not appear to alter sensitivity of cultured colon cancer cells to standard chemotherapeutic agents(132). Fiskus et al(133) observed that HL-60 cells selected for resistance to vorinostat, sodium butyrate, LAQ824, and panobinosat exhibited increased expression of HDACs 1, 2, and 4, yet lacked expression of HDAC 6. HL-60 cells resistant to HDACi were also resistant to etoposide, cytarabine and TRAIL, and exhibited increased proliferation in vitro and in vivo, suggesting that HDACi exposure may select for outgrowth of cancer cells with a more aggressive phenotype.

Conclusions and Future Directions

HDACi have emerged as major pharmacologic agents for cancer therapy. In all likelihood, these agents will be used in combination with standard treatment regimens. Efforts to further develop these agents should be focused on thorough evaluation of HDAC expression in

different human cancers, comprehensive analysis of the mechanisms of action of various classes of HDACi in vitro using array-based profiling techniques, and validation of recently identified prognosticators of response in clinical settings.

Synthesis of HDACi that selectively target HDACs relevant to cancer initiation/progression may enhance the anti-tumor effects while decreasing systemic toxicities of HDAC inhibition in cancer patients. For example, HDAC6 enhances oncogenic transformation(134), and modulates epithelial-mesenchymal transition in cancer cells(11); as such, selective inhibitors of HDAC6 may prove highly effective for cancer therapy. As HDAC inhibitors are further evaluated in cancer patients, it will be important for investigators remain cognizant of the potential immunosuppressive effects of these agents, given their ability to perturb T cell function and alter expression of inflammatory cytokines mediating innate antiviral and anti-tumor immunity(108;135–137).

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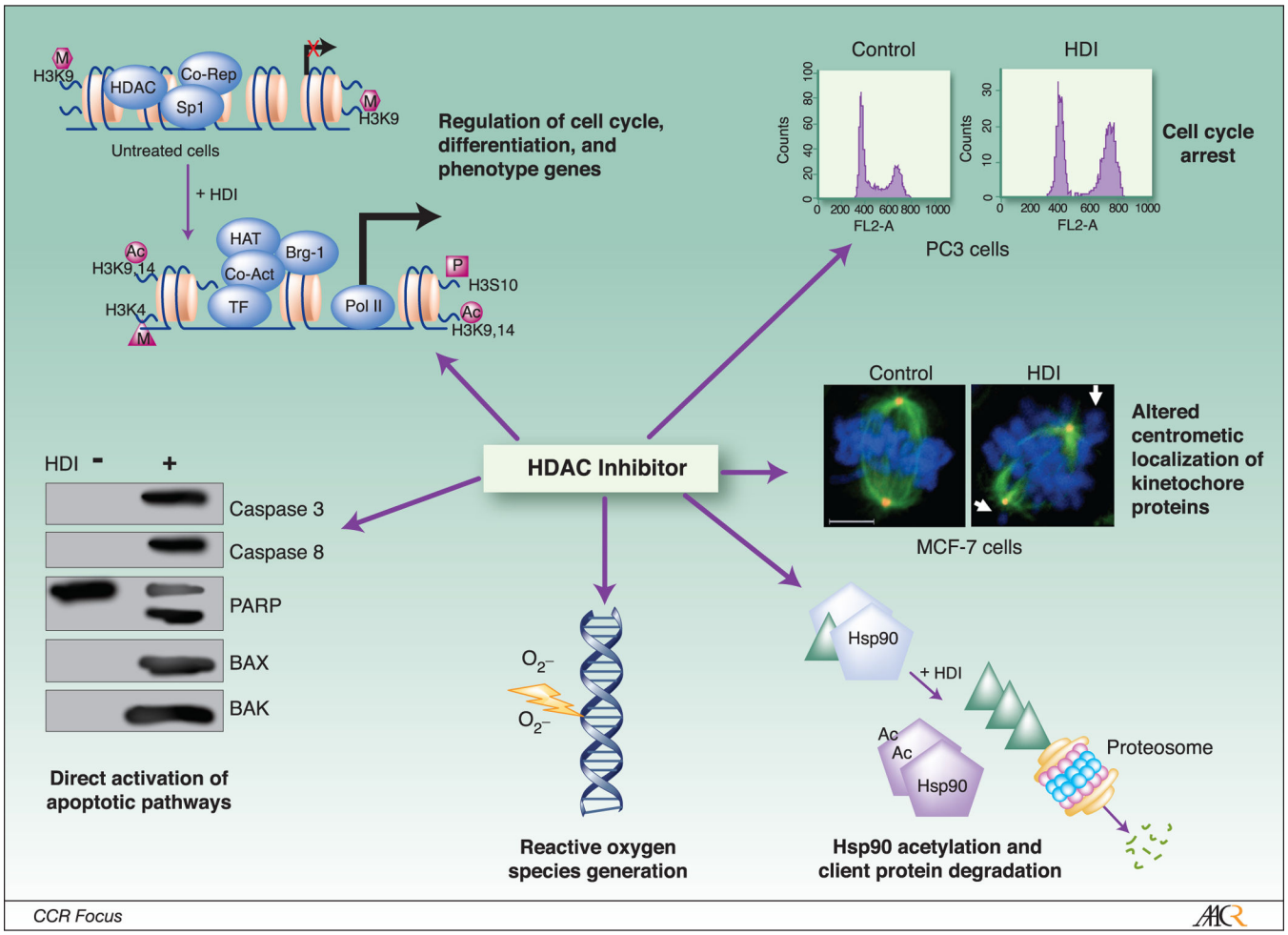

Figure 1:

Plieotropic mechanisms of cytotoxicity mediated by HDAC inhibitors in cancer cells. The kinetochore panel is reproduced with the permission of Landes Bioscience, from Robbins et al., *Cell Cycle*, 2005 (69).

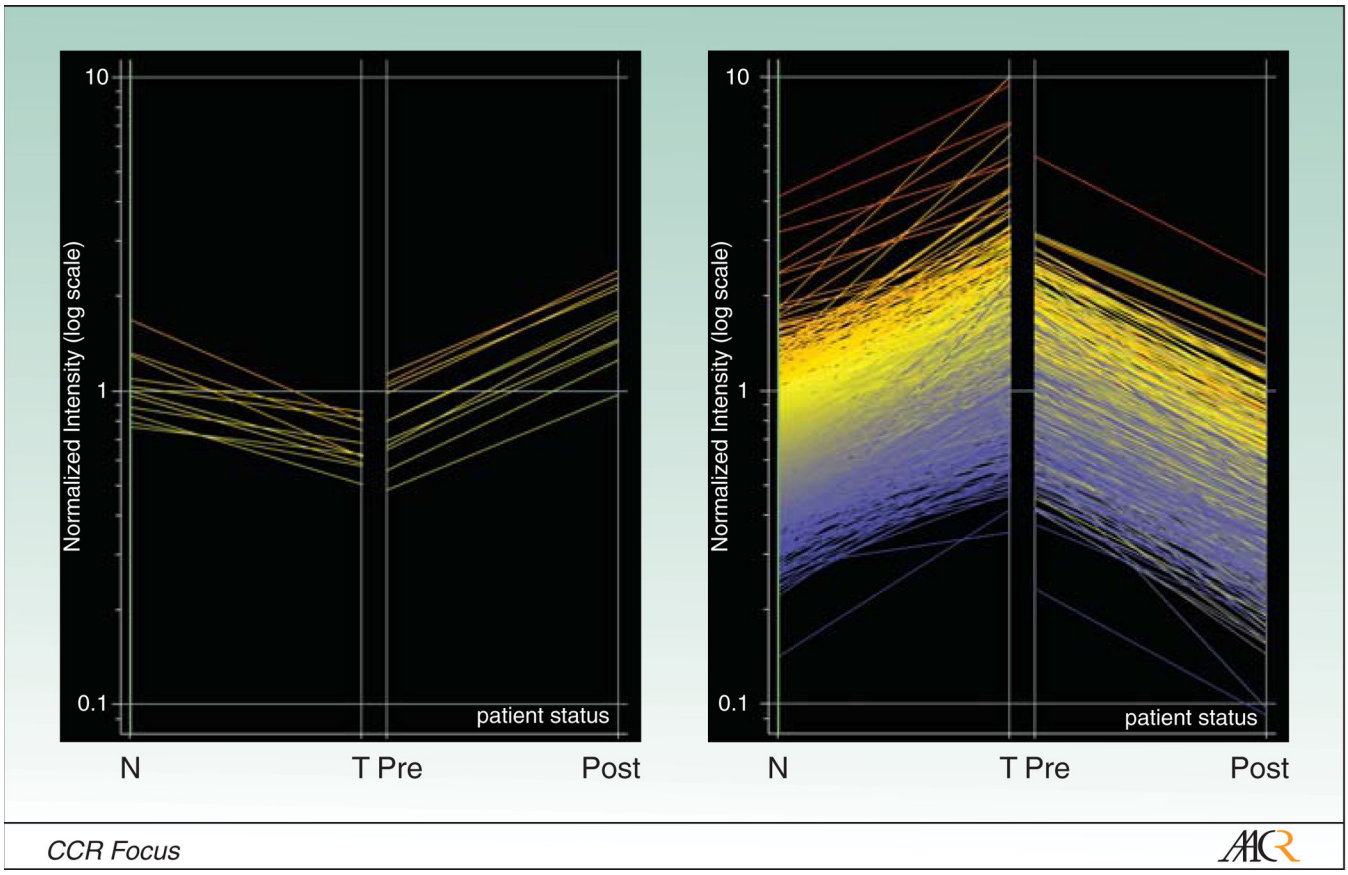

Figure 2:
Comparison of gene expression in pre/post treatment biopsies relative to paired lung cancer/normal bronchial epithelia. FK228 appears to “normalize” gene expression in lung cancer cells.

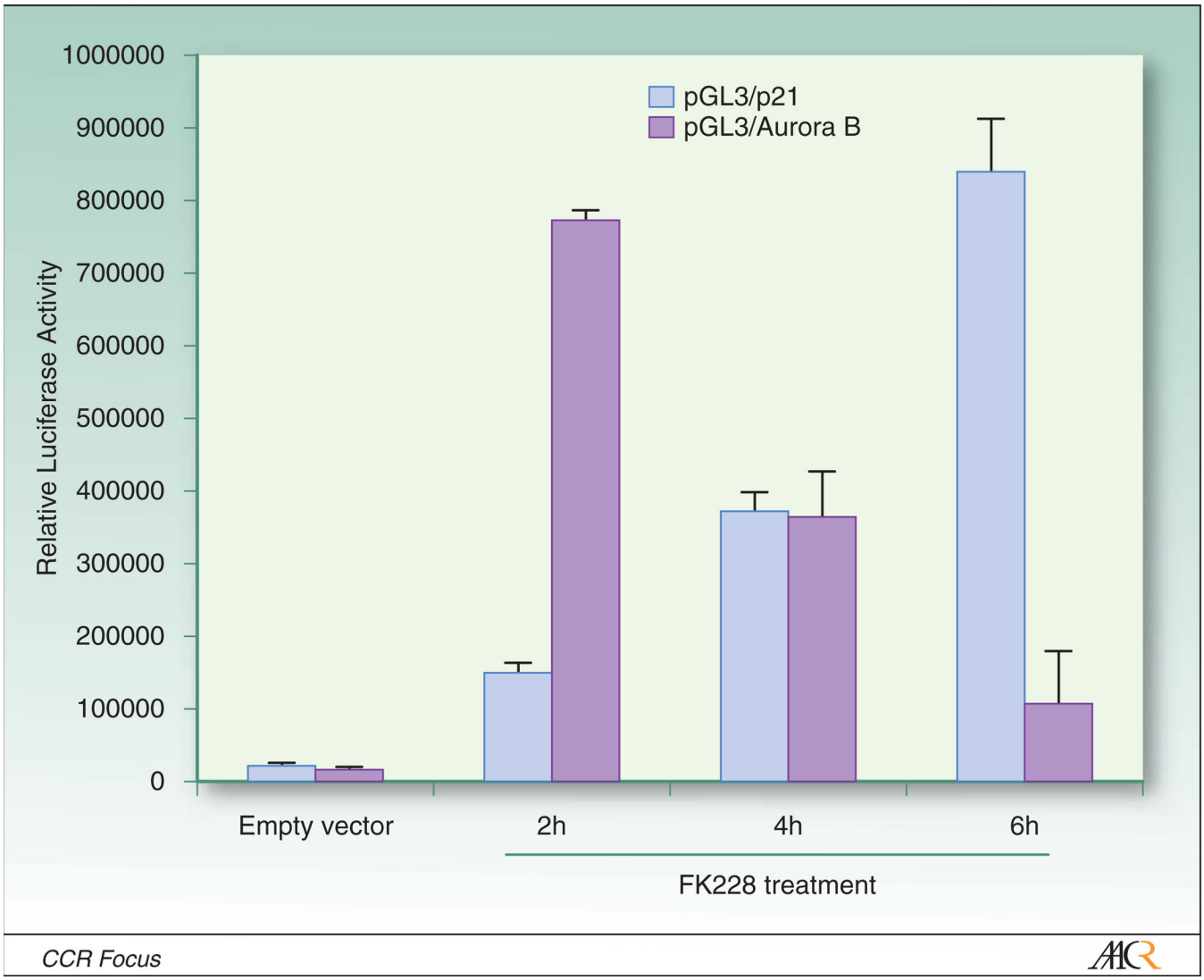

Figure 3:

Modulation of p21 and Aurora B expression in lung cancer cells following FK228 exposure. Calu-6 lung cancer cells were transiently transfected with p21 or Aurora B luciferase promoter-reporter constructs. Twenty-four hours later, cells were exposed to FK228 (25ng/ml). FK228 increased p21 reporter activity, while diminishing Aurora B promoter-reporter activity in a time-dependent manner. Full details and additional experiments contained in reference 44.

Table 1

Non-histone Cellular Proteins Targeted by HATS and HDACs

p53, p73, Hsp 90, C-MYC, H2A-2, E2F1, RUNX 3, Amod-7, STAT-3, p50, p65, HMG-A1, PLAGL2, p300, ATM, MYO-D, Sp1, β-catenin, pRb, GATA-1, YY-1, HIF-1α, STAT-1, FOX01, FOX04