Histone Deacetylase Inhibition in Hematological Malignancies

a report by
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Cancer has traditionally been perceived as a disease attributable to the accumulation of genetic abnormalities.[1] Recent research has identified the additional, prominent role of epigenetics in cancer pathogenesis. Epigenetic modifications differ from genetic mutations as they are largely reversible, covalent alterations of DNA or histone proteins. Still, they exert a dominant effect on chromatin structure and signaling, thus strongly influencing gene expression. Our understanding of the cancer epigenome is rapidly expanding due to advances in genomic techniques. As the principal regulatory components defining the epigenome are enzymes, an opportunity exists to develop targeted therapies. An emerging class of epigenetic cancer therapeutics is defined by the inhibitors of histone deacetylase (HDAC) proteins. HDAC inhibitors have already demonstrated a clinical utility in the treatment of advanced cutaneous T-cell lymphoma (CTCL). Basic and translational research has identified a compelling activity of these agents beyond CTCL, beyond cancer, and beyond even epigenetics.[2]

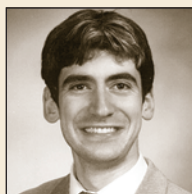## Acetylation—A Dynamic Regulatory Process

Acetylation is a type of post-translational modification that can induce structural and conformational changes in proteins in a manner analogous to phosphorylation. Much as kinases and phosphatases regulate the phosphorylation state of proteins, acetyltransferases and HDACs regulate their acetylation state.[3] For example, lysine acetylation is analogous to tyrosine phosphorylation in that both modifications are reversible and have functional consequences linked to disease biology in cancer: tyrosine auto-phosphorylation of the Abl kinase is causally linked to the development of chronic myeloid leukemia, while de-acetylation of the amino-terminal tail

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of histone proteins is linked to gene silencing in many cancers. As suggested by the name given to this class of proteins, the first deacetylases were found to act on histones, the round octomeric protein complexes tightly packaging DNA into nucleosomes.[4] More recently, a variety of other proteins have been identified that undergo reversible acetylation, including tubulin,[5] p53,[6] and Hsp90.[7] As each of these proteins is a validated target in cancer, inhibitors capable of modulating the protein acetylation state have become highly desirable.

HDACs and HDAC Inhibitors—History and Biology

The discovery of human HDACs illustrates the success of forward chemical genetics, a discovery process in which the biological activity of a small molecule leads to the identification of the cellular target and, when compelling, the optimization of a therapeutic. Trichostatin A is a natural substance that was found in 1987 to potently arrest growth and induce differentiation of leukemia cells.[8] Linking this antitumor activity to the induction of histone hyperacetylation three years later[9] sparked an interest in developing HDAC inhibitors as potential chemotherapeutics. However, the chemical optimization of trichostatin and other natural product inhibitors required the identification of the mammalian target protein, which proved elusive for several years. A breakthrough came in 1996 with the purification and characterization of the first human HDAC (HDAC1) by the laboratory of Stuart Schreiber.[10] The Schreiber laboratory was studying a structurally similar molecule, trapoxin, which was found to induce substantial phenotypic changes in cultured cancer cells. After chemically coupling trapoxin to an affinity matrix, the first human HDACs were pulled down, purified, and characterized as master regulators of gene expression. In the years that followed, additional HDACs were discovered, now defining a family of 18 enzymes divided into four classes by structure and function.[2]

Class I HDACs (1, 2, 3, and 8) are all about 50kD in size, the bulk of which is a conserved Zn-containing catalytic domain. They are typically located in the nucleus of cells, and primarily target acetylated histones *in vivo*. Class II HDACs (4, 5, 6, 7, 9, and 10) contain a Zn-binding catalytic domain and additional regulatory domains, making the resultant proteins considerably larger. They also tend to shuttle back and forth between the nucleus and cytoplasm, and have been demonstrated to target non-histone proteins. HDAC6 is unique among all HDACs as it contains two catalytically active domains.[11] Of relevance to cancer, HDAC6 has been demonstrated to target alpha-tubulin as well as the chaperone protein, heat shock protein 90 (Hsp90).[12,13] The only class IV HDAC is HDAC11, which shares homology with class I and II HDACs in its Zn+2 catalytic domain and primarily localizes to the nucleus. Class III HDACs (SIRT1, 2, 3, 4, 5, 6, and 7) possess little structural homology to the other HDAC classes and deacetylate target proteins using a co-factor NAD+-dependent chemistry. The functions of class III HDACs are not well characterized, though

they have been implicated in mitochondrial function and lifespan.

Pathways Through Which HDAC Inhibitors Affect Cancer Growth

The specific substrate proteins targeted by HDACs and thus affected by HDAC inhibitors are incompletely understood. Still, HDACs have been linked to a number of fundamental cancer processes (see *Table 1*).

Cell-cycle Arrest and Differentiation

Even prior to the identification of the cellular target, HDAC inhibitors have been known to arrest cells at the G1/S transition of the cell-cycle, allowing subsequent differentiation. This has largely been linked to the increased expression of p21WAF1, reducing phosphorylation of pRb.[14]

Repression of Pro-apoptotic Proteins

The best-characterized function of HDACs is their ability to repress transcriptional activity. HDAC inhibition has been linked to the de-repression of pro-apoptotic factors, including Fas, tumor necrosis factor-alpha-related apoptosis-inducing ligand, and death receptor 5.[15] Importantly, HDAC inhibitors have also been identified as activating the transcription of other target genes, suggesting that direct inhibition of co-repressor complexes is also accompanied by global alterations of chromatin structure, leading to activation or repression by other cis-regulatory elements.

Regulation of Hsp90 Activity

Hsp90 is a chaperone protein responsible for the proper folding and stabilization of a number of proteins, including oncogenic and anti-apoptotic factors. Researchers have recently shown that Hsp90 can be deacetylated by HDAC6, measurably affecting Hsp90 activity.[13] Inhibition of HDAC6 leads to Hsp90 acetylation and degradation of anti-apoptotic and pro-survival Hsp90-client proteins, including mutant FMS-like tyrosine kinase 3, Bcr-Abl, AKT, and c-Raf.[7]

Angiogenesis

HDAC inhibitors have been shown to decrease tumor angiogenesis in a variety of pre-clinical cancer models. This has been attributed to decreased expression of pro-angiogenesis genes such as eNos, VEGF, and bFGF.[16,17]

p53 Activation

p53 is one of the most well-characterized mediators of apoptosis in the cancer cell. HDAC inhibitors have been shown to induce hyperacetylation of both mutant and wild-type p53, leading to canonical p53-mediated pro-apoptotic responses.

HDAC Inhibitors as Single-agent Drugs—Variation on a Theme

With an industry-wide interest in developing HDAC inhibitors as drugs, a structural theme has emerged among lead compounds, which thus far target only class I, II, and IV enzymes. HDAC inhibitors possess three distinct elements: a chelation feature that binds to catalytic zinc at the active site; an aliphatic or structured 'linker' navigating the narrow hydrophobic pocket; and a hydrophobic 'capping' element docking the enzyme surface. Following this structural dogma a variety of HDAC inhibitors have been developed, of which dozens are in various stages of pre-clinical and clinical development. Though a definitive analysis of inhibitor potency for each enzyme has not been reported, these first-generation agents are generally non-selective. Available clinical data have identified single-agent activity against hematologic malignancies: non-Hodgkin's lymphoma (NHL), acute myeloid leukemia, and CTCL. Thus far, one

Table 1: Acetylation Impacts the Hallmarks of Cancer

Cell Cycle
Induction of p21 and p27; activation of p23; degradation of cyclin D1, cdk4
Metabolism
Protein trafficking; degradation of pAKT; mitochondrial function
Stemness
Heterochromatin structure; differentiation
Angiogenesis
HIF1-a, VEGF, Tie2, eNOS, bFGF
Oncogenes
EGFR, HER-2, Flt3, Bcr-Abl, Bcl-6
Apoptosis
TRAIL, DR5 induction; pAKT, Bcl-2 downregulation
Metastasis/Invasion
a-tubulin acetylation; MTA1 downregulation

HDAC inhibitors exert a therapeutic effect through multiple pathways in the cancer cell. HDAC inhibitors induce growth arrest, differentiation, and apoptosis, leading to decreased proliferation, increased cell death, and the potential for a therapeutic effect in cancer treatment.

deacetylase inhibitor has been approved by the US Food and Drug Administration (FDA). Vorinostat (Zolinza, suberoylanilide hydroxamic acid, SAHA; Merck Research Laboratories, Rahway, New Jersey) received approval for the treatment of CTCL based on performance in a phase II study of 74 patients with advanced disease (stage IIB or higher). Oral vorinostat demonstrated an overall response rate of 29.7%, with one observed complete response. Overall, the clinical experience with vorinostat suggests that HDAC inhibitors are likely to be well tolerated. Dose-limiting toxicities with HDAC inhibitors have proved to be generally uniform: fatigue, diarrhea, nausea, and thrombocytopenia.[18] A number of other molecules are in advanced clinical testing for hematological malignancies. Among these are molecules with markedly greater potency for deacetylases, including the synthetic LBH589 (Novartis Pharmaceuticals, Basel, Switzerland) and the natural product depsipeptide FK228 (Romidepsin; Gloucester Pharmaceuticals, Cambridge, Massachusetts). LBH589 has demonstrated compelling pre-clinical activity in models of multiple myeloma and is currently under testing in the setting of relapsed, refractory disease as a single agent administered orally.[19,20] The clinical potential of LBH589 has been suggested in a phase I study in patients with advanced CTCL.[21] An interim analysis of FK228 suggests activity in CTCL and relapsed peripheral TCL.[22] Higher potency agents such as these display markedly enhanced *in vitro* antineoplastic activity, but tolerability in large cohorts has yet to be determined. In particular, a key challenge for HDAC inhibitors concerns prolongation of cardiac repolarization, drug-induced torsades de pointes and sudden cardiac death.[23] Consequently, electrocardiographic monitoring is an essential component of pre-clinical and post-marketing studies and use in patients with underlying structural heart disease or dysrhythmia, or in patients who are taking QT-prolonging drugs, should be avoided.

HDAC Inhibitors in Combination Chemotherapeutics

HDAC inhibitors display potent antineoplastic activity as single agents in a variety of disease models. However, the use of HDAC inhibitors in combination with pre-existing chemotherapies appears to be the most promising application beyond hematological malignancies. A few promising combinations are currently in clinical trials, based on mechanistic hypotheses.

A particularly illustrative example is the combination of certain HDAC inhibitors with bortezomib in multiple myeloma. The proteasome inhibitor bortezomib is

a currently FDA-approved medication for relapsed and refractory multiple myeloma. However, while bortezomib shows broad potency against myeloma cell lines *in vitro*, it has a limited response rate as a single agent.[24] HDAC6 has been implicated as having a fundamental role in aggresome formation, an alternate pathway of protein degradation.[25] Consequently, combinations of HDAC inhibitors capable of inhibiting HDAC6 and bortezomib have been studied in models of multiple myeloma and other secretory malignancies, such as pancreatic cancer. Cell-based studies have revealed a synergistic effect of combination treatment with bortezomib and the selective HDAC6-inhibitor tubacin, validating this strategy.[26] These findings were replicated with LBH589, a pan-deacetylase inhibitor, prompting a clinical study in multiple myeloma.[19] Another contribution to the tumor-selective cytotoxicity of this combination is mediated by Hsp90 hyperacetylation, another consequence of HDAC6 inhibition. Hsp90 is a validated target in multiple myeloma (MM). Perturbation of Hsp90 function results in misfolded protein stress, degradation of oncoproteins, and apoptosis. Combination studies in MM with bortezomib are planned using other HDAC inhibitors as well, including vorinostat, FK228, and MS275 (Syndax Pharmaceuticals, San Diego, California). Many combination studies are warranted, based on biological hypotheses and pre-clinical studies. HDAC inhibition impairs nuclear hormone receptor signaling, both through inhibition of nuclear deacetylases and by increasing receptor degradation. Consequently, combinations of HDAC inhibitors and modulators of androgen or estrogen receptor function are desirable. Recently, hyperacetylation of the Ku protein was reported following HDAC inhibitor therapy *in vitro*. Acetylation of Ku impairs DNA binding and thus its function as a key component of the DNA double strand break repair complex.[27] Combination studies of HDAC inhibitors with anthracycline chemotherapeutics might therefore be pursued in sensitive tumors, such as MM, NHL, and prostate and ovarian cancer.

Future Directions

All of the HDAC inhibitors currently in clinical development inhibit multiple HDAC isoforms. Vorinostat has been shown to inhibit most class I and II HDACs at pharmacologically achievable concentrations. As the biology of individual isoforms is elucidated in the scientific literature, selective inhibitors will become increasingly desirable. Already, there is a pressing need for selective inhibitors of HDAC6, as above. Consequently, we have endeavored to develop highly potent and selective inhibitors of HDAC6 in our laboratory. Another goal of developing selective inhibitors concerns toxicity. The class effect of HDAC inhibitors on cardiac repolarization, constitutional symptoms, and platelet count suggests these are on-target toxicities. Isoform-selective HDAC inhibitors would therefore be expected to greatly expand the therapeutic window.

The use of HDAC inhibitors in the laboratory as chemical probes has revealed therapeutic opportunities beyond the treatment of cancer. Studies have demonstrated the induction of fetal hemoglobin in patients with thalassemia using butyrate, now known to be a weak HDAC inhibitor. Recent *in vitro* studies with potent HDAC inhibitors have validated this approach to induce gamma globin gene expression and support the study of these agents in beta-thalassemia and sickle cell disease. HDAC inhibitors also hold promise in neurodegenerative and psychiatric diseases. Recent laboratory studies of Huntington's disease using the tool HDAC6 inhibitor, tubacin, suggest that HDAC6 inhibition ameliorates the characteristic microtubule defect leading to impaired vesicle trafficking.[28] Other studies have suggested that HDAC inhibitors may also improve learning and memory.[29] Disparities between the deacetylases of micro-organisms also establish the feasibility of developing well-tolerated, antimicrobial deacetylase inhibitors.

In summary, deacetylases have emerged as appealing, tractable targets for therapeutic discovery and development in cancer. As the cellular biology of HDAC protein function unfolds and as second-generation, isoform-selective inhibitors are brought forward as therapeutics, we can expect a broad and meaningful clinical impact. ■

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