Meeting Report

Clinical translation of epigenetics in cancer: eN-CORe—a report on the second workshop

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Abstract

Recent advances in understanding the role that epigenetics plays in cancer pathogenesis and understanding the mechanisms through which these processes regulate gene expression have stimulated considerable interest in developing clinically viable antineoplastic agents that target enzymatic components of transcriptional regulatory complexes responsible for the establishment of pathologic epigenetic modifications that lead to deregulated gene expression in cancer. In January 2003, a workshop was held in Baltimore to discuss the therapeutic potential of several agents that can modify chromatin structure. A follow-up meeting on ''Clinical Translation of Epigenetics in Cancer'' was held in Charleston, SC, in January 2005. The aim of this workshop was to assess the progress that has been made over the past 2 years in bringing effective therapeutic protocols that use agents capable of reverting pathologic epigenetic changes into the clinic. The meeting was attended by ~70 investigators and included formal presentations, panel group discussions, and two breakout sessions that addressed targeted therapies in hematologic and solid tumors. The aim of this article is to summarize topics discussed at this workshop and highlight conclusions as to the immediate and long-term future of epigenetic therapy in cancer. [Mol Cancer Ther 2005;4(11):1810–9]

Received 7/6/05; revised 9/2/05; accepted 9/14/05.

Grant support: Pharmion, MGI Pharma, Celgene, Gloucester Pharmaceuticals, Merck, Novartis, Schering AG, and Waxman Foundation.

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Note: Report on ''Clinical Translation of Epigenetics in Cancer,'' Charleston, SC, January 2005.

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doi:10.1158/1535-7163.MCT-05-0230

Introduction

Although genetics have played a dominant role in cancer research, epigenetics (heritable changes in gene function that do not involve alterations in DNA sequence) has become equally important in this field. The potential reversibility of epigenetic modifications renders the enzymes that are responsible for establishment of tumorigenic epigenetic changes important targets for drug development. Central to the epigenetic control of gene expression is the histone code through which input signals can be translated into a heritable pattern of gene expression defining cellular output states (Fig. 1). The code is an array of post-translational modifications (acetylation, phosphorylation, methylation, ubiquitination, and sumoylation) of NH_2-terminal tails of core histone and to a lesser degree their globular domains. Cross-talk between different histone modifications, as well as DNA methylation, seems complex. In mammalian cells, histone H3 Lys^9 (H3K9) methylation and histone hypoacetylation are usually associated with methylated DNA, heterochromatin, and gene silencing. Histone hyperacetylation and H3K4 methylation are associated with unmethylated DNA, euchromatin, and gene expression (Fig. 2). The effects of DNA methylation and the histone code are due, at least in part, to modification-specific recruitment of factors, such as heterochromatin-associated proteins (HP1) and methyl-binding domain proteins, which establish and maintain higher order of chromatin structure.

Two nonexclusive models have emerged recently to explain the establishment of pathologic epigenetic changes. In a stochastic model, overexpression of a given component of the machinery responsible for writing epigenetic code may increase probability of its mistargeting, causing deregulated expression of a gene important for tumorigenesis. This is consistent with experimental findings indicating overexpression of some histone- and DNA-modifying enzymes in cancer. In the recruitment model, on the other hand, chromatin-modifying complexes are inappropriately targeted to regulatory regions of specific genes by fusion oncoproteins. This has been shown to play a role in some subtypes of acute myelogenous leukemia (AML), such as AML1/ETO and PML-retinoic acid receptor (RAR) α. Because in both cases aberrant gene expression patterns in cancer are a consequence of abnormal epigenetic changes established through inappropriate targeting of various histone- and DNA-modifying enzymes, the development of therapeutic approaches that pharmacologically target these enzymatic activities is an attractive strategy. Another plausible therapeutic

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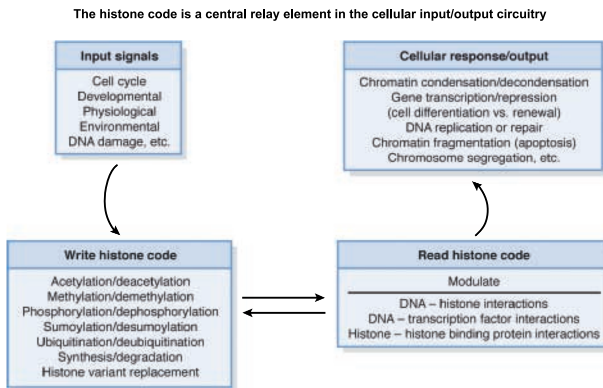

Figure 1. An input signal leads to alterations to the core histones that cause combinations of histone modifications (or replacement with histone variants). These changes to the core histones of the nucleosome modulate interactions between components of the chromatin complex, which in turn affect further modifications on the histones. The interplay between these two steps reaches a balance at particular loci, which may be transient or longer term in nature, and the new chromatin state elicits a cellular response, such as differentiation or gene expression.

intervention is the disruption of specific interactions between a DNA-targeting transcription factor oncoprotein and an associated complex containing such enzymatic activities. Both of these approaches were discussed during the meeting.

DNA Methylation, Histone Code, and Gene Expression

In humans, DNA methylation can occur in a fifth carbon position at a cytosine base located immediately 5′ to a guanosine (the CpG dinucleotide). Fifty percent of RNA polymerase II–dependent promoters possess clusters of CpG dinucleotides called CpG islands. In contrast to normal dividing cells, methylation of CpG islands is commonly observed in cancer cells. Promoter methylation is associated with transcriptional repression of adjacent genes. Genes silenced by DNA hypermethylation are usually involved in important developmental pathways and regulate multiple cell functions. The acquisition of promoter methylation occurs very early in cancer progression accompanied by other specific epigenetic changes that together inhibit transcription (see Fig. 2). Certain epigenetic modifications seem dominant to others in terms of regulating gene expression. For example, although hypermethylated genes may be silenced through recruitment of transcriptional corepressors, including histone deacetylases (HDAC), reexpression cannot be accomplished through HDAC inhibition alone. Treatment of cells with DNA methyltransferase (DNMT) inhibitors is associated with reversal of specific epigenetic marks. For example, reactivation of gene expression by azacytosine nucleosides in the *MLH* gene in colon cancer is specifically

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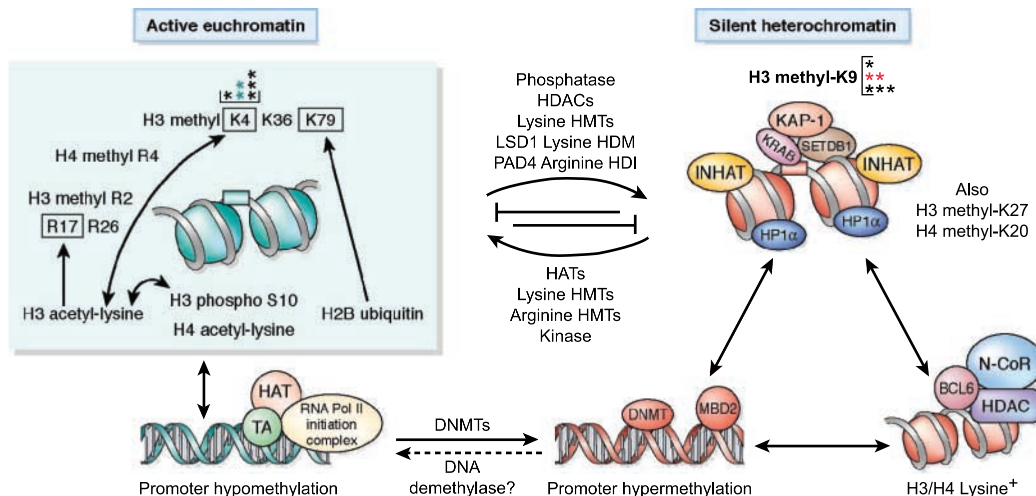

Figure 2. Schematic representation of epigenetics associated with active and silenced loci. *Left,* active euchromatin. Various biochemical markers found on the core histones are boxed in green. *Reciprocal arrows,* region where a particular marker can influence the acquisition of another; *single arrows,* a situation where the deposition of a marker enhances or has been shown to be necessary for the acquisition of another. The reciprocal arrows between promoter hypomethylation and euchromatic histone markers refer to the reinforcement of the active state that occurs as a result of histone acetyltransferases (*HAT*; e.g., gaining access to ''open'' chromatin and depositing acetyllysine markers that, in turn, provide anchorage sites for coactivators and components of the transcription initiation complex). *TA,* a transcriptional activator. *Right,* silent heterochromatin. The types of biochemical markers important in differentiating heterochromatin from active euchromatin are in red. *Lysine+*, positively charged, unacetylated histone H3 or H4. Recent evidence suggests that each marker can influence the acquisition of the other two (*reciprocal arrows*). Initiation of the heterochromatic state may occur through sequence-specific DNA-binding proteins that promote either histone methylation, such as KRAB domain zinc finger transcriptional repressors, or histone deacetylation, such as BCL-6, and subsequent recruitment of specific corepressors and the general silencing machinery. Gene silencing and formation of heterochromatin may proceed through changing the balance of dynamic processes (e.g., histone acetylation/deacetylation) or deposition of long-term markers, such as DNA or histone methylation. Degree of H3K9 methylation is indicated with asterisks, with dimethylation, which is preferably lost from a silenced locus following treatment with an azacytosine analogue, in red. Maintenance of the repressive heterochromatic state is achieved through binding of proteins to unmodified histone tails or to tails possessing a neutral charge (the INHAT complex), to specifically methylated residues (HP1α for H3K9), or to methylated CpG dinucleotide sequences (methyl DNA-binding proteins). The enzymes responsible for the transition between the active and silent chromatin states are indicated. *Dashed line,* activity of a putative DNA demethylase, which still remains to be identified.

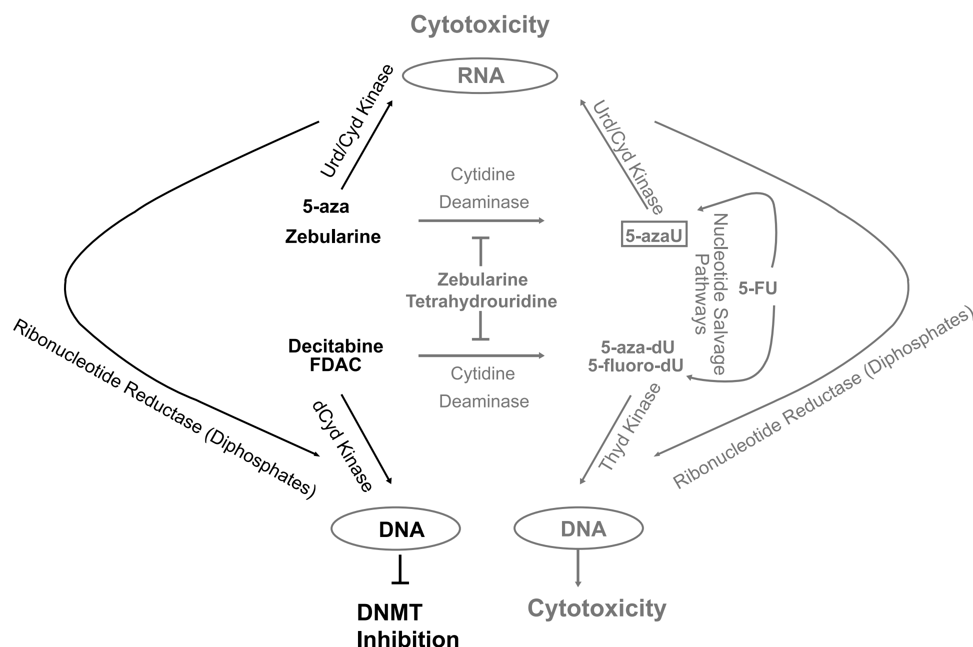

Figure 3. Pathways of nucleoside analogue metabolism. Demethylating agents, such as 5-azacytidine, decitabine, and 5-fluoro-2′-deoxycytidine, may be enzymatically converted to their uridine equivalents (*U* or *dU*) by cytidine deaminase, the activity of which is blocked by zebularine and tetrahydrouridine. Deoxyribose-containing compounds are sequentially phosphorylated by deoxycytidine (*dCyd*) or thymidine (*Thyd*) kinase and ribose-containing compounds by uridine/cytidine (*Urd/Cyd*) kinase to yield nucleotide triphosphates. Zebularine is assumed to follow the same pathway as 5-azacytidine. 5-FU, which lacks a sugar group, is metabolized initially through nucleotide salvage pathways. When 5-FU is converted to 5-fluoro-dUMP (*5-fluoro-dU*), it serves as a potent inhibitor of thymidylate synthase. The ribose moiety of nucleotide diphosphates may be converted to deoxyribose by ribonucleotide reductase. Ribose-containing compounds are incorporated into RNA, which is cytotoxic to the cell. Those compounds containing deoxyribose are incorporated into DNA, where the uridine nucleotides cause cytotoxicity, and the cytidine compounds can exert more specific activities by blocking DNMT through covalent trapping of the enzyme. Metabolic pathways leading to nucleoside analogues that do not inhibit DNA methylation are in gray. It should be noted that this is simplified schematic and the metabolic pathways *in vivo* are more complex.

associated with depletion of dimethylated H3K9 but not H3K9 trimethylation, associated with pericentric heterochromatin, nor with H3K27 methylation, a mark of facultative heterochromatin (Baylin).[5] Withdrawal of the DNMT inhibitor is associated with reestablishment of the silencing marks. The sequence of events (DNA or histone methylation) and enzymes that specifically act to reestablish DNA methylation in cancer cells remains poorly understood.

Coregulator recruitment by DNA-binding transcriptional activators and repressors seems highly specific in terms of promoters with which they associate in response to specific signaling pathways (Emerson).[5] It is likely that deregulated gene expression in cancer is associated with such specific interactions between transcription factors and their coregulators, allowing therapeutic targeting by small molecules. However, recruitment of corepressors to a heterochromatic nuclear region can be readily reestablished, possibly reflecting the extent of other epigenetic changes, such as degree of H3K9 methylation, increasing the challenge of targeting these mechanisms therapeutically (Rauscher).[5]

[5] Contributors whose data or presentation are included in this document are referenced by surname in the text and listed alphabetically in Appendix 1.

Current drugs that target DNMT are highly unstable. Metabolic pathways responsible for their catabolism are displayed in Fig. 3. A stable, potentially orally bioavailable analogue, zebularine, restored expression of a methylated *p16* tumor suppressor gene, reducing tumor growth (Jones).[5] This agent also inhibited mouse mammary tumor growth when given in drinking water (Sausville).[5] Other agents promoted as direct DNMT inhibitors (epigallocatechin gallate, psammaplin A, and procainamide), failed to restore expression of a methylated gene in cultured cells (Jones).[5] Treatment with DNMT inhibitors has been associated with activation of micro-RNA (miR-127 and miR-136) expression, potentially accounting for down-regulation of specific genes following treatment with azacytosine analogues (Jones).[5]

Specificity of nucleoside analogues for neoplastic cells may be due, at least in part, to a high level of expression of uridine/cytidine kinase in cancer cells (Jones; see Fig. 3).[5] Inhibition of deamination by the cytidine deaminase inhibitor tetrahydrouridine may enhance specific antineoplastic activities. This approach has been used with 5-fluoro-2′-deoxycytidine (Fig. 4), currently in phase I trials (Newman).[5] Zebularine can also inhibit cytidine deaminase and has shown synergy *in vitro* and *in vivo* with 2′-deoxy-5-azacytidine (decitabine; Momparler).[5]

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Molecular Monitoring of Epigenetic Changes and Gene Expression

Current translational studies of therapies targeting epigenetic changes vary widely in techniques used to monitor epigenetic changes and gene expression. Approaches include those monitoring individual silenced genes (such as methylation-specific PCR) and those monitoring epigenetic changes genome-wide. Changes in global methylation have been monitored in multiple DNA repeat sequences, such as Alu and long interspersed nucleotide elements following bisulfite modification and PCR (Yang).[5] Such sequences are heavily methylated. The PCR product, representing a pool of ~15,000 genomic loci, can be used for direct sequencing, selective restriction digestion, or pyrosequencing to quantify DNA methylation with a very high degree of reproducibility. Translational studies to date have been focused on methylation changes and changes in expression of genes during trials of DNMT inhibitors in myeloid malignancies. These techniques have detected changes in methylation and expression of specific genes, such as *p15*^{INK4B}, as well as temporary changes in global methylation following treatment with DNMT inhibitors. However, a clear relationship between molecular changes and clinical responses has not yet been established.

It is likely that future studies will use newer techniques based in genomics. Restriction landmark genomic scanning has been used to show widespread nonrandom CpG island methylation in cells from patients with chronic lymphocytic leukemia when compared with normal CD19-selected B cells (Plass).[5] CpG island microarrays have been used to identify aberrantly methylated CpG islands in acute promyelocytic leukemia cells; these were not reversed with retinoic acid treatment. Similar CpG island analysis in ovarian cancer revealed impressive tumor stage-specific methylation patterns (Futscher).[5] The feasibility of bringing these genomics-based approaches into translational studies based in clinical trials remains to be shown.

Molecular Mechanism of HDAC Inhibitors and Mechanism-Driven Combinatorial Applications of HDAC Inhibitors

Although HDAC inhibitors undergoing preclinical and clinical development all share the ability to inhibit that class of enzymes, the molecular mechanisms underlying their clinical activity may be complex. Histone acetylation is required for repairing DNA double-strand breaks by the nonhomologous end-joining pathway (Rassool).[5] Agents that induce double-strand break, such as ionizing radiation, cause phosphorylation of a variant histone, H2AX, which serves as a signal for activation of nonhomologous end-joining DNA repair pathway. Phosphorylation of H2AX by a phosphatidylinositol 3-kinase family member is also associated with enhanced histone acetylation; HDAC inhibitors were found to stimulate repair and possibly induce H2AX phosphorylation. Knockdown of

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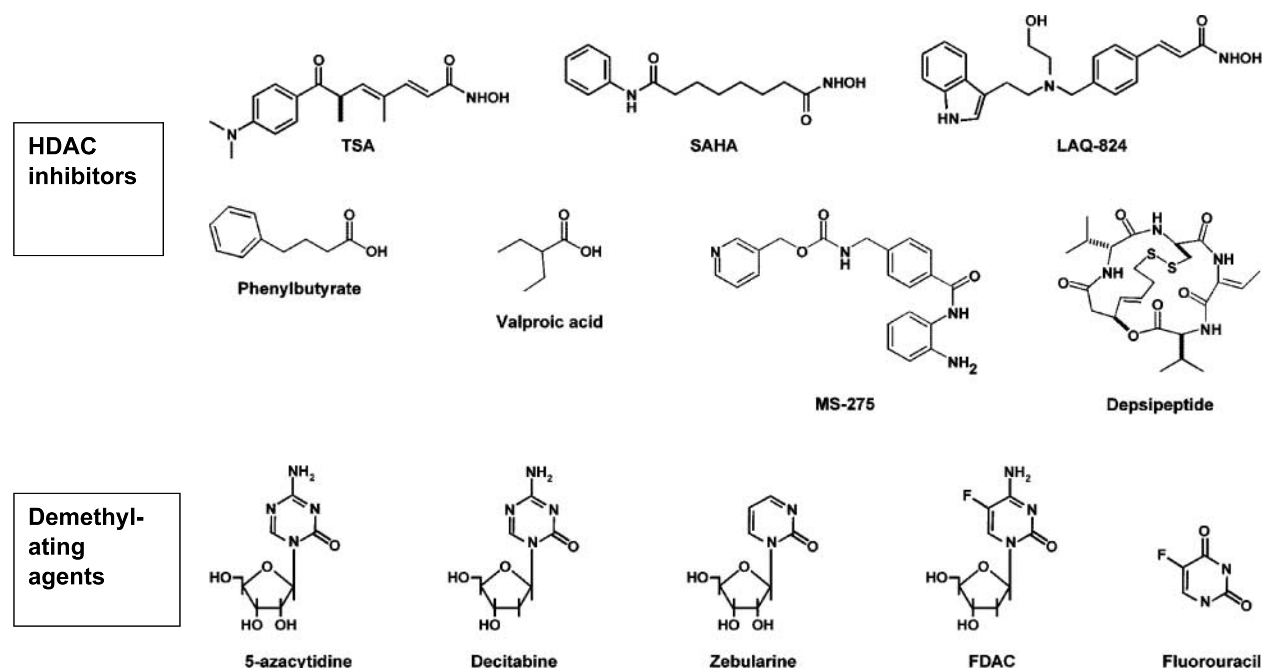

Figure 4. Selected compounds that form the basis of epigenetic therapy. The chemical structures of various HDAC inhibitor and demethylating agents are indicated. *Top,* examples of the hydroxamic acid – based HDAC inhibitor trichostatin A (*TSA*), SAHA, and LAQ-824, with structures of the short-chain fatty acids phenylbutyrate and valproic acid and the benzamide MS-275 and microbially derived depsipeptide. *Bottom,* structures of selected demethylating agents: 5-azacytidine, decitabine, zebularine, 5-fluoro-2′-deoxycytidine (*FDAC*), and 5-FU.

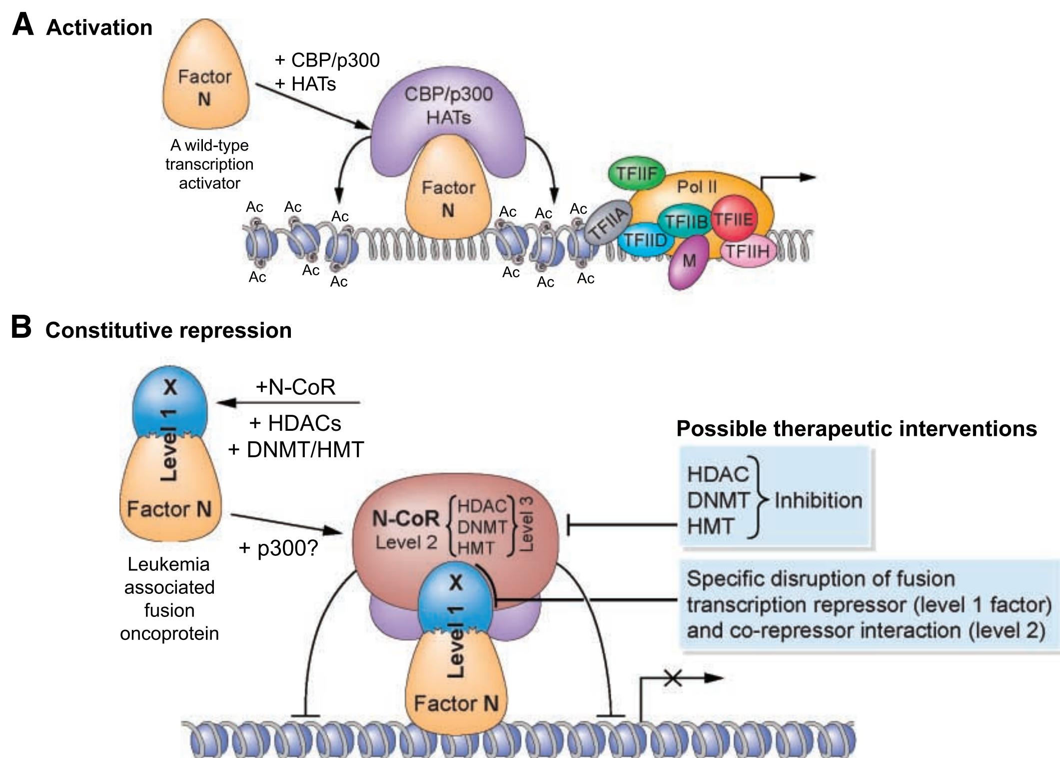

Figure 5. A model for the molecular mechanism of a transcription factor fusion oncoprotein action in leukemogenesis and possible modes of therapeutic intervention. **A,** a wild-type factor, such as AML1 or RARα, recruits histone acetyltransferase containing protein complexes to activate gene transcription and promote hematopoietic cell differentiation. Histone acetyltransferases add acetyl groups (*Ac*) to lysine residues in NH_2-terminal tails of core histones (H3 and H4), thus allowing formation of more open chromatin and markedly enhancing accessibility of a given promoter to the basal transcriptional machinery. **B,** in a fusion oncoprotein (*Level 1*), the ability of its X moiety to bind level 2 N-CoR [which serves as a platform for recruitment of level 3 proteins, such as HDAC, histone methyltransferase (*HMT*), and DNMT] turns the N factor into a constitutive repressor. Potential therapeutic interventions range from inhibition of enzymatic activities associated with transcriptional repression (level 3 and possibly least specific) to inhibiting directly the function of a level 1 fusion oncoprotein (e.g., PML-RARα with ATRA and/or arsenic trioxide), which would be the most specific. Developing small molecules that can disrupt interaction between level 1 and 2 proteins is a novel option that may also be highly specific.

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H2AX using small interfering RNA reduced trichostatin-dependent nonhomologous end-joining repair, highlighting the importance of H2AX for this process. Because treatment with HDAC inhibitor results in enhanced misrepair, HDAC inhibitors may kill cells by activating DNA damage response pathways. The lower frequency of mismatch repair in normal cells may provide HDAC inhibitor selectivity to malignant cells.

Tumor cell death in response to the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) and MS-275 was associated with an increase in reactive oxygen species and decreased scavenger activity (Marks).[5] One mechanism underlying the resistance of normal cells to the activity of SAHA could be their ability to induce thioredoxin in contrast to cancer cells. Consistently, knockdown of thioredoxin expression with small interfering RNA sensitized cells to SAHA-induced caspase-independent apoptosis.

SAHA induced the enzyme 15-lipoxygenase-1, which metabolizes linoleic acid to 13-*S*-Hydroxyoctadecadienoic acid. This metabolite is known to increase differentiation, inhibit cell growth, and mediate apoptosis. In colon carcinoma cells, SAHA treatment increased levels of alkaline phosphatase and induced apoptosis, all of which could be inhibited by caffeic acid, an inhibitor of the 15-lipoxygenase-1 enzyme, suggesting a role for 15-lipoxygenase-1 in mediating the effects of SAHA on colorectal cancer cell death (Lotan).[5] In mantle cell lymphoma, SAHA treatment led to very rapid decrease of cyclin D1 protein; inhibition of the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathway, which ultimately leads to decreased activities of translation initiation factors, decreased translation of cyclin D1 and led to subsequent apoptosis (Koeffler).[5]

The HDAC inhibitor LAQ-824 induces the cyclin-dependent kinase inhibitors p21 and p27 but also cooperates in apoptosis induction with Abl kinase inhibitors, such as imatinib, as well as heat shock protein 90 inhibitor 17-allyamino-17-demethoxygeldanamycin (Bhalla).[5] Enhanced acetylation of heat shock protein 90 was observed following treatment with the HDAC inhibitor possibly mediated through inhibition of HDAC6. LAQ-824 also induced tumor necrosis factor–related apoptosis-inducing ligand receptor expression and enhanced TRAIL-mediated apoptosis.

SAHA synergizes with the cyclin-dependent kinase inhibitor flavopiridol (Grant)[5] potentially through inhibition of SAHA-induced nuclear factor-κB activation and p21 up-regulation, steering the effects of SAHA from p21-dependent differentiation to apoptosis. Agents that can activate nuclear factor-κB pathway provided a switch from apoptosis to differentiation induction for several HDAC inhibitors; nuclear factor-κB can be activated by acetylation. HDAC inhibitors also induce reactive oxygen species leading to induction of ceramide through down-regulation of AKT/extracellular signal-regulated kinase signaling. Down-regulation of nuclear factor-κB also diminishes manganese superoxide dismutase/superoxide dismutase 2 activities leading to higher levels of reactive oxygen species. The observation that proteosome inhibition can have similar effects has led to clinical trials of SAHA plus bortezomib (Grant and L. Gore).[5]

HDAC inhibitors reverse epigenetically silenced RARβ2 expression and could thus potentially restore retinoid sensitivity to resistant epithelial tumors. Such a model has been developed using MS-275 with 13-*cis*-retinoic acid in urologic tumors *in vitro* and *in vivo* and in breast cancer models (Pili).[5] HDAC inhibitors have additive antiangiogenic activity when combined with vascular endothelial growth factor receptor inhibitors (LAQ-824 and PTK787; Pili),[5] leading to an ongoing clinical trial of the anti–vascular endothelial growth factor antibody bevacizumab and SAHA in renal cell cancer.

The HDAC inhibitor phenylbutyrate, in combination with IFN and indomethacin, inhibits the regrowth of human colon cancer cells after 5-fluorouracil (5-FU) treatment (Sung).[5] This led to a phase I study of 5-FU followed by phenylbutyrate or phenylbutyrate plus indomethacin. The addition of IFN increased toxicity (Sung and Waxman).[5]

Clinical Applications of DNA Methylation Inhibitors

Although the two clinically available DNMT inhibitors, 5-azacytidine and decitabine (Fig. 4), are >20 years old, the optimal dose schedule for reversal of promoter methylation for these agents has not been established. Decitabine is incorporated to DNA within 5 minutes following a single exposure; however, decitabine is S phase specific and may not be active against a small population of nondividing cancer stem cells (Issa).[5] The optimal decitabine concentration for reversal of DNA methylation is 0.2 to 2 μmol/L. Issa et al. have studied 15 mg/m^2 1-hour infusion repeated daily times 5, daily times 10, 20 $mg/m^2/d \times 5$ days, and 10 $mg/m^2/d \times 10$ days. High response rates have been seen in several of these dosing schemata, associated with transient decreases in long interspersed nucleotide methylation in patient samples. Changes in long interspersed nucleotide methylation did not differentiate responders from nonresponders in decitabine-treated patients with chronic myelogenous leukemia (CML). Prolonged exposure to lower doses of 5-azacytidine followed by the HDAC inhibitor sodium phenylbutyrate may be associated with an increased proportion of complete and partial responses in patients with myelodysplastic syndrome (MDS) and AML patients (S. Gore).[5] Changes in methylation of $p15^{INK4B}$ were observed in responding patients; however, the relationship between clinical response and methylation reversal will require larger scale phase II and III studies.

Other DNMT inhibitor/HDAC inhibitor combinations currently in phase I and II trials include decitabine plus valproic acid under investigation in myeloid malignancies (complete remission rate, 22%; Garcia-Manero)[5] and decitabine plus FK228 in myeloid malignancies (depsipeptide; Marcucci).[5]

DNMT inhibitors have also been combined with retinoids based in part on the frequency of methylation of the RARβ promoter in both hematologic malignancies and solid tumors. Decitabine plus all-*trans*-retinoic acid (ATRA) is under clinical investigation in AML (Luebbert).[5] The cellular retinol-binding protein 1 is silenced in human breast cancer cells; treatment with DNMT inhibitors or transfection of wild-type cellular retinol-binding protein 1 into these cells sensitizes these cells to ATRA (Waxman).[5] Methylation of RARβ2 promoter is higher in BEAS-2B immortalized bronchial epithelial cells (selected for resistance to ATRA) compared with wild-type cells. Consistently, some genes that are induced by ATRA in wild-type cells were not induced in the ATRA-resistant ones. Pretreatment of the resistant cells with decitabine followed by ATRA restored inducibility of RARβ2 and some differentiation-related genes. These findings can serve as the basis for design of clinical trials combining epigenetic modulators and ATRA (Petty and Dmitrovsky).[5]

Clinical Applications of HDAC Inhibitor

Cutaneous T-cell lymphoma has proven extremely sensitive to HDAC inhibitor, with major responses documented in to SAHA and FK228 (SAHA partial response rate, 95%; Frankel).[5] SAHA, a nonselective class I and II HDAC inhibitor, is undergoing extensive phase II testing in a variety of tumors, including multiple myeloma, cutaneous T-cell lymphoma, head and neck cancer, diffuse large B-cell lymphoma, and peripheral T-cell lymphoma (Frankel).[5] Activity has also been shown in AML in a phase I trial (Garcia-Manero).[5] The most commonly observed adverse effects were dehydration, nausea, diarrhea, and fatigue (Frankel).[5] The optimal dose schedule for this drug is not clear; moreover, the uncertainty of the molecular mechanism underpinning clinical response has made the design of laboratory correlative studies difficult.

FK228 has also shown significant activity in cutaneous T-cell lymphoma (17% complete remission, 33% partial response; Bates);[5] this agent showed less activity in chronic lymphocytic leukemia and AML at the dose schedule studied (Byrd).[5] FK228 was able to induce remissions in AML patients with core binding factor fusion genes, known to recruit HDAC, but not in cases of AML not associated with such molecular abnormalities (Brandt).[5]

Administration of the orally bioavailable MS-275 to patients with relapsed AML led to rapid histone hyperacetylation in peripheral blood mononuclear cells with

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Table 1. Clinical trials of single agents or combinatorial therapies targeting cancer epigenetics

Presenting author	Epigenetic therapy	Associated therapy
Susan Bates	Depsipeptide	None
Kapil Bhalla	LAQ-824	STI571
	LBH-589	17-Allyamino-17-demethoxygeldanamycin (heat shock protein 90 inhibitor)
Steven Brandt	Depsipeptide	None
John Byrd	Depsipeptide	None
Stanley Frankel	SAHA	None
Guillermo Garcia-Manero	SAHA	None
	Valproic acid	Decitabine
Steven Gore	Phenylbutyrate	5-Azacytidine
Steven Grant	NaB	Perifosin
	SAHA	Flavopiridol (cyclin-dependent kinase inhibitor)
	SAHA	Bortezomib
Jean-Pierre Issa	Decitabine	None
Philip Koeffler	SAHA	None
Reuben Lotan	SAHA	None
Michael Luebbert	Decitabine	ATRA
Selina Lugar	Valproic acid	None
Guido Marcucci	Depsipeptide	Decitabine
Ari Melnick	N-CoR/BCL-6 peptide	Trichostatin A
Richard Momparler	Decitabine	Zebularine
Ned Newman	5-Fluoro-2′-deoxycytidine	Tetrahydrouridine (cytidine deaminase inhibitor)
Jeffrey Petty	Decitabine	ATRA
Richard Piekarz	Depsipeptide	Ontak (interleukin-2-conjugated toxin)
Roberto Pili	MS-275	13-*cis*-Retinoic acid
	SAHA	(Avastin) anti–vascular endothelial growth factor antibody
Edward Sausville	MS-275	None
Max Sung	Phenylbutyrate	Indomethacin, 5-FU, IFN

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sustained presence due to a very long half-life (49 hours). Some bone marrow responses and hematologic improvement were observed (Sausville, Gojo, and Karp).[5] Four of 14 patients with MDS developed hematologic improvement in response to valproic acid (achieving serum concentrations of 60–100 μmol/L; Luger).[5] Although these results indicate that the HDAC inhibitor have single-agent activity in myeloid malignancies, the optimal dose schedules and optimal target patient populations remain to be determined.

Novel Targets and Therapeutic Strategies

Studies of the mechanisms underlying the action of leukemia-associated fusion oncoproteins highlighted a common mechanism by which a key transcriptional activator is converted to a constitutive repressor by acquisition of corepressor/HDAC–binding domains from the fused sequences encoded by the translocation partner gene (see Fig. 5). Agents capable of inhibiting enzymatic components of the corepressor complexes could reverse the differentiation blocks imposed by such fusion oncoproteins. Peptide-mediated inhibition of the interaction between N-CoR and BCL-6 in BCL-6-driven lymphoma was sufficient to induce apoptosis of BCL-6-positive diffuse large B-cell lymphoma cells both *in vitro* and *in vivo*. When application of the peptide was combined with use of the HDAC inhibitor trichostatin A, the effects were synergistic, perhaps reflecting an additional role for HDACs in the pathogenesis of non-Hodgkin's lymphoma (Melnick).[5]

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Table 1. Clinical trials of single agents or combinatorial therapies targeting cancer epigenetics (Cont'd)

Status	Target	Outcome/effect
Phase II	Cutaneous T-cell lymphoma, peripheral T-cell lymphoma	17% Complete remission, 33% partial response, 17% stabilization, 25% response rate
Preclinical	CML cells	p21/p27 expression ↑
Phase II	Refractory/relapsed AML	No sustained response observed
Phase I	AML, chronic lymphocytic leukemia	No effect at 13 mg/m²
Phase II	Cutaneous T-cell lymphoma, diffuse large B-cell lymphoma, peripheral T-cell lymphoma, head and neck cancer	Up to 95% partial response (cutaneous T-cell lymphoma)
Phase I	Acute lymphocytic leukemia, AML, chronic lymphocytic leukemia, CML, MDS	21% Response rate (AML only)
Phase I	Refractory AML, CML, MDS	N/A
Phase II	Refractory/relapsed AML/MDS	22% Complete remission, H3/H4 acetylation ↑
Phase I/II	AML/MDS	50% Response
Preclinical	Leukemic cells	AKT/extracellular signal-regulated kinase signaling ↓
		p21 expression ↑, nuclear factor-κB activity ↓ (preclinical)
		N/A
Phase I/II	MDS	85% Overall response
	AML/MDS, AML, CML	65% Overall response
Preclinical	Mantle cell lymphoma	Cyclin D1 protein ↓
Preclinical	Colon carcinoma cells	15-Lipoxygenase-1 ↑
Phase II	AML	No clear response established
Phase I	MDS	29% Response
Phase I	AML, CML	Differentiation, gene expression ↑ (preclinical)
Preclinical	Lymphoma	Apoptosis ↑ (enhanced with HDAC inhibitor)
Preclinical	Leukemia cells, stage IV non–small cell lung cancer	Cytidine deaminase ↓, synergistic activity
Phase I	Solid tumors	No response
Preclinical	Immortalized bronchial epithelial cells	Restoration of retinoic acid sensitivity
Preclinical	T-lymphoma cells	Sensitization to Ontak
Preclinical	Renal and prostate cancer cells	Restoration of retinoic acid sensitivity (preclinical)
Phase I/II	Renal cell cancer	N/A
Phase I	Relapsed AML	10 mg/m² maximum tolerated dose, no complete remission, partial response observed
Phase I	Colon cancer	Side effects and central nervous system toxicity observed

Abbreviation: N/A, not available.

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Methyl-binding domain proteins may confer specificity that can be exploited therapeutically. Methyl-binding domain 2 was specifically associated with the *GSTP1* gene in MCF-7 breast cancer cells, mediating gene silencing. Using a cell-based reporter screening assay for small molecules that could revert gene silencing by targeting methyl-binding domain 2 interaction with DNA, three structurally similar compounds were isolated, which are active at micromolar levels (Nelson).[5]

Corepressors and chromatin-modifying enzymes were recruited by AML1/ETO in the Kasumi AML cell line (Marcucci).[5] Treatment with the HDAC inhibitor FK228 led to a loss of association of HDAC1 and DNMT1 proteins with the *interleukin-3* promoter (an AML1/ETO target gene). Expression of a nuclear receptor corepressor SMRT isoform lacking a NH_2-terminal repression domain (SMRTβ) can potentiate response of acute promyelocytic leukemia cells to ATRA and overcome ATRA resistance (Miller),[5] indicating the potential importance of this domain in oncogenesis.

SET domain proteins (histone methyltransferases) are overexpressed in several cancers (Licht).[5] These include MMSET/NSD2 in multiple myeloma and EZH2 in prostate and breast cancers, potentially indicating a direct role in carcinogenesis through random association with chromatin.

Conclusions

In the past 2 years, clear progress has been made both in the basic understanding of epigenetic contribution to the oncogenic process and in evaluating clinical activities of

DNMT inhibitor and HDAC inhibitor. Following a successful phase III clinical trial in MDS, the Food and Drug Administration has approved 5-azacytidine for the treatment of all MDS subtypes. HDAC inhibitors, such as SAHA and FK228, have shown clinical activity in hematologic malignancies, particularly cutaneous T-cell lymphoma. Anecdotal reports of activity in solid tumors will potentially be confirmed in phase II trials.

There has also been progress in the development of clinical trials employing combinatorial approaches (see Table 1 for highlights). Although in many cases optimal dosing schedules still remain to be determined, some combinatorial use of HDAC inhibitor, DNMT inhibitor, and retinoids should lead to better outcomes in AML. Similarly, in solid tumors, studies addressing the mechanisms of action of HDAC inhibitor may lead to development of better combinatorial therapies. Combination of HDAC inhibitor, such as SAHA or MS-275, with other agents that increase levels of reactive oxygen species or DNA damage may lead to enhanced tumor cell apoptosis and better therapeutic results. Many activities of various HDAC inhibitor will remain tumor specific and these structurally diverse compounds will, in addition to causing hyperacetylation of histones, possess other specific mechanisms of action. Some authors have questioned the safety of manipulating chromatin and gene transcription in the absence of highly selective pharmacologic agents, raising the question of inducing expression of genes that could be detrimental and pointing to animal models in which such approaches have in fact led to the development of cancers. It is somewhat reassuring that no such events have been noted in clinical trials to date; indeed, the drugs under current investigation are significantly less toxic clinically than many antineoplastic drugs currently in the clinic. Particular attention to possible tumorigenesis will need to be paid as these approaches are studied in earlier-stage patients, patients with indolent malignancies, and patients with nonmalignant disorders such as hemoglobinopathies.

More informative assays are clearly needed to allow assessment of a given drug activity at a molecular level *in vivo* that correlates with patients' responses. Molecular and/or clinical predictors of clinical response need to be established to bring maximal benefit for a given drug (or drug combination) in a specific malignant condition. Little progress has been made in unraveling basic issues of specificity of drug action, particularly with respect to HDAC inhibitor. Are there specific HDACs expressed in a particular tumor type and can inhibitors specific for a given HDAC be synthesized? It also remains to be seen if translational end points can be developed that consistently correlate with dose efficacy for these agents. A lack of standardized reagents, cell lines, and preclinical models has also been identified as problematic.

Despite these drawbacks, clear progress has been made. It seems increasingly likely that HDAC inhibitors, as well as other agents targeting aberrant epigenetics, will be developed for clinical application in neoplastic diseases, most likely in combination with other more specifically acting drugs. The development of small molecular drugs that can specifically disrupt interactions between oncoproteins and their coregulators (such as BCL-6 and N-CoR) or DNA is highly promising. However, effective targeting of aberrant epigenetic modifications will require a more precise understanding of the relationships that exist between various epigenetic modifications and pathologic patterns of gene expression in addition to uncovering the mechanisms and molecular specificities that underlie these processes. A comprehensive understanding of the histone code in normal and cancer cells is critically important.

Appendix

Meeting Participants and Affiliations

Bates, Susan (National Cancer Institute, Bethesda, MD)

Baylin, Stephen (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD)

Bhalla, Kapil (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL)

Brandt, Steven (Vanderbilt University Medical Center, Nashville, TX)

Byrd, John (Ohio State University, Columbus, OH)

Dmitrovsky, Ethan (Dartmouth University School of Medicine, Hanover, NH)

Emerson, Beverly (Salk Institute, La Jolla, CA)

Frankel, Stanley (Merck & Co., Inc., Blue Bell, PA)

Futscher, Bernard (Arizona Cancer Center, University of Arizona, Tucson, AZ)

Garcia-Manero, Guillermo (University of Texas M.D. Anderson Cancer Center, Houston, TX)

Gojo, Ivana (University of Maryland, Baltimore, MD)

Gore, Lia (University of Colorado Cancer Center, Aurora, CO)

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Grant, Steven (Medical College of Virginia, Richmond, VA)

Jones, Peter (University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA)

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Koeffler, Philip (University of California at Los Angeles School of Medicine, Los Angeles, CA)

Licht, Jonathan (Mount Sinai School of Medicine, New York, NY)

Luebbert, Michael (University Medical Center, Freiburg, Germany)

Luger, Selina (University of Pennsylvania, Philadelphia, PA)

Marcucci, Guido (The Ohio State University James Cancer, Columbus, OH)

Marks, Paul (Memorial Sloan-Kettering Cancer Center, New York, NY)

Melnick, Ari (Albert Einstein College of Medicine, Bronx, NY)

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