In this issue of Clinical Cancer Research, Huang and colleagues, characterize the effects of novel histone demethylase inhibitors, either alone or in combination with inhibitors of DNA methyltransferases (DNMTI), on the proliferation and survival of transformed cells (1). A widely accepted view holds that the origins of cancer lie, at least in part, in the silencing of genes responsible for cell death and/or differentiation, a phenomenon that generally stems from perturbations in the transcriptional regulatory machinery. In the broadest sense, silencing of such genes occurs at three fundamentally different but closely interrelated levels. For example, silencing frequently results from DNA methylation at the site of CpG islands by DNA methyltransferases (DNMT; ref. 2). Silencing can also stem from mutations in chromatin remodeling complexes [i.e., nucleosomal remodeling factors (NURF)] such as SWI/SNF. Finally, silencing can result from multiple post-translational covalent histone modifications, which collectively comprise the histone code, and which include such processes as acetylation, methylation, SUMOylation, glycosylation, ubiquitylation, and phosphorylation (3). The therapeutic relevance of these events is underscored by accumulating evidence indicating that agents capable of reversing these processes—e.g., DNMTIs or histone deacetylase inhibitors (HDACI)—are clearly capable of inducing re-expression of silenced genes in association with induction of transformed cell death. Moreover, representative agents of these classes have recently been approved for the treatment of hematologic malignancies, e.g., DNMTIs such as 5-azacytidine in the case of myelodysplastic syndrome (MDS), and HDACIs in the case of cutaneous T-cell lymphoma. There is also evidence that these silencing mechanisms do not operate in a vacuum, but instead interact with each other, and HDACIs in the case of cutaneous T-cell lymphoma.

A novel oligoamine analog inhibitor of histone demethylases blocks colon tumor cell growth in association with histone methylation and gene re-expression. It also markedly potentiates the activity of hypomethylating agents in vitro and in vivo, suggesting that histone demethylase inhibitors may represent a valuable addition to the armamentarium of epigenetic agents. (Clin Cancer Res 2009;15(23):7111–3)
This report provides the first evidence that histone demethylase inhibitors, as previously reported in the case of HDACIs (4), cooperate with DNMTIs to promote gene re-expression in transformed cells, a phenomenon accompanied by pronounced inhibition of cell growth and induction of cell death. Such findings argue, albeit indirectly, that targeting a single epigenetic aberration, by itself, may be insufficient to achieve the desired therapeutic outcome. Instead, targeting multiple cooperating abnormalities may be required. However, as in the case of DNMT/HDAC inhibitory strategies, numerous questions remain to be answered. For example, in the present study, combined treatment of colon cancer cells with a DNMTI and a histone demethylase inhibitor resulted in re-expression of aberrantly silenced SFRPs. Although it is tempting to speculate that induction of antagonists of the Wnt signaling cascade, a pathway that has been implicated in colon carcinogenesis and in cancer stem cell survival (8), may be responsible for or contribute to the antiproliferative effects of the combination regimen, this will not be trivial. However, gene array analysis should help to resolve this important issue.

Another question relates to the possibility that so-called epigenetic modulators may simultaneously act as cytotoxic agents. For example, both 5-aza-2′-deoxycytidine and 5-azacytine, currently viewed primarily as DNMTIs, were initially developed because of their direct cytotoxic activities (9). Although combination strategies incorporating epigenetic modulators generally employ sub- or minimally toxic concentrations of these agents when administered individually, it is conceivable that their limited cytotoxicity might be substantially increased by alterations in gene expression (e.g., down-regulation of DNA repair or anti-apoptotic genes). In fact, the possibility that perturbations in gene expression may cooperate with more direct cytotoxic actions to promote cell death seems quite likely. Whether histone demethylase inhibitors exert direct cytotoxic actions in addition to their effects on the epigenome remains to be determined.

Aside from providing the first demonstration that blocking histone demethylation, as in the case of HDAC inhibition (4), may cooperate with DNMT inhibition to antagonize transformed cell growth both in vitro and in vivo, the present findings have broader therapeutic implications that extend beyond this particular setting. Although within the field of epigenetic therapy HDAC and DNMT inhibitors have received the bulk of...
attention to date, attention is currently beginning to focus on inhibitors of other proteins involved in epigenetic regulation, including, as described in the present study, histone demethylases, as well as histone methyltransferases and histone acetyltransferases, among numerous others. In addition, efforts to target other epigenetic components of the transcriptional control machinery e.g., co-repressor proteins, are well underway (10). Such considerations raise the possibility that in the future, combination chemotherapy regimens may consist of, instead of purely cytotoxic agents, multiple inhibitors (or activators) targeting distinct regulatory components of the epigenome (summarized in Fig. 1). In support of this notion, results of a recent study suggest that HDAC inhibitors enhance the lethal actions of histone methyltransferase inhibitors in human leukemia cells (11). Because our understanding of the epigenetic factors regulating gene expression, as well as our ability to target them, are advancing rapidly, the number of such regimens is potentially very large. Nevertheless, on the basis of, in part, the report by Huang and colleagues (1), such efforts seem well justified.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**


