Like most other types of cancers, human breast cancer occurs as a result of a multistep process that generally consists of initiation and progression resulting from uncontrolled cell proliferation and/or aberrant apoptosis as a consequence of cumulative genetic and/or epigenetic alterations in genome. Genetic alterations such as mutations or deletions or rearrangements of specific genes and/or chromosomal instability can inactivate normally expressed genes that would otherwise protect against breast cancer development. Another general mechanism by which expression of growth regulatory genes can be modified is so called “epigenetic alterations” which refer to high level modifications in chromatin structure above the genetic code (1,2). Importantly, epigenetic alterations, unlike mutation, deletion or loss of specific chromosomal regions, are generally reversible. Therefore, it should be theoretically possible to restore normal growth phenotypes by reversing aberrant epigenetic changes through treatment with epigenetic modifying drugs. Multiple primary and interconnected epigenetic mechanisms, such as DNA and histone modifications as well as non-coding RNA expression, have been elucidated (3). The impact of DNA methylation and histone modifications on cancer initiation and progression has been extensively investigated in preclinical models. In addition, many clinical trials using DNA methyltransferase (DNMT) inhibitors have shown clinical benefit in treatment of myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) (4,5). The use of drugs that inhibit histone deacetylases (HDAC) also holds great promise for cancer therapy. Several inhibitors of DNMTs or HDACs have already been approved by the US FDA for the clinical treatment of cutaneous T-cell lymphoma (CTCL) and multiple myeloma (6-8). Unfortunately, the results of initial clinical trials of DNMT inhibitors (DNMTi) and HDAC inhibitors (HDACi) in solid tumors including breast cancer have not been as rewarding. Nonetheless it is critically important to continue to explore the potential effects of epigenetic drugs as a means to improve therapy for epithelial cancers in solid tumor.

In a recent issue of Cancer Research, Pathania and colleagues characterized the in vitro and in vivo antineoplastic effect of a combination of the DNMT inhibitor, 5-azacytidine (5-AzaC), and the HDAC inhibitor, butyrate, on breast cancer stem-like cells (BCSCs) at a genomic level (9). The authors provided interesting evidence to show that Lin-CD49f-CD24+ cells isolated from tumor tissues of MMTV-Neu-Tg mice possessed tumor-propagating and metastatic potential when these cells were injected into the mammary fat pad of NOD/SCID mice. They further demonstrated that both transformed basal myoepithelial stem cells and luminal progenitor cells developed mammary tumors, and these cells were susceptible to combination treatment with DNMT and HDAC inhibitors (9). RNA-sequencing study identified a subset of genes, whose expression is regulated by DNMT and HDAC inhibitors, are potentially involved in regulation of basal stem cell-driven breast cancer phenotypes. Further analysis through the Ingenuity System Database (IPA) and UCSC cancer genome browser program showed that expression of RAD51AP1 and SPC25 was high in basal breast tumor tissues and cell lines and downregulated by 5-AzaC/butyrate.

DNA methylation and histone post translational modifications (PTMs) are two fundamental epigenetic
regulatory mechanisms that govern chromatin structure, gene transcription and other important biological processes.

The functional interaction between DNMTs and HDACs has emerged as a key research issue and a possible novel target for cancer therapy. In breast cancer, dysregulated DNA CpG methylation frequently cooperates with abnormal histone modifications to result collectively in an aberrant chromatin landscape and gene expression profile (2,5,10). Our early work showed that the HDAC inhibitor, Scriptaid, inhibited human breast tumor growth in vitro and in vivo and acted, in conjunction with the DNMT inhibitor (DNMTi) AZA, to re-express functional Estrogen Receptor Alpha (ERα) in ER-negative breast cancer cells (11). We also demonstrated that disruption of Hsp90 function by HDACi facilitated DNMT1 degradation through the ubiquitin-proteasome pathway in breast cancer cells (12). Another novel DNMTi, Zebularine, potentiated the inhibitory effect of HDACi on cell proliferation and colony formation in breast cancer cells (13). Studies from our laboratory and others consistently showed that combined treatment of ER negative breast cancer cells with DNMTi and HDACi restored response to endocrine therapy (14,15). The potential translation of these findings into clinical investigation is demonstrated by a “window” clinical trial showing that oral HDACi vorinostat (SAHA) administered to patients with primary breast cancer for 3 days preoperatively was associated with significant reduction in expression of proliferation-related genes such as Ki-67, STK15 and Cyclin B1 (16). Another phase II study assessed the activity of the DNMTi, 5-AzaC, and the HDACi, Entinostat, in patients with advanced breast cancer (17).

Nonetheless, while DNMTi and HDACi have shown promising results in treatment of hematological malignancies, these drugs have proven to be less effective against solid tumors including breast cancer. The likely explanations for the unsatisfactory efficacy of epigenetic agents in solid tumors may include poor pharmacokinetic properties, inadequate incorporation of drugs into tumor cells, lack of specificity in targeting chromatin modifiers and gene expression, and/or toxicity. In addition, insufficient knowledge about the basic mechanisms of epigenetic alterations in a neoplastic disease like breast cancer may impede the progress of future clinical application of the epigenetic agents. To enhance the potential of epigenetic drugs as effective anti-breast tumor agents, it is necessary to better understand how DNMT and HDAC activities are regulated in breast cancer. It is also critical to develop novel combinatorial strategies to improve the efficacy of the epigenetic drugs.

Since Al-Hajj et al. first reported the existence of CSCs in breast cancer (18), increasing lines of evidence have indicated that BCSCs have important implications in breast cancer initiation, progression and therapeutics (19). A recent study used whole genome promoter microarray to compare the DNA methylation portraits of human BCSCs versus non-BCSCs, and showed a distinct DNA methylation landscape in BCSCs as a key epigenetic mediator of their differentiation (20). Epigenetic silencing of the tumor suppressor breast cancer 1 (BRCA1) gene due to CpG island hypermethylation in breast cancer which contained expanded luminal progenitor cells was reported in a recent study (21). Another investigation revealed the promise of using the HDACi, Abexinostat, as a differentiation therapy targeting BCSCs (22). In addition, recent evidence showed that Enhancer of Zeste Homolog 2 (EZH2), an important member of the polycomb repressor complex, downregulated the DNA damage repair protein Rad51 which resulted in expansion of the BCSCs population (23). These results suggest that epigenetic alterations in BCSCs play important roles in governing their biological properties.

Compared to non-CSCs, CSCs generally exhibit elevated resistance to conventional chemotherapy and/or radiation therapy. Therapies using purely cytotoxic regimens commonly fail to hinder CSC propagation. There are vigorous research and clinical activities in identifying or developing novel agents and therapeutic approaches that specifically targets the small, phenotypically distinct CSC subpopulations in tumors. Ohm et al. proposed a model based on the findings that cancer stem/progenitor cells develop in a stepwise manner as a result of crosstalk between multiple epigenetic mechanisms including DNMT and histone modifications (24). Their findings suggested that targeting aberrant interaction of epigenetic modifiers may represent an effective therapeutic approach in blocking CSC initiation and progression. Pathania and colleagues provided the first evidence that the combined inhibition of DNMTs and HDACs effectively blocks mammary tumorigenesis and attenuates mammosphere-forming capacity of tumor-propagating cells by regulating the expression of key genes that are involved in development of basal stem cell-driven breast cancer (9). Such findings imply that targeting single epigenetic aberration might be insufficient to suppress BCSC expansion. Instead, targeting multiple interactive epigenetic abnormalities may be required to improve the therapeutic efficacy. However, many questions still remain to be answered about the use of DNMT/HDAC inhibitory
strategies in blocking BCSC expansion. For example, the detailed mechanisms underlying the effect of inhibition of DNMT/HDACs on BCSC propagation and other phenotypes are not clear. Alterations in DNA methylation and histone marks in BCSCs due to combination therapy have not been fully elucidated.

Since the impact of inhibition of DNMT and HDAC on global gene expression changes is likely to be very broad, it is critical to map out specific alterations of gene expression that are responsible for the antineoplastic activity of this strategy. Through a comprehensive RNA-seq analysis, Pathania and colleagues identified a subset of genes whose expression was differentially regulated by 5-AzaC/butyrate. These genes are extensively involved in regulation of cell division and cycle, mitosis, chromosome segregation, kinetochore formation, etc. Among the top tier candidate genes, RAD51AP1 and SPC25 were selected for further analysis and found to be highly expressed in basal breast cancer cells. Although these two genes were overexpressed in breast tumor tissues and their expression was downregulated by combination therapy, there was no evidence to indicate that expression of these genes is directly regulated by DNA methylation or histone acetylation. Further studies are needed to clarify the regulatory mechanisms of combination therapy on the transcriptional activity of the key genes involved in BCSC proliferation.

Since DNA methylation and histone acetylation are normal features of the genome, the use of inhibitors targeting DNMT/HDACs may induce changes in the epigenomic landscape that could result in toxicity. Although combination strategies generally use lower doses of epigenetic modulators than those employed when the agents are administered individually, it is possible that cumulative alterations in gene expression or genomic instability could considerably enhance toxicity. Also it remains to be determined whether the combination of DNMTi and HDACi exerts direct cytotoxic actions on BCSC in addition to the effects on the epigenome of tumor cells.

In summary, the recently published research article by Pathania et al. demonstrated for the first time that simultaneous blockade of the activity of two important epigenetic modifiers, DNMT and HDAC, significantly reduced BCSC propagation and increased overall survival in tumor-bearing animal models. These findings have significant implications for the hypothesis that dual inhibition of DNMT and HDAC may improve the therapeutic efficacy in refractory or drug resistant breast cancer. Although use of epigenetic therapy with HDAC and DNMT inhibitors for breast cancer patients has received a lot of attention, numerous technical and clinical obstacles still remain to be overcome. These issues include how to select breast tumor patients that may benefit from epigenetic treatments at the greatest extent, and how to quantitatively measure the therapeutic effect of epigenetic therapy. Pathania et al. have completed a valuable study to pave the way for a potential new strategy to eliminate CSCs in breast tumors using epigenetic approaches. The precise role of DNMT/HDAC in regulation of BCSC progression and therapeutic response, however, warrants further investigation.

**Acknowledgements**

The authors’ work has been supported by US Army Breast Cancer Research Breakthrough Awards (W81XWH-14-1-0237 to Y Huang; W81XWH-14-1-0238 to NE Davidson) and Breast Cancer Research Foundation (to NE Davidson).

**Footnote**

*Provenance:* This is an invited Editorial commissioned by the Section Editor Dr. Hongcheng Zhu, MD, PhD (Department of Radiation Oncology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

*Conflicts of Interest:* The authors have no conflicts of interest to declare.


**References**

5. Katz TA, Huang Y, Davidson NE, et al. Epigenetic...


