Epigenetic Approaches for Chemosensitization of Refractory Diffuse Large B-Cell Lymphomas

James J. Steinhardt and Ronald B. Gartenhaus

Summary: Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma, with the greatest challenge for improving patient survival being the management of chemorefractory disease upon relapse. Epigenetic dysregulation has been correlated with more-aggressive malignancies and chemoresistance. In this issue of Cancer Discovery, Clozel and colleagues show the potential for low-dose DNA methyltransferase inhibitors as both a rational and an effective neoadjuvant approach for chemosensitization in DLBCL. Cancer Discov. 3(9); 968–70. © 2013 AACR.

See related article by Clozel et al., p. 1002 (1).

Diffuse large B-cell lymphoma (DLBCL) is an aggressive and heterogeneous disease generally treated with the anthracycline-based regimen R-CHOP. Chemoresistance remains the primary obstacle for the eradication of DLBCL, which may be conferred through either intrinsic mechanisms related to enhanced drug inactivation/transport or acquired resistance through altered gene expression that prevents cell death. Epigenetic mechanisms have been explored in the context of acquired drug resistance, as epigenetic modification can result in altered gene expression without altering the DNA sequence itself. Epigenetics can be defined as the regulation of DNA architecture and the accessibility of chromosomal DNA to transcription factors, which is influenced by how compactly (heterochromatin) or relaxedly (euchromatin) the DNA is coiled around the nucleosome. DNA methylation may influence gene expression by either directly interfering with transcription factor binding to gene promoters or by encouraging the recruitment of histone deacetylases (HDAC) by methyl-CpG-binding proteins, thus remodeling the chromatin structure.

In this issue of Cancer Discovery, Clozel and colleagues (1) expand upon previous studies to establish DNA methylation as a critical component of epigenetic dysregulation during DLBCL lymphomagenesis, as well as a rational epigenetic mechanism to target in chemorefractory DLBCL, much as acetylation and methylation in the context of DLBCL will be briefly discussed as these epigenetic events are physically and functionally related to DNA methylation and exemplify personalized epigenetic therapies in DLBCL (2–4).

Acetylation of histones on conserved lysine residues in the N-terminal tail or on the core of the nucleosome removes a positive charge, generally resulting in relaxed, transcriptionally active DNA. HDACs catalyze the removal of acetyl groups back to coenzyme A, thus repressing gene expression. Methyl-CpG-binding proteins MBD2 and MeCP2 are able to recruit HDACs to the site of CpG island methylation, which can result in enhanced gene suppression (5). Nearly 70% of DLBCLs overexpress BCL6, a corepressor of numerous genes including EP300, a putative tumor suppressor (2). The differentiation of germinal center (GC) B cells can be inhibited by BCL6 proteins, which regulate and coordinate plasmacytic differentiation in conjunction with HDACs and transcription factors such as BLIMP1, PAX5, and XBP1. Cerchietti and colleagues (2) previously identified the BCL6-EP300 axis through chromatin immunoprecipitation (ChIP)-on-ChIP experiments and have subsequently shown the efficacy of HDAC inhibitors (HDACI) in inducing cell death in GCB-DLBCLs. Although HDACIs have yet to be explored as chemosensitizers in DLBCL, they have shown potential as potent chemo- and radiosensitizers in cell lines from lung, breast, ovary, esophageal, gastric, colon, thyroid, prostate, and pancreatic cancers and appear to be rational therapies for DLBCLs of GC origin (6).

Histone methylation, especially H3K9 and H3K27, is generally associated with transcriptional repression and often concurrent with deacetylation. EZH2 is the catalytic component of the polycomb repressive complex 2 (PRC2) responsible for the methylation of H3K27 and the repression of select...
genes. EZH2 mutations on residues Y641 and A677 were identified in 22% of DLBCL and 10% of follicular lymphoma patient samples, resulting in altered substrate preference and enhanced di- and trimethylation of H3K27 (3). A recent study showed a correlation between H3K27me3-mediated silencing through EZH2 overexpression, inactivation of pathways such as TGF-β, and chemoresistance in serous ovarian cancer (7). McCabe and colleagues recently used the S-adenosylmethionine (SAM)-competitive small-molecular inhibitor GSK126 and showed its selectivity for EZH2 activity in GCB–DLBCL and its ability to reactivate PRC2-suppressed genes. Pharmacologic inhibition of EZH2 has yet to be explored in the context of chemosensitization in DLBCL, but studies suggest that combinatorial epigenetic or neoadjuvant approaches may be rational for some hematologic malignancies.

DNA hypermethylation on CpG islands located in the promoters of tumor suppressors can be aberrantly methylated by DNA methyltransferase (DNMT)1, DNMT3A, and DNMT3B. A replication-coupled passive DNA demethylation process has been described, but the mechanism behind active DNA demethylation remains elusive. While no enzyme has been attributed to the catalysis of DNA demethylation, some research suggests that hydroxymethylation of 5-methylcytosine (5mC) to 5hmC may be an intermediate for the removal of methylated cytosine (8). TET proteins are capable of promoting demethylation by catalyzing 5mC to 5hmC, and a recent study using genome-wide profiling identified DNA hypermethylation signatures associated with DLBCLs harboring inactivating mutations in TET2 (9). Clozel and colleagues (1) used the pyrimidine nucleoside analog DAC to show a mechanism for chemosensitization of DLBCL (1). DAC and 5-azacytidine were initially used because of their intrinsic cytotoxic properties but are now known to be DNA-hypomethylating agents through their irreversible inhibition of DNMTs. During cellular replication, inhibition of efficient incorporation of methyl groups into newly synthesized DNA results in hypomethylation and global reactivation of genes. Clozel and colleagues (1) exploited this event in rapidly dividing tumor cells, believing they would be more susceptible to this augmented replication-coupled passive

Figure 1. A depiction of the model Clozel et al. proposed for the mechanism-based epigenetic chemosensitization of diffuse large B-cell lymphoma upon low-dose 5-aza-2-deoxycytidine, decitibine (DAC) treatment (1). Acquired resistance to anthracyclines may occur through DNMT1 methylation of CpG islands located in the promoter of genes such as SMAD1. Hypomethylation and reactivation of genes occurs after inhibition of DNMT1 by DAC. This hypomethylation, which is augmented in rapidly dividing cells, results in the reactivation of genes [e.g., SMAD1]. SMAD1 confers chemosensitization upon its phosphorylation, trimerization, translocation into the nucleus and transcription of target genes, including CDKN1A. BMP, bone morphogenic protein.
DNA demethylation. Their hypothesis was that low-dose DAC would result in the reactivation of tumor suppressor genes and pathways necessary for chemosensitization. Previous studies by Cerchietti and colleagues (2) have shown that DNMT1, which primarily maintains DNA methylation due to its preference for hemimethylated DNA, is highly expressed in normal GC B-cells (10). In addition to the genetic heterogeneity induced by AICDA-mediated mutagenesis during somatic hypermutation, GC B-cells are characterized by DNA methylation heterogeneity. This diverse repertoire of GC B-cells is under considerable pressure to maintain homogeneous DNA methylation patterning in all clonogenic progeny during rapid proliferation and clonal expansion, which requires high expression of DNMT1. Clonal populations may acquire aberrant DNA methylation, resulting in the inactivation of tumor suppressor genes and pathways, thus conferring a malignant phenotype.

In their study, Clozel and colleagues (1) identified SMAD1 inactivation mediated through aberrant DNA methylation as the primary culprit conferring chemoresistance in DLBCL. Interestingly, this finding agrees with previous observations that disruption of the TGF-β-related pathway through epigenetic mechanisms results in more stem-like tumors that are resistant to chemotherapies (7). Conversely, activation of TGF-β signaling has been established as a critical mediator of epigenetic mechanisms results in more stem-like tumors that are resistant to chemotherapies (7). Consequently, activation of TGF-β paradox has been attributed to the ability for specific SMAD trimers to associate with distinct transcription factors and DNA sequences, thus allowing each SMAD to regulate a unique repertoire of genes. SMAD1, along with SMAD5, mediates TGF-β-related BMP-dependent pathways of which tumor suppressor CDKN1A is a target gene (12). With the exception of indolent follicular lymphoma, SMAD1 activation upon TGF-β stimulation rarely occurs in B-cell malignancies, and overexpression of SMAD1 in DLBCL results in antiproliferative effects (13). Furthermore, Clozel and colleagues (1) observed the induction of the tumor-specific senescence with incomplete growth arrest (SWING) program upon SMAD1 induction and subsequent activation of DNA damage response gene CDKN1A (1, 14). Depicted in Figure 1 is a model Clozel and colleagues (1) proposed for the mechanism-based epigenetic chemosensitization of diffuse large B-cell lymphoma.

While many clinical trials using the maximally tolerated dose are showing a low therapeutic index for epigenetic therapies, low-dose administration of these agents is showing great promise in their ability to “prime” tumors for subsequent chemotherapy. In addition, the combination of HDACIs with demethylating agents has become a very attractive approach due to the physical and functional relationship between histones and DNA (15). This TGF-β paradox has been attributed to the ability for specific SMAD trimers to associate with distinct transcription factors and DNA sequences, thus allowing each SMAD to regulate a unique repertoire of genes. SMAD1, along with SMAD5, mediates TGF-β-related BMP-dependent pathways of which tumor suppressor CDKN1A is a target gene (12). With the exception of indolent follicular lymphoma, SMAD1 activation upon TGF-β stimulation rarely occurs in B-cell malignancies, and overexpression of SMAD1 in DLBCL results in antiproliferative effects (13). Furthermore, Clozel and colleagues (1) observed the induction of the tumor-specific senescence with incomplete growth arrest (SWING) program upon SMAD1 induction and subsequent activation of DNA damage response gene CDKN1A (1, 14). Depicted in Figure 1 is a model Clozel and colleagues (1) proposed for the mechanism-based epigenetic chemosensitization of diffuse large B-cell lymphoma.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES

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