The cancer epigenome: Concepts, challenges, and therapeutic opportunities

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Cancer biology is profoundly influenced by changes in the epigenome. Because the dynamic plasticity of the epigenome lends itself well to therapeutic manipulation, the past few years have witnessed an unprecedented investment in the development, characterization, and translation of targeted epigenetic therapies. In this review, I provide a broad context for recent developments that offer a greater understanding of how epigenetic regulators facilitate the initiation, maintenance, and evolution of cancer. I discuss newly developed epigenetic therapies and the cellular and molecular mechanisms that may govern sensitivity and resistance to these agents. I also review the rationale for future combination therapies involving existing and emerging epigenetic drugs.
of the hallmarks of cancer, EMT is underpinned by alterations in epigenetic regulators (22).

At the molecular level, these cell state transitions are largely mediated through the collaborative action of transcription factors and epigenetic regulators. The diversity of histone and DNA modifications introduces a complexity that can subtly alter transcription programs within the cell (4). These chromatin modifications, including acetylation, methylation, and phosphorylation, are not static entities but constitute a dynamically changing and complex landscape that evolves in a cell context-dependent fashion. Notably, chromatin modifications that activate or repress transcription are not always mutually exclusive, as evidenced by “bivalent domains” marking genes poised for transcription in normal and malignant cells (23). First described in embryonic stem cells, genes associated with bivalent promoters, which have the concurrent presence of both the activating histone modification H3K4me3 and the repressive modification H3K27me3, have been implicated in the development of cancer and are noted to undergo DNA hypermethylation at their CpG islands (23). The Polycomb (PcG) and Trithorax (TrxG) complexes—which are responsible for the methylation of histones H3K27 and H3K4, respectively—largely work in an opposing manner to repress or facilitate gene expression. Members of these two essential complexes are some of the most commonly mutated epigenetic regulators in cancer (6, 13). Some malignancies, such as germinal center–derived B cell lymphomas, contain mutations in the PcG protein EZH2 and in the TrxG member MLL2, alongside mutations in histone acetyltransferases such as CREBBP (cyclic adenosine monophosphate response element–binding protein (CREB) binding protein) and EP300 (E1A-binding protein) (24). These findings would suggest that impaired resolution of the germline B cell transcription program of these cells prevents maturation and is a seminal event in the initiation and maintenance of these malignancies.

Epigenetic dysregulation contributes to the origin of cancer

Cancer is thought to develop through clonal expansion of mutant premalignant stem and progenitor cells. These cells subsequently acquire further mutations that provide a subclonal growth and proliferative advantage, which ultimately manifests in a clinically diagnosed malignancy. This evolutionary model of cancer has been well studied in the hematopoietic system, where recent evidence has implicated epigenetic regulators as the primary targets that establish the fertile soil for malignant outgrowth. Several groups have established that clonal hematopoiesis, whereby hematopoietic stem and progenitor cell clones carrying somatic mutations give rise to the majority of blood cells, occurs in at least 10% of people older than 65 years of age (25–27). The three most common mutations driving clonal stem cell expansion occur in genes encoding the epigenetic regulators DNMT3A, TET2, and ASXL1 (25–27). These mutations confer a competitive self-renewal advantage to the hematopoietic stem cells harboring them, resulting in clonal expansion of these cells (28). The precise molecular mechanisms governing this increased capacity for self-renewal are still under investigation, but it is increasingly clear that clonal hematopoiesis is a prelude to a variety of hematopoietic malignancies, including myelodysplastic syndromes and acute myeloid leukemia (AML) (25–27). Moreover, there is accumulating evidence that preleukemic cells containing mutations in epigenetic regulators such as the de novo DNA methyltransferase DNMT3A can survive conventional chemotherapy and serve as the nidus for leukemia relapse (29). It remains to be established whether the lessons learned about the central role of epigenetic regulators in the origins of clonal malignant hematopoiesis are broadly applicable to other malignancies.

Cancer mutations in “dark matter” affect chromatin regulation

As we near completion of a comprehensive annotation of recurrent mutations in protein-coding genes, it is clear that we have just scratched the surface of the cancer genome. The mutation rate of the noncoding regulatory genome, or so-called “dark matter,” is nearly double that of coding regions, although what role these noncoding mutations play in driving oncogenesis is the subject of ongoing investigation (30). Such mutations occur in multiple gene promoters and enhancer elements and are found in a range of cancers (31, 32). A pioneering example was the discovery of mutations within the promoter region of TERT, the gene that encodes the catalytic subunit of telomerase, in more than 70% of melanomas (33, 34). Although it is well recognized that cancer cells have high telomerase activity, mutations within the coding region of the telomerase gene are not common in human cancer genomes. Interestingly, the TERTpromoter mutations appear to increase the expression of TERT by creating a de novo binding motif for the ETS family of transcription factors.

In addition to the promoter mutations, mutations that alter enhancer elements have also been discovered and demonstrated to be functionally relevant. Chromosomal translocations involving inv(3)(q21q26.2) or t(3:3)(q21;q26.2) result in AML with a poor prognosis; this is in part due to the aberrant and sustained expression of the proto-oncogene EVI1. Detailed studies have now revealed that the translocation leads to the relocation of a distal enhancer for the GATA2
transcription factor; this simultaneously results in GATA2 haploinsufficiency and the generation of a superenhancer that ectopically activates ETV1 (35, 36). “Superenhancers” have been defined as regulatory DNA elements with a high density of binding of transcriptional coactivators and other components of the transcription machinery. Every cell is purported to harbor a few hundred superenhancers that control genes associated with cell fate determination or the initiation and maintenance of cancer (37). It appears that malignant superenhancers, with their increased concentration of transcription coactivators, provide a unique sensitivity to epigenetic therapies (35, 37, 38). Oncogenic superenhancers have also been described in T-ALL (T cell acute lymphoblastic leukemia), where somatic mutations create new binding sites for the transcription factor MYB at a superenhancer upstream of the TAL1 oncogene (39).

An inherited predisposition to cancer may also involve superenhancers. The association of the single-nucleotide polymorphism rs2165101 G>T with the development of neuroblastoma appears to involve a key superenhancer that regulates the expression of the oncogene LMO1. The ancestral G allele results in a conserved GATA4 binding site within a superenhancer that sustains a high level of LMO1 expression (40). These data raise the intriguing prospects that (i) the development of certain cancers involves heritable or acquired mutations that create unique regulatory elements required for oncogenesis, and (ii) epigenetic therapies may provide a unique avenue to intervene in these malignancies.

**Cancer metabolism and its effects on the epigenome**

Several human cancers, particularly gliomas and AML, harbor mutations in isocitrate dehydrogenase (IDH1 and IDH2); these mutations confer neomorphic activity to the mutant enzyme, leading to accumulation of the oncometabolite 2-hydroxyglutarate (2HG) (41). In contrast to wild-type IDH, which converts isocitrate to α-ketoglutarate (αKG), IDH mutants preferentially metabolize αKG to the ω-oxo-anionomer of 2HG. Elevated 2HG levels appear central to the pathogenesis of IDH1 mutant malignancies, as 2-HG is a competitive inhibitor of the Fe(II)-dependent and 2-oxoglutarate (2OG)-dependent dioxygenases. Essential epigenetic regulators, such as the TET (ten-eleven translocation) family of proteins involved in DNA demethylation and the Jumonji-C domain family of histone demethylases, are examples of iron-dependent dioxygenases, whose catalytic activity at chromatin is profoundly altered by elevated 2HG levels after IDH1 or IDH2 mutations (41). In addition to mutations in IDH, other critical enzymes involved in the tricarboxylic acid (TCA) cycle, including succinate dehydrogenase (SDH) and fumarate hydratase (FH), have also been observed in cancer. Mutations in all these TCA cycle enzymes appear to induce a CpG island hypermethylation phenotype (CIMP) in tumor DNA. The functional consequences of IDH- and FH-mediated CIMP include a disruption of the normal chromatin topology, resulting in the aberrant juxtaposition of cis-regulatory elements alongside oncogenes (42). CIMP also appears to underpin an EMT phenotype that accounts for the aggressive nature of FH-deficient cancers (43).

The reciprocal interaction between the cancer metabolome and the epigenome is far more pervasive than the aberrations described above in the TCA enzymes. It is clear that nutrient levels regulate a dynamic interplay between key metabolic pathways that generate the substrates for the posttranslational modifications of chromatin, including histone and DNA methylation (44), histone acetylation (45), and O-linked N-acetylglucosamine (O-GlcNAc) transferase–catalyzed O-GlcNAcylation (46). This rapidly expanding area of investigation is likely to reveal new insights into the mechanisms of epigenetic dysregulation in cancer and may also provide new therapeutic avenues.

**Epigenetic therapies**

The central influence of epigenetics in regulating many of the hallmarks of cancer has garnered the interest and focus of scientists, clinicians, and the pharmaceutical industry with the aim of manipulating and resetting the cancer epigenome. These efforts have yielded a vast array of investigational small molecules that target epigenetic writers, readers, erasers and chromatin remodelers (Fig. 1). They have also fueled studies aimed at delineating the role of epigenetic regulators in human diseases unrelated to cancer.

The most clinically advanced epigenetic therapies in oncology are DNA hypomethylating agents (DNA methyltransferase inhibitors (DNMTi)) (47) and histone deacetylase (HDAC) inhibitors (48). DNA methylation of the 5-carbon on cytosine residues (5mC) in CpG dinucleotides is perhaps the best-characterized chromatin modification in cancer. There is irrefutable evidence that global alterations in DNA methylation occur in a broad range of cancers and are associated with repression of tumor suppressor genes, widespread shifts in chromatin architecture, and derepression of transposable elements within the genome (47). Remarkably, in the case of pediatric ependymomas, changes in DNA methylation can accurately classify these cancers into distinct prognostic subgroups even in the absence of any recurrent somatic DNA mutations (49). Although genome-wide mapping of the DNA methylation changes has been useful for categorizing a range of cancers into valuable prognostic groupings (50), to date this classification has not been very influential in directing therapeutic decisions, including the use of DNMTi such as azacitidine or decitabine.

Azacitidine is arguably the most successful epigenetic therapy. This agent has transformed the natural history of the myelodysplastic syndromes (MDS) (51), but there is ongoing debate about the molecular events that underpin this clinical success. Azacitidine reactivates the expression of certain aberrantly silenced genes in cancer cells, but a gene-specific signature that can guide the use of this drug in MDS and other cancers has remained elusive. Moreover, although much of our understanding of the mechanism of action of azacitidine has focused on its ability to cause DNA hypomethylation, this nucleoside analog preferentially incorporates into RNA and markedly alters RNA and protein metabolism (52). Recent observations suggest that part of the mechanism of action of DNMTi may relate to the fact that these drugs produce a cell-intrinsic stimulation of the immune system by reactivating endogenous retroviral elements (53, 54). These recent studies highlight an emerging theme in epigenetic cancer therapies: functional interaction with host immunity (55).

The past few years have also witnessed the emergence of exciting new therapies that specifically target epigenetic writers, readers, and erasers. These include BET (bromodomain and extraterminal) protein inhibitors, LSD1 (lysine-specific histone demethylase–1) inhibitors, mutant IDH1 and IDH2 inhibitors, EZH2 (enhancer of zeste 2) inhibitors, PRMT5 (protein arginine methyltransferase 5), and DOT1L (disruptor of telomeric silencing–1-like) inhibitors. All of these agents have entered clinical trials (Fig. 1). Although final data from these clinical studies are still being collected and analyzed, some broad lessons have begun to emerge.

Some of these epigenetic therapies have been used to specifically target a somatic mutation that is thought to be central to a particular malignancy. Examples include the use of BET inhibitors in patients with the rare NUT (nuclear protein in testis) midline carcinoma (NMCs), where the pathognomonic molecular abnormality involves a BRD4–NUT fusion protein or, less frequently, a BRD3–NUT fusion protein (56). Similarly, EZH2 mutations in lymphoma, and IDH1 or IDH2 mutations in gliomas and AML, have provided the rationale for targeted therapies in these malignancies. In the case of IDH1 or IDH2 mutant AML, therapies directly targeting the mutant enzyme responsible for the production of the oncometabolite 2HG have produced encouraging results in early clinical studies. Recent data from phase 1 trials have demonstrated an objective response in up to 40% of patients, with complete responses in up to 20% (57). As these studies have been performed primarily in heavily pretreated cohorts with relapsed and refractory AML, this degree of response for a single agent is a promising start for these targeted therapies.

Epigenetic therapies have also been used to target non-oncogene epigenetic dependencies in cancer (33). For instance, therapies directed against specific epigenetic writers such as DOT1L (58), epigenetic readers such as BET bromodomains (59, 60), and epigenetic erasers such as LSD1 (62) do not target a protein that is somatically mutated in cancer, but instead exploit malignant transcriptional dependencies in cancers such as AML. Because these epigenetic regulators are ubiquitously expressed, the therapeutic window relies on the premise that, relative to homeostatic transcriptional programs in normal cells, malignant cells have a greater need for the epigenetic regulator to sustain a malignant transcriptional program. Despite initial concerns about targeting ubiquitously expressed essential proteins, the early data...
suggest that these epigenetic therapies are relatively well tolerated; more important, they demonstrate single-agent efficacy in up to 15% of patients, with some complete responses noted (57). These results are both encouraging and perplexing. For example, the molecular rationale for DOT1L inhibitors in MLL-fusion protein leukemia is well established and appears to be applicable to the majority of the common MLL fusions in preclinical models of this disease. However, the clinical benefits appear to be not as extensive: 94 patients have received this therapy to date, and only two complete responses have been reported (57). Similarly, although BET bromodomain inhibitors have also been shown to induce complete responses in some patients with AML, it remains unclear why only certain patients respond and what precise molecular features are most likely to predict response (62).

The discrepancy between the promising preclinical data and modest clinical efficacy raises important issues for the field. First, it highlights the fact that the preclinical models used are not perfect surrogates for the challenges faced in the clinical arena. It also emphasizes the deficiencies in our ability to characterize and understand the preclinical activity of small-molecule drugs. When assessing a new small-molecule drug, ideally one would like to visualize the cellular localization of the compound, identify the protein targets that the molecule engages within a cell, and, for drugs that primarily target epigenetic proteins, understand where in the genome the drug is engaging its target at the chromatin interface (63). Once such drugs emerge as preclinical candidates and progress to evaluation in animal models, it is important to understand whether they preferentially accumulate in specific tissues. For cancer therapies, it would be especially advantageous to know whether there is a greater effect of the drug in cancer cells versus normal cells within the same tissue environment. New chemical tools, animal models, and innovative strategies are needed to address these issues.

Tumor heterogeneity and transcriptional plasticity

Across many malignancies, genetic and functional differences exist between subpopulations of cancer cells in a single patient. This “intra-tumor heterogeneity” can be driven by nongenetic influences. Normal differentiation provides an excellent paradigm for understanding this heterogeneity. Any given tissue comprises cells that are genetically identical yet exhibit marked phenotypic and functional differences. This balance between self-renewal and differentiation is orchestrated through various cell-intrinsic and -extrinsic cues. This paradigm also extends to malignant tissues, where it is clear that there are populations of cells capable of initiating and sustaining the tumor—so-called cancer stem cells—whereas other malignant cells are relatively short-lived and incapable of self-renewal. In a genetically identical context, this functional heterogeneity is driven by critical variations in transcription programs that are finessed by epigenetic regulators.
Epigenetic heterogeneity is far more dynamic than genetic heterogeneity, and it is likely that transcriptional plasticity driven by epigenetic regulators responding to environmental and therapeutic pressures underpins the failure of many cancer drugs to induce durable disease remission in patients. It has been hypothesized that therapeutic failures often arise from adaptive responses in cancer stem cells. Consistent with this idea, it was recently demonstrated in preclinical models that resistance to epigenetic therapies in AML emerges from a subpopulation of leukemia stem cells (64). This model of resistance was not driven by genetic evolution but rather by transcriptional plasticity, an important emerging theme of epigenetic resistance in cancer biology (64–66).

These studies have answered some crucial questions but have also raised new ones: Why are some leukemia stem cells sensitive and others resistant to the same therapeutic pressure? What drives this transcriptional plasticity? Is the adaptive response equal in different tissues? The transcriptional plasticity by which some cancers evade epigenetic therapies also potentially offers new opportunities. In the absence of gatekeeper mutations and genetic evolution, the therapeutic pressure exerted on a heterogeneous population of cancer cells by epigenetic therapies provides a unique bottleneck that helps to homogenize the adaptive response to the therapy by invoking the use of an alternative transcriptional program to sustain the cancer cells. Although this adaptive response is likely to be cell context–dependent, it may be predictable and therefore of use in exposing and exploiting new synthetic lethal dependencies. These strategies may result in new combination therapies that are more efficacious.

Combination therapies

All too often, an empirical approach drives the iteration of combination therapies in cancer. Urgent clinical need will continue to fuel this policy, but there have been some important lessons learned with epigenetic therapies that warrant a more cautious approach. Because normal and malignant epigenetic regulation is cell context–specific, empirical combinations of therapies that substantially alter the epigenome may potentially be detrimental. For example, monotherapy with a DNMTi extends the survival of many patients with MDS (53), and HDAC inhibitors in isolation have also shown some benefit in MDS. However, in contrast to the predicted synergy, several studies have now demonstrated that the empirical combination of these agents results in no discernible synergy and in fact may result in functional antagonism; several patients have had a poorer outcome with combination therapy than those treated with a DNMTi alone (67, 68). These findings highlight the need to thoroughly explore the molecular rationale for combination epigenetic therapies and experimentally demonstrate the synergistic effects of the combination therapy in appropriate preclinical models and primary human cancer cells. Several recent examples of this molecular approach, including the combination of BET inhibitors and DOT1L inhibitors (38) and a synthetic lethal strategy of combining IDH inhibitors with BCL2 inhibitors (69), have begun to emerge and set the stage for future combination therapy trials.

Developing new epigenetic therapies

There is now great interest in generating small molecules that are effective in modulating the cancer epigenome. At present, however, there is no clear strategy to establish what these therapeutic targets should be. Much of epigenetic drug discovery is being driven by what is possible from a medicinal chemistry viewpoint rather than what is needed. To aid in this process, a number of investigators have used genetic screens to identify novel targets that compromise the viability of cancer cells both in vitro and in vivo (70, 71). Although a comprehensive discussion on the merits of genetic knockout using methods such as CRISPR/Cas9 versus knockdown using RNA interference (RNAi) is beyond the scope of this review, some caveats need to be acknowledged when using such approaches to identify druggable epigenetic regulators. First, it is important to recognize that many epigenetic proteins function in the context of multiprotein member complexes, and a single epigenetic protein may have an essential scaffold/targeting/catalytic role in several diverse complexes (23). Therefore, genetic ablation of a single member may disrupt the entire complex and the "real" druggable target may not be the one identified in the screen. Furthermore, epigenetic proteins often contain several functional protein domains (Fig. 2). Some of these domains are used to bind DNA or posttranslational histone modifications (epigenetic reader domains). Other domains have catalytic activity to either deposit (epigenetic writer domain) or remove (epigenetic eraser domain) histone/DNA modifications (23). This is important because each of these domains may have a distinct role in epigenetic regulation. Therefore, identifying the precise domain responsible for the phenotype of interest is critical to informing rational drug design. An elegant strategy using genome editing to identify the protein domain most critical for drug development has recently been proposed. By designing guide RNAs against the coding regions of epigenetic writer, reader, or eraser domains, the authors could identify the most important functional domain because in-frame variants in the domain, which preserve the full-length protein, were equally deleterious to gene silencing of the epigenetic regulator (72). Perhaps the ideal method in future studies seeking to identify new epigenetic therapies is to use a combination of these strategies in sophisticated models of cancer (Fig. 2).

Conclusions and perspective

The recognition that epigenetic regulators play a central role in the initiation and maintenance of cancer and can be therapeutically targeted has presented a myriad of opportunities. Thus far, as is customary, all of the new epigenetic therapies have been tested in early-stage clinical trials in patients with relapsed and chemoresistant cancers. The fact that some of these patients have achieved a complete response, albeit transiently on single-agent therapy, offers hope for the future of these agents. It is unlikely that epigenetic therapies as single agents will provide a panacea for any aggressive malignancy, and therefore the future lies in rational patient selection and combination therapies. Even in aggressive and often incurable diseases such as AML, combination chemotherapy using two cytotoxic drugs is very effective in inducing a complete response. The problem lies in maintaining this response by eradicating the tumor-initiating cells that persist as minimal residual disease and serve as the nidus for a later relapse. The fact that mutations in epigenetic regulators are often early events seen in premalignant cells raises the possibility that therapies targeted to these mutations may have a role as maintenance therapies to consolidate the gains from combination chemotherapy. Epigenetic therapies may also achieve success when used to exploit synthetic lethal vulnerabilities exposed by therapeutic pressure from other targeted therapies or the de novo mutational landscape; for example, preclinical models of cancers containing SWI/SNF mutations have been shown to be more responsive to EZH2 inhibitors (25).

A rapidly increasing body of evidence demonstrates the interdependence of cancer epigenetics and cancer immunology. It is clear that epigenetic therapies induce an immunological response that contributes to their efficacy (55), and accumulating data also demonstrate that epigenetic therapies potentiate the effects of adoptive immunotherapies (73) and immune checkpoint inhibitors (74). As such, the next frontier—combinations of immunotherapies and epigenetic therapies—is set for clinical evaluation. Despite the challenges posed by the incessant evolution of cancer under therapeutic pressure, our increasing understanding of these diverse trajectories and the fundamental role that epigenetic regulation plays in this process has offered several innovative therapeutic opportunities. This field looks forward with optimism as we continue our quest to change the natural history of aggressive malignancies with combination therapies that include epigenetic agents as a cornerstone.

REFERENCES AND NOTES


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