REVIEW SUMMARY

CANCER

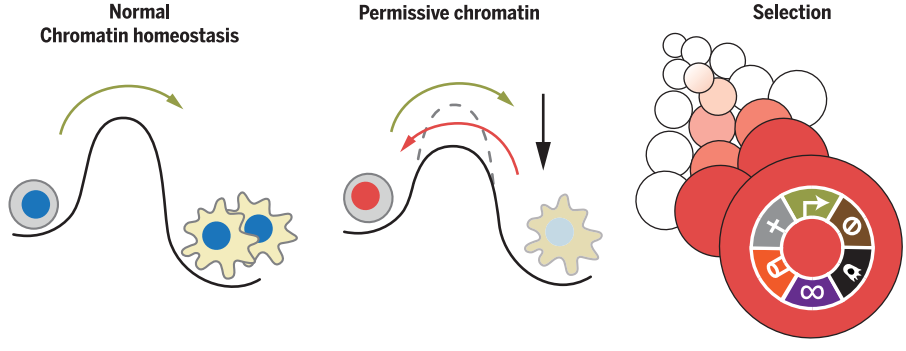
Epigenetic plasticity and the hallmarks of cancer

William A. Flavahan, Elizabeth Gaskell, Bradley E. Bernstein*

BACKGROUND: Chromatin is the essential medium through which transcription factors, signaling pathways, and other cues alter gene activity and cellular phenotypes. It assumes distinct conformations that reinforce regulatory activity or repression at a given locus, and reorganizes in response to appropriate intrinsic and extrinsic signals. The biologist Conrad Waddington famously conceptualized developmental specification as an epigenetic landscape in which differentiating cells proceed downhill along branching canals separated by walls that restrict cell identity. By restricting lineage-specific gene expression and phenotypes, chromatin affects the height of the walls between the canals in this epigenetic landscape.

Genetic, metabolic, and environmental stimuli that disrupt chromatin alter cellular states and responses, thereby predisposing individuals to a range of common diseases. Although cancer is typically considered a genetic disease, chromatin and epigenetic aberrations play important roles in tumor potentiation, initiation, and progression.

ADVANCES: We discuss how the stability of chromatin, or its "resistance" to change, is precisely titrated during normal development, and we propose that deviation from this norm is a major factor in tumorigenesis. We review genetic, environmental, and metabolic stimuli that disrupt the homeostatic balance of chromatin, causing it to become aberrantly restrictive or permissive. Stimuli that increase chromatin resistance may result in a restrictive state that blocks differentiation programs. Stimuli that decrease chromatin resistance may result in a permissive state, which we refer to as epigenetic plasticity. We propose that plasticity allows premalignant or malignant cells to stochastically activate alternate gene regulatory programs and/or undergo nonphysiologic cell fate transitions. Some stochastic changes will be inconsequential "passengers"; others will confer fitness and be selected as "drivers." As cancer cells divide, acquired epigenetic states may be maintained through cell division by DNA methylation, repressive chromatin, or gene regulatory circuits, giving rise to adaptive epiclones that fuel malignant progression.



Epigenetic plasticity, selection, and cancer. (**Left**) Normal chromatin and associated epigenetic mechanisms stabilize gene expression and cellular states while facilitating appropriate responses to developmental or environmental cues (blue nuclei represent normal cell state). Genetic, environmental, and metabolic insults that disrupt chromatin can lead to either restrictive or overly permissive chromatin states. (**Center**) Overly permissive chromatin results in epigenetic plasticity; this plasticity permits stochastic activation of alternate gene regulatory programs (red nuclei represent cancer-like cell state). (**Right**) Some stochastic changes will be inconsequential "passengers" while others will confer fitness and be selected as "drivers"; in this way, chromatin aberrations have the potential to fulfill each hallmark of cancer.

We highlight specific chromatin aberrations that confer epigenetic restriction or plasticity, and ultimately drive tumor progression via oncogene activation, tumor suppressor silencing, or adaptive cell fate transitions. Aberrations initiated by defined genetic stimuli, such as chromatin regulator gene mutations, are particularly informative regarding mechanism. Examples include gain-of-function mutations of the Polycomb repressor EZH2 that promote chromatin restriction and hinder differentiation, and metabolic enzyme mutations that disrupt the balance of DNA methylation. Changes in DNA methylation resulting from the latter have been tied to tumor suppressor silencing but may also result in stochastic insulator disruption and oncogene activation. We also carefully consider metabolic and environmental stimuli that disrupt chromatin homeostasis in the absence of genetic changes. Examples include links between folate metabolism and methylase activity, environmental factors that promote DNA hypermethylation in gastrointestinal tissues, and potential effects of microenvironmental stress on chromatin regulator expression. Purely epigenetic mechanisms may explain tumors that arise with few or no recurrent mutations, as well as heterogeneous functional phenotypes within tumors that lack genetic explanation. We conclude that chromatin and epigenetic aberrations can confer wide-ranging oncogenic properties and may fulfill all of cancer's hallmarks.

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OUTLOOK: Initial successes with epigenetic therapies suggest the potential of cancer epigenetics for major clinical impact. Yet realizing this promise will require a clearer understanding of epigenetic mechanisms of tumorigenesis. The identification of increasing numbers of oncogenic epigenetic lesions provides an opportunity to develop and test conceptual and mechanistic models of their functions. Progress will require new technologies for probing chromatin and epigenetic alterations with single-cell precision, as well as experimental models that faithfully recapitulate epigenetic states in tumors. We are optimistic that an improved understanding of epigenetic plasticity and restriction could advance diagnostic strategies for evaluating tumor stage and heterogeneity, and yield new therapeutic strategies for correcting epigenetic lesions or exploiting vulnerabilities of epigenetically altered cells. ■

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Chromatin and associated epigenetic mechanisms stabilize gene expression and cellular states while also facilitating appropriate responses to developmental or environmental cues. Genetic, environmental, or metabolic insults can induce overly restrictive or overly permissive epigenetic landscapes that contribute to pathogenesis of cancer and other diseases. Restrictive chromatin states may prevent appropriate induction of tumor suppressor programs or block differentiation. By contrast, permissive or "plastic" states may allow stochastic oncogene activation or nonphysiologic cell fate transitions. Whereas many stochastic events will be inconsequential "passengers," some will confer a fitness advantage to a cell and be selected as "drivers." We review the broad roles played by epigenetic aberrations in tumor initiation and evolution and their potential to give rise to all classic hallmarks of cancer.

A single human genome gives rise to hundreds of cell types and adapts to different developmental and environmental conditions with a vast repertoire of gene expression patterns. A mere 2% of the genome encodes proteins; the remaining 98% is replete with regulatory elements that underlie context-specific gene activity. The 6 billion bases of coding and noncoding DNA are wrapped about ~30 million nucleosomes, forming a massive, exquisitely regulated macromolecular complex termed chromatin. Chromatin is the essential medium through which transcription factors (TFs), signaling pathways, and other cues alter gene activity and cellular phenotypes (Fig. 1A) (*1*, *2*). Aberrations in chromatin are associated with a wide range of common diseases, including aging-related diseases, neuropsychiatric disorders, autoimmunity, and cancer.

Although cancer is typically considered a genetic disease, epigenetic aberrations play profound and ubiquitous roles. In fact, cancers are universally associated with abnormalities in gene expression, cellular identity, and responsiveness to internal and external cues (*3*–*6*). A major, unanticipated outcome of large-scale cancer genome sequencing projects is the finding that roughly 50% of human cancers harbor mutations in chromatin proteins (*7*, *8*). Malignant cells also exhibit genome-wide alterations in DNA methylation, chromatin structures, and regulatory element activities. In addition, many tumors exhibit deranged developmental programs indicative of differentiation block or epigenetic reprogramming (*6*, *9*, *10*).

The goal of this review is to synthesize current literature into a general mechanistic model for cancer epigenetics. The overriding premise is that specific genetic, environmental, and metabolic stimuli disrupt the homeostatic balance of chromatin, causing it to become either aberrantly restrictive or aberrantly permissive. Such stimuli may act in a premalignant cell to promote tumor initiation and/or in a malignant cell to accelerate tumor evolution and adaptation. This model can explain diverse oncogenic stimuli whose effects are mediated through chromatin aberrations. The ubiquity of such stimuli suggests that epigenetic defects contribute to diverse aspects of cancer biology and may in fact suffice to satisfy every hallmark of cancer (*11*, *12*).

Epigenetic homeostasis in healthy cells

The human genome comprises thousands of expansive genomic loci that contain genes as well as proximal and distal regulatory elements (promoters and enhancers, respectively) that control gene activity in specific cell types. The genome is packaged into chromatin, and these individual loci are organized into topologically associating domains (TADs) and bounded by insulators that ensure their independent and appropriate regulation (*13*–*15*). Examples include the β-globin locus, which orchestrates developmental stage-specific expression of globin genes; various developmental loci containing TF genes flanked by enhancers that specify their tissue-specific expression; and generich loci packed with housekeeping genes. The activity of a locus is intimately tied to its chromatin organization. Active genes and elements must be accessible to regulatory factors and transcriptional machinery, whereas inactive loci are sequestered within compact and inaccessible structures that prevent their inappropriate activity (*1*, *2*, *6*).

Context-specific repression of lineage-specific developmental genes is enforced by Polycomb repressors, such as the histone H3 Lys^{27} (H3K27) methyltransferase EZH2 (enhancer of zeste homolog 2) (*6*, *16*). Polycomb repression can be maintained through mitotic cell division by several mechanisms, including a conformational switch in the EZH2 complex that is stimulated by H3K27 methylated histones and results in increased enzyme activity (*17*). Repetitive sequences and gene deserts are silenced by heterochromatin structures, histone H3 Lys^{9} (H3K9) methylation, and lamin-associated factors (Fig. 1A). These repressive states can also be propagated through mitosis via functional interactions among histone modifications, DNA methylation, regulatory proteins, and noncoding RNAs (*1*, *2*).

Conversely, active loci may be sustained by TFs and chromatin modifying cofactors that bind promoters and enhancers, engage RNA polymerase, and stimulate transcriptional activity. These regulatory activities present a potent barrier to chromatin repression and compaction, which facilitates robust maintenance of the active state (*13*, *18*).

Because any single locus can assume different transcriptional states in different cellular contexts, the chromatin state must be capable of responding to appropriate cues and conditions. As discussed below, the likelihood that a locus will respond to a signal for change is dependent on the expression of TFs and their recruitment to the locus, as well as its local chromatin state and the global chromatin environment in the cell (Fig. 1B).

Chromatin homeostasis and Waddington's landscape

The biologist Conrad Waddington famously conceptualized developmental specification as an epigenetic landscape in which differentiating cells proceed downhill along branching canals (*19*). The canals are separated by walls that constrain lineage and cell identity. Decades of research since Waddington's prescient description have revealed that TFs are the predominant specifiers of cellular identity, and therefore of the topography of the canals (*13*, *18*). TF networks define and sustain the discrete cellular states represented by the canals.

Although chromatin regulators are critical partners for TFs, they play a secondary role in the definition of cell fates. Rather, a primary function of chromatin during development is to reinforce or stabilize these lineages and cell fates. In the context of Waddington's landscape, chromatin structures and regulators affect the height of the walls that partition canals and prevent cells from switching states. This central role for chromatin is strongly supported by genetic, cell biology, and biochemistry studies [as reviewed in (*6*, *18*, *20*)]. Here we highlight a few key concepts. *Drosophila* embryos that are genetically deficient in Polycomb repressors exhibit profound alterations in cell identity while the corresponding mutant cells can transdifferentiate across lineages (*21*). Polycomb repressors, heterochromatin factors, and other histone-modifying enzymes act as barriers that hinder cellular reprogramming (*22*–*25*). Suppression of these proteins facilitates the conversion of fibroblasts to induced pluripotent stem (iPS) cells. Repressive chromatin structures sequester genomic loci that are unused in a given lineage, including nonlineage TF genes, compacting their DNA and preventing their

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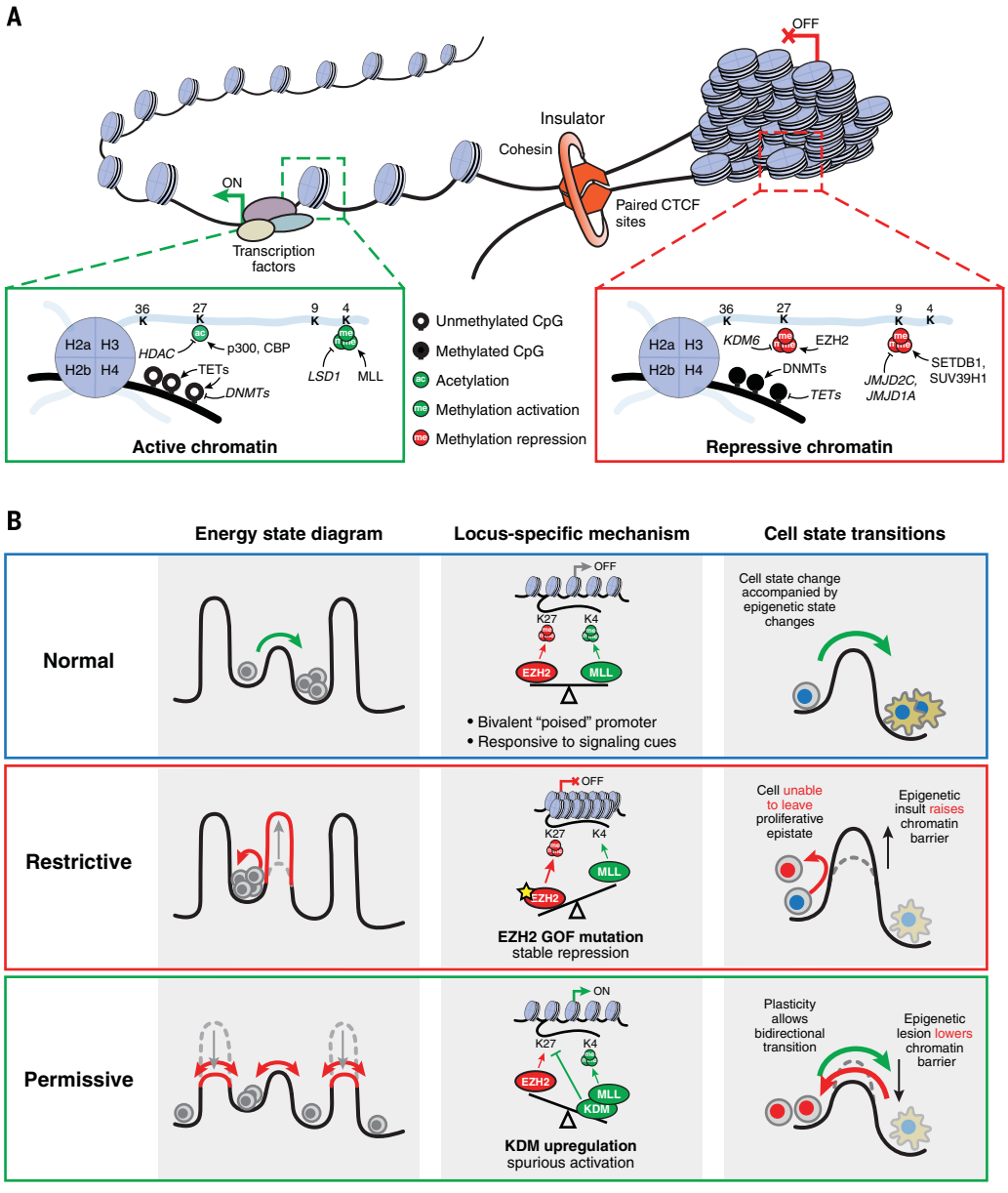

Fig. 1. Chromatin structure affects cellular identity and state transitions. (**A**) Chromatin can adopt active and repressive states. Active states are made accessible to transcription factors and other regulatory factors; they are enriched for histone modifications such as acetylation (H3K27ac) and trimethylation (H3K4me3). Repressive states are compact and are characterized by DNA hypermethylation, chromatin repressors, and specific histone methylation marks (H3K27me3, H3K9me3). CTCF and cohesin partition the genome into discrete regulatory units, termed TADs. (**B**) Chromatin networks reinforce cell states and affect responsiveness to intrinsic and extrinsic cues. Cells with perturbed chromatin networks fail to respond appropriately to such cues. Overly restrictive chromatin accentuates epigenetic barriers that prevent cell state transitions. Overly permissive chromatin lowers epigenetic barriers, allowing promiscuous sampling of alternate cell states. The opposing activities of the H3K27 methyltransferase EZH2 and the H3K4 methyltransferase MLL are given as an example; however, the concept holds for other regulators such as DNMTs and TET enzymes (see text for details). HDAC, histone deacetylase; DNMTs, DNA methyltransferases; CBP, CREB-binding protein; LSD1, lysine-specific histone demethylase 1A (KDM1A); JMJD2C, JmjC domain–containing histone demethylase 2C (KDM4C); SETDB1, SET domain bifurcated 1; SUV39H1, suppressor of variegation 3-9 homolog 1. Far right: Blue nuclei represent normal cell states; red nuclei represent cancer-like states.

spurious activation. Thus, by restricting changes in gene activity, chromatin increases the heights of energy walls between cell states and resists changes in cell identity.

Further evidence indicates that the magnitude of chromatin restriction can change during development. In embryonic stem (ES) cells, hyperdynamic nucleosome exchange hinders the establishment of repressive structures, leaving many developmental TF genes in a "bivalent" state with "active" and "repressive" histone marks that "poise" these genes for alternate fates (*1*, *15*, *26*). As developing cells commit along specific lineages, their chromatin becomes more restrictive (*18*, *20*, *22*, *27*–*29*). Progressive chromatin restriction correlates with reduction in cell fate potential and is likely to play a causal role in this regard (*18*, *29*). Hence, chromatin structure impedes changes to gene activity (or, more broadly, cellular state) with a developmentally and contextually appropriate degree of resistance.

On the basis of a growing body of evidence, we postulate that chromatin resistance must be precisely titrated at each stage of development, and that deviation from the norm is a major factor in tumorigenesis. We discuss genetic, environmental, and metabolic "stimuli" that cause such deviations. Certain stimuli may increase chromatin resistance, resulting in a restrictive state that blocks a differentiation program. Others may decrease chromatin resistance, resulting in a permissive state that allows stochastic induction of oncogenes or other adaptive programs.

Epigenetic restriction in tumorigenesis

The homeostatic chromatin network is predicated in large part on interplay among Polycomb-family repressors, trithorax-family activators, and nucleosome remodelers (*30*). Recurrent mutations in the genes encoding these factors are genetic stimuli likely to disrupt this homeostasis. We begin by considering stimuli that induce chromatin restriction through excessive repressor activity, repressive chromatin marks, and/or DNA methylation (Fig. 2). Gain-of-function EZH2 mutations are frequent in several lymphoma subtypes and have also been detected in melanoma (*31*). EZH2 is the catalytic subunit of Polycomb repressive complex 2 (PRC2), which plays broad roles in B cell development. It is highly active in germinal center B cells but is rapidly down-regulated upon differentiation, allowing activation of specification genes. The gain-of-function mutations create a hyperactive methyltransferase enzyme (*32*, *33*). Genome-wide analyses of EZH2 mutant lymphomas revealed

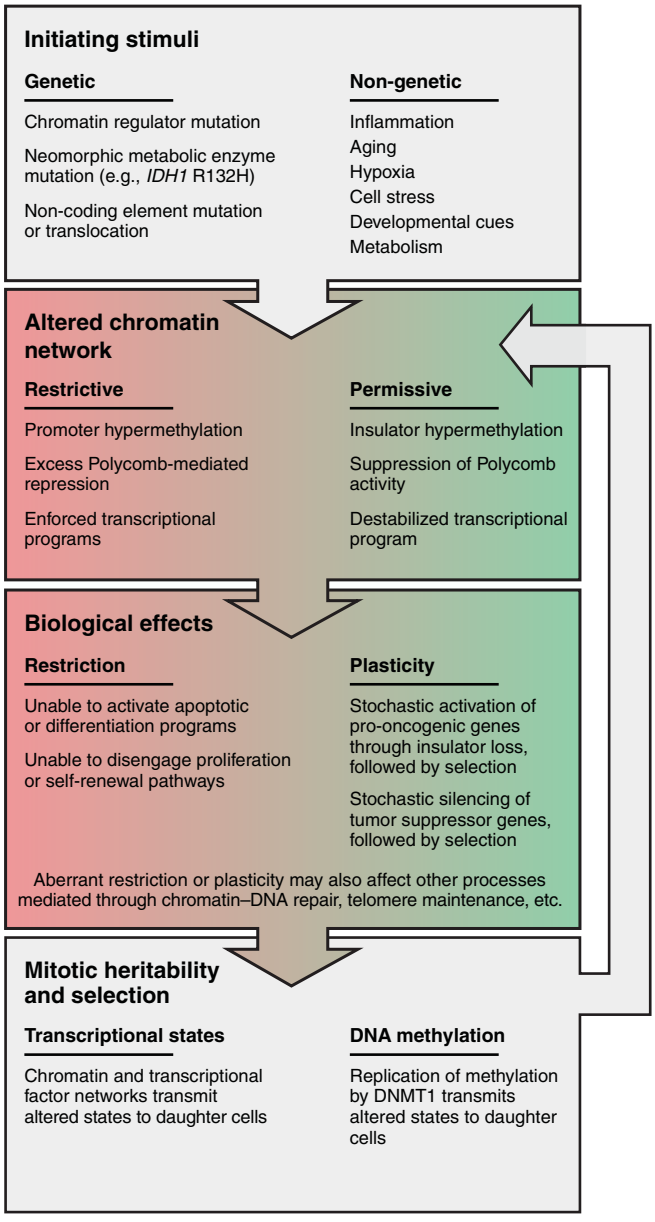

Fig. 2. Chromatin homeostasis is disrupted in cancer. Chromatin homeostasis may be disrupted by genetic stimuli (e.g., chromatin regulator mutations or regulatory element translocation) or non-genetic stimuli (e.g., aging, inflammation, hypoxia, etc.). Such stimuli can result in an overly permissive or overly restrictive chromatin network. Permissive states may allow stochastic oncogenic epigenetic changes such as silencing of tumor suppressor genes. Adaptive epigenetic changes that are mitotically heritable will be selected (Fig. 3) and may give rise to hallmarks of cancer (Fig. 4).

expansive H3K27 trimethylation and depletion of active chromatin marks over loci encoding terminal genes. The tumorigenic mutants thus appear to induce a restrictive state that prevents induction of differentiation genes and arrests B cell development such that the cells remain in a proliferative state (*34*, *35*).

PRC2 activity is opposed by demethylases that remove H3K27 methylation, by modifying enzymes that catalyze H3K27 acetylation or H3K4 methylation, and by nucleosome remodelers (*2*, *30*). Corresponding enzymes, including KDM6A/B, p300, MLL components, and ARID1A/B, are genetically inactivated in a wide range of cancers (*7*, *36*). The functional effects of the mutations remain poorly understood. In certain cases, they appear to shift the balance of chromatin toward PRC2 repression. For example, inactivating MLL2 and CBP/p300 mutations in lymphoma impede appropriate engagement of promoters or enhancers needed for differentiation, paralleling or potentially cooperating with EZH2 gain-of-function alleles (*34*, *37*, *38*).

In pediatric malignant rhabdoid tumors, homozygous inactivation of the gene encoding the remodeling enzyme SNF5 disables enhancers associated with mesenchymal differentiation genes, many of which are PRC2 targets (*6*, *31*). Chemical inhibitors of EZH2 lead to rhabdoid tumor regression in mouse xenograft models and are now in clinical trials. Genes encoding other SWI/SNF complex members, most notably ARID1A/B, are among the most frequently mutated in all human cancers (*36*). Their genetic inactivation may promote global chromatin restriction.

Chromatin restriction can also arise from alterations to the DNA methylation landscape. DNA methylation plays diverse roles in repetitive element silencing, in parent-of-origin allelic imprinting, and in transcriptional elongation and RNA splicing (*39*). In normal cells, cytosines in CpG islands and other CG-rich loci are largely unmethylated, whereas cytosines in CG-poor regions tend to be highly methylated. In many cancers, this pattern is profoundly distorted as CpG islands become hypermethylated and CG-poor regions become hypomethylated. The former aberration has been termed CpG island methylator phenotype (CIMP) and is perhaps the most widely studied epigenetic alteration in cancer, having been described in a wide range of phenotypically diverse tumors. CpG island hypermethylation can silence and/or prevent reactivation of the tumor suppressor p16 (*3*, *4*) and DNA mismatch repair genes [e.g., those encoding MLH1 and MSH2 (*3*, *4*, *40*)]. Although the generality and causality of DNA hyper- and hypomethylation in cancer remains controversial, these examples are consistent with a role for CpG island hypermethylation in epigenetic restriction.

Epigenetic plasticity and clonal selection in tumorigenesis

Whereas certain stimuli exert their oncogenic effects by epigenetic restriction, others induce a more permissive state that may be conceptualized as a lowering of the walls between canals in Waddington's landscape (Fig. 1B and Fig. 2). Permissive or "plastic" chromatin may allow premalignant or malignant cells to sample alternative transcriptional states, gene pathways, or developmental programs, a subset of which may be pro-oncogenic or otherwise adaptive. Critically, if an adaptive chromatin or transcriptional state change is propagated through mitosis, a new clone will arise and expand as a result of its increased fitness (Fig. 3).

In considering the epigenetic plasticity model, it is useful to draw an analogy with the genetic instability that is induced by carcinogens or DNA repair defects. In that genetic framework, increased mutation frequency leads to "driver" events (e.g., mutations that activate oncogenes) as well as "passenger" events that do not alter tumor cell fitness. Similarly, in the setting of epigenetic plasticity, we posit that some ensuing chromatin or transcriptional alterations will be drivers (e.g., will induce oncogene expression), while others will be passengers in that they fail to affect the expression of a consequential gene. Epigenetic alterations may occur individually over time or, alternatively, may arise as multiple simultaneous disruptions, analogous to the catastrophic genetic aberrations associated with "chromothrypsis" (*41*, *42*). Thus, our broad hypothesis is that certain stimuli induce epigenetic plasticity that allows premalignant or malignant cells to sample alternate chromatin or transcriptional states, a subset of which confer fitness and are maintained through cell division (Fig. 3). Indeed, many cancers exhibit marked cell-to-cell variability in gene expression and functional phenotypes (*43*). In the following sections, we describe potential stimuli for epigenetic plasticity that may promote tumor initiation

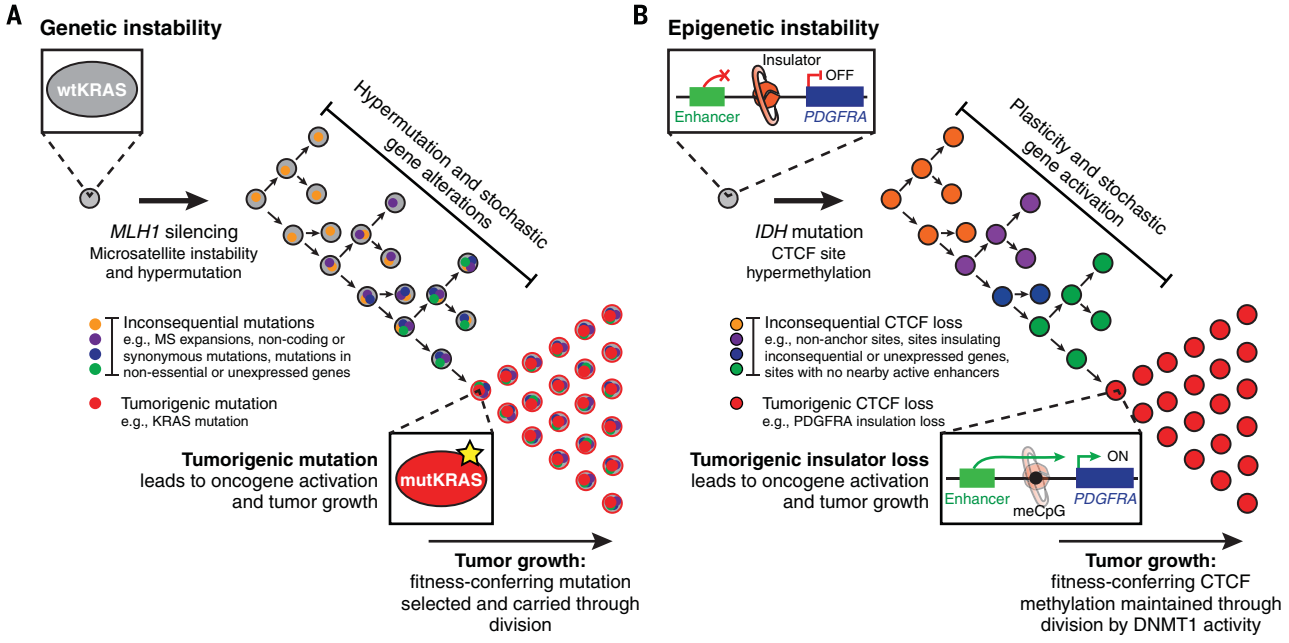

Fig. 3. Genetic and epigenetic evolution in cancer. (**A**) Genetic instability in tumor initiation. An initiating event (e.g., MLH1 silencing) causes stochastic hypermutation, leading to inconsequential "passenger" alterations as well as a "driver" mutation (e.g., in KRAS) that is selected. (**B**) Epigenetic instability in tumor initiation. An initiating event (e.g., IDH mutation) causes stochastic hypermethylation, leading to inconsequential "passenger" CTCF losses as well as a "driver" event that disrupts insulation of the *PDGFRA* oncogene and is selected. Selective pressure and mechanisms of epigenetic mitotic heritability may result in persistence of the altered states even if the initiating stimulus is removed.

and/or allow malignant cells to adapt to their environment.

Epigenetic plasticity: DNA methylation and disruption of oncogene insulation

As a first example, we focus on a genetic stimulus that destabilizes chromatin structure and thereby triggers epigenetic instability. Gain-of-function mutations in the gene encoding isocitrate dehydrogenase (IDH) are frequent initiating events in glioma, leukemia, and other tumors (*44*–*47*). Mutant IDH generates an oncometabolite that inhibits hydroxylases, including TET (ten-eleven translocation) enzymes, which catalyze DNA demethylation. Thus, the DNA in IDH mutant tumors is hypermethylated. Hypermethylation disrupts binding of the methylation-sensitive DNA binding protein CTCF. CTCF is critical for the establishment of chromosomal loops that partition the human genome into discrete functional domains and ensure that enhancers regulate their appropriate gene targets. CTCF thus acts as an "insulator" that protects genes from inappropriate activation by overly promiscuous enhancers.

Reduced CTCF binding in IDH mutant gliomas is associated with a global transcriptional signature indicative of insulator dysfunction (*48*). Specifically, the expression of proximal genes separated by a CTCF insulator is more highly correlated in IDH mutant tumors than in normal cell types (*48*, *49*). This suggests that IDH oncogenicity might be mediated by loss of gene insulation. Indeed, one consistently deregulated CTCF boundary is near *PDGFRA*, a prominent glioma oncogene encoding platelet-derived growth factor receptor A. Loss of this boundary allows a potent enhancer in a neighboring domain to aberrantly activate PDGFRA and drive proliferation of hypermethylated gliomas.

Although the PDGFRA insulator may be preferentially sensitive to disruption, the totality of findings is consistent with an epigenetic plasticity model. The human genome contains thousands of chromosomal loops, hundreds of which appear to be disrupted in IDH mutant tumors (*48*). This suggests that hypermethylation causes stochastic CTCF insulator disruption in a premalignant IDH mutant cell. The loss of any specific insulator may then be preserved through cell division, as a result of the epigenetic stability of DNA methylation (Fig. 3). Thus, a new "epigenetic clone" will arise with altered chromosomal topology and proximal gene activity. In most cases, transcriptional changes will be inconsequential "passengers" and the new clone will be maintained at low frequency or lost entirely. However, in a subset of cases, a "driver" transcriptional change will activate an oncogene or otherwise confer a fitness advantage to the clone. This adaptive clone will expand and, given appropriate conditions and subsequent hits, give rise to a tumor.

The proposed model of insulator loss is of general importance because many oncogenes are sequestered within insulated neighborhoods, presumably owing to their tumorigenic potential (*50*). Indeed, frequent mutations of CTCF motifs in the vicinity of oncogenes have been reported in colorectal, liver, and esophageal cancer (*50*, *51*). Furthermore, the genes encoding CTCF protein and its associated boundary factor cohesin are recurrently mutated in multiple tumor types (*52*–*55*). CTCF haploinsufficiency has also been shown to promote tumor formation in mice (*56*); CTCF haploinsufficiency in this setting also destabilizes DNA methylation, providing further support for the concept of interplay between DNA methylation and CTCF function. Thus, multiple genetic and epigenetic mechanisms can compromise CTCF-mediated genome topology, each with the potential to drive stochastic insulator dysfunction, epigenetic plasticity, and oncogene activation.

Epigenetic plasticity: Permissive chromatin states

In considering chromatin aberrations likely to confer plasticity, EZH2 and its substrate histone H3K27 are of particular interest. EZH2 can repress a wide range of genes but does so in a highly context-dependent manner (*6*). Thus, EZH2 gain-of-function mutations may be oncogenic in B cell lineages, whereas EZH2 loss-of-function mutations are tumorigenic in other settings. EZH2 is genetically inactivated in myelodysplastic syndromes (MDS), T cell acute lymphoblastic leukemia (T-ALL), and other cancers (*31*). In addition, somatic mutation of the histone substrate (e.g., the H3.3 $Lys^{27} \rightarrow$ Met "oncohistone") in pediatric brain tumors dominantly suppresses EZH2 function (*57*–*60*). Although the underlying mechanisms remain unclear, suppression of Polycomb activity by EZH2 inactivation or histone mutation may create an overly permissive chromatin state that allows spurious gene activation, prevents differentiation-associated silencing, or destabilizes other processes such as telomere regulation.

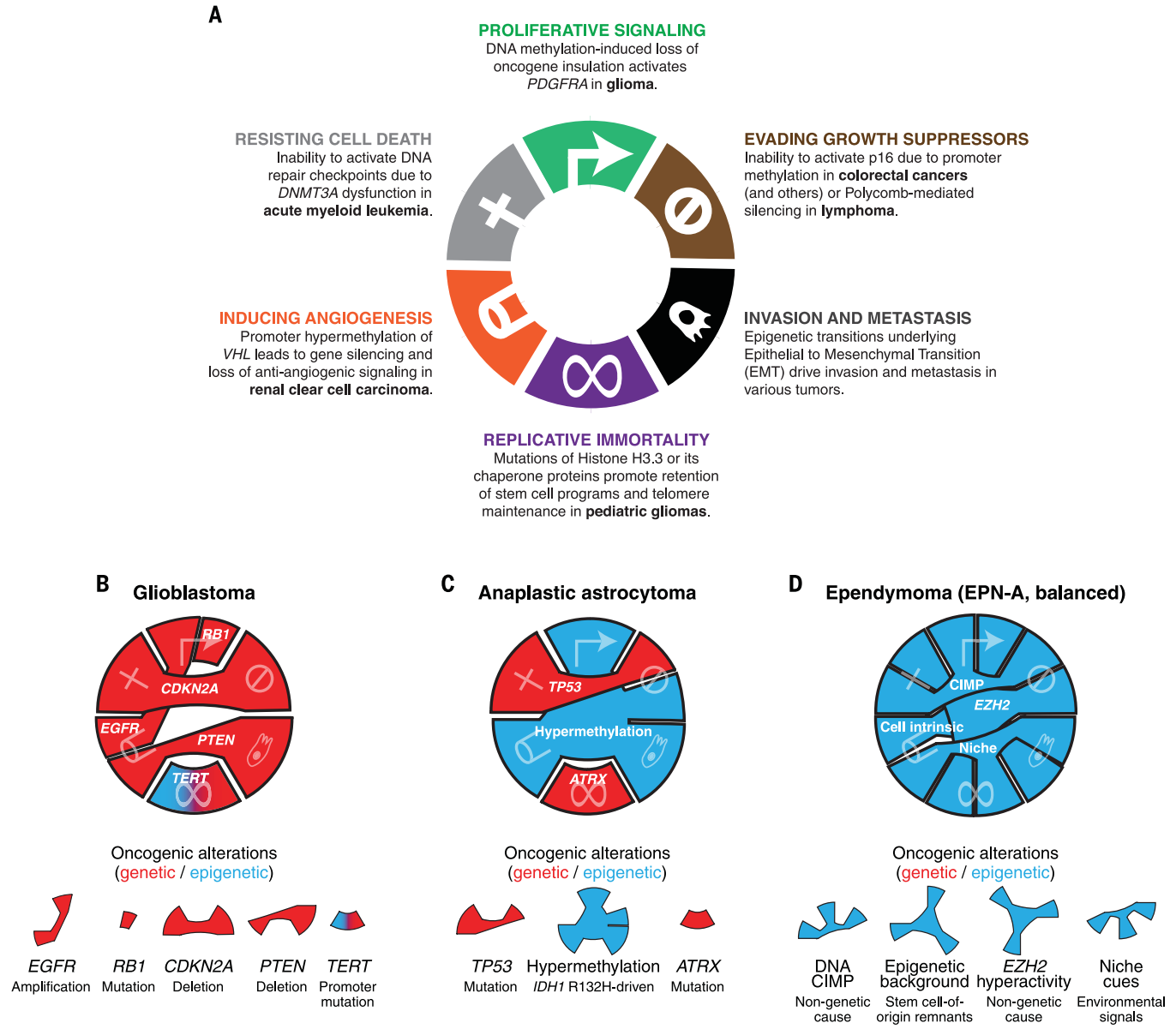

Fig. 4. Genetic and epigenetic mechanisms underlie the hallmarks of cancer. (**A**) Epigenetic mechanisms involving aberrant chromatin restriction or plasticity can give rise to each classic hallmark of cancer. [Adapted with permission from (*11*)] (**B** to **D**) Genetic and epigenetic mechanisms are important factors in the development of human cancer, but their relative contribution is dependent on tumor type. Three distinct tumors of the central nervous system illustrate this point, with the potential basis for each hallmark shown in red (genetic) or blue (epigenetic). Most hallmarks can be traced to genetic drivers in glioblastoma, a brain tumor that primarily affects adults; epigenetic factors predominate in pediatric tumors such as ependymoma, which exhibits DNA hypermethylation but lacks recurrent mutations (*72*, *73*). Anaplastic astrocytomas may exhibit examples of both genetic and epigenetic lesions, leading to different hallmarks.

Histone lysine demethylases (KDMs) have also been widely implicated in cancer. The human genome encodes more than 25 KDMs that target different lysine positions in the histone tails and thus differ in their regulatory functions (*61*). Families of related KDMs are up-regulated under stress conditions and are responsive to signals from the tumor microenvironment. Model organism studies have established roles for KDMs in erasing epigenetic memory, raising the possibility that cancer-associated deregulation of these enzymes may confer plasticity and facilitate reprogramming or adaptation (*62*, *63*). H3K4 demethylases (KDM5 family) enable lung and melanoma cell lines to evade antiproliferative therapies by adopting a slow-cycling persister state (*64*, *65*). H3K27 demethylases (KDM6 family) enable glioblastoma stem cells to tolerate similar drug pressures by regressing to a more "primitive" developmental state (*66*). KDM4 family enzymes, which demethylate H3K9 and H3K36, are up-regulated in many cancer types, where they deregulate heterochromatin, affect replication timing, and prime chromosomal copy number alterations (*67*).

Finally, compromised fidelity of DNA methylation patterning may also contribute to stochastic activation of self-renewal or cell proliferation genes. DNA methyltransferase mutations can lead to hypomethylation and aberrant activation of enhancers that drive leukemogenic gene expression patterns (*68*, *69*). Fidelity may also be perturbed by altered cellular contexts, including the increased demands of a rapidly replicating cancer genome. Accordingly, tumor cells can exhibit increased heterogeneity and variability of methylation at large numbers of CpG sites genome-wide (*70*).

DNA methylation instability has also been linked to stochastic activation of cancer-associated genes that are stably repressed in non-neoplastic tissue, including cell cycle–related and epithelial-mesenchymal transition–related genes (*71*).

Nongenetic stimuli of plasticity or restriction

The preceding sections are biased toward chromatin modifier mutations and other genetic stimuli whose relatively clear effects on chromatin provide the strongest support for our model. However, an essential element of our thesis is that oncogenic chromatin aberrations can also be induced by nongenetic (purely epigenetic) stimuli. In support of this concept, we note that chromatin state and stability can differ markedly between cells with identical genetic backgrounds, as a function of development (see above), metabolic conditions, aging, and environment. We also note that some pediatric tumors arise with very few or no detectable genetic mutations (e.g., ependymoma) (*72*, *73*). We next review nongenetic stimuli that alter chromatin state, followed by specific examples of pro-oncogenic epigenetic changes that arise in the absence of any genetic stimulus (Fig. 2).

Chromatin state is intimately tied to metabolic conditions. DNA- and histone-modifying enzymes use many metabolites as donors and cofactors, including α-ketoglutarate (α-KG), methyl donors in the folate pathway, and acetyl-CoA (*74*). Because the dissociation constants for these cofactors are close to their physiologic cellular concentrations, chromatin enzymes are exquisitely sensitive to shifts in metabolite levels. Physiologic methylation of repetitive DNA is dependent on folate (*75*), whereas maintenance of unmethylated CpG islands requires vitamin C, which is critical for demethylase activity (*76*). In stem cells, the maintenance of pluripotency is dependent on finely tuned levels of the methyl donor *S*-adenosylmethionine and the demethylase cofactor α-KG (*77*, *78*). In the adipocyte lineage, the AMPK (adenosine 5′-monophosphate–activated protein kinase)/α-KG axis regulates a master transcriptional regulator of brown fat differentiation and maintenance, PRDM16, by controlling demethylase activity at the *PRDM16* gene promoter (*79*).

Chromatin and methylation states also change during aging. Methylation signatures associated with aging have been identified in epigenomic studies and parallel some changes seen in cancer, such as increased CpG island methylation and global hypomethylation (*80*). Studies with model organisms have documented changes in global histone methylation patterns and established causal roles for the corresponding modifying enzymes in longevity (*80*, *81*). Finally, a recent single-cell transcriptomic study found that T cells from aging mice show a highly heterogeneous response to stimulation, consistent with increased epigenetic state variability or plasticity (*82*).

Understanding how epigenetic changes associated with metabolism, environment, and aging drive tumorigenesis remains a formidable challenge. However, concrete examples are emerging. DNA methylation is of particular interest as a mediator of nongenetic stimuli given its stability and mitotic heritability. Only a minority of cancers with aberrant methylation can be explained by an underlying genetic event. DNA methylation changes are particularly prevalent in colorectal cancer and may also be detected in premalignant hyperplastic polyps that have yet to acquire characteristic genetic mutations (*83*). Indeed, the epigenome of gastrointestinal cells appears particularly sensitive to environmental stimuli, such as inflammation or butyrate levels associated with diet and the gut microbiome (*84*). Cancer-specific rewiring of glucose metabolism (the so-called Warburg effect) can promote butyrate accumulation, resulting in histone deacetylase inhibition and increased cancer cell proliferation (*85*).

Promoter methylation is the best-studied epigenetic mediator of oncogenic effects. A key example is methylation and silencing of the promoter region of *MGMT*, the gene encoding DNA repair factor O^6-methylguanine-DNA methyltransferase. Silencing of the *MGMT* gene drives a hypermutator phenotype that generates many genetic subclones in the tumor (*86*). This methylation event underlies a field defect wherein multiple sporadic colorectal tumors arise within a larger region or "field" of tissue. This strongly suggests that the epigenetic aberration precedes the genetic change and likely arises in response to an environmental insult. Another notable example is methylation of the succinate dehydrogenase (SDH) gene promoter in gastrointestinal stromal tumors. Reduced SDH expression increases succinate levels and inhibits DNA demethylation, potentially reinforcing the initial methylation event and causing global hypermethylation (*87*). MLH1, MSH2, and PTEN methylation are also commonly observed without any obvious genetic trigger and are associated with poor prognosis (*3*, *4*, *40*, *88*).

A variety of studies have shown that signals from the tumor microenvironment influence cancer epigenomes. As discussed above, stress induced by the microenvironment and/or by therapeutic intervention up-regulates histone demethylases that reshape the chromatin landscape, potentially inducing plasticity and adaptation to therapy (*64*–*66*). Microenvironmental hypoxia has been shown to suppress DNA demethylase activity in breast cancer (*89*). Altered cellular contexts can also increase the rate of DNA methylation changes that affect enhancer activity and increase transcriptional plasticity in melanoma (*90*). Finally, the observation that nonmalignant cells in the tumor ecosystem can also undergo striking phenotypic changes suggests that microenvironmental effects on epigenetic states and plasticity may extend beyond the malignant compartment (*91*). These collective examples likely portend a far broader role for oncogenic epigenetic alterations that arise from nongenetic stimuli in tumorigenesis.

Relating genetic and epigenetic models of cancer

An expanded view of epigenetics raises important questions regarding the interplay between genetic and epigenetic oncogenic lesions (*8*). Such lesions may arise concurrently or stepwise, potentially in a defined order. In some cases, epigenetic changes precede characteristic oncogenic mutations (e.g., hypermethylation in premalignant colon polyps) (Fig. 3). Moreover, certain epigenetic lesions prime genetic lesions. For example, KDM4 overexpression is causally associated with chromosomal copy number aberrations, whereas silencing of the *MGMT* gene promoter causes increased mutational rates. It remains to be seen whether a genetically unstable cancer cell still requires such initiating epigenetic lesions once it has acquired downstream oncogenic mutations.

The converse case, wherein a genetic lesion disrupts epigenetic regulation, also occurs. Consider, for example, the epigenetic plasticity associated with IDH mutations (see above). The initiating genetic stimulus (IDH mutation) may become irrelevant once a downstream epigenetic event has occurred (stable oncogene induction). Such a "hit and run" mechanism has important therapeutic implications, as targeting the initiator of plasticity would be futile (Fig. 3). New diagnostic strategies that integrate genetic and epigenetic biomarkers might thus provide critical insight into cancer subtypes, prognosis, and therapeutic susceptibility.

The hallmarks of cancer

In two influential essays, Hanahan and Weinberg distilled a set of biological capabilities or "hallmarks" that must be acquired for development of a human cancer (*11*, *12*). These hallmarks framed efforts to define the mechanisms by which cells and tumor ecosystems progress through subsequent malignant stages. In large part, it was assumed that these mechanisms are fundamentally rooted in genetic alterations. However, pervasive alterations in chromatin state, methylation, and gene expression suggest that the contributions of epigenetic alterations must also be carefully considered (*3*, *92*).

How might epigenetic mechanisms contribute to each hallmark? As shown in Fig. 4A, cancer's hallmarks may be achieved through tumor suppressor silencing, oncogene activation by repurposed enhancers, or cell fate transitions. Proliferative signaling can be achieved by *PDGFRA* gene activation caused by disruption of chromatin insulators (*48*). Evasion of growth suppressors, such as p16/INK4a, can be mediated by promoter hypermethylation or EZH2 hyperactivity (*3*, *31*). Invasion and metastasis depend on cell fate transitions, such as the epithelial-mesenchymal transition, with epigenetic etiology (*90*, *93*). Replicative immortality may be driven by mutations in the gene encoding histone H3.3 or its chaperone proteins that promote telomerase-independent telomere lengthening (*57*, *94*), or by nongenetic mechanisms that simulate self-renewing stem cell states (*9*, *95*). Angiogenesis may be rooted in hypermethylation of the von Hippel–Lindau (VHL) tumor suppressor gene promoter (*96*). Finally, resisting cell death may be achieved by DNMT3A or IDH mutations that alter DNA damage responses (*97*, *98*) or epigenetic mechanisms that alter expression of apoptosis or prosurvival genes (*99*, *100*).

Clearly, our understanding of how epigenetic plasticity and alterations contribute to cancer's hallmarks lags that of the genetic culprits. Yet new epigenetic mechanisms are rapidly being discovered and, as with genetic lesions, may have potent oncogenic properties (*5*). As cancer cells divide, adaptive epigenetic states may be maintained by the stability of DNA methylation, repressive chromatin, or transcriptional regulatory programs. Thus, epigenetic plasticity and restriction should be considered alongside the more familiar genetic events that underlie each hallmark of cancer (Fig. 4, B to D).

Outlook for cancer biology and therapeutics

The altered epigenetic state of cancer cells suggests that epigenetic therapies could have a major clinical impact (*3*). However, realizing the promise of such therapies will require a deeper understanding of how epigenetic lesions drive cancers. Toward this end, the field must develop, test, validate (or refute) conceptual and mechanistic models for cancer epigenetics, and place them in context with prevailing genetic models.

Investigating the specific hypotheses presented here will require a new generation of assays and experimental models. First, new single-cell technologies are urgently needed to evaluate the state and variability of insulators, enhancers, methylation, and expression in situ within human tumors. We predict that such approaches will detect heterogeneous "passenger" changes at low frequency as well as higher-frequency "driver" events that are recurrent across tumors. Such changes will likely be accompanied by increased cell-to-cell variability in gene expression (e.g., in single-cell RNA sequencing) and other phenotypes. These technologies could also be applied to premalignant lesions such as benign colon polyps, with the goal being to ascertain whether stochastic epigenetic changes in individual cells represent early indicators of tumorigenic potential. Second, in vitro and in vivo tumor models that recapitulate the nature, dynamics, and heterogeneity of successive tumorigenic epigenetic alterations are needed. Current cancer models are biased toward genetic lesions (e.g., genetically engineered mouse models) and may not recapitulate aspects of the tumor microenvironment that may profoundly influence the epigenetic state of malignant cells. Such experimental systems will need to be complemented by new technologies capable of tracking epigenetic alterations, such as insulator loss, with temporal resolution. Here again, we predict that plasticity stimuli (e.g., a metabolic insult that disables DNA demethylation) will increase the rate at which epigenetic changes arise, yielding some epiclones with a fitness advantage that will enable them to overtake the cell population over the course of tumor development.

We are optimistic that a fuller understanding of epigenetic plasticity and restriction in cancer will advance diagnostic tools for detection of early epigenetic lesions and for evaluation of tumor stage and heterogeneity (*101*, *102*). It could also yield new therapeutic strategies for correcting epigenetic lesions or exploiting vulnerabilities of epigenetically altered cells. These new diagnostics and therapies would complement those rooted in cancer genetics. The road ahead is long but must be traveled to capture this major component of cancer biology and other human diseases.

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Epigenetic plasticity and the hallmarks of cancer

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Cancer epigenetics in the driver's seat

Recent cancer genome projects unexpectedly highlighted the role of epigenetic alterations in cancer development. About half of human cancers were found to harbor mutations in chromatin proteins. In a Review, Flavahan *et al.* propose that chromatin and epigenetic aberrations have the potential to confer on cells the full range of oncogenic properties represented in the classic "hallmarks" depiction of cancer. They suggest that genetic, environmental, and metabolic factors can make chromatin aberrantly permissive or restrictive. Permissive chromatin creates a state of "epigenetic plasticity," which can activate oncogene expression or cell fate changes that drive cancer development.

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