Interplay Between the Cancer Genome and Epigenome

Hui Shen and **Peter W. Laird**
USC Epigenome Center, University of Southern California, Room G511B, Mail code 9061, 1450 Biggy Street, Los Angeles, CA 90089-9061

Abstract

Cancer arises as a consequence of cumulative disruptions to cellular growth control, with Darwinian selection for those heritable changes which provide the greatest clonal advantage. These traits can be acquired and stably maintained by either genetic or epigenetic means. Here we explore the ways in which alterations in the genome and epigenome influence each other and cooperate to promote oncogenic transformation. Disruption of epigenomic control is pervasive in malignancy, and can be classified as an enabling characteristic of cancer cells, akin to genome instability and mutation.

Introduction

Cancer develops through successive disruptions to the controls of cellular proliferation, immortality, angiogenesis, cell death, invasion and metastasis. This evolutionary process requires new malignant traits to be stably encoded so that oncogenic events can accumulate in clonal lineages. Genetic mechanisms of mutation, copy number alteration, insertion, deletion and recombination are particularly well suited as vehicles of persistent phenotypic change. For this reason, cancer has long been viewed as a disease based principally on genetics. Nevertheless, genetic events occur at low frequency, and are thus not a particularly efficient means for malignant transformation. Some cancer cells overcome this bottleneck by acquiring DNA repair defects, thus boosting the mutation rate. Mechanisms of epigenetic control offer an alternative path to acquiring stable oncogenic traits. Epigenetic states are flexible yet persist through multiple cell divisions, and exert powerful effects on cellular phenotype. Although cancer cells have long been known to undergo epigenetic changes, genome-scale genomic and epigenomic analyses have only recently revealed the widespread occurrence of mutations in epigenetic regulators and the breadth of alterations to the epigenome in cancer cells (You and Jones, 2012). It is now clear that genetic and epigenetic mechanisms influence each other, and work cooperatively to enable the acquisition of the hallmarks of cancer (Hanahan and Weinberg, 2011).

Shaping the Epigenome

Epigenetic mechanisms allow genetically identical cells to achieve diverse stable phenotypes by controlling the transcriptional availability of various parts of the genome through differential chromatin marking and packaging. These embellishments include direct DNA modifications, primarily CpG cytosine-5 methylation (Jones, 2012), but also

plaird@usc.edu, (323) 442-7890.

hydroxylation, formylation and carboxylation (Ito et al., 2011), as well as nucleosome occupancy and positioning (Gaffney et al., 2012; Valouev et al., 2011), histone variants, and dozens of different histone modifications (Tan et al., 2011b), interacting proteins (Ram et al., 2011), and non-coding RNAs (Fabbri and Calin, 2010; Lee, 2012). These epigenetic marks do not act in isolation, but form a network of mutually reinforcing or counteracting signals. Genome-scale projects charting the human epigenome are rapidly extending our understanding of epigenetic marks and how they interact (Adams et al., 2012; Encode-Project-Consortium et al., 2012; Ernst et al., 2011).

A key facet of epigenetics is that these marks can be stably maintained, yet adapt to changing developmental or environmental needs. This delicate task is accomplished by *initiators*, such as long non-coding RNAs, *writers*, which establish the epigenetic marks, *readers*, which interpret the epigenetic marks, *erasers*, which remove the epigenetic marks, *remodelers*, which can reposition nucleosomes, and *insulators*, which form boundaries between epigenetic domains. Epigenetic writers are directed to their target locations by sequence context, existing chromatin marks and bound proteins, non-coding RNAs, and/or nuclear architecture. Those marks are then recognized by reader proteins to convey information for various cellular functions. The establishment, maintenance, and change of epigenetic marks are intricately regulated, with crosstalk among the marks and writers to help guide changes to the epigenetic landscape.

DNA Methylation

De novo methylation of DNA is catalyzed by the enzymes DNMT3A and DNMT3B, and is then maintained by the major DNA methyltransferase DNMT1, with participation from DNMT3A and DNMT3B (Jones and Liang, 2009). DNA methylation patterns are guided in part by primary DNA sequence context (Cedar and Bergman, 2012; Lienert et al., 2011) and influenced by germline variation (Gertz et al., 2011; Kerkel et al., 2008). Much of the mammalian genome consists of vast oceans of DNA sequence containing sparsely distributed, but heavily methylated CpG dinucleotides, punctuated by short regions with unmethylated CpGs occurring at higher density, forming distinct islands in the genome (Bird et al., 1985). These CpG islands (CGIs) are protected from DNA methylation in part by GC strand asymmetry and accompanying R-Loop formation (Ginno et al., 2012) and possibly also by active demethylation mediated by the TET family members (Williams et al., 2012). The unmethylated state of CpG islands in the germline, along with biased gene conversion, helps to preserve CpG islands, despite ongoing attrition of methylated CpG dinucleotides by cytosine deamination throughout most of the genome (Cohen et al., 2011). Transition zones between CpG islands and CpG oceans are called CpG shores, and display more tissue-specific variation in DNA methylation (Irizarry et al., 2009). CpG islands span the transcription start sites of about half of the genes in the human genome, largely representing genes that are either actively expressed or poised for transcription (Figure 1).

Methylated DNA is recognized by methyl-CpG binding domains (MBD) or C2H2 zinc fingers. The MBD-containing DNA methylation readers include MBD1, MBD2, MBD4 and MeCP2, whereas Kaiso (ZBTB33), ZBTB4 and ZBTB38 proteins use zinc fingers to bind methylated DNA. MBDs and Kaiso are believed to participate in DNA methylation mediated transcriptional repression of tumor suppressor genes with promoter DNA methylation.

Histone Modifications

Post-translational modifications of histones are coordinated by counteracting histone-methyltransferases (HMTs) and demethylases (e.g. KDMs), histone acetyltransferases (HATs) and deacetylases (HDACs), and writers and erasers of phosphorylation, as well as

many other modifications (Chi et al., 2010; Tan et al., 2011b). These histone modifiers generally act in complexes, such as the repressive Polycomb (PcG) and activating Trithorax (TrxG) group complexes, which counterbalance each other in the regulation of genes important for development, but which have also been implicated in cancer (Mills, 2010). Polycomb repressive complexes (PRCs) are guided to their targets in part by intrinsic signals in the genome sequence (Ku et al., 2008; Tanay et al., 2007). The histone H3K27me3 mark deposited by PRC2 provides docking sites for PRC1, whose enzymatic core unit RING1B monoubiquitinylates histone H2A at lysine 119 (H2AK119ub1) thereby blocking RNA polymerase II elongation. The Trithorax group complex, containing MLL, which lays down the H3K4 methylation mark, counteracts Polycomb function. The transcription factors encoding master regulators of differentiation and development are targeted by PRC2 in embryonic stem cells and held in a bivalent chromatin state poised for transcription, with both the activating H3K4me3 and the repressive H3K27me3 (Bernstein et al., 2006) (Figure 1). During differention the Trithorax demethylase, KDM6A/UTX removes the repressive H3K27me3 mark, allowing transcription elongation to proceed for genes required in that particular lineage, while genes not required in that cell type may acquire H3K9me3, which is recognized by readers like HP1 to reinforce a repressive state. Other histone marks have various readers with binding motifs including bromodomain, PHD domain, chromodomain, and tudor domain (Musselman et al., 2012) (Figure 2). Trithorax and Polycomb complexes recruit HATs and HDACs, respectively, to counteract each other, and the establishment of histone acetylation can block Polycomb binding (Mills, 2010).

Histone Variants

Histone variants provide an additional layer of regulation. The main histone genes have multiple copies in the genome and are expressed during S-phase. Single-copy variants are also expressed at other phases of the cell cycle and have distinct functions and/or locations. H2A has the largest number of variants, including H2A.Z, MacroH2A, H2A–Bbd, H2AvD, and H2A.X (Kamakaka and Biggins, 2005). The H3 variants include H3.3 and centromeric H3 (CenH3, or CENP-A), as well as a mammalian testis-specific histone H3 variant called H3.4. Nucleosomes containing H3.3 and H2A.Z are located at dynamic regions requiring nucleosome mobility and exchange, such as at actively expressed gene promoters (Jin et al., 2009). Wide presence of H2A.Z in embryonic stem cells (Zhu et al., 2013) suggests prevalent chromatin exchange, consistent with the emerging idea that the genome of ESC is generally kept highly accessible. During differentiation H2A.Z quickly redistributes. The mechanisms of recruitment have not been fully delineated, but various chromatin remodeler complexes and/or chaperones have been shown to be involved. For example, SRCAP is involved in H2A.Z loading into promoter/TSS, while H3.3 is loaded to telomeric/pericentric regions by the ATRX/DAXX complex and promoter/TSS by HIRA (Boyarchuk et al., 2011).

Nucleosome Positioning and Remodeling

The positioning of nucleosomes displays a weak 10-bp periodicity associated with minor sequence composition fluctuations in phase with the DNA helical repeat. Some nucleosomes are more consistently positioned in phased arrays anchored by sequence-specific binding of proteins such as CTCF or adjacent to nucleosome-free regions at transcription start sites (Gaffney et al., 2012; Valouev et al., 2011). CpG islands have been associated with transcription-independent nucleosome-depletion at mammalian promoters (Fenouil et al., 2012). ATP-dependent chromosome remodeling complexes are responsible for sliding of the nucleosomes, as well as insertion and ejection of histone octamers, processes important for transcriptional repression and activation, and other important cellular functions such as DNA replication and repair. The remodeling complexes can be divided into four families:

SWI/SNF, CHD (chromodomain and helicase-like domain), ISWI, and INO80 (including SWR1, or SRCAP in mammals).

Insulators

The CCCTC-binding factor CTCF (and its paralogue CTCFL/BORIS, expressed in the germline) are the only insulator proteins that have been identified so far in vertebrates. CTCF has a strong binding motif and there is extensive overlap of the occupied CTCF-binding sites among different cell types (Kim et al., 2007). CTCF binds to enhancer blocking elements to prevent enhancer interactions with unintended promoters ('enhancer blocking insulator'), and also demarcates active and repressive chromatin domains ('barrier insulator').

Nuclear Architecture

The genome can be compartmentalized based on nuclear architecture and associated genomic features into mostly heterochromatic late-replicating regions attached to the nuclear lamina at the nuclear periphery, and more gene-rich early-replicating regions closer to the nuclear interior (Encode-Project-Consortium et al., 2012; Meuleman et al., 2012). Lamina-associated sequences (LASs) enriched for a GAGA motif are bound by transcriptional repressors, and appear to contribute to the establishment of lamina associated domains (LADs) in the mammalian genome (Zullo et al., 2012).

Maintaining the Epigenetic State

The persistence of epigenetic traits in a growing tumor requires that the epigenome be faithfully copied during cell division. The chromatin structure is dismantled for passage of the replication fork (RF). Newly synthesized DNA and histone octamers are then assembled at the RF by Chromatin assembly factor I (CAF1), tethered to the RF by PCNA. Similarly, the dedicated maintenance DNA methyltransferase DNMT1, the euchromatic H3K9 methyltransferase G9a, among other epigenetic maintainers, are loaded to RFs and copy the epigenetic marks. The Trithorax and Polycomb complexes are recruited prior to replication and distributed evenly to the mother and daughter strands at the RF, and restore the correct marks on the daughter molecules during G1 (Petruk et al., 2012). The histone marks are self-reinforcing and self-propagating, as PcG, SUV39H1/2, SETDB1 and TrxG all bind to the marks that they are responsible for catalyzing, via an intrinsic reader domain or by interacting with a reader protein, thus helping to maintain the epigenetic state. Nucleosomes containing methylated DNA also stabilize DNMT3A/3B, which is a self-reinforcing mechanism for DNA methylation maintenance (Sharma et al., 2011).

Disruption of Epigenetic Control in Cancer

Most studies of cancer epigenetics have focused on DNA methylation, as the epigenetic mark that most easily survives various forms of sample processing, including DNA extraction, and even formalin fixation and paraffin embedding (Laird, 2010). However, other epigenetic marks also undergo broad changes, including long non-coding RNAs and miRNAs (Baer et al., 2013; Baylin and Jones, 2011; Dawson and Kouzarides, 2012; Sandoval and Esteller, 2012), and loss of K16 acetylation and K20 trimethylation at histone H4 (Fraga et al., 2005; Hon et al., 2012; Kondo et al., 2008; Seligson et al., 2005; Yamazaki et al., 2013). Loss of 5-methylcytosine in cancer cells was discussed more than three decades ago (Ehrlich and Wang, 1981), with global DNA hypomethylation reported in cancer cell lines (Diala and Hoffman, 1982; Ehrlich et al., 1982) and reduced levels of DNA methylation found at selected genes in primary human tumors compared to normal tissues (Feinberg and Vogelstein, 1983). The widespread loss of DNA methylation contrasted starkly with the subsequent finding of hypermethylation of CpG islands in cancer (Baylin et

al., 1986), including of promoter CpG islands of tumor-suppressor genes (Jones and Baylin, 2002). These seemingly contradictory findings have been widely reported for many types of cancer (Baylin and Jones, 2011).

The causal relevance of epigenetic changes in cancer was initially questioned but this concern has now largely been laid to rest. First, many known tumor-suppressor genes have been shown to be silenced by promoter CpG island hypermethylation (Jones and Baylin, 2002). Importantly, the finding that these silencing events are mutually exclusive with structural or mutational inactivation of the same gene, such as the case for *BRCA1* in ovarian cancer (TCGA, 2011) and for *CDKN2A* in squamous cell lung cancer (TCGA, 2012a), reinforces the concept that epigenetic silencing can serve as an alternative mechanism in Knudson's two-hit hypothesis (Jones and Laird, 1999). Second, mouse models of cancer have been shown to require epigenetic writers and readers for tumor development (Laird et al., 1995; Prokhortchouk et al., 2006; Sansom et al., 2003). Third, some DNA methylation changes appear to be essential for cancer cell survival, suggesting an acquired addiction to epigenetic alterations (De Carvalho et al., 2012). Finally, a plethora of significantly mutated epigenetic regulators have now been reported for many types of human cancer, as discussed further below.

Long-Range Coordinated Disruptions and Nuclear Architecture

The genome of undifferentiated embryonic stem cells is uniformly heavily methylated across CpG oceans, punctuated by unmethylated CpG islands. As stem cells differentiate and proliferate, the late-replicating lamin-associated domains (LADs) undergo progressive loss of DNA methylation within CpG oceans, and the LADs become recognizable as long partially methylated domains (PMDs), which become even more strikingly demarcated as hypomethylated domains in cancer cells (Berman et al., 2012; Hansen et al., 2011; Hon et al., 2012; Lister et al., 2009). This loss of DNA methylation is associated with an increase of repressive chromatin with large organized chromatin-lysine-(K) modification regions (LOCKs) (Hansen et al., 2011; Hon et al., 2012; Lister et al., 2009). CpG island hypermethylation is enriched in the hypomethylated domains, suggesting that these two events may be mechanistically linked, but confined to distinct areas of the genome near the nuclear periphery (Berman et al., 2012). These long regions of DNA hypomethylation and repressive chromatin are consistent with prior reports of coordinated epigenetic silencing events located across megabase distances, a phenomenon termed Long-Range Epigenetic Silencing (LRES) (Clark, 2007; Coolen et al., 2010).

It is noteworthy that the euchromatic part of the genome associated with the interior of the nucleus is generally much more epigenetically stable during cell differentiation, aging and malignant transformation. However, loss of the DNA methyltransferase Dnmt3a can promote tumor progression with uniform hypomethylation across the genome, and moderate deregulation of genes in euchromatic regions (Raddatz et al., 2012).

Disruption of Differentiation and Development

Differences between cell types are guided by the expression of tissue-specific transcription factors and consolidation of associated epigenetic states. Therefore, the epigenome of a cancer cell is determined in part by the cell of origin for that cancer and includes passenger hypermethylation events at genes not required in that particular lineage (Sproul et al., 2012). Epithelial to mesenchymal transition (EMT) of cancer cells is partly under reversible epigenetic control (Craene and Berx, 2012). For example, primary breast tumors display heterogeneous and unstable silencing of the *CDH1* (E-cadherin) gene, which facilitates the plasticity required during extravasation, metastasis and establishment of a solid tumor at the metastatic site (Graff et al., 2000).

It has long been debated whether cancer cells arise by dedifferentiation or instead originate from stem cells or early progenitors by a differentiation block. Polycomb repressors mark genes in stem cells encoding master regulators of differentiation and development, poised to either be turned on to coordinate differentiation of a lineage, or to be fully repressed if it is not needed in that particular lineage (Bernstein et al., 2006). These genes occupied by Polycomb repressors in stem cells are particularly prone to acquiring CpG island hypermethylation during cell proliferation, aging and particularly malignant transformation (Ohm et al., 2007; Schlesinger et al., 2007; Teschendorff et al., 2010; Widschwendter et al., 2007) (Figure 1). Although the genes affected by this process are primarily those not required or expressed in that particular cell lineage, cancer cells do also show evidence of silencing of genes essential for differentiation of their cell of origin (Berman et al., 2012; Easwaran et al., 2012; Gal-Yam et al., 2008; Mohn et al., 2008; Teschendorff et al., 2010). This predisposition of Polycomb target genes to aberrant permanent epigenetic silencing is consistent with a model in which stem cells slowly acquire irreversible silencing of poised master regulators required for successful differentiation. As a consequence, some stem cells lose their ability to properly differentiate while retaining their self-renewal capabilities, and become attractive candidates for malignant transformation by subsequent genetic and epigenetic events. One provocative implication of this model is that the first steps of oncogenesis may in some cases be an epigenetic defect affecting the differentiation capabilities of stem cells, as opposed to a gatekeeper mutation.

Hematopoietic cell lineages and their corresponding malignancies also offer insights into the role of epigenetics in differentiation and transformation. For example, the *DNMT3A* gene is commonly mutated in human cases of Acute Myeloid Leukemia (AML) (Ley et al., 2010; Yan et al., 2011), whereas loss of *Dnmt3a* in mice progressively impairs hematopoietic stem cell differentiation (Challen et al., 2012), suggesting that epigenetic perturbation can lead to differentiation block and subsequent malignant transformation.

CpG Island Methylator Phenotypes

Aberrant DNA methylation of promoter CpG islands in cancer was initially viewed as a spontaneous or stochastic event with selection for functionally relevant silencing events. However, the discovery of cases of colorectal cancer with an exceptionally high frequency of CpG island hypermethylation suggested a coordinated event, possibly attributable to an epigenetic control defect. This phenomenon was referred to as a "CpG Island Methylator Phenotype" (CIMP) (Toyota et al., 1999), analogous to the mutator phenotypes observed in mismatch repair deficient cancers. Although the existence of CIMP subsets of cancer was initially disputed (Yamashita et al., 2003), more recent genome-scale analyses have unambiguously documented distinct epigenetic subtypes for some types of cancer, such as colorectal cancer (Hinoue et al., 2012; TCGA, 2012b) and glioblastoma (Noushmehr et al., 2010), and not for others, such as serous ovarian cancer (TCGA, 2011). The most distinct examples of CIMP show exceptionally strong associations with other molecular or pathological features of the tumors, lending further validity to the biological relevance to this classification. For example, colorectal CIMP is very tightly associated with the V600E mutation of the *BRAF* oncogene (Weisenberger et al., 2006), while glioma CIMP (G-CIMP) is exceptionally tightly associated with mutation of the *IDH1* gene (Noushmehr et al., 2010). In the case of G-CIMP, *IDH1* mutation appears to be a causal contributor to the phenotype (Turcan et al., 2012), whereas *BRAF* mutation does not appear to be directly implicated in colorectal CIMP (Hinoue et al., 2009). The affected gene subsets,differ between colorectal CIMP and glioblastoma G-CIMP, and their predisposition to aberrant methylation appears to be distinct from the susceptibility of stem cell polycomb targets in lamin-attachment domains (Hinoue et al., 2012), which is generally not restricted to cancer subtypes. Despite a clear rationale for the association of *IDH1* mutation with G-CIMP, the mechanistic basis for

the coordinated hypermethylation events in most cases of CIMP is unknown, and will remain an active area of investigation.

Epigenetic Influences on Genomic Integrity

Mutation rates vary strikingly across the genome, with strong local influences of base composition on single nucleotide variation (SNV), and regional effects of sequence composition, chromatin structure, replication timing, transcription and nuclear architecture, among others on both SNVs and structural alterations (Hodgkinson and Eyre-Walker, 2011). Despite widespread misuse of the term in the literature, it should be recognized that mutation *rates* of a tumor cannot be inferred directly from observed mutation *numbers* or *frequencies* in a tumor without consideration of the number of cell divisions that have occurred since a shared reference genome, although comparisons across the genome obviate the need for Luria-Delbrück fluctuation modeling and analysis. Epigenetic mechanisms can influence both the rates at which lesions arise and the rates at which they are repaired. For example, the epigenetic mark 5-methylcytosine undergoes spontaneous deamination at higher rates than unmethylated cytosines (Wang et al., 1982), while epigenetic silencing of the *MLH1* mismatch repair gene increases mutation frequencies by several orders of magnitude, providing an adaptive advantage to mismatch repair deficient cancer cells.

Unmethylated and methylated cytosine residues both undergo spontaneous hydrolytic deamination but yield uracil and thymine respectively. Uracil is not a normal constituent base in DNA, and is repaired much more efficiently than thymine in a mismatch with guanine. As a consequence, the rate of C-to-T mutations in the context of CpG dinucleotides, most of which contain methylated cytosines, is about ten-fold higher than any other SNV in the human genome (Hodgkinson and Eyre-Walker, 2011). This effect is particularly pronounced in highly proliferative tissues because deamination of 5-methylcytosine in the parent strand just prior to DNA replication results in a full T:A base substitution that is not recognizable as a lesion for repair. Approximately a quarter of all *TP53* mutations in human cancer are thus attributable to this epigenetic mark (Olivier et al., 2010).

Regional Effects of Chromatin Organization

Chromatin regulators play a role in maintaining genomic integrity (Papamichos-Chronakis and Peterson, 2012) and regional chromatin structure has a major impact on mutation frequencies. Megabase regions of repressive chromatin, represented by the H3K9me3 mark are positively correlated with single nucleotide variations in cancer (Schuster-Bockler and Lehner, 2012), while open chromatin associated with DNAse I hypersensitive sites (DHS) have a lower inferred mutation rate, but this is partly due to evolutionary constraints on this compartment (Hodgkinson and Eyre-Walker, 2011). Transcription-coupled repair may also play a role in suppressing observed mutation frequencies in gene-rich euchromatic regions.

Other types of mutation and structural change also appear to be associated with chromatin states. For example, retrotransposition occurs more frequently in hypomethylated regions (Lee et al., 2012). Genes resistant to cancer-associated hypermethylation are more likely to have SINE and LINE retrotransposons near their transcription start sites than methylation-prone genes (Estecio et al., 2010). Severe hypomethylation appears to be associated with genomic instability. Mouse models of DNA methyltransferase deficiency display chromosomal instability (Eden et al., 2003), and germline mutations of the *DNMT3B* gene cause ICF syndrome, characterized by centromeric instability (Okano et al., 1999). Indeed, areas of hypomethylation in the human germline showed higher frequencies of structural mutability (Li et al., 2012). DNA breakpoints associated with somatic copy-number alterations are also enriched in hypomethylated domains (De and Michor, 2011).

Epigenetic Influences on DNA Repair

Depletion of DNA methyltransferases causes increased microsatellite instability (Guo et al., 2004; Kim et al., 2004), destabilization of repeats (Dion et al., 2008), and dramatically increased telomere length, telomeric recombination, and alternative telomere lengthening (Gonzalo et al., 2006). These effects of DNA methyltransferase depletion appear to be mediated in part by a drop in DNA repair proteins as part of DNA damage response (Loughery et al., 2011). The Dnmt1 protein has also been shown to be recruited to areas of irradiation-induced DNA damage, possibly to facilitate repair of epigenetic information following DNA repair (Mortusewicz et al., 2005). It is increasingly appreciated that chromatin can serve as a cellular sensor for DNA damage and other genomic events (Johnson and Dent, 2013).

Epigenetic silencing of DNA repair genes such as *MLH1*, *MGMT*, *BRCA1*, *WRN*, *FANCF*, and *CHFR* can boost mutation rates and promote genomic instability in cancer cells (Toyota and Suzuki, 2010). Familial cases of tumors with microsatellite instability (MSI) in Lynch syndrome result from germline mutations in mismatch repair genes, primarily *MSH2* and *MLH1*. However, most MSI-high tumors arise from an epigenetic defect in sporadic cases of cancer. Approximately 15% of sporadic cases of colorectal cancer display MSI as a consequence of epigenetic silencing of the *MLH1* mismatch repair gene by promoter CpG island hypermethylation (Herman et al., 1998) in the context of CIMP (Toyota et al., 1999; Weisenberger et al., 2006). MSI caused by epigenetic silencing of MLH1 has also been reported in other types of cancer, including about a quarter of sporadic endometrial cancers, (Simpkins et al., 1999). Germline variants of *MLH1* and *MSH2* can predispose to extensive somatic epigenetic silencing of these genes, and thereby increase cancer risk (Hitchins et al., 2011; Ligtenberg et al., 2009). Such familial cases of systemic epigenetic abnormalities can masquerade as germline transmission of epigenetic defects. True transgenerational epigenetic inheritance is evident in genomic imprinting, and in mouse models, but has been difficult to demonstrate directly in human populations, although there is indirect evidence for its existence (Daxinger and Whitelaw, 2012).

The O6-Methylguanine DNA methyltransferase (*MGMT*) enzyme repairs O6-alkylated guanine residues in genomic DNA. O6-methylguanine pairs with thymine, and would lead to a G to A transition during DNA replication if left unrepaired. *MGMT* promoter methylation in colorectal cancer is associated with G-to-A mutations in *KRAS* (Esteller et al., 2000b) and in *TP53* (Esteller et al., 2001). Alkylating agents such as Temozolomide are current standard of care for malignant glioblastoma (GBM), but are counteracted by MGMT-mediated repair of the alkylation damage. Epigenetic silencing of *MGMT* by promoter CpG island hypermethylation inactivates this repair pathway and renders the tumor more sensitive to the Temozolomide treatment (Esteller et al., 2000a; Hegi et al., 2005).

A Genetic Basis for Epigenetic Disruption in Cancer

The discovery of mutations in *SMARCB1/SNF5* driving malignant rhabdoid tumours first introduced genetic disruption of epigenetic control as a mechanism of oncogenesis (Versteege et al., 1998),. Mutations in epigenetic regulators continued to emerge from subsequent cancer studies, and have surged in recent large-scale sequencing efforts (Figure 3). Epigenetic control genes are mutated in about half of hepatocellular carcinomas (Fujimoto et al., 2012) and bladder cancer (Gui et al., 2011) and represent six of the twelve most significantly mutated genes in medulloblastoma (Pugh et al., 2012). It is conceivable that disruption of epigenetic control by mutation of a key regulator has the capacity to cause widespread changes to the transcriptome, multiplying the effect of the single genetic alteration. It should be recognized that some of the mutations reported for epigenetic regulators may be passenger events, particularly in tumors with high background mutation

rates. Therefore, we have emphasized hotspot mutations and genes recurrently mutated at significant frequencies. We focus here on somatic mutations, but germline variation have also been shown to play a role in cancer. For example, germline mutations in *BAP1* have been found to be linked to a tumor predisposition syndrome characterized by melanocytic tumors, mesothelioma, and uveal melanoma (Testa et al., 2011; Wiesner et al., 2011), and rare germline allelic forms of *PRDM9* have been found to be associated with childhood leukemia (Hussin et al., 2013).

DNA Methylation Writers and Erasers

The DNA methyltransferase *DNMT3A* is recurrently mutated in acute myeloid leukemia (AML) and other myeloid malignancies (Ley et al., 2010; Yan et al., 2011), as well as T-cell lymphoma (Couronne et al., 2012). The mutations often occur at a R882 hotspot, but nevertheless likely reflect loss of function of *DNMT3A*. Mutations in the DNA methylation eraser *TET2* have also been identified in the same cancer types (Abdel-Wahab et al., 2009; Langemeijer et al., 2009; Quivoron et al., 2011), and bone marrow from patients with *TET2* mutations show reduced levels of 5hmC (Ko et al., 2010). The isocitrate dehydrogenases *IDH1* and *IDH2* are also recurrently mutated in AML. IDH1 enzymes with the R132 hotspot mutation and IDH2 enzymes containing R140 or R172 mutations have lost the ability to produce alpha-ketoglutarate (α-KG), but instead convert α-KG to an aberrant metabolite 2-hydroxyglutarate (2-HG), a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases, such as the TETs and JmjC-domain containing histone demethylases (Lu et al., 2012; Xu et al., 2011). *IDH1/2* mutations are mutually exclusive with *TET2* mutations in AML, consistent with the inhibitory effect of 2-HG on TETs as a mediator of the effects of IDH1/2 mutations (Figueroa et al., 2010; Weissmann et al., 2012). The same hotspot mutation for *IDH1*, and less often *IDH2* is also found in gliomas and glioblastomas. Both glioblastomas with *IDH1* mutations (Noushmehr et al., 2010) and cases of AML with mutation of *IDH1* or *IDH2* (Figueroa et al., 2010) display CpG island methylator phenotypes.

Histone Gene Mutations

Mutations in histone variants H3.3 (*H3F3A*) or sometimes H3.1 (*HIST1H3B*) have been found in pediatric (Schwartzentruber et al., 2012; Wu et al., 2012) and adult brain tumors (Sturm et al., 2012) with K27M and G34R or G34V mutation hotspots. Tumors with G34 mutations display extensive DNA hypomethylation, particularly in subtelomeric regions (Sturm et al., 2012), perhaps contributing to alternative lengthening of telomeres (ALT) (Schwartzentruber et al., 2012). Mutations were also observed in the *ATRX* and *DAXX* genes, encoding proteins responsible for loading of the H3.3 variant into the telomere region (Schwartzentruber et al., 2012). Pancreatic neuroendocrine tumors (PanNETs) with *ATRX* and *DAXX* mutations also exhibit ALT (Heaphy et al., 2011). Since this phenotypic effect is associated with a H3.3 loading defect, the G34 mutations may also interfere with H3.3 loading. In contrast, tumors with K27M mutations did not display ALT, and these mutations may instead mimic dimethylated lysine 27, a repressive Polycomb mark, given that methionine is a natural mimic of this epigenetic mark (Hyland et al., 2011). H3.3 G34R mutations have also been reported in primitive neuroectodermal tumors of the CNS (Gessi et al., 2013), mirroring the defect in the ATRX-DAXX-H3.3 axis in other brain tumors and PanNETs.

Mutations in *HIST1H3B* and *HIST1H1C* have been found in diffuse large B-cell lymphoma (DLBCL), although the mutations do not occur in clusters (Lohr et al., 2012; Morin et al., 2011). These might be functionally different from the hotspot mutations seen in brain tumors. Focal deletion of a histone gene cluster at 6p22 is seen in near-haploid cases of acute lymphoblastic leukemia (Holmfeldt et al., 2013).

Histone Methylation Writers

The *MLL* gene, encoding one of the H3K4 methyltransferases has over 50 translocation fusion partners in different lineages of leukemia. These rearrangements account for 80% of the cases of infant leukemia and 5–10% of adult leukemia cases, and are generally associated with poor prognosis (Tan et al., 2011a). The primary mechanism has been attributed to the recruitment of inappropriate epigenetic factors to MLL targets, by fusions between recruitment proteins and the DNA-binding N-terminus of MLL. Target genes for these recruited complexes include the *HOX* genes, particularly *HOXA9*, whose upregulation is a key feature of MLL leukemia. *MLL* regulates the expression of HOX genes in normal pluripotent cells, but the oncogenic fusion proteins keep them from being turned off during differentiation and therefore impart stem-cell like properties. Targeted therapeutic strategies are emerging for AML with *MLL* fusions, including inhibition of menin (encoded by *MEN1*), *DOT1L*, *PRMT1*, the histone acetylation reader *BRD4*, and *LSD1* (Zeisig et al., 2012). In addition to the translocations, loss-of-function mutations of *MLL-MLL3* have been reported in many different types of cancer, including AML - possibly another way of disturbing the temporal control at promoters associated with pluripotency. *MLL2* is mutated at very high frequency in B-cell follicular lymphoma and diffuse large B-cell lymphoma, consistent with the gain-of-function mutations of *EZH2* in the same tumor types.

While menin is critical to the *oncogenic* effects of MLL fusion proteins in AML (Yokoyama and Cleary, 2008), loss of function mutations have been found in PanNETs (Jiao et al., 2011), consistent with a *tumor-suppressor* role, suggesting that cellular context is important.

The recurrent t(5;11)(q35;p15.5) translocation in AML results in the fusion of the H3K36 methyltransferase *NSD1* to nucleoporin-98 (*NUP98*), with elevated levels of H3K36me3 levels at *HOXA* genes and accompanying transcriptional activation. Translocations involving another dedicated H3K36 methyltransferase *WHSC1/MMSET/NSD2* are seen in 20% of multiple myelomas. Another H3K36 methyltransferase, *SETD2* is recurrently mutated in clear cell renal cell carcinomas (ccRCC) (Dalgliesh et al., 2010).

A recent study reconstructed the phylogenetic structure of molecular events in ccRCC with multiple spatially separated samples from the same tumors (Gerlinger et al., 2012). In both of the two patients studied, distinct *SETD2* inactivating mutations were found in different parts of the same tumor. Immunohistochemistry staining confirmed H3K36me3 loss in all the mutant tumors. This convergent somatic evolution indicates that failure to establish H3K36 methylation marks provides a strong selective advantage relatively late in ccRCC progression. A similar molecular convergence was found for *KDM5C*, an H3K4 demethylase, in one of the two patients. This, together with recurrent mutations in other epigenetic regulators, shows that epigenetic dysregulation, often mediated by genetic events, is important in advanced ccRCCs.

EZH2, the writer for the H3K27 methylation mark associated with Polycomb repression, has long been viewed as an oncogene in cancer. Indeed, gain-of-function mutations are seen in lymphomas. However, loss-of-function mutations in this gene have recently been described in other cancers. We discuss these divergent effects of *EZH2* mutations and other alterations to this pathway in more detail later (Figure 4).

Histone Methylation Erasers

Consistent with *EZH2* overexpression in various solid tumors, the corresponding eraser *KDM6A/UTX* is mutated in more than a dozen tumor types, with the highest frequency in bladder (Gui et al., 2011; van Haaften et al., 2009). The H3K9 demethylase *KDM4C/GASC1* is amplified in breast cancer and has been shown to drive transformation (Liu et al., 2009; Rui et al., 2010). Ectopic expression of this putative oncogene in vitro causes an

efficient decrease of H3K9me3 (Cloos et al., 2006). Its co-amplification with *JAK2* (Rui et al., 2010) -which phosphorylates H3Y41 and prevents binding of H3K9 methylation reader HP1 to the H3K9 methylation mark - in lymphoma makes for an interesting example of a single genetic event hitting two possible epigenetic regulators. Inhibiting the two co-amplified and cooperating gene products is efficient at killing these lymphoma cells.

Histone Acetylation Writers and Erasers

The counteracting histone acetyltransferases (HATs) and deacetylases (HDACs) are considered to be promiscuous, and often have important non-histone substrates such as *TP53*. There are three major families of HATs, namely the CBP/P300, GNAT, and the MYST families. *CREBBP* is mutated at high frequency in follicular lymphoma and diffuse large-cell B-cell lymphoma (DLBCL) (Morin et al., 2011; Pasqualucci et al., 2011) and in ALL (Mullighan et al., 2011), particularly relapsed hyperdiploid ALL (Inthal et al., 2012). Its paralogue *EP300* also undergoes frequent mutation (Gayther et al., 2000; Gui et al., 2011; Pasqualucci et al., 2011; Zhang et al., 2012) and loss of heterozygosity (LOH) in many different epithelial cancers.

HATs have also been implicated in gene fusions. The t(8;16)(p11;p13) translocation in AML fuses the N-terminal part of *MOZ*, the founding member of the MYST HAT family, to the major part of the *CBP* gene containing the acetylase domain. *MOZ* has also been found to be involved in fusions with *EP300* (Yang, 2004). These translocations generating chimeric oncoproteins with the DNA-binding domain of *MOZ* and the transcription-activating domain of another coactivator are associated with AML M5/M4. These results suggest that both disruption and redirection of HAT could contribute to cancer.

Reports of mutations in HDACs are rare. Rather, HDACs are often co-opted by other genetic alterations. A prime example is the PML-RARα translocation, responsible for 95% of the AML FAB-M3 (APL, Acute promyelocytic leukemia) cases. The leukemogenetic effect of this translocation is primarily mediated through aberrant recruitment of N-CoR/HDAC repressor complexes (Minucci and Pelicci, 2006). The retinoic acid receptor-alpha (RARα) part binds to retinoic acid-responsive elements (RAREs), while the PML moiety recruits the HDAC-containing repressive complex. All-trans retinoic acid (ATRA) targets RARα, dissociates these repressor complexes and effectively induces differentiation of the leukemic promyelocytes. Combination therapy of ATRA and arsenic trioxide shows excellent clinical response, and turned APL into a highly curable disease (Wang and Chen, 2008). Another fusion protein PLZF-RARα blocks differentiation by a similar mechanism. However, APL with this translocation is ATRA-resistant due to the higher affinity of the PLZF moiety to the N-CoR complex, but a combination of ATRA with HDAC inhibitors can fully reverse the transcriptional repression and induce terminal differentiation for this type of AML (Wang and Chen, 2008). Similarly, the RUNX1-ETO fusion, the AML1-ETO fusion, and the CBF-MYH11 protein from inv(16), all recruit HDACs, and efficacy of HDAC inhibitors has been demonstrated for all three (Zeisig et al., 2012).

Epigenetic Readers

The epigenetic readers add another layer of control to the epigenetic state, by serving as interpreters of the epigenetic state and relaying epigenetic signals. Many of the epigenetic writer/eraser/remodelers have intrinsic reader domains, or interact with dedicated readers to sense the presence or absence of particular epigenetic marks. Translocations joining *BRD4* or occasionally *BRD3*, both readers of the BET Bromodomain-containing family, to almost the entire length of the *NUT* gene define a lethal, poorly differentiated pediatric tumor, NUT midline carcinoma (NMC) (French et al., 2008). The BET family members targetable by BET inhibitors (Filippakopoulos et al., 2010), are lysine acetylation readers that bind

transcriptionally active chromatin as acetylated lysine readers, and are targetable by BET inhibitors (Filippakopoulos et al., 2010). Functional studies show that the BRD-NUT fusion oncoprotein binds avidly to acetylated histones, resulting in a differentiation block, potentially by interfering with transcriptional programs driving differentiation (French et al., 2008). *BRD3* is significantly mutated in lung adenocarcinomas (Imielinski et al., 2012), and *BRD8* in liver cancer (Fujimoto et al., 2012). In addition, the plant homeodomain (PHD)-domain containing gene *PHF6* is recurrently mutated in AML (Van Vlierberghe et al., 2011) and overall loss of this gene (mutation and/or deletion) is observed in TALL (Van Vlierberghe et al., 2010).

Chromatin Remodelers

A large number of SWI/SNF complexes exist in mammals and contribute to lineage- and tissue-specific gene expression (Wilson and Roberts, 2011). The two major types of SWI/SNF complexes are BAF (BRM-containing, or SWI/SNF-A) and PBAF (BRG1 containing, or SWI/SNF-B), defined by a core enzymatic unit being either SMARCA2 (BRM) or SMARCA4 (BRG1). Those complexes also contain other core units, such as SMARCB1/SNF5 and ARID1A/B, unique to BAF, and PBRM1 and BRD7, unique to PBAF. Truncating mutations in the *SMARCB1* gene are very common in malignant rhabdoid tumours (RTs) (Versteege et al., 1998), a rare yet lethal tumor diagnosed in children. The biallelic nature of the inactivation fits with a tumor suppressor role for *SMARCB1*. Familial cases of RTs are associated with inheritance of one defective *SMARCB1* allele. *SMARCB1* is also mutated in a few other cancers (Figure 1). *SMARCA4* mutation is also seen in familial cases of RT (Schneppenheim et al., 2010), indicating that it is indeed SWI/SNF dysfunction that is responsible for RT development. *SMARCA4* is also mutated Burkitt's Lymphoma, in a mutually exclusive manner with *ARID1A* mutations (Love et al., 2012), again suggesting a driver role for SWI/SNF mutations. Recurrent *SMARCA4* mutation is also seen in lung cancer (Imielinski et al., 2012) and medulloblastoma (especially the WNT subtype) (Jones et al., 2012; Parsons et al., 2011; Pugh et al., 2012; Robinson et al., 2012).

ARID1A mutations have been found in more than ten different tumor types, with the highest rate in the clear cell subtype of ovarian cancer, where it is mutated in more than half of the tumors (Jones et al., 2010; Wiegand et al., 2010). *ARID1A* is also mutated in the endometrioid subtypes of ovarian (Wiegand et al., 2010) and endometrial cancers (Guan et al., 2011). *ARID1B* and *ARID2* mutations are also seen in various cancer types, including liver cancer (Fujimoto et al., 2012) and melanoma (Hodis et al., 2012), among others. In addition, the polybromo-containing *PBRM1* in the *PBAF* complex was recently found to be the second most mutated gene in clear cell renal cell carcinomas (Varela et al., 2011). Another SWI/SNF gene, *SMARCE1*, is highly recurrently mutated in clear cell meningiomas (Smith et al., 2013). The exceptionally high mutation rate of SWI/SNF member in clear cell tumors from different tissues (ovary, kidney, and meninges) highlights an interesting possible link between clear cell tumors and SWI/SNF dysfunction.

ATRX, responsible for H3.3 incorporation at telomeres and pericentric heterochromatin is often mutated in pancreatic neuroendocrine tumours (PanNETs), the second most common malignancy of the pancreas (Jiao et al., 2011). Interestingly, there are also recurrent mutations in the associated chaperone *DAXX* in the same cancer type, and the two mutations are mutually exclusive. Mutation of these two genes are also found in GBM where they are mutually exclusive with the *H3F3A* mutations described earlier, with any of these three genes mutated in almost half of the tumors studied (Schwartzentruber et al., 2012). These mutations all lead to alternative lengthening of telomeres associated with increased genomic instability (Heaphy et al., 2011; Schwartzentruber et al., 2012). With the possible exception of *ATRX* and *DAXX*, most of the mutations in the SWI/SNF family members are not associated with genomic instability (Wilson and Roberts, 2011). Rather,

perturbed differentiation may be the major mechanism, as cells from different lineages co-exist within an individual rhabdoid tumor.

The CHD family chromatin remodelers can be divided into three classes. Class I (CHD1/2), Class II (CHD3/4, in the NuRD/Mi-2/CHD complex), and Class III (CHD5–9). The NuRD/Mi-2/CHD complexes are unique in that they have core enzymatic subunits with at least two distinct functions: ATP-dependent remodeling (CHD3 and CHD4), as well as histone deacetylase (HDAC1 and HDAC2) functions (Lai and Wade, 2011), and therefore couple two epigenetic processes in one complex for transcriptional repression. Their MBD and MTA subunits target the complex to different parts of the genome by binding methylated DNA (MBD) or other transcription factors (MTA), in physiological and pathological conditions. For example, the MTA-2 containing NuRD complex associates with TWIST in breast cancer, represses genes such as E-cadherin (*CDH1*), and contributes to EMT. *CHD4* is mutated in 17% of serous endometrial cancer (Le Gallo et al., 2012). Of the other CHDs, *CHD1* is the second most-frequently deleted gene in prostate cancer, defining an ETS-negative subtype (Grasso et al., 2012), with mutations reported as well (Berger et al., 2011).

Epigenetic Insulators

CTCF is located in 16q22.1 with LOH in breast and prostate cancers (Filippova et al., 1998) and Wilms' tumors (Mummert et al., 2005). CTCF mutation has also been reported in breast cancer (Filippova et al., 2002), and this mutation is found to be significant in a cohort of 510 tumors (TCGA, 2012c). Rare mutations in this gene have also been reported for prostate cancer (Filippova et al., 2002), Wilms' tumor (Filippova et al., 2002), AML (Dolnik et al., 2012), ALL (Mullighan et al., 2011; Zhang et al., 2012)), and endometrial cancers (Le Gallo et al., 2012). The functional implications of *CTCF* deletion/mutation, especially given the low frequency, have not been fully delineated yet, but abrogation of proper insulation might be one mechanism.

Genetic Disruption of a Central Epigenetic Control Circuit

Figure 4 illustrates the diverse ways in which a central epigenetic control circuit can be impacted in cancer. EZH2 catalyzes methylation at H3K27, as part of Polycomb Repressive Complex 2 (PRC2). Two other core subunits of PRC2 are EED and SUZ12, and other components such as JARID2 can be part of a PRC2 complex too. *EZH2* has long been thought to be oncogenic since it is overexpressed as a result of amplification of *EZH2* in breast, bladder and other cancers (Bracken et al., 2003), as well as genetic loss of miR101, which represses EZH2 in prostate cancer (Varambally et al., 2008). In line with this view, gain-of-function hotspot mutations (Y641 and A677,) in the SET-domain of *EZH2* have been found in a significant portion of lymphomas (Lohr et al., 2012; Morin et al., 2010; Morin et al., 2011; Pasqualucci et al., 2011). More convincingly, *EZH2* amplification and overexpression in two of the largest subgroups of medulloblastoma is mutually exclusive with mutation of the H3K27 demethylase *KDM6A*, suggesting that accumulation of H3K27me3 is a key step in these tumors (Robinson et al., 2012).

On the other hand, loss of function mutations of *EZH2* have also been found in a series of myeloid malignancies, including MDS, Multiple Myeloma, MPN and MDS/MPN, as well as in head-and-neck squamous cell carcinomas (HNSCCs) (Ernst et al., 2010; Nikoloski et al., 2010; Stransky et al., 2011), suggesting that PRC2 can also act as tumor suppressors. Aside from the myeloid malignancies, mutually exclusive recurrent deletion and loss of function mutations of *EZH2* and *SUZ12* have also been found in T-lineage acute lymphoblastic leukemia (T-ALL) (Ntziachristos et al., 2012) or of all three PRC2 subunits in early-T-cell-precursor ALL (Zhang et al., 2012) further substantiate a tumor suppressor role. Indeed, disruption of *EZH2* is sufficient to induce T-ALL in mice (Simon et al., 2012). PRC2

component mutations are much more common in early T-cell precursor ALL, a lymphoblastic leukemia with myeloid features. The Polycomb repressive deubiquitinase (PR-DUB) component *ASXL1* is also mutated in myeloid malignancies, and *ASXL1* mutation mediates myeloid transformation through loss of PRC2 repression (Abdel-Wahab et al., 2012), in line with the observed loss of function of PRC2 members in myeloid disorders. Mouse models also show that loss of *BAP1*, the enzymatic unit of PR-DUB, lead to myeloid transformation (Dey et al., 2012). This, together with a *BAP1* catalytic mutation found in a MDS patient lacking other MDS mutations (Dey et al., 2012) further lends credibility to the idea that loss of Polycomb repression drive myeloid disorders, while in B-cell lymphoma and solid tumors gain of Polycomb repression seem important.

EZH2 also exhibits PRC2-independent oncogenic activities. For example, in castration-resistant prostate cancer Akt-mediated phosphorylation of EZH2 at S21 can shift EZH2 from PRC2-dependent promoters to EZH2 'solo' promoters. This EZH2 activity is often associated with androgen receptor (AR), and activates gene expression at these loci (Xu et al., 2012).

These complex ways in which the H3K27me3 axis is disrupted in cancer suggest differential therapeutic approaches should be developed for 1) myeloid malignancies and early T-cell ALL, 2) lymphomas and some solid tumors, and possibly 3) hormone-associated cancers. In particular, caution should be used when considering EZH2 inhibitors for the first group of tumors, which feature genetic lesions leading to loss of PRC2 repression.

Conclusions

It is clear that the cancer genome and epigenome influence each other in a multitude of ways (Figure 5). They offer complementary mechanisms to achieve similar results, such as the inactivation of tumor-suppressor genes such as *BRCA1*, *CDKN2A*, *VHL*, and *RB1* by either deletion or epigenetic silencing, and they can work cooperatively, as in the case of CIMP and *BRAF* mutation in colorectal cancer, where CIMP appears to create a permissive context for *BRAF* mutation as early as in the precursor lesion (Hinoue et al., 2009; Yamamoto et al., 2012).

Many questions and challenges remain. For example, the explosion in the number of epigenetic regulator mutations identified in human cancer has underscored the importance of epigenetic control in tumor suppression, but the phenotypic consequences of these mutations remain largely uncharacterized. However, this plethora of newly identified mutations in epigenetic regulators opens entirely new avenues of therapeutic attack (Dawson and Kouzarides, 2012).

Another major question remaining in the field is the mechanistic basis for some well-recognized examples of disruption of epigenetic control, such as CIMP in colorectal cancer. Even in the case of G-CIMP in gliomas and its very tight association with IDH1 mutation, it is not clear what confers the gene specificity of the hypermethylation events. Correlated events such as CIMP create other challenges as well. In contrast to most mutations, CIMP-associated DNA methylation events are highly correlated, with a large number of recurrent alterations that appear to be passenger events without functional contribution to the cancer process. This high degree of correlation precludes the straightforward use of recurrence frequency among different tumors as a main filter criterion in the identification of functionally relevant epigenetic driver events. Therefore, the identification of epigenetic drivers must rely more on the analysis of transcriptional consequences, mutual exclusivity with other events in the same pathway within a tumor, and complementary mechanisms of inactivation of the same gene in other tumors, and most importantly, functional experimental

validation of an impact of the epigenetic gene inactivation on cellular proliferation, immortality, angiogenesis, cell death, invasion or metastasis.

Cancer epigenetics and genetics may in orm each other. enetics can shed light on the identity of epigenetic drivers by revealing mutual exclusivity with genetic aberrations in the same gene or pathway. Epigenetics may also provide insight into genetic drivers in a similar fashion. In addition, the understanding of epigenetic networks provides a framework to interpret the functional significance of lower-frequency drivers in the same pathway. The high frequency of epigenetic regulator mutations seen in various cancers, the hotspot nature of some mutations found, mutual exclusivity between different mechanisms affecting the same genes/pathways, clonal analysis highlighting convergent evolution, and validations in experimental systems all attest to the importance of mutations in epigenetic regulators in cancer, and strengthen the concept that disruption of epigenetic control is a common enabling characteristic of cancer cells.

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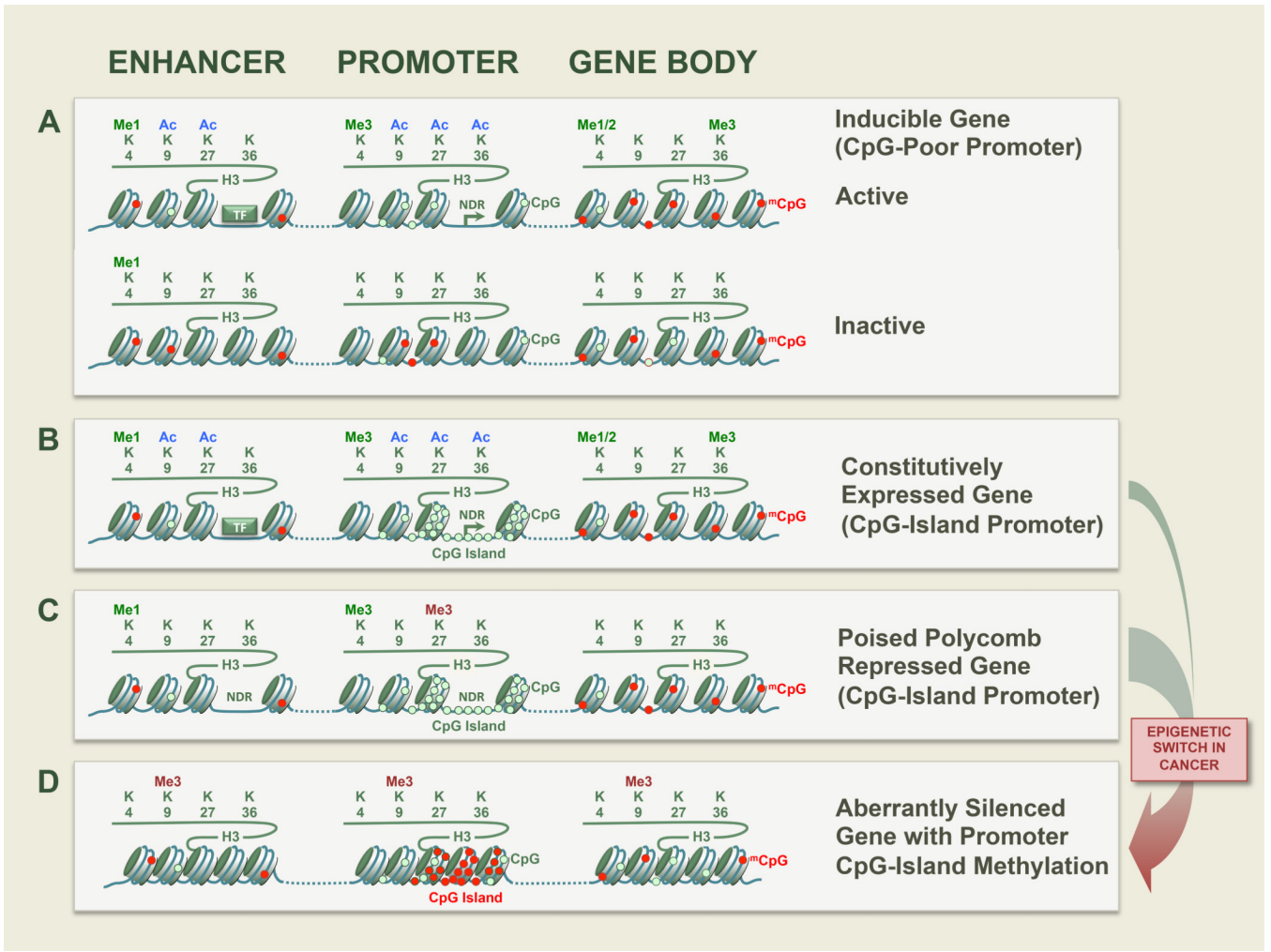
Figure 1. Representative Epigenetic States
Examples of representative epigenetic states are shown for several typical categories of genes and in different cellular contexts. A. CpG-poor promoters are often tissue-specific and/or reside in inducible genes which can be readily turned on or off. Transcription factor (TF) binding at regulatory elements and the promoter initiates nuclear depleted regions (NDR). B. Many genes with CpG island promoters are constitutively expressed housekeeping genes. C. Some genes with CpG island promoters, such as transcription factor master regulators of differentiation and development are repressed by the Polycomb complexes in stem cells and kept in a bivalent state with both active and repressive marks. D. Polycomb targets in stem cells are predisposed to cancer-specific promoter hypermethylation.
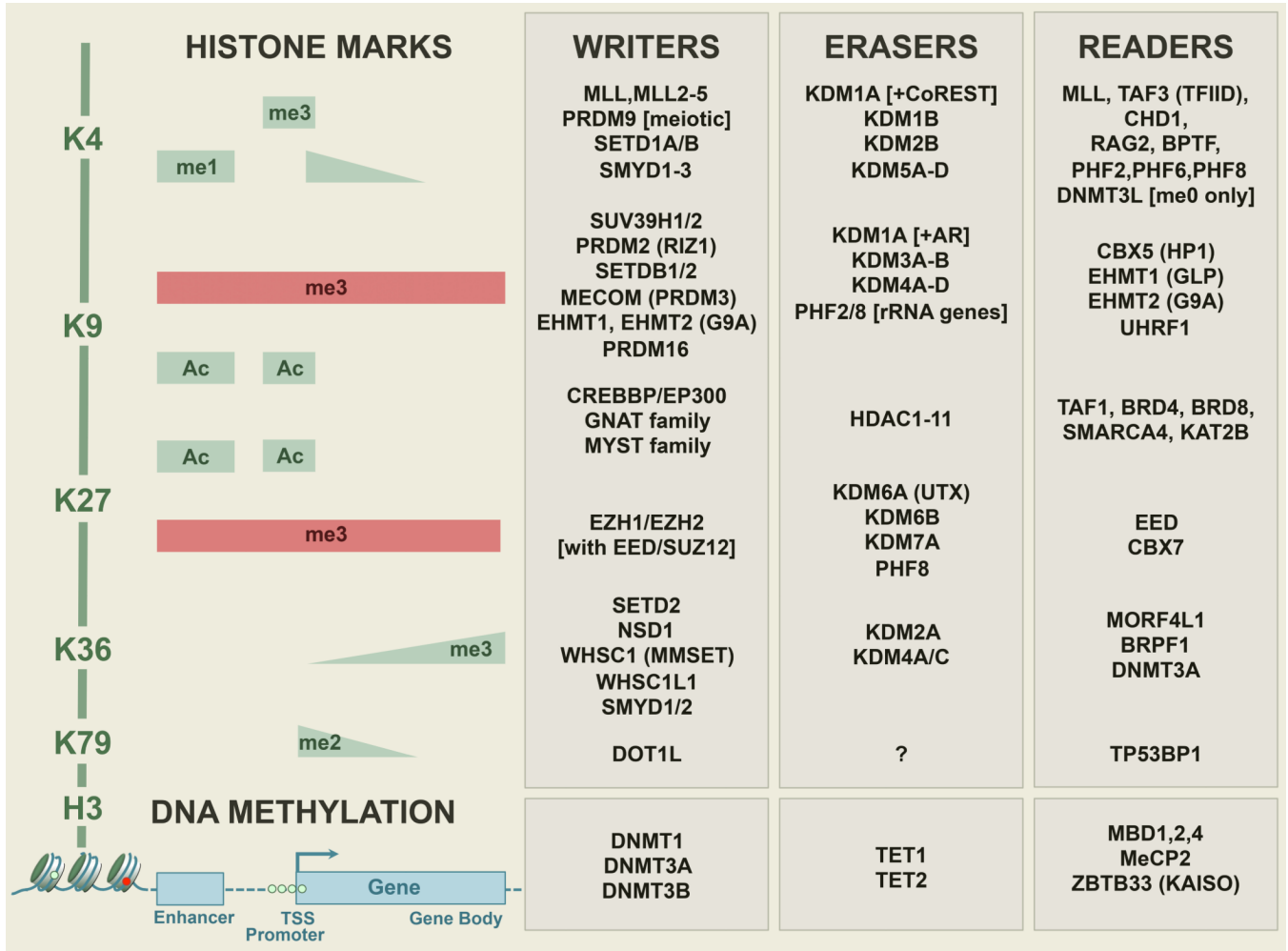
Figure 2. Histone H3 Lysine Writers, Erasers and Readers
Although many other important histone modifications also occur, only major histone H3 lysine modifications (Ac: Acetylation; me1: monomethylation; me3: trimethylation) with well-defined functions are shown above a representative gene. The distribution of the marks are shown as colored bars and wedges to indicate approximate abundance. Repressive marks are shown in red, and active marks in blue. Epigenetic regulators are listed to the right of each mark. Acetylation across different lysines share writers and erasers, while methylation usually has dedicated enzymes. Readers (which can also be writers and erasers themselves) recognize different chromatin states and propagate the signal in various ways, including self reinforcement or cross talk, transcriptional activation or repression, or DNA repair. Crosstalk can also occur between histone modification and DNA methylation, since *DNMT3A, DNMT3L, UHRF1* all contain reader domains for chromatin states.
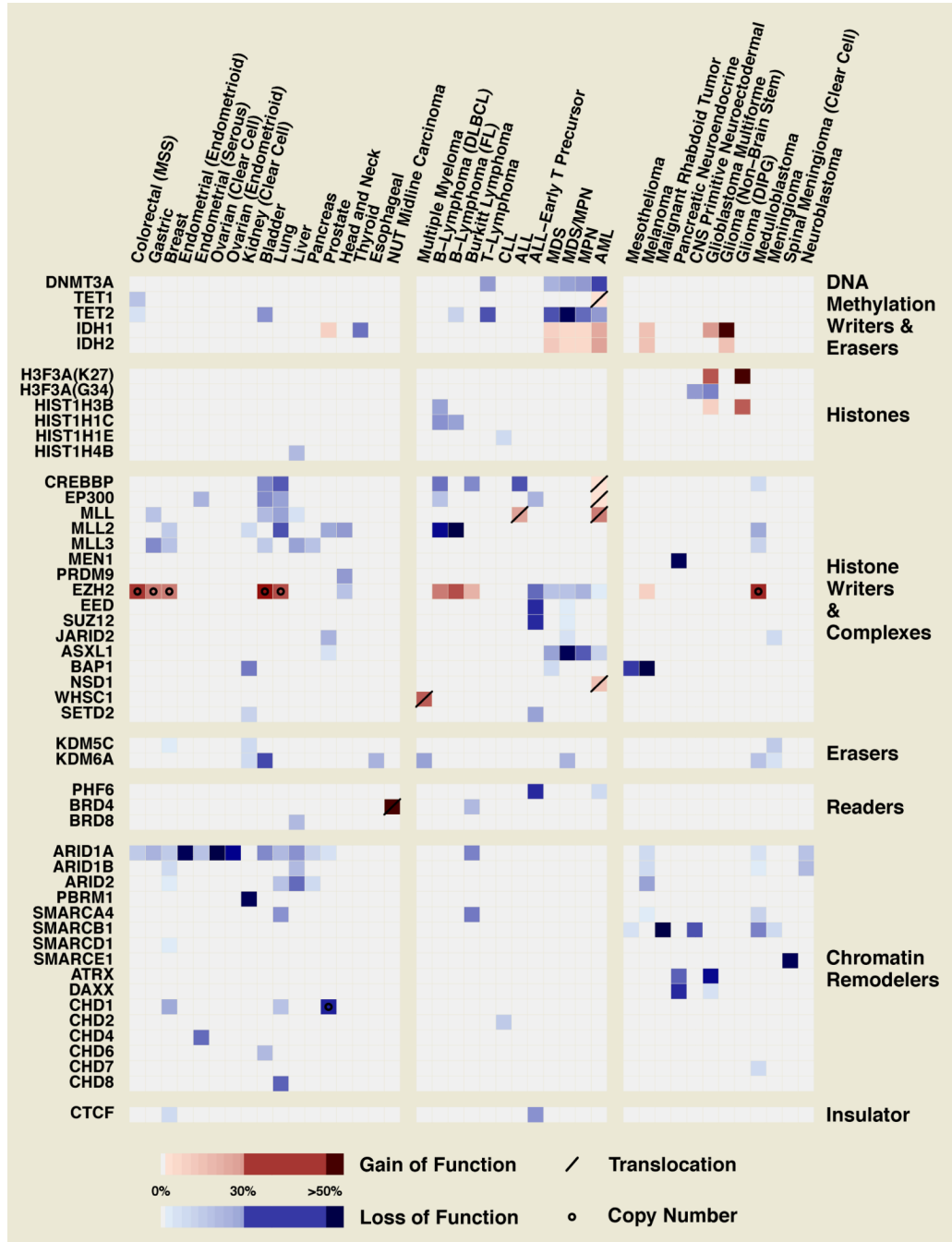
Figure 3. Genetic Alterations in Epigenetic Regulators

Mutations and other genetic alterations reported for selected epigenetic regulators are shown for various types of human cancer in a heatmap. Malignancies are grouped by epithelial, hematological and other cancers. Mutations, represented by colored cells, are deemed loss of function (blue) unless evidence for gain of function (either hypermorphic or neomorphic, red) has been shown. Other genetic alterations are plotted with different symbols, with a slash indicating translocation events and a dot indicating copy number alterations. Translocations that generate oncogenic fusion proteins are represented in red as well. The mutation frequencies, represented by the darkness of the shade, are based on recent whole genome-exome studies, with adjustments made where whole-genome/exome studies are not

available or have a small sample size. Different subtypes of lung cancers are combined without adjusting for subtype prevalence and certain mutations may only represent one subtype. Cells showing no entry may represent false negatives in our curation or in the literature, and cancer types highly covered with whole-genome/exome studies (e.g. breast cancer) might have fewer false negatives than those that are not. MSS/MSI – microsatellite stable/instable (MSI CRCs are excluded due to the high background mutation rate); DLBCL - Diffuse large B-cell lymphoma; FL – follicular lymphoma.
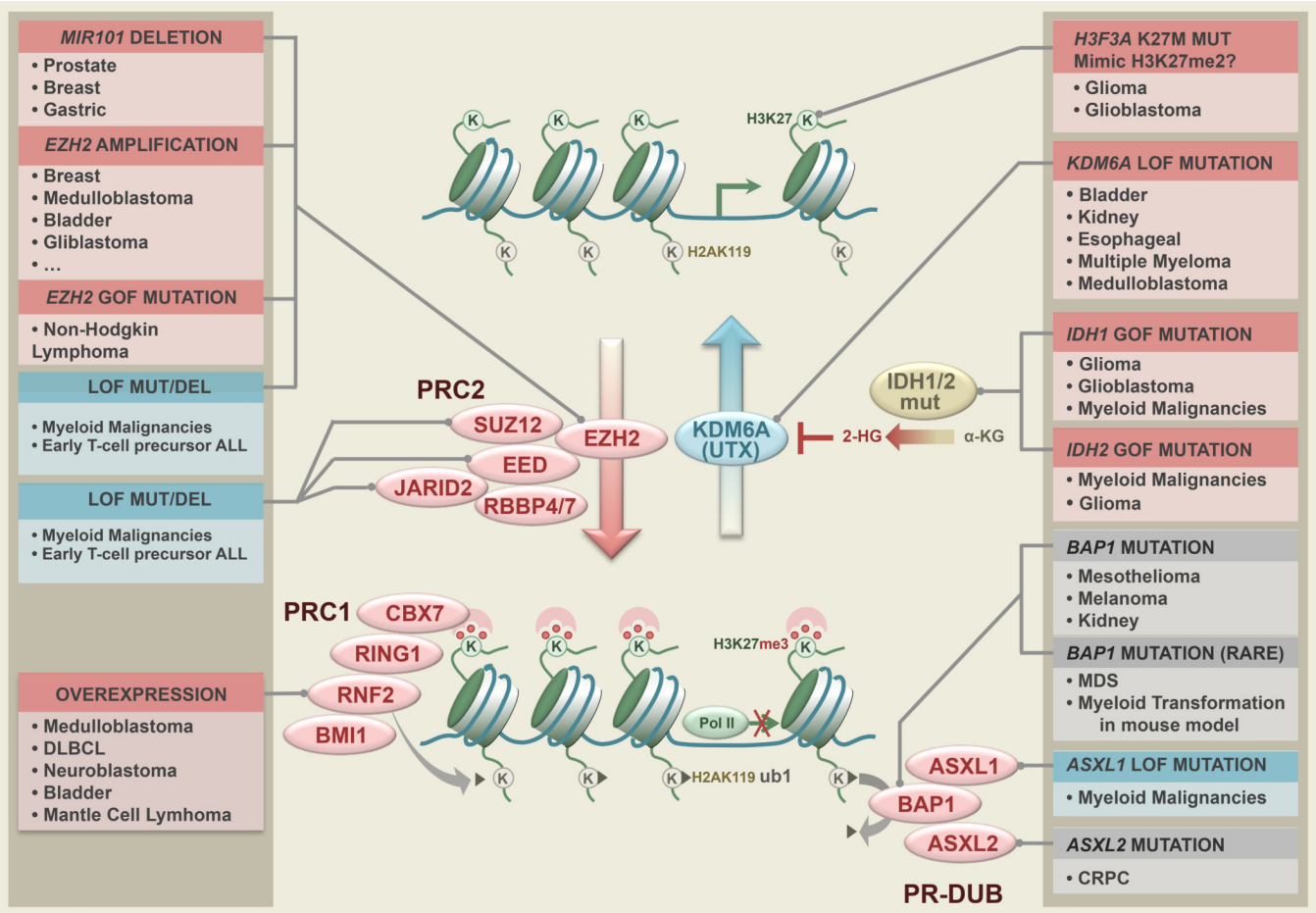
Figure 4. Genetic Disruption of Epigenetic Control at H3K27 in Cancer
The counteracting writer EZH2 and eraser KDM6A/UTX form a pair in regulating an important epigenetic mark, methylation at H3 lysine 27. EZH2 catalyzes the methylation process with help from other components in the Polycomb Repressive Complex 2 (PRC2), while KDM6A, part of the Trithorax complex, removes this repressive mark. The K27me3 mark attracts another Polycomb complex, PRC1, which ubiquitinates H2AK119, and thereby blocks PolII elongation. Another Polycomb complex, PR-DUB is also critical to the maintainance of the repression at a subset of the Polycomb genes, although it removes the H2AK119ub mark and thus counteracts PRC1 in that regard. Mutations and genetic alterations spanning a wide spectrum of human cancers hit this epigenetic pathway. Solid tumors show possibly neomorphic histone K27 mutations (mimicking H3K27me2), UTX mutation, EZH2 amplification and/or overexpression due to genomic loss of the repressive microRNA miR101, as well as amplification/overexpression of the PRC1 member BMI1, and lymphoma exhibits gain-of-function mutations of EZH2, consistent with a gain of Polycomb repression (red boxes) in the affected malignancies. In contrast, myeloid malignancies and ALL, particularly early T-cell precursor ALL show mutations that could sabotage Polycomb repression (blue boxes). Mouse models show that loss of *BAP1*, the enzymatic unit of PR-DUB, leads to myeloid transformation, although *BAP1* mutation in MDS is rare. Gray boxes indicate that the effect on H3K27me3 is not clear.
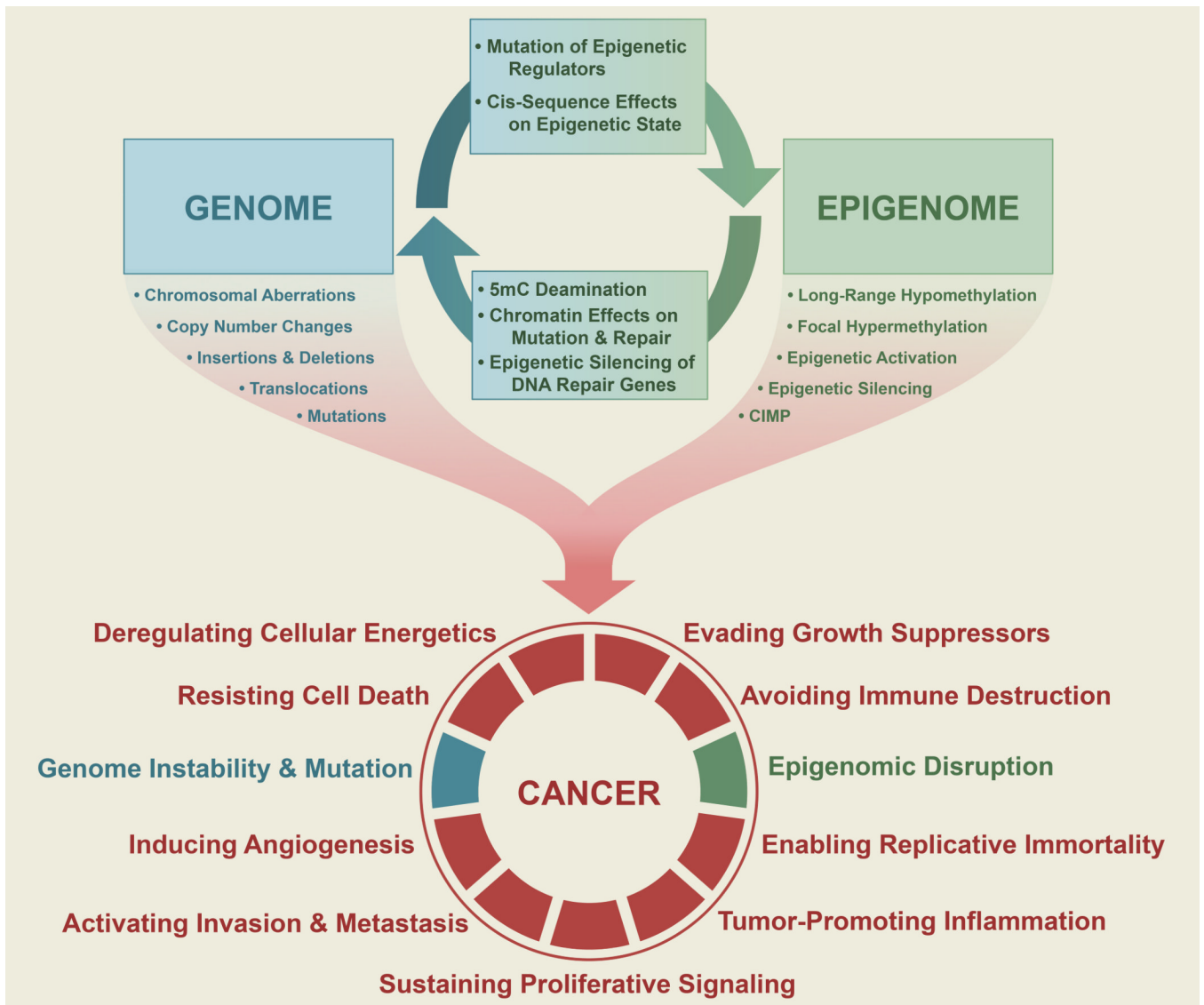
Figure 5. Interplay Between the Cancer Genome and Epigenome
The genome and epigenome influence each other, as the genome provides the primary sequence information and encodes regulators of epigenetic states, while the epigenome controls the accessibility and interpretation of the genome. Changes in one can influence the other, forming a partnership in producing genetically or epigenetically encoded phenotypic variation subject to Darwinian selection for growth advantage, and thus eventually achieving the hallmarks of cancer (Hanahan and Weinberg, 2011). Genetic instability and mutation, and epigenomic disruption can be considered enabling characteristics of cancer cells.