Published in final edited form as:

Nat Rev Cancer. 2016 June ; 16(6): 359–372. doi:10.1038/nrc.2016.41.

Beyond transcription factors: how oncogenic signaling reshapes the epigenetic landscape

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Abstract

Cancer, once thought to be caused largely by genetic alterations, is now considered to be a mixed genetic and epigenetic disease. The epigenetic landscape, which is dictated by covalent DNA and histone modifications, is profoundly altered in transformed cells. These abnormalities may arise due to mutations in or altered expression of chromatin modifiers. Recent reports on the interplay between cellular signaling pathways and chromatin modifications add another layer of complexity to the already complex regulation of the epigenome. In this Review, we discuss these new studies and how the insights they provide can contribute to a better understanding of the molecular pathogenesis of neoplasia.

Signal transduction pathways convert environmental stimuli to changes in cell behavior, and are thus central to the control of all biological processes. Not surprisingly, deregulation of these pathways contributes to the development of many diseases, and is particularly important in the context of cancer. Extensive studies over the past decades have identified genetic alterations in components of many signaling pathways involved in cancer, and have also led to the development of therapeutics that can successfully target some of these pathways, most notably the use of tyrosine kinase inhibitors in chronic myeloid leukemia¹, HER2 (also known as ERBB2) inhibitors in breast cancer², BRAF inhibitors in melanoma³ and Janus kinase 2 (JAK2) inhibitors in myeloproliferative neoplasms⁴.

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Conflict of interest

No Competing Interest.

Canonical signaling pathways usually involve a cascade of protein phosphorylation events, ultimately resulting in the activation (or inactivation) of transcriptional regulators and changes in target gene expression. The kinases that comprise these pathways are largely cytoplasmic, and are therefore not generally considered to directly affect chromatin structure; yet numerous kinases, including AKT^{5, 6} and JAK2⁷, have been shown to modify histones or proteins that control chromatin structure. Thus, although diagrams typically show these complex signaling pathways ending at transcription factors, they can also directly regulate histone proteins, and histone or DNA modifying enzymes.

The N-terminal tails of histones undergo extensive posttranslational modifications, including phosphorylation, acetylation, methylation, ubiquitination and SUMOylation⁸. These modifications affect chromatin configuration and chromatin-DNA interactions, and subsequently regulate numerous cellular processes, including transcriptional regulation, DNA damage repair, DNA replication, genomic imprinting and X chromosome inactivation. Some histone modifications, such as histone H3-lysine 14 acetylation (H3K14Ac), H3K27Ac and H3K4 trimethylation (H3K4me3), are found at actively transcribed promoters or active enhancers, whereas others, such as H3K9me3 and H3K27me3, are considered to represent repressive marks for transcription (Table 1 summarizes histone modifications and their functions on transcriptional regulation). However, it is the combination of histone modifications and DNA methylation at regulatory regions in the genome, in concert with regulation by non-coding RNAs, that determines whether genes are expressed or not, and at what level. For example, many promoters in embryonic stem cells (ESCs) harbor a specific histone modification pattern that combines the activating histone mark H3K4me3 and the repressive H3K27me3 mark⁹. These ‘bivalent domains’ silence the developmental genes in ESCs, while keeping them poised for activation, and provide a key example of how gene expression can be determined by the combined effects of different histone marks on the same promoter.

Cancer genetic studies have focused on mutations that contribute to cell transformation, either as ‘gain-of-function’ or ‘loss-of-function’ genetic events. In the past few decades, many studies have demonstrated the impact of epigenetic events on cancer development, adding another dimension to our understanding of cancer. Almost 30 years ago, it was discovered that tumor cells had lower levels of genome-wide **CpG island** methylation compared with their normal counterparts¹⁰. This global DNA hypomethylation is thought to contribute to genomic instability, due in part to the activation of **transposable elements** and loss of genomic **imprinting**. DNA hypermethylation is also a common feature of cancer, sometimes at the promoter regions of tumor suppressor genes, leading to loss of expression of key gatekeeper genes, such as *CDKN2A* (cyclin-dependent kinase inhibitor 2A) and *BRCA1* (breast cancer 1)^{11, 12}. Cancer-associated perturbations in histone modifications often occur at individual gene promoters or enhancers in a given cancer, resulting in the improper activation or repression of genes that could contribute to cancer development. However, some common events, such as the global loss of both H4K16ac and H4K20me3 are observed in many types of human cancer¹³. Unlike the irreversible character of genetic events, epigenetic alterations are potentially reversible. In fact, some epigenetic drugs, most notably some histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors,

have been approved by the US Food and Drug Administration (FDA) for the treatment of hematological malignancies¹⁴.

Due to the complex regulation of the human epigenome, the cause of aberrant epigenetic modifications remains difficult to determine for most cancers. Nevertheless, the interplay between upstream signaling pathways and chromatin modifications represents an important molecular mechanism that leads to specific alterations in the cancer epigenome. This Review summarizes recent findings into how such cross talk can contribute to the development of cancer.

Regulation of histone phosphorylation by upstream signalling pathways

Histone phosphorylation is involved in a variety of cellular processes. Phosphorylation of H3S10 and H3S28 is associated with two seemingly paradoxical functions, transcriptional activation and chromosome condensation^{15, 16}. Whereas phosphorylation of H3S10 and H3S28 by mitotic kinases such as Aurora kinase B is required for the initiation of chromosome condensation and the onset of mitosis¹⁶, phosphorylation at these sites by signal transduction kinases, such as ribosomal S6 kinase 2 (RSK2) and mitogen- and stress-activated kinase 1 and 2 (MSK1 and 2), promotes the transcriptional activation of **immediate early genes**, such as *FOS*, *JUN* and *MYC*^{17, 18}. Mitotic histone H3 phosphorylation is a highly coordinated spatio-temporal event, initiating at pericentromeric heterochromatin regions at the onset of mitosis, reaching maximal abundance during metaphase, and then decreasing rapidly upon the transition to anaphase. Unlike the global H3 phosphorylation seen during mitosis, interphase H3 phosphorylation occurs rapidly in response to extracellular stimuli, but targets only a small fraction of nucleosomes, thus affecting only the local chromatin structure¹⁹.

Other signaling kinases, including I- κ B kinase- α (IKK α)²⁰, JUN N-terminal kinase (JNK)²¹ and PIM1²², have also been reported to phosphorylate H3 at either S10 or S28 and control gene transcription. IKK α , a crucial regulator of nuclear factor- κ B (NF- κ B) signaling that controls the stability of I κ B protein (the inhibitor of NF- κ B) in the cytoplasm, has been shown to shuttle into the nucleus and phosphorylate H3S10 at NF- κ B-responsive promoters, thus directly affecting the cytokine-induced expression of NF- κ B-regulated genes²⁰. Whereas JNK-mediated H3S10 phosphorylation contributes to the differentiation of stem cells into neurons²¹; PIM1 forms a complex with MYC on MYC-responsive genes, where it phosphorylates H3S10 and facilitates MYC-induced gene expression and cellular transformation²². In addition to cell cycle progression and control of gene transcription, H3S10 phosphorylation by protein kinase C- δ (PKC δ) has been shown to play a role in apoptosis-induced chromatin condensation²³.

The mechanisms by which phosphorylation at these two serine residues in histone H3 affects gene expression and mitosis are still unclear. H3S10 and H3S28 phosphorylation have been shown to recruit the scaffolding protein 14-3-3- ϵ and ζ to the promoters of immediate early genes, allowing the transcription initiation machinery to assemble there; the recruitment of 14-3-3 proteins is greatly enhanced when H3K14 (on the histone H3 'tail') is simultaneously acetylated^{24, 25}. Since both H3S10 and H3S28 reside within an 'ARKS' sequence motif,

researchers have speculated that dynamic serine phosphorylation might affect the readout of the more stable methyl marks on the adjacent lysine residues. Indeed, H3S10 phosphorylation has been shown to displace heterochromatin protein 1 γ (HP1 γ) from the adjacent methylated H3K9 residue, impairing its ability to propagate heterochromatin during both transcriptional activation and mitosis^{26, 27}. In contrast, phosphorylation of H3S28 not only prevents binding of polycomb-repressive complexes to methylated H3K27, but it also induces a switch from methylation to acetylation at this lysine residue²⁸.

We are beginning to learn how aberrant histone phosphorylation contributes to cellular transformation. For example, mutating H3S10 or H3S28 to alanine blocks the epidermal growth factor (EGF)-induced transformation of mouse epidermal cells in culture²⁹, likely due to reduced transactivation of EGF target genes, including *Fos* and *Jun*. Similarly, inhibiting MSK1 activity reduces the activation of immediate early genes in response to oncogenic RAS-MAPK signaling, impairing tumor cell growth in soft agar colony assays³⁰. Both of these observations support that phosphorylation of H3S10 and H3S28 are important in tumorigenesis. However, neither report examined the changes in chromatin condensation that occur during mitosis when phosphorylation of H3S10 and H3S28 is inhibited.

Another example of crosstalk between histone phosphorylation and lysine methylation was reported in two studies by Metzger and colleagues^{31, 32}. They showed that histone H3 is phosphorylated at T6 by PKC β 1 and at T11 by protein-kinase C-related kinase 1 (PRK1; also known as PKN1), two phospholipid-dependent kinases that are involved in cell proliferation and migration and are overexpressed in several human cancers³³. These phosphorylation events can be androgen-driven and facilitate the transactivation of androgen receptor (AR) target genes in prostate cancer cell lines. Interestingly, the ability of lysine demethylases, such as lysine-specific histone demethylase 1 (LSD1, also known as KDM1A) and Jumonji domain-containing protein 2C (JMJD2C; also known as KDM4C), to remove the methyl marks from H3K4 and H3K9 is affected by H3T6 and H3T11 phosphorylation^{31, 32}. H3T6 phosphorylation blocks the demethylation of H3K4me1 and H3K4me2 by LSD1, whereas phosphorylation of H3T11 accelerates the demethylation of H3K9me3 by JMJD2C. Given the positive role of H3K4 methylation and the inhibitory effect of H3K9 methylation on gene expression, these changes to methylation would be expected to promote transcription and enhance the strength of AR-dependent gene expression. In fact, high H3T6 and H3T11 phosphorylation levels correlates with high Gleason scores (and worse prognosis) for prostate cancer patients. In addition, inhibition of PRK1 and PKC β 1 greatly impairs the *in vitro* growth of prostate cancer cells, supporting at least one role of histone H3 threonine phosphorylation in promoting AR-dependent transformation^{31, 32}.

In addition to serine and threonine phosphorylation, histone H3 is also phosphorylated at tyrosine residues by JAK2³⁴. JAK2 signaling plays an important role in normal hematopoiesis, as it is downstream of several cytokine receptors. These pathways can be hijacked in cancer through mutations in cytokine receptor genes or in the tyrosine kinase genes themselves. For example, JAK2-V617F, a constitutively activated form of JAK2, is found in more than half of all patients with **myeloproliferative neoplasms** (MPNs)³⁵. Dawson and colleagues³⁴ found that a substantial proportion of the cellular JAK2 protein is

in the nuclei of hematopoietic cells, where it phosphorylates histone H3Y41 (Figure 1). Phosphorylation of H3Y41 excludes HP1 from chromatin, an event linked to the transcriptional activation of at least one JAK2-target gene, LIM domain only 2 (*LMO2*), a gene that is essential for normal hematopoietic development and that has been implicated in leukemogenesis^{36, 37}. Direct signaling of JAK2-V617F to chromatin was also shown to mediate ESC self-renewal via regulation of *NANOG* expression³⁸. Using genome-wide chromatin immunoprecipitation-sequencing (ChIP-seq) technology, the same group identified many more genes that are regulated through phosphorylation of H3Y41³⁹. Furthermore, the detection of H3Y41 phosphorylation in JAK2-deficient gamma-2A cells indicates that tyrosine kinases other than JAK2 are also capable of phosphorylating H3Y41³⁴.

Even though histone H3 seems to be the major target of upstream signaling kinases, other histones such as H2B can also be phosphorylated at multiple serine and threonine residues, providing additional mechanisms that can regulate cell behavior. For example, AMP-activated protein kinase (AMPK), a crucial regulator of cell metabolism, controls stress-elevated gene transcription via phosphorylation of H2BS36 in both the promoters and transcribed regions of these genes⁴⁰. In contrast, phosphorylation of H2BS14 by mammalian STE20-like protein kinase 1 (MST1; also known as STK4) is associated with apoptotic chromatin condensation⁴¹.

Together these recent studies show that histone phosphorylation is a dynamic nuclear event involved in transcriptional regulation, chromatin condensation, apoptosis and DNA replication. In addition, histone phosphorylation can regulate DNA repair (which we describe further below). Table 2 summarizes signaling kinases that can target histone sites, and Baek⁴² provides more detailed information on the role of kinases in regulating histone modifications.

Regulation of histone-modifying enzymes

Histone modifying enzymes can be categorized into ‘writers’ and ‘erasers’ that add or remove covalent posttranslational modifications to histones, respectively (Box 1). Many of these writer or eraser enzymes are either mutated or aberrantly expressed in cancer, where they may play a pivotal and targetable role.

Regulation of Polycomb group proteins: EZH2 and BMI1

Polycomb group (PcG) proteins were initially identified as key factors that regulate developmental pathways⁴³, maintaining the repression of homeobox gene expression and antagonizing the activities of trithorax proteins during embryogenesis. PcG proteins were later found to be crucial for controlling gene expression, and essential for many cellular processes, including cell-fate decisions during embryogenesis⁴⁴, maintenance of embryonic stem cell pluripotency⁴⁵ and preservation of adult stem cell self-renewal⁴⁶. These proteins are structurally and functionally diverse and are found in two major multimeric protein complexes, PcG repressive complex 1 (PRC1) and PRC2 (Box 2).

Misregulation of these PRCs has now been implicated in the initiation and development of a variety of cancers. The PRC2 component enhancer of zeste homologue 2 (EZH2) is frequently overexpressed in many advanced stage or metastatic solid tumors, including prostate and breast cancer^{47, 48}. Both gain- and loss-of-function mutations in EZH2 are found in human cancer. The methyltransferase activity of EZH2 is altered by mutations at Y641 in lymphomas^{49, 50}; these mutations enhance its catalytic efficiency for the di- to trimethylation reaction of H3K27, but ablate its ability to catalyze the formation of monomethylation on unmethylated H3K27 residue⁵¹. In contrast, loss-of-function mutations are found in patients with **myelodysplastic syndrome** (MDS) or acute myeloid leukemia (AML)⁵². Given the crucial role of EZH2 in stem cell maintenance and tumorigenesis, these mutations are likely to play a pathogenic role.

Bmi1 (also known as *Pcgf4*), which encodes a central component of many PRC1 complexes, was first identified as a proto-oncogene capable of inducing lymphomas in mice in cooperation with *Myc*^{53, 54}. BMI1 plays an important role in hematopoietic and neural stem cell self-renewal^{55, 56}, and upregulation of *BMI1* has been detected in AML and chronic myeloid leukemia (CML)⁵⁷, as well as many solid tumors, including squamous cell carcinomas⁵⁸, neuroblastoma⁵⁹ and bladder cancer⁶⁰. The oncogenic potential of BMI1 has been associated with its ability to repress the *CDKN2A* locus, blocking the expression of the two proteins encoded by this locus, the cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitors p16^{INK4A}, and the p53 activator p14^{ARF} (p19^{ARF} in mouse)⁶¹.

There is growing evidence that signaling pathways can regulate the functions of EZH2 and BMI1 (Figure 2). Starting from the observation that cells with higher levels of activated AKT have lower levels of H3K27me, Cha *et al.*⁵ demonstrated that AKT phosphorylates EZH2 on S21, repressing its methyltransferase activity toward H3K27 by impairing its recruitment to chromatin. Inhibition of AKT activity increases global H3K27me3, and decreases the expression of the EZH2 target gene homeobox A9 (*HOXA9*). EZH2 phosphorylation changes its substrate specificity, directing its methyltransferase activity towards non-histone substrates. The authors further showed that a phospho-mimic form of EZH2 enhanced cell growth in vitro and tumor development in vivo, indicating the important role of this phosphorylation event in its transforming ability. The EZH2-containing PRC2 complex is found in the cytoplasm, where it can function in actin polymerization and signal transduction⁶²; it is still unknown which non-histone substrates of EZH2 are important for its transforming ability.

Multiple signaling pathways have been shown to phosphorylate and regulate BMI1 activity. The association of BMI1 with chromatin is cell cycle-regulated: BMI1 is hyperphosphorylated during G2/M, when it disassociates from chromatin, but it is dephosphorylated and retained on chromatin during G1/S⁶³. Although the enzyme responsible for BMI1 phosphorylation was not identified in this study, it is likely to be a cell cycle-regulated kinase. BMI1 is also a downstream target of the RAS-MAPK pathway⁶⁴. MAPKAP kinase 3 (3pK; also known as MAPKAPK3), a convergence point of both the RAS-ERK and RAS-p38-MAPK signaling pathways, phosphorylates BMI1, resulting in its disassociation from chromatin, de-repression of the *CDKN2A* locus and upregulation of p14^{ARF} expression.

We and two other groups^{6,65,66} have reported that BMI1 is phosphorylated by PI3K-AKT signaling, although the consequences of phosphorylation on BMI1 function varied between these reports. Kim *et al.*⁶⁵ showed that AKT-mediated phosphorylation of BMI1 prevents its degradation, resulting in BMI1 accumulation in the nucleus of neural stem cells (NSCs), leading to an increase in histone H2A ubiquitination and a decrease in p21 expression. BMI1 has been reported by several groups to play a crucial role in DNA damage repair^{67, 68}. Using prostate cancer as a model, the van Lohuizen group reported that phosphorylation of BMI1 by AKT is required for DNA-damage-induced ubiquitination (Ub) of H2A and γ -H2AX at sites of double-strand breaks and for efficient DNA repair⁶⁶. However, neither the global level of H2A-Ub nor the expression of BMI1 target genes is altered by this phosphorylation. We found that AKT phosphorylates BMI1 on S316⁶, an AKT-consensus site, triggering the disassociation of the PRC1 complex from chromatin, resulting in decreased global H2A mono-ubiquitination, and increased expression of p16^{INK4A} and p19^{ARF} in mouse embryonic fibroblasts (MEFs). BMI1 plays an important role in the self-renewal of hematopoietic stem cells (HSCs), where AKT signaling is inactive^{55, 69–71}. We found that AKT-mediated phosphorylation of BMI1 inhibits its ability to promote HSC self-renewal. This observation may explain why progenitor cells that have active AKT signaling have limited self-renewal and why constitutively activated AKT leads to HSC depletion^{72, 73}.

Together, these studies indicate that BMI1 phosphorylation occurs following oxidative stress, DNA damage and HSC differentiation, suggesting that BMI1 is a convergence point for multiple signaling pathways. The different functional outcomes of BMI1 phosphorylation may reflect both site-specific and cell context-specific variations. For example, AKT is capable of phosphorylating BMI1 at distinct serine residue(s) in different cell types under different conditions, resulting in enhanced or diminished BMI1 function^{6,65,66}. Furthermore, given the importance of BMI1 in cellular senescence⁷⁴, its inhibition by oncogenic pathways such as RAS-MAPK and PI3K-AKT could be a potential mechanism underlying oncogene-driven senescence.

Regulation of histone lysine demethylases

Two evolutionarily conserved families of histone lysine demethylases have been identified: the LSD family and the Jumonji (JMJC) family. LSDs, which include LSD1 and LSD2 (also known as KDM1B), catalyze the demethylation of mono- and dimethylated H3K4 and H3K9⁷⁵. The JMJC demethylases, which contain 30 members, have activity against methylated H3K4, H3K9, H3K27, H3K36 and H4K20⁷⁵.

Recurrent genetic alterations, including mutations, gene amplifications and deletions of lysine demethylases have been noted in cancer. Mutations in *KDM6A* (also known as *UTX*), encoding an H3K27 demethylase, are seen in a variety of solid and hematological cancers⁷⁶, whereas *KDM4A* (also known as *JMJD2*), encoding an H3K9 demethylase, is frequently amplified and overexpressed in **oesophageal squamous cell carcinoma, lung sarcomatoid carcinoma and desmoplastic medulloblastoma**⁷⁷. Similarly, LSD1 is overexpressed in multiple cancer types, and high LSD1 expression correlates with poor clinical outcomes in these cancers^{78–81}.

In addition to being affected by somatic mutations, the activity of these erasers is also regulated by upstream oncogenic signaling pathways, which provide another way of altering their function. For instance, the expression of JMJD3 (also known as KDM6B) is induced by oncogenic RAS-RAF signaling, leading to demethylation of H3K27me3 at the *CDKN2A* locus, and increased p16^{INK4A} expression⁸² (Figure 2). These results suggest an important role for JMJD3 in triggering oncogene- or stress-induced senescence, two fail-safe mechanisms that help prevent malignant transformation. Indeed, JMJD3 expression is significantly reduced in lung and liver cancer, and in hematological malignancies⁸². Furthermore, the *TP53* (which encodes p53) and *JMJD3* genes are located at 17p, a region frequently lost in a variety of human cancers⁸³. This suggests that JMJD3 may play a tumor-suppressor role, similar to the known tumor suppressor role played by p53.

Regulation of histone arginine methylation

In addition to lysine methylation, histones are methylated on arginine residues, a reaction that is catalyzed by the protein arginine methyltransferases (PRMTs). Nine PRMTs have been identified in mammalian cells, and classified into two major groups based on their ability to catalyze either the asymmetric (type I enzymes) or the symmetric (type II enzymes) di-methylation of arginine residues in histone and non-histone proteins^{84, 85}. PRMT7 seems to exhibit type III enzymatic activity – namely the ability to catalyze the formation of monomethylated but not di-methylated arginine⁸⁶. Arginine methylation of proteins has been implicated in various cellular processes, including transcriptional regulation, signal transduction, DNA repair and RNA processing⁸⁷.

PRMT5 is the major type II arginine methyltransferase in mammalian cells, catalyzing symmetric dimethylation of histones H2A, H3 and H4^{88–90}, as well as of non-histone proteins, including p53, methyl-CpG-binding domain protein 2 (MBD2) and the Sm ribonucleoproteins^{91–93}. The role of PRMT5 in lymphomagenesis, leukemogenesis and in the genesis of solid tumors is rapidly being unraveled. PRMT5 is overexpressed in leukemia and lymphoma, as well as in many types of solid tumors, suggesting a potential oncogenic role⁹⁴. The Sif group first reported that the level of PRMT5 was elevated in primary lymphoma samples and cell lines, where it represses the expression of the retinoblastoma tumor suppressor proteins: RB1, RBL1 and RBL2⁹⁵. PRMT5 upregulation is induced by the ectopic expression of several oncogenes, including *NOTCH1*, the mixed-lineage leukaemia (*MLL*)-fusion protein *MLL-AF9* (also known as *KMT2A-MLLT3*) and *MYC*⁹⁶. In addition to repressing the expression of tumor suppressor genes, upregulation of PRMT5 also maintains proper pre-mRNA splicing in lymphoma cells⁹⁷. Treating mantle cell lymphoma cells with PRMT5-specific inhibitors shows promising *in vitro* and *in vivo* anti-tumor activities⁹⁸.

PRMT5 was originally identified in a yeast two-hybrid assay as JAK binding protein 1⁹⁹. We therefore investigated the interaction between PRMT5 and JAK2 given the high frequency of the JAK2-V617F mutation in patients with MPNs, which suggested that this mutation might generate a specific gain-of-function, rather than simply constitutively activating JAK2 kinase activity⁷. Indeed, we found that JAK2-V617F interacts more strongly with PRMT5 than wild-type JAK2, and that it gained the ability to phosphorylate PRMT5 and downregulate its

histone methyltransferase activity by disrupting the interaction between PRMT5 and its co-factor methylome protein 50 (MEP50). Treating JAK2-V617F-harboring cells with JAK2 inhibitors rapidly upregulated global H2A and H4 R3 symmetric dimethylation, providing an example of how an oncogenic tyrosine kinase can affect a specific chromatin modification by altering the activity of a histone modifying enzyme. However, JAK2 inhibitors did not affect the symmetric methylation of all proteins examined, suggesting differential sensitivities of various PRMT5 substrates in the cell. This observation may reflect the intracellular localization where the phosphorylation occurs (for example, in the cytoplasm or nucleus), or the differential effects of phosphorylation on the various PRMT5 complexes found in the cell. We have also recently shown that PRMT5 is essential for the proliferation and survival of adult hematopoietic stem and progenitor cells, in part due to its role in regulating multiple cytokine signaling pathways¹⁰⁰.

Whereas we showed that phospho-PRMT5 (catalyzed by JAK2-V617F) no longer binds MEP50, Aggarwal *et al.*¹⁰¹ showed that the mutant cyclin D1-T286A-CDK4 complex, which is found in some **non-Hodgkin lymphomas**, phosphorylates MEP50 and increases PRMT5 methyltransferase activity toward histone H4R3 and H3R8. This leads to decreased expression of the ubiquitin ligases cullin 4A (CUL4A) and CUL4B, and stabilization of the DNA replication factor CDT1, which triggers DNA re-replication and enhances genomic instability. In a recent report by the same group, PRMT5 activation via MEP50 phosphorylation was shown to impair p53 signaling, further promoting cyclin D1-T286A-mediated transformation⁹⁶.

The activity of other PRMTs is also controlled by phosphorylation. For instance, PRMT4, originally identified as a co-activator for the nuclear hormone receptors¹⁰² is phosphorylated at S217 and S228, likely by a mitotic kinase; this disrupts its interaction with the methyl donor **S-adenosylmethionine** (SAM), and represses its methyltransferase activity^{103, 104}. These studies suggest a potential role of PRMT4 regulation in cell cycle progression, however neither study identified the kinase(s) involved.

Regulation of histone acetylation

Two highly homologous histone lysine (K) acetyltransferases (KATs, formerly called HATs), p300 and CREB binding protein (CBP; also known as CREBBP), are recruited to chromatin by sequence-specific transcription factors, leading to histone acetylation, localized chromatin remodeling and ultimately, the activation of specific gene expression programs¹⁰⁵. Both enzymes can function as tumor suppressors. For example, patients with **Rubinstein–Taybi syndrome** (RTS), which is caused by germline mutations that disrupt CBP or p300 function, have an increased predisposition to develop tumors of neural and developmental origins¹⁰⁶. Somatic loss-of-function mutations are also seen in the genes encoding these two enzymes in lymphomas and in solid tumors, including colorectal cancer and gastric cancer¹⁰⁷. Chromosome translocations that fuse *CREBBP* (the gene encoding CBP) with the monocytic leukemia zinc finger gene (*KAT6A* also known as *MOZ* or *MYST3*) are found in t(8;16) AML¹⁰⁸; these translocations generate haploinsufficiency for CBP, in addition to the gain-of-functions intrinsic to the fusion proteins they generate.

Both p300 and CBP are phosphorylated at multiple serine, threonine and tyrosine residues by various signal transduction pathways (Figure 3). The functional consequences of these phosphorylation events are diverse, impacting the enzymatic activity, protein stability and protein-protein interactions of these two enzymes. p300 was first shown to be phosphorylated by cyclin-CDK2, and this phosphorylation is blocked by the adenovirus E1A proteins¹⁰⁹. CDK1 phosphorylates p300 at S1038 and S2039 during mitosis, leading to p300 degradation, which contributes to cell cycle progression by facilitating chromatin condensation in mitotic lung cancer cells¹¹⁰. Whereas phosphorylation of p300 by AKT (at S1834) enhances its HAT activity and its recruitment to gene promoters¹¹¹, its phosphorylation by PKC α , β II and δ (at S89) impairs its ability to activate transcription^{112, 113}. AMPK also phosphorylates p300 (at S89), reducing its affinity for nuclear receptors (NRs), which leads to decreased transcription of NR target genes. Nevertheless, S89 phosphorylation does not affect the interactions of p300 with non-nuclear hormone receptor transcription factors, such as p53 and GATA4¹¹⁴. ATM phosphorylates p300 at S106, leading to protein stabilization¹¹⁵. Furthermore, p300 phosphorylation by PKA or p38 MAPK impairs protein stability, triggering protein degradation by the ubiquitin-proteasome pathway^{116,117}.

Phosphorylation of CBP at its C-terminus, by ERK1 and the cyclin E-CDK2 complex (which functions at the G1/S boundary), increases its enzymatic activity toward histones^{118,119}. Whereas, the CaM kinase IV (CaMKIV) phosphorylates CBP at its N-terminus (S302) and stimulates its acetyltransferase activity in cultured neuron cells¹²⁰. Phosphorylation of CBP (at S1382 and S1386) by IKK α enhances its HAT activity, and switches its binding preference from p53 to NF- κ B¹²¹. This enhances transcription of NF- κ B-target genes and decreases transcription of p53-target genes, thereby promoting cell proliferation, and possibly tumor progression. A positive correlation between IKK α activity and CBP phosphorylation was found in various cancer cell lines, indicating that CBP phosphorylation may promote NF- κ B-mediated tumorigenesis¹²¹. Given the diverse functions of p300 and CBP, it appears that the availability of these proteins can be a rate-limiting factor for many biological processes, including transcriptional regulation and cell cycle progression. Thus the tight regulation of the activity of p300 and CBP by various signal transduction pathways provides a crucial layer of control for these cellular events¹²².

The aberrant expression of and mutations in genes encoding histone deacetylases (HDACs) have also been linked to tumorigenesis, and the more specific targeting of these enzymes is a promising therapeutic approach for some types of cancer. HDAC function is regulated by phosphorylation: the class II HDACs (HDAC4, 5, 7 and 9) are phosphorylated by multiple signaling kinases at their N-terminal regulatory regions, and phosphorylation-dependent interaction with 14-3-3 proteins triggers the nuclear export of HDACs, leading to de-repression of their target genes¹²³. Casein kinase 2 (CK2) appears to be the major signaling kinase that phosphorylates the class I HDACs, HDAC1 and HDAC2 under hypoxic conditions¹²⁴. Phosphorylation-dependent activation of these HDACs is involved in the downregulation of **von Hippel-Lindau protein (VHL)** and subsequent stabilization of hypoxia inducible factor 1 α (HIF1 α). Therefore, targeting CK2 in hypoxic tumors may be a potential therapeutic approach.

DNA damage and chromatin modification

The DNA damage response (DDR) represents a major signaling pathway that maintains the integrity and accuracy of genetic information, and suppresses mutagenic events that could be tumorigenic¹²⁵. The DDR is intimately related to changes in chromatin structure, via phosphorylation of histones and histone modifiers (Figure 4). Phosphorylation of H2AX at S139 (which is generally referred to as γ H2AX) by the PI3K-like family of kinases (that include ataxia-telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR) and the DNA-dependent protein kinase catalytic subunit (DNA-PK_{CS}) appears to be the first histone modification following DNA damage¹²⁶. γ H2AX not only recruits molecules that sense or signal the presence of DNA breaks, but also histone modifiers, including ubiquitin ligases and histone methyltransferases, to further modify the local chromatin structure and activate cellular processes that lead to repair of the DNA damage. Other histone phosphorylation events induced by DNA damage include H2B phosphorylation (at S14) by MST1, leading to chromatin condensation and apoptosis⁴¹, and H4 phosphorylation (at S1) by CK2, which facilitates DNA repair and blocks local H4 acetylation by the NuA4 histone acetyltransferase complex^{127, 128}.

The decision of cells to undergo cell cycle arrest and repair the damaged DNA, or undergo cell death after DNA damage occurs, reflects the integration of a variety of upstream signals. For instance, H2AX is constitutively phosphorylated at Y142 by the tyrosine kinase WSTF (also known as BAZ1B)¹²⁹. Upon DNA damage, this tyrosine residue can either remain phosphorylated or be dephosphorylated (by the phosphatases eyes absent homologue 1 (EYA1) and EYA3)¹³⁰. Intriguingly, the phosphorylation status of Y142 affects the function of the nearby S139 residue, a site phosphorylated in response to DNA damage. Dephosphorylation of Y142 allows the recruitment of DNA repair complexes by phosphorylated S139, leading to DNA repair and cell survival. In contrast, phosphorylated Y142 facilitates the recruitment of pro-apoptotic factors by phosphorylated S139, leading to cell death. Thus, phosphorylation at Y142 on H2AX acts as a key switch that regulates cell decisions following DNA damage¹³⁰. Phosphorylation at H3T11 is another mechanism that regulates such decisions¹³¹. This residue is phosphorylated by CHK1 during the cell cycle, and its phosphorylation generally decreases upon DNA damage due to the disassociation of CHK1 from chromatin. This reduces the recruitment of the HAT GCN5 (also known as KAT2A) to the promoters of cell cycle regulatory genes, such as *CDK1* and *CCNB1* (which encodes cyclin B), leading to less H3K9 acetylation at these promoters and decreased gene expression. Thus, the reduced phosphorylation of H3T11 contributes to transcriptional repression and cell cycle arrest following DNA damage.

In addition to histones, histone-modifying enzymes can also be post-translationally regulated by the DDR. For example, the DDR triggers phosphorylation of the H3K4 methyltransferase *MLL* on S516 by ATR, disrupting its interaction with S-phase kinase-associated protein 2 (SKP2) E3 ubiquitin ligase and preventing its degradation¹³². At S-phase, the accumulated *MLL* methylates H3K4 on the β -globin origin of DNA replication, which can impair the loading of cell division control protein 45 (CDC45) onto chromatin, delaying the initiation of DNA replication. Interestingly, oncogenic *MLL* fusions (such as *MLL-AF4* and *MLL-*

AF9), function as dominant negative mutants to block this S-phase checkpoint, representing a potential mechanism of MLL fusion-mediated transformation¹³².

The various histone modifications induced by DDR are crucial for DNA damage sensing and signaling, repair of the lesion and cell cycle arrest and release of the cell back into the cycle¹³³. Mutations in genes that encode DDR proteins can result in a number of genomic instability syndromes, including **Fanconi anemia**, **Ataxia-telangiectasia**, and **Bloom's syndrome**, that lead to an increased risk of leukemia and other cancers, demonstrating an important role of DDR in the pathogenesis of cancer¹³⁴. Moreover, induction of DNA damage is one important strategy for cancer treatment; therefore, drugs that target the enzymes responsible for these modifications could be very promising therapeutics¹³⁵.

Regulation of DNA methylation

DNA methylation is established in mammalian cells by two types of DNA methyltransferases (DNMTs). The *de novo* DNMTs, DNMT3A and DNMT3B act on hemimethylated or unmethylated DNA, and are essential for establishing DNA methylation patterns during development. The 'maintenance DNMT', DNMT1 acts preferentially on hemi-methylated DNA, and ensures the propagation of tissue-specific methylation patterns¹³⁶.

Alterations in DNA methylation are a hallmark of cancer¹¹ (Box 3), and aberrant regulation of DNA methylation is commonly seen in the hematopoietic malignancies, including recurrent mutations in DNMT3A and in the DNA methylcytosine hydroxylase TET2 in patients with MPN, MDS and AML¹³⁷. Recent studies have demonstrated that mutations in these genes are found in a significant fraction of normal elderly individuals with age-associated clonal haematopoiesis^{138, 139}; this data, and data on variant allele frequency suggest that mutations in these enzymes are likely primary events that lead to transformation. As many signalling pathways have been implicated in directly controlling DNA methylation^{140,141}, the co-occurrence of oncogenic tyrosine kinase mutations with mutations in DNMT3A (for example. FLT3-ITD and DNMT3A-R882C or -R822H in a subset of AMLs¹³⁷) could represent the synergistic triggering of aberrant DNA methylation in cancer.

Regulation of DNMT activity via phosphorylation

Ectopic expression of DNMT1 in NIH 3T3 fibroblasts transforms these cells¹⁴², suggesting that it has a potential oncogenic role. Overexpression of DNMT1 has been reported in some types of cancer, including AML¹⁴³, breast cancer¹⁴⁴ and colon cancer¹⁴⁵, however, many tumors have normal levels of DNMT1 expression. A number of studies have reported that DNMT1 is phosphorylated on multiple sites within its N-terminus (DNMT1 is phosphorylated at S154 by CDK1, 2 and 5), and this phosphorylation is important in regulating its enzymatic activity and protein stability¹⁴⁶. SET domain-containing protein 7 (SETD7) methylates DNMT1 at K142, which triggers its degradation, however, this methylation is blocked by AKT, which phosphorylates DNMT1 on S143, leading to its stabilization¹⁴⁷. DNMT1 is positively regulated by oncogenic RAS signaling at multiple levels. Elevated RAS signaling may induce DNMT1 expression through c-JUN activation, as

several AP-1 binding sites (which are binding motifs for JUN) are found in the 5' upstream regulatory regions of *DNMT1*¹⁴⁸. The converse is also true, forced expression of GAP (also known as RASA1), a negative regulator of RAS activity, decreases *DNMT1* expression and global levels of DNA methylation, which can reverse cellular transformation¹⁴¹. In contrast, both PI3K-AKT and PKC-dependent signaling can phosphorylate DNMT1 (disrupting the DNMT1-proliferating cell nuclear antigen (PCNA)-UHRF1 replication complex), which leads to global hypomethylation and genomic instability¹⁴⁹. DNMT1 can also be phosphorylated by CK1δ and CK1ε, which impairs its affinity for DNA¹⁵⁰. Thus, multiple signaling pathways regulate DNMT1 in a cell type- and context-specific manner, resulting in increases or decreases in its methyltransferase activity, and ultimately, can result in alterations in DNA methylation patterns (Figure 5).

In addition to DNMT1, the activity of DNMT3A is also regulated by phosphorylation, as its phosphorylation by CK2 is required for the recruitment of DNMT3A to heterochromatic regions to silence repetitive elements in cells¹⁵¹.

Regulation of promoter hypermethylation of tumor suppressor genes in cancer

The constitutive activation of HRAS or KRAS has been shown to lead to promoter hypermethylation and to transcriptional silencing of numerous tumor suppressor genes, including those encoding the cell death-inducing protein FAS, the apoptosis-promoting protein PAR4, the RAS effector RASSF2 and *O*-6-methylguanine-DNA methyltransferase (MGMT)¹⁵².

Transforming growth factor-β (TGFβ) signaling is hyperactive in breast cancer cells during the **epithelial-mesenchymal transition** (EMT), and the increased promoter DNA methylation triggered by this signaling pathway silences genes involved in cell adhesion and tight-junction formation, such as cadherin 1 (*CHD1*), cingulin (*CGN*), claudin 4 (*CLDN4*) and kallikrein related peptidase 10 (*KLK10*; also known as *NES1*)¹⁵³.

Nuclear hormone receptor signaling also regulates DNA methylation at specific target gene promoters. For instance, the resistance of human cancer cells to the growth inhibitory effects of retinoic acid (RA) is associated with promoter hypermethylation of the retinoic acid receptor β2 gene (*RARB2*)¹⁵⁴. Inhibition of RA signaling induces a repressed chromatin state at *RARB2* and its transcriptional silencing. In contrast, loss of **oestrogen receptor** (ER) signaling in breast cancer cells results in epigenetic silencing of ER target genes, including the **progesterone receptor** (PR; encoded by *PGR*), largely due to the recruitment of the PRC2 complex and HDAC1 to the promoters of these genes. This leads to the progressive accumulation of DNA methylation at these promoters, thus reactivation of *PGR* expression requires both ER signaling and DNA demethylation¹⁵⁵.

The available evidence shows that oncogenic signaling shapes the DNA methylation pattern in cancer cells by controlling the expression levels of DNMTs; regulating the enzymatic activity or protein stability of DNMTs, directly affecting the binding of DNMTs to DNA, where they can regulate the local DNA methylation levels. Given the crosstalk between DNA methylation and histone modifications¹⁵⁶, the signaling pathways that contribute to

cancer-specific DNA methylation patterns can also affect global and local histone modifications.

Conclusions

We have summarized recent findings on the crosstalk between cellular signaling pathways and epigenetic elements, with a focus on how the epigenetic landscape in cancer cells is affected by aberrant growth control signaling. This regulation appears to be varied and complex, but it plays a crucial role in the numerous biological processes that are involved in cell transformation.

During normal development, appropriate temporal and spatial changes in the pattern of DNA methylation and histone modifications are essential to ensure that each cell within the body differentiates to its desired cell fate. This generally unidirectional process involves the step-wise silencing of genes associated with developmental potential and the activation of tissue-specific gene programs. Thus, alterations in epigenetic modifications can disrupt normal developmental pathways and interfere with cell differentiation, ultimately leading to cellular transformation¹⁵⁷.

Relatively little is known about how the abnormal cancer epigenome is established, although several potential mechanisms have been proposed. First, it has been shown that epigenetic modifier genes are expressed in a tissue-specific pattern and are differentially expressed in tumor cells compared to their normal counterparts (e.g. increased *EZH2* expression in aggressive breast or prostate cancer)¹⁵⁸. Second, the genetic alterations that lead to loss-of-function or gain-of-function mutations in epigenetic modifying enzymes are observed in the large subset of cancers that have an aberrant epigenetic landscape¹⁵⁹. Third, non-coding RNAs have been shown to play a role in controlling the distribution of epigenetic marks¹⁶⁰. Finally, as reviewed in this article, a variety of oncogenic kinases can affect DNA and histone modifications via either direct phosphorylation of histones or phosphorylation of epigenetic-modifying enzymes.

These discoveries have several implications for the field of cancer epigenetics. They raise the possibility that the aberrant epigenomes seen in tumor cells without epigenetic mutations could be caused by oncogenic mutations that alter upstream signaling pathways. For example, the MPN-associated mutant JAK2-V617F gains the ability to inhibit PRMT5 function and decrease H2AR3 and H4R3 symmetric methylation; thus JAK2-V617F-positive cells may have decreased levels of global H2AR3meS2 and H4R3meS2 compared to normal cells, even though both cell types may have identical levels of PRMT5 protein.

Traditionally, oncogenic kinases were thought to affect gene expression primarily through activation or repression of downstream transcription factors. By gaining the ability to regulate histone and DNA modifications, oncogenic kinases can affect the overall and local chromatin structure and have a profound influence on gene expression and on genomic stability, with the latter potentially leading to more aberrations within the cancer cell genome. The crosstalk between genetics and epigenetics is likely an important mechanism that underlies the aberrant cell behaviors that result in tumorigenesis.

One key feature of epigenetic regulation is its inheritance, the passing of ‘epigenetic memory’ to the next generation of cells. This ensures the long-term maintenance of epigenetic patterns even after the initiating developmental and environmental cues have been removed. In contrast, most signaling transduction pathways are much more dynamic, and are fine-tuned by numerous kinases and phosphatases to ensure quick responses to exogenous stimuli. However, the modulation of chromatin marks by upstream signaling pathways allows these pathways to leave their ‘fingerprints’ on gene expression profiles, which may remain even after the initiating signal is removed (Figure 6). This may have important therapeutic implications; treating tumor cells with kinase inhibitors may not be sufficient to restore the deregulated gene expression programs that are found in these cells, and combining kinase inhibitors with epigenetic drugs that can directly attack the epigenetic aberrancies found in cancer cells may lead to superior therapeutic outcomes.

Despite the recent major advances in the field described in this Review, our understanding of how the cancer epigenome is affected by abnormal oncogenic signaling pathways is still at a nascent stage. As the link between signaling networks and epigenetic modifications solidifies, we hope to obtain a better understanding of how changes in the epigenetic landscape establish and maintain the cancer cell’s identity.

Acknowledgments

We thank Feng-chun Yang and Lluís Morey for their helpful advice, and Delphine Prou for her assistance with manuscript preparation. The SDN laboratory is supported by grants from National Cancer Institute Grant RO1 CA166835-01, FL is supported by American Cancer Society Institute Research Grant Pilot Project Award, and WL is supported by National Natural Science Foundation of China Grant 81470334.

Glossary

Myeloproliferative neoplasms (MPN)

A group of diseases that affect normal blood cell production in the bone marrow, which manifests as an overproduction of one or more blood cell types (red cells, white cells or platelets)

Myelodysplastic syndrome (MDS)

A haematological medical condition also known as myelodysplasia. It is characterized by ineffective production of all blood cells and a disorder of the haematopoietic stem cells in the bone marrow, and relates only to cells of the myeloid lineage

Esophageal squamous cell carcinoma

A type of cancer that begins in the flat squamous cells lining the esophagus. Smoking and heavy alcohol use increase the risk of this disease

Lung sarcomatoid carcinoma

A unique type of lung carcinoma, which contains cytological and tissue architectural features that are usually characteristic of sarcoma

Desmoplastic medulloblastoma

A subtype of medulloblastoma with a biphasic pattern of compact sheets of undifferentiated cells alternating with islands of more loosely cohesive cells. This cancer generally occurs in adolescence and young adults and has a better prognosis than medulloblastoma

Non-Hodgkin lymphomas

Lymphomas other than Hodgkin disease, classified as nodular or diffuse depending on the tumor pattern and cell type

S-adenosyl methionine

A common co-substrate involved in methyl group transfers, transsulfuration and aminopropylation

Rubinstein–Taybi syndrome

A condition characterized by short stature, moderate to severe intellectual disability, distinctive facial features, and broad thumbs and first toes. Mutations in the genes *CREBBP* and *EP300* (encoding CBP and p300) are seen in some people with this condition

Von Hippel-Lindau protein

An E3 ubiquitin protein ligase that functions as part of the VCB-CUL2 complex, and is classified as a tumor suppressor

Epithelial-mesenchymal transition

The process by which epithelial cells lose cell-cell junctions and baso-apical polarity and acquire plasticity, mobility, invasive capacity, stemlike characteristics, and resistance to apoptosis

Estrogen receptor

The receptor activated by the hormone estrogen. ER can translocate into the nucleus and bind to DNA to regulate gene expression

Progesterone receptor

The intracellular receptor activated by the steroid hormone progesterone. It is often overexpressed in breast cancer

CpG island

It is a DNA region with at least 200 base pair, and a CG percentage which is higher than 50 %. Methylation of the CpG sites within the gene promoter can lead to gene silencing

Transposable elements

It is a DNA sequence that can change its position within a genome. It can create or reverse mutations and alter the genome size of the cell

Imprinting

It is an epigenetic phenomenon by which some genes can be expressed in a parent-of-origin-specific manner. If the gene inherited from the father is imprinted, this gene is silenced, then only the gene from the mother can be expressed

Immediate early genes

They are genes which can be activated rapidly and transiently in response to different kinds of cellular stimuli. These genes stand for a response mechanism which is activated at the transcription level before the synthesis of new proteins

Fanconi anemia

It is an inherited blood disorder which results in bone marrow failure. It prevents the bone marrow from producing enough hematopoietic cells for human body to function normally

Ataxia-telangiectasia

It is an inherited disease which affects several body systems, including the immune system, and the patients with A-T have a high rate of cancer

Bloom's syndrome

It is a rare autosomal recessive disorder which is characterized by short stature, predisposition to the cancer development and genomic instability. This disorder is induced by the BLM gene mutations which lead to the formation of the mutated DNA helicase protein

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Box 1**Histone modifying enzymes**

Epigenetic histone regulators are classified based on their broad function.

- Epigenetic writers are enzymes that lay down epigenetic marks on histone tails.
- Epigenetic erasers are enzymes that revert histone modifications.
- Epigenetic readers are proteins that recognize epigenetic marks.

Most histone modification patterns are established by histone acetyltransferases (HATs) or histone methyltransferases (HMTs), which can be further classified as lysine methyltransferases and arginine methyltransferases according to their target residue. Histone tails can also be modified by kinases, ubiquitin ligases and E2 SUMO-conjugating enzymes. These enzymes are usually specific to particular amino acid motifs and target both core histones (such as H3) and histone variants (such as H3.3)¹⁶¹.

Epigenetic marks are generally dynamic and can be modified or removed by a variety of enzymes. Histone lysine acetylation and methylation are removed by histone deacetylases (HDACs) and histone demethylases, respectively. Histone phosphatases can target phosphorylated serine, threonine and tyrosine in histone tails. The removal of histone mono-ubiquitination is carried out by deubiquitinating enzymes¹⁶¹.

The key function of histone modifications is to signal to recruit effectors and epigenetic readers to the local chromatin; these proteins ultimately determine the transcriptional outcome of certain epigenetic modifications¹⁶².

Box 2**Components of PRC1 and PRC2 complexes**

The core PRC2 complex, which is conserved from *Drosophila melanogaster* to mammals, comprises four components: EZH1 or EZH2, SUZ12, EED and RBAP46 (also known as RBBP7) or RBAP48 (also known as RBBP4). EZH1 and EZH2 are histone methyltransferases, catalyzing H3K27 methylation¹⁶³.

The PRC1 complex is more variable than PRC2 and is formed by four different orthologs, CBX (polycomb), PCGF (polycomb group factor), HPH (human polyhomeotic homolog), and an E3 ubiquitin ligase protein (RING) that catalyzes the monoubiquitination of histone H2A on lysine 119 (H2AK119ub)¹⁶⁴.

H3K27 trimethylation and H2AK119 mono-ubiquitination are major repressive marks that inhibit transcription and help to regulate the expression of development-related transcription factors, such as those encoded by clusters of HOX genes, as well as other genes that control cell proliferation, such as *CCND2* (encoding cyclin D1)¹⁶⁵.

Box 3**DNA epigenetic modifications that can be altered in cancer**

There are a number of different types of DNA epigenetic modification that have shown changes in cancer^{166, 167}

1. Large organized chromatin lysine modifications (LOCKS), which are usually heterochromatic, are reduced or lost in cancer.
2. Long blocks of DNA (median size: 28 kb) found around LOCKs and lamin-associated domains (LADs), which usually have high levels of DNA methylation, are hypomethylated in cancer.
3. Many tumor suppressor genes, such as *CDNK2A* and *BRCA1*, are linked to hypermethylated CpG islands in cancer¹⁶⁸.
4. The sharp boundaries between CpG island and shores are lost in cancers, and DNA methylation is remarkably hypervariable at shores or boundaries in these cancer cells.

Figure 1. Histone modifications regulated by the JAK-STAT pathway

In addition to activating its downstream transcription factors such as signal transducer and activator of transcription factors (STATs) the receptor-associated Janus kinase 2 (JAK2) can also affect chromatin structure via direct and indirect mechanisms. In response to extracellular stimuli, both wild type and JAK2-V617F (shown as JAK2VF) can shuttle into the nucleus and phosphorylate histone H3 at Y41. This leads to exclusion of heterochromatin protein 1 (HP1) from chromatin and activation of gene transcription at JAK2 target genes such as *LMO2*. In contrast, oncogenic JAK2-V617F, but not wild type JAK2, can phosphorylate the arginine methyltransferase PRMT5, resulting in the dissociation of the PRMT5-MEP50 complex, the downregulation of PRMT5 methyltransferase activity, and a decrease in symmetric dimethylation of histones H2A and H4 at R3. Me, methyl; P, phosphate.

Figure 2. Regulation of Polycomb group protein repressive complexes by signaling pathways Polycomb repressive complex 1 (PRC1) (composed of BMI1, CBX and RING1A or RING1B) and PRC2 (composed of EZH2, SUZ12 and EED) are key transcriptional regulators that control gene expression from early embryogenesis through birth to adulthood. These complexes are subject to intensive regulation from upstream signaling pathways, including the PI3K-AKT and RAS-MAPK pathways. Activated AKT phosphorylates both EZH2 and BMI1, two crucial components of the PRC2 and PRC1 complexes, respectively. Phosphorylation of EZH2 inhibits H3K27 methylation by the PRC2 complex, and phosphorylation of Bmi1 decreases the association of the PRC1 complex with chromatin, leading to decreased H2AK119ub. BMI1 can also be phosphorylated by activated 3pK, leading to the impaired recruitment of PRC1 to chromatin. Furthermore, MAPK signaling upregulates the expression of the histone demethylase JMJD3, which removes the H3K27 methyl mark that is added by the PRC2 complex. These changes then contribute to upregulation of the *CDKN2A* products p16^{INK4A}, p14^{ARF} (human) and p19^{ARF} (mouse), leading to oncogene-induced cell senescence. Me, methyl; P, phosphate; Ub, ubiquitin.

Figure 3. The extensive regulation of the CBP and p300 histone acetyltransferase activity by signaling kinases

Both CREB binding protein (CBP) and p300 are phosphoproteins, and they are regulated by a number of signaling pathways. These kinase-dependent and site-specific phosphorylation events variably affect the acetyltransferase activity of these histone lysine acetyltransferases (KATs) via changes in protein stability and protein-protein interactions. AMPK, AMP-activated protein kinase; Bro, bromodomain; CaMKIV, calcium/calmodulin dependent protein kinase IV; CDK, cyclin-dependent kinase; IKK α , I- κ B kinase- α ; KIX, kinase-inducible domain interacting; NF- κ B, nuclear factor- κ B; NR, nuclear receptor; PK, protein kinase.

Figure 4. The DNA damage response modulates chromatin modifications

DNA damage triggers a variety of changes in chromatin modifications via direct phosphorylation and/or dephosphorylation of histones such as histone H2 and H3, as well as the phosphorylation of histone modifiers such as mixed lineage leukaemia 1 (MLL1), ultimately leading to cell cycle arrest and repair of DNA damage, or apoptosis-mediated cell death. Ac, acetyl; DNA-PK, DNA-dependent protein kinase; Me, methyl; MST1, mammalian STE20-like kinase 1; P, phosphate.

Figure 5. Oncogenic signaling regulates DNMT1 activity

Multiple signaling cascades, including RAS-MAPK, PI3K-AKT, protein kinase C (PKC), casein kinase 1 (CK1) and cyclin dependent kinases (CDKs), phosphorylate DNA methyltransferase 1 (DNMT1) in a cell type-specific and context-dependent manner, resulting in increases or decreases in cellular DNA methylation. RAS signaling upregulates DNMT1 expression, consistent with its transforming activity. Me, methyl; P, phosphate.

Figure 6. The epigenetic memory of signaling

Although canonical signaling pathways are usually drawn as converting extracellular stimuli to the activation of downstream transcription factors, we believe that changes in the target gene expression also require changes in the activities of epigenetic enzymes, which can consolidate the patterns of gene expression that are triggered by specific signals. Thus, although some of these changes are transient and can be easily reversed by removing the signal, others are more persistent and may not be reversed after the initial signal is removed. By phosphorylating histone and/or DNA modifiers, or the histones themselves, signaling pathways can leave their 'fingerprints' on chromatin structure, indicating their earlier presence in the cell. Me, methyl; P, phosphate; RTK, receptor tyrosine kinase.

Table 1

Major histone modifications and their function in transcription regulation.

Modifications	Position		Enzymes	Function in Transcription
Methylation	H3	K4	MLL1-5, SETD1A, SETD1B, SETD7, ASH1L	Activation
		K9	SUV39H1, G9A, SETBD1	Repression
		K27	EZH1, EZH2	Repression
		K36	SETD2, ASH1L, NSD1	Activation
	H2A	K79	DOT1L	Activation
		R2, R17, R26	PRMT4	Activation
		R8	PRMT5	Repression
		R3	PRMT1, PRMT5	Activation and/or repression
Acetylation	H4	K20	SETD8, SUV4-20H1, SUV4-20H2	Repression
		R3	PRMT1, PRMT5	Activation and/or repression
	H3	K9,K14,K18, K23, K27	KAT2A (GCN5), KAT2B (PCAF), p300, CBP	Activation
		K5,K8,K12, K16	KAT5 (Tip60), KAT7, p300, CBP, ATF2	Activation
Ubiquitination	H2B	K120	UBE2E1, RNF20, RNF40	Activation and/or repression
	H2A	K119	RNF2 (RING1B)	Repression

CBP, CREB binding protein; MLL, mixed lineage leukaemia; PRMT1: protein arginine methyltransferase 1; SETD1A: SET domain containing 1A; SUV39H1: suppressor of variegation 3–9 homolog 1; ASH1L: (absent, smaller homeotic)-like; KAT2A: lysine acetyltransferase 2A; RNF20: ring finger protein 20; UBE2E1: Ubiquitin conjugating enzyme E2 E1.

Table 2

Direct phosphorylation of histones by upstream signaling kinases.

Function	Histone	Site	Kinase	Upstream signalling	Ref
Transcription regulation	H3	S10, S28	RSK2 MSK1, MSK2	p42 and p44-MAPK p38-MAPK	17, 18
	H3	S10	IKK α	Cytokines, NF- κ B	20
	H3	S10	JNK	MAPK	21
	H3	S10	PIM1	JAK-STAT	22
	H3	T6	PKC β 1	Phospholipase, DAG	32
	H3	T11	PRK1	Rho family of small G proteins	31
	H3	Y41	JAK2	Cytokines,	34
	H2B	S32	RSK2	p42 and p44-MAPK	169
	H2B	S36	AMPK	Metabolic stress, LKB1	40
	H2B	T37	WEE1	Cell cycle	170
Chromatin condensation -apoptosis	H3	S10	PKC6	Apoptosis, caspases	23
	H3	T45	PKC6	Apoptosis, caspases	171
	H2B	S14	MST1	Hippo pathway, YAP PI3K-AKT SAPK-JNK	34
				Apoptosis, caspases	
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	H4	S1	CK2	DNA damage	127
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AMPK, AMP-activated protein kinase; CK2, casein kinase 2; DAG, diacylglycerol; DNA-PK, DNA-dependent protein kinase; IKK α , I- κ B kinase α ; JAK, Janus kinase; MSK, mitogen- and stress-activated kinase; MST1, STE20-like kinase; NF- κ B, nuclear factor- κ B; PKC, protein kinase C; RSK, ribosomal S6 kinase; WSTF, Williams syndrome transcription factor.