Control of Breast Cancer Pathogenesis by Histone Methylation and the Hairless Histone Demethylase

Megan H Trager¹, Bindeshwar Sah², Zhongming Chen²,³, and Liang Liu²,³*

¹ Columbia University Vagelos College of Physicians and Surgeons, New York, NY 10032
² The Hormel Institute, University of Minnesota, Austin, MN 55912
³ Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55912

*To whom correspondence should be addressed:
The Hormel Institute
University of Minnesota
Austin, MN
55912
Phone: 507-437-9627
Email: LIU00965@umn.edu

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Abstract

Breast cancer is a highly heterogeneous disease, encompassing many subtypes that have distinct origins, behaviors and prognoses. Although traditionally seen as a genetic disease, breast cancer is now also known to involve epigenetic abnormalities. Epigenetic regulators, such as DNA methyltransferases and histone modifying enzymes, play essential roles in gene regulation and cancer development. Dysregulation of epigenetic regulator activity has been causally linked with breast cancer pathogenesis. Hairless (HR) encodes a 130 kDa transcription factor that is essential for development and tissue homeostasis. Its role in transcription regulation is partly mediated by its interaction with multiple nuclear receptors, including thyroid hormone receptor, retinoic acid receptor-related orphan receptors, and vitamin D receptor. HR has been studied primarily in epidermal development and homeostasis. Hr-mutant mice are highly susceptible to UV- or carcinogen-induced skin tumors. Besides its putative tumor suppressor function in skin, loss of HR function has also been implicated to increase leukemia susceptibility and promote the growth of melanoma and brain cancer cells. HR has also been demonstrated to function as a histone H3 lysine 9 (H3K9) demethylase. Recent genomics studies have identified HR mutations in a variety of human cancers including breast cancer. The anticancer function and mechanism of action by HR in mammary tissue remains to be investigated. Here, we review the emerging role of HR, its histone demethylase activity and histone methylation in breast cancer development and potential for epigenetic therapy.

Keywords Breast cancer, histone methylation, histone demethylase, Hairless, oncogenesis
Introduction

According to the latest data from the International Agency for Research on Cancer, breast cancer surpassed lung cancer in 2020 to become the most commonly diagnosed cancer worldwide. In the US alone, approximately 42,000 women die from breast cancer each year (1). Despite decades of intense research, significant gaps exist in our understanding of breast cancer etiology and associated genetic and epigenetic defects, which hinders breast cancer treatment progress (2-4). This is partly due to the fact that breast cancer is a highly heterogeneous disease, encompassing many subtypes that have distinct origins, behaviors and prognoses (5). Four major subtypes of breast cancer have been described including basal-like, luminal A, luminal B, and HER2+ breast cancer, while additional subtypes may exist (6,7). These subtypes differ significantly in their origins, behavior, and prognoses (8), underscoring the importance of precision medicine in guiding individualized treatment to improve patient survival and quality of life.

Cancer genetics and genomics studies have identified many high-risk breast cancer predisposing genes among familial breast cancer cases such as BRCA1 and BRCA2 and medium- to low-risk genes such as CHEK2, ATM, PALB2, BRIP1, TP53, PTEN, CDH1, and MLL3 (9,10). However, only about 10% of breast cancer patients have a clear family history that can be linked to known pathogenic mutations in these predisposition genes, while the majority of breast cancers are thought to occur sporadically with undefined causes (9). A major challenge facing breast cancer precision medicine is the lack of comprehensive understanding of breast cancer-associated genetic and non-genetic factors. Additional etiological factors that promote the initiation, growth and progression of both familial and sporadic breast cancers remain to be identified. Such knowledge will enable targeted therapeutics directed at the specific mechanisms underpinning each breast cancer subtype.
Breast cancer research has focused extensively on genetic alterations. There is growing interest and emphasis on elucidating the role of epigenetic alterations in breast cancer, which may provide new mechanistic insights into breast cancer pathogenesis (11). Genetic mutations affecting the enzymatic activity of epigenetic regulators, such as DNA methyltransferases and histone modifying enzymes, have been linked with cancer and other developmental disorders (12-14). Epigenetic regulators also modulate the interaction between genes and the environment to influence disease pathogenesis, thus carrying a great potential for addressing the missing heritability of breast cancers with environmental origins. More importantly, epigenetic alterations are often reversible, providing unique opportunities for cancer epigenetic therapies. Indeed, preclinical and clinical testing of inhibitors of epigenetic regulators demonstrates that such epigenetic drugs are effective when used alone or in combination with other therapies (15-21). However, mechanistic insights concerning the cause or consequence of epigenetic alterations in cancer are still limited. More studies are needed to determine how epigenetic regulators contribute to breast cancer development to understand the role of epigenetic abnormalities in breast cancer.

Here, we will review the mounting evidence supporting a versatile role of histone methylation in breast cancer development. We will discuss the function of the *hairless* (*HR*) gene, which encodes an epigenetic regulator with histone H3K9 demethylase activity, and its transcriptional regulatory and tumor suppressive activities in breast cancer. We will also discuss the therapeutic potential of targeting histone methylation as a new epigenetic therapy for breast cancer treatment.
Epigenetic regulators and histone methylation in cancer development

Eukaryotic DNA is packaged into chromatin. The repeating unit of chromatin is the nucleosome, which contains 145-147 base pairs of DNA wrapped around core histones (H2A, H2B, H3 and H4). Chromatin is further condensed into high-order chromosomes, a process that is believed to impede gene transcription by default. Genes located within highly condensed chromatin regions are often transcriptionally inactive and require chromatin remodeling to alter chromatin conformation and facilitate gene activation (22). The mammalian genome encodes multiple epigenetic regulators, including DNA methyltransferases and histone modifying enzymes, which modulate gene expression epigenetically by altering the chromatin conformation to either promote or suppress gene transcription (23,24).

Histone C-terminal tails often undergo post-translational modifications that can dynamically influence chromatin conformation and gene transcription, DNA replication and repair (13,25). Post-translational modification of histones by acetylation or methylation plays an essential role in development and tissue homeostasis (26,27). Acetylation of lysine residues in core histones produces a negative charge, resulting in a less tight interaction between DNA and histones to facilitate transcription. Histone methylation, on the other hand, does not alter the overall charge of the affected residues. Instead, histone methylation marks are recognized by a variety of effector protein factors (reader proteins) to modulate gene expression and activity.

Histone methylation usually occurs on basic residues including arginines, lysines and histidines, although histidine methylation is thought to be rare and has not been well
characterized compared to lysine or arginine methylation (28). Lysine and arginine residues both contain amino groups that confer basic and hydrophobic characteristics. Lysine can be mono- (me1), di- (me2), or tri- (me3) methylated with a methyl group replacing each hydrogen of its ε-NH3+ group. On the other hand, arginine can be mono- or di-methylated on its NH2+ group (29). Arginine methylation is catalyzed by protein arginine methyltransferase, while lysine methylation involves specific lysine methyltransferases (KMT) that often contain an evolutionarily conserved SET domain (30). The most extensively studied histone lysine methylation sites include H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79, and H4K20 (31). The different forms and sites of histone lysine methylation occur in spatiotemporal-specific manners, which exert diverse effects on gene regulation in context-dependent manners (32).

Unlike the relatively straightforward functional relationship between histone acetylation and gene activation, the role of histone methylation in gene regulation is complex. Some individual methylation modifications can have different roles (activating or silencing) depending on the genetic context (32-34). H3K9me1, for example, is found to be associated with both actively transcribed and repressed genes (7,32); whereas H3K9me2 and H3K9me3 are often associated with transcriptional repression or heterochromatin (26,32,35). It is possible that histone methylation serves as the chromatin marks, which influence genome organization and gene activity in the presence of other reader proteins including transcription factors. Readers of lysine methylation and epigenetic changes interpret the modifications in a context dependent fashion, and binding of histone readers to their target post-translational modifications is influenced by interactions with the surrounding residues (36,37).
Based on the number of total lysines in the core histones H3 and H4, which can each exist in un-, mono-, di- or tri-methylated forms, there are potentially billions of possible lysine methylation patterns in the human genome. The functional implications of such complex histone methylation patterns in development and human diseases are poorly understood. Extensive efforts are required to elucidate the importance of highly conserved patterns of histone modifications. Based on the prevalence of lysine methylation and their role in gene regulation, it is predictable that genetic mutations affecting the genes encoding the enzymes regulating lysine methylation have profound impact on development and disease pathogenesis (12,14,38,39).

**Histone H3K9 methyltransferases and demethylases**

Several H3K9 methyltransferases have been identified in mammalian cells, including SUV39H1/H2 (KMT1A/1B), G9A (KMT1C), G9A-like protein (GLP, or KMT1D), SETDB1/2 (KMT1E/1F), and PR domain containing protein family members (PRDM3/16). SUV39H1/H2 catalyze H3K9me2/me3, which are often found in heterochromatin or transcriptionally silent regions (40). SETDB1 catalyzes H3K9me1 at the pericentromeric region and provides a substrate for SUV39H1/H2 to produce H3K9me3 (41). The heterodimer of G9A and GLP is the major KMT that catalyzes H3K9me1/me2 in euchromatin regions (42). *In vitro* studies suggest that G9A or GLP homodimers also exhibit H3K9me1/me2 activity (43). PRDM3/16 are known to exhibit H3K9me1 activity (44), but whether PRDM protein-mediated H3K9 methylation is direct or indirect awaits further experimental verification.

Previously, lysine methylation was considered to be a permanent and irreversible modification due to the high thermodynamic stability of the N-CH₃ bond (25). However, this view has changed since the discovery of histone demethylase
enzymes. Dozens of lysine demethylases (KDMs) have been reported to date (45-47), which are classified into two main groups: the amine-oxidase type lysine-specific demethylases (LSDs) and the highly conserved Jumonji C (JmjC) domain-containing histone KDMs. LSD1 and LSD2 (KDM1A/B) are flavin adenine dinucleotide (FAD)-dependent amine oxidases that can demethylate mono- or dimethylated H3K4/K9. The JmjC KDMs, on the other hand, can remove methyl groups from all three lysine methylation states. The human genome encodes more than 30 JmjC KDMs including KDM3 (JHDM2), KDM4 (JHDM3), KDM6a (UTX), and KDM7A/B (KIAA1718/PHF8). Three KDM3 family proteins (KDM3A–C) can demethylate H3K9me1/me2 and regulate hormone-dependent transcriptional activation (48,49). KDM4 family proteins can demethylate H3K9me2 and H3K9me3 in addition to H3K36me2 and H3K36me3 (48,49). Both KIAA1718 and PHF8 harbor a plant homeodomain that binds H3K4me3 and demethylates either H3K9me2 or H3K27me2. The presence of H3K4me3 on the same peptide as H3K9me2 makes the doubly methylated peptide a better substrate of PHF8 while diminishing the H3K9me2 demethylase activity of KIAA1718 without adversely affecting its H3K27me2 activity (50). PHF8 depletion also leads to increased H4K20me1/H3K9me1 at transcription start sites (TSS) and H3K9me2 at non-TSS sites, respectively, suggesting differential substrate specificities at different target locations.

Both KMTs and KDMs have been implicated in oncogenesis. These enzymes may be either overexpressed or inactivated in different cancer types (51,52). Genetic mutations affecting the enzymatic activity of KMTs or KDMs have been linked with cancer and other developmental disorders (12-14). The effect of alteration in each
specific KMT/KDM depends on the type of cancer involved. As the first KDM identified, LSD1 has been extensively studied. It can both activate and repress gene expression. Similarly, LSD1 can exhibit either pro-tumor or anti-tumor activity in breast cancer development (53, 54), highlighting a context-dependent role in regulating different biological processes possibly by using different functional domains. The potential role of different KMTs and KDMs in breast cancer is summarized in Table 1 based on previous studies detailed in Cheng and colleagues’ 2019 review (55).

Furthermore, genomic histone methylation levels have been associated with clinical outcomes and progression of cancers (51). For example, immunohistochemical analyses have shown that low levels of H3Kme2 are associated with decreased survival in lung and renal carcinoma (56). Other cancers with altered levels of global histone modifications include prostate (57), lung (56), kidney (56), pancreas (58), hepatocellular carcinoma (59), and breast (60) among others. Different cancers can have opposite alterations in histone modifications; H3K9me3 is increased in gastric adenocarcinomas but decreased in prostate cancer compared to normal tissue (51), which highlights the complex function of histone methylation in cancer development. It has been proposed that the patterns of histone methylation may provide prognostic information. In addition to the global level of histone methylation and alterations in KMT and KDM activity, reader proteins of histone lysine methylation (effector proteins) have also been implicated in development of various cancers (51), which will not be discussed in this review.
Transcriptional regulation by hairless

The HR gene encodes a transcription factor that regulates multiple pathways involved in cell proliferation, apoptosis, and inflammation. The HR gene structure and function are highly conserved between human, rat, and mouse, containing a nuclear localization signal and a zinc finger domain for DNA binding (Figure 1A) (61,62). HR is essential for normal skin development and hair follicle cycling. (63,64) Both humans and rodents with mutations in HR suffer from congenital hair loss and epidermal abnormalities. (65,66) Mice with loss of function Hr mutations develop irreversible hair loss around postnatal day 18, followed by epidermal hyperplasia and hyperkeratosis (67-69). Intriguingly, re-expressing Hr in Hr-null mice can restore hair growth (70), providing strong evidence that Hr is necessary and sufficient for re-initiation of hair growth. Human patients with atrichia with papular lesions (APL) harbor inactivating mutations in HR and exhibit similar skin and hair disorders (64,71). APL patients initially have normal hair growth after birth, but the hair sheds within a few years and does not grow back (72-74). The hairless phenotype is attributed to defective proliferation and migration of the hair follicle stem cells, which fail to respond to various signaling molecules in the absence of HR function (75).

As a transcription factor, HR exerts its effects on gene transcription regulation though several mechanisms. The HR protein contains four motifs consisting of hydrophobic amino acids (two LXXLL where L is leucine and X is any amino acid; two form ØXXØØ motifs where Ø can be leucine, isoleucine, or valine) (61,62), which mediates its interactions with other proteins (Figure 1A). HR has been shown to interact with various steroid receptors including vitamin D receptor (VDR), thyroid hormone receptor (TR), and receptor-related orphan receptors (ROR) (69). Breast cancers are known to express VDR and TR, thus the interaction between HR and steroid receptors provides a potential role for HR in regulating
breast cancer pathogenesis. VDR is expressed in most breast cancer cell lines (76), and an upregulation of VDR protein in breast carcinomas in comparison to healthy breast tissue (77). It has been suggested that expression of VDR in breast tumors may be a prognostic factor, with increased levels of VDR associated with lower risk of death (78,79). TR is also expressed in breast carcinomas and has been associated with prognosis. Jerzak and colleagues examined expression of thyroid hormone receptor alpha (THRα1 and THRα2) in samples of patients with breast cancer and found high expression of THRα1 and THRα2 in 74% and 40% of samples, respectively. High THRα2 expression was associated with improved overall survival (80). In rodents, the interaction between HR and TR occurs strongly in the absence of thyroid hormone, and HR was shown to repress the transactivation activity of TRα and TRβ (61,81,82).

Other KDMs including KDM8, KDM2A, and JMJD3 interact with the nuclear hormone receptors that are expressed in breast tumors. For example, KDM8 is a H3K36me2 demethylase that is involved in the regulation of tumor metabolism and cell cycle in breast cancer cells (83). KDM8 interacts with the androgen receptor activating the AR response in the absence of androgen (84). Recent evidence has been emerging surrounding the positive role of androgens in breast cancer treatment for women with estrogen-receptor positive metastatic disease. Hickey and colleagues showed that AR activation lead to potent antitumor activity in multiple scenarios including resistance to ER and CDK4/6 inhibitors (85). JMJD3 is a H3K27 demethylase involved in estrogen signaling (86). JMJD3 has been associated with breast cancer progression. Xun et al. showed that ectopic expression of JMJD3 suppresses the stem cell-like characteristics of breast cancer cells (87). Taken together, HR and other KDMs may regulate breast cancer development and progression via their interactions with nuclear hormone receptors.
Additional to the steroid receptors, other interacting partners of HR include TP53, RBP3, and histone deacetylase (HDAC) 1-3 based on the STRING Protein Interaction database (Figure 1B) (88). There is clear evidence showing that HR interacts with HDACs, which partly accounts for its role in transcriptional repression (62,89-91). A recent study shows that HR recognizes and binds to TP53-resposne elements, therefore regulating several key TP53 target genes (PUMA, GADD45A, CDKN1A) and downstream effectors (BIRC5 and STMN1) (88,92). These findings suggest a possible crosstalk between HR and p53 tumor suppressor activities. Lastly, the HR protein exhibits KDM activity that can modulate gene activity epigenetically, which is discussed in detail below (93).

The HR protein contains a JmjC domain, a highly conserved motif among over 30 human proteins that catalyze demethylation of histones through an oxidative reaction using iron and alpha-ketoglutarate as cofactors (93,94). HR contains an atypical JmjC domain and is structurally related to KDM3A (48). The metal-binding motifs of KDM3A and HR differ only by substitution of a cysteine residue for the first histidine in the HR JmjC domain. In vitro demethylation assays showed that partial HR protein containing the JmjC domain can demethylate H3K9me1 in a dose-dependent manner, but with a weaker effect on H3K9me2 and no effect on H3K9me3 or other methylated histone substrates (93). Additional demethylation experiments showed that full-length HR can reduce the level of H3K9me1 and H3K9me2 in total native histones purified from HeLa cells. When human HeLa cells were transfected with either wild-type HR or HR with mutations in the metal-binding motif, immunofluorescence studies showed that expression of the mutant HR had no effect on H3K9 methylation while wild-type HR led to a dramatic loss of H3K9 methylation (93), confirming the H3K9 KDM activity of HR in human cells. To determine if missense mutations in the HR JmjC domain in APL patients could alter the HDM activity of HR, we introduced single-point mutations in the HR JmjC domain. In vitro demethylation analysis showed that two of the APL patient mutations in the HR JmjC domain (D1012N and V1056M, Figure 1A)
significantly reduced its H3K9 demethylase activity, suggesting that the KDM activity of HR may be linked to the clinical manifestation of APL patients. Taken together, these findings provided strong evidence that HR demethylase activity is necessary for hair cycling and skin homeostasis.

HR contains a zinc-finger domain that is highly conserved among mammalian species (95). Comparison with other zinc-finger proteins showed that this motif is similar to the GATA transcription factors, and thus it was proposed that HR may have DNA binding ability (75). By ChIP-seq analysis, we identified approximately 46 genomic regions that HR directly interacts with in human HEK293 kidney cells. The identified HR target genes cluster into 3 major functional categories, including hair biology, neural activity, and oncogenesis, which are consistent with the predicted biological functions of HR. Among these newly identified targets, HR may regulate cell growth, migration and survival through COL25A1, COL6A1, CSNK2A2, DLEC1, FADD, FGF13, GPD1L, PREX2 and PVT1 (96). Demethylation of H3K9me1 often leads to gene repression, whereas demethylation of H3K9me2 leads to gene activation, consistent with the bi-modal transcriptional regulation by HR in gene-specific or context-dependent manners (67,96,97).

**HR mutations and H3K9 methylation alterations in cancer development**

There are several naturally occurring loss-of-function Hr-mutant mouse strains including the albino SKH1 mice, the Hr/hr mice, and the Hr/hr-J mice (68). Loss of HR function in SKH1 and Hr/hr mice, is due to a proviral insertion into Hr exon 6 (98,99), and due to missense Hr mutations in Hr/hr-J mice (100,101). Iversen and colleagues first showed that Hr/hr mice had increased skin tumor incidence compared to wild-type littermates when exposed to a
chemical carcinogenesis protocol (102). Recent studies from our lab and others have confirmed that Hr deficiency alone drastically increases skin cancer susceptibility in response to UV irradiation or topical treatment with carcinogen 7,12-dimethylbenz(a)anthracene (103-105). While previous studies on HR have mostly focused on understanding its role in skin development and hair follicle cycling, HR is widely expressed in other tissues including the brain and mammary tissues where its function is unknown (75). Cancer genomics studies have revealed HR somatic alterations in a variety of human cancers. Surveying different cancer genomics datasets including The Cancer Genome Atlas, International Cancer Genome Consortium, the Catalog of Somatic Mutations in Cancer and cBioPortal databases identified approximately 200 missense mutations across the HR gene locus in skin, colon, stomach, brain, breast, prostate, ovaries, and uterus among other tissues (106-110). Importantly, a subset of the missense mutations is common among different cancer types (Figure 1B).

Interrogation of cancer genomics datasets also revealed prevalent HR deletion or mutations in breast cancers (Figure 2A). Of 816 cases of breast cancer, 49 (~6%) of cases of invasive tumors harbored HR deletions or missense mutations (111). Multiple recurring HR mutations have been identified in human cancers, including G337D, R750Q, R927C, and P1046L in invasive breast tumors (Figure 2B). In addition to somatic mutations, copy number analysis of HR by digital PCR identifies frequent HR deletions in human cutaneous squamous cell carcinomas (Liu et al., unpublished). Consistent with this observation, querying the Cancer Cell Line Encyclopedia mutation database revealed that 17 out of 59 human breast cancer cell lines harbor HR gene copy number loss (Table 2). Most of these HR-deficient cell lines are derived from HER2+ or basal-like subtypes based on the PAM50 gene signature (112,113), highlighting that loss of HR may be linked to the development of aggressive breast cancer subtypes. Additional to mutations and deletions, surveying TCGA breast
cancer datasets also reveals a significant decreases of HR mRNA expression in luminal/HER2+ positive breast tumors (data not shown).

Despite the strong evidence suggesting that mutational inactivation of HR promotes tumorigenesis, the mechanism underlying the putative tumor suppressor function of HR is not well understood. In skin carcinogenesis, Kim et al reported that there is a constitutive NF-κB activation in Hr-mutant epidermis, leading to activation of several downstream effectors including cyclin D1, cyclin E, Bcl-2, and the pro-inflammatory protein Cox-2 that contribute to uncontrolled epidermal proliferation and promote skin tumor development (103). It is proposed that HR is a crucial UV response gene and its loss creates a permissive environment for skin tumorigenesis (103). The human HR gene is located on chromosome 8p, a region that is frequently lost in breast and other cancer types (114). Analysis of the genes near the HR locus in the affected chromosome 8p region suggests no other known tumor suppressor genes except HR (unpublished observations). As mentioned above, the interaction between HR and TP53 may be one of the mechanisms by which HR suppresses cancer development, although further research is needed to elucidate the underlying mechanism and pathway (115).

Based on its activity in H3K9 demethylation, it is possible that loss of HR function perturbs genomic histone methylation, although no studies have explored this important epigenetic pathway in the tumor suppressive function of HR. As described above, G9A and GLP are the major KMTs that catalyze H3K9me1 and H3K9me2. Consistent with their opposite activities in H3K9 methylation, G9A is implicated to function as an oncogene whereas HR is implicated to function as a tumor suppressor gene. G9A is aberrantly upregulated in various human cancers and is found to be associated with enhanced cancer cell proliferation and metastasis. In addition to its role in H3K9 methylation, G9A can also methylate the TP53
protein and exhibit a negative regulation of UHRF1 and JAK2 transcription (116,117). In breast cancer cells, Yang et al reported a novel mechanism by which G9A regulates breast cancer growth by modulating iron homeostasis through the repression of ferroxidase hephaestin (HEPH) activity (118). Although G9A/GLP and HR all target H3K9me1/me2, it remains to be determined whether and how many common target genes they share in the genome.

**Therapeutic targeting of H3K9 methylation in breast cancer**

Dysregulation of KMTs or KDMs leads to altered histone methylation patterns, which may promote cancer pathogenesis via dysregulation of oncogenes and tumor suppressor genes to cause uncontrolled cell proliferation, invasion and metastasis. Analyses of the large genomic datasets have shown that KMTs and KDMs are often mutated in cancer and represent 5% of the driver mutations identified in breast cancer (119). Acquired resistance to cancer treatment has also been associated with increased KMT expression (120,121). Thus, it has been suggested that KMTs may serve as potential biomarkers or therapeutic targets for cancer patients. Among the breast cancer subtypes, basal-like breast cancers have the highest frequency of KMT mutations, amplifications, or deletions, while luminal A tumors have the lowest mutation frequency in KMT genes (122).

Epigenetic alterations are often reversible, providing unique opportunities for cancer epigenetic therapies by using various drugs to target epigenetic regulators. 5-azaC (Vidaza) and 5-aza-dC (Dacogen), which are inhibitors of DNA methyltransferases, are among the first epigenetic drugs applied to treat blood cancers (myelodysplastic syndrome and myelomonocytic leukemia). Shortly after, HDAC inhibitors SAHA (Zolinza) and romidepsin
(Istodax) received FDA approval for treating cutaneous or peripheral T cell lymphoma patients. While 5-azaC and 5-aza-dC are used as first-line chemotherapy for the treatment of leukemia patients, vorinostat and romidepsin are largely restricted for treatment of T cell lymphoma refractory to other therapies (123). Following this initial success, over 20 epigenetic drugs have entered clinical testing and validation, which led to approvals of three HDAC inhibitors (belinostat, panobinostat, and chidamide) (124). In 2015, panobinostat (Farydak) received FDA approval for patients with refractory multiple myeloma that do not respond to other treatments. A major progress in cancer epigenetic therapy is success with the combination of epigenetic therapy with immunotherapy that produced promising potential in treating solid tumors including advanced breast cancer and metastatic lung cancer (125,126).

The success with epigenetic drugs targeting DNA methylation and histone acetylation has sparked widespread interest and efforts to explore epigenetic writers, erasers and readers involved in histone methylation as drug targets. There are over 100 KMTs in human cells that use S-adenosyl methionine (SAM) as the substrate for methylation reactions. This offers a conceptual basis for KMT inhibitor design by using potent SAM-mimetics. Tazemetostat (Tazverik) is an orally available, small molecule selective inhibitor of EZH2, a H3K27 KMT. Activating mutations in EZH2 are found in human cancer patients (127,128). Tazemetostat was first approved by the FDA for the treatment of metastatic or locally advanced epithelioid sarcoma in January 2020. In June 2020, the FDA granted accelerated approval to tazemetostat for treatment of adult patients with relapsed or refractory follicular lymphoma whose tumors are positive for EZH2 mutations. Of note, prolonged EZH2 inhibition has been associated with tumor progression, therefore further studies should be conducted to understand the dual role of the drug in cancer growth and progression (129). Pinometostat is a first-in-class small-molecule inhibitor of DOT1L, a H3K79 KMT, and has been shown in
phase I clinical study to exhibit therapeutic potential for targeting DOT1L in genetically defined acute leukemia with recombinant MLL (130).

In addition to DOT1L and EZH2, G9A has also been actively explored as an epigenetic drug target due to its frequent upregulation in multiple human cancers. In a xenograft mouse model, knockdown of G9A suppressed breast tumor cell growth (131). Further, silencing of G9A led to re-expression of cell-adhesion molecules such as E-cadherin (132), suggesting that G9A may regulate the epithelial-to-mesenchymal transition to promote breast cancer progression. Paradoxically, G9A has been shown to function as a coactivator for p21 transcription to trigger apoptosis (133). This is consistent with the observations that down-regulation of G9A in breast cancer tissue is associated with breast cancer progression and metastasis (134), suggesting that G9A may also exhibit tumor suppressive activity in certain settings. Further studies on G9A are necessary to understand its context-dependent role in breast cancer development and progression.

There are at least 8 different compounds that have been developed and tested to inhibit G9A and H3K9 methylation in human cells (Table 3). BIX-01294 is the first selective small molecule inhibitor of G9A discovered by high-throughput screening of 125,000 preselected compounds (43). The mechanism of action by BIX-01294 is through its competitive binding to the enzyme against the lysine substrate (135). BIX-01294 exhibits relatively high toxicity, which prompted further studies to develop other selective small molecule inhibitors with less toxicity for In vivo studies. Among them, UNC0642 exhibits both a high selectivity and potency in inhibiting G9A and reducing cellular H3K9me1/me2 levels in cell-based assays, while having excellent In vivo pharmacokinetic properties that enable preclinical testing in animal studies (136). Mechanistically, UNC0642 also functions as a substrate competitive inhibitor with a high selectivity for G9A and GLP over dozens of other KMTs (136). Despite
these progresses, the small molecule inhibitors of G9A and H3K9 methylation have not reached the stage of clinical testing.

Recently, a hybrid KDM inhibitor (MC3324) was developed as a pan-KDM inhibitor by coupling the chemical properties of tranylcypromine, a LSD1 inhibitor, with the 2-OG competitive moiety developed for JmjC KDM inhibition (137). Benedetti et al. showed that MC3324 induced significant growth arrest and apoptosis of ER-positive breast cancer cells that was associated with significant increases in H3K4me2 and H3K27me3 (138). The antitumor action of MC3324 is linked to its ability to epigenetically down-regulate ER and ER-responsive genes in breast cancer cells. Benedetti et al. further demonstrated a tumor-selective potential of MC3324 in both xenograft mouse and chicken embryo models, with insignificant toxicity and good oral efficacy (138). This hybrid pan-KDM inhibitor represents an exciting step in developing effective and selective epigenetic drugs for breast cancer treatment. Additionally, there are ongoing studies exploring engineered DNA binding domains to achieve selective targeting of specific genomic loci such as regulatory elements and non-coding genes. Engineered zinc-finger protein repressors have been shown to epigenetically silence oncogenes and decrease growth of breast cancer cells both in vitro and in mouse models (139,140).

**Conclusions**

The hallmarks of sporadic breast cancer include both genetic mutations and epigenetic alterations in DNA methylation and histone modifications. Compared to DNA methylation and histone acetylation, histone methylation is much more complex and involves a significantly large number of factors that write, erase, and read the histone methylation marks to modulate genome organization and gene activity. Many KMTs and KDMs appear to have bi-modal activities in either promoting or suppressing gene transcription in context-
dependent manners. Not surprisingly, some of the well-studied KMTs and KDMs, such as LSD1 and G9A, have been implicated as oncogenes in certain settings but tumor suppressors in other settings. Despite the opposite roles of KMTs and KDMs in writing and erasing histone methylation marks, small molecular inhibitors targeting both KMTs and KDMs are the major focus of current epigenetic drug development for cancer therapy. Many of the drugs developed to date show some promising potential in cell-based assays and preclinical models, but they remain to enter clinical testing. A better understanding of the specific mechanisms underlying epigenetic alterations will likely facilitate precision targeting of the driver epigenetic factors and pathways to improve therapeutic efficacy. We also discussed the function and mechanism of HR, an atypical JmjC KDM targeting H3K9me1/me2. There is strong evidence that HR functions as a tumor suppressor in skin tumorigenesis, but HR function in breast cancer has not been reported. Preliminary studies in our lab suggest a strong link of HR dysregulation to breast cancer development. Future studies are necessary to characterize the role of HR mutation and H3K9 demethylation in breast cancer pathogenesis, tumor growth and progression. These studies will offer new insights into how genetic and epigenetic alterations cooperate to drive breast cancer development and may enable epigenetic therapy by targeting the H3K9 methylation pathway in breast cancer treatment.
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Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References.
References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *Ca-Cancer J Clin.* 2020;70(1):7-30.
2. Turashvili G, Brogi E. Tumor Heterogeneity in Breast Cancer. *Front Med (Lausanne).* 2017;4:227.
3. Ellsworth RE, Blackburn HL, Shriver CD, Soon-Shiong P, Ellsworth DL. Molecular heterogeneity in breast cancer: State of the science and implications for patient care. *Semin Cell Dev Biol.* 2017;64:65-72.
4. Harris EER. Precision Medicine for Breast Cancer: The Paths to Truly Individualized Diagnosis and Treatment. *Int J Breast Cancer.* 2018;2018:4809183.
5. Dai X, Cheng H, Bai Z, Li J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *J Cancer.* 2017;8(16):3131-3141.
6. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Storløkken K, Perou CM. Molecular portraits of human breast tumours. *Nature.* 2000;406(6797):747-752.
7. Michalak EM, Visvader JE. Dysregulation of histone methyltransferases in breast cancer - Opportunities for new targeted therapies? *Mol Oncol.* 2016;10(10):1497-1515.
8. Baslan T, Kendall J, Volyanskyy K, McNamara K, Cox H, D'Italia S, Ambrosio F, Riggs M, Rodgers L, Leotta A, Song JY, Mao Y, Wu J, Shah R, Gularte-Merida R, Chadalavada K, Nanjungud G, Varadan V, Gordon A, Curtis C, Krasnitz A, Dimitrova N, Harris L, Wigler M, Hicks J. Novel insights into breast cancer copy number genetic heterogeneity revealed by single-cell genome sequencing. *Elife.* 2020;9.
9. Ellis MJ, Perou CM. The Genomic Landscape of Breast Cancer as a Therapeutic Roadmap. *Cancer Discovery.* 2013;3(1):27-34.
10. Apostolou P, Fostira F. Hereditary Breast Cancer: The Era of New Susceptibility Genes. *Biomed Res Int.* 2013.
11. Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. *Science.* 2017;357(6348).
12. Albert M, Helin K. Histone methyltransferases in cancer. *Semin Cell Dev Biol.* 2010;21(2):209-220.
13. Pedersen MT, Helin K. Histone demethylases in development and disease. *Trends Cell Biol.* 2010;20(11):662-671.
14. D’Oto A, Tian Q-W, Davidoff AM, Yang J. Histone demethylases and their roles in cancer epigenetics. *J Med Oncol Ther.* 2016;1(2):34-40.
15. Jones PA, Ohtani H, Chakravarty A, De Carvalho DD. Epigenetic therapy in immunoncology. *Nat Rev Cancer.* 2019;19(3):151-161.
16. Bates SE. Epigenetic Therapies for Cancer. *N Engl J Med.* 2020;383(7):650-663.
17. Kim Y, Lee HM, Xiong Y, Sciakey N, Hulbert SW, Cao X, Everitt JI, Jin J, Roth BL, Jiang YH. Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of Prader-Willi syndrome. *Nat Med.* 2017;23(2):213-222.
18. Kaniskan HU, Konze KD, Jin J. Selective inhibitors of protein methyltransferases. *J Med Chem.* 2015;58(4):1596-1629.
19. Rowbotham SP, Li F, Dost AFM, Marsh BP, Pessina P, Anbarasu CR, Brainson CF, Tuminello SJ, Lieberman A, Ryeom S, Schlaeger TM, Aronow BJ, Watanabe H, Wong KK, Kim CF. H3K9 methyltransferases and demethylases control lung tumor-propagating cells and lung cancer progression. *Nat Commun.* 2018;9(1):4559.

20. Cao YP, Sun JY, Li MQ, Dong Y, Zhang YH, Yan J, Huang RM, Yan X. Inhibition of G9a by a small molecule inhibitor, UNC0642, induces apoptosis of human bladder cancer cells. *Acta Pharmacol Sin.* 2019;40(8):1076-1084.

21. McCabe MT, Mohammad HP, Barbash O, Kruger RG. Targeting Histone Methylation in Cancer. *Cancer J.* 2017;23(5):292-301.

22. Jenuwein T, Allis CD. Translating the histone code. *Science.* 2001;293(5532):1074-1080.

23. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell.* 2007;128(4):669-681.

24. Chi P, Allis CD, Wang GG. Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer.* 2010;10(7):457-469.

25. Cloos PA, Christensen J, Agger K, Helin K. Erasing the methyl mark: histone demethylases at the center of cellular differentiation and disease. *Genes Dev.* 2008;22(9):1115-1140.

26. Pedersen MT, Helin K. Histone demethylases in development and disease. *Trends Cell Biol.* 2010;20(11):662-671.

27. Feinberg AP, Koldobskiy MA, Gondor A. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nat Rev Genet.* 2016;17(5):284-299.

28. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet.* 2012;13(5):343-357.

29. Blanc RS, Richard S. Arginine Methylation: The Coming of Age. *Mol Cell.* 2017;65(1):8-24.

30. Green JP, Karras DJ. Update on Emerging Infections: News From the Centers for Disease Control and Prevention. *Annals of Emergency Medicine.* 2012;59(1):53-54.

31. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. *Exp Mol Med.* 2017;49(4):e324.

32. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell.* 2007;129(4):823-837.

33. Kouzarides T. Chromatin modifications and their function. *Cell.* 2007;128(4):693-705.

34. Vakoc CR, Sachdeva MM, Wang H, Blobel GA. Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol Cell Biol.* 2006;26(24):9185-9195.

35. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. *Genes Dev.* 2011;25(8):781-788.

36. Yun M, Wu J, Workman JL, Li B. Readers of histone modifications. *Cell Research.* 2011;21(4):564-578.

37. Musselman CA, Lalonde M-E, Côté J, Kutateladze TG. Perceiving the epigenetic landscape through histone readers. *Nature structural & molecular biology.* 2012;19(12):1218-1227.

38. Raghuraman S, Donkin I, Versteyhe S, Barres R, Simar D. The Emerging Role of Epigenetics in Inflammation and Immunometabolism. *Trends Endocrin Met.* 2016;27(11):782-795.
39. Ballestar E, Li TL. New insights into the epigenetics of inflammatory rheumatic diseases. *Nat Rev Rheumatol*. 2017;13(10):593-605.

40. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature*. 2001;410(6824):120-124.

41. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, Nakatani Y, Dent SY, Almouzni G. The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep*. 2009;10(7):769-775.

42. Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, Fukuda M, Takeda N, Niida H, Kato H, Shinkai Y. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev*. 2002;16(14):1779-1791.

43. Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, Rea S, Mechtler K, Kowalski JA, Homon CA, Kelly TA, Jenuwein T. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell*. 2007;25(3):473-481.

44. Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritsch C, Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M, Son J, Reuber D, Lachner M, Jenuwein T. Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. *Cell*. 2012;150(5):948-960.

45. Cloos PA, Christensen J, Agger K, Maiolica A, Rappilber J, Antal T, Hansen KH, Helin K. The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature*. 2006;442(7100):307-311.

46. Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet*. 2006;7(9):715-727.

47. Klose RJ, Yamane K, Bae Y, Zhang D, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature*. 2006;442(7100):312-316.

48. Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell*. 2006;125(3):483-495.

49. Okada Y, Scott G, Ray MK, Mishina Y, Zhang Y. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. *Nature*. 2007;450(7166):119-+.

50. Horton JR, Upadhyay AK, Qi HH, Zhang X, Shi Y, Cheng X. Enzymatic and structural insights for substrate specificity of a family of jumonji histone lysine demethylases. *Nat Struct Mol Biol*. 2010;17(1):38-43.

51. Varier RA, Timmers HT. Histone lysine methylation and demethylation pathways in cancer. *Biochim Biophys Acta*. 2011;1815(1):75-89.

52. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, Han J, Wei X. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther*. 2019;4:62.

53. Fang Y, Liao G, Yu B. LSD1/KDM1A inhibitors in clinical trials: advances and prospects. *J Hematol Oncol*. 2019;12(1):129.

54. Hu X, Xiang D, Xie Y, Tao L, Zhang Y, Jin Y, Pinello L, Wan Y, Yuan GC, Li Z. LSD1 suppresses invasion, migration and metastasis of luminal breast cancer cells via
activation of GATA3 and repression of TRIM37 expression. Oncogene. 2019;38(44):7017-7034.

55. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, Han J, Wei X. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Signal Transduct Target Ther. 2019;4:62-62.

56. Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, Tze S, Wang Q, Chia D, Goodglick L, Kurdistani SK. Global levels of histone modifications predict prognosis in different cancers. Am J Pathol. 2009;174(5):1619-1628.

57. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdistani SK. Global histone modification patterns predict risk of prostate cancer recurrence. Nature. 2005;435(7046):1262-1266.

58. Manuyakorn A, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Guha C, Dawson DW. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. J Clin Oncol. 2010;28(8):1358-1365.

59. Magerl C, Ellinger J, Braunschweig T, Kremmer E, Koch LK, Höller T, Büttner R, Lüscher B, Güttgemann I. H3K4 dimethylation in hepatocellular carcinoma is rare compared with other hepatobiliary and gastrointestinal carcinomas and correlates with expression of the methylase Ash2 and the demethylase LSD1. Hum Pathol. 2010;41(2):181-189.

60. Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, Soria D, Garibaldi JM, Paish CE, Ammar AA, Grainge MJ, Ball GR, Abdelghany MK, Martinez-Pomares L, Heery DM, Ellis IO. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. Cancer Res. 2009;69(9):3802-3809.

61. Potter GB, Beaudoin GM, 3rd, DeRenzo CL, Zarach JM, Chen SH, Thompson CC. The hairless gene mutated in congenital hair loss disorders encodes a novel nuclear receptor corepressor. Genes Dev. 2001;15(20):2687-2701.

62. Thompson CC. Hairless is a nuclear receptor corepressor essential for skin function. Nucl Recept Signal. 2009;7:e010-e010.

63. Potter GB, Beaudoin GM, DeRenzo CL, Zarach JM, Chen SH, Thompson CC. The hairless gene mutated in congenital hair loss disorders encodes a novel nuclear receptor corepressor. Gene Dev. 2001;15(20):2687-2701.

64. Ahmad W, Haque WFU, Brancolini V, Tsou HC, Haque SU, Lam H, Aita VM, Owen J, deBlauiere M, Frank J, Cserhalmi-Friedman PB, Leask A, McGrath JA, Peacocke M, Ahmad M, Ott J, Christiano AM. Alopecia universalis associated with a mutation in the human hairless gene. Science. 1998;279(5351):720-724.

65. Ahmad W, Panteleyev AA, Christiano AM. The molecular basis of congenital atrichia in humans and mice: mutations in the hairless gene. J Investig Dermatol Symp Proc. 1999;4(3):240-243.

66. Panteleyev AA, Ahmad W, Malashenko AM, Ignatieva EL, Paus R, Sundberg JP, Christiano AM. Molecular basis for the rhino Yurlovo (hr(rhY)) phenotype: severe skin abnormalities and female reproductive defects associated with an insertion in the hairless gene. Exp Dermatol. 1998;7(5):281-288.
67. Zarach JM, Beaudoin GMJ, Coulombe PA, Thompson CC. The co-repressor hairless has a role in epithelial cell differentiation in the skin. *Development*. 2004;131(17):4189-4200.

68. Benavides F, Oberyaszyn TM, VanBuskirk AM, Reeve VE, Kusewitt DF. The hairless mouse in skin research. *J Dermatol Sci*. 2009;53(1):10-18.

69. Thompson CC. Hairless is a nuclear receptor corepressor essential for skin function. *Nucl Recept Signal*. 2009;7:e010.

70. Beaudoin GM, 3rd, Sisk JM, Coulombe PA, Thompson CC. Hairless triggers reactivation of hair growth by promoting Wnt signaling. *Proc Natl Acad Sci U S A*. 2005;102(41):14653-14658.

71. Ahmad W, Irvine AD, Lam H, Buckley C, Bingham EA, Panteleyev AA, Ahmad M, McGrath JA, Christiano AM. A missense mutation in the zinc-finger domain of the human hairless gene underlies congenital atrichia in a family of Irish travellers. *Am J Hum Genet*. 1998;63(4):984-991.

72. Cichon S, Anker M, Vogt IR, Rohleder H, Pützstück M, Hillmer A, Farooq SA, Al-Dhafri KS, Ahmad M, Haque S, Rietschel M, Propping P, Kruse R, Nöthen MM. Cloning, genomic organization, alternative transcripts and mutational analysis of the gene responsible for autosomal recessive universal congenital alopecia. *Hum Mol Genet*. 1998;7(11):1671-1679.

73. Ahmad W, Faiyaz ul Haque M, Brancolini V, Tsou HC, ul Haque S, Lam H, Aita VM, Owen J, deBlaquiere M, Frank J, Cserhalmi-Friedman PB, Leask A, McGrath JA, Peacocke M, Ahmad M, Ott J, Christiano AM. Alopecia universalis associated with a mutation in the human hairless gene. *Science*. 1998;279(5351):720-724.

74. Sprecher E, Bergman R, Szargel R, Raz T, Labay V, Ramon M, Baruch-Gershoni R, Friedman-Birnbaum R, Cohen N. Atrichia with papular lesions maps to 8p in the region containing the human hairless gene. *Am J Med Genet*. 1998;80(S2):35-40.

75. Maatough A, Whitfield GK, Brook L, Hsieh D, Palade P, Hsieh JC. Human Hairless Protein Roles in Skin/Hair and Emerging Connections to Brain and Other Cancers. *J Cell Biochem*. 2018;119(1):69-80.

76. Frampton RJ, Suva LJ, Elsman JA, Findlay DM, Moore GE, Moseley JM, Martin TJ. Presence of 1,25-dihydroxyvitamin D3 receptors in established human cancer cell lines in culture. *Cancer Res*. 1982;42(3):1116-1119.

77. Friedrich M, Axt-Fliedner R, Villena-Heinsen C, Tilgen W, Schmidt W, Reichrath J. Analysis of vitamin D receptor (VDR) and retinoid X-receptor alpha in breast cancer. *Histoch J*. 2002;34(1-2):35-40.

78. Huss L, Butt ST, Borgquist S, Elebro K, Sandsveden M, Rosendahl A, Manjer J. Vitamin D receptor expression in invasive breast tumors and breast cancer survival. *Breast Cancer Research*. 2019;21(1):84.

79. Ditsch N, Toth B, Mayr D, Lenhard M, Gallwas J, Weissnabecher T, Dannecker C, Friese K, Jeschke U. The association between vitamin D receptor expression and prolonged overall survival in breast cancer. *J Histochem Cytochem*. 2012;60(2):121-129.

80. Jerzak KJ, Cockburn J, Pond GR, Pritchard KL, Narod SA, Dhesy-Thind SK, Bane A. Thyroid hormone receptor α in breast cancer: prognostic and therapeutic implications. *Breast Cancer Res Treat*. 2015;149(1):293-301.
81. Thompson CC, Bottcher MC. The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. *Proc Natl Acad Sci U S A*. 1997;94(16):8527-8532.

82. Potter GB, Zarach JM, Sisk JM, Thompson CC. The thyroid hormone-regulated corepressor hairless associates with histone deacetylases in neonatal rat brain. *Mol Endocrinol*. 2002;16(11):2547-2560.

83. Hsia DA, Tepper CG, Pochampalli MR, Hsia EY, Izumiya C, Huerta SB, Wright ME, Chen HW, Kung HJ, Izumiya Y. KDM8, a H3K36me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation. *Proc Natl Acad Sci U S A*. 2010;107(21):9671-9676.

84. Wang H-J, Pochampalli M, Wang L-Y, Zou JX, Li P-S, Hsu S-C, Wang B-J, Huang S-H, Yang P, Yang JC, Chu C-Y, Hsieh C-L, Sung S-Y, Li C-F, Tepper CG, Ann DK, Gao AC, Evans CP, Izumiya Y, Chuu C-P, Wang W-C, Chen H-W, Kung H-J. KDM8/JMJD5 as a dual coactivator of AR and PKM2 integrates AR/EZH2 network and tumor metabolism in CRPC. *Oncogene*. 2019;38(1):17-32.

85. Hickey TE, Selth LA, Chia KM, Laven-Law G, Milioli HH, Roden D, Jindal S, Hui M, Finlay-Schultz J, Ebrahimie E, Birrell SN, Stelloo S, Iigo R, Alexandre S, Caldon CE, Abdel-Fatah TM, Ellis IO, Zwart W, Palmieri C, Sartorius CA, Swarbrick A, Lim E, Carroll JS, Tilley WD. The androgen receptor is a tumor suppressor in estrogen receptor-positive breast cancer. *Nature Medicine*. 2021;27(2):310-320.

86. Biddie SC, John S. Minireview: Conversing with chromatin: the language of nuclear receptors. *Mol Endocrinol*. 2014;28(1):3-15.

87. Xun J, Wang D, Shen L, Gong J, Gao R, Du L, Chang A, Song X, Xiang R, Tan X. JMJD3 suppresses stem cell-like characteristics in breast cancer cells by downregulation of Oct4 independently of its demethylase activity. *Oncotarget*. 2017;8(13):21918-21929.

88. Brook L, Palade P, Maatough A, Whitfield GK, Emeterio LS, Hsieh D, Hsieh JC. Hairless regulates p53 target genes to exert tumor suppressive functions in glioblastoma. *J Cell Biochem*. 2019;120(1):533-543.

89. Li H, Leo C, Schroen DJ, Chen JD. Characterization of receptor interaction and transcriptional repression by the corepressor SMRT. *Mol Endocrinol*. 1997;11(13):2025-2037.

90. Burke LJ, Banalhamad A. Co-repressors 2000. *Faseb J*. 2000;14(13):1876-1888.

91. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev*. 2000;14(2):121-141.

92. Brook L, Whitfield GK, Hsieh D, Bither RD, Hsieh JC. The Mammalian Hairless Protein as a DNA Binding Phosphoprotein. *Journal of Cellular Biochemistry*. 2017;118(2):341-350.

93. Liu L, Kim H, Casta A, Kobayashi Y, Shapiro LS, Christiano AM. Hairless is a histone H3K9 demethylase. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology*. 2014;28(4):1534-1542.

94. Mosammaparast N, Shi Y. Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. *Annu Rev Biochem*. 2010;79:155-179.

95. Lowry JA, Atchley WR. Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain. *J Mol Evol*. 2000;50(2):103-115.
96. Liu L, Kim H, Casta A, Kobayashi Y, Shapiro LS, Christiano AM. Hairless is a histone H3K9 demethylase. *FASEB J.* 2014;28(4):1534-1542.

97. Chuma M, Endo-Umeda K, Shima S, Yamada S, Makishima M. Hairless modulates ligand-dependent activation of the vitamin D receptor-retinoid X receptor heterodimer. *Biol Pharm Bull.* 2012;35(4):582-587.

98. Schaffer BS, Grayson MH, Wortham JM, Kubicek CB, McCleish AT, Prajapati SI, Nelson LD, Brady MM, Jung I, Hosoyama T, Sarro LM, Hanes MA, Rubin BP, Michalek JE, Clifford CB, Infante AJ, Keller C. Immune Competency of a Hairless Mouse Strain for Improved Preclinical Studies in Genetically Engineered Mice. *Mol Cancer Ther.* 2010;9(8):2354-2364.

99. Stoye JP, Fenner S, Greenoak GE, Moran C, Coffin JM. Role of Endogenous Retroviruses as Mutagens - the Hairless Mutation of Mice. *Cell.* 1988;54(3):383-391.

100. Ahmad W, Panteleyev AA, Henson-Apollonio V, Sundberg JP, Christiano AM. Molecular basis of a novel rhino (hr(hrChr)) phenotype: a nonsense mutation in the mouse hairless gene. *Exp Dermatol.* 1998;7(5):298-301.

101. Ahmad W, Panteleyev AA, Sundberg JP, Christiano AM. Molecular basis for the rhino (hrhr-BJ) phenotype: a nonsense mutation in the mouse hairless gene. *Genomics.* 1998;53(3):383-386.

102. Iversen U, Iversen OH. The sensitivity of the skin of hairless mice to chemical carcinogenesis. *Cancer Res.* 1976;36(4):1238-1241.

103. Kim H, Casta A, Tang X, Luke CT, Kim AL, Bickers DR, Athar M, Christiano AM. Loss of hairless confers susceptibility to UVB-induced tumorigenesis via disruption of NF-kappaB signaling. *PLoS One.* 2012;7(6):e39691.

104. Ha W, Hinde A, Xie L, Trager MH, Liu L. Biomarker function of HMGA2 in ultraviolet-induced skin cancer development. *Exp Dermatol.* 2020;29(10):1021-1026.

105. Neagu M, Caruntu C, Constantin C, Boda D, Zurac S, Spandidos DA, Tsatsakis AM. Chemically induced skin carcinogenesis: Updates in experimental models (Review). *Oncol Rep.* 2016;35(5):2516-2528.

106. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding L, Zhang W, Mills GB, Kucherlapati R, Mardis ER, Levine DA. Integrated genomic characterization of endometrial carcinoma. *Nature.* 2013;497(7447):67-73.

107. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. *Cancer Discovery.* 2012;2(5):401.

108. Bettegowda C, Agrawal N, Jiao Y, Wang Y, Wood LD, Rodriguez FJ, Hruban RH, Gallia GL, Binder ZA, Riggins CJ, Salmasi V, Riggins GJ, Reitman ZJ, Rasheed A, Keir S, Shinjo S, Marie S, McLendon R, Jallo G, Vogelstein B, Bigner D, Yan H, Kinzler KW, Papadopoulos N. Exomic sequencing of four rare central nervous system tumor types. *Oncotarget.* 2013;4(4):572-583.

109. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, Bowry B, Shen H, Hayat S, Fieldhouse R, Lester SC, Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB, Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW, Hoadley KA, King TA, Perou CM. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell.* 2015;163(2):506-519.
110. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, Beltran H, Abida W, Bradley RK, Vinson J, Cao X, Vats P, Kunju LP, Hussain M, Feng FY, Tomlins SA, Cooney KA, Smith DC, Brennan C, Siddiqui J, Mehra R, Chen Y, Rathkopf DE, Morris MJ, Solomon SB, Durack JC, Reuter VE, Gopalani A, Gao J, Loda M, Lis RT, Bowden M, Balk SP, Gaviola G, Sougnez C, Gupta M, Yu EY, Mostaghel EA, Cheng HH, Mulcahy H, True LD, Plymate SR, Dvinge H, Ferraldeschi R, Flohr P, Miranda S, Zafeiriou Z, Tunariu N, Mateo J, Perez-Lopez R, Demichelis F, Robinson BD, Schiﬀman M, Nanus DM, Tagawa ST, Sigaras A, Eng KW, Elemento O, Sboner A, Heath EI, Scher Hl, Pienta KJ, Kantoff P, de Bono JS, Rubin MA, Nelson PS, Garraway LA, Sawyers CL, Chinnaian AM. Integrative clinical genomics of advanced prostate cancer. Cell. 2015;161(5):1215-1228.

111. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, Bowlby R, Shen H, Hayat S, Fieldhouse R, Lester SC, Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB, Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW, Hoadley KA, King TA, Network TR, Perou CM. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. Cell. 2015;163(2):506-519.

112. Barretina J, Caponigro G, Stranksy N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehar J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jane-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway C, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P, Jr., de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winkler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Schlegel R, Garraway LA. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483(7391):603-607.

113. Jiang GL, Zhang SJ, Yazdanparast A, Li M, Pawar AV, Liu YL, Inavolu SM, Cheng LJ. Comprehensive comparison of molecular portraits between cell lines and tumors in breast cancer. BMC Genomics. 2016;17.

114. Cai Y, Crowther J, Pastor T, Abbasi Asbagh L, Baietti MF, De Troyer M, Vazquez I, Talebi A, Renzi F, Dehairs J, Swinnen JV, Sablina AA. Loss of Chromosome 8p Governs Tumor Progression and Drug Response by Altering Lipid Metabolism. Cancer Cell. 2016;29(5):751-766.

115. Soussi T, Wiman KG. TP53: an oncogene in disguise. Cell Death & Differentiation. 2015;22(8):1239-1249.

116. Son HJ, Kim JY, Hahn Y, Seo SB. Negative regulation of JAK2 by H3K9 methyltransferase G9a in leukemia. Mol Cell Biol. 2012;32(18):3681-3694.

117. Huang J, Dorsey J, Chukov S, Perez-Burgos L, Zhang X, Jenuwein T, Reinberg D, Berger SL. G9a and Gip methylate lysine 373 in the tumor suppressor p53. J Biol Chem. 2010;285(13):9636-9641.

118. Wang YF, Zhang J, Su Y, Shen YY, Jiang DX, Hou YY, Geng MY, Ding J, Chen Y. G9a regulates breast cancer growth by modulating iron homeostasis through the repression of ferroxidase hephaestin. Nat Commun. 2017;8(1):274.

119. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, Van Loo P, Ju YS, Smid M, Brinkman AB,
Morganella S, Aure MR, Lingjærde OC, Langerød A, Ringnér M, Ahn S-M, Boyault S, Brock JE, Broeks A, Butler A, Desmedt C, Dirix L, Dronov S, Fatima A, Foekens JA, Gerstung M, Hooijer GJK, Jang SJ, Jones DR, Kim H-Y, King TA, Krishnamurthy S, Lee HJ, Lee J-Y, Li Y, McLaren S, Menzies A, Mustonen V, O’Meara S, Pauporté I, Pivot X, Purdie CA, Raine K, Ramakrishnan K, Rodríguez-González FG, Romieu G, Siewerts AM, Simpson PT, Shepherd R, Stebbings L, Stefansson OA, Teague J, Tommassi S, Troilleux I, van den Eynden GG, Vermeulen P, Vincent-Salomon A, Caldas C, Veer LV, Tutt A, Knappskog S, Tan BKT, Jonkers J, Borg Å, Ueno NT, Sotiriou C, Viari A, Futreal PA, Campbell PJ, Span PN, Van Laere S, Lakhani SR, Eysjord JE, Thompson AM, Birney E, Stunnenberg HG, van de Vijver MJ, Martens JWM, Børresen-Dale A-L, Richardson AL, Kong G, Thomas G, Stratton MR. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534(7605):47-54.

120. Borley J, Brown R. Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer. *Annals of Medicine*. 2015;47(5):359-369.

121. Magnani L, Brunelle M, Gévry N, Lupien M. Chromatin landscape and endocrine response in breast cancer. *Epigenomics*. 2012;4(6):675-683.

122. Liu L, Kimball S, Liu H, Holowatyj A, Yang Z-Q. Genetic alterations of histone lysine methyltransferases and their significance in breast cancer. *Oncotarget; Vol 6, No 4*. 2014.

123. Berdasco M, Esteller M. Clinical epigenetics: seizing opportunities for translation. *Nat Rev Genet*. 2019;20(2):109-127.

124. Ganesan A, Arimondo PB, Rotm MG, Jeronimo C, Berdasco M. The timeline of epigenetic drug discovery: from reality to dreams. *Clin Epigenetics*. 2019;11(1):174.

125. Connolly RM, Li H, Jankowitz RC, Zhang Z, Rudek MA, Jeter SC, Slater SA, Powers P, Wolff AC, Fetting JH, Brufsky A, Piekarz R, Ahuja N, Laird PW, Shen H, Weisenberger DJ, Cope L, Herman JG, Somlo G, Garcia AA, Jones PA, Baylin SB, Davidson NE, Zahnow CA, Stearns V. Combination Epigenetic Therapy in Advanced Breast Cancer with 5-Azacitidine and Entinostat: A Phase II National Cancer Institute/Stand Up to Cancer Study. *Clin Cancer Res*. 2017;23(11):2691-2701.

126. Mazzone R, Zwergel C, Mai A, Valente S. Epi-drugs in combination with immunotherapy: a new avenue to improve anticancer efficacy. *Clin Epigenetics*. 2017;9:59.

127. Bradley William D, Arora S, Busby J, Balasubramanian S, Gehling Victor S, Nasveschuk Christopher G, Vaswani Rishi G, Yuan C-C, Hatton C, Zhao F, Williamson KE, Iyer P, Méndez J, Campbell R, Cantone N, Garapaty-Rao S, Audia James E, Cook Andrew S, Dakin LA, Albrecht Brian K, Harmange J-C, Daniels Danette L, Cummings Richard T, Bryant Barbara M, Normant E, Trojer P. EZH2 Inhibitor Efficacy in Non-Hodgkin’s Lymphoma Does Not Require Suppression of H3K27 Monomethylation. *Chemistry & Biology*. 2014;21(11):1463-1475.

128. Kim KH, Roberts CWM. Targeting EZH2 in cancer. *Nature Medicine*. 2016;22(2):128-134.

129. de Vries Nienke A, Hulsman D, Akhtar W, de Jong J, Miles Denise C, Blom M, van Tellingen O, Jonkers J, van Lohuizen M. Prolonged Ezh2 Depletion in Glioblastoma Causes a Robust Switch in Cell Fate Resulting in Tumor Progression. *Cell Reports*. 2015;10(3):383-397.

130. Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, Savona MR, Jongen-Lavrenic M, Altman JK, Thomson B, Blakemore SJ, Daigle SR, Waters NJ, Suttle AB,
Clawson A, Pollock R, Krivtsov A, Armstrong SA, DiMartino J, Hedrick E, Lowenberg B, Tallman MS. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood*. 2018;131(24):2661-2669.

131. Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, Evers BM, Zhou BP. G9a interacts with Snail and is critical for Snail-mediated E-cadherin repression in human breast cancer. *The Journal of Clinical Investigation*. 2012;122(4):1469-1486.

132. Wozniak RJ, Klimecki WT, Lau SS, Feinstein Y, Futscher BW. 5-Aza-2’-deoxycytidine-mediated reductions in G9A histone methyltransferase and histone H3 K9 dimethylation levels are linked to tumor suppressor gene reactivation. *Oncogene*. 2007;26(1):77-90.

133. Oh ST, Kim KB, Chae YC, Kang JY, Hahn Y, Seo SB. H3K9 histone methyltransferase G9a-mediated transcriptional activation of p21. *FEBS Lett*. 2014;588(5):685-691.

134. Si W, Huang W, Zheng Y, Yang Y, Liu X, Shan L, Zhou X, Wang Y, Su D, Gao J, Yan R, Han X, Li W, He L, Shi L, Xuan C, Liang J, Sun L, Wang Y, Shang Y. Dysfunction of the Reciprocal Feedback Loop between GATA3- and ZEB2-Nucleated Repression Programs Contributes to Breast Cancer Metastasis. *Cancer Cell*. 2015;27(6):822-836.

135. Chang Y, Zhang X, Horton JR, Upadhaya AK, Spannhoff A, Liu J, Snyder JP, Bedford MT, Cheng X. Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. *Nat Struct Mol Biol*. 2009;16(3):312-317.

136. Liu F, Barsyte-Lovejoy D, Li F, Xiong Y, Korboukh V, Huang XP, Allali-Hassani A, Janzen WP, Roth BL, Frye SV, Arrowsmith CH, Brown PJ, Vedadi M, Jin J. Discovery of an in vivo chemical probe of the lysine methyltransferases G9a and GLP. *J Med Chem*. 2013;56(21):8931-8942.

137. Rotili D, Tomassi S, Conte M, Benedetti R, Tortorici M, Ciossani G, Valente S, Marrocco B, Labella D, Novellino E, Mattevi A, Altucci L, Tumber A, Mai A. Pan-histone demethylase inhibitors simultaneously targeting Jumonji C and lysine-specific demethylases display high anticancer activities. *J Med Chem*. 2014;57(1):42-55.

138. Benedetti R, Dell’Aversana C, De Marchi T, Rotili D, Liu NQ, Novakovic B, Boccella S, Di Maro S, Cosconati S, Baldi A, Nimeneus E, Schulz J, Hoglund U, Maione S, Papulino C, Chianese U, Iovino F, Federico A, Mai A, Stunnenberg HG, Nebbioso A, Altucci L. Inhibition of Histone Demethylases LSD1 and UTX Regulates ERAlpha Signaling in Breast Cancer. *Cancers (Basel)*. 2019;11(12).

139. Falahi F, Huisman C, Kazemier HG, van der Vlies P, Kok K, Hospers GAP, Rots MG. Towards Sustained Silencing of HER2/neu in Cancer By Epigenetic Editing. *Molecular Cancer Research*. 2013;11(9):1029.

140. Stolzenburg S, Beltran AS, Swift-Scanlan T, Rivenbark AG, Rashwan R, Blancafort P. Stable oncogenic silencing in vivo by programmable and targeted de novo DNA methylation in breast cancer. *Oncogene*. 2015;34(43):5427-5435.

141. Chen J, Luo Q, Yuan Y, Huang X, Cai W, Li C, Wei T, Zhang L, Yang M, Liu Q, Ye G, Dai X, Li B. Pygo2 associates with MLL2 histone methyltransferase and GCN5 histone acetyltransferase complexes to augment Wnt target gene expression and breast cancer stem-like cell expansion. *Mol Cell Biol*. 2010;30(24):5621-5635.

142. Kim JH, Sharma A, Dhar SS, Lee SH, Gu B, Chan CH, Lin HK, Lee MG. UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells. *Cancer Res*. 2014;74(6):1705-1717.
143. Fenizia C, Bottino C, Corbetta S, Fittipaldi R, Floris P, Gaudenzi G, Carra S, Cotelli F, Vitale G, Caretti G. SMYD3 promotes the epithelial-mesenchymal transition in breast cancer. Nucleic Acids Res. 2019;47(3):1278-1293.

144. Cho MH, Park JH, Choi HJ, Park MK, Won HY, Park YJ, Lee CH, Oh SH, Song YS, Kim HS, Oh YH, Lee JY, Kong G. DOT1L cooperates with the c-Myc-p300 complex to epigenetically derepress CDH1 transcription factors in breast cancer progression. Nat Commun. 2015;6:7821.

145. Liu B, Zhang X, Song F, Zheng H, Zhao Y, Li H, Zhang L, Yang M, Zhang W, Chen K. MiR-502/SET8 regulatory circuit in pathobiology of breast cancer. Cancer Lett. 2016;376(2):259-267.

146. Shen C, Wang D, Liu X, Gu B, Du Y, Wei FZ, Cao LL, Song B, Lu X, Yang Q, Zhu Q, Hou T, Li M, Wang L, Wang H, Zhao Y, Yang Y, Zhu WG. SET7/9 regulates cancer cell proliferation by influencing β-catenin stability. Faseb J. 2015;29(10):4313-4323.

147. Montenegro MF, Sánchez-Del-Campo L, González-Guerrero R, Martínez-Barba E, Piñero-Madrona A, Cabezas-Herrera J, Rodríguez-López JN. Tumor suppressor SET9 guides the epigenetic plasticity of breast cancer cells and serves as an early-stage biomarker for predicting metastasis. Oncogene. 2016;35(47):6143-6152.

148. Reijm EA, Timmermans AM, Look MP, Meijer-van Gelder ME, Stobbe CK, van Deurzen CHM, Martens JWM, Sleijfer S, Foekens JA, Berns P, Jansen M. High protein expression of EZH2 is related to unfavorable outcome to tamoxifen in metastatic breast cancer. Ann Oncol. 2014;25(11):2185-2190.

149. Tryndyak VP, Kovalchuk O, Pogribny IP. Loss of DNA methylation and histone H4 lysine 20 trimethylation in human breast cancer cells is associated with aberrant expression of DNA methyltransferase 1, Suv4-20h2 histone methyltransferase and methyl-binding proteins. Cancer Biol Ther. 2006;5(1):65-70.

150. Mo W, Liu Q, Lin CC, Dai H, Peng Y, Liang Y, Peng G, Meric-Bernstam F, Mills GB, Li K, Lin SY. mTOR Inhibitors Suppress Homologous Recombination Repair and Synergize with PARP Inhibitors via Regulating SUV39H1 in BRCA-Proficient Triple-Negative Breast Cancer. Clin Cancer Res. 2016;22(7):1699-1712.

151. Khanal P, Kim G, Lim SC, Yun HJ, Lee KY, Choi HK, Choi HS. Prolyl isomerase Pin1 negatively regulates the stability of SUV39H1 to promote tumorigenesis in breast cancer. Faseb J. 2013;27(11):4606-4618.

152. Ryu TY, Kim K, Kim SK, Oh JH, Min JK, Jung CR, Son MY, Kim DS, Cho HS. SETDB1 regulates SMAD7 expression for breast cancer metastasis. BMB Rep. 2019;52(2):139-144.

153. Chen L, Vasilatos SN, Qin Y, Katz TA, Cao C, Wu H, Tasdemir N, Levine KM, Oesterreich S, Davidson NE, Huang Y. Functional characterization of lysine-specific demethylase 2 (LSD2/KDM1B) in breast cancer progression. Oncotarget. 2017;8(47):81737-81753.

154. Chen JY, Luo CW, Lai YS, Wu CC, Hung WC. Lysine demethylase KDM2A inhibits TET2 to promote DNA methylation and silencing of tumor suppressor genes in breast cancer. Oncogenesis. 2017;6(8):e369.

155. Kottakis F, Foltopoulou P, Sanidas I, Keller P, Wronska A, Dake BT, Ezell SA, Shen Z, Naber SP, Hinds PW, Mcniel E, Kuperwasser C, Tsichlis PN. NDY1/KDM2B functions as a master regulator of polycomb complexes and controls self-renewal of breast cancer stem cells. Cancer Res. 2014;74(14):3935-3946.
156. Wade MA, Jones D, Wilson L, Stockley J, Coffey K, Robson CN, Gaughan L. The histone demethylase enzyme KDM3A is a key estrogen receptor regulator in breast cancer. *Nucleic Acids Res*. 2015;43(1):196-207.

157. Wang W, Oguz G, Lee PL, Bao Y, Wang P, Terp MG, Ditzel HJ, Yu Q. KDM4B-regulated unfolded protein response as a therapeutic vulnerability in PTEN-deficient breast cancer. *J Exp Med*. 2018;215(11):2833-2849.

158. Luo W, Chang R, Zhong J, Pandey A, Semenza GL. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. *Proc Natl Acad Sci U S A*. 2012;109(49):E3367-3376.

159. Yang GJ, Wang W, Mok SWF, Wu C, Law BYK, Miao XM, Wu KJ, Zhong HJ, Wong CY, Wong VKW, Ma DL, Leung CH. Selective Inhibition of Lysine-Specific Demethylase 5A (KDM5A) Using a Rhodium(III) Complex for Triple-Negative Breast Cancer Therapy. *Angew Chem Int Ed Engl*. 2018;57(40):13091-13095.

160. Wang W, Lim KG, Feng M, Bao Y, Lee PL, Cai Y, Chen Y, Zhang H, Marzese D, Hoon DSB, Yu Q. KDM6B Counteracts EZH2-Mediated Suppression of IGFBP5 to Confer Resistance to PI3K/AKT Inhibitor Treatment in Breast Cancer. *Mol Cancer Ther*. 2018;17(9):1973-1983.

161. Zhao Z, Sun C, Li F, Han J, Li X, Song Z. Overexpression of histone demethylase JMJD5 promotes metastasis and indicates a poor prognosis in breast cancer. *Int J Clin Exp Pathol*. 2015;8(9):10325-10334.

162. Abu-Jamous B, Buffa FM, Harris AL, Nandi AK. In vitro downregulated hypoxia transcriptome is associated with poor prognosis in breast cancer. *Mol Cancer*. 2017;16(1):105.

163. Cao C, Vasilatos SN, Bhargava R, Fine JL, Oesterreich S, Davidson NE, Huang Y. Functional interaction of histone deacetylase 5 (HDAC5) and lysine-specific demethylase 1 (LSD1) promotes breast cancer progression. *Oncogene*. 2017;36(1):133-145.

164. Wang Y, Zhang H, Chen Y, Sun Y, Yang F, Yu W, Liang J, Sun L, Yang X, Shi L, Li R, Li Y, Zhang Y, Li Q, Yi X, Shang Y. LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell*. 2009;138(4):660-672.

165. Li LL, Xue AM, Li BX, Shen YW, Li YH, Luo CL, Zhang MC, Jiang JQ, Xu ZD, Xie JH, Zhao ZQ. JMJD2A contributes to breast cancer progression through transcriptional repression of the tumor suppressor ARHI. *Breast Cancer Res*. 2014;16(3):R56.

166. Kaniskan H, Konze KD, Jin J. Selective inhibitors of protein methyltransferases. *J Med Chem*. 2015;58(4):1596-1629.

167. Greiner D, Bonaldi T, Eskeland R, Roemer E, Ihmof A. Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. *Nat Chem Biol*. 2005;1(3):143-145.

168. Liu F, Chen X, Allali-Hassani A, Quinn AM, Wasney GA, Dong A, Basyte D, Kozieradzki I, Senisterra G, Chau I, Siarheyeva A, Kireev DB, Jadhav A, Herold JM, Frye SV, Arrowsmith CH, Brown PJ, Simeonov A, Vedadi M, Jin J. Discovery of a 2,4-diamino-7-aminoalkoxyquinazoline as a potent and selective inhibitor of histone lysine methyltransferase G9a. *J Med Chem*. 2009;52(24):7950-7953.

169. Liu F, Chen X, Allali-Hassani A, Quinn AM, Wigle TJ, Wasney GA, Dong A, Senisterra G, Chau I, Siarheyeva A, Norris JL, Kireev DB, Jadhav A, Herold JM, Janzen WP, Arrowsmith CH, Frye SV, Brown PJ, Simeonov A, Vedadi M, Jin J. Protein lysine methyltransferase G9a inhibitors: design, synthesis, and structure activity
relationships of 2,4-diamino-7-aminoalkoxy-quinazolines. *J Med Chem*. 2010;53(15):5844-5857.

170. Chang Y, Ganesh T, Horton JR, Spannhoff A, Liu J, Sun A, Zhang X, Bedford MT, Shinkai Y, Snyder JP, Cheng X. Adding a lysine mimic in the design of potent inhibitors of histone lysine methyltransferases. *J Mol Biol*. 2010;400(1):1-7.

171. Vedadi M, Barsyte-Lovejoy D, Liu F, Rival-Gervier S, Allali-Hassani A, Labrie V, Wigle TJ, Dimaggio PA, Wasney GA, Siarheyeva A, Dong A, Tempel W, Wang SC, Chen X, Chau I, Mangano TJ, Huang XP, Simpson CD, Pattenden SG, Norris JL, Kireev DB, Tripathy A, Edwards A, Roth BL, Janzen WP, Garcia BA, Petronis A, Ellis J, Brown PJ, Frye SV, Arrowsmith CH, Jin J. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nat Chem Biol*. 2011;7(8):566-574.

172. Konze KD, Pattenden SG, Liu F, Barsyte-Lovejoy D, Li F, Simon JM, Davis IU, Vedadi M, Jin J. A chemical tool for in vitro and in vivo precipitation of lysine methyltransferase G9a. *ChemMedChem*. 2014;9(3):549-553.

173. Yuan Y, Wang Q, Paulk J, Kubicek S, Kemp MM, Adams DJ, Shamji AF, Wagner BK, Schreiber SL. A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. *ACS Chem Biol*. 2012;7(7):1152-1157.

174. Sweis RF, Pliushchev M, Brown PJ, Guo J, Li F, Maag D, Petros AM, Soni NB, Tse C, Vedadi M, Michaelides MR, Chiang GG, Pappano WN. Discovery and development of potent and selective inhibitors of histone methyltransferase g9a. *ACS Med Chem Lett*. 2014;5(2):205-209.
Figure Legends

Figure 1.  **A**: Schematic depiction of major HR functional domains including a nuclear localization signal (NLS), a zinc finger (ZF), four protein-protein interaction domains (IDs), and a JmjC domain in the C-terminus. Recurrent HR mutations identified in three or more human cancer types are indicated (top). Two APL patient mutations at amino acids 1012 and 1056 are also depicted (bottom). **B**: Illustration of top five HR-interacting proteins based on STRING interaction network database, including the critical tumor suppressor TP53 and several histone deacetylases HDAC1 – 3. There is experimental evidence supporting HR-HDACs interaction, but HR-TP53 interaction awaits further experimental validation.

Figure 2.  **A**: Frequency of HR deletion, amplification, and mutation in human breast cancers based on data from various genomics databases. According to TCGA, HR deletion is a predominant genetic alteration in breast cancers, consistent with our unpublished targeted sequencing results. **B**: HR mutations identified in invasive breast cancers based on the cBioPortal for Cancer Genomics database. Among them, G337D, R927C and P1046L are found in other human cancer types as depicted in Figure 1A.
Table 1. Tumor-promoting or -suppressing function of KMTs and KDMs that is implicated in breast cancer development.

| Promoters     | Suppressors     | Dual             |
|---------------|-----------------|------------------|
| KMT1C(G9a) (118) | KMT2C (MLL3)    | KMT1A(SUV39H1)   |
| KMT2B(MLL2) (141) | KMT5C (SU4-20H2) | (150,151)        |
| KMT2D(MLL4) (142) | (149)           | KMT1E(SETDB1) (152) |
| KMT3C (SMYD2)  | KMT7 (SET7) (146) |                 |
| KMT3E (SMYD3) (143) |                 |                 |
| KMT4 (DOT1L) (144) |                 |                 |
| KMT5A (SET8) (145) |                 |                 |
| KMT7 (SET7) (146,147) |                 |                 |
| EZH1 (KIAA0388) (148) |                 |                 |
| KMT2C (MLL3)    | KMT5C (SU4-20H2) | KMT1A(SUV39H1)   |
| KMT1A(SUV39H1) (150,151) | KMT1E(SETDB1) (152) |                 |
| KMT1E(SETDB1) (152) |                 |                 |
| KMT2C (MLL3)    | KMT5C (SU4-20H2) | KMT1A(SUV39H1)   |
| KMT1A(SUV39H1) (150,151) | KMT1E(SETDB1) (152) |                 |
| KMT1E(SETDB1) (152) |                 |                 |
| KMT2C (MLL3)    | KMT5C (SU4-20H2) | KMT1A(SUV39H1)   |
| KMT1A(SUV39H1) (150,151) | KMT1E(SETDB1) (152) |                 |
| KMT1E(SETDB1) (152) |                 |                 |
| KMT2C (MLL3)    | KMT5C (SU4-20H2) | KMT1A(SUV39H1)   |
| KMT1A(SUV39H1) (150,151) | KMT1E(SETDB1) (152) |                 |
| KMT1E(SETDB1) (152) |                 |                 |
Table 2. A list of breast cancer cell lines with HR copy number loss.

| Cell Line | Histology Subtype       | PAM50 Subtype | Source  |
|-----------|-------------------------|---------------|---------|
| HCC1599   | Ductal Carcinoma        | Basal-like    | ATCC    |
| HCC70     | Ductal Carcinoma        | Basal-like    | ATCC    |
| CAL148    | Ductal Carcinoma        | Basal-like    | ATCC    |
| HS343T    | Adenocarcinoma          | Basal-like    | ATCC    |
| EVSAT     | Carcinoma Met           | ND*           | DSMZ    |
| HS281T    | Adenocarcinoma          | Basal-like    | ATCC    |
| HCC202    | Ductal Carcinoma        | Her2Amp       | ATCC    |
| CAL120    | Ductal Carcinoma        | Basal-like    | DSMZ    |
| ZR7530    | Ductal Carcinoma Met    | Her2Amp       | ATCC    |
| HCC38     | Ductal Carcinoma        | Basal-like    | ATCC    |
| HCC1428   | Carcinoma Met           | Luminal B     | ATCC    |
| MDA MB231 | Carcinoma Met           | Basal-like    | ATCC    |
| MDA MB436 | Carcinoma Met           | Basal-like    | ATCC    |
| HS274T    | Carcinoma               | Basal-like    | ATCC    |
| HMC18     | Carcinoma Met           | ND*           | HSRRB   |
| HCC1806   | Ductal Carcinoma        | Basal-like    | ATCC    |
| HCC1954   | Ductal Carcinoma        | Her2Amp       | ATCC    |

*ND: not determined
Table 3. H3K9 KMT inhibitors in development for cancer therapies and associated mechanisms of action (166).

| Reference | Compound | KMT and methylation target | Selectivity | IC$_{50}$ | Mechanism of action |
|-----------|----------|-----------------------------|-------------|----------|---------------------|
| Greiner (2005) (167) | Chaetocin | KMT1A, G9a, H3K9me1/me2 | Low | 0.8 μM | Mixed disulfide linkages formed between cysteine residues of enzyme and inhibitor. |
| Chang (2009) (135) | BIX-01338 | G9a, GLP and other KMTs | Not selective | 5-15 μM | Unknown |
| Chang (2009) (135) | BIX-01294 | G9a and GLP, H3K9me2 | Selective | 1.7 μM | Binds to the substrate binding groove of the enzyme to prevent enzyme and substrate (SAH) interaction. |
| Liu (2009) (168) | UNC0224 | G9a and GLP, H3K9me2 | Selective | 15 – 30 nM | Occupation of the G9a lysine binding channel by the 7-dimethylaminopropoxy group |
| Liu (2010) (169) | UNC0321 | G9a and GLP, H3K9me2 | Selective | 63 pM | Same as UNC0638 but with a longer ethoxyl chain instead of the 3-carbon chain of UNC0638. |
| Chang (2010) (170) | E72 | G9a and GLP | Selective | 100 nM | a lysine mimic added to the BIX-01294 structure to inhibit substrate binding |
| Vedadi (2011) (171) | UNC0638 | G9a and GLP, H3K9me1/me2 | Selective | 15 – 20 nM | Competition with the lysine substrate. This inhibitor occupies the substrate binding groove and does not interact with the SAM binding pocket |
| Liu (2013) (136) | UNC0642 | G9a and GLP, H3K9me1/me2 | Selective | < 2.5 nM | Same as UNC0638 but with optimized In vivo In vivo pharmacokinetic properties. |
| Konze (2014) (172) | UNC0965 | G9a and GLP, H3K9me1/me2 | Selective | 15 – 20 nM | a biotinylated derivative of UNC0638 |
| Yuan (2012) (173) | BRD4770, BRD9539 | G9a, GLP, PRC2–EZH2, H3K9me2/me3 | Less selective | 6.3 nM | SAM-competitive inhibitor. |
| Sweis (2014) (174) | A-366 | G9a and GLP, H3K9me1/me2 | Selective | 3.3 - 38 nM | Substrate-competitive inhibitor. |
Figure 1.

A.

B.
Figure 2.

A.

![Bar graph showing alteration frequency (%) across different BC types: Deletion, Mutation, Amplification. The x-axis represents BC types such as TCGA, InvBC, and others.]

B.

| BC type                          | change   | mutation |
|----------------------------------|----------|----------|
| Invasive breast carcinoma        | G337D    | Missense |
| Invasive ductal carcinoma        | R927C    | Missense |
| Invasive ductal carcinoma        | P368Afs*13 | FS ins  |
| Mixed ductal and lobular BC      | P368Afs*13 | FS ins  |
| Invasive breast carcinoma        | R747H    | Missense |
| Invasive ductal carcinoma        | P1046L   | Missense |
| Invasive ductal carcinoma        | E448G    | Missense |
| Invasive breast carcinoma        | S366C    | Missense |