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Review

The Role of H3K4 Trimethylation in CpG Islands Hypermethylation in Cancer

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Abstract: CpG methylation in transposons, exons, introns and intergenic regions is important for long-term silencing, silencing of parasitic sequences and alternative promoters, regulating imprinted gene expression and determining X chromosome inactivation. Promoter CpG islands, although rich in CpG dinucleotides, are unmethylated and remain so during all phases of mammalian embryogenesis and development, except in specific cases. The biological mechanisms that contribute to the maintenance of the unmethylated state of CpG islands remain elusive, but the modification of established DNA methylation patterns is a common feature in all types of tumors and is considered as an event that intrinsically, or in association with genetic lesions, feeds carcinogenesis. In this review, we focus on the latest results describing the role that the levels of H3K4 trimethylation may have in determining the aberrant hypermethylation of CpG islands in tumors.

Keywords: H3K4 trimethylation; DNA hypermethylation; acute myeloid leukemia; cancer

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1. Histone Lysine Methylation

Epigenetic landscapes functionally define the chromatin architecture and they are shaped by the coordinated activity of "writers", "readers" and "erasers". "Writers" introduce covalent chemical modifications into DNA and histone tails, the "erasers" modulate the amount of these modifications and the "readers" recognize and bind the chemical modifications which induce functional effects in the chromatin architecture and DNA binding of transcription factors (TFs). Among the writers, histone methyltransferases catalyze the introduction of methyl groups in specific lysine and arginine residues at the amino terminal ends of the histone core [1], mainly at histones H3 and H4. Lysine methylation involves the ε-amine group of lysine at different positions of H3. Methylation events at K4, K9, K27, K36 and K79 are the most studied and characterized. Lysine can be mono-, di- or trimethylated. The level and state of histone lysine methylation depends not only on the activity of histone methyltransferases (KMTs) but also on the counteracting activity of histone lysine demethylases (KDMs). The variety of methylation sites and differentially methylated states describes the level of complexity of signaling mediated by histone lysine methylation, which is involved in transcription regulation, gene silencing, genome stability and RNA processing.

2. Histone Lysine 4 Methyltransferases

The enzymes responsible for histone lysine methylation (KMTs) contain a common active domain known as Su(var)3–9, Enhancer of zeste and Trithorax (SET), originally identified in yeast (SET1). Three SET1 homologs were subsequently identified in *Drosophila melanogaster*, including dSet1, Trithorax (Trx) and Trithorax-related [2], and 23 canonical SET-containing histone KMTs and one seven-beta-strand (7βS)-containing domain KMT (hDOT1L) with proven methyltransferase activity in mammals [3–5]. Some KMTs are highly selective. Each KMT methylates a specific lysine but not others located at different positions in the H3 polypeptide chain. For instance, the KMT that methylates H3K36 does not methylate H3K4, and the only KMT able to methylate H3K79 is hDOT1L [6–10]. In

addition, a lysine can be specifically targeted by multiple enzymes. This redundancy allows specific activities to occur in a context-dependent manner. For instance, the same lysine may be modified by a different enzyme as a function of the histone's genomic localization (enhancer versus promoter regions) but also to generate different methylation states (dimethylation versus trimethylation). KMT activity depends also on the specific lysine methylation state to add new methyl groups [11–15]. H3K4 methylation is one of the most studied and characterized histone lysine methylations. H3K4 can be mono-(H3K4me1), di-(H3K4me2) or tri-(H3K4me3). In mammals, H3K4 methylation is catalyzed by six SET domain-containing KMTs, namely SET1A/KMT2F, SET1B/KMT2G, MLL1/KMT2A, MLL2/KMT2B, MLL3/KMT2C and MLL4/KMT2D. Each of these enzymes is a component of multimeric complexes that may or may not contain other proteins such as WDR5, RbBP5, ASH2L and DPY30 [3]. These complexes are not redundant, as their activity marks H3K4 not only at functionally distinct loci but also at specific target genes determining different methylation states related to the recruitment of distinct "readers" [16,17]. For instance, multimeric complexes containing MLL1 and MLL2 trimethylate H3K4 at the promoter region of Hox gene clusters, which require the correct transcriptional regulation for hematopoietic development [18,19]. MLL2 is responsible for the tri-methylation of H3K4 of bivalent domains which is necessary for a mechanism aiming to maintain a paused transcriptional state in a targeted gene [20,21]. MLL3 and MLL4 monomethylate H3K4 located at the enhancer regions involved in cell type-specific gene expression [22–25]. Recent studies have revealed that the activity of KMT complexes is stimulated by the monoubiquitylation of histone H2B, and that distinct subunits components may have a role in determining the levels and state of H3K4 methylation [26–28].

3. Histone Lysine 4 Demethylases

To date, more than 30 KDM family members have been reported, and most of them contain a Jumonji domain, with the exception of KDM1A and KDM1B [29]. As for KMTs, KDMs target methylated lysines in H3, mainly at K4, K27, K9, K36 and K56, and in H4 at K20. KDMs demethylate specific lysines and not others located in different positions of the histone polypeptide chain. For instance, H3K27me3 is demethylated by KDM6B, which is not able to demethylate H3K4me3. KDMs may have distinct genomic localization and biological effects [30]. In mammals, H3K4 demethylation is catalyzed by the Jumonji, AT-rich interactive domain 1 (KDM5) and lysine-specific histone demethylase (KDM1) protein families. The KDM5 family is composed of four members designated KDM5A–D, and these enzymes are 2-oxoglutarate-dependent dioxygenases which require Fe^{2+} and O_2 for their function in order to undergo the hydroxylation necessary to remove methyl groups [31]. All members contain conserved domains of five types: the ARID (DNA-binding domain), C5HC2 zinc finger, Jumonji C (JmjC), Jumonji N (JmjN) and plant homeodomain finger (PHD) (histone-binding domain) domains [32]. The KDM1 family is composed of the KDM1A member and its homolog, KDM1B, which are both Flavin Adenine Dinucleotide (FAD) -dependent histone lysine demethylases [33,34]. The KDM1A consists of three domains: the amine oxidase domain, the FAD binding domain and the SWIRM domain. In particular, the FAD binding domain consists of a Tower domain, which interacts with RE1-Silencing Transcription factor (REST), a transcription factor essential for demethylation activity [35]. KDM1B, however, does not bind REST [36,37]. KDM5A-D and KDM1A-B proteins have histone demethylases activity towards particular histone H3K4 methylation states; for instance, KDM5A demethylates H3K4me3/2 and processively H3K4me1, and KDM1 demethylates H3K4me1/2, with KDM1A also demethylating H3K9 [38–44]. KDM5A, KDM5C and KDM1A proteins form complexes with transcriptional repressors such as REST and KMTs establishing repressive chromatin marks [45,46]. Members of the KDM5 andKDM1 families may differ in their functions and biological effects. The KDM5A-D proteins are associated with transcriptional repression, as H3K4me3 is considered to be a transcriptional activating signal, since it is globally distributed, mainly at the promoters of the transcribed genes, and seems fundamental for recruiting the preini-

tiation factor Transcription Factor IID (TFIID) to certain gene promoters, even if loss of H3K4me3 does not always affect gene transcription [47]. However, KDM5A and B proteins may interact with different partners or complexes with transcriptional repressive functions such as Polycomb Repressive Complex 2 [46,48]. KDM5A interacts with the SIN3B-containing deacetylase and the nucleosome remodeling and deacetylase (NuRD) complexes [49]. KDM5B interacts with NuRD and KDM1A [50,51], whereas KDM5C interacts with the repressive H3K9 and H3K27 methyltransferase G9a in complex with histone deacetylases (HDACs) and REST [52]. Moreover, KDM5B protein may interact directly with HDACs mediating their recruitment to specific sites [53]. However, the activity of KDM5A–D also seems related, in some cases, to transcriptional activation, although it is not clear if this effect depends on demethylase activity or not [54,55]. As for the KDM5 protein family, KDM1 demethylase activity is also related to transcriptional repression. However, KDM1A, as it can demethylate H3K9, may be associated with transcriptional activation [56–58]. For instance, when KDM1A interacts with androgen and estrogen nuclear hormone receptors (AR and ER), it can demethylate H3K9me1/2, thus facilitating gene transcription [59,60]. Moreover, a neuron-specific isoform of KDM1An (also known as LSD1n) can target H3K20me2 controlling transcriptional elongation of a neuronal gene network [61]. Garcia-Bassets et al. [62] reported that 80% of the promoters occupied by KDM1A were bound to RNA polymerase II, suggesting that KDM1A was associated more often with active genes rather than the inactive genes. The formation of a protein complex including KDM1A, Rest corepressor (CoREST) and Growth factor independence (GFI) 1 proteins is also noteworthy [63]. This complex target represses a gene regulatory network that is necessary for normal hematopoiesis. KDM1A–GFI interaction may be disrupted by pharmacological molecules rescuing blast cell differentiation in acute myeloid leukemia with MLL translocations [64] and restoring the normal H3K4me3 state at targeted gene promoters. KDM1A is also found to be associated with long non-coding RNAs (LncRNAs) such as HOX Transcript Antisense RNA(HOTAIR), TElomeric Repeat-containing RNA (TERRA) and Steroid receptor RNA activator (SRA) [65]. Several non-histone proteins have been recognized as targets of KDM1A activity such as p53 [66], MYPT1 [67], E2F1 [68], and HIF-1α [69], which determine different effects on protein stability. JARID1 and LSD demethylases are involved in various cellular processes, including cell proliferation, embryonic mesenchymal transition, stemness, differentiation, cell motility, autophagy and senescence [70,71], and their dysregulation is also closely associated with embryonic development [72], human cancer development and other diseases [73].

4. K4 Methylated Histone H3's Genomic Distribution and Function

The genomic distribution of methylated H3K4 has been studied both in simple eukaryotes, such as yeast, and in higher eukaryotes. In both cases, the distribution of methylated H3K4 is tightly associated with the state of methylation. In budding yeast, H3K4me3 localizes at gene promoter regions, H3K4me2 is mainly distributed within gene bodies and H3K4me1 tends to accumulate towards the 3' end of genes [74]. In multicellular eukaryotes, genome-wide analyses of methylated H3K4 distribution show that H3K4me3 is predominantly localized at gene promoter regions, centered on the transcriptional start sites [75–79], while H3K4me2 tends to localize downstream of the H3K4me3 peak, and H3K4me1 is considered a marker of enhancer regions [80–86], although there is increasing evidence of H3K4me1's role at gene promoters. H3K4me3 marks actively transcribed genes [75,87,88], in addition genes marked by the "broadest H3K4me3 domains" show increased transcriptional consistency [89]. Based on the observations described above, H3K4me3 has been proposed to sustain gene transcription [90,91]. However, the specific state of H3K4 methylation seems to have a role in involving distinct effectors regulating gene expression with different effects. For instance, Sims et al. [92] showed that the ATP-remodeling enzyme CHD1, which recruits the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex [93] and sustains RNA polymerase II activity, recognizes H3K4me3. The bromodomain PHD finger transcription factor (BPTF), which is a component of the Nucleosome

Remodeling Factor (NURF) complex, also recognizes and binds H3K4me3 [94]. Transcription factor IID (TFIID), through its PHD domain-containing TAF3 subunit, is recruited by H3K4me3, allowing more efficient preinitiation complex formation [95]. Several histone acetyltransferase-containing complexes such as SAGA, NuA3 and HBO1 may be recruited by H3K4me3 [96,97]. On the other hand, H3K4me2 was shown to bind to the Set3 complex. The Set3 complex induces histone deacetylation in 5′ transcribed regions. The resulting deacetylation slows gene activation. Set3C may repress internal cryptic promoters, but in different regions of genes from the Set2/Rpd3S pathway. In addition, Set3C induces transcription of some genes by repressing an overlapping antagonistic anti-sense transcript. Set3C histone deacetylase activity can combine with ncRNA transcription to delay or attenuate gene activation [98]. Although, as previously reported, H3K4me1 marks active enhancer regions when associated with H3K27ac, H3K4me1 at gene promoters has been shown to constrain the recruitment of H3K4me3-interacting reader proteins regulating the activity of corresponding genes [85].

5. H3K4me3 and H3K27me3 Overlapping: Genomic Distribution and Function

In the last two decades, the genomic distribution of modified histones has been extensively investigated, especially in relation to functionally distinct genomic compartments. The methodological approaches differ, and the continuous development of techniques capable of large-scale analysis has made it possible to obtain a precise picture of the distribution of modified histones in functionally different genomic regions. However, the functional consequences of these different distributions are still debated, so that more in-depth studies are necessary. For the purposes of this review, we will analyze the distribution and related functions of H3K4 and H3K27 methylation. In 2005, Bernstein et al. [81] and Kim et al. [99] conducted pioneering studies on genomic H3K4me2/3 distribution using human cancer cell lines and mouse fibroblasts cell lines. However, a year later, Bernstein et al. [100] observed that in mouse embryonic stem cells (mESCs), the majority of transcriptional start sites were found to be marked by H3K4me3. In the same study, they showed that H3K27me3 had a broader distribution but 75% of the H3K27me3 sites spanning transcriptional start sites (TSSs) were also marked by H3K4me3. These genomic locations were defined as bivalent domains. Genes that were marked by a bivalent domain at their 5′ end were found to be expressed at low levels, despite the presence of H3K4me3. Bernstein et al. also showed that by promoting differentiation toward the neuronal lineage, some bivalent genes became expressed and lost the H3K27me3 mark. These observations are fundamental to a model in which mainly developmental genes are marked by bivalent domains to pause or activate, or even permanently inactivate transcription as differentiation proceeds. Similar results were obtained in the work of Azaura et al. [101], which exploited replication timing as a surrogate for chromatin accessibility and the transcriptional status of genes. Mikkelsen et al. [102] combined the Chromatin Immunoprecipitation (ChIP) assay with next-generation sequencing (ChIP-seq) to analyze the genome-wide distribution of H3K4me3 and H3K27me3, and found that virtually all promoters with high CpG density (CpG islands) in mouse embryonic stem cells (ES) were marked by H3K4me3 and that a percentage of these promoters also exhibited H3K27me3. In addition, Mikkelsen et al. [102], and later Mohn et al. [103], showed that bivalent domains are not exclusive features of mouse embryonic stem cells (mESCs) but they also exist in differentiated cells, although to a lesser extent, as, during differentiation, bivalent domains are resolved in one direction or the other. Pan et al. [104], Zhao et al. [105] and Cui et al. [106] showed that bivalent domains were also present in cultured human embryonic stem cells (hESCs) and that the resolution of bivalency is required for lineage restriction [107,108]. However, stem cells in developing embryos are only transiently pluripotent, raising the question as to whether bivalency and other characteristics of embryonic stem cell chromatin are present in developing organisms as well. Bivalent domains also exist in pluripotent epiblast cells of early post-implantation embryos in mice [109]. However, one possibility is that bivalent domains might reflect the cellular heterogeneity of the samples analyzed rather than co-occurrence on individual

nucleosomes or H3 histones. Several studies [104,110–113] suggest that bivalent domains exist and are not an artefact arising from culture conditions or heterogeneous samples.

Bernstein et al. [100] provided the first evidence that bivalent domains strongly correlate with CpG islands (CGI) in embryonic stem cells. CGI are a common feature of gene promoters in vertebrate genomes. They are defined by an elevated GC content, a ratio of observed to expected CpG dinucleotides of more than 0.6, 1 kb of length on average in promoter regions, and an unmethylated state in contrast to the methylated state of the vast majority of intragenic and intergenic CpG dinucleotides. Seventy percent of all promoters contain CGI [114–116], and all H3K4me3s mark CGI, whereas H3K27me3 show a broader distribution [102,104] with not all H3K27me3s marking CGI. These observations support the hypothesis that CGI may have a role in the establishment of bivalent domains. Artificially introduced CGI are able to recruit H3K4 and -27's methylation activities [117]. The ability of histone K4 methyl transferases to target unmethylated CpGs depends on the presence of a CXXC domain or zinc finger CXXC (ZF-CXXC) DNA-binding domain. This domain characterizes MLLs [118,119], whereas it is absent in SET1A/B KMTs. However, in this case, the ability to target unmethylated CGI is mediated by a CXXC-containing protein called CFP1 (CXXC finger protein 1) [117,120,121], which is a component of SET1A/B KMT complexes. In addition, Eberl et al. [122] showed that the plant homeodomain finger (PHD) contained in CFP11 may target H3K4me3, favoring a mechanism that is able to sustain the accumulation of H3K4me3 at specific sites. An alternative mechanism for KMT recruitment to CGI is suggested by studies showing that Host cell factor 1, a component of KMT complexes, binds O-linked b-N-acetylglucosamine (O-GlcNAc) transferase (OGT) [123]. The transferase, in turn, interacts with the Ten–Eleven Trans-location (TET) family of proteins [124], which includes well-known players in maintaining the unmethyated state of CpG dinucleotides. Histone variants which are expressed throughout the cell cycle and deposited independently of DNA replication might also play a role in the establishment of bivalent marks at the gene promoter CpGi [125,126]. CGI may also guide the recruitment of the H3K27 methylating enzyme. In embryonic stem cells, H3K27me3-associated CGI are bivalent [102,127]. Polycomb repressive complex 2 (PRC2), through the EZH2 component, is responsible for H3K27 methylation. However, PRC2 components do not contain DNA-binding domains. Several authors [128–134] have shown that different proteins, such as Jarid2, PHF1 and MTF2, might be responsible for the recruitment of the PRC2 complex, and thus of EZH2, to GC-rich sequences. In addition, EZH2 and other PRC2 complexes interact with long ncRNAs [135] and short ncRNAs originating in proximity to or spanning CGI promoters [108–136].

6. CpG Dinucleotide: Unmethylated and Methylated Status

The human genome contains ∼29 million CpG dinucleotides, each of which may exist in the methylated or unmethylated state. Introns, 3′ untranslated regions and intergenic sequences are severely depleted in CpGs, whereas coding exons have a relatively higher density [137]. In contrast, CpG dinucleotides tend to form clusters in correspondence with mammalian gene promoters. These clusters are defined as CpG islands (CGI) and cover almost 75% of all genes, whereas the remaining 25% of genes are characterized as CpG-poor promoters. Whole genome methylation profiles [138–140] have shown that tandem and dispersed transposons tend to be heavily methylated, and exons, introns and intergenic regions tend to be heterogeneously methylated within a population of cells, while CpG-rich promoter regions are almost exclusively unmethylated in all tissue types.

CpG methylation in transposons, exons, introns and intergenic regions is important for long-term silencing, silencing of parasitic sequences and alternative promoters, regulating imprinted gene expression and determining X chromosome inactivation [141,142]. CGI, although rich in CpG dinucleotides, are unmethylated and remain so during all phases of mammalian embryogenesis and development, except in specific cases [143,144]. The biological mechanisms that contribute to the maintenance of the unmethylated state of CGI remain elusive, but the modification of established DNA methylation patterns is a

common feature in all types of tumors and is considered as an event that intrinsically, or in association with genetic lesions, feeds carcinogenesis. In tumors, methylation events occur at promoter CGI, CpG shores, enhancers and insulators [145–149]. This aberrant DNA hypermethylation of such regulatory genomic regions is generally correlated with the repression of tumor suppressor [150–153], metastasis [154] and DNA repair genes [155,156], leading to the conclusion that DNA hypermethylation is directly responsible for the observed gene silencing [157–159]. However, recent experimental data on the characterization of the methylome of normal tissues and derived cancer types suggested that aberrant DNA hypermethylation represents a secondary event stabilizing gene inactivation, as most aberrantly hypermethylated genes in cancer are already repressed in the tissue of origin [160–165]. Nevertheless, DNA hypermethylation represents a mechanism directly affecting the expression of important tumor suppressor genes [166–172].

7. Mechanisms of the Protection of CpG Islands

The mechanisms that might generate a protective effect against CGI methylation are still not clear. The prevalent view is that the frequency of CpG dinucleotides and the content of C and G represent a key feature required to prevent methylation at CGI [117,173–176] (Figure 1). However, these observations must be integrated with others showing that specific transcription factor binding is linked to protection of the underlying DNA sequence [173,174,177–180] (Figure 1). Sequence features and specific transcription factor binding may also determine the recruitment of other proteins that might integrate their functions. In this view, all proteins containing a CXXC domain, such as CFP1, MLL1, MLL2, KDM2A, KDM2B, TET1 and TET3 [181] (Figure 1), which specifically targets them to CGI, might have a putative role in protecting CGI from methylation per se or because they recruit effectors able to block DNA methylation, triggering additional mechanisms. For instance, CFP1 recruits SET1A/B KMTs to CGI, which trimethylate H3K4, and trimethylated H3K4 has a specific role in inhibiting DNA methyltrasferases. H3K4me3 tends to be inversely correlated with DNA methylation [182–184]. The exclusive nature of this association of H3K4me3 with CGI methylation is related to its role in regulating DNA methyltransferase activity (Figure 1).

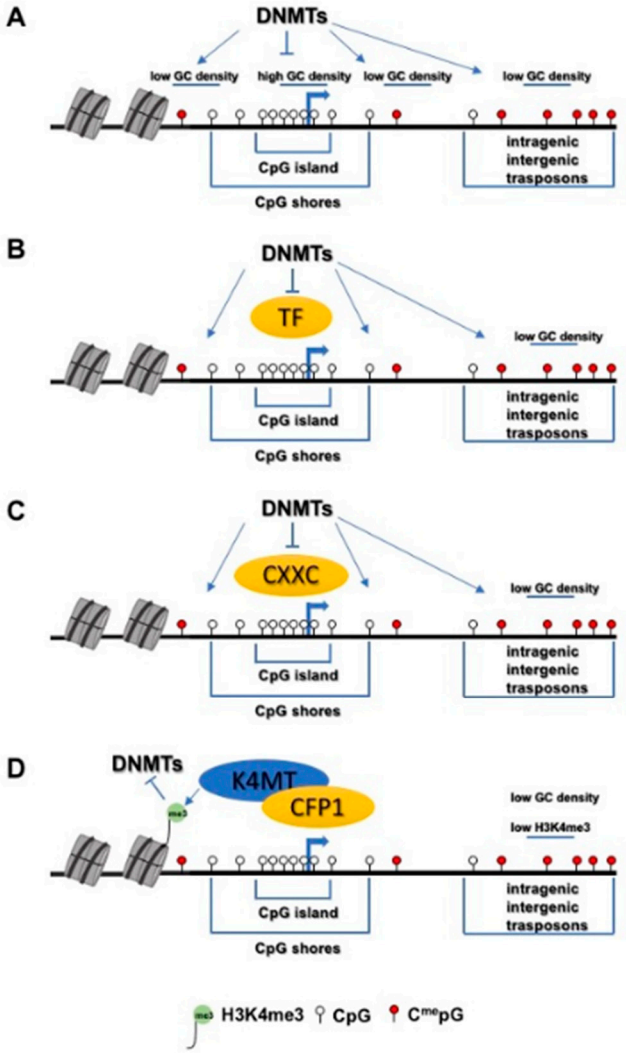

Figure 1. Schematic models of CpG islands protection. (**A**): effect of GC density and CpG frequency on de novo DNA methyltranserases (DNMTs); (**B**): effect of transcription factors on de novo DNMTs; (**C**): effect of CXXC domain containing protein on de novo DNMTs; (**D**): example of the effect produced by the recruitment oh histone H3K4 methyltransferases (K4MT) by CXXC domain containing proteins (CFP1) on the activity of de novo DNMTs.

8. Mechanisms of CGI Hypermethylation in Cancer and the Role of H3K4me3

In cancer, CGI undergo methylation events defined as aberrant DNA hypermethylation. What causes the loss of the protective effect against methylation or, in other words, what interrupts a mechanism that appears to be based on sequence identity? It has been proposed that aberrant hypermethylation of CGI regions may be the consequence of the modified activity of DNA methyltransferases (DNMTs) [185–191]; aberrant recruitment of mutated transcription factors [192]; mutations in demethylating enzymes, such as ten-eleven translocation enzymes (TETs) and their associated cofactor pathways (isocitrate dehydrogenase; IDH1/2) [193–197]; and changes in chromatin architecture depending on post-translational histone modifications. Specific, global and local histone modifications are often associated with distinctive DNA methylation patterns [198]. Several studies investigating the molecular basis of DNA hypermethylation propose an "instructive mechanism" of aberrant DNA methylation in tumors that relies on histone modifications characterizing chromatin in embryonic and adult stem cells. Accordingly, CGI prone to hypermethylation in tumors are embedded in chromatin enriched in H3K27me3 only or H3K27me3 in association with an H3K4me3 mark at the same locus (bivalent domain) in human embryonic and adult stem cells [161,199–201]. This instructive model is supported by the observation

that EZH1/2, a component of the PRC2 complex, is responsible for H3K27 methylation and may recruit de novo DNA methyltransferases (DNMTs) [202,203]. However, in hESCs as well as mESCs, H3K27me3 is mainly located in bivalent domains coinciding with unmethylated CGI [102,199,204,205]. This observation might indicate that H3K27me3 and DNA methylation are mutually exclusive. However, several independent studies have revealed a causal association among PRC2 recruitment, H3K27me3 and DNA hypermethylation during carcinogenesis [206–209]. A possible explanation for this apparent contradiction arises from the observation that TET1 and TET2 have been found to be associated with the PRC2 complex at CpGi in mESCs [210] and in cell lines overexpressing TETs 182]. TET enzymes catalyze hydroxylation of 5-methylcytosine and the active demethylation process, thus maintaining the unmethylated state of CGI.

Recent studies also indicate that methylation of CGI is related to the methylation status of H3K4 (Figure 2); the levels of methylated H3K4 (H3K4me3) tend to be inversely correlated with DNA methylation [183,184].

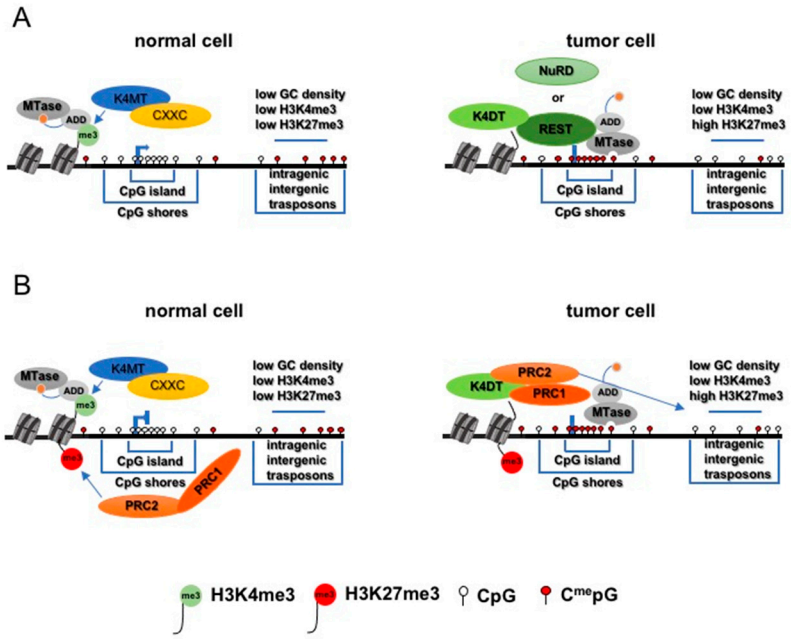

Figure 2. Effect of diminution of H3K4me3 levels on the activity of de novo methyltransferases (DNMTs) in tumor cell. (**A**): Promoter CpG islands marked by H3K4me3 in normal cells are protected by the H3K4me3 induced auto-inhibition of DNMTs. In tumor cell, the aberrant binding of repressive complexes (REST or NuRD) at promoter regions and the consequent recruitment of H3K4me3 demethylases (K4DT) causes the demethylation of H3K4me3 restoring the activity of de novo DNMTs and CpG island hypermethylation. (**B**): Promoter CpG islands marked by bivalent domains in normal cells are protected by the H3K4me3 induced auto-inhibition of DNMTs. In tumor cell, the aberrant recruitment of histone H3K4 demethylases (K4DT) by Polycomb Repressive Complexes (PRC2-PRC1) at promoter regions causes the demethylation of H3K4me3 restoring the activity of de novo DNMTs and CpG island hypermethylation.

This mutually exclusive nature of the association of H3K4me3 with CGI methylation might be related to its role in regulating methyltransferase activity. The ATRX–DNMT3–DNMT3L domain (ADD) in the de novo DNA methyltransferase (DNMT) DNMT3a, for example, recognizes the unmethylated form of H3K4 (H3K4me0), which stimulates methyltransferase activity [211]. Through structural and biochemical analyses, the ADD domain of DNMT3a has been shown to also interact with its catalytic domain (CD), but in the presence of H3K4me3, DNMT3a loses the ability to bind and methylate DNA [212]. Thus, H3K4me0 and H3K4me3 have opposite effects on DNMT3a activity [213]. However, there is no evidence that an H3K4me3's protective effect against aberrant methylation

exists or is lost in tumors. Our studies [214] conducted on an acute myeloid leukemia cancer model suggest a direct role of the deregulation of H3K4me3 levels in determining the hypermethylation of the corresponding CGI.

In our study, we obtained, by Restriction Landmark Genome Scanning (RLGS) DNA methylation profiles of acute myeloid leukemia samples from patients and acute myeloid leukemia (AML) cell lines. We integrated methylation data with publicly available chromatin ChIP-seq data for H3K4me3 and H3K27me3 promoter CGI occupation in hESCs or hematopoietic stem and/or progenitor cells (hHSC/MPP). We observed that, in most cases, hypermethylated CGI in AML display H3K27me3 occupancy, even in the context of a bivalent domain in hESCs and hHSC/MPP. However, the analyses of specific hypermethylated CGI revealed a chromatin context characterized by a significant reduction in the H3K4me3 signal, with a concomitant increase in unmethylated H3K4 levels as opposed to a non-significant increase in the H3K27me3 mark, particularly in AML patient samples. Thus, we concluded that the loss of the normal levels of H3K4me3 in favor of increased levels of unmethylated H3K4 removes the de novo DNMTs auto-inhibition and promotes, as a consequence, aberrant CpGi hypermethylation. We also showed that the diminution of H3K4me3 levels is associated with the defective expression and recruitment patterns of specific writers, erasers and readers to CGI. Our proposal of the critical role of maintaining correct H3K4me3 levels to protect CGI from methylation was also suggested in recent works by Clarck [215] and Meehan [216]. Skvortsova [215] analyzed the pattern of H3K4me1 marked nucleosomes in embryonic stem cells and normal epithelial cells, and the mode of promoter CpG island hypermethylation in cancer. They observed that depletion or enrichment of H3K4me1 levels at the borders of CGI results in loss or gain of DNA methylation encroachment, and that H3K4me3, in contrast to H3K4me1 and H3K27me3, is highly enriched across the body of unmethylated CGI. In cancer cells, H3K4me3 is lost from DNA hypermethylated islands and is notably absent from the internal borders of the islands that undergo DNA methylation encroachment. Thus, relative enrichment of H3K4me1 and H3K4me3 represent a critical interface in predicting CGI DNA hypermethylation in cancer. The H3K4me1/H3K4me3 ratio was shown to be statistically significantly higher at CGI that become hypermethylated in cancer compared with CGI that remain unmethylated.

Moreover, H3K4me1-marked DNA exhibits low basal cytosine methylation in normal cells but a greater shift toward higher methylation levels in cancer cells in comparison with H3K27me3-marked DNA. On the other hand, Dunican et al. [216], using the concept of bivalent domains, revealed the existence in mESCs and hESCs of two subgroups of gene promoters, defined as HighBiv and LowBiv promoters, characterized by a high ratio of H3K27me3/H3K4me3 (enrichment in H3K27me3) and by a low ratio of H3K27me3/H3K4me3 (enrichment in H3K4me3), respectively. These two groups were associated with a different distribution of MLL2 that was enriched in LowBiv promoters, and with a different expression pattern showing that LowBiv promoters are highly expressed, although in the context of the typical reduced expression of bivalent domains. The analysis of DNA methylation levels in HighBiv and LowBiv regions in breast and colon cancer cell lines compared with normal cells showed that HighBiv promoters were more highly methylated than LowBiv promoters in cancer cell lines. In addition, they found little evidence of DNA hypermethylation at promoters that are characterized as H3K4me3-only or H3K27me3-only. Dunican et al. [216] suggested that a possible explanation for these results is that in LowBiv promoters, higher levels of H3K4me3 inhibit de novo methyltransferase activity. Dunican et al. [216] concluded that it could be "useful to investigate whether there is also a link between low level H3K4me3 and de novo DNA methylation at other discrete sets of CGI in cancer". In this context, our study provides evidence that H3K4me3 levels play an important role in maintaining the unmethylated state of CGI in normal cells and that the H3K4me3-methylation-dependent protection of CGI is altered in tumor cells.

9. Conclusions and Perspectives

The modification of the H3K4 methylation state still represents an understudied event among the hypotheses describing how the unmethylated state of CGI is maintained in a normal cell or modified in a cancer cell, despite the fact that H3K4 methylation represents a powerful determinant of the activity of DNMTs 211-213]. To date, the connection between H3K4 trimethylation and the unmethylated state of CGI is derived from studies conducted with the aim of evaluating its genomic distribution [81,99–106]. Although these studies represent milestones in our understanding of the topography of the genomic distribution of H3K4 trimethylation, they do not represent a proof of concept of the role that H3K4 trimethylation may have in protecting CGI. There is no direct evidence that H3K4me3's protective effect against aberrant methylation is lost in tumor cells. In our study, for the first time, to our knowledge, we demonstrated that in AML patient samples and AML cell lines, although based on a limited number of target CGI, DNA hypermethylation is associated with the loss of H3K4me3 and the acquisition of unmethylated H3K4. However, these results are not a proof of concept. Further studies need to be conducted to reveal, the genome-wide genomic distribution of unmethylated H3K4 and the significance of its association with hypermethylated CGI in cancer cells. In addition, a critical goal is to unveil the mechanisms underlying a change in the methylation state of H3K4 and the activity of DNMTs.

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References

1. Greer:, E.L.; Shi, Y. Histone methylation: A dynamic mark in health, disease and inheritance. *Nat. Rev. Genet.* **2012**, *13*, 343–357. [CrossRef] [PubMed]
2. Mohan, M.; Herz, H.-M.; Smith, E.R.; Zhang, Y.; Jackson, J.; Washburn, M.P.; Florens, L.; Eissenberg, J.C.; Shilatifard, A. The COMPASS Family of H3K4 Methylases in Drosophila. *Mol. Cell. Biol.* **2011**, *31*, 4310–4318. [CrossRef] [PubMed]
3. Shilatifard, A. The COMPASS family of histone H3K4 methylases: Mechanisms of regulation in development and disease pathogenesis. *Ann. Rev. Biochem.* **2012**, *81*, 65–95. [CrossRef] [PubMed]
4. Falnes, P.Ø.; Jakobsson, M.E.; Davydova, E.; Ho, A.; Małecki, J. Protein lysine methylation by seven-β-strand methyltransferases. *Biochem. J.* **2016**, *15*, 1995–2009. [CrossRef] [PubMed]
5. Husmann, D.; Gozani, O. Histone lysine methyltransferases in biology and disease. *Nat. Struct. Mol. Biol.* **2019**, *26*, 880–889. [CrossRef]
6. Sun, X.J.; Wei, J.; Wu, X.-Y.; Hu, M.; Wang, L.; Wang, H.-H.; Zhang, Q.-H.; Chen, S.-J.; Huang, Q.-H.; Chen, Z.; et al. Identification and Characterization of a Novel Human Histone H3 Lysine 36-specific Methyltransferase. *J. Biol. Chem.* **2005**, *280*, 35261–35271. [CrossRef]
7. Strahl, B.D.; Grant, P.A.; Briggs, S.D.; Sun, Z.-W.; Bone, J.R.; Caldwell, J.A.; Mollah, S.; Cook, R.G.; Shabanowitz, J.; Hunt, D.F.; et al. Set2 Is a Nucleosomal Histone H3-Selective Methyltransferase That Mediates Transcriptional Repression. *Mol. Cell. Biol.* **2002**, *22*, 1298–1306. [CrossRef]
8. An, S.; Yeo, K.J.; Jeon, Y.H.; Song, J.-J. Crystal Structure of the Human Histone Methyltransferase ASH1L Catalytic Domain and Its Implications for the Regulatory Mechanism. *J. Biol. Chem.* **2011**, *286*, 8369–8374. [CrossRef]
9. Ng, H.H.; Feng, Q.; Wang, H.; Erdjument-Bromage, H.; Tempst, P.; Zhang, Y.; Struhl, K. Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* **2002**, *16*, 1518–1527. [CrossRef]
10. Feng, Q.; Wang, H.; Ng, H.H.; Erdjument-Bromage, H.; Tempst, P.; Struhl, K.; Zhang, Y. Methylation of H3-Lysine 79 Is Mediated by a New Family of HMTases without a SET Domain. *Curr. Biol.* **2002**, *12*, 1052–1058. [CrossRef]
11. Kuo, A.J.; Cheung, P.; Chen, K.; Zee, B.M.; Kioi, M.; Lauring, J.; Xi, Y.; Park, B.H.; Shi, X.; Garcia, B.A.; et al. NSD2 Links Dimethylation of Histone H3 at Lysine 36 to Oncogenic Programming. *Mol. Cell* **2011**, *44*, 609–620. [CrossRef] [PubMed]
12. Edmunds, J.W.; Mahadevan, L.C.; Clayton, A.L. Dynamic histone H3 methylation during gene induction: HYPB/Setd2 mediates all H3K36 trimethylation. *EMBO J.* **2008**, *27*, 406–420. [CrossRef] [PubMed]
13. Schotta, G.; Sengupta, R.; Kubicek, S.; Malin, S.; Kauer, M.; Callén, E.; Celeste, A.; Pagani, M.; Opravil, S.; De La Rosa-Velazquez, I.A.; et al. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. *Genes Dev.* **2008**, *22*, 2048–2061. [CrossRef] [PubMed]
14. Beck, D.B.; Oda, H.; Shen, S.S.; Reinberg, D. PR-Set7 and H4K20me1: At the crossroads of genome integrity, cell cycle, chromosome condensation, and transcription. *Genes Dev.* **2012**, *26*, 325–337. [CrossRef] [PubMed]

15. Kuo, A.J.; Song, J.; Cheung, P.; Ishibe-Murakami, S.; Yamazoe, S.; Chen, J.K.; Patel, D.J.; Gozani, O. The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. *Nature* **2012**, *484*, 115–119. [CrossRef] [PubMed]
16. Hyun, K.; Jeon, J.; Park, K.; Kim, J. Writing, erasing and reading histone lysine methylations. *Exp. Mol. Med.* **2017**, *49*, e324. [CrossRef]
17. Crump, N.T.; Milne, T.A. Why are so many MLL lysine methyltransferases required for normal mammalian development? *Cell. Mol. Life Sci.* **2019**, *76*, 2885–2898. [CrossRef]
18. Yang, W.; Ernst, P. Distinct functions of histone H3, lysine 4 methyltransferases in normal and malignant hematopoiesis. *Curr. Opin. Hematol.* **2017**, *24*, 322–328. [CrossRef]
19. Yang, W.; Ernst, P. SET/MLL family proteins in hematopoiesis and leukemia. *Int. J. Hematol.* **2017**, *105*, 7–16. [CrossRef]
20. Harikumar, A.; Meshorer, E. Chromatin remodeling and bivalent histone modifications in embryonic stem cells. *EMBO Rep.* **2015**, *16*, 1609–1619. [CrossRef]
21. Jiang, Y.; Dominguez, P.M.; Melnick, A.M. The many layers of epigenetic dysfunction in B-cell lymphomas. *Curr. Opin. Hematol.* **2016**, *23*, 377–384. [CrossRef] [PubMed]
22. Froimchuk, E.; Jang, Y.; Ge, K. Histone H3 lysine 4 methyltransferase KMT2D. *Gene* **2017**, *627*, 337–342. [CrossRef] [PubMed]
23. Wang, C.; Lee, J.E.; Lai, B.; Macfarlan, T.S.; Xu, S.; Zhuang, L.; Liu, C.; Peng, W.; Ge, K. Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 11871–11876. [CrossRef] [PubMed]
24. Lee, J.E.; Wang, C.; Xu, S.; Cho, Y.W.; Wang, L.; Feng, X.; Baldridge, A.; Sartorelli, V.; Zhuang, L.; Peng, W.; et al. H3K4 mono- and di-methyltransferase MLL4 is required for enhancer activation during cell differentiation. *eLife* **2013**, *2*, e01503. [CrossRef] [PubMed]
25. Hu, D.; Gao, X.; Morgan, M.A.; Herz, H.M.; Smith, E.R.; Shilatifard, A. The MLL3/MLL4 branches of the COMPASs family function as major histone H3K4 monomethylases at enhancers. *Mol. Cell. Biol.* **2013**, *33*, 4745–4754. [CrossRef]
26. Kwon, M.; Park, K.; Hyun, K.; Lee, J.-H.; Zhou, L.; Cho, Y.-W.; Ge, K.; Skalnik, D.G.; Muir, T.W.; Kim, J. H2B ubiquitylation enhances H3K4 methylation activities of human KMT2 family complexes. *Nucleic Acids Res.* **2020**, *48*, 5442–5456. [CrossRef]
27. Shinsky, S.A.; Monteith, K.E.; Viggiano, S.; Cosgrove, M.S. Biochemical reconstitution and phylogenetic comparison of human SET1 family core complexes involved in histone methylation. *J. Biol. Chem* **2015**, *290*, 6361–6375. [CrossRef]
28. Hsu, P.L.; Li, H.; Lau, H.T.; Leonen, C.; Dhall, A.; Ong, S.E.; Chatterjee, C.; Zheng, N. Crystal structure of the COMPASS H3K4 methyltransferase catalytic module. *Cell* **2018**, *174*, 1106–1116. [CrossRef]
29. Shi, Y.G.; Tsukada, Y. The discovery of histone demethylases. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a017947. [CrossRef]
30. Kang, M.K.; Mehrazarin, S.; Park, N.-H.; Wang, C.-Y. Epigenetic gene regulation by histone demethylases: Emerging role in oncogenesis and inflammation. *Oral Dis.* **2017**, *23*, 709–720. [CrossRef]
31. Højfeldt, J.W.; Agger, K.; Helin, K. Histone lysine demethylases as targets for anticancer therapy. *Nat. Rev. Drug Discov.* **2013**, *12*, 917–930. [CrossRef] [PubMed]
32. Pilka, E.S.; James, T.; Lisztwan, J.H. Structural definitions of Jumonji family demethylase selectivity. *Drug Discov. Today* **2015**, *20*, 743–749. [CrossRef] [PubMed]
33. Shi, Y.; Lan, F.; Matson, C.; Mulligan, P.; Whetstine, J.R.; Cole, P.A.; Casero, R.A.; Shi, Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **2004**, *119*, 941–953. [CrossRef] [PubMed]
34. Janardhan, A.; Kathera, C.; Darsi, A.; Ali, W.; He, L.; Yang, Y.; Luo, L.; Guo, Z. Prominent role of histone lysine demethylases in cancer epigenetics and therapy. *Oncotarget* **2018**, *28*, 34429–34448. [CrossRef] [PubMed]
35. Lee, M.G.; Wynder, C.; Cooch, N.; Shiekhattar, R. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* **2005**, *437*, 432–435. [CrossRef] [PubMed]
36. Ciccone, D.N.; Su, H.; Hevi, S.; Gay, F.; Lei, H.; Bajko, J.; Xu, G.; Li, E.; Chen, T. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **2009**, *461*, 415–418. [CrossRef]
37. Yang, Z.; Jiang, J.; Stewart, M.D.; Qi, S.; Yamane, K.; Li, J.; Zhang, Y.; Wong, J. AOF1 is a histone H3K4 demethylase possessing demethylase activity-independent repression function. *Cell Res.* **2010**, *20*, 276–287. [CrossRef]
38. Yamane, K.; Tateishi, K.; Klose, R.J.; Fang, J.; Fabrizio, L.A.; Erdjument-Bromage, H.; Taylor-Papadimitriou, J.; Tempst, P.; Zhang, Y. PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. *Mol. Cell* **2007**, *25*, 801–812. [CrossRef]
39. Christensen, J.; Agger, K.; Cloos, P.A.C.; Pasini, D.; Rose, S.; Sennels, L.; Rappsilber, J.; Hansen, K.H.; Salcini, A.E.; Helin, K. RBP2 belongs to a family of demethylases, specific for tri- and dimethylated lysine 4 on histone 3. *Cell* **2007**, *128*, 1063–1076. [CrossRef]
40. Iwase, S.; Lan, F.; Bayliss, P.; de la Torre-Ubieta, L.; Huarte, M.; Hank, H.Q.; Whetstine, J.R.; Bonni, A.; Roberts, T.M.; Shi, Y. The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell* **2007**, *128*, 1077–1088. [CrossRef]
41. Klose, R.J.; Yan, Q.; Tothova, Z.; Yamane, K.; Erdjument-Bromage, E.; Tempst, P.; Gilliland, D.G.; Zhang, Y.; Kaelin, W.J., Jr. The retinoblastoma binding protein RBP2 is an H3K4 demethylase. *Cell* **2007**, *128*, 889–900. [CrossRef] [PubMed]
42. Lee, M.G.; Norman, J.; Shilatifard, A.; Shiekhattar, R. Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein. *Cell* **2007**, *128*, 877–887. [CrossRef] [PubMed]
43. Secombe, J.; Li, L.; Carlos, L.; Eisenman, R.N. The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. *Genes Dev.* **2007**, *21*, 537–551. [CrossRef] [PubMed]

44. Seward, D.J.; Cubberley, G.; Kim, S.; Schonewald, M.; Zhang, L.; Tripet, B.; Bentley, D.L. Demethylation of trimethylated histone H3 Lys4 in vivo by JARID1 JmjC proteins. *Nat. Struct. Mol. Biol.* **2007**, *14*, 240–242. [CrossRef]
45. Tahiliani, M.; Mei, P.; Fang, R.; Leonor, T.; Rutenberg, M.; Shimizu, F.; Li, J.; Rao, A.; Shi, Y. The histone H3K4 demethylase SMCX links REST target genes to X- linked mental retardation. *Nature* **2007**, *447*, 601–605. [CrossRef]
46. Pasini, D.; Hansen, K.H.; Christensen, J.; Agger, K.; Cloos, P.A.; Helin, K. Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-repressive complex 2. *Genes Dev.* **2008**, *22*, 1345–1355. [CrossRef]
47. Vermeulen, M.; Mulder, K.W.; Denissov, S.; Pim Pijnappel, W.W.M.; van Schaik, F.M.A.; Varier, R.A.; Baltissen, M.P.A.; Stunnenberg, H.G.; Mann, M.; Timmers, H.T.M. Selective Anchoring of TFIID to Nucleosomes by Trimethylation of Histone H3 Lysine 4. *Cell* **2007**, *131*, 58–69. [CrossRef]
48. Zhang, Y.; Qian Li, J.L. Coordinated regulation of retinoic acid signaling pathway by KDM5B and polycomb repressive complex 2. *J. Cell Biochem.* **2014**, *115*, 1528–1538. [CrossRef]
49. Nishibuchi, G.; Shibata, Y.; Hayakawa, T.; Hayakawa, N.; Ohtani, Y.; Sinmyozu, K.; Tagami, H.; Nakayama, J. Physical and functional interactions between the histone H3K4 demethylase KDM5A and the nucleosome remodeling and deacetylase (NuRD) complex. *J. Biol. Chem.* **2014**, *289*, 28956–28970. [CrossRef]
50. Li, Q.; Shi, L.; Gui, B.; Yu, W.; Wang, J.; Zhang, D.; Han, X.; Yao, Z.; Shang, Y. Binding of the JmjC demethylase JARID1B to LSD1/NuRD suppresses angiogenesis and metastasis in breast cancer cells by repressing chemokine CCL14. *Cancer Res.* **2011**, *71*, 6899–6908. [CrossRef]
51. Scibetta, A.G.; Santangelo, S.; Coleman, J.; Hall, D.; Chaplin, T.; Copier, J.; Catchpole, S.; Burchell, J.; Taylor-Papadimitriou, J. Functional analysis of the transcription repressor PLU-1/JARID1B. *Mol. Cell Biol.* **2007**, *27*, 7220–7235. [CrossRef] [PubMed]
52. Verrier, L.; Vandromme, M.; Trouche, D. Histone demethylases in chromatin cross-talks. *Biol. Cell* **2011**, *103*, 381–401. [CrossRef] [PubMed]
53. Barrett, A.; Santangelo, S.; Tan, K.; Catchpole, S.; Roberts, K.; Spencer-Dene, B.; Hall, D.; Scibetta, A.; Burchell, J. Breast cancer associated transcriptional repressor PLU-1/JARID1B interacts directly with histone deacetylases. *Int. J. Cancer* **2007**, *121*, 265–275. [CrossRef] [PubMed]
54. Lloret-Llinares, M.; Pérez-Lluch, S.; Rossell, D.; Morán, T.; Ponsa-Cobas, J.; Auer, H.; Corominas, M.; Azorín, F. dKDM5/LID regulates H3K4me3 dynamics at the transcription-start site (TSS) of actively transcribed developmental genes. *Nucleic Acids Res.* **2012**, *40*, 9493–9505. [CrossRef] [PubMed]
55. He, R.; Kidder, B.L. H3K4 demethylase KDM5B regulates global dynamics of transcription elongation and alternative splicing in embryonic stem cells. *Nucleic Acids Res.* **2017**, *45*, 6427–6441. [CrossRef] [PubMed]
56. Laurent, B.; Ruitu, L.; Murn, J.; Hempel, K.; Ferrao, R.; Xiang, Y.; Liu, S.; Garcia, B.A.; Wu, H.; Wu, F.; et al. A specific LSD1/KDM1A isoform regulates neuronal differentiation through H3K9 demethylation. *Mol. Cell* **2015**, *57*, 957–970. [CrossRef]
57. Metzger, E.; Wissmann, M.; Yin, N.; Müller, J.M.; Schneider, R.; Peters, A.H.F.M.; Günther, T.; Buettner, R.; Schüle, R. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **2005**, *437*, 436–439. [CrossRef]
58. Wang, J.; Scully, K.; Zhu, X.; Cai, L.; Zhang, J.; Prefontaine, G.G.; Krones, A.; Ohgi, K.A.; Zhu, P.; Garcia-Bassets, I.; et al. Opposing LSD1 complexes function in developmental gene activation and repression programmes. *Nature* **2007**, *446*, 882–887. [CrossRef]
59. Perillo, B.; Ombra, M.N.; Bertoni, A.; Cuozzo, C.; Sacchetti, S.; Sasso, A.; Chiariotti, L.; Malorni, A.; Abbondanza, C.; Avvedimento, E.V. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science* **2008**, *319*, 202–206. [CrossRef]
60. Cai, C.M.; He, H.H.; Gao, S.; Chen, S.; Yu, Z.; Gao, Y.; Chen, S.; Chen, M.W.; Zhang, J.; Ahmed, M.; et al. Lysine-specific demethylase 1 has dual func- tions as a major regulator of androgen receptor transcriptional activity. *Cell Rep.* **2014**, *9*, 1618–1627. [CrossRef]
61. Wang, J.; Telese, F.; Tan, Y.; Li, W.; Jin, C.; He, X.; Basnet, H.; Ma, Q.; Merkurjev, D.; Zhu, X.; et al. LSD1n is an H4K20 demethylase regulat- ing memory formation via transcriptional elongation control. *Nat. Neurosci.* **2015**, *18*, 1256–1264. [CrossRef] [PubMed]
62. Garcia-Bassets, I.; Kwon, Y.S.; Telese, F.; Prefontaine, G.G.; Hutt, K.R.; Cheng, C.S.; Ju, B.G.; Ohgi, K.A.; Wang, J.; Escoubet-Lozach, L.; et al. Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. *Cell* **2007**, *128*, 505–518. [CrossRef] [PubMed]
63. Saleque, S.; Kim, J.W.; Rooke, H.M.; Orkin, S.H. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. *Mol. Cell* **2007**, *27*, 562–572. [CrossRef] [PubMed]
64. Maiques-Diaz, A.; Spencer, G.J.; Lynch, J.T.; Ciceri, F.; Williams, E.L.; Amaral, F.M.R.; Wiseman, D.H.; Harris, W.J.; Li, Y.; Sahoo, S.; et al. Enhancer activation by pharmaco- logic displacement of LSD1 from GFI1 induces differentiation in acute myeloid leukemia. *Cell Rep.* **2018**, *22*, 3641–3659. [CrossRef] [PubMed]
65. Majello, B.; Gorini, F.; Sacca, C.D.; Amente, S. Expanding the role of the histone lysine-specific demethylase LSD1 in cancer. *Cancers* **2019**, *11*, 324. [CrossRef]
66. Huang, J.; Sengupta, H.; Espejo, A.B.; Lee, M.G.; Dorsey, J.A.; Richter, M.; Opravil, S.; Shiekhattar, R.; Bedford, M.T.; Jenuwein, T.; et al. p53 is regulated by the lysine demethylase LSD1. *Nature* **2007**, *449*, 105–108. [CrossRef]
67. Cho, H.S.; Suzuki, T.; Dohmae, N.; Hayami, S.; Unoki, M.; Yoshimatsu, M.; Toyokawa, G.; Takawa, M.; Chen, T.; Kurash, J.K.; et al. Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. *Cancer Res.* **2011**, *71*, 655–660. [CrossRef]

68. Kontaki, H.; Talianidis, I. Lysine methylation regulates E2F1- induced cell death. *Mol. Cell* **2010**, *39*, 152–160. [CrossRef]
69. Baek, S.H.; Kim, K.I. Regulation of HIF-1 alpha stability by lysine methylation. *BMB Rep.* **2016**, *49*, 245–246. [CrossRef]
70. Lan, F.; Nottke, A.C.; Shi, Y. Mechanisms involved in the regulation of histone lysine demethylases. *Curr. Opin. Cell Biol.* **2008**, *20*, 316–325. [CrossRef]
71. Ambrosio, S.; Sacca, C.D.; Majello, B. Epigenetic regulation of epithelial to mesenchymal transition by the Lysine-specific. demethylase LSD1/KDM1A. *Biochim. Biophys. Acta Gene Regul. Mech.* **2017**, *1860*, 905–910. [CrossRef] [PubMed]
72. Whyte, W.A.; Bilodeau, S.; Orlando, D.A.; Hoke, H.A.; Frampton, G.M.; Foster, C.T.; Cowley, S.M.; Young, R.A. Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. *Nature* **2012**, *482*, 221–225. [CrossRef] [PubMed]
73. Amente, S.; Lania, L.; Majello, B. The histone LSD1 demethylase in stemness and cancer transcription programs. *Biochim. Biophys. Acta* **2013**, *1829*, 981–986. [CrossRef] [PubMed]
74. Soares, L.M.; He, P.C.; Chun, Y.; Suh, H.; Kim, T.S.; Buratowski, S. Determinants of Histone H3K4 Methylation Patterns. *Mol. Cell* **2017**, *68*, 773–785. [CrossRef] [PubMed]
75. Barski, A.; Cuddapah, S.; Cui, K.; Roh, T.-Y.; Schones, D.E.; Wang, Z.; Wei, G.; Chepelev, I.; Zhao, K. High-resolution profiling of histone methylations in the human genome. *Cell* **2007**, *129*, 823–837. [CrossRef] [PubMed]
76. Wang, Z.; Zang, C.; Rosenfeld, J.A.; Schones, D.E.; Barski, A.; Cuddapah, S.; Cui, K.; Roh, T.-Y.; Peng, W.; Zhang, M.Q.; et al. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Gen.* **2008**, *40*, 897–903. [CrossRef]
77. Nie, Y.; Liu, H.; Sun, X. The patterns of histone modifications in the vicinity of transcription factor binding sites in human lymphoblastoid cell lines. *PLoS ONE* **2013**, *8*, e6002. [CrossRef]
78. Siggens, L.; Ekwall, K. Epigenetics, chromatin and genome organization: Recent advances from the ENCODE project. *J. Intern. Med.* **2014**, *276*, 201–214. [CrossRef]
79. Liu, X.; Wang, C.; Liu, W.; Li, J.; Li, C.; Kou, X.; Chen, J.; Zhao, Y.; Gao, H.; Wang, H.; et al. Distinct features of H3K4me3 and H3K27me3 chromatin domains in pre-implantation embryos. *Nature* **2016**, *537*, 558–562. [CrossRef]
80. Schneider, R.; Bannister, A.J.; Myers, F.A.; Thorne, A.W.; Crane-Robinson, C.; Kouzarides, T. Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. *Nat. Cell Biol.* **2004**, *6*, 73–77. [CrossRef]
81. Bernstein, B.E.; Kamal, M.; Lindblad-Toh, K.; Bekiranov, S.; Bailey, D.K.; Huebert, D.J.; McMahon, S.; Karlsson, E.K.; Kulbokas, E.J.; Gingeras, T.R.; et al. Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* **2005**, *120*, 169–181. [CrossRef] [PubMed]
82. Heintzman, N.D.; Stuart, R.K.; Hon, G.; Fu, Y.; Ching, C.W.; Hawkins, R.D.; Barrera, L.O.; Van Calcar, S.; Qu, C.; Ching, K.A.; et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* **2007**, *39*, 311–318. [CrossRef] [PubMed]
83. Heintzman, N.D.; Hon, G.C.; Hawkins, R.D.; Kheradpour, P.; Stark, A.; Harp, L.F.; Ye, Z.; Lee, L.K.; Stuart, R.K.; Ching, C.W.; et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* **2009**, *459*, 108–112. [CrossRef] [PubMed]
84. Calo, E.; Wysocka, J. Modification of enhancer chromatin: What, how, and why? *Mol. Cell* **2013**, *49*, 825–837. [CrossRef] [PubMed]
85. Cheng, J.; Blum, R.; Bowman, C.; Hu, D.; Shilatifard, A.; Shen, S.; Dynlacht, B.D. A role for H3K4 monomethylation in gene repression and partitioning of chromatin readers. *Mol. Cell* **2014**, *53*, 979–992. [CrossRef] [PubMed]
86. Vavouri, T.; Lehner, B. Human genes with CpG island promoters have a distinct transcription-associated chromatin organization. *Genome Biol.* **2012**, *13*, R110. [CrossRef] [PubMed]
87. Guenther, M.G.; Levine, S.S.; Boyer, L.A.; Jaenisch, R.; Young, R.A. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* **2007**, *130*, 77–88. [CrossRef]
88. Dong, X.; Greven, M.C.; Kundaje, A.; Djebali, S.; Brown, J.B.; Cheng, C.; Gingeras, T.R.; Gerstein, M.; Guigó, R.; Birney, E.; et al. Modeling gene expression using chromatin features in various cellular contexts. *Genome Biol.* **2012**, *13*, R53. [CrossRef]
89. Benayoun, B.A.; Pollina, E.A.; Uçar, D.; Mahmoudi, S.; Karra, K.; Wong, E.D.; Devarajan, K.; Daugherty, A.C.; Kundaje, A.B.; Mancini, E.; et al. H3K4me3 breadth is linked to cell identity and transcriptional consistency. *Cell* **2014**, *158*, 673–688. [CrossRef]
90. Kusch, T. Histone H3 lysine 4 methylation revisited. *Transcription* **2012**, *3*, 310–314. [CrossRef]
91. Cruz, C.; della Rosa, M.; Krueger, C.; Gao, Q.; Horkai, D.; King, M.; Field, L.; Houseley, J. Tri-methylation of histone H3 lysine 4 facilitates gene expression in ageing cells. *Elife* **2018**, *7*, e34081. [CrossRef] [PubMed]
92. Sims, R.J., III; Chen, C.F.; Santos-Rosa, H.; Kouzarides, T.; Patel, S.S.; Reinberg, D. Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *J. Biol. Chem.* **2006**, *280*, 41789–41792. [CrossRef] [PubMed]
93. Pray-Grant, M.; Daniel, J.; Schieltz, D.; Yates, J.R., III; Grant, P.A. Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature* **2005**, *433*, 434–438. [CrossRef] [PubMed]
94. Wysocka, J.; Swigut, T.; Xiao, H.; Milne, T.A.; Kwon, S.Y.; Landry, J.; Kauer, M.; Tackett, A.J.; Chait, B.T.; Badenhorst, P.; et al. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* **2006**, *442*, 86–90. [CrossRef]
95. Lauberth, S.M.; Nakayama, T.; Wu, X.; Ferris, A.; Tang, Z.; Hughes, S.S.; Roeder, R.G. H3K4me3 Interactions with TAF3 Regulate Preinitiation Complex Assembly and Selective Gene Activation. *Cell* **2013**, *152*, 1021–1036. [CrossRef]
96. Saksouk, N.; Avvakumov, N.; Champagne, K.S.; Hung, T.; Doyon, Y.; Cayrou, C.; Paquet, E.; Ullah, M.; Landry, A.J.; Côté, V.; et al. HBO1 HAT complexes target chromatin throughout gene coding GENE regions via multiple PHD finger interactions with histone H3 tail. *Mol. Cell* **2009**, *33*, 257–265. [CrossRef]

97. Martin, B.J.E.; McBurney, K.L.; Maltby, V.E.; Jensen, K.N.; Brind'Amour, J.; Howe, L.J. Histone H3K4 and H3K36 Methylation Independently Recruit the NuA3 Histone Acetyltransferase in Saccharomyces cerevisiae. *Genetics* **2017**, *205*, 1113–1123. [CrossRef]
98. Kim, T.S.; Xu, Z.; Clauder-Münster, S.; Steinmetz, L.M.; Buratowski, S. Set3 HDAC Mediates Effects of Overlapping Noncoding Transcription on Gene Induction Kinetics. *Cell* **2012**, *150*, 1158–1169. [CrossRef]
99. Kim, T.H.; Barrera, L.O.; Zheng, M.; Qu, C.; Singer, M.A.; Richmond, T.A.; Wu, Y.; Green, R.D.; Ren, B. A high-resolution map of active promoters in the human genome. *Nature* **2005**, *436*, 876–880. [CrossRef]
100. Bernstein, B.E.; Mikkelsen, T.S.; Xie, X.; Kamal, M.; Huebert, D.J.; Cuff, J.; Fry, B.; Meissner, A.; Wernig, M.; Plath, K.; et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **2006**, *125*, 315–326. [CrossRef]
101. Azuara, V.; Perry, P.; Sauer, S.; Spivakov, M.; Jørgensen, H.F.; John, R.M.; Gouti, M.; Casanova, M.; Warnes, G.; Merkenschlager, M.; et al. Chromatin signatures of pluripotent cell lines. *Nat. Cell Biol.* **2006**, *8*, 532–538. [CrossRef] [PubMed]
102. Mikkelsen, T.S.; Ku, M.; Jaffe, D.B.; Issac, B.; Lieberman, E.; Giannoukos, G.; Alvarez, P.; Brockman, W.; Kim, T.-K.; Koche, R.P.; et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **2007**, *448*, 553–560. [CrossRef] [PubMed]
103. Mohn, F.; Weber, M.; Rebhan, M.; Roloff, T.C.; Richter, J.; Stadler, M.B.; Bibel, M.; Schubeler, D. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol. Cell* **2008**, *30*, 755–766. [CrossRef] [PubMed]
104. Pan, G.; Tian, S.; Nie, J.; Yang, C.; Ruotti, V.; Wei, H.; Jonsdottir, G.A.; Stewart, R.; Thomson, J.A. Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell* **2007**, *1*, 299–312. [CrossRef]
105. Zhao, X.D.; Han, X.; Chew, J.L.; Liu, J.; Chiu, K.P.; Choo, A.; Orlov, Y.L.; Sung, W.-K.; Shahab, A.; Kuznetsov, V.A.; et al. Whole-genome mapping of histone H3 Lys4 and 27 trimethylations reveals distinct genomic compartments in human embryonic stem cells. *Cell Stem Cell* **2007**, *1*, 286–298. [CrossRef]
106. Cui, K.; Zang, C.; Roh, T.-Y.; Schones, D.E.; Childs, R.W.; Peng, W.; Zhao, K. Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* **2009**, *4*, 80–93. [CrossRef]
107. Zardo, G.; Cimino, G.; Nervi, C. Epigenetic plasticity of chromatin in embryonic and hematopoietic stem/progenitor cells: Therapeutic potential of cell reprogramming. *Lukemia* **2008**, *22*, 1503–1518. [CrossRef]
108. Zardo, G.; Ciolfi, A.; Vian, L.; Starnes, L.M.; Billi, M.; Racanicchi, S.; Maresca, C.; Fazi, F. Polycombs and microRNA-223 regulate human granulopoiesis by transcriptional control of target gene expression. *Blood* **2012**, *119*, 4034–4046. [CrossRef]
109. Rugg-Gunn, P.J.; Cox, B.J.; Ralston, A.; Rossant, J. Distinct histone modifications in stem cell lines and tissue lineages from the early mouse embryo. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10783–10790. [CrossRef]
110. Roh, T.; Cuddapah, S.; Cui, K.; Zhao, K. The genomic landscape of histone modifications in human T cells. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15782–15787. [CrossRef]
111. Brookes, E.; de Santiago, I.; Hebenstreit, D.; Morris, K.J.; Carroll, T.; Xie, S.Q.; Stock, J.K.; Heidemann, M.; Eick, D.; Nozaki, N.; et al. Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ESCs. *Cell Stem Cell* **2012**, *10*, 157–170. [CrossRef] [PubMed]
112. Xie, W.; Ling, T.; Zhou, Y.; Feng, W.; Zhu, Q.; Stunnenberg, H.G.; Grummt, I.; Tao, W. The chromatin remodeling complex NuRD establishes the poised state of rRNA genes characterized by bivalent histone modifications and altered nucleosome positions. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 8161–8166. [CrossRef] [PubMed]
113. Voigt, P.; Leroy, G.; Drury, W.J., 3rd; Zee, B.M.; Son, J.; Beck, D.B.; Young, N.L.; Garcia, B.A.; Reinberg, D. Asymmetrically modified nucleosomes. *Cell* **2012**, *151*, 181–193. [CrossRef] [PubMed]
114. Deaton, A.M.; Bird, A. CpG islands and the regulation of transcription. *Genes Dev.* **2011**, *25*, 1010–1022. [CrossRef]
115. Illingworth, R.; Kerr, A.; Desousa, D.; Jorgensen, H.; Ellis, P.; Stalker, J.; Jackson, D.; Clee, C.; Plumb, R.; Rogers, J.; et al. A novel CpG island set identifies tissue-specific methylation at developmental gene loci. *PLoS Biol.* **2008**, *6*, e22. [CrossRef]
116. Illingworth, R.S.; Gruenewald-Schneider, U.; Webb, S.; Kerr, A.R.W.; James, K.D.; Turner, D.J.; Smith, C.; Harrison, D.J.; Andrews, R.; Bird, A.P. Orphan CpG islands identify numerous conserved promoters in the mammalian genome. *PLoS Genet.* **2010**, *6*, e1001134. [CrossRef]
117. Thomson, J.P.; Skene, P.J.; Selfridge, J.; Clouaire, T.; Guy, J.; Webb, S.; Kerr, A.R.W.; Deaton, A.; Andrews, R.; James, K.D.; et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* **2010**, *464*, 1082–1086. [CrossRef]
118. Birke, M.; Schreiner, S.; García-Cuéllar, M.-P.; Mahr, K.; Titgemeyer, F.; Slany, R.K. The MT domain of the proto-oncoprotein MLL binds to CpG-containing DNA and discriminates against methylation. *Nucleic Acids Res.* **2002**, *30*, 958–965. [CrossRef]
119. Bach, C.; Mueller, D.; Buhl, S.; Garcia-Cuellar, M.P.; Slany, R.K. Alterations of the CxxC domain preclude oncogenic activation of mixed-lineage leukemia 2. *Oncogene* **2009**, *28*, 815–823. [CrossRef]
120. Lee, J.H.; Voo, K.S.; Skalnik, D.G. Identification and characterization of the DNA binding domain of CpG-binding protein. *J. Biol. Chem.* **2001**, *276*, 44669–44676. [CrossRef]
121. Clouaire, T.; Webb, S.; Skene, P.; Illingworth, R.; Kerr, A.; Andrews, R.; Lee, J.-H.; Skalnik, D.; Bird, A. Cfp1 integrates both CpG content and gene activity for accurate H3K4me3 deposition in embryonic stem cells. *Genes Dev.* **2012**, *261*, 1714–1728. [CrossRef] [PubMed]
122. Eberl, H.C.; Spruijt, C.G.; Kelstrup, C.D.; Vermeulen, M.; Mann, M. A map of general and specialized chromatin readers in mouse tissues generated by label-free interaction proteo- mics. *Mol. Cell* **2013**, *49*, 368–378. [CrossRef] [PubMed]

123. Capotosti, F.; Guernier, S.; Lammers, F.; Waridel, P.; Cai, Y.; Jin, J.; Conaway, J.W.; Conaway, R.C.; Herr, W. O-GlcNAc transferase catalyzes site-specific proteolysis of HCF-1. *Cell* **2011**, *144*, 376–388. [CrossRef] [PubMed]
124. Vella, P.; Scelfo, A.; Jammula, S.; Chiacchiera, F.; Williams, K.; Cuomo, A.; Roberto, A.; Christensen, J.; Bonaldi, T.; Helin, K.; et al. Tet proteins connect the O-linked N-acetylglucosamine transferase Ogt to chromatin in embryonic stem cells. *Mol. Cell* **2013**, *49*, 645–656. [CrossRef] [PubMed]
125. Hu, G.; Cui, K.; Northrup, D.; Liu, C.; Wang, C.; Tang, Q.; Ge, K.; Levens, D.; Crane-Robinson, C.; Zhao, K. H2A.Z facilitates access of active and repressive complexes to chromatin in embryonic stem cell self-renewal and differentiation. *Cell Stem Cell* **2013**, *12*, 180–192. [CrossRef]
126. Wang, Y.; Long, H.; Yu, J.; Dong, L.; Wassef, M.; Zhuo, B.; Li, X.; Zhao, J.; Wang, M.; Liu, C.; et al. Histone variants H2A.Z and H3.3 coordinately regulate PRC2-dependent H3K27me3 deposition and gene expression regulation in mES cells. *BMC Biol.* **2018**, *16*, 107. [CrossRef]
127. Ku, M.; Koche, R.P.; Rheinbay, E.; Mendenhall, E.M.; Endoh, M.; Mikkelsen, T.S.; Presser, A.; Nusbaum, C.; Xie, X.; Chi, A.S.; et al. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet.* **2008**, *4*, e1000242. [CrossRef]
128. Kim, H.; Kang, K.; Kim, J. AEBP2 as a potential targeting protein for Polycomb Repression Complex PRC2. *Nucleic Acids Res.* **2009**, *37*, 2940–2950. [CrossRef]
129. Peng, J.C.; Valouev, A.; Swigut, T.; Zhang, J.; Zhao, Y.; Sidow, A.; Wysocka, J. Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **2009**, *139*, 1290–1302. [CrossRef]
130. Shen, X.; Kim, W.; Fujiwara, Y.; Simon, M.D.; Liu, Y.; Mysliwiec, M.R.; Yuan, G.-C.; Lee, Y.; Orkin, S.H. Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells. *Cell* **2009**, *139*, 1303–1314. [CrossRef]
131. Landeira, D.; Sauer, S.; Poot, R.; Dvorkina, M.; Mazzarella, L.; Jørgensen, H.F.; Pereira, C.F.; Leleu, M.; Piccolo, F.M.; Spivakov, M.; et al. Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to develop- mental regulators. *Nat. Cell Biol.* **2010**, *12*, 618–624. [CrossRef] [PubMed]
132. Li, G.; Margueron, R.; Ku, M.; Chambon, P.; Bernstein, B.E.; Reinberg, D. Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev.* **2010**, *24*, 368–380. [CrossRef] [PubMed]
133. Pasini, D.; Bracken, A.P.; Jensen, M.R.; Lazzerini Denchi, E.; Helin, K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* **2004**, *23*, 4061–4071. [CrossRef] [PubMed]
134. Simon, J.A.; Kingston, R.E. Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping tran- scriptional traffic, and staying put. *Mol. Cell* **2013**, *49*, 808–824. [CrossRef]
135. Rinn, J.L.; Chang, H.Y. Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem.* **2012**, *81*, 145–166. [CrossRef] [PubMed]
136. Kanhere, A.; Viiri, K.; Araújo, C.C.; Rasaiyaah, J.; Bouwman, R.D.; Whyte, W.A.; Pereira, C.F.; Brookes, E.; Walker, K.; Bell, G.W.; et al. Short RNAs are transcribed from repressed polycomb target genes and interact with polycomb repressive complex-2. *Mol. Cell* **2010**, *38*, 675–688. [CrossRef] [PubMed]
137. Louie, E.; Ott, J.; Majewski, J. Nucleotide frequency variation across human genes. *Genome Res.* **2003**, *13*, 2594–2601. [CrossRef]
138. Lister, R.; Mukamel, E.A.; Nery, J.R.; Urich, M.; Puddifoot, C.A.; Johnson, N.D.; Lucero, J. Global epigenomic reconfiguration during mammalian brain development. *Science* **2013**, *341*, 1237905. [CrossRef]
139. Edwards, J.R.; O'Donnell, A.H.; Rollins, R.A.; Peckham, H.E.; Lee, C.; Milekic, M.H.; Chanrion, B.; Fu, Y.; Su, T.; Hibshoosh, H.; et al. Chromatin and sequence features that define the fine and gross structure of genomic methylation patterns. *Genome Res.* **2010**, *20*, 972–980. [CrossRef]
140. Stadler, M.; Murr, R.; Burger, L.; Ivanek, R.; Lienert, F.; Schöler, A.; van Nimwegen, E.; Wirbelauer, C.; Oakeley, E.J.; Gaidatzis, D.; et al. DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* **2011**, *480*, 490–495. [CrossRef]
141. Mohandas, T.; Sparkes, R.S.; Shapiro, L.J. Reactivation of an inactive human X chromosome: Evidence for X inactivation by DNA methylation. *Science* **1981**, *211*, 393–396. [CrossRef] [PubMed]
142. Greenberg, M.V.C.; Bourchis, D. The diverse roles of DNA methylation in mammalian development and disease. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 590–607. [CrossRef] [PubMed]
143. Borgel, J.; Guibert, S.; Li, Y.; Chiba, H.; Schübeler, D.; Sasaki, H.; Forné, T.; Weber, M. Targets and dynamics of promoter DNA methylation during early mouse development. *Nat. Genet.* **2010**, *42*, 1093–1100. [CrossRef] [PubMed]
144. Auclair, G.; Guibert, S.; Bender, A.; Weber, M. Ontogeny of CpG island methylation and specificity of DNMT3 methyltransferases during embryonic development in the mouse. *Genome Biol.* **2014**, *15*, 545. [CrossRef] [PubMed]
145. Irizarry, R.A.; Ladd-Acosta, C.; Wen, B.; Wu, Z.; Montano, C.; Onyango, P.; Cui, H.; Gabo, K.; Rongione, M.; Webster, M.; et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat. Genet.* **2009**, *41*, 178–186. [CrossRef] [PubMed]
146. Pfeifer, G.P. Defining driver DNA methylation changes in human cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1166. [CrossRef]
147. Taberlay, P.C.; Statham, A.L.; Kelly, T.K.; Clark, S.J.; Jones, P.A. Reconfiguration of nucleosome-depleted regions at distal regulatory elements accompanies DNA methylation of enhancers and insulators in cancer. *Genome Res.* **2014**, *9*, 1421–1432. [CrossRef]
148. Doi, A.; Park, I.H.; Wen, B.; Murakami, P.; Aryee, M.J.; Irizarry, R.; Herb, B.; Ladd-Acosta, C.; Rho, J.; Loewer, S.; et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat. Genet.* **2009**, *41*, 1350–1353. [CrossRef]

149. Ji, H.; Ehrlich, L.I.; Seita, J.; Murakami, P.; Doi, A.; Lindau, P.; Lee, H.; Aryee, M.J.; Irizarry, R.A.; Kim, K. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* **2010**, *467*, 338–342. [CrossRef]
150. Greger, V.; Debus, N.; Lohmann, D.; Hopping, W.; Passarge, E.; Horsthemke, B. Frequency and parental origin of hypermethylated RB1 alleles in retinoblastoma. *Hum. Genet.* **1994**, *94*, 491–496. [CrossRef]
151. Herman, J.G.; Latif, F.; Weng, Y.; Lerman, M.I.; Zbar, B.; Liu, S.; Samid, D.; Duan, D.S.; Gnarra, J.R.; Linehan, W.M. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 9700–9704. [CrossRef]
152. Stirzaker, C.; Millar, D.S.; Paul, C.L.; Warnecke, P.M.; Harrison, J.; Vincent, P.C.; Frommer, M.; Clark, S.J. Extensive DNA methylation spanning the Rb promoter in retinoblastoma tumors. *Cancer Res.* **1997**, *57*, 2229–2237. [PubMed]
153. Merlo, A.; Herman, J.G.; Mao, L.; Lee, D.J.; Gabrielson, E.; Burger, P.C.; Baylin, S.B.; Sidransky, D. 5′ CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat. Med.* **1995**, *1*, 686–692. [CrossRef] [PubMed]
154. Graff, J.R.; Gabrielson, E.; Fujii, H.; Baylin, S.B.; Herman, J.G. Methylation patterns of the E-cadherin 5′CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. *J. Biol. Chem.* **2000**, *275*, 2727–2732. [CrossRef] [PubMed]
155. Costello, J.F.; Futscher, B.W.; Kroes, R.A.; Pieper, R.O. Methylation-related chromatin structure is associated with exclusion of transcription factors from and suppressed expression of the O-6-methylguanineDNA methyltransferase gene in human glioma cell lines. *Mol. Cell Biol.* **1994**, *14*, 6515–6521. [CrossRef] [PubMed]
156. Herman, J.G.; Umar, A.; Polyak, K.; Graff, J.R.; Ahuja, N.; Issa, J.P.; Markowitz, S.; Willson, J.K.; Hamilton, S.R.; Kinzler, K.W.; et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6870–6875. [CrossRef] [PubMed]
157. Boyes, J.; Bird, A. Repression of genes by DNA methylation depends on CpG density and promoter strength: Evidence for involvement of a methyl-CpG binding protein. *EMBO J.* **1992**, *11*, 327–333. [CrossRef]
158. Veigl, M.L.; Kasturi, L.; Olechnowicz, J.; Ma, A.; Lutterbaugh, J.D.; Periyasamy, S.; Li, G.-M.; Drummond, J.; Modrich, P.L.; Sedwick, W.D.; et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 8698–8702. [CrossRef]
159. Veeck, J.; Ropero, S.; Setien, F. BRCA1 CpG island hypermethylation predicts sensitivity to poly(adenosine diphosphate)-ribose polymerase inhibitors. *J. Clin. Oncol.* **2010**, *28*, e563–e564. [CrossRef]
160. Sproul, D.; Nestor, C.; Culley, J.; Dickson, J.; Dixon, J.M.; Harrison, D.J.; Meehan, R.R.; Sims, A.H.; Ramsahoye, B.H. Transcriptionally repressed genes become aberrantly methylated and distinguish tumors of different lineages in breast cancer. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4364–4369. [CrossRef]
161. Hinoue, T.; Weisenberger, D.J.; Lange, C.P.; Shen, H.; Byun, H.; van den Berg, D.; Malik, S.; Pan, F.; Noushmehr, H.; van Dijk, C.M.; et al. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome Res.* **2012**, *22*, 271–282. [CrossRef] [PubMed]
162. Houshdaran, S.; Hawley, S.; Palmer, C.; Campan, M.; Olsen, M.N.; Ventura, A.P.; Knudsen, B.S.; Drescher, C.W.; Urban, N.D.; Brown, P.O.; et al. DNA Methylation Profiles of Ovarian Epithelial Carcinoma Tumors and Cell Lines. *PLoS ONE* **2010**, *5*, e9359. [CrossRef] [PubMed]
163. Keshet, I.; Schlesinger, Y.; Farkash, S.; Rand, E.; Hecht, M.; Segal, E.; Pikarsky, E.; A Young, R.; Niveleau, A.; Cedar, H.; et al. Evidence for an instructive mechanism of de novo methylation in cancer cells. *Nat. Genet.* **2006**, *38*, 149–153. [CrossRef] [PubMed]
164. Pike, B.L.; Greiner, T.C.; Wang, X.; Weisenburger, D.D.; Hsu, Y.-H.; Renaud, G.; Wolfsberg, T.G.; Kim, M.; Weisenberger, D.J.; Siegmund, K.D.; et al. DNA methylation profiles in diffuse large B-cell lymphoma and their relationship to gene expression status. *Leukemia* **2008**, *22*, 1035–1043. [CrossRef]
165. Sproul, D.; Meehan, R.R. Genomic insights into cancer-associated aberrant CpG island hypermethylation. *Brief Funct. Genom.* **2013**, *12*, 174–190. [CrossRef]
166. Fueyo, J.; Gomez-Manzano, C.; Bruner, J.M.; Saito, Y.; Zhang, B.; Zhang, W.; Levin, V.A.; Yung, W.K.A.; Kyritsis, A.P. Hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas. *Oncogene* **1996**, *13*, 1615–1619.
167. Kawano, S.; Miller, C.W.; Gombart, A.F.; Bartram, C.R. Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. *Blood* **1999**, *94*, 1113–1120.
168. Haraldsdottir, S.; Hampel, H.; Wu, C.; Weng, D.Y.; Shields, P.G.; Frankel, W.L.; Pan, X.; de la Chapelle, A.; Goldberg, R.M.; Bekaii-Saab, T. Patients with colorectal cancer associated with Lynch syndrome and MLH1 promoter hypermethylation have similar prognoses. *Genet. Med.* **2016**, *18*, 863–868. [CrossRef]
169. Esteller, M.; Hamilton, S.R.; Burger, P.C.; Baylin, S.B.; Herman, J.G. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.* **1999**, *59*, 793–797.
170. Galamb, O.; Kalmár, A.; Péterfia, B.; Csabai, I.; Bodor, A.; Ribli, D.; Krenács, T.; Patai, Á.V.; Wichmann, B.; Barták, B.K.; et al. Aberrant DNA methylation of WNT pathway genes in the development and progression of CIMP-negative colorectal cancer. *Epigenetics* **2016**, *11*, 588–602. [CrossRef]
171. Saunderson, E.A.; Stepper, P.; Gomm, J.J.; Hoa, L.; Morgan, A.; Allen, M.D.; Jones, J.L.; Gribben, J.G.; Jurkowski, T.P.; Ficz, G. Hit-and-run epigenetic editing prevents senescence entry in primary breast cells from healthy donors. *Nat. Commun.* **2017**, *8*, 1450. [CrossRef] [PubMed]

172. Zardo, G.; Tiirikainen, M.I.; Hong, C.; Misra, A.; Feuerstein, B.G.; Volik, S.; Collins, C.C.; Lamborn, K.R.; Bollen, A.; Pinkel, D.; et al. Integrated genomic and epigenomic analyses pinpoint biallelic gene inactivation in tumors. *Nat. Genet.* **2002**, *32*, 453–458. [CrossRef] [PubMed]
173. Mendenhall, E.M.; Koche, R.P.; Truong, T.; Zhou, V.W.; Issac, B.; Chi, A.S.; Ku, M.; Bernstein, B.E. GC-Rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet.* **2010**, *6*, e1001244. [CrossRef] [PubMed]
174. Lienert, F.; Wirbelauer, C.; Som, I.; Dean, A.; Mohn, F.; Schubeler, D. Identification of genetic elements that autonomously determine DNA methylation states. *Nat. Genet.* **2011**, *43*, 1091–1097. [CrossRef]
175. Krebs, A.R.; Dessus-Babus, S.; Burger, L.; Schubeler, D. High-throughput engineering of a mammalian genome reveals building principles of methylation states at CG rich regions. *eLife* **2014**, *3*, e04094. [CrossRef]
176. Wachter, E.; Quante, T.; Merusi, C.; Arczewska, A.; Stewart, F.; Webb, S.; Bird, A. Synthetic CpG islands reveal DNA sequence determinants of chromatin structure. *eLife* **2014**, *3*, e03397. [CrossRef]
177. Dickson, J.; Gowher, H.; Strogantsev, R.; Gaszner, M.; Hair, A.; Felsenfeld, G.; West, A.G. VEZF1 elements mediate protection from DNA methylation. *PLoS Genet.* **2010**, *6*, e1000804. [CrossRef]
178. Brandeis, M.; Frank, D.; Keshet, I.; Siegfried, Z.; Mendelsohn, M.; Nemes, A.; Temper, V.; Razin, A.; Cedar, H. Sp1 elements protect a CpG island from de novo methylation. *Nature* **1994**, *371*, 435–438. [CrossRef]
179. Macleod, D.; Charlton, J.; Mullins, J.; Bird, A.P. Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. *Genes Dev.* **1994**, *8*, 2282–2292. [CrossRef]
180. Jara-Espejo, M.; Line, S.R. DNA G-quadruplex stability, position and chromatin accessibility are associated with CpG island methylation. *FEBS J.* **2019**, *287*, 483–495. [CrossRef]
181. Long, H.K.; Blackledge, N.P.; Klose, R.J. ZF-CxxC domain-containing proteins, CpG islands and the chromatin connection. *Biochem. Soc. Trans.* **2013**, *41*, 727–740. [CrossRef] [PubMed]
182. Kong, L.; Tan, L.; Lv, R.; Shi, Z.; Xiong, L.; Wu, F.; Rabidou, K.; Smith, M.; He, C.; Zhang, L.; et al. A primary role of TET proteins in establishment and maintenance of De Novo bivalency at CpG islands. *Nucleic Acids Res.* **2016**, *44*, 8682–8692. [CrossRef] [PubMed]
183. Weber, M.; Hellmann, I.; Stadler, M.B.; Ramos, L.; Pääbo, S.; Rebhan, M.; Schübeler, D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* **2007**, *39*, 457–466. [CrossRef] [PubMed]
184. Okitsu, C.Y.; Hsieh, C.L. DNA methylation dictates histone H3K4 methylation. *Mol. Cell Biol.* **2007**, *27*, 2746–2757. [CrossRef] [PubMed]
185. El-Deiry, W.S.; Nelkin, B.D.; Celano, P.; Yen, R.W.; Falco, J.P.; Hamilton, S.R.; Baylin, S.B. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. *Proc. Natl. Acad. Sci. USA* **1991**, *8*, 3470–3474. [CrossRef]
186. Robertson, K.D.; Keyomarsi, K.; Gonzales, F.A.; Velicescu, M.; Jones, P.A. Differential mRNA expression of the human DNA methyltransferases (DNMTs) 1, 3a and 3b during the G(0)/G(1) to S phase transition in normal and tumor cells. *Nucleic Acids Res.* **2000**, *28*, 2108–2113. [CrossRef]
187. Ibrahim, A.E.; Arends, M.J.; Silva, A.L.; Wyllie, A.H.; Greger, L.; Ito, Y.; Vowler, S.L.; Huang, T.H.-M.; Tavaré, S.; Murrell, A.; et al. Sequential DNA methylation changes are associated with DNMT3B overexpression in colorectal neoplastic progression. *Gut* **2011**, *60*, 499–508. [CrossRef]
188. Laird, P.W.; Jackson-Grusby, L.; Fazeli, A.; Dickinson, S.L.; Jung, W.E.; Li, E.; Weinberg, R.A.; Jaenisch, R. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* **1995**, *81*, 197–205. [CrossRef]
189. Ley, T.J.; Ding, L.; Walter, M.J.; McLellan, M.D.; Lamprecht, T.; Larson, D.E.; Kandoth, C.; Payton, J.E.; Baty, J.; Welch, J.; et al. DNMT3A mutations in acute myeloid leukemia. *N. Engl. J. Med.* **2010**, *363*, 2424–2433. [CrossRef]
190. Walter, M.J.; Ding, L.; Shen, D.; Shao, J.; Grillot, M.; McLellan, M.; Fulton, R.; Schmidt, H.; Kalicki-Veizer, J.; O'Laughlin, M.; et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia* **2011**, *25*, 1153–1158. [CrossRef]
191. Yan, X.J.; Xu, J.; Gu, Z.H.; Pan, C.-M.; Lu, G.; Shen, Y.; Shi, J.-Y.; Zhu, Y.-M.; Tang, L.; Zhang, X.-W.; et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nat. Genet.* **2011**, *43*, 309–315. [CrossRef] [PubMed]
192. Di Croce, L.; Raker, V.A.; Corsaro, M.; Fazi, F.; Fanelli, M.; Faretta, M.; Fuks, F.; Lo Coco, F.; Kouzarides, T.; Nervi, C.; et al. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* **2002**, *295*, 1079–1082. [CrossRef] [PubMed]
193. Wu, X.; Zhang, Y. TET-mediated active DNA demethylation: Mechanism, function and beyond. *Nat. Rev. Genet.* **2017**, *18*, 517–534. [CrossRef] [PubMed]
194. Ko, M.; Huang, Y.; Jankowska, A.M.; Pape, U.J.; Tahiliani, M.; Bandukwala, H.S.; An, J.; Lamperti, E.D.; Koh, K.P.; Ganetzky, R.; et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* **2010**, *468*, 839–843. [CrossRef]
195. Figueroa, M.E.; Abdel-Wahab, O.; Lu, C.; Ward, P.S.; Patel, J.; Shih, A.; Li, Y.; Bhagwat, N.; Vasanthakumar, A.; Fernandez, H.F.; et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* **2010**, *18*, 553–567. [CrossRef]
196. Yamazaki, J.; Jelinek, J.; Lu, Y.; Cesaroni, M.; Madzo, J.; Neumann, F.; He, R.; Taby, R.; Vasanthakumar, A.; Macrae, T.; et al. TET2 mutations affect non-CpG Island DNA methylation at enhancers and transcription factor-binding sites in chronic myelomonocytic leukemia. *Cancer Res.* **2015**, *75*, 2833–2843. [CrossRef] [PubMed]

197. Rasmussen, K.D.; Jia, G.; Johansen, J.V.; Pedersen, M.T.; Rapin, N.; Bagger, F.O.; Porse, B.T.; Bernard, O.A.; Christensen, J.; Helin, K. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. *Genes Dev.* **2015**, *29*, 910–922. [CrossRef] [PubMed]
198. Schlesinger, Y.; Straussman, R.; Keshet, I.; Farkash, S.; Hecht, M.; Zimmerman, J.; Eden, E.; Yakhini, Z.; Ben-Shushan, E.; Reubinoff, B.E.; et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nat. Genet.* **2007**, *39*, 232–236. [CrossRef]
199. Widschwendter, M.; Fiegl, H.; Egle, D.; Mueller-Holzner, E.; Spizzo, G.; Marth, C.; Weisenberger, D.J.; Campan, M.; Young, J.; Jacobs, I.; et al. Epigenetic stem cell signature in cancer. *Nat. Genet.* **2007**, *39*, 157–158. [CrossRef]
200. Ohm, J.E.; McGarvey, K.M.; Yu, X.; Cheng, L.; Schuebel, K.E.; Cope, L.; Mohammad, H.P.; Chen, W.; Daniel, V.C.; Yu, W.; et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. *Nat. Genet.* **2007**, *39*, 237–242. [CrossRef]
201. Easwaran, H.; Johnstone, S.E.; Van Neste, L.; Ohm, J.; Mosbruger, T.; Wang, Q.; Aryee, M.J.; Joyce, P.; Ahuja, N.; Weisenberger, D.; et al. A DNA hypermethylation module for the stem/progenitor cell signature of cancer. *Genome Res.* **2012**, *22*, 837–849. [CrossRef] [PubMed]
202. Viré, E.; Brenner, C.; Deplus, R.; Blanchon, L.; Fraga, M.; Didelot, C.; Morey, L.; van Eynde, A.; Bernard, D.; Vanderwinden, J.-M.; et al. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **2006**, *439*, 871–874.
203. Wu, X.; Gong, Y.; Yue, J.; Qiang, B.; Yuan, J.; Peng, X. Cooperation between EZH2, NSPc1-mediated histone H2A ubiquitination and Dnmt1 in HOX gene silencing. *Nucleic Acids Res.* **2008**, *36*, 3590–3599. [CrossRef] [PubMed]
204. Brinkman, A.B.; Gu, H.; Bartels, S.J.; Zhang, Y.; Matarese, F.; Simmer, F.; Marks, H.; Bock, C.; Gnirke, A.; Meissner, A.; et al. Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. *Genome Res.* **2012**, *22*, 1128–1138. [CrossRef]
205. Court, F.; Arnaud, P. An annotated list of bivalent chromatin regions in human ES cells: A new tool for cancer epigenetic research. *Oncotarget* **2017**, *8*, 4110–4124. [CrossRef]
206. Chinaranagari, S.; Sharma, P.; Chaudhary, J. EZH2 dependent H3K27me3 is involved in epigenetic silencing of ID4 in prostate cancer. *Oncotarget* **2014**, *5*, 7172–7182. [CrossRef]
207. Stern, J.L.; Paucek, R.D.; Huang, F.W.; Ghandi, M.; Nwumeh, R.; Costello, J.C.; Cech, T.R. Allele-Specific DNA Methylation and Its Interplay with Repressive Histone Marks at Promoter-Mutant TERT. *Genes Cell Rep.* **2017**, *21*, 3700–3707. [CrossRef]
208. Statham, A.L.; Robinson, M.D.; Song, J.Z.; Coolen, M.W.; Stirzaker, C.; Clark, S.J. Bisulfite sequencing of chromatin immunoprecipitated DNA (BisChIP- seq) directly informs methylation status of histone-modified DNA. *Genome Res.* **2012**, *22*, 1120–1127. [CrossRef]
209. Cai, Y.; Lin, J.R.; Zhang, Q.; O'Brien, K.; Montagna, C.; Zhang, Z.D. Epigenetic alterations to Polycomb targets precede malignant transition in a mouse model of breast cancer. *Sci. Rep.* **2018**, *8*, 5535. [CrossRef]
210. Neri, F.; Incarnato, D.; Krepelova, A.; Rapelli, S.; Pagnani, A.; Zecchina, R.; Parlato, C.; Oliviero, S. Genome-wide analysis identifies a functional association of Tet1 and Polycomb repressive complex 2 in mouse embryonic stem cells. *Genome Biol.* **2013**, *14*, R91. [CrossRef]
211. Ooi, S.K.; Qiu, C.; Bernstein, E.; Li, K.; Jia, D.; Yang, Z.; Erdjument-Bromage, H.; Tempst, P.; Lin, S.P.; Allis, C.D.; et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **2007**, *448*, 714–717. [CrossRef] [PubMed]
212. Otani, J.; Nankumo, T.; Arita, K.; Inamoto, S.; Ariyoshi, M.; Shirakawa, M. Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX–DNMT3–DNMT3L domain. *EMBO Rep.* **2009**, *10*, 1235–1241. [CrossRef] [PubMed]
213. Guo, X.; Wang, L.; Li, J.; Ding, Z.; Xiao, J.; Yin, X.; He, S.; Shi, P.; Dong, L.; Li, G.; et al. Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* **2015**, *517*, 640–644. [CrossRef] [PubMed]
214. Scalea, S.; Maresca, C.; Catalanotto, C.; Marino, R.; Cogoni, C.; Reale, A.; Zampieri, M.; Zardo, G. Modifications of H3K4 methylation levels are associated with DNA hypermethylation in acute myeloid leukemia. *FEBS J.* **2020**, *287*, 1155–1175. [CrossRef]
215. Skvortsova, K.; Masle-Farquhar, E.; Luu, P.-L.; Song, J.Z.; Qu, W.; Zotenko, E.; Gould, C.M.; Du, Q.; Peters, T.J.; Colino-Sanguino, Y.; et al. DNA Hypermethylation Encroachment at CpG Island Borders in Cancer Is Predisposed by H3K4 Monomethylation Patterns Cancer Cell 2019, 35, 297–314. *Cancer Cell* **2019**, *35*, 297–314. [CrossRef]
216. Dunican, D.S.; Mjoseng, H.K.; Duthie, L.; Flyamer, I.M.; Bickmore, W.A.; Meehan, R.R. Bivalent promoter hypermethylation in cancer is linked to the H327me3/H3K4me3 ratio in embryonic stem cells. *BMC Biol.* **2020**, *18*, 25. [CrossRef]