



Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

Examining the impact of gene variants on histone lysine methylation[☆]Capucine Van Rechem, Johnathan R. Whetstine^{*}

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ARTICLE INFO

Article history:

Received 17 February 2014
 Received in revised form 14 May 2014
 Accepted 15 May 2014
 Available online xxxxx

Keywords:

Somatic mutation
 SNP
 KMT
 KDM
 EZH2
 Histone

ABSTRACT

In recent years, there has been a boom in the amount of genome-wide sequencing data that has uncovered important and unappreciated links between certain genes, families of genes and enzymatic processes and diseases such as cancer. Such studies have highlighted the impact that chromatin modifying enzymes could have in cancer and other genetic diseases. In this review, we summarize characterized mutations and single nucleotide polymorphisms (SNPs) in histone lysine methyltransferases (KMTs), histone lysine demethylases (KDMs) and histones. We primarily focus on variants with strong disease correlations and discuss how they could impact histone lysine methylation dynamics and gene regulation. This article is part of a Special Issue entitled: Methylation Multifaceted Modification – looking at transcription and beyond.

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1. Introduction

Chromatin is the highly ordered structure responsible for the compaction of the eukaryotic genetic information and the regulation of processes such as DNA replication, mitosis and gene expression. The basic unit of chromatin is the nucleosome, which is composed of DNA wrapped around an octamer of histones. The histone octamer is comprised of two copies each of H2A, H2B, H3, and H4. Histone H1 is positioned in between nucleosomes and influences the higher order structure. Histones are proteins composed of a globular domain and an unstructured tail that extends away from the nucleosome core. The histone tails are extensively post-translationally modified leading to the regulation of biological processes, including transcription, cell cycle progression and DNA repair [1].

To date, there are at least 16 different classes of histone modifications that have been described [2]. One of the most studied is methylation. A main difference between this modification and others is the complexity in the number of methyl groups that can be added per residue. For example, lysine methylation can occur in a mono- (me1), di- (me2) and trimethylated (me3) state. Furthermore, the degree of methylation and the lysine being modified are associated with different consequences. For example, H3 lysine 4 monomethylation (H3K4me1) marks enhancers, while H3K4me3 peaks around the transcriptional

start site of genes that are active or going to be expressed. In contrast, H3K9me3 enrichment at promoters is associated with repression, while enrichment in the body of genes is associated with induction (Fig. 1). These few examples highlight the importance of the lysine and degree of methylation. This modification state provides a mechanism to intricately regulate the genome and associated processes [2,3]. This topic is covered in an accompanying review in this issue (Wozniak and Strahl).

Although histone methylation has been known since the 1960's [3], it took ~40 years for the enzymes responsible for the addition and removal to be discovered. The first histone lysine methyltransferase (KMT) was described in 2000 by Jenuwein and colleagues (KMT1A/SUV39H1) [4], and Shi and colleagues reported the first histone lysine demethylase (KDM) in 2004 (KDM1A/LSD1) [5]. Subsequent to these studies, the field of lysine methylation underwent massive expansion and numerous lysine modifying enzymes were discovered [3]. In addition to these discoveries, numerous genome-wide studies have been conducted to visualize the methylation landscape. These studies have also highlighted the importance of methylation placement in relation to gene bodies as well as regions within the genome as a whole [3]. The combined knowledge of factors modulating lysine methylation as well as the genomic distribution has now allowed the scientific community to gain more molecular insights into the regulation of nuclear processes. These data have highlighted the importance of methylation from yeast to man and established that methylation dynamics are a critical parameter to consider in disease [6].

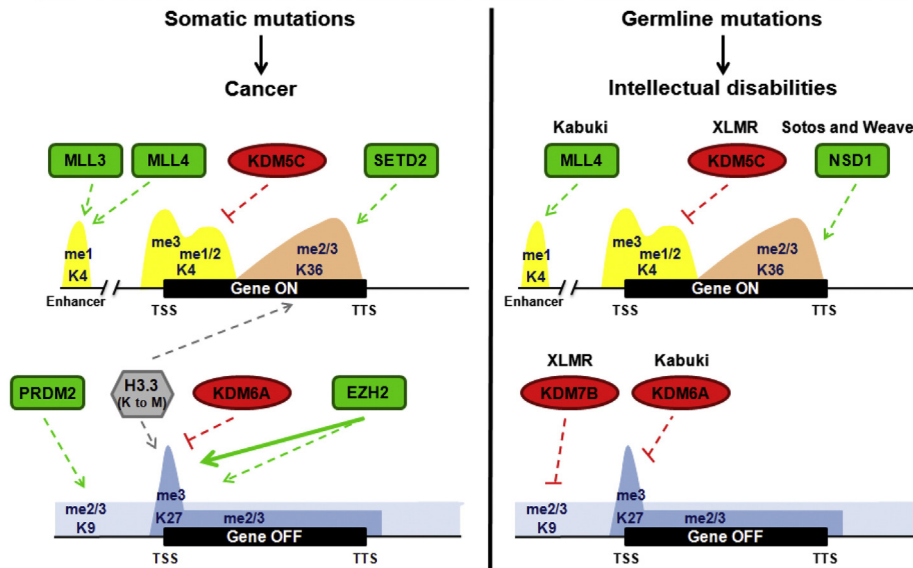
Recent evidence from cancer genome sequencing studies and disease associated genome-wide association studies (GWAS) have begun to highlight the importance of genes involved in modulating the chromatin microenvironment (i.e., lysine methylation) in cancer

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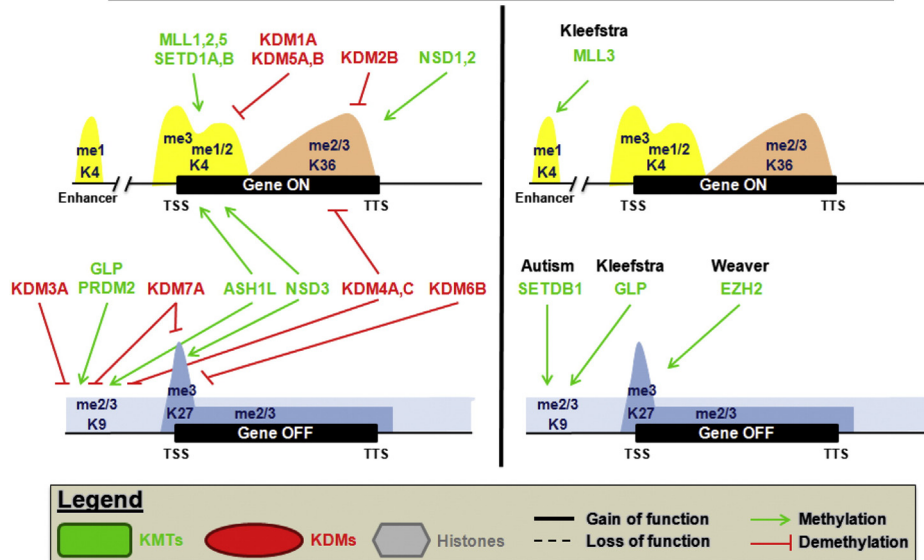
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A. Putative role for mutations with high rate and/or function in diseases



B. Putative role for additional mutations observed in diseases



Legend

KMTs	KDMs	Histones	Gain of function	Methylation
			Loss of function	Demethylation

Fig. 1. Putative mechanisms for how KMTs, KDMs and histone mutations could impact transcriptional regulation. A. Mutations observed at high rate and/or associated with a functional impact in disease. These mutations are discussed in the text. B. Mutations observed in diseases at low frequency and for which no causal role or association has been reported. We apologize to all those whose work we could not depict or cite due to space limitation.

and other genetic diseases. These studies are beginning to suggest that mutations in chromatin modulators may in fact be driver mutations. Driver mutations in cancer are causally implicated in the development of the disease and are the primary focus for targeted therapy. As defined by Stratton et al. [7], a driver mutation has conferred growth advantage to the cancer cell and has been positively selected by the microenvironment of the tissue in which the cancer arises. On the contrary, passenger mutations have not been selected, have not conferred growth advantage and have therefore not contributed to cancer development. These mutations will be a consequence of disease progression. However, these mutations can become drivers when the microenvironment changes (i.e., following chemotherapy) [7]. Thus, “passenger” mutations could be critical in long term treatment of diseases. This area needs more development and will require samples being collected before, during and after therapies are complete. These studies will be vital to our understanding of how chromatin associated factors affecting cancer progression are functioning in the progression of the disease.

In addition to somatic mutations, the selection of germline variants could also result in chromatin modulators becoming important regulators in disease progression and therapeutic response. Single nucleotide polymorphisms (SNPs) are defined as a single base change in a DNA sequence that occurs in a significant proportion of a population ($\geq 1\%$) [8]. Non-synonymous SNPs (i.e., changing the amino acid sequence of a protein) are less frequent in genes known to modulate diseases [9]. While SNPs can be associated with diagnosis and correlated with the risk of a disease, they can also contribute to the outcome without being a cause of the disease itself [10,11]. Therefore, there is a need to consider the impact these polymorphisms within methylation modifying enzymes have on regulation of methylation, the genomic landscape as well as on potential disorders.

In this review, we describe existing research regarding mutations and SNPs in KMTs, KDMs and histones. We focus primarily on variants with disease correlations and also enumerate diseases with observed variations (somatic mutation or SNP) in genes encoding KMTs, KDMs

and histones (Table 1). These genetic variations are then discussed in relation to the histone lysine methylation state they modulate and how the variation impacts or possibly effects gene regulation (Fig. 1).

2. Mutations

Several recent genome-wide sequencing studies have revealed that chromatin modifier genes have significantly higher somatic mutation rates than other genes across different cancer types [12–15]. Interestingly, mutations have also been described for chromatin regulators in syndromes characterized by intellectual disabilities. These mutations are germline mutations (for a review see [16]). While most of the research is still at the discovery stages, some modifiers and readers have been implicated as disease events in both cancer and intellectual disabilities and the mechanism of the dysfunction has begun to be elucidated [2,16]. This section focuses on these relationships in the context of histone mutations and enzymes that modify lysine methylation.

2.1. Histone mutations

A variety of histone mutations have been described across multiple cancer types (Table 1) [13,14,17–27]. In fact, recurrent mutations are found within one of the histone 3 genes: H3F3A (Fig. 1; [17,22], reviewed in [28]). H3F3A encodes the H3.3 replication-independent histone H3 variant, which is enriched at actively transcribed genes and telomeres [29]. H3F3A K27M is a common mutation in pediatric and young adult gliomas and to a lesser extent in anaplastic astrocytoma [17,22–26]. Introduction of H3.3K27M dramatically reduces the level of lysine 27 trimethylation (H3K27me₃) through inhibition of the H3K27me₃ KMT- KMT6/EZH2 (Enhancer of Zeste Homolog 2) [25, 30]. Furthermore, mutations of other histone lysine residues to methionine also inhibit methyltransferases containing the catalytic SET domain. The methionine competes with substrate binding and turnover so methylation is reduced in cis and trans [30]. Most recently, H3.3K36M recurrent mutations have been described in 95% of chondroblastomas, predominantly in the H3F3B gene [27]. Cells expressing the transgene H3.3K36M harbored a strong decrease in global H3K36me₃ levels [30]. Interestingly, this mutation has been shown to promote site-specific copy gains, suggesting that it could be contributing to copy alterations in these tumors, which is something that needs further evaluation [31].

Pediatric gliomas also exhibit H3.3 mutations in glycine 34 to valine (G34V) or arginine (G34R); however, the frequency is less than the K27M mutation [22,25]. G34, which has not been shown to be posttranslationally modified, is adjacent to K36. This leads to the hypothesis that a mutation targeting G34 could alter the methylation of K36. Indeed, mutations of G34 lead to a decrease in the methylation of K36 on the same H3.3 molecule but do not inhibit KMTs in trans and thus do not alter global K36 methylation state [25,30]. Interestingly, G34 is part of a critical di-glycine motif that is required for KDM4A/JMJD2A to demethylate H3K36me₃ (see accompanying review Del Rizzo and Trievel). Therefore, this mutation could be impacting both the addition and removal of this methylation state and the overall distribution across the genome. Consistent with this notion, a recent study demonstrated that a cell line derived from a patient carrying the G34V mutation presented altered enrichment of H3K36me₃ through the genome. Gene ontology analysis of activated genes demonstrated enrichment for the processes involved in forebrain and cortex development as well as differentiation of neurons and regulation of cell proliferation. Interestingly, the most significant H3K36me₃ enriched and expressed gene was MYCN, which provides an alternative mechanism for overexpressing this oncogene [32]. However, it has yet to be determined whether the alteration of K36 methylation drives the gene changes or if the genes change expression levels and thus alter the enrichment of H3K36me₃.

Overall, these recurrent mutations could be affecting both H3K27 and K36 methylation by altering gene expression. Specifically, these alterations could impact gene expression through aberrant methylation at promoters modulated by H3K27me₃ levels (i.e., bivalent genes; [33]) or gene bodies (see accompanying review Wozniak and Strahl). While H3K36me₃ is present within the body of transcribed genes, H3K27me₃ is found at inactive promoters and throughout the body of repressed genes (Fig. 1), implying that mutations targeting any of these residues could alter the proper regulation of transcription (e.g., elongation and/or splicing). Since K27 and K36 methylation are diametrically opposed in the genome, there is the possibility that the mutations within these residues will allow the opposing modification to prevail and impact gene expression. However, we cannot exclude the impact that altering H3K27/36me₃ patterns would have on genome organization since both modifications are associated with large domains throughout the genome [3]. Another formal possibility is they may not affect one another at all. The K27M mutation would also block the ability of K27 to be acetylated, which is a critical feature associated with enhancers [34]. This mutation would impact the enhancer elements and could in turn affect the gene expression and cellular fate. This mechanism would be independent of imbalanced H3K36 methylation. Future studies evaluating these possibilities will begin to shed light on the molecular contributions these mutations have in cancer.

2.2. H3K27 methylation targeted mutations

Histone mutations are not the only way to affect H3K27 methylation levels. One of the most well characterized histone methyltransferases mutated in cancer is the PRC2 (Polycomb Repressive Complex 2) member KMT6/EZH2 (Fig. 1 and Table 1). Loss of function mutations have been observed in 6% of myelodysplastic-myeloproliferative neoplasm patients (MDS; [35]) in the heterozygous and homozygous states. Some patients that initially present as heterozygous loss of function mutations progressed to the homozygous state [36]. Patients with loss of function mutations were associated with poor prognosis [36]. Several non-recurrent loss of function EZH2 mutations have also been described in T cell acute lymphoblastic leukemia [37,38].

EZH2 is also altered by activating somatic mutations (Fig. 1 and Table 1). The recurrent Y641H mutation occurs as a heterozygous mutation and has been observed in 22% of diffuse large B cell lymphoma (DLBCL) and 7% of follicular lymphoma (FL) [39]. The heterozygosity is critical for the gain of function phenotype. While EZH2 efficiently monomethylates H3K27, EZH2-Y641H is deficient in monomethylation but has increased efficiency for di- and trimethylation reactions. The wild type and Y641H enzymes cooperate to produce a hypertrimethylated H3K27 state [40]. Structural homology modeling lead to the hypothesis that mutation of this tyrosine would shift the product equilibrium, while reducing the steric crowding in the active site, favoring efficient trimethylation [40,41]. Indeed, this mutation increased the preference for dimethyl peptide in vitro [41].

Both gain and loss of function EZH2 mutations are early events in the disease process and maintained throughout the transformation of the disease [36,42]. These data raise the possibility that cellular states or originating cell background could be a major determinant in whether an activating or loss of function mutation would be selected for during tumorigenesis. They also raise the question of how many other chromatin regulators will have both gain and loss on function mutations. This will only be resolved as mutations within the chromatin regulators are systematically annotated and assayed in vitro and in vivo.

The gain of function mutations suggest that increasing H3K27 methylation could be important in tumorigenesis. Consistent with these possibilities, somatic mutations have been observed in the H3K27 demethylase KDM6A/UTX in multiple cancers [18,43–51] (Table 1). In addition to the somatic mutations, germline mutations in KDM6A have been observed in Kabuki syndrome [52,53]. Kabuki syndrome is a pediatric congenital disorder characterized by growth

Table 1

KMTs, KDMs and histones mutations and single nucleotide polymorphism (SNP) observed in diseases. *Abbreviations:* AML (Acute Myeloid Leukemia), ccRCC (clear cell Renal Cell Carcinoma), CLL (Chronic Lymphocytic Leukemia), CML (Chronic Myeloid Leukemia), CMML (Chronic Myelomonocytic Leukemia), CRPC (Castrate Resistant Prostate Cancer), DLBCL (Diffuse Large B Cell Lymphoma), ESCC (Esophageal Squamous Cell Carcinoma), ETP-ALL (Early T-cell Precursor Acute Lymphoblastic Leukemia), FL (Follicular Lymphoma), GBM (glioblastoma multiforme), HCC (Hepatocellular Carcinoma), HNSCC (Head and Neck Squamous Cell Carcinoma), MB (Medulloblastoma), MCL (Mantle Cell Lymphoma), MDS (myelodysplastic syndrome), MM (Multiple Myeloma), NHLs (Non Hodgkin's Lymphoma), NSCLC (Non Small Cell Lung Cancer), OSCC (Oral Squamous Cell Carcinoma), SCLC (Small Cell Lung Cancer), SCC (Squamous Cell Carcinoma). * KMT2B/MLL2 gene ID 9757 is located on chromosome 19, KMT2D/MLL4 gene ID 8085 is located on chromosome 12; # indicates coding SNP. This table is illustrative of variants observed in the literature and might not be fully inclusive. We apologize to all those whose work we could not depict or cite due to space limitation.

Protein	Mutation	SNP	Protein	Mutation	SNP
KMTs	KMT1B/SUV39H2 KMT1C/G9a/EHMT2	Diabetes, lung cancer Breast cancer, chronic hepatitis B, colorectal cancer Beast cancer	KDMs	KDM1A/LSD1 KDM2B/FBXL10	Lymphoblastic leukemia, medulloblastoma Gastric adenocarcinoma
	KMT1D/GLP/EHMT1 KMT1E/SETDB1	Kleefstra syndrome, ganglioglioma Autism, plausible candidate gene for melanoma susceptibility		KDM3A/JHDM2A/JMJD1A KDM3C/JMJD1C	MB Juvenile idiopathic arthritis
	KMT2A/MLL	Gastric adenocarcinoma, liver cancer, bladder cancer, MM, AML, HCC		KDM4A/JMJD2A	Breast cancer
	KMT2B/MLL2 *	HCC, OSCC, bladder urothelial carcinoma, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, ccRCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma		KDM4B/JMJD2B	Lung cancer (cell lines)
	KMT2C/MLL3	Kleefstra syndrome, MB, AML, colorectal cancer, gastric adenocarcinoma, liver cancer, bladder cancer, bile duct cancer, highly aggressive tumors, pancreatic cancer, breast cancer, glioblastoma, HNSCC, ccRCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma, adenoid cystic carcinoma, prostate cancer, MM, HCC		KDM4C/JMJD2C	MB Autism spectrum disorders, esophageal cancer
	KMT2D/MLL4 *	Kabuki syndrome, MB, MDS, CLL, prostate cancer, CRPC, bladder cancer, DLBCL, ETP-ALL, FL, MCL, ccRCC, AML, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma, childhood B-cell ALL, MM, HCC, OSCC		KDM5A/JARID1A#	MB, AML Ankylosing spondylitis
	KMT2E/MLL5 KMT2F/SETD1A	CRPC, breast cancer Gastric adenocarcinoma		KDM5B/JARID1B/PLU1 KDM5C/JARID1C/SMCX	MB, breast cancer Renal carcinoma, ccRCC, autism spectrum disorder, X-linked mental retardation, bladder urothelial carcinoma, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma, OSCC, high grade glioma

KMT2G/SETD1B	CRPC			KDM6A/UTX	lung cancer (cell lines), Leukemia, Kabuki syndrome, MB, prostate cancer, bladder cancer, renal carcinoma, ccRCC, adenoid cystic carcinoma, urothelial carcinoma, CMML, AML, MM, ESCC, SCLC, T-ALL, colorectal adenocarcinoma, endometrial adenocarcinoma, glioblastoma, NSCLC, breast adenocarcinoma, HNSCC, uterine corpus endometrial carcinoma, childhood B-cell ALL, CRPC	Graves' disease
KMT2H/ASH1L KMT3A/SETD2	Lung cancer (cell lines), CRPC, high grade glioma Renal carcinoma, high-grade gliomas, ccRCC, ETP-ALL, synovial sarcoma, bladder urothelial carcinoma, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, AML, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma			KDM6B/JMJD3 KDM7A/JHDM1D/KIAA1718	MB, near haploid lymphoblastic leukemia MB	
KMT3B/NSD1	AML, Sotos syndrome, Weaver syndrome, Beckwith-Wiedemann syndrome, ALL, bladder urothelial carcinoma, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, ccRCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, uterine corpus endometrial carcinoma, OSCC			KDM7B/PHF8		X-linked mental retardation
KMT3E/SMYD3		colorectal cancer, breast cancer, HCC, NSCLC, ESCC	Histones	H1.2	Bladder urothelial carcinoma, breast adenocarcinoma, colon and rectal carcinoma, GBM, HNSCC, ccRCC, lung adenocarcinoma and SCC, ovarian serous carcinoma, NHLs, DLBCL	
KMT3F/WHSC1L1/NSD3 KMT3G/WHSC1/NSD2/ MMSET KMT4/DOT1L	MM MCL, MM, pediatric ALL, low grade glioma			H1.3 H1.4	DLBCL Adenoid cystic carcinomas, DLBCL	
KMT6/EZH2	Prostate cancer, DLBCL, FL, myelofibrosis, myeloid malignancies, T-ALL, HCC, MDS, CLL, AML, ETP-ALL, CMML, Weaver syndrome, bladder urothelial carcinoma, breast adenocarcinoma, GBM, HNSCC, ccRCC, lung adenocarcinoma and SCC, uterine corpus endometrial carcinoma	Osteoarthritis of the hip, adolescent idiopathic scoliosis and pubertal height velocity		H2A.1	Adenoid cystic carcinomas, DLBCL	
KMT8A/PRDM2/RIZ1 #	Gastric carcinoma, endometrial carcinoma, colorectal carcinoma, pancreatic cancer, DLBCL	Cholangiocarcinoma, colorectal cancer, HCC, lung cancer, metastatic colorectal cancer		H3.1	Pediatric diffuse intrinsic pontine gliomas, nonbrainstem pediatric glioblastomas, DLBCL, OSCC, osteosarcoma	
KMT8B/PRDM9	Lung cancer (cell lines)	Breast cancer, leukemia, bone mineral density, lung cancer, Parkinson's disease		H3.3	Childhood ALL, pediatric diffuse intrinsic pontine gliomas and non-Brainstem glioblastomas, anaplastic astrocytomas, high-grade thalamic gliomas from young adults, osteosarcoma, chondrosarcoma, giant cell tumor of bone, chondroblastoma	
				H3.5	GBM, HNSCC, lung adenocarcinoma and SCC, uterine corpus endometrial carcinoma	
				H4	DLBCL, HCC	

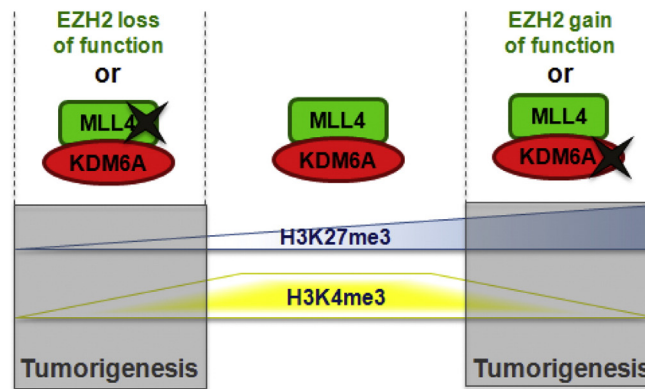
deficiency, a characteristic facial appearance and intellectual disabilities. Of note, germline mutations have also been observed in renal cancer, suggesting that KDM6A may contribute to susceptibility in rare instances [43,54].

KDM6A mutations were observed in 7–8% of chronic myelomonocytic leukemia (CML) and ~10% of derived acute myeloid leukemia (AML). Interestingly, EZH2 mutations were found in 6–8% of CML and 5–9% of AML. The mutations in EZH2 and KDM6A were not observed in the same patient samples, which suggest these mutations are mutually exclusive. However, a larger patient cohort is necessary to demonstrate this conclusively [44,45]. Because EZH2 and KDM6A modulate H3K27 methylation balance, mutations in either member may be sufficient to tip the balance and promote tumorigenesis

(Figs. 1 and 2A). This raises the issue of how to achieve the correct balance when using KMT and KDM inhibitors for cancer therapy. It may be difficult to re-establish the balance when treating with these inhibitors and not tip the balance in the opposite direction. For example, in the case of a gain of function of EZH2 it would then be necessary to treat in such a manner to reserve some enzymatic activity. It would be beneficial to study this in cancer cell line and mouse models over time.

A large study of KDM6A mutations in 1390 cancer samples implicates KDM6A as an important factor in multiple cancer types. The highest prevalence of mutations was found in multiple myeloma (MM, 10%) and esophageal squamous cell carcinoma (ESCC, 8%). Interestingly, a potential mutual exclusion was observed in MM with another methyltransferase KMT3G/NSD2 [43]. This observation raises

A. Disruption of H3K27 and H3K4 methylation in cancer



B. Models for H3K4/K9 methylation crosstalk in XLMR

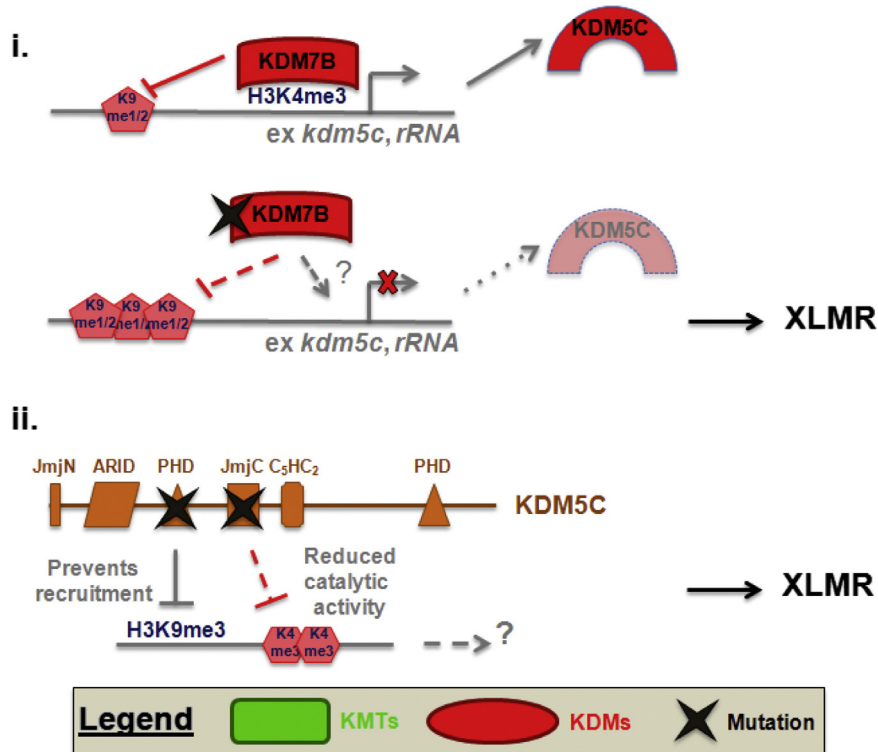


Fig. 2. Crosstalk between histone methylation in diseases. A. Mutations in EZH2 or MLL4 disrupt the H3K27 and H3K4 trimethylation crosstalk leading to tumorigenesis. B. Mutations in KDM7B or KDM5A disrupt H3K9 and H3K4 methylation leading to XLMR. (i) Schematic depicting how mutation could impact KDM7B recruitment or loss of activity. ? represents the loss of recruitment by methylation or ZNF711. (ii) Schematic depicting how mutations could impact KDM5C function.

an interesting question about the genetic relationship between these two enzymes, which should be further evaluated.

KDM6A is the most frequently mutated chromatin modifying gene in transitional cell carcinoma (TCC, the most common type of bladder cancer) with a frequency of 21%. Many of the mutations are predicted to truncate the catalytic domain, which would result in loss of enzymatic activity. KDM6A mutations in TCC are likely to be associated with early developmental stages of cancer as the mutation frequency was statistically associated with lower tumor grade and correlated with a lower stage [46]. Interestingly, the KDM6A mutations in adenoid cystic carcinoma (ACC, salivary gland cancers) are localized around the catalytic domain and appear to abrogate the demethylase activity [18]. These observations emphasize the selection for enzyme suppression, which raises some interesting questions about what will happen should KDM6A inhibitors be developed and clinically applied.

KDM6A is found in two of the H3K4 methylation MLL complexes (MLL3 and MLL4) [55], which are responsible for H3K4 methylation and the regulation of enhancer methylation [56]. Interestingly, MLL members are frequently mutated in human cancers as well (see H3K4 section). Since these proteins are found in complex with one another, such observations would support the notion that these protein complexes are important in tumorigenesis (Fig. 2A) [55]. For example, MLL4 and KDM6A are mutated in clear cell renal cell carcinoma (CCRCC) [47], in castrate resistant prostate cancer (CRPC) [48], and so far in different tumors in 8% and 4–18% of medulloblastoma (MB), respectively [49–51]. These observations suggest that these tumors could have similar molecular profiles and may be similar to one another. Taken together, these data raise the importance of knowing the biochemical characteristics of the enzymes so that the cancers can be better understood at a molecular level. This aspect will be further discussed in subsequent sections.

EZH2 and KDM6A have antagonistic functions on H3K27me3. However, both are involved in regulating gene transcription (Fig. 1), especially in gene programs linked to differentiation [57]. The disruption of either of these proteins could have an impact on the same pathways and/or mechanisms. For example, one of the potential transcriptional roles of the PRC2 complex is to control engaged RNA Polymerase II during promoter escape or elongation by interacting with the RNAs transcribed by genes with paused Polymerase [58]. On the other hand, KDM6A has been directly linked to transcriptional elongation through its interaction with RNA Polymerase II via Spt6 [59]. Furthermore, PRC2 specifically resides at genes encoding developmental regulators (e.g., HOX genes) and its absence leads to embryonic stem cell differentiation defects and a delay in the loss of pluripotency [60]. On the other hand, KDM6A is needed for the re-establishment of pluripotency [61] and upon overexpression leads to demethylation of H3K27me3 at HOX gene promoters, reduced PRC2 complex association and transcriptional activation [62]. Moreover, EZH2 and KDM6A regulate adipogenesis and osteogenesis through a H3K27 methylation switch at the promoter of master regulatory genes. For example, EZH2 promotes adipogenic differentiation, while KDM6A promotes osteogenesis [63]. Therefore, the mutations within these proteins could alter transcriptional initiation or elongation to generate variation in differentiation pathways.

2.3. H3K4 methylation targeted mutations

There are 5 MLL family members, MLL 1 to 5, also known as KMT2A to E. Among this family, MLL3 and MLL4 are the most frequently mutated members (Table 1). For example, MLL3 is most frequently mutated in gastric adenocarcinoma [64], cholangiocarcinoma [65], pancreatic adenocarcinoma [66], colorectal cancer [67,68], non-small cell lung cancer (NSCLC) [13,69] and bladder urothelial carcinoma (BLCA) [13]. On the other hand, MLL4 is frequently mutated in MB [70,71], DLBCL [15,19,20], FL [20,72], early T-cell precursor ALL [73], BLCA, head and neck squamous cell carcinoma and lung squamous cell carcinoma [13].

The majority of MLL4 mutations in DLBCL and FL are at the heterozygous state, but approximately half of the cases had two MLL4 mutations in trans, leading to a complete loss of MLL4 function in the tumor cells of patients [20]. Heterozygous somatic inactivation of MLL4 in DLBCL suggests a role for MLL4 as a haploinsufficient tumor suppressor, although in some patients complete inactivation of MLL4 is required [15,39]. A second, although less common mechanism, by which MLL4 function is inactivated is by loss of heterozygosity (LOH) [20]. Interestingly, truncating mutations in one allele of MLL4 have a pathogenic effect in Kabuki syndrome [74]. These data raise the possibility that MLL4 protein dosage is a critical parameter in both cancer and Kabuki syndrome.

MLL4 is mutated in 60% of the Kabuki syndrome patients. Truncating mutations were found throughout the entire coding region, especially before the catalytic domain. Non-truncating mutations were mainly located around the LXXLL and PHD domains [52]. For example, two exons in MLL4 exhibit a higher mutation frequency, exon 39 encoding a region with LXXLL motifs and exon 48 encoding the last PHD domain and a part of the FYRN domain [74–76]. The localization of these mutations would suggest that the disruption of MLL4 catalytic activity and/or targeting is important to the development of the disease. Furthermore, the patients harboring MLL4 mutations may represent a clinical subgroup within the Kabuki syndrome population because they were usually more severely affected (facial appearance and growth retardation) than the patients without MLL4 mutations [77,78]. These data really highlight the selection for mutations in such genes and suggest that imbalance H3K4 methylation is a critical parameter in promoting these diseases.

In the case of MB and hepatocellular carcinoma (HCC), MLL4 is most likely a driver mutation [70,79] and the presence of a similar allelic frequency in two mantle cell lymphoma tumor samples from two distinct topographic sites or at two time points from the same patients suggests that MLL4 mutations present an early event [80]. On the contrary, the non-uniform representation of MLL4 variants in FL tumor cell subpopulations indicates that they are most likely late events during FL disease progression [72]. The early or late relationship could be the impact of the cell of origin or cell fate programs being executed. This possibility is of particular interest when considering the role of MLL4 in modulating enhancers. Indeed, the MLL4 complex methylates enhancers, which activate them to control gene expression of proteins involved in signaling cascades, embryonic development and cell differentiation [56,81]. These relationships should be further explored.

MLL proteins are part of distinct complexes. MLL1 and MLL2 are related to the Trithorax complex in *Drosophila*, whereas MLL3 and MLL4 are related to the Trithorax-like complex [55]. MLL3 and MLL4 have recently been demonstrated to monomethylate H3K4 at enhancers [56,81] and MLL2 is required for H3K4me3 at bivalent promoters of developmentally regulated genes [82]. Bivalent promoters are characterized by the presence of both H3K27me3 and H3K4me3 marks and exhibit low expression in ES cells. Following differentiation, bivalent promoters resolve into H3K27me3 marked promoters at genes regulating the function of unrelated lineages (non-expressed genes), and H3K4me3 at promoters of markers of the specific lineage (expressed genes) [33]. These data highlight how altered MLL complex formation or enzymatic activity could impact H3K4 methylation dynamics, cell fate and cellular responses. These data are particularly interesting when considering that mutations exist in both MLL3/4 and KDM6A, which can be found in the same multisubunit complex (i.e., COMPASS) (Fig. 2A) [55]. It is interesting to see that proteins within the same complex are frequently mutated. These observations may suggest that specific genes/pathways are mis-regulated and promote/cause certain cancer types or promote/cause other genetic disorders (e.g., Kabuki syndrome). Future studies evaluating the impact that mutations within MLL3/4 and KDM6A have on gene regulation and transcription will be informative in our understanding of how they contribute to various diseases. Studying their impact in diverse cell

lineages will also be of interest to determine if a unique series of gene networks are resolved and possibly associated with diseases of certain cellular lineages.

2.4. H3K36 methylation targeted mutations

H3K36 methylation is highly correlated with actively transcribed genes. The three states of methylation (mono-, di-, tri-) are present across transcribed regions of active genes. The level of H3K36me3 correlates with the level of gene expression [33,83]. Although this modification might also play a role in transcriptional repression, this mechanism is less well understood [83].

KMT3B/NSD1 mono and dimethylates H3K36. However, NSD1 inactivation or loss also affects H3K36me3, which is likely due to a lack of substrate availability for the trimethyltransferase KMT3A/SETD2 [83]. It is interesting to note here that the alteration of an enzyme can lead to more defects than its specific targets. For example, NSD1 affects transcriptional initiation through binding to promoters and the consequent methylation throughout the promoter proximal region of genes and facilitates recruitment of RNA Polymerase II at the promoter [83]. On the other hand, SETD2 interacts with the polymerase during elongation. In yeast, the SETD2 homolog, Set2, is important for the prevention of aberrant transcriptional initiation within the coding region [83]. Thus, the alteration of H3K36 methyltransferases in cancer and genetic disorders could lead to defects in both the transcription initiation and elongation of important genes (Fig. 1).

In the case of NSD1, there are clear links between this gene and certain genetic disorders. Depending on the cohort analyzed, NSD1 has been described to be mutated or deleted in 30–100% of patients with Sotos and Weaver syndromes [84–91]. Sotos and Weaver syndromes are genetic disorders characterized by excessive physical growth during the first years of life, by a variety of intellectual disabilities as well as a host of facial characteristics [92]. NSD1 mutations are also observed in 5% of the unexplained Beckwith–Wiedemann syndrome patients (a genetic disease with a distinct overgrowth condition that is typically explained by the deregulation of imprinted growth-regulatory genes within the 11p15 region) [93]. In Sotos and Weaver syndromes, the most common NSD1 germline alterations are deletions and truncation mutations. An analysis of 530 subjects with diverse phenotypes such as facial dysmorphism, learning disabilities and childhood overgrowth showed that 99% of NSD1-positive individuals (with NSD1 mutations or 5q35 microdeletions encompassing NSD1) have Sotos syndrome and that 93% of Sotos syndrome patients present NSD1 abnormalities (83% with mutations and 10% with microdeletions). The strongest phenotype associated with NSD1 abnormalities was dysmorphism (99%) and learning disabilities (97%), while the individuals with microdeletions had more severe intellectual disabilities [94]. The fact that Sotos patients with a single mutation in the SET, PHD domains 1 through 6 or PWWP2 domains produce the same phenotype as the 5q35 microdeletion that eliminates the *nsd1* gene suggests that each of these domains plays an essential role and likely represent a loss-of-function.

Eleven of the twelve mutations described in the PHD domains 4 through 6 strongly reduced the binding of NSD1 to methylated H3K4 and H3K9. Interestingly, eight out of nine mutations within PHD domains 4 and 6 reduced the binding to the transcription factor Nizp1 [95]. Nizp1 is responsible for recruitment of NSD1 to RNA polymerase II promoters, leading to transcriptional repression [96]. This repressive role for NSD1 is observed for a downstream Ras effector molecule RASIP1. NSD1 depletion increases the expression of RASIP1, which was also increased in Sotos syndrome patients with altered NSD1 [97]. These data highlight the repressive role NSD1 can have and also illustrate how mutations could increase pathways associated with disease. Taken together, these data suggest that both the positively and negatively regulated targets could be critical components in the development of NSD1-related diseases.

The methyltransferase responsible for most global H3K36me3 is SETD2 [83]. SETD2 is mutated in 15% of high grade pediatric gliomas and 8% of adult gliomas. These gliomas exhibit a substantial decrease in total H3K36me3 levels by western blot, suggesting that the mutations are loss-of-function [98]. This has also been described in CCRCC, where intron retention was dramatically increased in the H3K36me3-deficient tumors at transcripts marked by H3K36me3 in the normal kidney and by nucleosome depletion in the tumors. Many of the affected genes are part of cancer-associated pathways. Aberrant RNA processing such as intron retention, variation in exon utilization or generation of previously unannotated splice forms were detected more frequently in highly expressed genes [99]. Future studies exploring the impact H3K36 methylation-associated mutations or alterations have on gene expression, splicing, cellular growth and proliferation will be very interesting and will have a profound impact on our understanding of various cancers and diseases.

2.5. H3K9 methylation targeted mutations

Although H3K9me3 has a role in alternative splicing with a specific enrichment in the body of certain genes, H3K9 methylation is mostly correlated with the absence of transcription. Indeed, H3K9me2/3 covers the body of non-expressed genes (Fig. 1). Furthermore, large domains of H3K9me2 are enriched in developmentally regulated genes, which are thought to prevent their expression in differentiated cells [3].

When compared to other chromatin regulators, the H3K9 modifying enzymes have fewer reported mutations. The H3K9/27 and H4K20 demethylase KDM7B has been reported to have mutations in X-linked mental retardation (XLMR) patients [100,101], whereas two methyltransferases targeting H3K9 have been shown to be mutated in diseases. The H3K9 methyltransferase KMT1E/SETDB1 presents a recurrent germline mutation in five families with autism spectrum disorder [102]; while, KMT8A/PRDM2 contains somatic mutations in 44–57% of gastric carcinomas, 33% of endometrial cancers, 26–37% of colorectal carcinomas and 10% of pancreatic cancers [103–108].

The currently identified PRDM2 mutations reside in polyA tracks within the coding sequence of the gene and were specific for tumors with microsatellite instability (MSI) [103–108]. MSI is defined by hyper mutability of microsatellites repeats, which results from impaired DNA mismatch repair (MMR). This pathway repairs errors due to DNA replication at repeated regions. Microsatellites are one to four base long repeats distributed throughout the entire genome. MSI can result in cancer through mutations in oncogenes or tumor suppressor genes such as TGFBR2 or BAX [109]. Two mechanisms have been described resulting in MSI: mutation in the MMR pathway or silencing of genes from this pathway resulting from DNA methylation [110,111]. It is not clear how methylation of H3K9 could be linked to MSI. One possibility would be a role for this mark in the MMR pathway. While a recent study linked H3K36me3 and SETD2 in proper MMR [112], H3K9 methylation has not been implicated in this pathway as of yet. A second hypothesis is that oncogenes linked to the MMR pathway are repressed by H3K9 methylation by PRDM2. The alteration of this enzyme could lead to tumorigenesis due to the misregulation of oncogene(s). A third possibility is the lack of H3K9 methylation could disrupt DNA methylation leading to the destabilization of repeats and MSI. Indeed, H3K9me2/3 is important for the maintenance of DNA methylation [113]. However, another possibility is the mutations within PRDM2 are not a cause but a consequence of MSI, and therefore, H3K9 methylation has a passenger, rather than a driver function. This possibility would not preclude H3K9 methylation imbalance from emerging as a contributor under certain selective pressures. Future studies will need to be conducted to establish if all or some of these possibilities are affiliated with the diseases.

The deregulation of H3K9 methylation has been described in several malignancies [6]. For example, a loss of H3K9 methylation has been associated with MB, together with a decreased expression of the KMT

responsible for the deposition of this mark (KMT1C/G9a) and an amplification of the H3K9 demethylases KDM4B/JMJ2B and KDM4C [114]. Although very few mutations have been observed in H3K9 methylation modifying enzymes to date, further studies might uncover mutations related to disruption of H3K9 methylation in certain cancers, low frequency, less abundant cancers or in tumors subjected to therapies such as genotoxic stress. We must also consider the possibility that mutations throughout the promoters, gene bodies or 3'-untranslated regions of enzymes could contribute to an overall phenotype versus a specific amino acid being frequently mutated. Should this occur, there would be a recurrent phenotype, not a specific event. Lastly, mutations maybe less selected for in cancer, whereas, gains or losses of genes maybe more prevalent. As an example, the H3K9 methyltransferase SETDB1 is amplified in melanoma and lung cancer [115,116].

Overall, H3K9 methylation is involved in the arrangement of chromatin and global nuclear architecture, which impacts gene regulation, nuclear arrangement and cell cycle progression [3]. Therefore, future studies need to explore the potential role for mutations or altered gene function for proteins impacting H3K9 in disease.

H3K9 methylation defects are also implicated in XLMR (Fig. 1). For example, the H3K9/27 and H4K20 demethylase KDM7B/PHF8 [3] is truncated in some XLMR families [101,117]. Several mutations have been observed in the catalytic domain [101,117], with at least one that impairs demethylase activity [118–120]. Loss of KDM7B activity affects neuronal differentiation in mice [119] and regulates cell survival in zebrafish brain and jaw development [120]. KDM7B is targeted to the chromatin via its PHD domain and binds specifically H3K4me3 positive regions [100,118,119]. Interestingly, KDM7B interacts with two MLL complexes [118] and its catalytic activity toward H3K9me1/2 at rRNA genes and consequent transcriptional activation is stimulated by the adjacent H3K4me3 [118,121], which suggest another opportunity for crosstalk between H3K4 methylation dynamics and KDM7B function. There is also another interesting link between H3K9 and H3K4 methylation in XLMR. KDM7B interacts with the XLMR protein ZNF711 that binds to some KDM7B target genes, including the H3K4 tridemethylase KDM5C (Fig. 2B). The knock-down of either KDM7B or ZNF711 decreases KDM5C transcription [100]. KDM5C loss of function mutations have been described in XLMR [122,123]. Interestingly, KDM5C mutations have also been reported in CCRCC [47].

KDM5C binds H3K9me3 through its PHD domain. XLMR-related point mutations have been shown to reduce catalytic activity and disrupt binding to H3K9me3 peptides (Fig. 2B) [124]. Furthermore, KDM5C activity is important in neuronal survival and dendritic development [124]. These data highlight an interesting crosstalk between the H3K4 and H3K9 methylation pathways within the same genetic disease. On the one hand, the recruitment of KDM5C to H3K9 methylation and its catalytic activity towards H3K4; and on the other hand, by the recruitment of KDM7B to H3K4 methylation and its catalytic activity toward H3K9 (Fig. 2B). Thus, mutations in either KDM5C or KDM7B may yield similar phenotypes and could therefore be considered a phenotypic group for mutational frequency analysis. These relationships emphasize the need to consider the target and molecular relationships between the genes that are mutated in cancer and diseases. These relationships will ultimately give us the molecular fingerprint that could allow patient stratification.

3. Single nucleotide polymorphism (SNP)

The first systematic studies of single nucleotide polymorphisms (SNPs) in the coding region of human genes revealed that among all SNPs, half are predicted to be non-coding SNPs. The synonymous (silent, without modification of the amino acid in the protein) and the non-synonymous SNPs within the coding region (coding SNP or cSNPs) are equally represented. While the average diversity is one difference per 1200 bp when comparing two human genomes, the average gene contains approximately four cSNPs [125,126]. There is a

high fraction of non-synonymous SNPs that are predicted to affect the structure and thus probably function of the protein [127]. For example, these variants may affect protein stability or folding, ligand binding or post-translational modifications [127]. This suggests that some cSNPs might have a phenotype. While, at least in cancer, personalized therapy against individual mutations is starting to be considered as necessary if not essential, few studies have evaluated the relationship between SNPs within KMTs and KDMs and response to therapy. Even as it has become clear that KDMs and KMTs are involved in diseases, and that SNPs in these enzymes can correlate with disease (Table 1), it remains unclear how these SNPs affect the function or regulation of the enzymes.

SNPs located outside of the coding region of genes could impact gene function through a multitude of mechanisms. For example, SNPs in introns could impact splice site recognition, binding of proteins which regulate alternative splicing or affect mRNA stability. SNPs could also be located in promoter or enhancer elements and affect binding of transcription factors. In fact, many SNPs correlated with disease are found in regions with histone modifications consistent with enhancer function [128]. In agreement with this observation, a SNP in the regulatory region of KMT3E/SMYD3, creates a third E2F binding site resulting in increased SMYD3 expression. The frequency of the allele with three binding sites was higher in individuals with colorectal, HCC and breast cancers. Furthermore, elevated expression of SMYD3 is important in carcinogenesis of the colon, liver and breast [129]. The ability of SNPs to impact transcription suggests they could be important discriminators in response to chemotherapies or disease progression. Indeed, a SNP in EZH2 associated with progression free survival and overall survival in metastatic colorectal cancer patients treated with first-line irinotecan-based chemotherapy [130,131].

One of the issues of studying SNPs and their potential implication in disease mechanism and/or response to therapy is deciding which SNP to look at. Based on the fact that non-synonymous cSNPs are more likely to affect gene function, models have been developed for the action of missense cSNPs providing a basis for understanding the impact of a cSNP at the molecular level, and thus an approach of predicting which cSNP are potentially involved in disease [127,132]. A computational method evaluating candidate SNPs that are likely to affect the protein and their function identified a SNP in MLL1 (Q1198P), which is predicted to alter the solvent accessibility of seven residues within MLL1 [133]. Although further studies are necessary to definitively link this SNP and any potential effect on MLL function/stability to any disease, such methods could be helpful to identify important cSNPs for study.

So far, very few cSNPs in KMTs and KDMs have been associated with diseases (Table 1). A KDM5A/JARID1A SNP is associated with ankylosing spondylitis [134]. PRDM2 N283E, located between the conserved E1A-related motifs CR1 and CR2, presents a biased distribution between leukemic cells and normal subjects. The T allele encoding an Asparagine (N283) is present in the homozygous state in 81% of leukemic samples and in 10% of the normal samples [135]. However, it is not clear if these SNPs exert any functional consequences on KDM5A or PRDM2 catalytic activity or targeting to genomic locations. Understanding how cSNPs impact the function of KMTs and KDMs and if these functionally important cSNPs associate with disease or allow stratification of patient outcome is an important area of future research.

4. Conclusion

Recent advances in sequencing technologies have allowed chromatin modifying enzymes to emerge as important genes mutated in diseases such as cancer. This review emphasizes that chromatin modifier genes, especially enzymes involved in lysine methylation, are mutated at the same intermediate frequency as most cancer genes (i.e., 2–20%) [136]. An emerging theme from the genome-wide sequencing studies in various diseases is the presence of common mutations in related protein complex components or in enzymes targeting a certain amino acid residue such as H3K4 and H3K27. However, a few major questions

remain: 1) if larger patient cohorts are evaluated, will mutations within chromatin modifying enzymes co-occur in patient samples or will they be mutually exclusive? 2) Are mutations within genes associated with specific complexes or certain lysines able to connect cancer subtypes or predict tumor or patient responses? As genome-wide sequencing data expands and information about treatment responses and patient outcomes are annotated these questions can be addressed.

The catalog of cancer genes is still under construction and is far from complete as many more sequenced patient samples will be necessary in order to have the power to detect genes mutated at lower frequency (<2%) [136]. Genes mutated at a lower frequency may still be important in cancer when considering phenotypic recurrence across enzymes involved in methylation balance (e.g., KDM6A and MLL4 loss of function mutations or the KDM7B and KDM5C mutations in XLMR). These alterations could alter the same pathways, and in turn, result in the same molecular and cellular phenotypes. In that regard, the trithorax-related histone modifications could be considered to have a more pronounced role because the combined mutations within interactors or enzymes modulating the methylation state would increase the tumor frequency. Future studies that explore these types of relationships will be essential in our understanding of how to molecularly classify tumors and to completely appreciate the impact of mutations affecting lysine methylation on disease.

The recent exponential increase in the amount of data generated by new sequencing technologies has increased the power to detect variants that increase risk factor for a specific disease. For decades, SNPs have been linked to and involved in genetic diseases. Another important path that research needs to take is to study the implication of variants in response to treatment and/or with disease outcome. This analysis would facilitate the development of personalized therapy. In that regard, GWAS methodology might prove useful, allowing large scale comparison of patients with or without resistance to therapy. These data should be applied to KMTs and KDMs, which is a clearly under developed area.

In conclusion, it is becoming clear that KMTs and KDMs are altered in many diseases, at both germline and somatic levels, and that these enzymes are associated with disease mechanisms. However, while a number of recent studies uncovered the alteration of the genes encoding KMTs and KDMs, a lot of work is left to do in order to understand the mechanisms by which KMTs and KDMs are involved in the onset of diseases and response to treatments. Additionally, years of research have proven the importance of studying SNPs in relationship to genetic diseases; however, the understanding of the importance and functional impact of SNPs in KMTs and KDMs is still in the nascent stages. Improving our understanding of SNPs and mutations in KMTs and KDMs will help to assess risk and develop personalized medicine.

Acknowledgements

We are grateful to Dr. Joshua C. Black for his comments and suggestions. The studies conducted in this manuscript were funded by the following agencies: American Cancer Society Basic Scholar Grant, MGH Proton Beam Federal Share Grant (CA059267) and NIH R01GM097360 to J.R.W. A post-doctoral fellowship was provided by the Fund for Medical Discovery (C.V.R.). This research was supported in part by a grant from the Marsha Rivkin Center for Ovarian Cancer Research (C.V.R.).

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