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Gain-of-function mutations in chromatin regulators as an oncogenic mechanism and opportunity for drug intervention

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Abstract

Purpose of review—Somatic gain-of-function mutations that drive cancer pathogenesis are well-established opportunities for therapeutic intervention, as demonstrated by the clinical efficacy of kinase inhibitors in kinase-mutant malignancies. Here, we discuss recently discovered gain-of-function mutations in chromatin regulatory machineries that promote the pathogenesis of cancer. The current understanding of underlying molecular mechanisms and the therapeutic potential for direct chemical inhibition will be reviewed.

Recent findings—Point mutations that increase the catalytic activity of EZH2 and NSD2 histone methyltransferases are found in distinct subsets of B cell neoplasms, which promote cell transformation by elevating the global level of H3K27 tri-methylation or H3K36 di-methylation, respectively. In addition, mutations in histone H3 have been identified in certain pediatric cancers which cause reprogramming of H3K27 and H3K36 methylation by dominantly interfering with histone methyltransferase activity. Finally, chromosomal translocations involving chromatin regulator genes can lead to the formation of fusion oncoproteins that directly modify chromatin as their mechanism of action.

Summary—While relatively rare in aggregate, gain-of-function mutations in chromatin regulators represent compelling therapeutic targets in genetically-defined subsets of cancer patients. However, a broader clinical impact for epigenetic therapies in oncology will require an increased understanding of how non-mutated chromatin regulators function as cancer-specific dependencies.

Keywords

EZH2; NSD2; H3K27M; epigenetics; chromatin

Introduction

Modification of chromatin structure is a key regulatory mechanism that contributes to gene-specific transcriptional control. The chromatin regulatory apparatus encompasses a diverse

array of enzymatic (histone modifiers and nucleosome remodeling complexes) and non-enzymatic (e.g. chromatin reader proteins) machineries that function in concert with sequence-specific DNA binding proteins to influence gene expression. Most chromatin regulators are required for normal mammalian development and several genetic disorders in humans have been linked to mutations in genes that encode chromatin regulatory proteins.

Comprehensive cancer genome sequencing has revealed that chromatin regulators are among the most commonly mutated set of genes in this disease [1]. While the majority of these mutations appear to be loss-of-function changes that inactivate tumor suppressor activities, a subset of such mutations can be classified as gain-of-function, in that they confer a new or enhanced activity in a dominant manner [1]. The earliest of such examples were identified in acute leukemia, where chromosomal translocations lead to the formation of fusion proteins with aberrant regulatory attributes [2] (Table I). Here, we review the recent progress in identifying and understanding gain-of-function alterations in chromatin regulators as drivers of human cancer.

Gain-of-function EZH2 mutations in B-cell lymphomas

EZH2 is a catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), which can mono-, di-, or tri-methylate histone H3 at lysine 27 (H3K27me1, me2, or me3) to facilitate transcriptional repression [21]. The biological functions of PRC2 include the regulation of cell differentiation and proliferation programs, which occurs by maintaining a large number of genes in a silent or poised state of expression [21]. Levels of EZH2 in the cell are under precise transcriptional and post-transcriptional control; with a deficiency or an overproduction of H3K27 methylation being capable of altering cellular identity [22]. In addition, a substantial body of evidence shows that perturbations of H3K27 methylation can also promote malignant transformation [22]. EZH2 is often overexpressed in diverse solid tumors and is associated with metastatic progression, suggesting an oncogenic function [23–25]. In contrast, genetic inactivation of EZH2 and other PRC2 subunits occurs at a significant frequency in myelodysplastic syndrome and in T-cell acute lymphoblastic leukemias, which implies that a minimal level of H3K27me3 is needed to prevent cancer formation in some tissues [26–30].

A landmark study in 2010 reported heterozygous missense mutations in *EZH2* as a recurrent event in B-cell lymphomas, which were identified in ~22% of germinal center B-cell type diffuse large B-cell lymphomas (GCB-type DLBCL) and in ~10% follicular lymphomas [31]. The most common site of EZH2 mutations was Y641, but changes have also been found at A677 and A687, which are all residues in the catalytic SET domain [31–33] (Figure 1). The finding of recurrent heterozygous mutations in specific conserved residues was suggestive of a gain-of-function mechanism, particularly since prior studies had shown that Y641 corresponds to a key residue that restricts catalytic activity in other SET domain-containing proteins [34,35]. Indeed, EZH2-mutant lymphoma cell lines exhibited higher levels of global H3K27me3 when compared to EZH2 wild-type cells, further implicating a gain of catalytic function [32,36,37].

In biochemical assays using reconstituted PRC2 complexes, wild-type EZH2 preferentially catalyzes K27 mono-methylation of unmodified H3 peptides and is less active in performing di- and tri-methylation [36–38]. In contrast, it has been shown that Y641-mutant EZH2 displays enhanced tri-methylation activity but diminished capacity to perform mono-methylation [31,36–38]. The heterozygous expression of a wild-type and Y641-mutant alleles of EZH2 would therefore lead to a cooperative effect in driving increased conversion of H3K27me0 to H3K27me3 [32,36,37]. While A677 and A687 mutations exhibit some biochemical distinctions from Y641 *in vitro*, these mutations also stimulate EZH2 catalytic activity to promote increases in cellular H3K27me3 [32,33]. Structural studies of the EZH2 SET domain showed that Y641 hydrogen bonds with the ϵ -amino group of substrate lysine residues, in a conformation that is also stabilized by A677 [39]. Hence, the Y641 and A677 residues would be predicted to block rotation of mono-methylated lysine reaction product to prevent subsequent di- and tri-methylation, with side chain substitutions at these residues allowing enhanced rates of di- or tri-methylation. These results indicate that EZH2 mutations found in lymphoma lead to increased levels of H3K27me3, presumably as a mechanism of cell transformation.

How might elevations in H3K27 tri-methylation promote B cell transformation? Repressive chromatin reader proteins that recognize the H3K27 methylation (e.g. Cbx7) exhibit significantly greater affinity for H3K27me3 as compared to H3K27me1/me2 peptides [40]. This would suggest that EZH2 mutations cause stronger transcriptional repression than the wild-type enzyme. Using chromatin immunoprecipitation coupled to massively parallel DNA sequencing (ChIP-seq), it has been shown that EZH2 Y641 causes an increased abundance of H3K27me3 at the promoters that correspond to the normal targets of wild-type EZH2 in germinal center B cells [41,42]. This suggests that EZH2 mutants act to strengthen the normal role of EZH2 in regulating the germinal center reaction. Mouse knockout experiments have shown that EZH2 is required for the formation of normal germinal centers, where it acts to repress cell cycle checkpoints and differentiation-related genes (e.g. *IRF4*, *PRDM1*, and *CD138*), presumably to allow sufficient time for immunoglobulin affinity maturation [41,43]. Importantly, the expression of EZH2 is normally extinguished upon completion of the germinal center reaction, which allows terminal differentiation of B cells [41,43]. Hence, mutant-EZH2 would be expected to block the differentiation of germinal center B cells and prevent their transition into plasma/memory B cells, which has been confirmed in various experimental systems [41]. It is worth noting that EZH2 Y641-mediated differentiation blockade results in a hyperplasia of lymphoid tissues, but not a full lymphoma phenotype, which requires additional cooperating genetic lesions (e.g. *BCL2* overexpression) [41].

As an oncogene in B cell lymphoma, the enzymatic activity of mutant EZH2 presents an attractive target for cancer therapy. Several independent groups have reported selective small-molecule inhibitors of the EZH2 methyltransferase activity, which exhibit similar potency for wild-type and mutant forms of the enzyme [42,44–46]. These molecules have been shown to deplete H3K27 methylation in normal and malignant cells without significant effects on other histone methyl-marks [42,44–46]. Remarkably, EZH2-mutant lymphomas are uniquely sensitive to these compounds, while diverse EZH2 wild-type cell lines can

effectively tolerate reduced levels of H3K27 methylation [42,44–46]. This effect has been validated in tumor xenograft mouse models, with complete inhibition of B-cell lymphoma tumor growth occurring at dosing regimens that have limited toxicity to normal tissues [42].

While PRC2 has functions in normal hematopoiesis and is a tumor suppressor in certain hematopoietic cancers, it was found that EZH2 inhibitors did not lead to significant changes normal blood parameters [42,47]. Based on these promising pre-clinical findings, Phase I clinical trials were recently initiated (NCT01897571 and NCT02082977 at clinicaltrials.gov) that are investigating EZH2 inhibition in B cell lymphomas. Collectively, this flurry of research on EZH2-mutant lymphoma has revealed one of the most compelling epigenetic drug targets in oncology.

Gain-of-function NSD2 alterations in multiple myeloma and pediatric B-cell acute lymphoblastic leukemia

NSD2 (also known as MMSET/WHSC1) is a histone H3K36 mono- and dimethyltransferase, which exhibits oncogenic activity in various cellular contexts. NSD2 and its homologs NSD1 and NSD3 share a similar domain architecture that features a catalytic SET domain and various chromatin reader modules (PHD and PWWP) that interact with modified histones [48]. While early studies suggested that NSD2 methylates multiple lysine substrates on core histones, recent work has shown decisively that H3K36me2 is the key modification performed by NSD2 *in vitro* and *in vivo* [49–51]. Since H3K36me2 is a modification primarily found in active chromatin locations, it is likely that the principal function of NSD2 is to promote gene transcription [51,52], however, a repressive function for NSD2 has also been described in certain settings [50,53,54].

The gene encoding NSD2 was originally discovered based on its involvement in recurrent chromosomal translocations, t(4;14)(p16;q32) found in ~15% of multiple myeloma (MM), a cancer of terminally differentiated plasma B cells [55]. This rearrangement places the NSD2 and FGFR3 genes under the control of IgH enhancers, resulting in their overexpression [55–58]. While evidence suggests that both NSD2 and FGFR3 can have oncogenic properties, NSD2 appears to be the major oncogene in t(4;14) MM [56,58,59]. Indeed, knockdown of NSD2 in t(4;14) MM cell lines via RNA interference reduces clonogenic cell growth, induces apoptosis, and reduces tumorigenicity in xenograft mouse models [60–62].

It has been shown that the catalytic SET domain of NSD2 is required for its oncogenic functions in MM [51,62]. Indeed, t(4;14) MM cells exhibit substantially higher levels of global H3K36me2 than non-rearranged MM lines [51,62,63]. While the location of H3K36me2 is normally confined to intragenic locations in most cell types, when NSD2 is overexpressed H3K36me2 can be found broadly across the genome at intergenic locations [51,62]. The functional impact of H3K36me2 at intergenic sites is not well understood at present, but this might lead to activation of distal enhancer elements through transcription factor interactions [50]. Nevertheless, the net output of H3K36 hyper-dimethylation is the upregulated expression of several proto-oncogenes that are normally silent, such as regulators of apoptosis, cell cycle, and self-renewal [51,62,64]. The PHD domains of NSD2 are also required for its oncogenic properties, which may facilitate NSD2 recruitment to its

target genes [64]. One of the important downstream effects of NSD2 overexpression is to increase the protein levels of c-MYC, which occurs through an indirect microRNA-mediated mechanism [53]. NSD2 has also been shown to interact directly with the NFkB transcription factor and is essential to coactivate its target genes related to inflammatory processes [65]. H3K36 methylation of nucleosomes has been shown to inhibit the enzymatic activity of PRC2 and t(4;14) MM cell lines exhibit a global deficiency in H3K27me3 [62,66,67]. Hence, an antagonism with PRC2-mediated repression may represent an additional element of NSD2-mediated transformation.

Recent studies have identified a recurrent point mutation (E1099K) in the SET domain of NSD2 that occurs in ~8% of B cell acute lymphoblastic leukemia [63,68–70] (Figure 1B). To date, this mutation has been found primarily in pediatric B-ALL cases and not in adult B-ALL or other forms of myeloid or T-lymphoid leukemia [63,68]. Biochemical studies have shown that E1099K leads to enhanced H3K36 methyltransferase activity as compared to wild-type NSD2 [63,68]. Furthermore, NSD2 E1099K+ B-ALL lines exhibit increased H3K36me2 at levels that are comparable to those observed in t(4;14) MM lines. [63,68]. Hence, it would appear that t(4;14) and E1099K alterations represent two independent means of achieving NSD2-mediated H3K36 hyper-dimethylation. Based on the proven feasibility of pharmacological inhibition of SET domains illustrated above, NSD2 now emerges as a promising target for chemical inhibition in E1099K+ and t(4;14)+ B cell malignancies. Interestingly, NSD2 is also overexpressed in many other non B-lymphoid malignancies [51,71], suggesting that NSD2 inhibition could have a broader anti-cancer activity.

Gain-of-function histone H3 mutations in pediatric gliomas, chondroblastomas, and giant cell tumors of bone

Somatic mutations in genes encoding histone H3 have been uncovered recently in several forms of childhood cancer. Mutations in histone H3 (both the replication variant H3.1 and the replication-independent H3.3) were first identified in pediatric gliomas and led to amino acid substitutions at two different positions: K27M and G34R/G34V [72–74]. Subsequently, a genomic analysis of pediatric chondroblastoma (a benign bone tumor) identified H3.3 K36M mutations in ~95% of cases [75]. A related cancer, known as giant cell tumor of bone, also carries H3.3 mutations in ~92% of cases, but exclusively the G34W/G34L substitutions [75] (Figure 1C). The presence of a single mutation on one copy of H3 out of the 32 alleles present in a diploid genome and the specific recurrent site of mutation strongly suggested a gain-of-function mechanism.

It was recognized that H3K27M gliomas exhibited a dramatic deficiency of global H3K27 methylation, despite the fact that the mutated H3 protein only represents ~5% of the total H3 in the cell [76–78]. In efforts to explain this observation, it was demonstrated that H3K27M acts as a potent inhibitor of EZH2 methyltransferase activity, thus preventing the enzyme from efficiently modifying the wild-type complement of H3 in the cell [76–78]. Remarkably, introducing a single H3K27M transgene into cells by transfection is sufficient to trigger a global loss of H3K27me2 and H3K27me3 on wild-type histones [76–78]. Mechanistically, H3K27M appears to bind the SET domain EZH2 and interfere with its

catalytic function directly [76]. At the genomic level, H3K27M triggers a global reduction of H3K27me3 at most sites and results in increased transcriptional output of genes associated with neurogenic development, however a small subset of locations in the genome actually become H3K27 hyper-tri-methylated in association with strengthened repression [77]. H3K27M also leads to loss of DNA methylation at many associated sites, which further leads to transcriptional upregulation [78]. A similar mechanism appears to be at play for H3K36M, which leads to a global decrease in H3K36me3, presumably through direct inhibition of SETD2/HYPB, the major tri-methylase for H3K36 in human cells [76]. The G34-mutant of H3 can also inhibit SETD2-mediated H3K36 methylation in cis on the mutant H3 protein, but does not inhibit methylation of wild-type H3 in trans [76,77]. Hence, it is uncertain at present whether G34 mutations function through a loss- or gain-of function mechanism. It is possible, that H3G34 substitutions lead to altered localization of H3K36me3 in the cell, rather than global loss of this mark [79].

There exists a striking dichotomy between the oncogenic effects of elevated H3K27me3 and H3K36me2 described above in B cell cancers and the tumor protective role of H3K27me3 and, presumably, H3K36me3 in pediatric bone and brain cancers. Thus, a picture emerges across these different malignancies where an exquisite precision in the levels and localization of H3K27 and H3K36 methylation is vital for the regulation of differentiation and proliferation programs and to navigate the delicate balance between normal development and neoplasia. An important objective for future investigation will be to determine whether H3-mutant cancers harbor unique epigenetic dependencies as a consequence of their altered pattern of histone methylation.

Fusion oncoproteins involving the chromatin regulatory machinery

The first known examples of genetic aberrations involving chromatin regulators are the fusion oncoproteins generated via chromosomal translocation, which universally function through gain-of-function mechanisms [2]. In Table I, we provide a list of known chromatin regulator fusion oncoproteins and their associated malignancies. While many such oncoproteins are rare, a few represent important genetic subtypes of malignancy. The H3K4 methyltransferase MLL can form fusion proteins with a diverse array partner proteins, which occur at a frequency of 5–10% in acute myeloid and lymphoblastic leukemias [20]. The resulting MLL-fusion protein has lost its H3K4 methyltransferase activity and instead recruits alternative effector complexes, such as the H3K79 methyltransferase DOT1L. Chemical inhibition of DOT1L is a promising therapeutic approach in MLL-fusion leukemia, now under investigation in clinical trial (NCT02141828) [80]. BRD4-NUT fusion proteins occur in a rare form of squamous cell carcinoma and retain the chromatin reader bromodomains of BRD4 [81]. Chemical inhibition of the BRD4 bromodomains also represents a promising therapeutic approach in this disease (NCT01587703) [82].

Concluding remarks

Epigenetic dysfunction is a molecular hallmark of human cancer, which in certain instances is the direct consequence of mutational changes in chromatin regulatory proteins. In the cases described above where gain-of-function changes occur in chromatin regulators, direct

or indirect chemical inhibition presents a promising therapeutic avenue. However, the overall rarity of chromatin regulator gain-of-function mutations indicates that such approaches will only impact on a small group of cancer patients. Basic research that investigates how cancer cells become dependent on non-mutated chromatin regulators may reveal a broader impact for translating epigenetic therapies into oncology. In this regard, the clinical investigation of inhibitors that suppress the activity of chromatin regulator oncoproteins (e.g. EZH2 and NSD2) will provide an important framework for all future studies of epigenetic therapies in oncology.

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Key Bullet Points

- Mutations linked to tumorigenesis often are found in chromatin regulator genes, with a subset leading to gain-of-function alterations
- Mutations in the SET domain of EZH2 are common in certain forms of B cell lymphoma and lead to increased H3K27 tri-methylation in cells
- Gain-of-function alterations of NSD2 in B-ALL and multiple myeloma lead to global increase H3K36 di-methylation.
- Gain-of-function mutations in histone H3 can deregulate histone methyltransferase activity to promote different types of pediatric cancer.

Figure 1. Gain of function mutations of EZH2, NSD2, and histone H3 found in human cancer
 Domain architecture is depicted for each protein. WD:WD repeat; HMG, High Mobility Group box; PWWP, Pro-Trp-Trp-Pro motif; PHD, PHD finger (Plant Homeo Domain); SET, Su(var)3–9, Enhancer-of-zeste and Trithorax domain;

Table 1

Chromatin regulator fusion oncoproteins.

Translocation	Fusion protein	Associated malignancy
t(15;19)(q14;p13.1)	BRD4-NUT	NUT midline carcinoma [3]
t(9;15)(q34.2;q14)	BRD3-NUT	NUT midline carcinoma [4]
t(8;15)(p12;q15)	NSD3-NUT	NUT midline carcinoma [5]
t(X;18)(p11.2;q11.2)	SS18-SSX	Synovial sarcoma [6]
t(11;17)(p15;p13)	NUP98-PHF23	AML [7]
t(11;15)(p15;q35)	NUP98-JARID1A	AML [8]
t(5;11)(q35;p15.5)	NUP98-NSD1	AML [9]
t(8;11)(p11;p15)	NUP98-NSD3	AML [10]; MDS [11]
t(8;16)(p11;p13)	MOZ-CBP	AML [12]
t(8;22)(p11;q13)	MOZ-p300	AML [13]
inv(8)(p11;q13)	MOZ-TIF2	AML [14]
t(10;16)(q22;p13)	MORF-CBP	AML [15]; MDS [16]
t(9;22)(p13;q13)	PAX5-BRD1	B-ALL [17]
t(1;10)(p13;q11)	TRIM33-RET	NSCLC [18]; PTC [19]
t(11q23)	MLL-X	AML, B-ALL [20]

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; B-ALL: B-cell acute lymphoblastic leukemia; NSCLC: non-small cell lung carcinoma; PTC: Papillary thyroid carcinoma