Invited Mini Review

The role of EZH1 and EZH2 in development and cancer

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Polycomb Repressive Complex 2 (PRC2) exhibits key roles in mammalian development through its temporospatial repression of gene expression. EZH1 or EZH2 is the catalytic subunit of PRC2 that mediates the mono-, di- and tri-methylation of histone H3 lysine 27 (H3K27me1/2/3), H3K27me2/me3 being a hallmark of facultative heterochromatin. PRC2 is a chromatin-modifying enzyme that is recruited to a limited number of "nucleation sites", spreads H3K27 methylation and fosters chromatin compaction. EZH1 and EZH2 exhibit differences in their expression patterns, levels of histone methyltransferase activity (HMT) in the context of PRC2, and DNA/nucleosome binding activity. This suggests that their roles in heterochromatin formation are disparate. Dysregulation of PRC2 activity leads to aberrant gene expression and is implicated in cancer and developmental diseases. In this review, we discuss the distinct function of PRC2/EZH1 and PRC2/EZH2 in the early and late developmental stages. We then discuss the cancers associated with PRC2/EZH1 and PRC2/EZH2. [BMB Reports 2022; 55(12): 595-601]

INTRODUCTION

Chromatin is largely divided into heterochromatin (highly condensed and transcriptionally repressed) and euchromatin (loosely packed and transcriptionally active). Heterochromatin is further categorized as being constitutive or facultative. In contrast to constitutive heterochromatin, facultative heterochromatin can convert into euchromatin and vice versa (Fig. 1). Thus, this region has the potential for gene expression and its state changes dynamically depending on cell type and develop-

mental signals. The regulation of facultative heterochromatin is therefore key to cellular commitment to a given lineage and the maintenance of this identity through cell divisions. The platform for generating such facultative heterochromatin arises from the presence of specific histone post-translational modifications (PTMs). PRC2 catalyzes mono-, di- and tri-methylated lysine 27 of histone H3 (H3K27me1/-me2/-me3), and H3K27me2/3 is a hallmark of facultative heterochromatin (1). Unlike other histone methylation, which is carried out by a variety of histone methyltransferases, H3K27 methylation is solely catalyzed by PRC2 in mammalian cells (Fig. 1). Therefore, it gives PRC2 a greater role in the control of chromatin states. How is it possible for a single enzymatic complex to play such a significant part in temporospatial gene repression? One of the answers is that PRC2 forms complexes by one of two distinct catalytic core subunits, EZH1 or EZH2, and different combinations of accessory proteins. The expression of these subunits and accessory proteins varies throughout development, and the activities of different PRC2 subcomplexes differ. In this study, we concentrate on how PRC2 evolved to acquire the ability for dynamic regulation by the two catalytic subunits, EZH1 and EZH2.

PRC2 AND HISTONE H3K27 METHYLATION

PRC2 has three core subunits: two isoforms of enhancer of zeste (EZH1/2), embryonic ectoderm development (EED), and suppressor of zeste 12 (SUZ12). Each core subunit shows a distinct function. EZH1/2 methylates H3K27, EED recognizes H3K27me3, and SUZ12 plays a role as a structural platform (1-3). These three core subunits together form the PRC2 core complex and are indispensable for histone methyltransferase (HMT) activity. H3K27me3 binds to the aromatic cage of EED ("read") and allosterically activates HMT activity of PRC2 ("write") (4). The EZH1/2 subunit contains a SET domain, a canonical motif for histone lysine methyltransferase, that possesses HMT activity, and methylates H3K27 (5-7). The binding of H3K27me3 to the aromatic cage of EED leads to the physical interaction between EZH2-SRM and EZH2-SET-I, and the interaction stabilizes the PRC2's SET domain structure, therefore enhancing the catalytic activity of PRC2 (8). This "read-and-write" mechanism induces allosteric activation of PRC2 and is critical for the maintenance of epigenetic modifications at newly synthe-

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sized histones (4, 9-11). SUZ12 interacts with both EED and EZH1/2 and is essential to the formation of the PRC2 complex and for its catalytic activity (12). SUZ12 is also important for the interaction with PRC2 accessory proteins (3). Although it is not necessary for HMT activity, the retinoblastoma-associated protein 46 or 48 (RBAP46/48 also known as RBBP7 and RBBP4, respectively) binds to the complex through the N-terminal part of SUZ12 (13) and leads to an increase in its HMT activity with oligonucleosome substrates (12).

In addition to the core subunits, PRC2 also interacts with non-core subunits or accessory proteins, such as AEBP2, Polycomb-like proteins (PCLs), EPOP, JARID2, PALI, EZHIP/CATACOMB, etc (14-20). These accessory proteins bind to the N-terminal regions of SUZ12. Each non-core subunit assists PRC2 to focus on H3K27 methylation activity at specific sites in the genome (21). Also, these non-core PRC2 subunits are collectively required for PRC2's target site specificity. The review for PRC2 accessory proteins can be found elsewhere (1, 14, 15, 22).
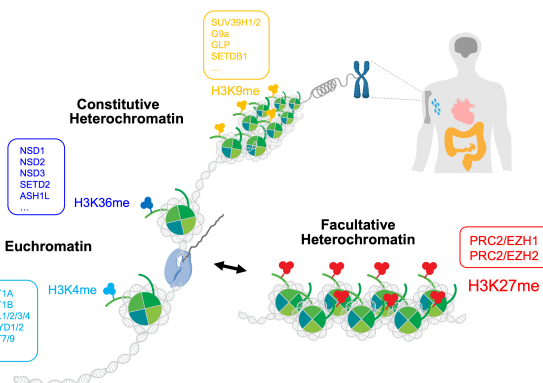

Fig. 1. Chromatin structure and histone methylations. The representative histone methylations and their histone methyltransferases for euchromatin, facultative heterochromatin and constitutive heterochromatin are shown.

TWO CATALYTIC SUBUNITS OF PRC2, EZH1 AND EZH2

Biochemical properties of EZH1 versus EZH2

The human EZH1 and EZH2 genes diverged from the Drosophila gene E(Z) over the course of evolution. Because the sequence of E(Z) is more comparable to human EZH2, EZH1 may have a new function. The SET domain at the C-terminal region of EZH1 and EZH2 is highly conserved while the N-terminal region is less conserved. Allosteric activation and nucleosome binding activity are major factors which boost PRC2 activity (Fig. 2A). The Stimulatory Responsive Motif (SRM) domain of EZH1/2 located at the N-terminal region is critical for allosteric activation, and the SANT1/2 and MCSS domains of EZH1/2 are important for the nucleosome binding activity of PRC2 (9, 23). Allosteric activation of PRC2/EZH2 is more efficient than that of PRC2/EZH1. On the other hand, PRC2/EZH1 possesses a higher nucleosome/DNA binding activity relative to PRC2/EZH2 (23-25). Moreover, PRC2/EZH1 can compact chromatin robustly both *in vitro* and *in vivo*, independent of its catalytic activity (25).

The effect of allosteric activation is different at PRC2/EZH1 and PRC2/EZH2 (23). This is caused by the presence of different residues in their SRM domains (EZH2 H129 versus the corresponding EZH1 C130). PRC2/EZH2 showed a higher catalytic activity than that of PRC2/EZH1 in the presence of H3K27me3. The PRC2 complex with an EZH2 mutant that mimics the structure of EZH1 SRM (EZH2 H129C) showed a significant decrease in catalytic activity both *in vitro* and *in vivo*. These results are suggestive of the biochemical mechanism that lies behind the differential response to allosteric activation between PRC2/EZH1 and PRC2/EZH2 (23). It was also confirmed that PRC2/EZH2 is more sensitive to the EED inhibitors than PRC2/EZH1, due to the differential response to allosteric activation (23).

Despite the low catalytic activity of PRC2/EZH1 compared to PRC2/EZH2, PRC2/EZH1 has a higher nucleosome and DNA binding activity (23, 26). EZH1 possesses basic-rich motifs at SANT1L (RKRKR motif) and the MCSS-SANT2L loop (RRRRR motif) which enhance the interaction with nucleosomal DNA
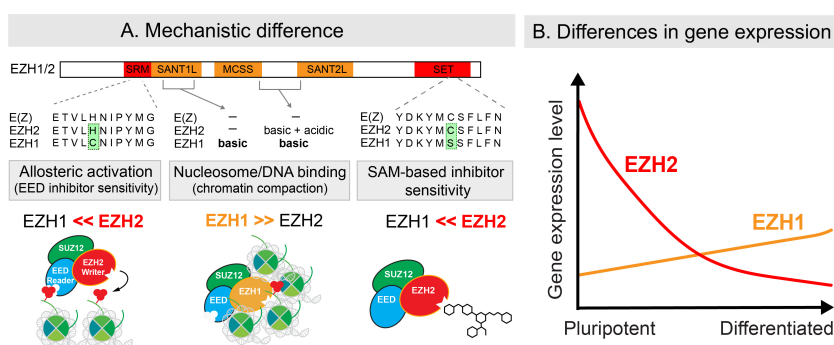

Fig. 2. Two writers of PRC2, EZH1 and EZH2. (A) The mechanistic difference between PRC2/EZH1 and PRC2/EZH2 is highlighted. The sequence of Drosophila E(Z), EZH2 and EZH1 is shown. (B) The expression level of EZH1 and EZH2 during differentiation is shown.

(Fig. 2A) (26). On the other hand, EZH2 lacks basic motif at SANT1L and has both basic and acidic motifs at the MCSS-SANT2L loop (Fig. 2A). The high nucleosome binding activity was reduced when the MCSS-SANT2L loop of EZH1 was substituted with that of EZH2, suggesting the importance of nucleosome binding activity through these basic-rich motifs. Moreover, a recent structural study of the PRC2/EZH1-nucleosome showed that chromatin condensation was facilitated by PRC2/EZH1 when compared to PRC2/EZH2, indicating that PRC2/EZH1 compacts chromatin more efficiently than PRC2/EZH2 (26). It is likely that PRC2/EZH1 can create a dense chromatin environment that will aid in the catalysis of H3K27 methylation (27).

The residue difference in the SET domain (EZH1 S664 vs EZH2 C663) resulted in differential responses to a SAM-based EZH2 inhibitor (Fig. 2A). A cysteine 663 to serine substitution in EZH2 showed a low response to an EZH2 inhibitor whereas a serine 664 to cysteine substitution in EZH1 showed a higher response to the EZH2 inhibitor (23, 28).

Further research into the variations in PRC2/EZH1 and PRC2/EZH2 activities will aid in the development of EZH1- or EZH2-specific inhibitors to treat the associated human diseases (see below).

Gene expression of EZH1 and EZH2 in development
EZH1 and EZH2 can regulate PRC2 activities differently during development because of their differential expression level and different intrinsic activities (see above).

EZH2 is highly expressed in proliferative cells, such as embryonic stem cells, as its expression is controlled by E2F, a cell cycle-dependent transcription factor (29). In general, the level of EZH2 expression subsequently declines throughout cell differentiation (Fig. 2B). The greater catalytic activity of PRC2/EZH2 is likely more optimal for constitutively dividing cells to restore the level of H3K27 methylation after DNA replication (Fig. 3, left). The "read-and-write" mechanism of PRC2/EZH2 ensures the recognition of pre-existing H3K27me3 ("read") and methylates H3K27 ("write") on newly synthesized histones.

The expression of EZH1 is relatively consistent and low throughout development (Fig. 2B). However, EZH1 expression level increases in the post-developmental stage. It has been demonstrated that EZH1 expression increases in the striatum of

mice after two weeks, indicating that EZH1 may have a role in the development of neuronal cells (30). Whether the increase of EZH1 expression in differentiated stages is either common or cell-type specific, needs to be investigated. Because PRC2/EZH1 possesses a higher nucleosome binding activity and chromatin compaction ability, we speculate that non-dividing cells in the post-developmental stage prefer PRC2/EZH1 to sustain the compaction of established repressive chromatin domains (Fig. 3, right). By forming dense chromatin domains via PRC2/EZH1, it could locally boost PRC2/EZH2 activity despite its low amount in differentiated cells.

The role of PRC2/EZH1 and PRC2/EZH2 in development
The role of EZH1 and EZH2 has been studied in mice and many other types of cellular systems. EZH2 knockout (EZH2-KO) mouse embryos are deformed and underdeveloped as compared to wild-type (WT) littermates and die at the early developmental stage (31). On the other hand, EZH1-KO mice are viable and did not show any overt developmental defect. These results suggest that EZH2 is required for normal development whereas EZH1 is redundant. Indeed, the EZH2-KO in mouse embryonic stem cells (mESCs) showed a significant reduction in H3K27me2 and H3K27me3 levels, while EZH1-KO in mESCs showed intact levels of H3K27me1/2/3, indicating that PRC2/EZH2 is essential for maintaining the levels of H3K27me2 and H3K27me3 in mESCs while PRC2/EZH1 is not necessary (23, 32).

On the other hand, conditional EZH2 deletion in differentiated cells displayed a different aspect from undifferentiated cells. Conditional EZH2 knockout (EZH2-cKO) in adult mouse intestinal epithelium resulted in a minor drop in H3K27me2 and H3K27me3 levels. This suggests that PRC2/EZH2 becomes less important, while PRC2/EZH1 becomes more important, in maintaining the levels of H3K27me2/3 in differentiated cells (33). The combined data suggest that the contribution of PRC2/EZH1 and PRC2/EZH2 on H3K27 methylation may differ depending on the developmental stage of cells.

EZH2 is involved in most early embryonic events regardless of cell lineages, however, it becomes less critical after a certain stage of differentiation. One study showed that early cardiac inactivation of *EZH2* disrupts heart development, while late cardiac inactivation of *EZH2* did not cause an overt pheno-
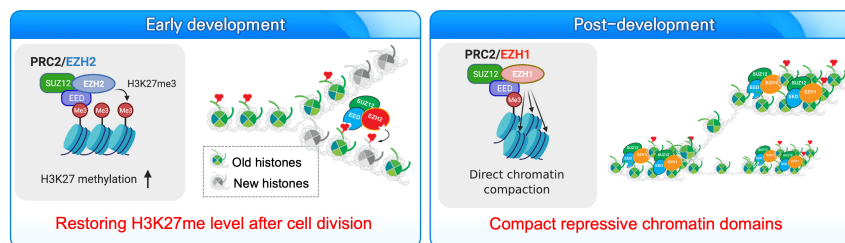

Fig. 3. The distinct role of PRC2/EZH2 and PRC2/EZH1 relies on their intrinsic properties. PRC2/EZH2 exhibits higher catalytic activity required for restoring H3K27 methylation after cell division. PRC2/EZH1 exhibits higher nucleosome binding activity which facilitates chromatin compaction and its maintenance at the post-developmental stage. Some images were created with BioRender.com.

type, suggesting EZH2 is not essential in late cardiac development (34). The study also showed that EED conditional knockout in the late stage of cardiac development exhibited developmental defects. EZH1 is therefore most likely necessary for late cardiac development. Despite the relevance of EZH1 function in late development, EZH1-KO mice did not exhibit a significant developmental phenotype. Most phenotypic defects caused by EZH1 loss occur in the absence of EZH2. In adult tissues, absence of both EZH1 and EZH2 exhibits severe defect compared to EZH1 or EZH2 single depletion in maintaining hematopoietic stem cells, maintaining the health of hair follicles, and preventing neurodegeneration (35-39). This might result from compensation of EZH2 which several studies reported. In presence of EZH2, EZH1 loss may not affect phenotype dramatically, due to the compensation of EZH2. However, in absence of both EZH1 and EZH2, when compensation is eliminated, it exhibits severe defect compared to EZH1 or EZH2 single depletion. Altogether, it highlights EZH1 requirement in the absence of EZH2 (35-39).

Nonetheless, a recent study suggests that EZH1 has a non-redundant function in heart regeneration (40). EZH1 and EZH2 knockout experiments in myocardial infarction (MI) mouse models revealed that only EZH1 (not EZH2) is necessary for heart regeneration. A MI mouse model, with an EZH1-KO, showed the presence of more fibrotic scar tissue and a decreased heart function than a MI mouse model with an EZH2 conditional knockout. Even with the rescue of EZH2, the EZH1 knockout mice did not show improvement in heart function and fibrosis. In contrast, EZH1 rescue in EZH1 knockout mice resulted in the reduction of the fibrotic area in injured hearts and an improvement of heart function. These findings indicate that EZH1 has a non-redundant role in heart regeneration. Further investigation is required to unveil the mechanism of EZH1-mediated gene regulation in heart regeneration.

EZH1 AND EZH2 IN CANCER

PRC2 plays a pleiotropic role in cancer. While the majority of cancers are associated with gain-of-function mutations or the overexpression of EZH2, PRC2 loss-of-function by mutations in EZH2, EED, or SUZ12 can also result in cancer. In general, aberrant PRC2 activity changes global histone H3K27 methylation, which leads to the reduction of facultative heterochromatin and the gain of euchromatin. Oncogenes may consequently be activated and/or tumor suppressor genes may be rendered inactive, which promotes carcinogenesis.

Several PRC2 inhibitors are undergoing clinical trials. Recently, tazemetostat (Tazverik^{TM}), which targets the catalytic site of EZH2, has been clinically approved for patients diagnosed with follicular lymphoma and epithelioid sarcoma with gain-of-function PRC2/EZH2 (41). To overcome the toxicity of the current EZH2 inhibitors, modified EZH2 inhibitors, EED inhibitors and EZH1/2 dual inhibitors are undergoing clinical trials.

In the next part of this study, we concentrate on two examples of gain-of-function PRC2/EZH2 malignancies that have received clinical approval for using EZH2 inhibitor, tazemetostat (Fig. 4) and a case for gain-of-function PRC2/EZH1 in thyroid cancer.

Follicular lymphoma with gain-of-function "hotspot" mutations of PRC2/EZH2

Gain-of-function mutations of EZH2, such as mutations in EZH2 Y646, A682 and A692, have been found in many types of human cancers and are usually correlated with a poor prognosis and metastasis (42-45). EZH2^{Y646X} (X = F, N, H, S and C) mutations have been discovered in more than 22% of Diffuse Large B-Cell Lymphoma (DLBCL), 15% of Follicular Lymphoma (FL) and 1% of melanoma (46-48). These tumors have an EZH2 heterozygous mutation. Biochemical studies revealed that EZH2^{Y646X}, EZH2^{A682G}, and EZH2^{A692V} mutations all lead to an increased level of H3K27me3 due to the altered substrate specificity (48-50). These PRC2/EZH2 mutants inefficiently catalyze unmodified and mono-methylated substrates, while the catalytic activity toward di-methylated H3K27 increases significantly compared to the WT (Fig. 4B). In the case of WT, EZH2 Y646 (Y641 in mouse) is present within the substrate binding pocket and it restricts the additional methylation of H3K27me2 by forming a limited space (49). However, the mutant case's substitution of a tyrosine residue at EZH2^{Y646} with smaller residues expands the area for effective catalysis from H3K27me2 to H3K27me3. EZH2 A682 is located behind the Y646 residue, so mutation of A682 to a smaller residue also results in the enlarged space for catalyzing H3K27me3. Recently, it was reported that EZH2$^{Y641F/WT}$ mutant mice (equivalent to EZH2 Y646F in human) cause the redistribution of repressive H3K27me3 modification, rather than monotonically
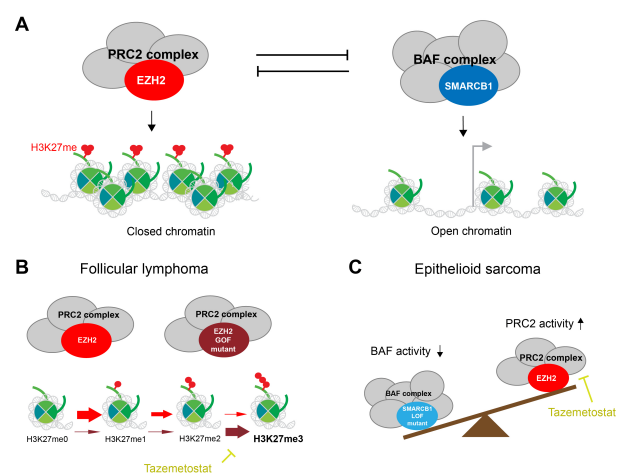

Fig. 4. PRC2 and cancer. (A) The relationship between PRC2/EZH2 and BAF complex. (B) Follicular lymphoma with EZH2 gain-of-function mutant. (C) Epithelioid sarcoma with increased PRC2 activity.

increase H3K27me3 across the genome (51). This may be because the PRC2/EZH2^{Y646X} mutant is extremely active on di-methylated chromatin but only weakly methylates unmethylated or mono-methylated chromatin (Fig. 4B). Although the efficient H3K27me3 catalysis in the PRC2/EZH2 mutant is widely reported, it is still unclear how the mutant poorly methylates unmethylated or mono-methylated H3K27.

EZH2 inhibitors have been discovered for treating cancers with gain-of-function PRC2/EZH2. Tazemetostat, an EZH2 inhibitor has been approved as an effective drug for FL with EZH2 gain-of-function mutations in June 2020 (41). Tazemetostat inhibits PRC2 enzymatic activity through a SAM competitive mechanism, especially blocking SAM binding to the EZH2-SET domain. Tazemetostat was studied in a phase II clinical trial consisting of 99 FL patients, 45 with an EZH2 mutation and 54 without. EZH2 mutations include Y646X [X = F, N, S, H, C], A682G, and A692V which enhance the catalysis of H3K27me3 as mentioned above. It was found that 69% of patients with an EZH2 mutation, and 34% of patients without an EZH2 mutation, showed an overall response rate (ORR) when treated with tazemetostat. Moreover, 98% of patients with an EZH2 mutation and 71% of patients without an EZH2 mutation showed a reduction in tumor size. Lastly, the median response duration time was 10.9 months for patients with an EZH2 mutation and 13.0 months for patients without an EZH2 mutation. Following the phase II clinical trial, tazemetostat was approved for FL patients under accelerated approval based on the overall response rate and duration of the response.

Epithelioid sarcoma with BAF mutation, an antagonist of PRC2

The BRG/BRM-associated (BAF) complex, a mammalian switch/sucrose non-fermentable (SWI/SNF) complex, is an ATP-dependent chromatin remodeler that activates transcription by enhancing the accessibility of transcription factors (52, 53). The BAF complex antagonizes PRC2 recruitment and its catalytic activity by evicting PRC2 from the chromatin (Fig. 4A) (54, 55). Due to this antagonism, researchers have started to focus on inhibiting PRC2/EZH2 whose activity has been enhanced in BAF-deficient tumors (Fig. 4C) (56, 57). Over 90% of the cases of epithelioid sarcoma, and 98% of the cases of malignant rhabdoid tumor (MRT) and atypical teratoid/rhabdoid tumor (AT/RT), show protein loss of one of the BAF subunits, SMARCB1/INI1. Preclinical studies with tazemetostat revealed that it can effectively induce regression of SMARCB1/INI1 deficient tumors *in vitro* and in xenograft models (57). In 2020, tazemetostat was approved for the treatment of epithelioid sarcoma patients with SMARCB1/INI1 deficiency (58).

Although tazemetostat is approved for epithelioid sarcoma patients, the dosage of the inhibitor must be further investigated for the treatment of other BAF-deficient cancers. Recent research has shown that the BAF complex can promote PRC2-mediated repression instead of always opposing PRC2 (59). This study suggests that PRC2, that is evicted by the BAF complex, binds to the PRC2-enriched chromatin domain which will increase the level of H3K27me3 and facilitate gene repression. It indicates that the PRC2 activity is not always enhanced in BAF-deficient malignancies due to the pleiotropic effect of the BAF complex on PRC2 recruitment. Therefore, further investigation is required to navigate PRC2 redistribution on chromatin following tazemetostat treatment for epithelioid sarcoma with gain-of-function PRC2/EZH2.

Gain-of-function PRC2/EZH1 in thyroid cancer

Although PRC2-associated cancers are mainly related to EZH2, recurrent EZH1 mutations are found in thyroid cancer and these mutants exhibit gain-of-function activity (60-62). The initial study indicated that approximately 27% of EZH1^{Q571R} mutations were found in autonomous thyroid adenomas and had an impact on thyroid cell proliferation (60). Moreover, thyroid adenoma with EZH1^{Q571R} exhibited an increased level of H3K27me3 (60). A later study revealed two hotspot EZH1 mutations, EZH1^{Q571R} and EZH1^{Y642F}, which were predominantly discovered in benign thyroid cancers but rarely detected in malignant tumors (62). Another study demonstrated that EZH1^{Q571R} and EZH1^{Y642F} were driver mutations for follicular thyroid carcinoma and follicular adenoma (61). Y642 of EZH1 corresponds to Y646 of EZH2. Therefore, it is likely that the EZH1^{Y642F} mutant may function similarly to the EZH2^{Y646F} mutant, as described above. EZH1^{Q571R} is a mutant that only occurs in EZH1 but not EZH2, suggesting an EZH1-specific mechanism for this mutant. We speculate that EZH1^{Q571R} could regulate nucleosome binding affinity, as EZH2^{Q570} interacts with nucleosomal DNA (63). Further structural and functional studies are required to understand the mechanisms and biological functions underlying tumorigenesis of thyroid cancer, mediated by the EZH1^{Q571R} mutation.

CONCLUSION

PRC2 is central for temporospatial gene regulation and its misregulation could cause various human diseases, including cancer and developmental diseases. Dissecting the function of PRC2/EZH1 and PRC2/EZH2 will be useful for developing new treatments. While the function of PRC2/EZH2 has been broadly studied, the function of PRC2/EZH1 still needs to be investigated. It is crucial to investigate the biological significance of PRC2/EZH1's stronger nucleosome binding and chromatin compaction ability.

The activity of PRC2/EZH1 and PRC2/EZH2 is also influenced by many other factors, including 3D chromatin structure, nuclear lamina, other histone modifiers and chromatin remodelers. Examining the interconnections between them is necessary to have a thorough grasp of the cell-specific epigenome. We can better comprehend molecular interactions in a variety of cell types because of advanced tools like single-cell-based sequencing. Cryo-EM, and other technologies that can elucidate protein structures, help us to understand previously unknown molecular protein features and make significant

progress in the development of novel therapeutic approaches based on protein structure. Future studies will concentrate on how to understand the cell-specific epigenome and develop treatments tailored towards individual patients, using the convergence of these various technologies.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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