Biochemical and Biophysical Research Communications 464 (2015) 256–262

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A new kinetochore component CENP-W interacts with the polycomb-group protein EZH2 to promote gene silencing

Wansoo Koh, Byoungwoo Park, Soojin Lee*

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea

ARTICLE INFO

Article history:
Received 10 June 2015
Accepted 20 June 2015
Available online 23 June 2015

Keywords:
PRC2
EZH2
CENP-W
Kinetochore
Gene silencing
Oncogene

ABSTRACT

Polycomb recessive complex 2 (PRC2) plays a central roles in chromatin compaction and remodeling. EZH2, the catalytic subunit of PRC2, is frequently overexpressed in many human tumors. Together with another essential core component, SUZ12, EZH2 trimethylates histone H3 on lysine 27 (H3K27me3). CENP-W was originally identified as a putative oncogene overexpressed in various human tumors, and later characterized as an essential factor for the formation of functional kinetochore during mitosis. In this study, we found that CENP-W associates with EZH2 to subsequently enhance the protein stability of EZH2. Chromatin immunoprecipitation revealed that ectopically expressed CENP-W bound the promoter of EZH2 target genes to enhance EZH2-mediated transcriptional repression, possibly by facilitating the recruitment of EZH2 to its target genes. Collectively, this study suggests CENP-W is a novel kinetochore component that may be involved in the EZH2-mediated silencing machinery.

1. Introduction

Epigenetic marking and chromatin modification is highly associated with the transcriptional regulation of tumor-associated genes during cell transformation [1]. Polycomb group proteins (PcG) were originally discovered as negative regulators of HOX gene expression in Drosophila, and were later found to play central roles in chromatin compaction and remodeling [2]. The PcG enhancer of zeste homolog 2 (EZH2), together with suppressor of zeste 12 (SUZ12), and embryonic ectoderm development (EED), form the polycomb recessive complex 2 (PRC2), which trimethylates histone H3 Lys 27 (H3K27me3) [2].

Aberrant expression of EZH2 has been observed in various cancers, such as breast, bladder, and prostate cancer, emphasizing its pivotal role in human malignancy [3]. A derepression study using an EZH2-specific inhibitor revealed that EZH2 target genes are enriched for roles in growth inhibition and apoptosis, demonstrating that EZH2 contributes to selectively inactivate tumor suppressive genes in cancers [4]. Recent studies revealed several other polypeptides co-fractionated with the core components of the PRC2 complex [2]. Recognized chromatin modifiers, such as DNA methyltransferase1 and histone deacetylase 1 (HDAC1), were also determined to transiently associated with PRC2 complex, and potentially regulate its activity [2].

CENP-W is newly identified centromeric component that was originally discovered as an unknown gene commonly overexpressed in various tumors [5]. CENP-W has key roles during mitosis, especially in kinetochore complex formation, as a prerequisite factor for the recruitment of other kinetochore components [6,7]. A recent study revealed that the histone-fold containing CENP-T-W and CENP-S-X complexes co-assemble to form a unique nucleosome-like structure in centromeres [8]. In addition, the nucleolar association of CENP-W contributes to facilitate the recruitment of kinetochore components during mitotic prophase [9]. Although nucleolus is recognized as the most active site of gene transcription [10], ironically, a shell of highly condensed heterochromatic DNA, consisting of repetitive major satellite (pericentric) and minor satellite (centric) repeats, also surround nucleoli [10]. In this study, we proposed that the centromeric nucleolus-associated protein, CENP-W, may function in transcriptional repression by associating with PRC2 machinery.

Abbreviations: PRC2, polycomb repressive complex 2; CENP-W, centromere protein W; EZH2, enhancer of zeste homolog 2; SUZ12, suppressor of zeste 12; CENP-T, centromere protein T; HDAC, histone deacetylase; HOXA9, homeobox A9.

* Corresponding author.
E-mail address: leesoojin@cnu.ac.kr (S. Lee).

http://dx.doi.org/10.1016/j.bbrc.2015.06.136
0006-291X/

2. Materials and methods

2.1. Cells, plasmids, and siRNAs

The 293T cells and HeLa-FLAG-CENP-W stable cells [9] were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Invitrogen). Transient transfection was performed using either Lipofectamin™ (Invitrogen) or polyethylenimine reagent (Sigma). Molecular cloning of FLAG-CENP-W and GST-CENP-W was described previously [9]. *EZH2* (NM_004456) clone was obtained from 21C Frontier Human Gene Bank (Korea) and subcloned into pcDNA3-HA construct (a gift from Dr. Changhoon Kim, Korea Basic Science Institute, Korea). To examine the transcriptional repression, we used pGH250 (pSV_2-GAL4-DNA binding domain vector) and $(GAL4)_5$-TK-Luc derived from pGL2 (Promega) by insertion of the thymidine kinase (TK) core promoter (−105 to +52). (kindly provided by Dr. Jin-Hyun Ahn, Sungkyunkwan University, Korea) [11]. GAL4-DB-fused CENP-W was constructed by inserting cDNA encoding CENP-W into pGH250 using BglII and XbaI. The luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega). The CENP-W knockdown experiment was performed as previously described [9].

2.2. Protein binding assays, antibodies, and fluorescence microscopy

Protein interactions were examined using in vivo co-immunoprecipitation and glutathione *S*-transferase (GST)-pull-down as previously described [9]. To produce recombinant proteins, genes were subcloned into the pET28a(+) (Novagen) or pGEX-4T-3 (GE Healthcare) bacterial expression vector and the recombinant proteins were expressed in BL21 *Escherichia coli* (Novagen) following induction with 0.2 mM IPTG for 3 h. Ni sepharose resin (GE healthcare) was used for isolating His-tagged proteins. Anti-FLAG and anti-Myc antibodies were purchased from Sigma–Aldrich. Anti-HP1α, anti-EZH2, and anti-H2K27me3 antibodies were obtained from Cell Signaling. Fluorescence microscopy was performed with the same protocol as previously presented [9], and the imaging was done using an Olympus IX70 fluorescence microscope at 200 × magnification.

2.3. Generation of adenoviral shRNA vector for CENP-W

To produce adenoviral shRNA particles, we first constructed an entry vector using the backbone of pENTR-CMV-tag2B (Invitrogen), of which the CMV promoter was replaced by H1 promoter. The hairpin RNA encoding CENP-W siRNA (5′–gat ccc c CAG ATA AAG CGG AAG GCT C ttc aag aga GAG CCT TCC GCT TTA TCT G ttt tta–3′) was synthesized and then inserted into pENTR-shRNA using BglII and HindIII. A recombination between pAD/PL/DEST and pENTR-shCENP-W was performed using LR clonase (Invitrogen) and the resulting vector, pAd-shCENP-W, was transfected into 293A cells to produce adenoviral particles. The virus titer was calculated after infection in 293A cells.

2.4. Chromatin immunoprecipitation (ChIP) assay

The ChIP assays were performed following as described previously [12]. Briefly, harvested cells were cross-linked with 0.5% formaldehyde, and sonicated for 30 min with alternating pulses and pauses for 10 s each. After pre-clearing, ChIP was performed overnight at 4 °C using appropriate antibodies, and captured by the addition of Protein G Dynabeads (Novex). The immunoprecipitated DNA was used for PCR using a specific primer set presented previously for the transcriptional start site of pTK-Luc reporter [13], and *HOXA9* (homeobox A9) [14]. Quantitative PCR was carried out with CFX Connect Real-Time PCR Detection System (Bio-Rad) using IQ SYBR Green Supermix (Bio-Rad).

3. Results

3.1. CENP-W may participate in transcriptional repression

We have long suspected CENP-W as a transcriptional regulator based on an accumulation of evidence collected from various experiments. After cloning the CENP-W cDNA, we discovered that CENP-W localized to the nucleus, and its amino acid sequence shares no significant homology with any known proteins, except a weak homology with DR1 (down-regulator of transcription 1; 27% sequence identity) [5]. Additional insights came from studying the apoptotic activity of CENP-W. We have previously reported that CENP-W overexpression is sufficient to induce apoptosis in SKOV-3 human ovarian cancer cells and Zebrafish embryos [15]. To test if CENP-W overexpression could also accelerate chemical-induced cell death, cell viability was measured after treatment with different cytotoxic agents. Unfortunately, CENP-W overexpression only had minor effects on cytotoxicity, as cell viability was either unaffected following actinomycin D treatment (Fig. 1A), or slightly increased after rapamycin (Fig. 1A) or H_2O_2 (data not shown). However, to our surprise, CENP-W overexpression significantly reduced trichostatin A (TSA)-induced cell death (Fig. 1A). Given that TSA is a well-known HDAC inhibitor that can facilitate the transcriptional reactivation of various genes, including dormant tumor suppressors [16], these results suggest that CENP-W may repress the transcriptional activation of HDAC target genes.

In addition, we previously observed an atypical localization pattern of ectopically expressed CENP-W that initially localized in nucleoli, becomes gradually dispersed throughout the nucleus, and is finally observed as numerous punctures with approximately half of the CENP-W signal still localized to the nucleolus [9]. However, the transiently expressed CENP-W is frequently observed in the perinucleolar boundary, when either transfected alone (Fig. 1B) or co-transfected with B23, a nucleolar marker protein (Fig. 1C), which led us to question if CENP-W might be associated with perinucleolar heterochromatin region. To test this possibility, we co-transfected CENP-W with a representative heterochromatin-associated protein, heterochromatin protein 1-α (HP1α), into NIH3T3 cells and observed a substantial co-localization at the boundary of nucleoli (Fig. 1D), supporting that CENP-W may associate with the perinucleolar heterochromatin rings.

To clarify the role of CENP-W in transcriptional regulation, we used GAL4-luciferase reporter system. We co-transfected a plasmid encoding CENP-W/GAL4 DNA-binding domain (DB) fusion protein along with a (GAL4)5-TK-Luc reporter plasmid [11]. The luciferase activity imparted by GAL4-DB alone gradually decreased following increasing substitutions with GAL4-DB-CENP-W plasmid (Fig. 1E), indicating that the CENP-W/GAL4-DB fusion protein inhibited transcription from a GAL4-responsive promoter. Moreover, we found that the transcriptional repression activity of CENP-W was substantially overcome with the addition of TSA (Fig. 1F), suggesting that CENP-W inhibits transcription by modulating chromatin structure. Next, since multiple studies have shown that TSA actively de-repress the transcriptional repression of EZH2 target genes [17,18], we tested the possibility of a functional link between the PRC2 components and CENP-W. Based on a report that treatment with the HDAC inhibitor, LBH589, specifically induced depletion of PRC2 complex members [19], we examined the protein levels of PRC2 components after incubation with LBH589 in the HeLa-CENP-W stable cell line. As shown in Fig. 1G,

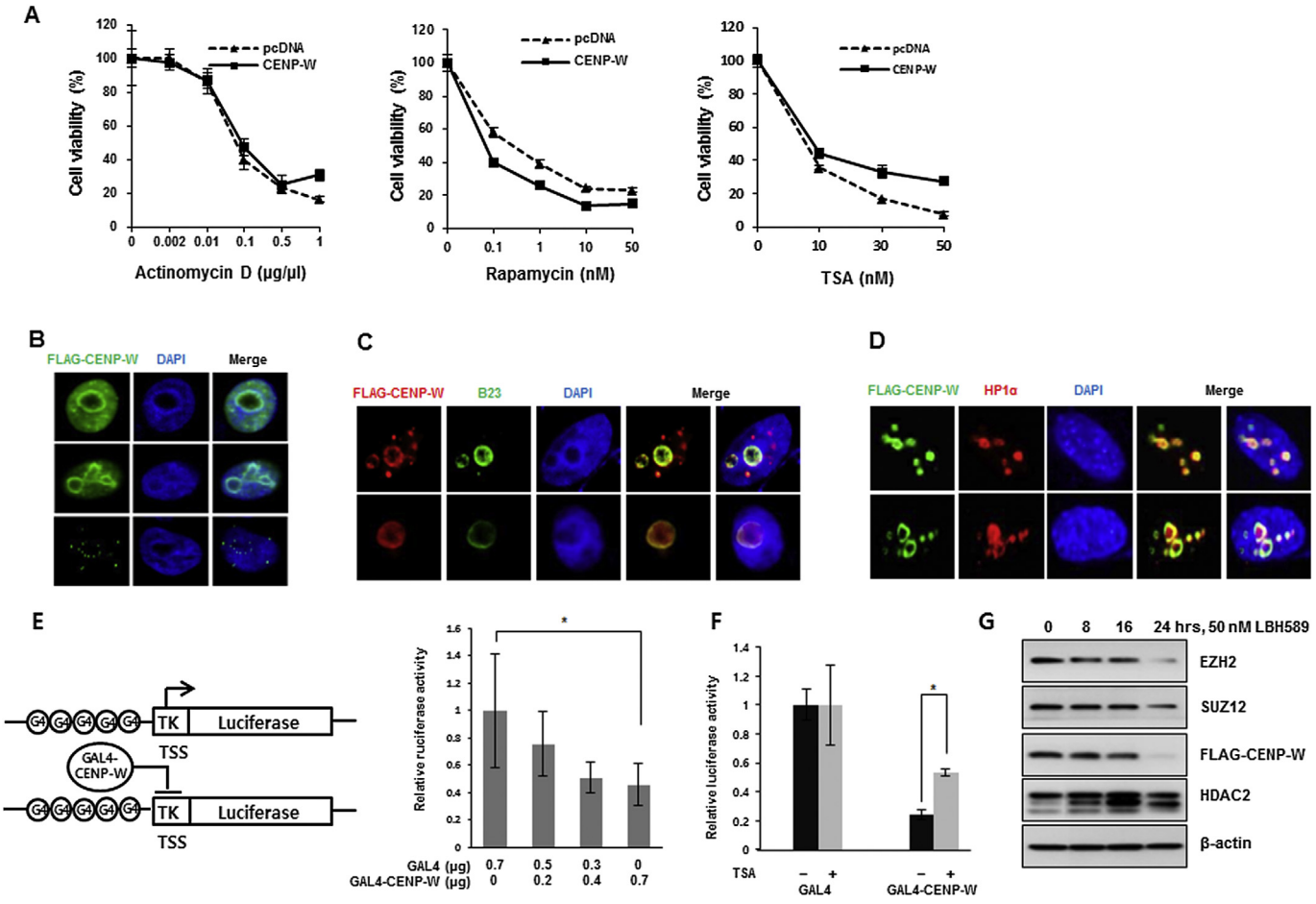

Fig. 1. CENP-W may function as transcriptional repressor. (A) Cell viability after treatment of various cytotoxic agents. After transfection of either pcDNA3-FLAG-CENP-W or vector control, HeLa cells were incubated with actinomycin D, rapamycin, or TSA for 24 h. The viable cells were counted with hemocytometer using Trypan Blue exclusion [15]. (B) Localization of ectopically expressed CENP-W. HeLa cells were immunostained with anti-FLAG antibody 48 h after transfection with pcDNA3-FLAG-CENP-W. (C) CENP-W is localized at the nucleolar periphery. HeLa cells were transfected with FLAG-CENP-W and double-immunostained with anti-FLAG and anti-B23 antibodies. (D) Co-localization of CENP-W with HP1α. NIH3T3 cells were transfected with FLAG-CENP-W and co-immunostained using anti-FLAG and anti-HP1α antibodies. (E) Transcriptional repression by GAL4-CENP-W fusion protein. Cells were transfected with different combinations of GAL4 or GAL4-CENP-W along with $(GAL4)_5$-TK-Luc. At 24 h post-transfection, cells were harvested and luciferase activity was measured. The error bar represents ±S.D. values between three separate reactions. $^*p < 0.001$. (F) TSA inhibits CENP-W-mediated transcriptional repression. Cells transfected with either GAL4-DB-CENP-W or GAL4-DB control were incubated with 100 nM TSA for 24 h. Relative luciferase activity of GAL4-CENP-W-transfected cells was calculated compared to the activity of GAL4-transfected controls. Data is presented as the mean ± SD ($^*p < 0.001$). (G) CENP-W is destabilized by LBH589. The protein level of the PRC2 components and CENP-W were monitored in HeLa-CENP-W cells after incubation of histone deacetylase inhibitor, LBH589 (50 nM).

the protein levels of EZH2 was reduced by LBH589. Moreover, a significant destabilization of CENP-W was observed in LBH589-treated cells, suggesting that CENP-W may be associated with the PRC2 complex.

3.2. *CENP-W interacts with EZH2*

To clarify the functional implication between EZH2 and CENP-W, we examined protein–protein interaction with ectopically expressed HA-EZH2 and FLAG-CENP-W in 293T cells by immunoprecipitation. CENP-W was only found in anti-EZH2 pulldowns, and not in control fraction (Fig. 2A). Reciprocally, HA-tagged EZH2 co-isolated with FLAG-CENP-W following immunoprecipitation with anti-FLAG antibody (Fig. 2B). We then examined this interaction between recombinant proteins produced using bacterial expression system in order to support a direct interaction between EZH2 and CENP-W. We constructed two bacterial expression plasmids, pET28a-EZH2 and pGEX-4T-3-CENP-W, to produce His-EZH2 and GST-CENP-W, respectively. Each protein was produced in *E. coli* and Ni-pulldown was performed using cell lysates. Notably, GST-CENP-W co-sedimented with His-EZH2 (Fig. 2C), demonstrating that these proteins are likely to interact directly in cells. To show this interaction at endogenous level, we performed co-immunoprecipitations with anti-EZH2 antibody. Due to the unavailability of an antibody capable of detecting endogenous CENP-W protein, we opt to use HeLa-FLAG-CENP-W stable cells [9]. CENP-W was co-fractionated with endogenous EZH2 (Fig. 2D), indicating that these proteins may be present in the same complex in cells.

To identify the CENP–W binding region of EZH2, we constructed several HA-EZH2 deletion mutants and performed co-immunoprecipitations on lysates from 293T cells that express EZH2 mutants and FLAG-CENP-W. As shown in Fig. 2E, central region (amino acids 334–527) which contains NLS sequence, was sufficient for binding with CENP-W. We also performed domain mapping using FLAG-CENP-W deletion constructs. For this, 293T cells were co-transfected with HA-EZH2 and CENP-W mutants, and immunoprecipitations were performed with anti-HA antibody. Although CENP-W is relatively small protein consisting of 88 amino acids, the central portion (amino acids 31–60) seems to be important for interacting with EZH2 (Fig. 2F).

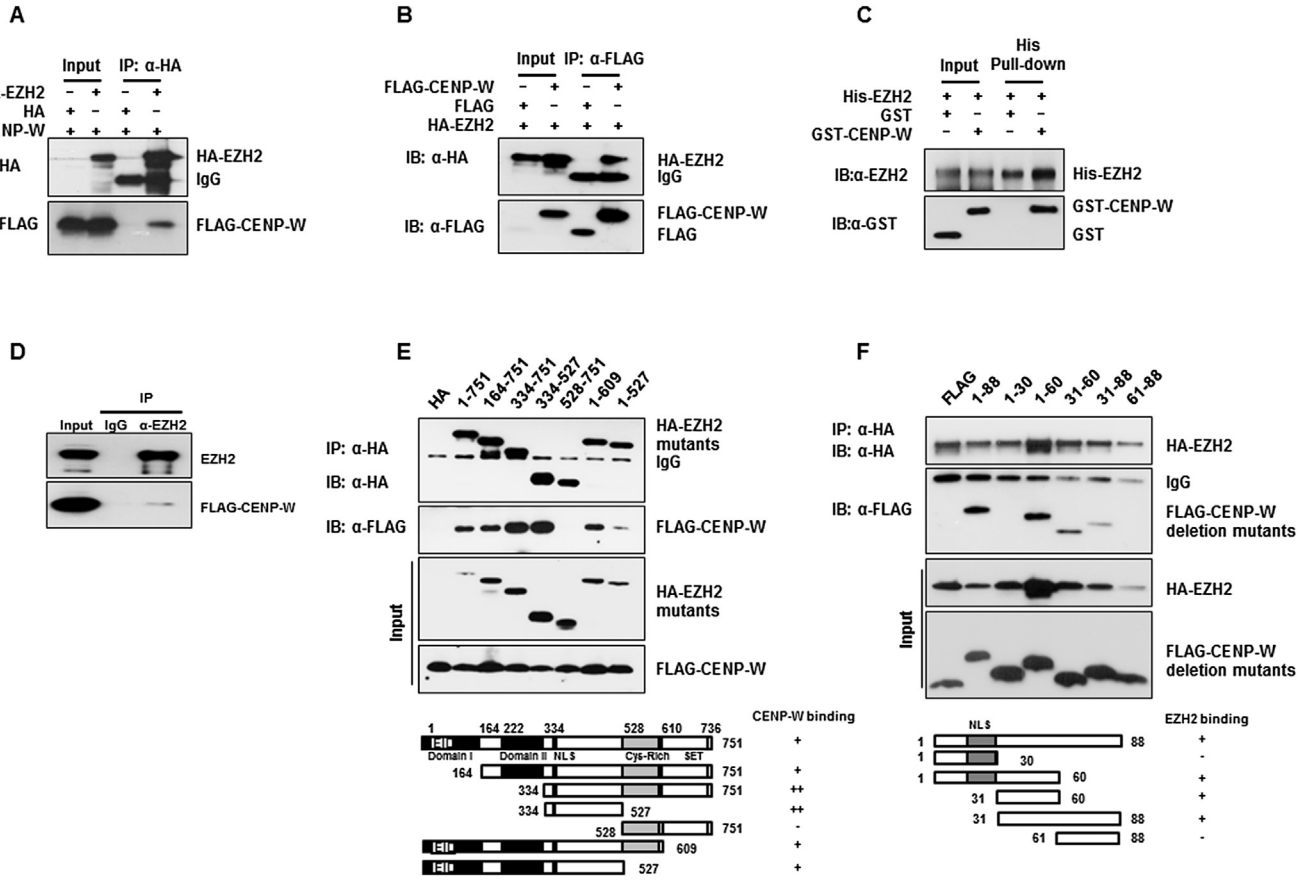

Fig. 2. CENP-W interacts with EZH2. (A) Co-immunoprecipitation between EZH2 and CENP-W. After 293T cells were transfected with HA-EZH2 and FLAG-CENP-W, co-immunoprecipitation was performed using anti-HA antibody. (B) Reciprocal co-immunoprecipitation. Cells transfected with HA-EZH2 and FLAG-CENP-W were subjected to immunoprecipitation using anti-FLAG antibody. (C) Interaction between recombinant proteins. His-EZH2 and GST-CENP-W proteins were expressed in *E. coli*, and His-EZH2 was collected using Ni Sepharose resin. (D) Complex formation of EZH2 and CENP-W at endogenous levels. EZH2 was immunoprecipitated using anti-EZH2 antibody from HeLa-FLAG-CENP-W cells. (E) Determination of EZH2 domain important for CENP–W binding. Various HA-tagged EZH2 domain mutants were generated as bait for co-immunoprecipitation, and co-fractionated FLAG-CENP-W was visualized by western blotting. (E) Domain mapping of CENP-W for EZH2 binding. After transfection into 293T cells, EZH2 was immunoprecipitated and co-isolated CENP-W mutants were identified using western blotting with anti-FLAG antibody.

3.3. *CENP-W increased EZH2 protein stability*

While performing the co-immunoprecipitation experiments, we noticed that EZH2 expression level was influenced by CENP-W co-expression. To evaluate this effect more clearly, we transfected increasing amounts of CENP-W plasmid along with constant amount of GFP-EZH2 plasmid. Western blotting with anti-EZH2 antibody revealed that both ectopically expressed GFP-EZH2 and endogenous EZH2 were significantly stabilized by CENP-W co-expression in HeLa and 293T cells (Fig. 3A). GFP was monitored as a transfection control. Conversely, we examined the effect of EZH2 on CENP-W, but no detectable change was observed (Fig. 3B). To confirm the previous results, we next performed a knockdown experiment using CENP-W siRNA in HeLa-FLAG-CENP-W cells. Notably, the EZH2 protein level were profoundly decreased in CENP-W-depleted cells (Fig. 3C). Moreover, H3K27 trimethylation was also decreased following CENP-W knockdown, indicating that CENP-W may influence the stability and the histone methylation activity of PRC2 complex. We also examined the EZH2 protein level by double-immunostaining after transfection with siCENP-W. A clear reduction in EZH2 protein was observed in CENP-W-suppressed cells, demonstrating a significant correlation between the EZH2 and CENP-W protein levels (Fig. 3D).

To conclusively demonstrate the effect of CENP-W on EZH2 stability, we examined the half-life of endogenous EZH2 protein. As shown in Fig. 3E, the EZH2 half-life was significantly increased by CENP-W co-expression. Based on the domain mapping results in Fig. 2F, we analyzed the effect of CENP-W mutants on EZH2 stability. Co-transfection with wild-type CENP-W increased the HA-EZH2 half-life (middle panel of Fig. 3F), as compared to the FLAG-transfected control (left panel); however, no effect on half-life was observed in cells with co-expression of CENP-W mutant (1–30 aa), which failed to interact with EZH2 (right panel of Fig. 3F). Finally, we examined the effect of CENP-W on various HA-EZH2 mutant constructs. We selected two deletion mutants–HA-EZH2 (1–609) and HA-EZH2 (528–751) that bound or did not bind CENP-W, respectively. Both wild-type and the EZH2 binding mutant (1–609) were clearly stabilized by CENP-W co-transfection (Fig. 3G); however, half-life of non-binding mutant (528–751) was not increased by CENP-W expression. Collectively, these data indicate that EZH2 stability was increased by CENP-W through protein–protein interaction.

3.4. *CENP-W enhanced EZH2-mediated transcriptional repression*

To examine if CENP-W could modulate EZH2-mediated transcriptional repression, we used a GAL4-TK-Luc reporter system. For this purpose, a mammalian expression vector encoding a EZH2/GAL4 DNA-BD fusion protein was generated and co-transfected with the GAL4-TK-Luc reporter in 293T cells. The luciferase activity observed in transfected cells reduced in proportion to the

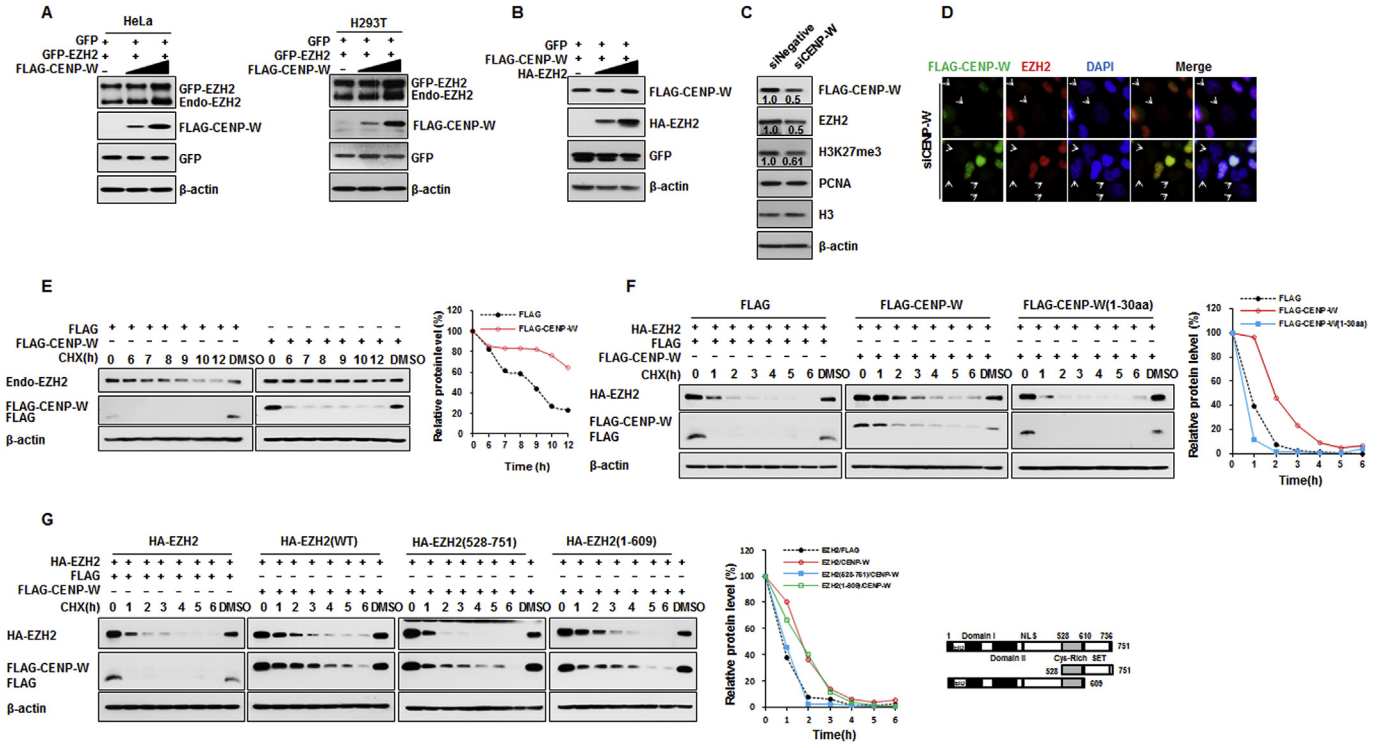

Fig. 3. CENP-W enhanced EZH2 stability. (A) Protein level of EZH2 is increased by the ectopic expression of CENP-W. HeLa or 293T cells were transfected with increasing amount of pcDNA3-FLAG-CENP-W, along with pEGFP-EZH2. (B) CENP-W protein was not affected by EZH2 overexpression. FLAG-CENP-W was transfected to 293T cells along with increasing amount of HA-EZH2. (C) Protein level of EZH2 in CENP-W knockdown cells. Protein levels were examined after treatment of CENP-W siRNA (100 nM) for 72 h in HeLa-FLAG-CENP-W cells. (D) Immunostaining of EZH2 upon CENP-W depletion. HeLa-CENP-W cells were incubated with CENP-W siRNA (100 nM) for 48 h, then double-immunostained with anti-FLAG and anti-EZH2 antibodies. The white arrows indicate cells exhibiting highly decreased levels in both EZH2 and CENP-W proteins. (E) Determination of half-life of endogenous EZH2 after CENP-W overexpression. After transfection with FLAG-CENP-W or vector control, HeLa cells were incubated with cyclohexamide (100 μg/mL) for the indicated times. (F) Half-life of EZH2 after transfection with mutant CENP-W. HA-EZH2 was co-transfected into 293T cells with wild-type, or CENP-W EZH2-nonbinding mutant (1–30 aa). At 24 h post-transfection, cells were incubated with cyclohexamide (100 μg/mL) and harvested at indicated time points. (G) Determination of half-life of mutant EZH2. The binding (1–609 aa) or non-binding (528–751 aa) EZH2 mutants and the wild-type were co-expressed with FLAG-CENP-W in 293T cells before cyclohexamide treatment.

increasing amount of transfected GAL4-EZH2 (Fig. 4A), demonstrating that EZH2 inhibits GAL4-mediated transcription. We then co-transfected FLAG-CENP-W plasmid along with GAL4-EZH2 into 293T cells, and examined the cellular luciferase activity. Notably, EZH2-mediated transcriptional inhibition was further reduced with the addition of CENP-W (Fig. 4B), suggesting that CENP-W may enhance the EZH2-mediated transcriptional repression. To evaluate if CENP-W participates in transcriptional repression through a physical association with the TK promoter, we performed chromatin immunoprecipitation (ChIP) analysis using anti-FLAG antibody after FLAG-CENP-W and GAL4-EZH2 expression. The subsequent PCR amplifications showed that both EZH2 and CENP-W bound to the TK promoter (Fig. 4C). We then examined if CENP-W is involved in the repression of well-documented EZH2 target gene, *HOXA9* (homeobox A9) [14]. ChIP was carried out to enrich EZH2- or CENP-W-bound DNA fragments using anti-EZH2 or anti-FLAG antibody in HeLa-CENP-W cells. Then, PCR was performed using primer set specific for the promoter (A) or intron region (B) of *HOXA9* gene. As shown in Fig. 4D, CENP-W specifically bound to the promoter region of *HOXA9*, supporting that CENP-W may participate in the PRC2-mediated gene silencing in cells.

Finally, taken that CENP-W is histone motif-containing DNA-binding protein, we examined if CENP-W could facilitate the recruitment of EZH2 to target DNA. After 293T cells were transfected with FLAG-CENP-W, ChIP was performed using anti-EZH2 antibody. The subsequent PCR showed that *HOXA9*-incorporated EZH2 is highly enhanced by CENP-W co-transfection (Fig. 4E). To further demonstrate the role of CENP-W in EZH2 targeting, we also transfected a mutant CENP-W (aa 31–88) which binds with EZH2 (Fig. 2F), but is defective for DNA binding. The transfection of the DNA-nonbinding mutant no longer promotes EZH2 targeting into its target (Fig. 4E), indicating that DNA-binding activity of CENP-W is required to facilitate target recognition of EZH2. Then, we infected HeLa cells with adenoviral particles harboring CENP-W shRNA. Subsequent ChIP assays revealed that the amount of EZH2 binding to the *HOXA9* promoter decreased in CENP-W-depleted cells (Fig. 4F). Finally, we performed ChIP using anti-H3K27me3 antibody to analyze the presence of *HOXA9* promoter in enriched DNA. The PCR result showed that H3K27 trimethylation at the *HOXA9* promoter decreased after CENP-W knockdown (Fig. 4G). Collectively, our data indicate that CENP-W may enhance the EZH2-mediated gene silencing and promote H3K27 trimethylation by incorporating with the target DNA.

4. Discussion

CENP-W was originally identified as a putative oncogene, which is activated in many human tumor biopsies. Initial identification of the transcriptional repressor signature in CENP-W led us to question whether CENP-W could be functionally associated with chromatin repression. In this study, we demonstrate that CENP-W interacts and stabilizes the PRC2 component, EZH2, and promotes gene silencing by physically associating with known EZH2 target genes.

Although PRC2 is known to mediate the silencing of a broad range of genes associated with differentiation of ES cells, as well as cancer progression [20], it is still not clear how PRC2 obtained

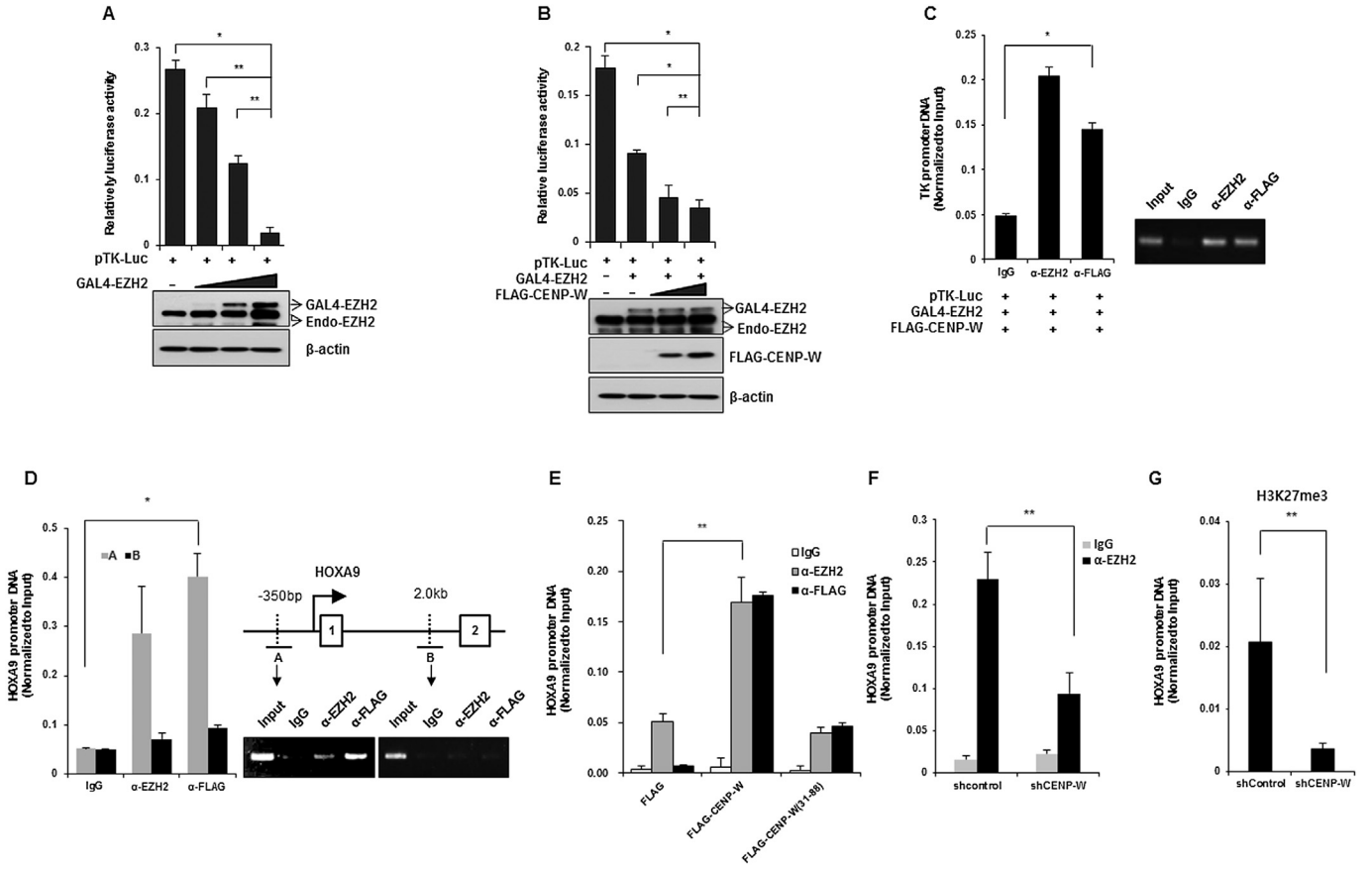

Fig. 4. CENP-W may participate in EZH2-mediated transcriptional repression. (A) EZH2 effectively inhibits transcription of GAL4-TK-Luc reporter gene. Luciferase activity was examined after transfection of increasing amount of GAL4-DB-EZH2 plasmid along with GAL4-responsive pTK-Luc. The expression of EZH2 was monitored using anti-EZH2 antibody. The error bars represent ±SD values between samples. $^{*}p < 0.001$, $^{**}p < 0.05$. (B) CENP-W expression may further repress EZH2-mediated transcription. Luciferase reporter activity was determined after increasing amount of FLAG-CENP-W was co-transfected with GAL4-DB-EZH2 ($^{*}p < 0.001$, $^{**}p < 0.05$). (C) ChIP of CENP-W. After transfection with GAL4-DB-EZH2, pTK-Luc, and FLAG-CENP-W, 293T cell lysates were subjected to ChIP using anti-EZH2 or anti-FLAG antibody. Primers corresponding to TK promoter region were used for subsequent PCR analysis for immunoprecipitated DNA ($^{*}p < 0.001$). (D) ChIP analysis of *HOXA9* gene. ChIP was performed using HeLa-CENP-W cells, and the immunoprecipitated DNA was analyzed by a real-time PCR using primer set specific for the promoter (A) or intron region (B) of *HOXA9* gene ($^{*}p < 0.001$). (E) CENP-W may facilitate the recruitment of EZH2 to the target genes. After transfection of CENP-W wild-type or deletion mutant (aa 31–88) to 293T cells, ChIP was performed using anti-EZH2 antibody. A primer set for *HOXA9* gene was used for the subsequent PCR ($^{**}p < 0.05$). (F) CENP-W knockdown decreased the target binding of EZH2. After transduction with adenoviral CENP-W shRNA, HeLa cells were subjected to ChIP analysis using anti-EZH2 antibody. The bound DNA was used for PCR analysis using primers for *HOXA9* gene. $^{**}p < 0.05$. (G) CENP-W suppression reduced H3K27 trimethylation. After CENP-W depletion using adenoviral shRNA, ChIP was carried out with anti-H3K27me3 antibody. The precipitated DNA was used in PCR for *HOXA9* gene ($^{**}p < 0.05$).

target specificity since none of the core PRC2 components possess a DNA binding domain [21]. For that reason, it was suggested that the specificity of transcriptional repression must be achieved by other factors, such as DNA binding components, long noncoding RNAs, or combination [21].

As a DNA-binding protein, CENP-W forms unique histone-like structures in centromere along with other kinetochore components [8]. Our results propose that a novel DNA-binding protein, CENP-W, may play a role in recruitment of PRC2 complex by incorporating with EZH2 target genes. The fact that EZH2 is frequently amplified in human cancers and is highly associated with tumor aggression [22,23], may also suggest a functional collaboration between CENP-W and EZH2 in the silencing of tumor suppressor genes during tumor development.

Acknowledgments

This research was supported by Basic Science Research Program by the Ministry of Education, Science and Technology of Korea (2013R1A1A3A04005274) and research fund of Chungnam National University.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.136.

References

[1] A. Sparmann, M. van Lohuizen, Polycomb silencers control cell fate, development and cancer, Nat. Rev. Cancer 6 (2006) 846–856.
[2] R. Margueron, D. Reinberg, The polycomb complex PRC2 and its mark in life, Nature 469 (2011) 343–349.
[3] H. Hock, A complex polycomb issue: the two faces of EZH2 in cancer, Genes Dev. 26 (2012) 751–755.
[4] J. Tan, X. Yang, L. Zhuang, X. Jiang, W. Chen, P.L. Lee, R.K. Karuturi, P.B. Tan, E.T. Liu, Q. Yu, Pharmacologic disruption of polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells, Genes Dev. 21 (2007) 1050–1063.
[5] S. Lee, J. Gang, S.B. Jeon, S.H. Choo, B. Lee, Y.G. Kim, Y.S. Lee, J. Jung, S.Y. Song, S.S. Koh, Molecular cloning and functional analysis of a novel oncogene, cancer-upregulated gene 2 (CUG2), Biochem. Biophys. Res. Commun. 360 (2007) 633–639.
[6] T. Hori, M. Amano, A. Suzuki, C.B. Backer, J.P. Welburn, Y. Dong, B.F. McEwen, W.H. Shang, E. Suzuki, K. Okawa, I.M. Cheeseman, T. Fukagawa, CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore, Cell 135 (2008) 1039–1052.

[7] H. Kim, M. Lee, S. Lee, B. Park, W. Koh, D.J. Lee, D.S. Lim, Cancer-upregulated gene 2 (CUG2), a new component of centromere complex, is required for kinetochore function, Mol. Cells 27 (2009) 697–701.
[8] T. Nishino, K. Takeuchi, K.E. Gascoigne, A. Suzuki, T. Hori, T. Oyama, K. Morikawa, I.M. Cheeseman, T. Fukagawa, CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold, Cell 148 (2012) 487–501.
[9] Y. Chun, B. Park, W. Koh, S. Lee, Y. Cheon, R. Kim, L. Che, New centromeric component CENP-W is an RNA-associated nuclear matrix protein that interacts with nucleophosmin/B23 protein, J. Biol. Chem. 286 (2011) 42758–42769.
[10] C. Guetg, R. Santoro, Formation of nuclear heterochromatin: the nucleolar point of view, Epigenetics 7 (2012) 811–814.
[11] J.H. Ahn, E.J. Brignole 3rd, G.S. Hayward, Disruption of PML subnuclear domains by the acidic IE1 protein of human cytomegalovirus is mediated through interaction with PML and may modulate a RING finger-dependent cryptic transactivator function of PML, Mol. Cell Biol. 18 (1998) 4899–4913.
[12] S. Tanaka, S. Miyagi, G. Sashida, T. Chiba, J. Yuan, M. Mochizuki-Kashio, Y. Suzuki, S. Sugano, C. Nakaseko, K. Yokote, H. Koseki, A. Iwama, Ezh2 augments leukemogenicity by reinforcing differentiation blockage in acute myeloid leukemia, Blood 120 (2012) 1107–1117.
[13] K.H. Hansen, A.P. Bracken, D. Pasini, N. Dietrich, S.S. Gehani, A. Monrad, J. Rappsilber, M. Lerdrup, K. Helin, A model for transmission of the H3K27me3 epigenetic mark, Nat. Cell Biol. 10 (2008) 1291–1300.
[14] S. Chen, L.R. Bohrer, A.N. Rai, Y. Pan, L. Gan, X. Zhou, A. Bagchi, J.A. Simon, H. Huang, Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2, Nat. Cell Biol. 12 (2010) 1108–1114.
[15] S. Lee, W. Koh, H.T. Kim, C.H. Kim, Cancer-upregulated gene 2 (CUG2) overexpression induces apoptosis in SKOV-3 cells, Cell Biochem. Funct. 28 (2010) 461–468.
[16] S. Shankar, R.K. Srivastava, Histone deacetylase inhibitors: mechanisms and clinical significance in cancer: HDAC inhibitor-induced apoptosis, Adv. Exp. Med. Biol. 615 (2008) 261–298.
[17] F. Sun, E. Chan, Z. Wu, X. Yang, V.E. Marquez, Q. Yu, Combinatorial pharmacologic approaches target EZH2-mediated gene repression in breast cancer cells, Mol. Cancer Ther. 8 (2009) 3191–3202.
[18] S. Varambally, S.M. Dhanasekaran, M. Zhou, T.R. Barrette, C. Kumar-Sinha, M.G. Sanda, D. Ghosh, K.J. Pienta, R.G. Sewalt, A.P. Otte, M.A. Rubin, A.M. Chinnaiyan, The polycomb group protein EZH2 is involved in progression of prostate cancer, Nature 419 (2002) 624–629.
[19] W. Fiskus, M. Pranpat, M. Balasis, B. Herger, R. Rao, A. Chinnaiyan, P. Atadja, K. Bhalla, Histone deacetylase inhibitors deplete enhancer of zeste 2 and associated polycomb repressive complex 2 proteins in human acute leukemia cells, Mol. Cancer Ther. 5 (2006) 3096–3104.
[20] I. Aldiri, M.L. Vetter, PRC2 during vertebrate organogenesis: a complex in transition, Dev. Biol. 367 (2012) 91–99.
[21] S. Kaneko, R. Bonasio, R. Saldana-Meyer, T. Yoshida, J. Son, K. Nishino, A. Umezawa, D. Reinberg, Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin, Mol. Cell 53 (2014) 290–300.
[22] B.D. Karanikolas, M.L. Figueiredo, L. Wu, Polycomb group protein enhancer of zeste 2 is an oncogene that promotes the neoplastic transformation of a benign prostatic epithelial cell line, Mol. Cancer Res. 7 (2009) 1456–1465.
[23] K. Collett, G.E. Eide, J. Arnes, I.M. Stefansson, J. Eide, A. Braaten, T. Aas, A.P. Otte, L.A. Akslen, Expression of enhancer of zeste homologue 2 is significantly associated with increased tumor cell proliferation and is a marker of aggressive breast cancer, Clin. Cancer Res. 12 (2006) 1168–1174.