EMBO reports

scientific report

PELP1 is a reader of histone H3 methylation that facilitates oestrogen receptor-α target gene activation by regulating lysine demethylase 1 specificity

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Histone methylation has a key role in oestrogen receptor (ERα)-mediated transactivation of genes. Proline glutamic acid and leucine-rich protein 1 (PELP1) is a new proto-oncogene that functions as an ERα co-regulator. In this study, we identified histone lysine demethylase, KDM1, as a new PELP1-interacting protein. These proteins, PELP1 and KDM1, were both recruited to ERα target genes, and PELP1 depletion affected the dimethyl histone modifications at ERα target genes. Dimethyl-modified histones H3K4 and H3K9 are recognized by PELP1, and PELP1 alters the substrate specificity of KDM1 from H3K4 to H3K9. Effective demethylation of dimethyl H3K9 by KDM1 requires a KDM1–ERα–PELP1 functional complex. These results suggest that PELP1 is a reader of H3 methylation marks and has a crucial role in modulating the histone code at the ERα target genes.

Keywords: ER; H3 methylation; KDM1; PELP1

EMBO *reports* (2010) **11**, 438–444. doi:10.1038/embor.2010.62

INTRODUCTION

Emerging evidence suggests that histone methylation, an epigenetic phenomenon, has a vital role in many neoplastic processes (Kouzarides, 2007). Histone lysine residues can be mono-, di- or trimethylated (Leader *et al*, 2006), and such modifications are shown to enable the recruitment of specialized proteins that facilitate defined chromatin-associated functions (Roth *et al*, 2001). The recent discovery of lysine-specific demethylase 1 (KDM1, also designated as LSD1) suggests that histone methylation is reversible (Shi *et al*, 2004). Recent studies showed that KDM1 can demethylate both H3K4 and H3K9 (Metzger *et al*, 2005); however, the molecular mechanisms and effectors that modulate the substrate specificity of KDM1 remain unknown and are an active area of investigation in many laboratories.

The human oestrogen receptor (ERα) is a key transcriptional regulator of oestrogen (E2) biology, which is implicated in breast cancer initiation and progression. Oestrogen stimulation promotes histone modifications at ERα target promoters and histone methylation imposes ligand dependency at ER target genes (Garcia-Bassets *et al*, 2007). KDM1 is recruited to a significant fraction of ER target genes, and is shown to be required to demethylate histones H3K9Me1 and H3K9Me2 at the promoter enhancer region to enable transcription by ER (Perillo *et al*, 2008). We have shown previously that proline glutamic acid- and leucine-rich protein 1 (PELP1) is an ERα co-regulator (Vadlamudi *et al*, 2001). PELP1 has no known enzymatic activity, however, it predominantly localizes to the nuclear compartment and associates with chromatin-modifying enzymes (Vadlamudi & Kumar, 2007). In this study, we present evidence that PELP1 acts as a molecular link that facilitates ERα-mediated histone methyl modifications by recognizing dimethyl modifications and by altering the substrate specificity of KDM1.

RESULTS AND DISCUSSION

PELP1 is a new KDM1-interacting protein

Our ongoing yeast-based library screening identified KDM1 as a potential PELP1-binding protein. To verify that the observed interaction between PELP1 and KDM1 in the yeast screen also occurs in mammalian cells, we treated MCF7 (Michigan Cancer Foundation 7) cells with E2, and the interaction of endogenous PELP1 with KDM1 was analysed by reciprocal immunoprecipitation assay. We observed that KDM1 interacts with PELP1 (Fig 1A). Immunoprecipitation of His-tagged PELP1 from MCF7 cells also revealed that PELP1 interacts with KDM1 (Fig 1B). As PELP1 recruits to ERα target genes on ligand stimulation (Nair *et al*,

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Received 10 November 2009; revised 25 March 2010; accepted 26 March 2010; published online 7 May 2010

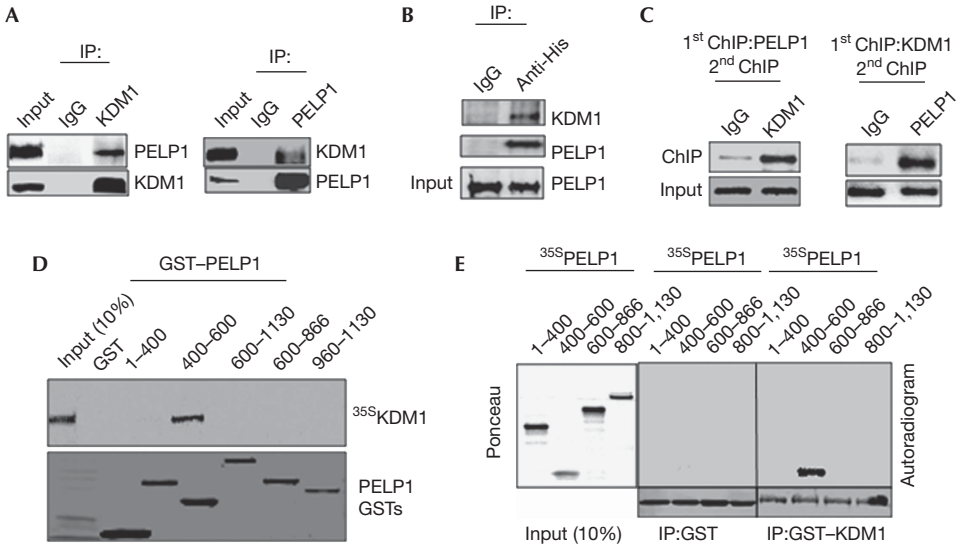

Fig 1 | PELP1 is a new KDM1-interacting protein. MCF7 cells were treated with E2 for 60 min. (**A**) The interaction of PELP1 and KDM1 was analysed by immunoprecipitation. (**B**) MCF7 cells expressing His-tagged PELP1 were treated with E2 and the interaction of His-tagged PELP1 with KDM1 was analysed by immunoprecipitation. (**C**) MCF7 cells were treated with E2, and the recruitment of PELP1 and KDM1 on TFF1 proximal promoter was analysed by ChIP and reChIP. (**D**) *In vitro* synthesized and radiolabelled KDM1 protein was incubated with various deletions of PELP1 and binding was analysed by autoradiography. (**E**) *In vitro* synthesized and radiolabelled PELP1 peptides were incubated with GST–KDM1 and binding was analysed by autoradiography. ChIP, chromatin immunoprecipitation; E2, oestrogen; GST, glutathione-*S*-transferase; KDM1, lysine demethylase 1; MCF7, Michigan Cancer Foundation 7; PELP1, proline glutamic acid- and leucine-rich protein 1; TFF1, trefoil factor family 1.

2004), we examined whether PELP1 and KDM1 are co-recruited to the same region in ERα target promoters. We performed a sequential chromatin immunoprecipitation (ChIP) experiment in which the first ChIP was carried out using the PELP1 or KDM1 antibodies, and then the eluted chromatin was reChIPed with either the KDM1 or the PELP1 antibody. Results from this experiment showed that PELP1 and KDM1 were co-recruited to the ERα target promoter (Fig 1C). To map the PELP1 and KDM1 interaction further, we tested the ability of an *in vitro*-translated ^{35}S-labelled full-length KDM1 protein to bind to various domains of PELP1 fused to glutathione *S*-transferase (GST). The GST–PELP1 fusion containing 400–600 amino acids (aa), but not other GST–PELP1 fusions, efficiently interacted with KDM1 (Fig 1D). Conversely, the GST–KDM1 protein specifically interacted with the *in vitro*-translated PELP1 400–600aa protein (Fig 1E). Collectively, these *in vitro* and *in vivo* results suggest that PELP1 interacts with KDM1 and that PELP1 400–600aa is the KDM1-binding region.

PELP1 influences histone H3 methylation

Co-transfection of PELP1 and KDM1 substantially increased the ability of E2 to stimulate oestrogen response element (ERE)-driven transcription (Fig 2A) compared with the ability of transfection with either PELP1 or KDM1 alone. However, transfection of PELP1 with the KDM1^{K661A} mutant (Lee *et al*, 2005) abolished the PELP1-mediated increase in ERα co-activation (supplementary Fig S1 online). These results suggest that KDM1 enzymatic activity is important for PELP1-mediated ERα co-activation functions. As recent studies showed that KDM1 is recruited to a substantial number of ERα target genes, we initially examined whether PELP1 is also recruited to the ERα target genes that exhibit KDM1 recruitment. A ChIP assay using five randomly selected ERα–KDM1 target genes revealed that PELP1 was also recruited to those ERα target genes (Fig 2B).

To examine whether, PELP1–KDM1 interactions have a role in modulating H3 methyl modifications at ERα target genes, we examined the status of H3 methylation at these target genes by using MCF7 cells that stably express PELP1 short hairpin RNA (shRNA). The expression of PELP1 shRNA reduced endogenous PELP1 to ~80% of the normal level (supplementary Fig S1 online). The MCF7 and MCF7-PELP1 shRNA cells were treated with E2 for various periods of time and chromatin was immunopreciptated using H3K4me2 and H3K9me2 antibodies. The E2 treatment of control MCF7 cells resulted in an increase in methylation of H3K4me2, an activation mark, with a concomitant decrease in methylation of H3K9me2, a repressive mark, at the trefoil factor family 1 (TFF1) promoter (Fig 2C, left panel). However, these changes were not seen in the MCF7-PELP1 shRNA cells treated with E2 (Fig 2C, right panels). Similar defects in histone H3 methylation were also observed at other ERα–KDM1 target genes GREB1C, DSCR3 and DDX17 (supplementary Fig S2 online).

To eliminate the possibility that the defects seen in H3 methylation were not due to defects in ERα recruitment, we examined the kinetics of ERα recruitment in PELP1 shRNA clones. We observed that the association of ERα with target genomic sites when stimulated by a ligand in PELP1 shRNA cells was not affected (Fig 2D). These results suggest that the observed changes in specific histone methylation pattern are clearly due to down-stream events in ERα signalling and are in part mediated by PELP1. Accordingly, reverse transcriptase–quantitative PCR (RT–qPCR) analysis revealed a lower expression of ERα target genes in PELP1

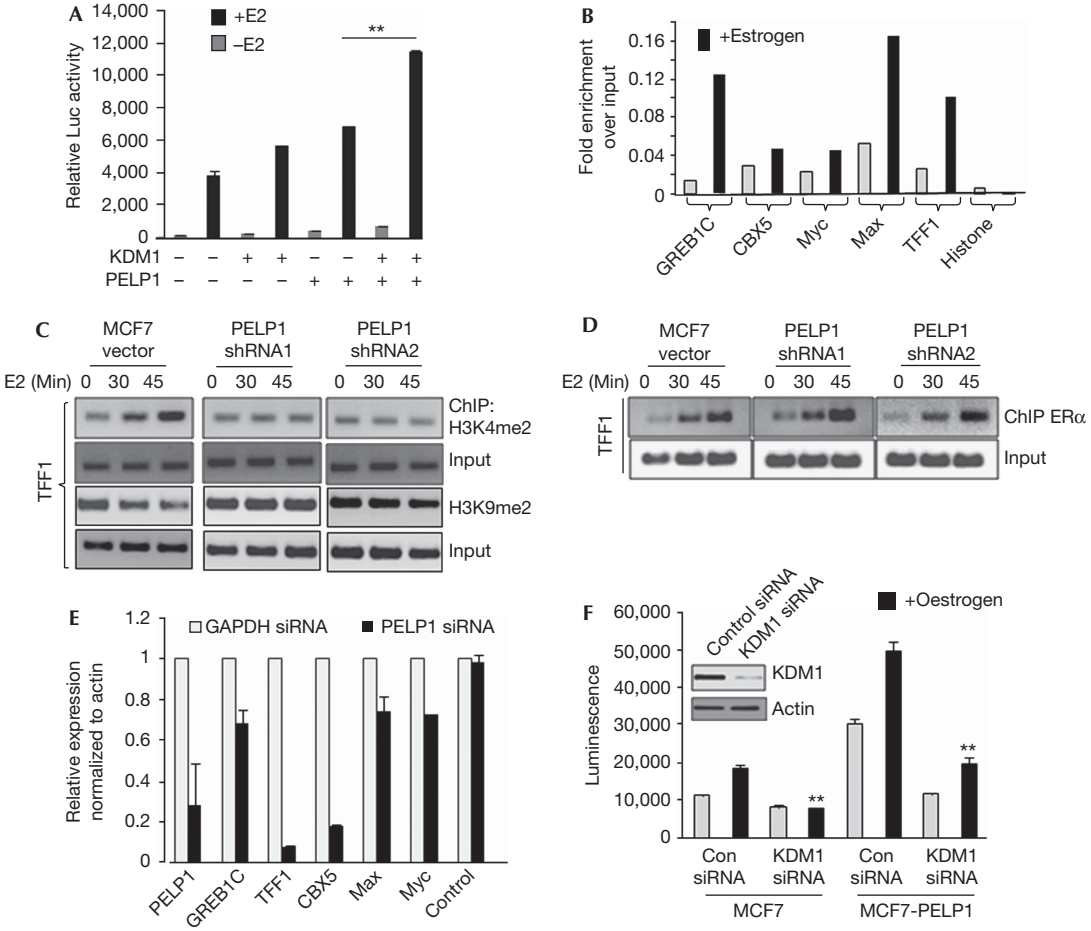

Fig 2 | PELP1 status influences histone H3 methylation at ERα target promoters. (**A**) MCF7 cells were transfected with an ERE-reporter gene with or without KDM1 and PELP1 expression vectors. After 72 h, cells were stimulated with or without E2 for 12 h and then luciferase activity was measured. (**B**) ChIP qPCR analysis of PELP1 recruitment at several ERα–KDM1 target genes. (**C**) MCF7 cell expressing vector or PELP1 shRNA1 or 2 were treated with oestrogen (E2) for the indicated times and ChIP analysis was performed using H3K9me2- or H3K4me2-specific antibodies and the status of methylation was analysed by using PCR with TFF1 gene-specific primers. (**D**) ChIP was performed using the ERα antibody. (**E**) MCF7 cells were transiently transfected with control or PELP1-specific siRNA; total RNA was isolated and expression of ERα–KDM1 target genes was analysed by RT–qPCR. (**F**) MCF7 and MCF7-PELP1 model cells were transfected with control (Con) or KDM1-specific siRNA. After 48 h, cells were placed in a 96-well plate, treated with or without E2, and cell viability was measured after 72 h using the Cell Titer Glo ATP assay. Insert shows the efficiency of KDM1 knockdown. Error bars indicate ± s.e.m. $^{**}P<0.001$. ChIP, chromatin immunoprecipitation; E2, oestrogen; ERE, ostrogen response element; GST, glutathione-*S*-transferase; KDM1, lysine demethylase 1; MCF7, Michigan Cancer Foundation 7; PELP1, proline glutamic acid- and leucine-rich protein 1; qPCR, quantitative PCR; RT–qPCR, reverse transcriptase–quantitative PCR; siRNA, small interfering RNA; shRNA, short hairpin RNA; TFF1, trefoil factor family 1.

knockdown cells than in control cells (Fig 2E). Previous studies showed that PELP1 is a proto-oncogene and its overexpression enhances cell proliferation (Vadlamudi & Kumar, 2007). We then determined whether KDM1 is needed for a PELP1-mediated increase in cell proliferation. As expected, we observed substantially greater proliferation in MCF7-PELP1 cells when compared with the proliferation in control MCF7 cells. Interestingly, treatment of MCF7 cells with KDM1-specific small interfering RNA (siRNA; Fig 2F) or pargyline, a KDM1 inhibitor (supplementary Fig S1D online), substantially reduced PELP1-mediated cell proliferation. These results suggest that PELP1–KDM1 interactions have a dominant role in modulating the histone methylation code at ERα target genes and that KDM1 is needed for PELP1-mediated ERα co-activation functions.

PELP1 is a reader of dimethyl-modified histones

PELP1 is a unique co-regulator that contains a 70-aa region comprising glutamic and/or aspartic acids. Earlier studies suggested that such a region might be involved in binding to basic proteins such as histones (Nair *et al*, 2004). As PELP1 interacts with KDM1, which is a histone demethylase, we determined whether PELP1 recognizes methylated histones. We performed a peptide pull-down assay using biotinylated H3K4 or H3K9 peptides that were either unmodified, mono-, di- or tri-methyl modified. We used baculovirus-expressed, purified full-length PELP1 as the source. After incubation of recombinant PELP1 with the peptides, peptides were pulled down using avidin beads and bound PELP1 was eluted and detected by

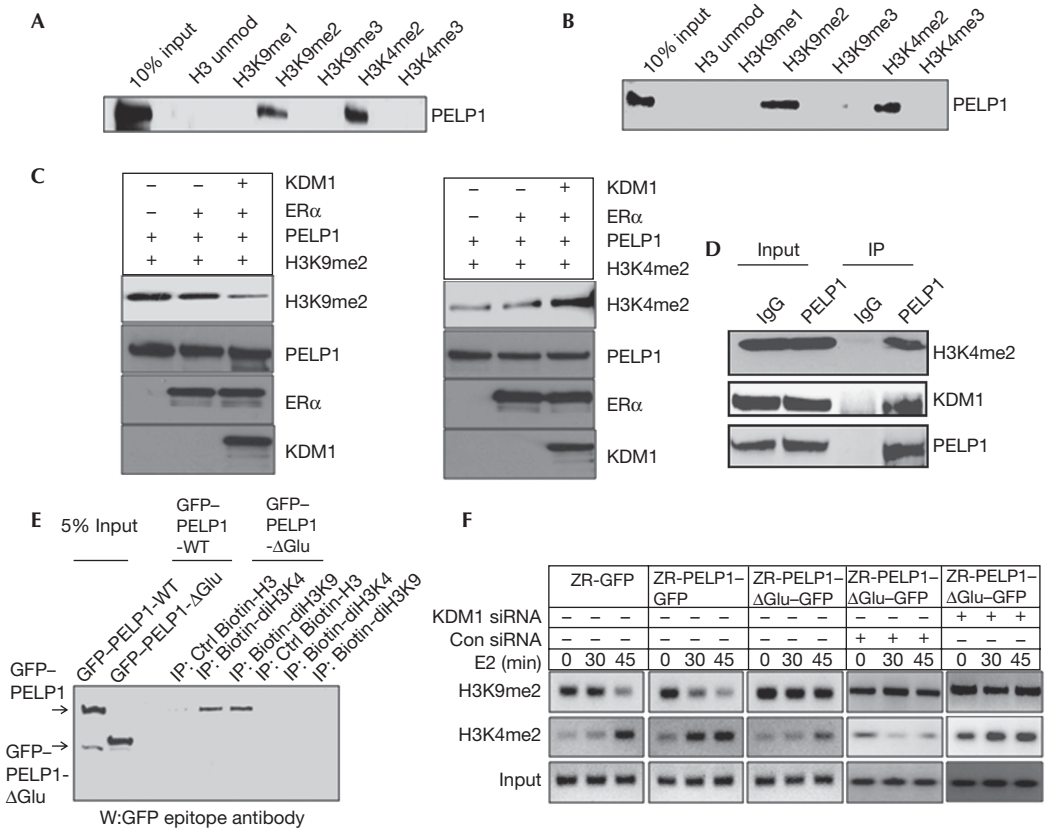

Fig 3 | PELP1 specifically associates with dimethyl-modified histones. Peptide pull-down assays were performed by using (**A**) purified PELP1 or (**B**) MCF7 cell nuclear extracts using unmodified or methylated peptides. PELP1 interaction with histones or modified histones was visualized by using western blot analysis. (**C**) Peptide pull-down assays were performed with purified PELP1 in the presence or absence of KDM1, ERα (**D**) MCF7 cells were treated with E2 for 30 min, nuclear extracts were immunoprecipitated with PELP1 antibody, and the presence of H3K4me2 and KDM1 was analysed by using western blotting. (**E**) GFP–PELP1-WT- and GFP–PELP1-ΔGlu-expressing nuclear lysates were incubated with control or H3-dimethyl peptides and biotin pull-down assays were performed. (**F**) ZR75 (ZR) cells expressing GFP vector, GFP–PELP1-WT or GFP–PELP1-ΔGlu were treated with control siRNA or KDM1-specific siRNA. After 72 h, cells were treated with E2. ChIP analysis was performed using H3K9me2- or H3K4me2-specific antibodies and the status of methylation was analysed by PCR using TFF1 gene-specific primers. ChIP, chromatin immunoprecipitation; E2, oestrogen; GFP, green fluorescent protein; KDM1, lysine demethylase 1; MCF7, Michigan Cancer Foundation 7; PELP1, proline glutamic acid- and leucine-rich protein 1; siRNA, small interfering RNA; TFF1, trefoil factor family 1; WT, wild type.

western blotting. Under these conditions, PELP1 recognized dimethyl H3K9 and H3K4 peptides. No binding was observed to either mono- or tri-methyl modified or unmodified H3 peptides (Fig 3A). We repeated this experiment using MCF7 nuclear extracts as the source of PELP1, which also showed that PELP1 recognizes dimethyl H3K9 and H3K4 peptides (Fig 3B). We confirmed the appropriate methyl modification of biotin-H3 peptides used in the assay by dot blotting and western blotting with methyl mark-specific antibodies. By using pull-down assays of various deletion mutants of PELP1, we observed that PELP1 800–960aa, containing a glutamic acid (Glu)-rich region, was the H3 methyl peptide-binding region (supplementary Fig S3A–C online). To examine which methylated site (K4 or K9) is bound by PELP1 when this factor is recruited on ERα–KDM1 target promoters, we performed peptide-binding assay in the presence of ERα and KDM1. The results showed that PELP1 prefers dimethyl K9 (DiK9) in the absence of ERα. In the presence of ERα, its binding affinity to DiK9 decreases, whereas the presence of KDM1 and ERα increases the binding affinity of PELP1 to DiK4 (Fig 3C). Accordingly, immunoprecipitation of E2-treated nuclear extracts, showed PELP1 interactions with H3K4me2 *in vivo* (Fig 3D). These results indicate that PELP1 has the potential to function as a reader of H3 methylation, and its affinity to dimethyl H3K4 and H3K9 sites, in part, is dictated by PELP1-associated proteins.

To confirm the significance of Glu-region of PELP1 in the recognition of dimethyl peptides, we constructed a PELP1 mutant that lacked the Glu region (aa 886–990). The ZR75 cells stably expressing PELP1 wild type (PELP1-WT) and PELP1 Glu mutant (PELP1-ΔGlu; pooled clones) were established. Both PELP1-WT and PELP1-ΔGlu localized to the nuclear compartment and migrated to the expected lengths in a western blot using a green fluorescent protein (GFP) antibody (supplementary Fig S4A,B online). The ChIP assays demonstrated that PELP1-ΔGlu, similarly to PELP1-WT, was recruited to the E2 target gene TFF1

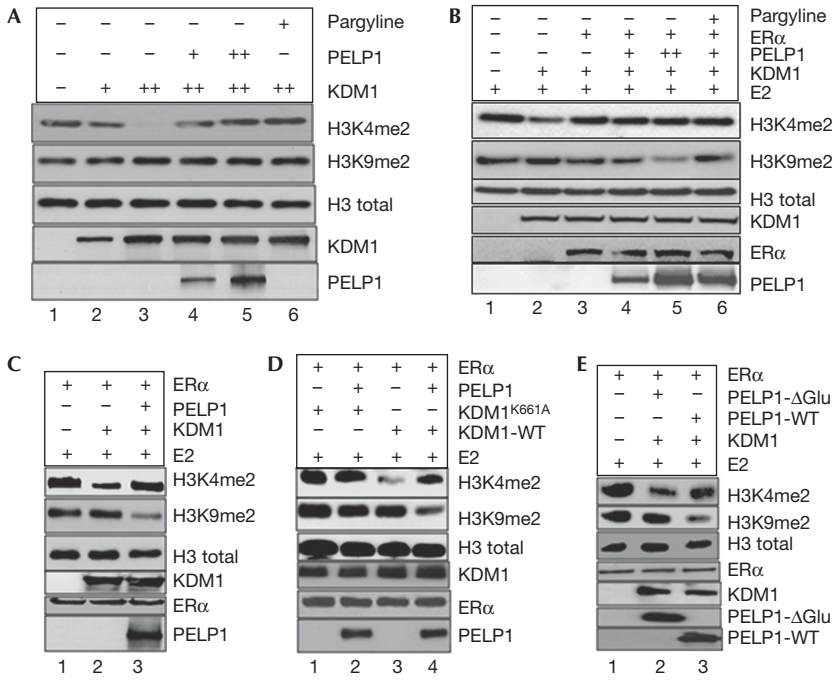

Fig 4 | PELP1 alters the substrate specificity of KDM1. (**A**) Total histones purified from HeLa cells were incubated with purified KDM1. In some reactions, either the PELP1 protein or the KDM1 inhibitor pargyline (10^{-3} M) was added to a standard *in vitro* demethylation assay. Western blot analysis was performed by using the indicated H3 and H3-methyl-specific antibodies. (**B**) Core histones were incubated in the presence of E2 with immunopurified KDM1, either with the ERα or ERα + PELP1 proteins, and then the histone demethylation assay was performed. (**C**) Core histones were incubated in the presence of E2 with *Escherichia coli*-purified KDM1, either with ERα or ERα + PELP1 proteins and then a histone demethylation assay was performed. Western blots were developed with the indicated histone methyl-specific antibodies. The presence of KDM1, ERα and PELP1 in the complexes was verified using western blotting with ERα, PELP1 and KDM1 antibodies. (**D**) A histone demethylation assay was performed as described in (**C**) in the presence of either KDM1-WT or KDM1^{K661A}. (**E**) Histone demethylation was performed as described in (**C**) in the presence of either PELP1-WT or PELP1-ΔGlu. ChIP, chromatin immunoprecipitation; E2, oestrogen; GFP, green fluorescent protein; KDM1, lysine demethylase 1; MCF7, Michigan Cancer Foundation 7; PELP1, proline glutamic acid- and leucine-rich protein 1; WT, wild type.

(supplementary Fig S4C,D online). However, peptide pull-down assays using nuclear extracts of PELP1-WT and PELP1-ΔGlu revealed that deletion of the Glu-rich region abrogates the ability of PELP1 to recognize dimethyl H3K9 and H3K4 peptides (Fig 3E). In ERE luciferase reporter assays, PELP1-ΔGlu interfered with the E2-mediated activation of reporter gene functioning as a dominant negative mutant (supplementary Fig S4E online). These results suggest that the PELP1 Glu-rich region has a crucial role in the recognition of histone dimethyl marks. In agreement with this possibility, E2 stimulation of PELP1-ΔGlu cells resulted in neither an increase in H3K4 methylation nor a decrease of H3K9 methylation. However, vector- or PELP1-WT-transfected cells showed efficient demethylation of H3K9 at TFF1 (Fig 3F) and at other ERα target gene accumulation of diH3K9 marks correlated with decreased expression of ERα target genes (supplementary Fig S5 online). Knockdown of KDM1 in PELP1-ΔGlu cells facilitated the E2-mediated increase in H3K4 methylation (Fig 3F, right panel). Reversal of the H3K4 phenotype by KDM1 siRNA and accumulation of DiK9 methylation in PELP1-ΔGlu cells independently validate the earlier observation that PELP1 in PELP1 shRNA cells has a role in histone methyl modifications; they also confirm that PELP1-mediated recognition of histone methyl marks might be crucial for E2-mediated histone methyl modifications at target promoters.

PELP1 regulates KDM1 substrate specificity

As PELP1 showed unique specificity to dimethyl-modified H3K9 and H3K4 (Fig 3) and interacted with KDM1—an enzyme that demethylates both H3K9Me2 and H3K4Me2—we hypothesized that PELP1 might regulate the substrate specificity of KDM1. We used a well-established *in vitro* methylation assay (Lan *et al*, 2007) with purified KDM1 as the enzyme and HeLa core histones as the substrate. As expected, KDM1 specifically demethylated H3K4 in a dose-dependent manner (Fig 4A). Interestingly, inclusion of purified PELP1 in this assay inhibited KDM1-mediated demethylation of H3K4me2 (Fig 4A). We confirmed the specificity of the KDM1 enzyme in these reaction conditions with addition of pargyline, which inhibited KDM1-mediated demethylation of H3K4me2, suggesting that the enzyme is active. However, under these conditions, KDM1 failed to show any demethylation activity to H3K9me2. Recent studies showed that H3K9me2 provides ligand dependency for activation of E2 target genes (Garcia-Bassets *et al*, 2007), KDM1 has dual specificity (H3K9me2 versus H3K4me2; Metzger *et al*, 2005) and a ligand-bound

nuclear receptor might facilitate the switch of KDM1 functions. We, therefore, hypothesized that PELP1, as an ERα co-regulator, might function as the necessary link of liganded ERα signalling and a KDM1–ERα–PELP1 complex might facilitate the removal of H3K9 dimethyl inhibitory marks in the presence of E2. We performed histone demethylation assays *in vitro* using KDM1 and ERα from two different sources. First, we used exogenously expressed and immunoprecipitated Myc-tagged KDM1 as the source of KDM1 enzyme (Fig 4B). HeLa histones and KDM1 were incubated with or without purified ERα and, in some reactions, exogenously purified PELP1 was added. The KDM1–ERα–PELP1 complex demethylated H3K9me2 in a dose-dependent manner *in vitro*, whereas neither KDM1 nor KDM1–ERα alone demethylated H3K9me2 (Fig 4B, lane 5). Pargyline blocked the demethylation of H3K9 by KDM1–ERα–PELP1, suggesting that the observed demethylation was specific for KDM1 (Fig 4B, last lane). Interestingly, KDM1 alone could facilitate the removal of dimethyl H3K4 but failed to demethylate dimethyl H3K9 (Fig 4B, lane 2). These results indicate that an ERα–PELP1 complex alters the substrate specificity of KDM1, and effective demethylation of dimethyl H3K9 by KDM1 would require a KDM1–ERα–PELP1 functional complex. To eliminate the potential artefacts that might have occurred due to the use of immunopreciptaed KDM1, we repeated the demethylation assays using purified KDM1 enzyme as a GST fusion from *Escherichia coli* (Fig 4C). The addition of KDM1, ERα and PELP1 proteins altered the substrate specificity of KDM1 from H3K4me2 demethylase to H3K9me2 demethylase. Similarly, a PELP1-mediated alteration in KDM1 specificity requires functional KDM1 demethylase activity (Fig 4D) and an intact Glu region in PELP1 (Fig 4E). Collectively, these results suggest that PELP1 has a role in defining KDM1 substrate specificity and has the potential to modulate histone methyl code at ERα target genes.

In summary, our results indicate that PELP1 is a new KDM1-interacting protein and PELP1–KDM1 interactions have an essential role in histone methyl modifications at ERα target genes. The results from this study suggest that PELP1 uniquely recognizes dimethyl-modified histones, and thus acts as a reader of histone methyl modifications for liganded ERα. Our results also imply that liganded ERα uses co-regulator PELP1 to remove inhibitory methyl marks. As PELP1 interacts with dimethyl H3 and also with ERα, one possibility would be that ligand-bound ERα uses LXXLL motifs to interact with the co-regulator PELP1. The ERα–PELP1 complex could then identify the specific methyl marks and facilitate potential recruitment of downstream demethylases, such as KDM1, to assist the removal of inhibitory methyl marks. It is also possible that PELP1-associated factors, including ERα and KDM1, might alter the structural conformation or folding of the Glu-rich region in PELP1, and thus might alter the specificity of PELP1 recognition of dimethyl H3K9 to H3K4. Even though ERα has a key role in bringing PELP1 to the target genes, and might facilitate in the recognition of methyl-binding sites, functional assays suggest that PELP1 is a reader of histone marks for KDM1. Alterations in transcription programmes commonly occur in cancer progression and nuclear receptors, such as ERα, have a central role in this process, as they have a direct impact on gene expression (Lupien & Brown, 2009). On the basis of the fact that PELP1 expression is deregulated in hormonal cancers, and that PELP1 has a role in regulating KDM1 substrate specificity, we predict that PELP1 deregulation has the potential to alter histone methylation at ERα target genes, contributing to hormone-driven tumour progression.

METHODS

Cell culture and reagents. The MCF7 and ZR75 cells were obtained from American Type Culture Collection (ATCC). The PELP1 and Myc tagged antibodies were purchased from Bethyl Laboratories (Montgomery, TX, USA). Dimethyl, H3K4, H3K9 and KDM1 antibodies and biotin peptides were purchased from Upstate (Chicago, IL, USA). The PELP1 and KDM1 SMARTpool siRNA duplexes were purchased from Dharmacon (Lafayette, CO, USA).

Cell extracts, western blots and immunoprecipitation. Cell lysates for western blot analysis were prepared as described previously (Nair *et al*, 2004). Nuclear extracts were prepared using Nuclear IP kit (Active Motif, Carlsbad, CA, USA). Immunoprecipitation was performed for 2 h at 4 °C using 1 μg antibody per milligram of protein.

Plasmids and reporter gene assays. The following plasmids have been described previously: GST–KDM1 (Shi *et al*, 2005), Myc–KDM1 (Metzger *et al*, 2005), T7–PELP1 (Vadlamudi *et al*, 2001), PELP1 deletions (Nair *et al*, 2004) and GFP–PELP1 (Nagpal *et al*, 2008). The GFP–PELP1 full-length mutant that lacks the Glu-rich region (886–990aa) was created using a PCR-driven protocol. The KDM1^{K661A} mutation was created by site-directed mutagenesis. Plasmid transfections were performed using FuGENE6 (Roche Applied Bioscience, Indianapolis, IN, USA). The ERE luciferase reporter gene assays were carried out using 100 ng of ERE Luc reporter, and 50 ng of PELP1 or KDM1 expression vectors. The luciferase assay was performed using the Promega reporter assay kit (Madison, WI, USA).

GST pull-down assay. The GST pull-down assays were performed by incubating equal amounts of GST, GST–PELP1 or GST–KDM1 deletions immobilized on GST beads with ^{35}S-labelled, *in vitro*-synthesized PELP1 peptides or KDM1 peptide. The mixtures were incubated for 2 h at 4 °C and washed six times with Nonipet-40 lysis buffer. Bound proteins were eluted with sodium dodecyl sulphate buffer, and visualized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and autoradiography.

Peptide pull-down assay. For peptide pull-down assays, biotinylated-H3 peptides, in unmodified, mono-, di- or tri-methyl modified forms, H3K9 or H3K4 (Upstate) were incubated with baculovirus-expressed, purified full-length PELP1 or nuclear extracts from MCF7. After incubation with the peptides, the peptides were pulled down using avidin beads and bound PELP1 was eluted and detected by western blotting.

Demethylation assay. *In vitro* demethylation assays were performed using total histones or methylated histone peptides and by using purified KDM1 with or without purified PELP1 and with or without ERα, as described previously (Lan *et al*, 2007). Purification of GST–KDM1 was performed as described earlier (Shi *et al*, 2005). Purified ERα was purchased from Invitrogen (Carlsbad, CA, USA). Purification of GST–PELP1 was performed as described previously (Nagpal *et al*, 2008).

Chromatin immunoprecipitation. The ChIP and ReChIP analyses were performed as described previously (Nair *et al*, 2004) and the primer sequences are given in the supplementary information online.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

We thank T. Baba and Y. Shi for providing glutathione-*S*-transferase–lysine demethylase 1 expression vector. This study was supported by grants: National Institutes for Health CA095681 and Komen-KG090447 (to R.K.V.).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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