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Histone H4 Lys 20 methyltransferase SET8 promotes androgen receptor-mediated transcription activation in prostate cancer

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ABSTRACT

Histone methylation status in different lysine residues has an important role in transcription regulation. The effect of H4K20 monomethylation (H4K20me1) on androgen receptor (AR)-mediated gene transcription remains unclear. Here we show that AR agonist stimulates the enrichment of H4K20me1 and SET8 at the promoter of AR target gene *PSA* in an AR dependent manner. Furthermore, SET8 is crucial for the transcription activation of *PSA*. Co-immunoprecipitation analyses demonstrate that SET8 interacts with AR. Therefore, we conclude that SET8 is involved in AR-mediated transcription activation, possibly through its interaction with AR and H4K20me1 modification.

1. Introduction

The N-terminal tails of histones are subjected to multiple covalent modifications such as phosphorylation, acetylation, ubiquitination, and methylation, which affect the chromatin packing status and then govern numerous genomic functions, including DNA replication, gene transcription, and DNA repair [1,2]. Histone methylations are more stable and have an important role in various aspects of chromatin function. Methylation of H3K27 and H3K9 is associated with gene silencing, whereas H3K4 and H3K36 methylation has been linked to transcriptional activation [3]. Lys-20 is the unique lysine residues on histone H4 which could be methylated in mammalian cells. The effect of H4K20 monomethylation (H4K20me1) on gene transcription needs to be further investigated.

SET domain-containing protein 8 (SET8, also known as PR-SET7, SETD8, and KMT5A) is a member of the SET domain-containing methyltransferase family that specifically catalyzes the addition of H4K20me1 [4]. SET8 has been implicated in a variety of biological processes, such as transcriptional regulation, genomic stability and cell-cycle progression through its H4-Lys-20-specific methylase activity [5]. However, the function of SET8 in transcription regulation remains controversial. SET8/H4K20me1 has been shown to be associated with silent chromatin [6] and promotes transcriptional repression by L3MBTL1 [7]; however, SET8 has also been identified as a transcription activation marker in several studies. H4K20me1 was reported to associate with Pol II and was increased in the promoter and coding regions of transcriptional active genes [8]. SET8 also was found to mediate the transcriptional activation of Wnt target genes [9].

Androgen receptor (AR) is a ligand-activated transcription factor that regulates eukaryotic gene expression and affects multiple bioprocesses such as cellular proliferation and differentiation in target tissues [10,11]. Transcriptional regulation by AR is associated with various chromatin modification factors, including histone modification enzymes [12]. Up to now, the function of SET8/H4K20me1 in AR signaling has not yet been investigated. In this study, we performed ChIP detection and found that H4K20me1 and SET8 are enriched at the promoter of AR target gene *PSA* (*prostate specific antigen*) after dihydrotestosterone (DHT) stimulation. Conversely, after knockdown of SET8 protein level, this enrichment was greatly decreased. We further found that SET8 was crucial for the transcription activation of AR target gene *PSA*. We also conducted Co-immunoprecipitation analyses and demonstrated that there are direct interaction between SET8 and AR.

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2. Materials and methods

2.1. Plasmids and reagents

SET8 cDNA was synthesized and amplified from total RNA of HEK293 cells by RT-PCR. TRIZOL reagent (Invitrogen) and Reverse Transcription System (K1621, Thermo Scientific) are used for RNA isolation and first strand cDNA synthesis, respectively. The SET8-FLAG construct was generated by cloning SET8 cDNA into pCDNA 3.1 vector with flag tag. Antibodies used in the study were AR (ab74272, Abcam), H4K20me1 (17-651, millipore), SET8 (Sab103612, Sigma), IgG (SC-2345, Santa Cruz), actin (SC-47778, Santa Cruz). The sequences of siRNAs used were described previously [13]: si-Ctr, 5′-UUCUCCGAACGUGUCACGU-3′; si-SET8, 5′-GGAAGAGAACUCAGUUACA-3′. RT-PCR primers for *PSA* mRNA assay were: Forward: GCATCAGGAACAAAAGCGTGA; Reverse: CCTGAGGAATCGATTCTTCAG. Dihydrotestosterone (DHT) was purchased from Sigma.

2.2. Cell culture and transfection

LNCap and 22RV-1 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. LNCaP and 22RV-1 cells were cultured in phenol-red-free RPMI1640 supplemented with 10% double-stripped fetal calf serum (dsFCS), when the cells were seeded for stimulating with DHT. Transfection of Cells with plasmids are carried out by FuGENE® HD transfection reagent. Transfection of siRNA was performed by lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

2.3. Gene reporter assay

Cells were co-transfected with PSA-TK-LUC reporter and SET8-FLAG plasmid or Si-SET8 by lipofectamine 2000. Luciferase activity was measured using a Dual-Luciferase (Promega) according to the manufacturer's protocol. Each experiment was performed in triplicate.

2.4. Immunoprecipitation analysis

Immunoprecipitation and Western blotting analysis were performed essentially as described by our previous publication [14]. Briefly, Cells (1×10^7) were lysed in lysis buffer (20 mM Hepes pH7.4; 150 mM NaCl; 0.2% Tween 20; 1.5 mM $MgCl_2$; 1 mM EGTA; 2 mM DTT; 50 mM NaF; 500 μM Na_3VO_4; 1 mM PMSF; 1 μg/ml aprotinin; 1 μg/ml leupeptin) for 1 h at 4 °C. Cell lysates were centrifuged at 13,000 rpm for 15 min at 4 °C to remove insoluble material. Specific antibodies suitable were used to immunoprecipitation overnight at 4 °C. Immune complexes are collected on protein-A agarose beads. Immunoprecipitates were washed 3 times in lysis buffer and once in base buffer (10 mM Hepes, pH7.4; 10 mM $MgCl_2$; 50 mM NaCl; 1 mM DTT; 1 mM PMSF). Proteins were eluted by boiling the beads in sample buffer containing SDS and DTT for 10 min at 95 °C. Immunoreactive bands were analyzed using Western blotting.

2.5. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) experiments were performed according to the procedure described previously with mini modification [15]. Briefly, cells were cross-linked for 10 min at 37 °C, and then sonicated in lysis buffer. Supernatant of lysates were incubated with specific antibodies and beads overnight at 4 °C. The beads were washed thoroughly before reversing cross-link. Purified DNA fragment was quantified by quantitative RT-PCR and normalized by input DNA. Primers used for ChIP are: Promoter-F, AGGGATCAGGGAGTCTCACA; Promoter-R, GCTAGCACTTGCTGTTCTGC.

2.6. Colony formation assay

After transfection with SET8-FLAG or control plasmid for 48 h, the transfected LNCaP cells were trypsinized and seeded into 6-well plate about 100 cells per well. Then the cells were allowed to grow for 5–7 days to form colonies, which were then stained with crystal violet (0.4 g/l).

3. Results

3.1. DHT stimulates H4K20me1 and SET8 enrichment at the PSA promoter in prostate cancer cells

In order to explore the role of SET8 involved in AR mediated transcription regulation, we performed ChIP assay to detect the recruitment of H4K20me1 and SET8 at the androgen response element located in the promoter of AR target gene *PSA*. We treated prostate cells LNCap with 10nM AR agonist DHT for 16 h and then harvested the cells to perform ChIP detection. Enrichment of AR on *PSA* promoter was conducted as positive control and that of IgG as negative control. Consistent with previous reports [16,17], DHT treatment increased the enrichment of AR at *PSA* promoter. We excitedly found that DHT stimulated approximately 16-fold increase for H4K20me1 enrichment at the promoter of *PSA* comparing with that of IgG. SET8 enrichment was also found an about 2-fold increase at the promoter of *PSA* with IgG as control. These data suggest that there are a specific H4K20 methylation and SET8 association on androgen response element at the promoter of *PSA* in a ligand-dependent manner (see Fig. 1).

3.2. SET8 is responsible for H4K20me1 methylation at PSA promoter in prostate cancer cells

SET8 is the only known enzyme that specifically catalyzes the H4K20 monomethylation [18,19]. So we tested the effect of SET8

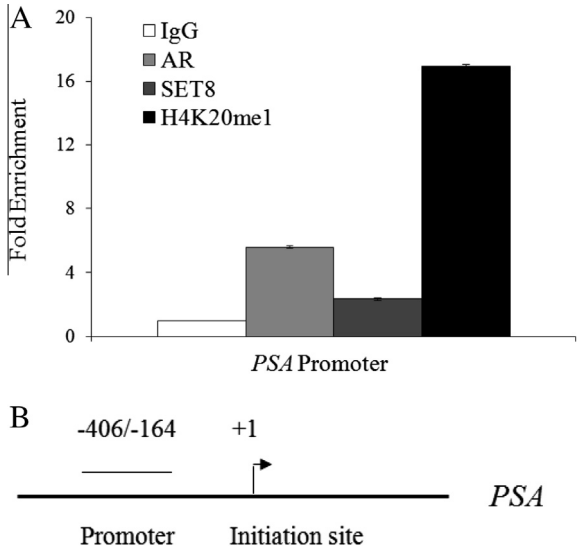

Fig. 1. DHT stimulates H4K20me1 and SET8 enrichment at the *PSA* promoter. (A) ChIP assay with specific antibodies was detected after treatment with 10 nM DHT for 16 h; (B) Regions that primers targeted *PSA* promoter for ChIP assay are indicated. All graphs are shown in mean ± S.D. of at least three independent experiments.

on H4K20me1 enrichment at *PSA* promoter. Transfection of LNCaP cells with Si-SET8 caused an efficient and specific decrease of the endogenous SET8 expression, but did not affect the expression level of AR (Fig. 2A). Companying with SET8 knockdown, DHT induced H4K20me1 enrichment at *PSA* promoter was greatly decreased in LNCaP cells, as shown in Fig. 2B. This result suggests that enrichment of H4K20me1 at *PSA* promoter is controlled by SET8.

3.3. SET8 is involved in AR mediated transcription activation in prostate cancer cells

We further detected the effects of SET8 on AR-mediated transcription activation through AR target gene *PSA*. Upregulation of SET8 expression with transfection of SET8-FLAG leaded to an increase of endogenous *PSA* mRNA level in a dose-dependent manner in LNCaP cells (Fig. 3A). We also performed luciferase assay with PSA-TK-LUC reporter plasmid. LNCap cells were co-transfected with a SET8-FLAG and PSA-TK-LUC reporter and the PSA-TK-LUC reporter gene expression level was obviously increased paralleling with the expression level of SET8. Instead, knockdown of SET8 expression decreased the PSA-TK-LUC reporter gene expression level. These results suggested that SET8 promotes AR regulated transcription activation.

3.4. SET8 interacts with AR and promotes LNCaP cell proliferation

In order to investigate the mechanism of SET8 involved in AR-mediated transcription activation, we performed a co-immunoprecipitation assay to detect the interaction of SET8 and AR. As shown in Fig. 4A, endogenous SET8 and AR interaction with each other in AR positive RV22-1 and LNCaP cells. These results suggest that SET8 involved in AR-mediated transcription activation may be through its interaction with AR. To access whether SET8 governs AR-dependent cell growth, we transfected LNCaP cells with SET8-FLAG and conducted colony formation experiments. Compared with control cells, SET8-FLAG transfected LNCaP cells markedly increased the AR-induced proliferation (Fig. 4B). These results show the physiological importance of SET8 in the control of AR induced cell proliferation.

4. Discussion

In this study, we show that H4K20me1 and SET8 are enriched at the promoter of AR target gene *PSA* under stimulation of AR. Immunoprecipitation analyses demonstrate that SET8 interacts with AR, either directly or as part of a complex including other binding partners. We also find that knockdown of SET8 decreased the H4K20me1 level at *PSA* promoter and the expression level of SET8 is proportional to the mRNA level of *PSA* in prostate cancer LNCaP cells. All these data suggest that H4K20me1 methyltransferase SET8 promotes AR-mediated transcription activation, which may be carried through its interaction with AR and its H4K20me1 enzymatic activity at the promoter region of AR target genes.

Function of H4K20me1 in chromatin regulation remains controversial. H4K20me1 has been shown to be associated with silent chromatin and transcriptional repression [6,7]. In other studies, SET8 has also been identified as a transcription activation marker [8,9]. Our data show that monomethylation of H4K20 by SET8 plays an active role in AR-mediated transcription regulation which

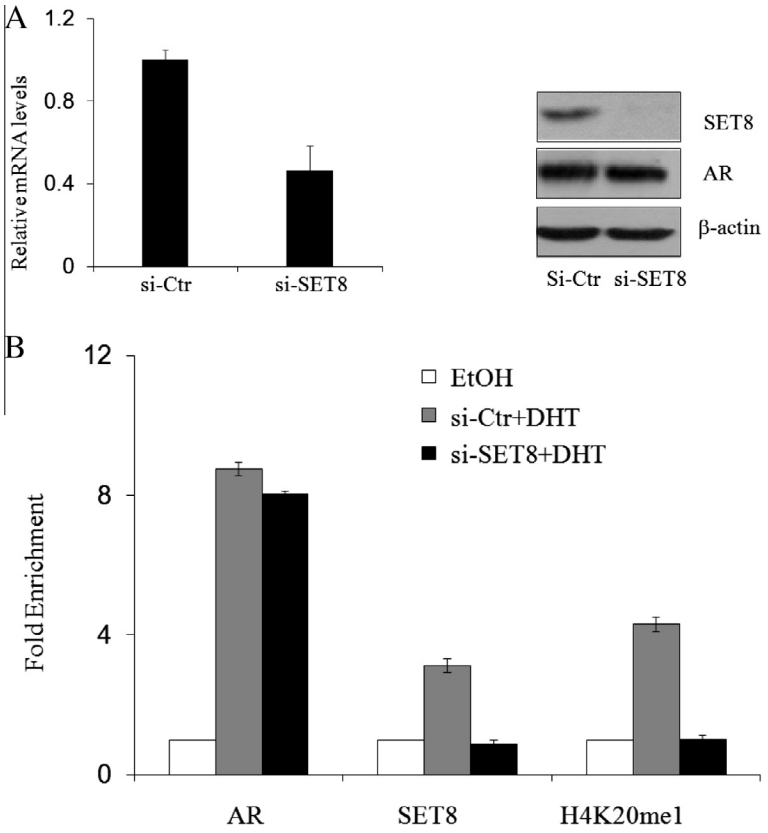

Fig. 2. SET8 is responsible for H4K20me1 methylation at *PSA* Promoter. (A) Efficiency of SET8 knockdown using siRNA, LNCap cells were transfected with Si-Ctr and Si-SET8 for 48 h, then mRNA and protein expression level of SET8 was detected by quantitative RT-PCR and Western blotting, respectively. (B) After transfected with Si-Ctr and Si-SET8 for 48 h, the LNCaP cells were treated with 10 nM DHT for 16 h, and then harvested the cells to perform ChIP assay targeted ARE I of *PSA* with specific antibodies AR, SET8, and H4K20me1. All graphs are shown in mean ± S.D. of at least three independent experiments.

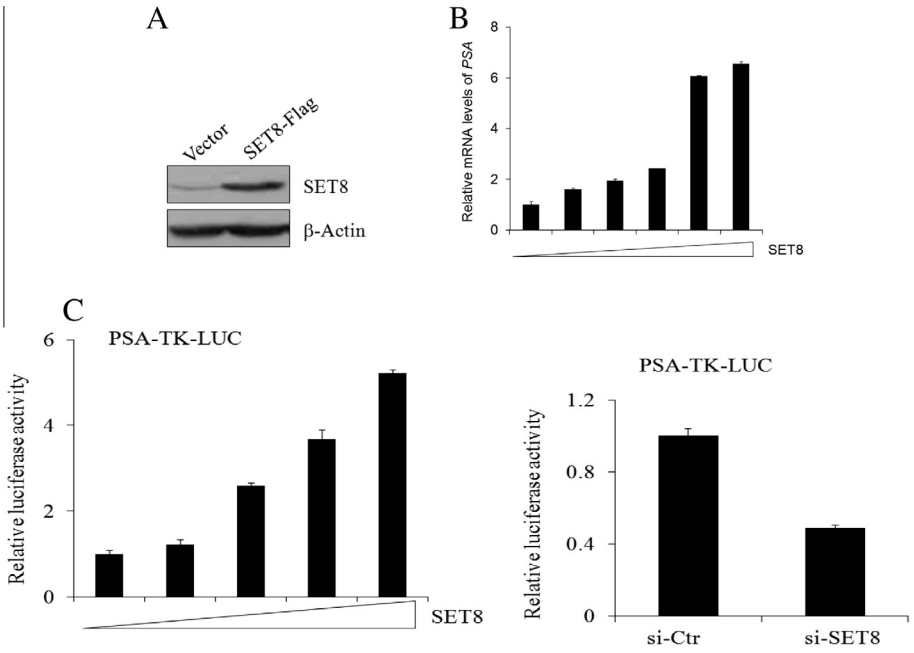

Fig. 3. SET8 is involved in AR medicated transcriptional activation. (A) Effect of SET8 overexpression on endogenous *PSA* mRNA level. LNCap cells were transfected with increasing SET8-FLAG plasmid and the mRNA level of *PSA* was detected by quantitative RT-PCR; (B) Luciferase assay for *PSA*. LNCap cells were co-transfected with PSA-TK-LUC reporter and SET8-FLAG plasmid or Si-SET8. Luciferase activity was measured by a Dual reporter kit. All graphs are shown in mean ± S.D. of at least three independent experiments.

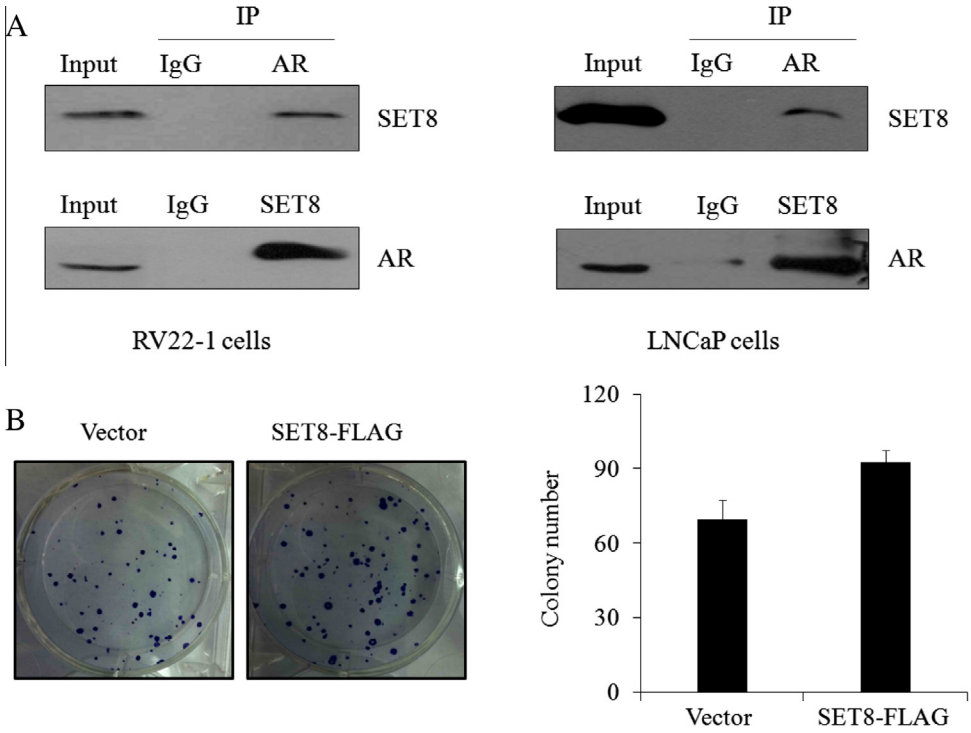

Fig. 4. SET8 interacts with AR and promotes cell proliferation. (A) SET8 interacts with AR. Co-immunoprecipitation of SET8 and AR in RV22-1 cells and LNCap cells. (B) SET8 promotes LNCaP cells proliferation. Colony formation experiments were performed as shown in Section 2. All graphs are shown in mean ± S.D. of at least three independent experiments.

indicates that H4K20me1 is a chromatin activation marker, at least in AR-mediated signaling.

AR signaling regulates multiple processes such as gene transcription, cellular proliferation and differentiation etc., in its target tissues including brain, testis and prostate [10,11]. Abnormal amplification of the androgen receptor as well as deregulation of AR gene expression has been shown to be associated with carcinogenesis of prostate cancer [12]. Our finding that SET8 promotes

AR-mediated transcription and cell proliferation suggests histone H4K20 monomethylase SET8 may be a promising therapeutic target in the cancers which AR plays a pivotal physiological role.

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