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Original article

LSD1 collaborates with EZH2 to regulate expression of interferon-stimulated genes

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ABSTRACT

Histone methylation is a complicate and dynamic epigenetic modification that regulates gene transcription, chromosomal structure and cell differentiation. Here, we discovered the interaction between the H3K4 demethylase, lysine specific demethylase 1 (LSD1, an important component of CoREST repressor complex) and the H3K27 methyltransferase, enhancer of zeste homolog 2 (EZH2, an essential component of PRC2). Immuno-precipitation and GST-pull down assay were performed to observe the interaction between the proteins. The MCF-7 cells were cultured and transfected with the siRNA. The mRNA and proteins were examined by using the real-time polymerase chain reaction (RT-PCR) and western blot assay, respectively. HPLC and LC–MS/MS analysis were performed to purify the proteins. RT-PCR-based quantitative ChIP analysis were performed. LSD1 interacts with histone modification protein EZH2 in MCF-7 cells. LSD1 and EZH2 target a few common genes. LSD1 knockdown and EZH2 knockdown affect protein expression. LSD1 knockdown and EZH2 knockdown affect the proteins involving in IFN signaling pathway. LSD1 and EZH2 modify histone methylation at IRF9 gene locus. We systematically analyzed the proteins that are affected by either LSD1 or EZH2 knockdown with proteomic approaches and identified that the interferon pathway and some other pathways are commonly affected. The interaction between LSD1 and EZH2 stabilizes the binding of LSD1 to the promoter region of IRF9, which is a key transcription factor of the interferon pathway. In conclusion, our study revealed that the coordination between histone demethylases and methyl-transferases might serve as a double lock system to suppress the expression of interferon stimulated genes.

1. Introduction

Histone lysine methylation plays an important role in the regulation of gene expression, chromosome maintenance and cell cycle. Depending on the particular lysine residue and degree of methylation, histone lysine methylation constitutes regulatory signals that mediates either negative or positive regulation of gene expression. Genes with active transcription are generally associated with the methylations at histone lysine 4, 36 and 79 (H3K4, H3K36 and H3K79), while genes with transcription repression are usually marked with methylations at histone lysine 9 and 27 (H3K9 and H3K27) [1]. Methylation of lysine residues within the histone is regulated by histone methyltransferases (KMTs) and histone demethylases (KDMs), both of which have high specificities for the lysine residues and degree of methylation [2].

Enhancer of zeste homolog 2 (EZH2), a KMT for H3K27 di/trimethylation (H3K27me2/me3), is an essential component of the polycomb repressive complex 2 (PRC2), which maintains the transcriptional repression of genes involved in differentiation, stem and somatic cell plasticity and response to environmental stimuli [3]. In addition to maintaining H3K27me2/me3, PRC2 also interacts with the other epigenetic regulators, including histone deacetylases, histone demethylases and DNA methyltransferases, and coordinately regulates gene transcription and chromatin compaction [4–6]. Notably, a detailed structural analysis reveals that the binding of PRC2 to H3 is inhibited if H3K4 is trimethylated, which suggests that the H3K4 demethylation is essential for the binding of PRC2 to H3 [7].

Lysine specific demethylase 1 (LSD1), the first identified histone demethylase, associates with other co-repressors, such as histone deacetylase 1/2 (HDAC1/2) and CoREST forming a core ternary complex, and mediates the demethylation of di- or mono-

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methylated H3K4 to repress transcription [8–10]. LSD1 interacts with T-cell acute lymphocytic leukemia protein (TAL1), a critical transcription factor required for hematopoiesis. The dynamic regulation of TAL1-associated LSD1/HDAC1 complex is crucial for the onset of erythroid differentiation [11]. In addition, LSD1 and CoREST could form a stable complex with SFMBT1 (Scm [sex comb on midleg] with four MBT [malignant brain tumor] domains 1), and could regulate the expression of histone genes during the cell cycle [12]. The association of LSD1 with Polycomb group proteins demonstrates a synergetic mechanism of transcription regulation.

Type I interferons (IFNs) induce expression of interferon-stimulated genes (ISGs) that have potent antiviral and growth-inhibitory effects, which forms the first line of defense against viral infections [13]. In response to IFNs, signal transducers and activator of transcription (STATs) are phosphorylated and interact with IFN response factor 9 (IRF9) to form the transcription factor IFN-stimulated gene factor 3 (ISGF3). ISGF3 binds to the IFN-stimulated response elements (ISREs) that are commonly located at the promoters of certain ISGs and initiate their transcription [14]. Notably, IFNs also induce the expression of STAT2 and IRF9, which also combine with STAT1 to form the unphosphorylated ISGF3 (U-ISGF3 [15]). U-ISGF3 prolongs the expression of a subset of ISGs and restricts HCV chronic replication [16]. Combined theoretical analysis and experimental investigation reveals that the amount of IRF9 is a crucial determinant for amplification and dynamics of IFN-mediated signal transduction [17]. The activity of histone deacetylases (HDACs) is required for the expression of certain ISGs, suggesting an important role of epigenetic modification on regulating IFN-induced gene expression [18–20].

In this study, we systematically investigated the expression of genes that are affected by LSD1 and EZH2 knockdown through mass-spectrometry. The present study identified that the expression of genes in IFN-mediated signaling pathway, such as IRF9, which is significantly affected by the perturbation of LSD1 and EZH2 expression. These results highlight the importance of histone methylation in controlling the expression of ISGs.

2. Materials and methods

2.1. Immuno-precipitation assay

For Immuno-precipitation assay (IP), 20 μl IP antibodies, including anti-human LSD1 (ab17721, Abcam, UK), anti-human EZH2 (Catalogue No. 5246S, Cell Signaling Technology, USA), were added to 500 μg nuclear extract (dilution ratio vary with the target protein in different cell lines). The antigen-antibody mixture were rotated slowly overnight at 4 °C. Next, 50 μl protein G/A agarose beads were incubated with the mixture at 4 °C for 5 h. After centrifuging mixture for 10 min at 2000 r/min at 4 °C, agarose beads-antigen-antibody complexes were collected (the IgG supernatant should be collected for Input). Complexes were washed with the pre-cooling RIPA buffer for three times (15 min for each time) and supernatant was completely absorbed and mixed with 60 μl 1 × sample buffer (added β-mercaptoethanol and DTT). Then, the mixtures were boiled for 5 min. Finally, the IP results were detected by using the western blotting assay.

2.2. GST-pull down assay

For GST-pull down assay, 2 μg plasmids were transfected into 50 μl BL21 competent cells and incubated for 10 min at 4 °C. The competent cells were besmeared on solid LB medium, which contain ampicillin and were cultured for overnight at 37 °C. The monoclonal was picked into the 10 μl liquid LB medium and cultured overnight at 37 °C, then transferred the bacteria into 300 μl liquid LB medium. When the OD600 achieves to 0.5–0.6, the bacteria was induced with 300 μl IPTG for 5 h. Then added 100 μl glutathione-agarose beads to the supernatant after the sonication. The beads were retreated with 1 ml pre-cooling PBS containing 0.1% Triton. The GST fusion protein linked with beads was added after the purification to nuclear extract, then the mixture was incubated overnight at 4 °C. The GST-pull down results were detected by western blotting assay.

2.3. Immunofluorescence assay

Cells were seeded on glass slides in 24-well plates (about 11000 cells each well), and cultured over 36 h. Cells were fixed on the slides with 4% paraformaldehyde for 15 min. The slides were washed with PBS for 3 times (5 min for each time), then incubated in 0.3% TritonX-100 for 10 min, washed with PBS for 3 times again. Then, the slides were blocked with 500 μl 1% BSA for 1 h, and incubated with LSD1 antibody (LSD1, Abcom, ab17721, 0.005 mg/ml) overnight at 4 °C. The slides were washed with PBS, and incubated with secondary antibody (Life technologies, #Alexa Fluor 488 donkey anti-rabbit, 0.005 mg/ml, total volume was 500 μl). The slides were stained with EZH2 antibody (EZH2, Cell Signaling Technology, 5246s, 0.005 mg/ml) and the secondary antibody (Life technologies, Alexa Fluor 555 donkey anti-mouse, 0.005 mg/ml, total volume was 500 μl) with the same procedure. The staining was mounted with 6-diamidino-2-phenylindole (DAPI). Fluorescence photography was captured with laser scanning confocal microscopy (Cat. No. LSM710, LSCM, ZEISS).

2.4. Cell culture and siRNA

The MCF-7 cells were cultured for more than six generations with high glucose (4.5 g/l) Dulbecco's modified Eagle's medium (DMEM). The glutamine and sodium pyruvat labeled DMEM medium (lysine 12C6 and arginine 12C6, 14N4, K0R0) was used in both siRNA groups. The cells were cultured until 80% confluence (about $\sim 5 \times 10^8$ cells in 15 cm dishes) and then treated with siRNAs. For the siRNA experiment, the "light" labeled cells were transfected with siRNA EZH2 (Catalogue No. E-004218-00-0010, Dharmacon) or LSD1 (Catalogue No. E-004218-00-0010, Dharmacon) and the "heavy" labeled cells were transfected with "NC" (Catalogue No. P-002099-01-20, Dharmacon). After treatment, the cells were cultured in SILAC media for another 48 h and then harvested, washed twice with ice-cold PBS. After snap freezing in liquid nitrogen, the cell pellets were sent for protein extraction containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C with 95% air and 5% CO_2. Heavy labeling DMEM medium (lysine 13C6 and arginine 13C6, 15N4, K6R10) was used in control group cell culture and light.

2.5. Western blotting analyses

The whole cell extracts were prepared by using ice-cold whole cell extraction buffer (WCEB) containing 25 mM β-glycerophosphate (pH 7.3), 5 mM EDTA, 2 mM EGTA, 5 mM β-mercaptoethanol, 1% Triton X-100, 0.1 M NaCl, and a protease inhibitor mixture (Roche Applied Science). Then the extracted proteins were quantified by using the Bradford methods. Equal amount of proteins (50 μg) were separated by SDS–polyacrylamide gel electrophoresis (PAGE). Primary antibodies, including anti-human LSD1 (Catalogue No. ab17721, Abcam, UK), anti-human EZH2 (5246S, Cell Signaling Technology, USA), anti-human SUZ12 (Catalogue No. ab175187, Abcam, UK), anti-human EED (Catalogue No. ab4499, Abcam, UK), anti-human HDAC1 (Catalogue No. ab7078, Abcam, UK), anti-human HDAC2(Catalogue No. ab7079, Abcam, UK) were used and were detected by using the horseradish