Lysine-Specific Demethylase Epigenetically Regulates Human and Plant Phenomena

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Summary Histone methylation in eukaryotes is one of the key posttranslational modifications in epigenetic regulation. Human lysine specific demethylase 1 (LSD1), which demethylates not only histone H3 lysines 4 and 9 but also non-histone proteins, is involved in the regulation of developmental processes. LSD1 also acts as an oncogene to promote cancer cell proliferation. *Arabidopsis thaliana* has four LSD homologs, which are involved in flowering, root development, circadian rhythm, DNA damage, pathogen resistance and shoot regeneration. Here, we summarize the roles of LSD family members in various biological processes.

Key words Histone demethylase, Histone modification, LSD, Cancer, Flowering, Shoot regeneration.

Epigenetic regulation is involved in heterochromatin formation (Bansal and Kaur 2015, Sato *et al.* 2015, Myoshu and Iwasa 2016, Walia *et al.* 2016, Choosangjaew *et al.* 2017, Klassmann and Martins dos Santos 2017, Patawang *et al.* 2017, Ayata *et al.* 2018) and chromatin organization (Matsunaga 2016, Hoshino *et al.* 2017). Methylation of histone H3 lysine residues is one of the most important epigenetic modifications. The level of histone methylation is reversibly controlled by histone methyltransferase and demethylase, and greatly affects the chromatin status and transcriptional regulation (Horton *et al.* 2017). Histone demethylases belong to either the lysine specific demethylase (LSD) or Jumonji C (JmjC) family (Shi and Tsukada 2013). Human LSD1 (also known as KDM1A) was the first identified histone demethylase, and has H3K4 demethylase activity (Shi *et al.* 2004). LSD1 possesses a SWIRM domain and a flavin-dependent amine oxidase (AO) domain. It recognizes and binds to DNA through the SWIRM domain and removes H3K4me1/2 by oxidizing the amine in a FAD-dependent manner through the AO domain (Da *et al.* 2006). In contrast, LSD2, a paralog of LSD1, has a zinc-finger domain in addition to the SWIRM and AO domains. While it has high homology to LSD1, its function is different (Fang *et al.* 2010). LSD1 demethylates the promoter and enhancer regions of genes while LSD2 demethylates gene body regions.

LSD1 has been reported to be involved in various cellular processes such as the control of stemness and differentiation, autophagy, senescence, neurodegenerative

diseases and metabolism (Hosseini and Minucci 2017). Because LSD1 is highly expressed in various types of cancer, inhibition of LSD1 has been the focus of epigenetic therapies to suppress cancer cell proliferation and invasion (Zheng *et al.* 2016).

LSD1 was originally identified as a transcriptional repressor that demethylates H3K4me1/2, the active mark. LSD1 forms a complex with HDAC1/2 and the corepressor CoREST, removes H3K4me1/2 in promoters and enhancers, and thus represses transcription (Metzger *et al.* 2005). The LSD1-CoREST-HDAC complex suppresses differentiation into non-neuronal cells and neural progenitor cells by demethylating H3K4 and deacetylating H3 and H4 of the neuronal genes (Lee *et al.* 2005) (Fig. 1a). In hematopoiesis, the LSD1-CoREST-HDAC complex interacts with two zinc finger proteins, growth factor independence (Gfi)-1 and Gfi-1b, to perform H3K4 demethylation of the target gene of Gfi-1/1b (Saleque *et al.* 2007). In addition, inhibition of LSD1 enhanced H3K4me and expression of epithelial transcription factors, promoting epithelial differentiation (Egolf *et al.* 2019).

Although LSD1 was identified as a transcriptional repressor, it has been reported to act as a transcriptional activator by demethylating H3K9me1/2, the repressive mark. Through interaction with an androgen receptor and an estrogen receptor, LSD1 demethylates H3K9me of target genes of these nuclear receptors (Perillo *et al.* 2008) (Fig. 1b). Additionally, it has been reported that an LSD1 splicing variation changes its tissue specificity and substrate, and the LSD1 isoform acts as an H3K9 and H3K20 demethylase in neuronal differentiation (Zibetti *et al.* 2010, Wang *et al.* 2015).

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Fig. 1. LSD epigenetically regulates biological phenomena in animals and plants. (a) Suppression of neuron differentiation by LSD1-CoREST-HDAC complex. The complex reduced H3K4me and histone acetylation of neuronal genes. (b) Activation of transcription with nuclear receptors. LSD1 demethylates H3K9me interacting with androgen receptor (AR) and estrogen receptor (ER). (c) Cancer formation. LSD1 demethylates p53K370me and inhibit coactivator binding. (d) Flowering. FLD-HDA5/6 complex reduced H3K4me and histone acetylation of *FLC*. (e) Circadian rhythm. LDL1/2-HDA6 reduced H3K4me and histone acetylation of *TOC1* interacting with CCA1/LHY. (f) Gene priming for shoot regeneration. LDL3 demethylates H3K4me on body regions of target genes.

Furthermore, LSD1 has demethylase activity not just toward histones but also lysine residues of other proteins, including p53, DNMT1, STAT3, E2F1, RB1, MEFD2, MTA1, ER α , HSP90, HIF-1 α and other transcription factors related to stemness and cancer-related transcription, and the effect of demethylation varies among proteins (Amente *et al.* 2013). p53, a tumor suppressor that regulates the progression of the cell cycle and apoptosis, is methylated at K370 by SMYD2, and coactivator p53 Binding Protein 1 (53BP1) recognizes and binds to p53. LSD1 inhibits the activation of p53 by demethylating K370me/2 (Huang *et al.* 2007, Scoumanne and Chen 2007) (Fig. 1c). HIF1 α , a transcription factor that promotes cancer metastasis, invasion, and angiogenesis, is methylated at K32 and K391 by SET domain methyltransferase and degraded in an ubiquitin-dependent manner. LSD1 contributes to the stabilization of HIF1 α by demethylating K32 and K391 (Bao *et al.* 2018).

The dicot plant species *A. thaliana* has four LSD homologs; LSD1-LIKE (LDL)1, LDL2, LDL3 and FLOWERING LOCUS D (FLD). These proteins all have AO and SWIRM domains (Jiang *et al.* 2007). Flowering is epigenetically regulated by temperature and day length (Shibuta and Matsunaga 2019). FLD, LDL1 and LDL2 demethylate H3K4me in the promoter and enhancer of

FLOWERING LOCUS C (FLC), a MADS-box transcriptional regulator that inhibits the floral transition and plays a central role in flowering control, and thus reduce its expression level (Jiang *et al.* 2007). Furthermore, FLD interacts with HDA5 and HDA6 and is involved in the deacetylation of H3 and H4 in *FLC* and its homologues *MADS AFFECTING FLOWERING1*, 4 and 5 (*MAF1*, 4 and 5) (Yu *et al.* 2011, Luo *et al.* 2015) (Fig. 1d). Additionally, LDL1 and LDL2 demethylate H3K4me in *FLOWERING WAGENINGEN (FWA)*, which is a flowering repressor (Jiang *et al.* 2007).

A recent study has shown that LDL1 and LDL2 are involved in the control of circadian rhythms that affect many biological processes, including the timing of flowering. In this study, LDL1 and LDL2 were found to regulate *TIMING OF CAB EXPRESSION1 (TOC1)*, a clock gene expressed at night, by reducing H3K4 methylation and H3 acetylation through interactions with HDA6 and clock genes expressed at noon; CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) (Hung *et al.* 2018) (Fig. 1e). The plant LSD family is also involved in development, regeneration and stress responses. LDL1 regulates primary root elongation and lateral root formation by repressing auxin-related genes (Singh *et al.* 2012). Furthermore, LDL1 and

LDL2 repress the expression of *ABA DEFICIENT 2 (ABA2)* and *ABSCISIC ACID INSENSITIVE 3 (ABI3)*, which are involved in abscisic acid production and signaling, and *DELAY OF GERMINATION1 (DOG1)*, which is involved in seed dormancy, thereby suppressing seed dormancy (Zhao *et al.* 2015).

In addition, FLD is involved in disease responses through the FLC-independent pathway (Banday and Nandi 2018). FLD demethylates H3K4me in *WRKY6* and *WRKY29* by interacting with GLUTATHIONE-S-TRANSFERASE THETA 2 (GSTT2), and thus contributes to the acquisition of pathogen resistance. LDL1 is also involved in responses to DNA damage (Diaz and Pecinka 2017). RAD54, a DNA repair and recombination protein, accumulates at damaged sites by recognizing H3K4me2. LDL1 removes RAD54 at damaged sites by demethylating H3K4me2 during homologous recombination repair and thereby maintains genome stability (Hirakawa *et al.* 2019).

LDL3, the role of which has never been mentioned before, was recently reported to function in gene priming during *de novo* shoot regeneration *via* callus, a pluripotent cell mass (Ishihara *et al.* 2019). LDL3 reduces the H3K4me2 level of genes responsible for shoot regeneration during callus formation. While LDL3-mediated H3K4 demethylation does not immediately affect gene expression, it creates a ready state for gene activation when external cues induce shoot regeneration (Fig. 1f). Thus, LDL3 is an epigenetic regulator for gene priming to maintain competency for shoot regeneration.

As mentioned above, LSDs are crucial regulators in various biological processes in animals and plants. Like its interaction with HDAC, future identification of complexes including LSD or its substrates will reveal novel roles for LSD in various epigenetic phenomena.

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