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Short communication

Histone deacetylation-mediated cellular dedifferentiation in *Arabidopsis*

Kyounghee Lee[a], Ok-Sun Park[b], Su-Jin Jung[b], Pil Joon Seo[a,b,*]

[a] Department of Bioactive Material Sciences and Research Center of Bioactive Materials, Chonbuk National University, Jeonju 561-756, Republic of Korea
[b] Department of Chemistry and Research Institute of Physics and Chemistry, Chonbuk National University, Jeonju 561-756, Republic of Korea

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ABSTRACT

Chromatin structure determines the accessibility of transcriptional regulators to target DNA and contributes to regulation of gene expression. Posttranslational modifications of core histone proteins underlie the reversible changes in chromatin structure. Epigenetic regulation is closely associated with cellular differentiation. Consistently, we found that histone deacetylation is required for callus formation from leaf explants in *Arabidopsis* . Treatment with trichostatin A (TSA) led to defective callus formation on callus-inducing medium (CIM). Gene expression profiling revealed that a subset of HDAC genes, including *HISTONE DEACETYLASE 9* (*HDA9*), *HD-TUINS PROTEIN 1* (*HDT1*), *HDT2*, *HDT4*, and *SIRTUIN 1* (*SRT1*), was significantly up-regulated in calli. In support of this, genetic mutations of *HDA9* or *HDT1* showed reduced capability of callus formation, probably owing to their roles in regulating auxin and embryonic and meristematic developmental signaling. Taken together, our findings suggest that histone deacetylation is intimately associated with the leaf-to-callus transition, and multiple signaling pathways are controlled by means of histone modification during cellular dedifferentiation.

1. Introduction

Eukaryotic genomic DNA is wrapped around histone octamers, two sets of H2A, H2B, H3, and H4, and exists as a form of chromatin. The core histones are subject to various post-translational modifications, such as methylation, acetylation, ubiquitination, and phosphorylation (Kim et al., 2015). The epigenetic regulation facilitates stable but reversible gene expression and is involved in a variety of developmental processes in higher eukaryotes (Fischer et al., 2006; Gan et al., 2013; Pikaard and Mittelsten-Scheid, 2014).

Genome-wide global regulation of chromatin structure is closely associated with cellular differentiation and dedifferentiation. In mammals, differentiated cells have a closed chromatin structure, whereas pluripotent dedifferentiated cells have an open chromatin structure (Gaspar-Maia et al., 2011). Plants have a remarkable capability to induce unorganized pluripotent cell masses, so called calli, from differentiated plant organs, and the chromatin state in the plant genome might also be dynamically modified depending on the status of cellular differentiation (Ikeuchi et al., 2013; Miguel and Marum, 2011).

Several lines of evidence support the importance of genome-wide reprogramming of histone modifications for cellular dedifferentiation in plants (Furuta et al., 2011; He et al., 2012). H3 lysine 27 trimethylation (H3K27me3) is a representative repressive mark established by polycomb group (PcG) proteins. The founding *Drosophila* polycomb repressive complex 2 (PRC2) consists of Enhancer of zeste [E(z)] histone methyltransferase, Extra sex comb (ESC) WD40 domain protein, Suppressor of zeste 12 [Su(Z) 12], and nucleosome-remodeling factor 55 (NURF55). The *Arabidopsis* genome contains multiple homologs of the core PRC2 components: three E(z) [CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA)], three SU(Z) 12 [EMBRYONIC FLOWER 2 (EMF2), FERTILIZATION-INDEPENDENT SEED 2 (FIS2), and VERNALIZATION RESPONSE 2 (VRN2)], one putative ESC [FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)], and five NURF55 [MULTICOPY SUPPRESSOR OF IRA 1–5 (MSI1-5)] (Derkacheva et al., 2013; Derkacheva and Hennig, 2014; Kim and Sung, 2014). Multiple combinations of the components facilitate formation of diverse types of PRC2 complexes. Notably, *clf swn* double- and *emf2* single-mutants have reduced capability for forming calli from leaf explants (He et al., 2012), probably because

Abbreviations: AGL15, AGAMOUS-LIKE 15; ARR, ARABIDOPSIS RESPONSE REGULATOR; BBM, BABY BOOM; CIM, callus-inducing medium; HDAC, HISTONE DEACETYLASE; HDT, HD-TUINS PROTEIN; LBD, LATERAL ORGAN BOUNDARIES DOMAIN; LEC1, LEAFY COTYLEDON 1; PcG, polycomb group; PRC, polycomb repressive complex; SRT1, SIRTUIN 1; TSA, trichostatin A.

* Corresponding author at: Department of Chemistry, Chonbuk National University, Jeonju 561-756, Republic of Korea.
E-mail address: pjseo1@jbnu.ac.kr (P.J. Seo).

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H3K27me3 accumulation is required to eliminate leaf identity. In contrast to the leaf-to-callus transition, mutations in PcG members result in the loss of differentiated cell identity in roots through the activation of embryonic regulators, such as *LEAFY COTYLEDON1 (LEC1), LEC2, and FUSCA3* (*FUS3*) (Bouyer et al., 2011; Makarevich et al., 2006), indicating the dual mode of PcG action in cellular dedifferentiation depending on the plant organs.

PRC1 is subsequently recruited and stably maintains the repressive status of corresponding genes established by PRC2 (Berke and Snel, 2015). The *Arabidopsis* PRC1-like complex contains the chromodomain protein LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1)/TERMINAL FLOWER 2 (TFL2), which recognizes H3K27me3 (Exner et al., 2009), and BMI1a, BMI1b, BMI1c, RING1a and RING1b, which catalyze H2A monoubiquitination (Shen et al., 2014). Alternatively, the PRC1 complex may initiate gene repression and the subsequent PRC2-mediated H3K27 methylation maintains the repressive effects (Calonje, 2014; Yang et al., 2013), supporting the intimate relationship between PRC1 and PRC2 for gene suppression. Accordingly, similar to the role of the PRC2 complex in cellular dedifferentiation, genetic mutants that harbor defects in *BMI1a*, *BMI1b*, *RING1a*, or *RING1b* show spontaneous callus formation from root tissues with ectopic expression of meristematic and embryonic genes (Bratzel et al., 2010; Chen et al., 2010).

Despite the importance of epigenetic regulation in cellular dedifferentiation, the biological relevance of other chromatin marks in callus formation is still elusive. In this study, we demonstrate that histone acetylation state is also crucial for pluripotent callus formation. Treatment with TSA, a potent chemical inhibitor of HDAC, led to reduced capability of cellular dedifferentiation. Expression of a subset of HDAC genes was up-regulated in calli, and consistently, genetic mutants with defects in *HDA9* or *HDT1* exhibited reduced callus formation. Our findings provide insights into the biological significance of dynamic histone acetylation states in cellular dedifferentiation in *Arabidopsis* .

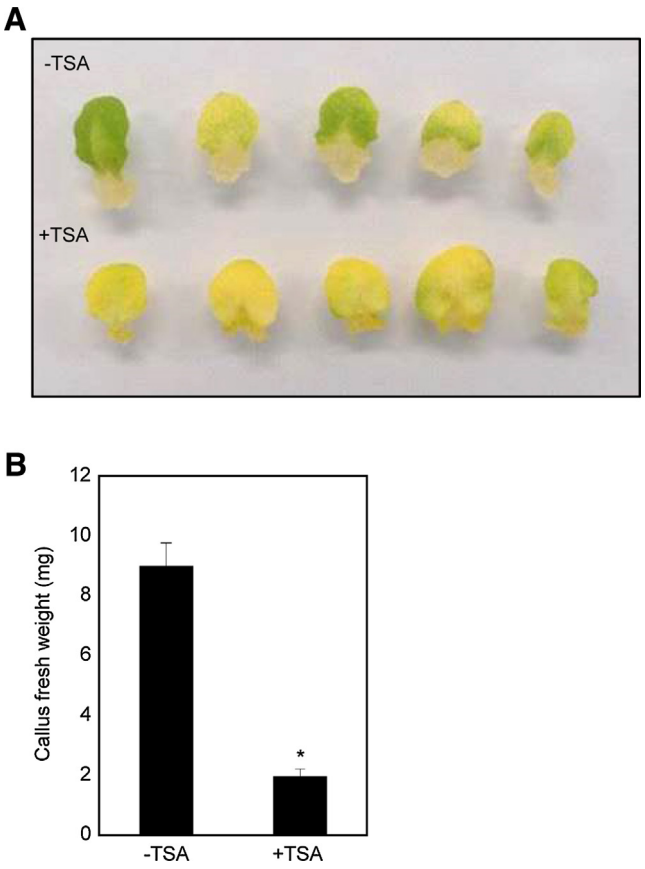

Fig. 1. Biological relevance of histone deacetylation in callus formation (A) Effects of TSA on callus formation. Leaf explants from third-leaves of two-week-old wild-type Columbia-0 (Col-0) plants were used to induce calli on CIM in the presence or absence of TSA. The plates were incubated for 2 weeks and photographed. (B) Impaired callus formation upon the TSA treatment. Thirty calli were collected to measure fresh weight. Bars indicate the standard error of the mean. Statistically significant differences are indicated by asterisks ($^{*}P < 0.05$).

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana (Columbia-0 ecotype) was used for all experiments unless otherwise specified. Plants were grown under long-day conditions (LDs; 16-h light/8-h dark cycles) with cool white fluorescent light (100 μmol photons m^{-2} s^{-1}) at 23 °C. The *hda9-1* mutant (SALK_120967) was previously reported (Kang et al., 2015). The *hdt1-1* (GK_355H03) mutant was isolated from a T-DNA insertional mutant pool deposited in the Nottingham *Arabidopsis* Stock Centre (NASC; http://arabidopsis.info).

For callus induction, leaf explants of two-week-old plants were placed on CIM (B5 medium supplemented with 0.5 mg/ml 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.05 μg/ml kinetin), followed by incubation at 22 °C in the dark for 2 weeks (Fan et al., 2012). To determine the effects of TSA on callus formation, TSA (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO), and 1 μM TSA was added to CIM.

2.2. Quantitative real-time RT-PCR analysis

Total RNA was extracted using TRI reagent (TAKARA Bio, Singa, Japan) according to the manufacturer's recommendations. Reverse transcription (RT) was performed using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Dr. Protein, Seoul, South Korea) with oligo(dT18) to synthesize first-strand cDNA from 2 μg of total RNA. Total RNA samples were pretreated with an RNAse-free DNAse. cDNAs were diluted to 100 μL with TE buffer, and 1 μL of diluted cDNA was used for PCR amplification.

Quantitative RT-PCR reactions were performed in 96-well blocks using the Step-One Plus Real-Time PCR System (Applied Biosystems). The PCR primers used are listed in Supplementary Table S1. The values for each set of primers were normalized to the *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1* (*eIF4A*) gene (At3g13920). All RT-qPCR reactions were performed with biological triplicates using total RNA samples extracted from three independent replicate samples. The comparative $\triangle\triangle C_T$ method was employed to evaluate relative quantities of each amplified product in the samples. The threshold cycle (C_T) was automatically determined for each reaction with the analysis software set using default parameters. The specificity of the RT-qPCR reactions was determined using melting curve analysis of the amplified products with the standard method employed by the software.

2.3. Immunoblot analysis

Harvested plant materials were ground in liquid nitrogen, and total cellular extracts were suspended in SDS-PAGE sample loading buffer. Protein samples were analyzed using SDS/PAGE (10% gels) and blotted onto Hybond-P+ membranes (Amersham-Pharmacia). Proteins were immunologically detected using anti-H3ac or anti-H4ac antibodies (Millipore, Billerica, MA, USA).