Biochemical and Biophysical Research Communications 496 (2018) 1134–1140

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpression of histone methyltransferase NSD in *Drosophila* induces apoptotic cell death via the Jun-N-terminal kinase pathway

Yuji Jeong [a], Taejoon Kim [a, b], Suyeun Kim [a], Yoon-Ki Hong [c], Kyoung Sang Cho [a, b], Im-Soon Lee [a, b, *]

[a] Department of Biological Sciences, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
[b] CHANS Research Center, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
[c] SK Chemicals, 310 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13494, Republic of Korea

ARTICLE INFO

Article history:
Received 9 January 2018
Accepted 25 January 2018
Available online 2 February 2018

Keywords:
NSD
H3K36 HMTase
Drosophila
JNK
Apoptosis
Sotos syndrome 1

ABSTRACT

The nuclear receptor-binding SET domain protein gene (*NSD*) family encodes a group of highly conserved SET domain-containing histone lysine methyltransferases that are important in multiple aspects of development in various organisms. The association of *NSD1* duplications has been reported with growth retardation diseases in humans. In this study, to gain insight into the molecular mechanisms by which the overexpression of *NSD1* influences the disease progression, we analyzed the gain-of-function mutant phenotypes of the *Drosophila NSD* using the *GAL4/UAS* system. Ubiquitous overexpression of *NSD* in the fly caused developmental delay and reduced body size at the larval stage, resulting in pupal lethality. Moreover, targeted overexpression in various developing tissues led to significant phenotype alterations, and the gain-of-function phenotypes were rescued by *NSD* RNAi knockdown. We also demonstrated that *NSD* overexpression not only enhanced the transcription of pro-apoptotic genes but also activated caspase. The atrophied phenotype of *NSD*-overexpressing wing was strongly suppressed by a loss-of-function mutation in *hemipterous*, which encodes a *Drosophila* Jun N-terminal kinase. Taken together, our findings suggest that NSD induces apoptosis via the activation of JNK, and thus contributes to the understanding of the molecular mechanisms involved in *NSD1*-related diseases in humans.

1. Introduction

Histone methyltransferases (HMTases) add methyl groups to specific histone lysine (Lys) or arginine residues. H3K36 HMTases, which modify the methylation status of Lys36 on histone 3 (H3), contain the suppressor of variegation 3-9 and enhancer of zeste and trithorax (SET) domains, which are conserved from yeast to mammals. In yeast, all H3K36 mono-, di-, and tri-methylations are carried out by Set2 [1]. However, more complex eukaryotes require two groups of enzymes to react with the various methyl states of H3K36 residues depending on the nature of the substrate. The first group, which mainly catalyzes H3K36 trimethylation, includes MET-1 in *C. elegans*, Hypb/Set2 in *Drosophila*, and HYPB/Setd2 in mammals [2–4]. The second group of enzymes includes maternal-effect sterile 4 (MES-4) in *C. elegans* and nuclear receptor-binding SET domain (NSD) proteins in *Drosophila* and mammals [3,5,6], which catalyze global mono- and/or di-methylation of H3K36 *in vivo* [3,7,8].

The MES-4/NSD proteins are conserved among various animal species. In contrast to mammals, which have one family of three related HMTases (NSD1, NSD2 [MMSET/WHSC1], and NSD3 [WHSC1L1]), *Drosophila* has a single homolog, NSD. As evidenced by the growing number of human diseases linked to human NSD family proteins, which play a critical role in chromatin integrity, mutations in the human *NSD1/2/3* genes underlie various developmental disorders and malignancies [6,9–11]. Heterozygous loss-of-function mutations within the two genes, *NSD1* and *NSD2*, have been reported to be linked to developmental diseases such as Sotos syndrome 1 (SOTOS1, OMIM 117550) and Wolf-Hirschhorn syndrome (WHS, OMIM 194190), respectively [12–15]. SOTOS1 is an autosomal dominant childhood disease, and children with this syndrome exhibit overgrowth with additional features such as characteristic dysmorphisms, advanced bone age, and macrocephaly [16]. Interestingly, however, duplications of *NSD1* have

* Corresponding author. Department of Biological Sciences, Konkuk University, Seoul 05029, Republic of Korea.
E-mail address: islee@konkuk.ac.kr (I.-S. Lee).

https://doi.org/10.1016/j.bbrc.2018.01.156
0006-291X/

been proposed to cause short stature, developmental delay, and microcephaly, which are opposite phenotypes to SOTOS1 [17–21], implying contrasting effects on body development in the roles of *NSD1* depending on its copy number. In addition, it is noteworthy that *NSD1* duplication was recently reported in a patient with Silver-Russell syndrome (SRS, OMIM 180860), which is a growth retardation syndrome [22].

Although current studies have been mainly focused on the roles of human NSD-related genes in histone modification and subsequent chromatin dynamics, the downstream signaling pathways that are associated with the genes involved in tumorigenesis or organ development remain to be determined. Thus, we focused on the *Drosophila melanogaster* NSD, and sought to determine the function of NSD in developing tissues. We examined the phenotypes of flies that ectopically overexpress NSD using the *GAL4/UAS* system in developing tissues, and further analyzed the gain-of-function mutant flies to determine which signaling pathway is involved in the phenotypes induced by NSD overexpression.

2. Materials and methods

2.1. Fly strains

A *NSD* line (DP00238) was obtained from Vienna Drosophila Resource Center (Vienna, Austria), and daughterless (*Da*)-*GAL4*, engrailed (*en*)-*GAL4*, glass multimer reporter (*GMR*)-*GAL4*, patched (*ptc*)-*GAL4*, pannier (*pnr*)-*GAL4*, *UAS-Drosophila* inhibitor of apoptosis protein 1 (*DIAP1*), *UAS-puckered*, and *UAS-2×enhanced green fluorescent protein* (*EGFP*) flies were obtained from the Bloomington *Drosophila* Stock Center. *MS1096-GAL4* and *hemipterous*[1] were gifts from Dr. M. Freeman (MRC Laboratory of Molecular Biology, UK) and Dr. S. Noselli (CNRS, France), respectively.

2.2. Overexpression of NSD using the GAL4/UAS system

To overexpress *NSD*, we used a modular misexpression system based on a P-element vector carrying a GAL4-regulated promoter. In the *NSD* line, in which the P-element was inserted in the first exon of *NSD*, GAL4-dependent transcription begins within the P-element and extends into the *NSD* gene, indicating that this insertion mutant can be used to study *NSD* gain-of-function mutants. *GMR-GAL4, MS1096-GAL4, ptc-GAL4, pnr-GAL4*, or *Da-GAL4* flies were used to overexpress NSD in various developing tissues.

External eye morphologies were observed by scanning electron microscopy (Seoul National University, Korea).

2.3. Scoring of developmental progression of flies

After crossing *Da-Gal4* virgin females with *UAS-NSD* males, eggs were laid on grape plates with yeast pastes at 25 °C for 16 h, and collected. Fifty hatched first instar larvae that woke up after 24 h of receiving were transferred into vials with standard fly food, and kept at 25 °C. The developmental stage of each larva was evaluated by checking its mouth hook and tracheal morphology every 24 h, as previously described [23].

2.4. Acridine orange staining and BrdU-labeling experiments

The eyes and wing imaginal discs of stage L3 larvae were dissected in phosphate-buffered saline (PBS). The discs were then incubated for 5 min in a 1.6×10^{-6} M solution of acridine orange (Aldrich), and rinsed briefly in PBS. The samples were then examined under an Axiophot2 fluorescence microscope (Carl Zeiss).

2.5. Preparation of RNA and RT-qPCR

Total RNA was isolated from flies according to the TRIzol method. Subsequently, cDNA was synthesized with the Maxime kit (iNtRON Biotechnology), and RT-qPCR was performed with the SYBR Green PCR Master Mix (Applied Biosystems). RT-qPCR was performed using a StepOne Real-time PCR system (Applied Biosystems). Quantification was performed using the delta-delta Ct method and normalized to *Rp49* transcript levels and the control. Each experiment was repeated at least 5 times ($n \geq 5$).

2.6. Primers

RT-qPCR was performed using primer pairs for *NSD* (5′-ATCCATCGTGTGGGCATATT-3′ for the forward and 5′-GAACAACAGCAAGAAGGCCT-3′ for the reverse) and *Rp49* (5′-TACAGGCCCAAGATCGTGAA-3′ for the forward and 5′-GTTCGATCCGTAACCGATGT-3′ for the reverse). RT products were amplified by 30 cycles of PCR, and confirmed on agarose gels.

2.7. Immunohistochemistry

Fly wing imaginal discs were dissected and fixed with 4% paraformaldehyde in PBS and then washed with PBT (PBS + 0.1% Tween 20) and blocked with 2% normal goat serum in PBT. The discs were incubated 16 h with rabbit anti-cleaved caspase-3 (Cell signaling, 1:100), anti-phospho-histone 3 (Promega, 1:200), 4D9 anti-engrailed (Corey Goodman, University of California, 1:200), anti-dimethyl-histone H3 (Lys36) (Millipore, 1:200), and Trimethyl-histone H3 (Lys36) (Cell Signaling, 1:200) antibodies and then washed using PBT. Subsequently, the samples were incubated with an FITC- or rhodamine-labeled goat anti-rabbit IgG (H + L) secondary antibody (1:200 in PBT) for 1 h at room temperature. The samples were observed with a LSM710 laser-scanning confocal microscope (Carl Zeiss).

3. Results

3.1. Ubiquitous overexpression of NSD causes pupal lethality in D. melanogaster

The *Drosophila* NSD protein consists of 1427 amino acids with characteristic motifs including PWWP, the plant homeodomain (PHD), and SET [Su(var)3-9, Enhancer-of-zeste, Trithorax] domains (Fig. 1A). As the sizes of the human NSD family proteins vary, their C-terminal regions containing PHD and SET domains were compared (Supplementary data 1), and the results showed similar percentages in amino acid homology with the *Drosophila* counterpart (40.2%, 39.7%, or 39.5%, respectively) (Fig. 1A).

To examine the effect of ubiquitous overexpression of NSD in the whole body during development, *Da-GAL4* flies were crossed with a P-element insertion line, the *NSD* fly (DP00238) (Fig. 1B). RT-qPCR analysis of the resultant progenies from the cross with the *NSD* line confirmed that the relatively high expression of *NSD* mRNA was maintained from the embryo to the third day larva compared to those of wild-type flies (Fig. 1C).

Next, we assessed the effect of ubiquitous overexpression of NSD on the survival rates and the body sizes of offspring. The survival rates of the fly offspring from the *NSD* line with the *Da-GAL4* driver were markedly reduced and appeared to be lethal during the larval and pupal stages (Fig. 1D). When comparing the sizes of the larvae on the third day after hatching, the average size of the *NSD*-overexpressing flies was 40% smaller than that of the control (Fig. 1E), resembling growth retardation seen in the