A Histone H3 Lysine 36 Trimethyltransferase Links Nkx2-5 to Wolf-Hirschhorn Syndrome

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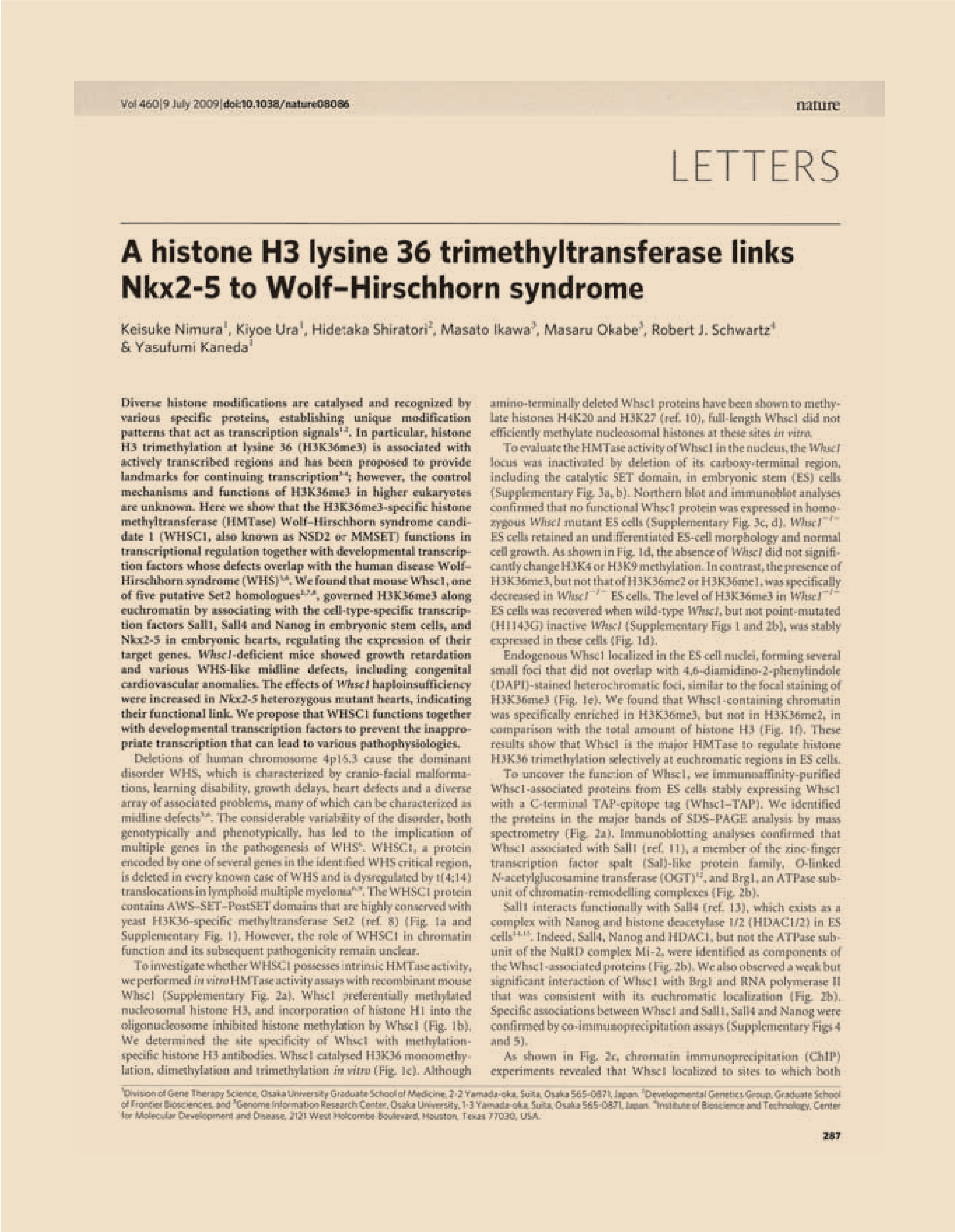

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LETTERS

A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf–Hirschhorn syndrome

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Diverse histone modifications are catalysed and recognized by various specific proteins, establishing unique modification patterns that act as transcription signals[1,2]. In particular, histone H3 trimethylation at lysine 36 (H3K36me3) is associated with actively transcribed regions and has been proposed to provide landmarks for continuing transcription[3,4]; however, the control mechanisms and functions of H3K36me3 in higher eukaryotes are unknown. Here we show that the H3K36me3-specific histone methyltransferase (HMTase) Wolf–Hirschhorn syndrome candidate 1 (WHSC1, also known as NSD2 or MMSET) functions in transcriptional regulation together with developmental transcription factors whose defects overlap with the human disease Wolf–Hirschhorn syndrome (WHS)[5,6]. We found that mouse Whsc1, one of five putative Set2 homologues[5,7,8], governed H3K36me3 along euchromatin by associating with the cell-type-specific transcription factors Sall1, Sall4 and Nanog in embryonic stem cells, and Nkx2-5 in embryonic hearts, regulating the expression of their target genes. *Whsc1*-deficient mice showed growth retardation and various WHS-like midline defects, including congenital cardiovascular anomalies. The effects of *Whsc1* haploinsufficiency were increased in *Nkx2-5* heterozygous mutant hearts, indicating their functional link. We propose that WHSC1 functions together with developmental transcription factors to prevent the inappropriate transcription that can lead to various pathophysiologies.

Deletions of human chromosome 4p16.3 cause the dominant disorder WHS, which is characterized by cranio-facial malformations, learning disability, growth delays, heart defects and a diverse array of associated problems, many of which can be characterized as midline defects[5,6]. The considerable variability of the disorder, both genotypically and phenotypically, has led to the implication of multiple genes in the pathogenesis of WHS[6]. WHSC1, a protein encoded by one of several genes in the identified WHS critical region, is deleted in every known case of WHS and is dysregulated by t(4;14) translocations in lymphoid multiple myeloma[6,9]. The WHSC1 protein contains AWS–SET–PostSET domains that are highly conserved with yeast H3K36-specific methyltransferase Set2 (ref. 8) (Fig. 1a and Supplementary Fig. 1). However, the role of WHSC1 in chromatin function and its subsequent pathogenicity remain unclear.

To investigate whether WHSC1 possesses intrinsic HMTase activity, we performed *in vitro* HMTase activity assays with recombinant mouse Whsc1 (Supplementary Fig. 2a). Whsc1 preferentially methylated nucleosomal histone H3, and incorporation of histone H1 into the oligonucleosome inhibited histone methylation by Whsc1 (Fig. 1b). We determined the site specificity of Whsc1 with methylation-specific histone H3 antibodies. Whsc1 catalysed H3K36 monomethylation, dimethylation and trimethylation *in vitro* (Fig. 1c). Although amino-terminally deleted Whsc1 proteins have been shown to methylate histones H4K20 and H3K27 (ref. 10), full-length Whsc1 did not efficiently methylate nucleosomal histones at these sites *in vitro*.

To evaluate the HMTase activity of Whsc1 in the nucleus, the *Whsc1* locus was inactivated by deletion of its carboxy-terminal region, including the catalytic SET domain, in embryonic stem (ES) cells (Supplementary Fig. 3a, b). Northern blot and immunoblot analyses confirmed that no functional Whsc1 protein was expressed in homozygous *Whsc1* mutant ES cells (Supplementary Fig. 3c, d). *Whsc1*$^{-/-}$ ES cells retained an undifferentiated ES-cell morphology and normal cell growth. As shown in Fig. 1d, the absence of *Whsc1* did not significantly change H3K4 or H3K9 methylation. In contrast, the presence of H3K36me3, but not that of H3K36me2 or H3K36me1, was specifically decreased in *Whsc1*$^{-/-}$ ES cells. The level of H3K36me3 in *Whsc1*$^{-/-}$ ES cells was recovered when wild-type *Whsc1*, but not point-mutated (H1143G) inactive *Whsc1* (Supplementary Figs 1 and 2b), was stably expressed in these cells (Fig. 1d).

Endogenous Whsc1 localized in the ES cell nuclei, forming several small foci that did not overlap with 4,6-diamidino-2-phenylindole (DAPI)-stained heterochromatic foci, similar to the focal staining of H3K36me3 (Fig. 1e). We found that Whsc1-containing chromatin was specifically enriched in H3K36me3, but not in H3K36me2, in comparison with the total amount of histone H3 (Fig. 1f). These results show that Whsc1 is the major HMTase to regulate histone H3K36 trimethylation selectively at euchromatic regions in ES cells.

To uncover the function of Whsc1, we immunoaffinity-purified Whsc1-associated proteins from ES cells stably expressing Whsc1 with a C-terminal TAP-epitope tag (Whsc1–TAP). We identified the proteins in the major bands of SDS–PAGE analysis by mass spectrometry (Fig. 2a). Immunoblotting analyses confirmed that Whsc1 associated with Sall1 (ref. 11), a member of the zinc-finger transcription factor spalt (Sal)-like protein family, *O*-linked *N*-acetylglucosamine transferase (OGT)[12], and Brg1, an ATPase subunit of chromatin-remodelling complexes (Fig. 2b).

Sall1 interacts functionally with Sall4 (ref. 13), which exists as a complex with Nanog and histone deacetylase 1/2 (HDAC1/2) in ES cells[14,15]. Indeed, Sall4, Nanog and HDAC1, but not the ATPase subunit of the NuRD complex Mi-2, were identified as components of the Whsc1-associated proteins (Fig. 2b). We also observed a weak but significant interaction of Whsc1 with Brg1 and RNA polymerase II that was consistent with its euchromatic localization (Fig. 2b). Specific associations between Whsc1 and Sall1, Sall4 and Nanog were confirmed by co-immunoprecipitation assays (Supplementary Figs 4 and 5).

As shown in Fig. 2c, chromatin immunoprecipitation (ChIP) experiments revealed that Whsc1 localized to sites to which both

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The following is a comment on the published paper shown on the preceding page.

A Histone H3 Lysine 36 Trimethyltransferase Links Nkx2-5 to Wolf-Hirschhorn Syndrome

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Introduction

Deletions of human chromosome 4p16.3 cause a dominant disorder known as Wolf-Hirschhorn syndrome (WHS), which is characterized by cranio-facial malformations, mental retardation, growth delays, heart defects, and a diverse array of associated problems, many of which can be characterized as midline defects. The considerable variability of the disorder, both genotypically and phenotypically, has led to the implication of multiple genes in the pathogenesis of WHS (*1*). WHSC1, one of several genes in the identified WHS critical region, is deleted in every known case of WHS and is dysregulated by t(4;14) translocations in lymphoid multiple myeloma (*2*). The WHSC1 protein contains AWS-SET-PostSET domains that are highly conserved with yeast H3K36-specific methyltransferase Set2. However, the role of WHSC1 in chromatin function and its subsequent pathogenicity remain unclear.

Whsc1 methylates histone H3 on lysine 36.

We performed *in vitro* HMTase activity assays using recombinant mouse Whsc1 to determine the site specificity of Whsc1 using methylation-specific histone H3 antibodies. Whsc1 catalyzed H3K36 mono-, di-, and tri-methylation *in vitro* (Fig. 1a). We next examined the HMTase activity of Whsc1 in the nucleus, the *Whsc1* locus was inactivated by deletion of its C-terminal region, including the catalytic SET domain, in ES cells. The absence of *Whsc1* did not significantly change H3K4 or H3K9 methylation. In contrast, the presence of H3K36me3, but not that of H3K36me2 or H3K36me1, was specifically reduced in *Whsc1*$^{-/-}$ ES cells. The level of H3K36me3 in *Whsc1*$^{-/-}$ ES cells was recovered when wild-type *Whsc1*, but not point-mutated (H1143G) inactive *Whsc1*, was stably expressed in these cells (Fig. 1b).

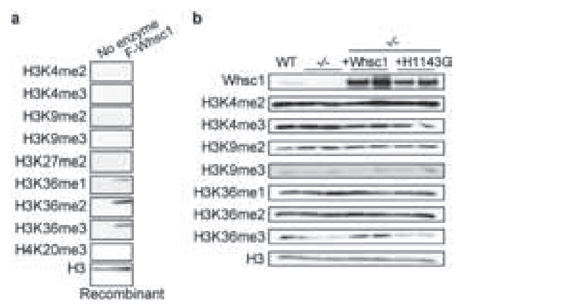

Figure 1. Whsc1 methylates histone H3 on lysine 36. **a,** Whsc1 catalyzes H3K36 methylation *in vitro*. Reconstituted oligonucleosomes using recombinant core histones were used as substrates for HMTase assays and analyzed by immunoblotting with the indicated specific histone H3 antibodies. **b,** Methylation status of histone H3 in wild-type or *Whsc1*$^{-/-}$ ES cells and in *Whsc1*$^{-/-}$ ES cells stably expressing either Whsc1 or mutant Whsc1 (H1143G).

Whsc1 associates with transcription factors to repress abnormal transcription.

To uncover the function of Whsc1, we immunoaffinity-purified Whsc1-associated proteins from ES cells stably expressing Whsc1 with a C-terminal TAP-epitope tag (Whsc1-TAP). We identified the proteins in the major bands of SDS-PAGE analysis by mass spectrometry (Fig. 2a). Immunoblotting analyses confirmed that Whsc1 associated with Sall1, a member of the zinc finger transcription factor spalt (Sal)-like protein family, Sall4, Nanog, *O*-linked N-acetylglucosamine transferase (OGT), HDAC1 and Brg1, an ATPase subunit of chromatin-remodeling complexes but not the ATPase subunit of the NuRD complex Mi-2 (Fig. 2b).

Chromatin immunoprecipitation (ChIP) experiments revealed that Whsc1 localized to Nanog-Sall4 cobinding sites within the coding regions of estrogen-related receptor *Esrrb* and T-box transcription factor *Tbx3*. We found that the accumulation of H3K36me3 was significantly reduced around these Nanog-Sall4 cobinding sites in *Whsc1*$^{-/-}$ ES cells and that the levels of nuclear transcripts from these regions at *Esrrb* and *Tbx3* were increased upon deletion of *Whsc1* (Fig. 2c and d).

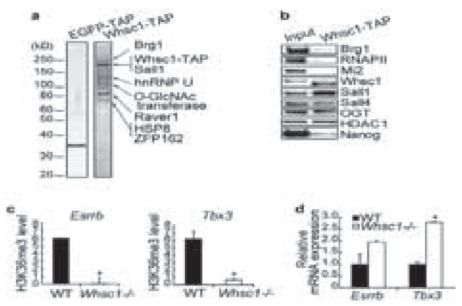

Figure 2. Whsc1 associates with transcription factors to repress abnormal transcription. **a,** Silver staining of Whsc1-TAP-associated proteins purified from ES cells stably expressing Whsc1-TAP. Whsc1-TAP-associated proteins as identified by mass spectrometry are indicated on the right. **b,** Purified Whsc1-TAP-associated proteins were analyzed by immunoblotting using the antibodies indicated. **c,** H3K36me3 occupancy on the *Esrrb* or *Tbx3* gene in wild-type (WT) and *Whsc1*$^{-/-}$ ES cells was analyzed by ChIP experiments. Error bars indicate SD., n = 3. **d,** Quantitative RT-PCR analysis of *Esrrb* and *Tbx3* pre-mRNA in the nucleus. Error bars indicate SD, n = 3.

The *Whsc1* gene is required for normal mouse development.

We generated *Whsc1*-deficient mice to elucidate the developmental and pathological role of Whsc1. Genotyping the offspring produced by interbreeding *Whsc1*$^{+/-}$ mice revealed significantly lower numbers of *Whsc1*$^{-/-}$ and *Whsc1*$^{+/-}$ mice compared to their expected Mendelian ratios at birth (Fig.3a). The majority of *Whsc1* heterozygotes survived and were fertile. However, the growth rates of *Whsc1*$^{+/-}$ mice were highly variable, and some of these mice exhibited severe growth defects,