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Original Article

Recombinant Protein Expression and Purification of the Human HMTase MMSET/NSD2

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Abstract

Chromatin remodelers that include histone methyl transferases (HMTases) are becoming a focal point in cancer drug development. The NSD family of three HMTases, NSD1, NSD2/MMSET/WHSC1, and NSD3/WHSC1L are *bona fide* oncogenes found aberrantly expressed in several cancers, suggesting their potential role for novel therapeutic strategies. Several histone modifiers including HMTase have clear roles in human carcinogenesis but the extent of their functions and regulations are not well understood, especially in pathological conditions. The extents of the NSDs biological roles in normal and pathological conditions remain unclear. In particular, the substrate specificity of the NSDs remains unsettled and discrepant data has been reported. NSD2/MMSET is a focal point for therapeutic interventions against multiple myeloma and especially for t(4;14) myeloma, which is associated with a significantly worse prognosis than other biological subgroups. Multiple myeloma is the second most common hematological malignancy in the United States, after non-Hodgkin lymphoma. Herein, as a first step before entering a pipeline for protein x-ray crystallography, we cloned, recombinantly expressed and purified the catalytic SET domain of NSD2. Next, we demonstrated the catalytic activities, *in vitro*, of the recombinantly expressed NSD2-SET on H3K36 and H4K20, its biological targets at the chromatin.

Keywords : Epigenetic therapy of cancer; Histone-lysine methyltransferase; NSD2; MMSET; Recombinant protein expression

Introduction

NSD1, NSD2/MMSET/WHSC1 and NSD3/WHSC1L1 composethe nuclear receptor binding SET domain (NSD) family classified into the histone methyltransferase (HMTase) KMT3 family. The NSDs are histone modifiers that participate in maintaining the chromatin. The NSDs predominantly methylate histone H3 lysine 36 (H3K36) and histone H4 lysine 20 (H4K20) at the chromatin (Lucio-Eterovic et al. 2010 Pei et al. 2011; Wagner and Carpenter, 2012). Lysine methylation or any of the other histone modifications, such as phosphorylation, acetylation and citrullination participates to the dynamic remodeling of the chromatin and ultimately contribute to regulating the transcription. Epigenetic marks on H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20 play primary roles in chromatin remodelling and contribute to the histone code that remains obscure (Strahl and Allis 2000).

The NSDs are large multi-domains proteins with four zinc finger domains, two PWWP domains, and a catalytic SET domain (Figure1). The function of PHD1-3 and PWWP1-2 domains of the three NSDs at the chromatin remains elusive. However, the recent study by He *et al.* demonstrated the role of the conserved PHD4 domain to localize the NSDs on histone H3, strongly suggesting that PHD4 functions as a histone-lysine mark recognition module to position the catalytic SET domain on its lysine substrate (He et al. 2013). The PHD4 domain is located 36-residues distance upstream of the postSET subdomain. Due to its immediate vicinity to the postSET subdomain, the PHD4 may be structurally part of the catalytic SET domain as well. Similarly, the PWWP2 domain is also conserved across NSDs and is downstream the preSET subdomain, but at a greater 72-residues distance compared to PHD4-postSET (36-residues). However, the role of this PWWP2 domain remains unclear.

A rapidly increasing amount of evidence highlights the importance of epigenetic deregulation in numerous carcinogenetic events (Morishita and di Luccio, 2011a). The amplification of NSD1 is found in multiple myeloma, lung cancer, neuroblastomas and glioblastomas. The amplification of either NSD1 or NSD2 triggers the cellular transformation. NSD2/MMSET (multiple myeloma SET) is associated with tumor aggressiveness or prognosis in most types of cancers including prostate cancer and multiple myeloma (Asangani et

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al. 2012; Brito et al. 2009; Ezponda et al. 2012; Kim et al. 2008; Klein et al. 2009; Yang et al. 2012b). NSD2 is overexpressed in solid tumors especially breast cancer, myeloma and glioblastoma, resulting in aberrantly high global levels of H3K36me2 (Asangani et al. 2012; Brito et al. 2009; Chesi et al. 1998; Ezponda et al. 2012; Hudlebusch et al. 2011; Kassambara et al. 2009; Kim et al. 2008; Klein et al. 2009; Marango et al. 2008; Martinez-Garcia et al. 2011; Pei et al. 2011; Yang et al. 2012a). Overexpression of NSD2 in prostate cancer causes epigenetic aberrations leading tothe metastatic phenotype (Ezponda et al. 2012). NSD3 plays a role in lung cancer andis found amplified in breast cancer cell lines and primary breast carcinomas (Angrand et al. 2001; Rosati et al. 2002). NSD2/MMSET is a focal point for therapeutic interventions against multiple myeloma and especially for t(4;14) myeloma, which is associated with a significantly worse prognosis than other biological subgroups (Mirabella et al. 2013). Multiple myeloma is the second most common hematological malignancy in the United States, after non-Hodgkin lymphoma.

Chromatin remodelers that include HMTases are becoming a focal point in cancer drug development (Allen et al. 2013; Brueckner et al. 2007; Kristensen et al. 2009; Lane and Chabner 2009; Mack 2010; Malmquist et al. 2012; Spannhoff et al. 2009a). Chromatin remodelling inhibitors targeting DNA methyltransferases (DNMTs), histone methyltransferases and deacetylases (HDACs) are being pursued for novel cancer chemotherapies as well as for chemoprevention. Several DNA-methylation and histone deacetylase inhibitors are currently in clinical trial stages(Lane and Chabner 2009; Spannhoff et al. 2009c). The NSDs and specifically NSD2 are deregulated in several human cancers and are considered as valuable drug-targets (Asangani et al. 2012; Brito et al. 2009; Chesi et al. 1998; Ezponda et al. 2012; Hudlebusch et al. 2011; Kassambara et al. 2009; Kim et al. 2008; Klein et al. 2009; Marango et al. 2008; Martinez-Garcia et al. 2011; Pei et al. 2011; Yang et al. 2012a). Although the NSD proteins are instrumental in the development and progression of numerous cancers, their *modi operandi* are not yet fully understood. Inhibitors of HMTases are scarceand very few compounds have been reported to be selective and specific to their target. Most HMTase inhibitors are still the early stage of drug-discovery (Morishita and di Luccio 2011a, b). Notably, GlaxoSmithKline Inc. and Epizyme Inc. have made significant progress on the discovery of HMTase DOT1L and EZH2 potent inhibitors (Knutson et al. 2013; Verma et al. 2012) (Basavapathruni et al. 2012; Daigle et al. 2013). DOT1L is apart from other HMTases as it does not contain a SET domain (Min et al. 2003). The HMTase inhibitors, BIX-01294 and BIX-01338, have been shown to be effective on G9a with an IC_{50} of 3 M and 5 M, respectively (Spannhoff et al. 2009b). In addition, Chaetocin inhibits Su(var)3-9 with an IC_{50} of 0.8 μM (Spannhoff et al. 2009b). Importantly, Liu et al. completed a seminal work on the G9a inhibitors such as UNC0224, with an IC_{50} of 15 nM (Liu et al. 2009). Both Epizyme Inc., GlaxoSmithKline Inc and research groups on G9a and EZH2 have reported HMTase inhibitors with IC_{50} in the low nano molar range.

The catalytic mechanism of lysine-HMTase proceeds through a linear S_N2 nucleophilic attack between the cofactor S-Adenosylmethionine and the Lysine-NH_3 substrate (Morishita and di Luccio, 2011a). The SAM binds into a small cavity immediately adjacent to the histone-tail large binding groove where the lysine substrate extends deep inside a channel at the interface between both binding areas (Martinez-Garcia et al. 2011). Previously, we demonstrated that the SET domain of NSD1 accommodates a 7-amino acid peptide, similarly as it was further identified in SET8 (Morishita and di Luccio 2011b) (Kudithipudi et al. 2012). In addition, we demonstrated the opening mechanism of the SET domain of NSD1 through the rotation of a small loop at the interface between the SET and postSET subdomain (Morishita and di Luccio 2011b). This regulatory-loop is likely to participate in both the substrate recognition and the catalytic mechanism by acting as a seat belt for the lysine-substrate. The regulatory-loop sits on top of the lysine-substrate by strongly anchoring the histone-tail in the SET domain (Kudithipudi et al. 2012). The histone-tail binding area involves regions from both the SET and postSET subdomains (Figure 1). The sequences for the SET domain are highly conserved amongst the NSDs, thus it is likely that NSD2 and NSD3 proceed through the same mechanism as described for NSD1 (Figure 2). Notably, the NSDs are apart from other known HMTases with both NSD2 and NSD3 being close sibling (Figure 2).

In this study, as a first step toward better understanding the biological functions of NSD2/MMSET, we cloned, recombinantly expressed and purified the catalytic SET domain of NSD2. We demonstrate the catalytic activities, *in vitro*, of the recombinantly expressed NSD2-SET on H3K36 and H4K20 human histone marks.

Materials and Methods

Cloning

The SET domain of human *NSD2* gene (NSD2-SET, 873 bp,

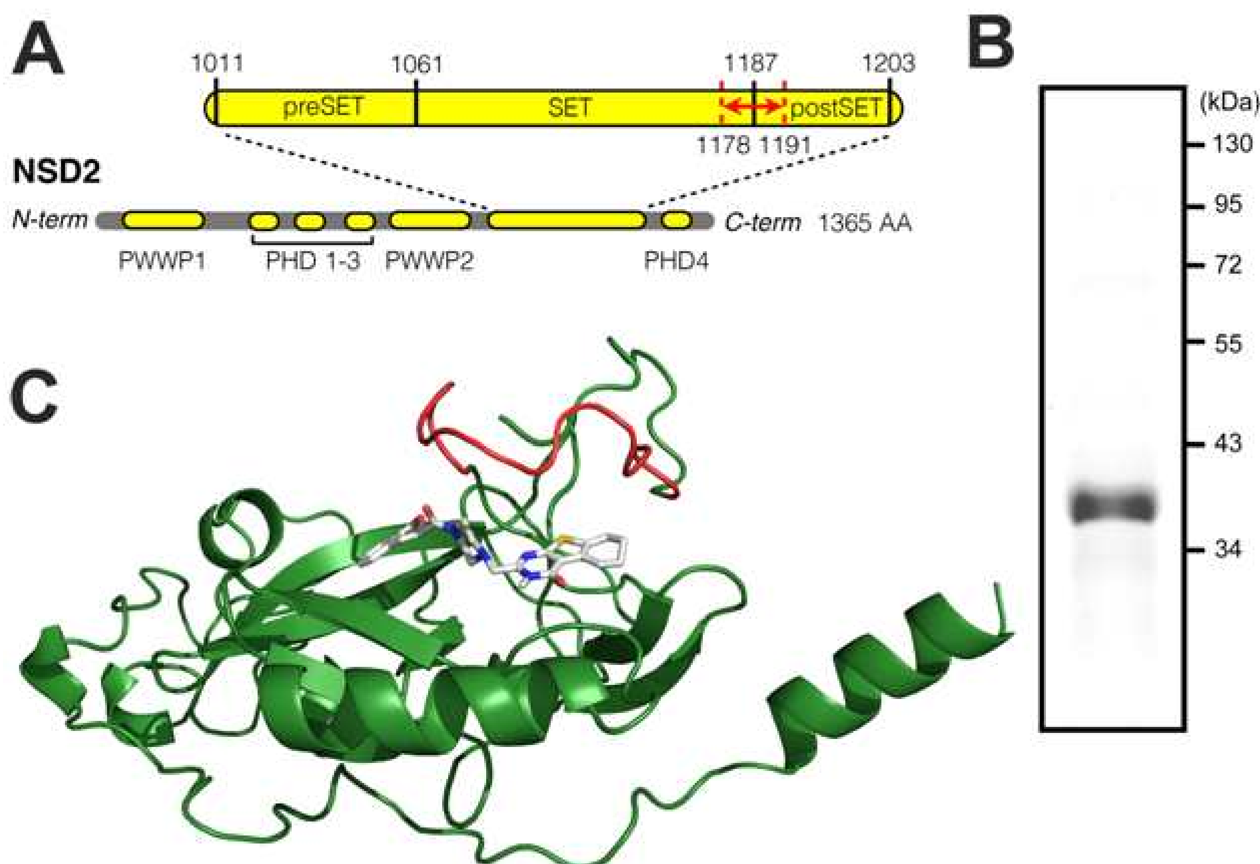

Figure 1. Architecture of NSD2/MMSET and model of the opened SET domain with LEM-07

(A) Schematic of the primary structure of NSD2: PWWP domain; PHD zinc fingers domain; SET histone methyl transferase (HMTase) with the preSET and postSET domains. The regulatory loop closing onto the histone-binding site is indicated in red. (B) Commassie staining on SDS-PAGE gel of the recombinant expressed NSD2-SETafter purification.

2844-3717 nt; 291 a.a., 948-1239 a.a) was amplified by PCR using human liver cDNA library (TAKARA, Japan) as template. The forward and reverse primers are PK162, 5'- GGC-AGCCATATG(*Nde*I)CAGGGGGTCAGAGGGATCGGAAG AG -3' and PK163, 5'- GAAGCACTCGAG(*Xho*I)CTCTGACTGCC-TCTTCCCTTCCCC -3', respectively. The PCR-amplified NSD2-SET DNA fragment was digested with *NdeI* and *XhoI* and inserted into the multi cloning site of the protein expression Intein-tagging vector, pTYB2, (New England Biolabs, USA). The sequence was verified correct by sequencing (Solgent, Korea).

Protein Expression and Purification

Escherichia coli expression strain, BL21, transformed with pTYB2 plasmid harbouring NSD2-SET was grown in LB medium containing 100 µg/mL ampicillin and the expression of recombinant NSD2-SET was induced with 250 µM isopropyl 1-thio-D-galactopyranoside (IPTG) for 4 h at 15℃. *E. coli* cells were harvested and lysed by freeze-and-thaw method and incubation in buffer A [20 mM Tris (pH 8.0), 500 mM NaCl, and 0.1 mM EDTA] containing 0.1% Triton X-100 and 10 mM phenylmethanesulfonylfluoride (PMSF) along with 20 cycles of sonication on ice. The resulting cell extract containing NSD2-SET-Intein-chitin-binding domain fusion protein was passed over an affinity column of chitin beads and washed with 100-column volumes of buffer A with 0.1% Triton X-100, followed by 20-column volumes of buffer A without Triton X-100. To remove bacterial chaperones bound to the recombinant proteins, the recombinant NSD2-SET-bound chitin beads were washed with 10-bed volumes of buffer A containing 10 mM adenosine triphosphate (ATP) and 2.5 mM $MgCl_2$. Following the removal of bacterial chaperones, the affinity column was washed with 20-bed volumes of buffer A. NSD2-SET proteins was cleaved off from the chitin beads by

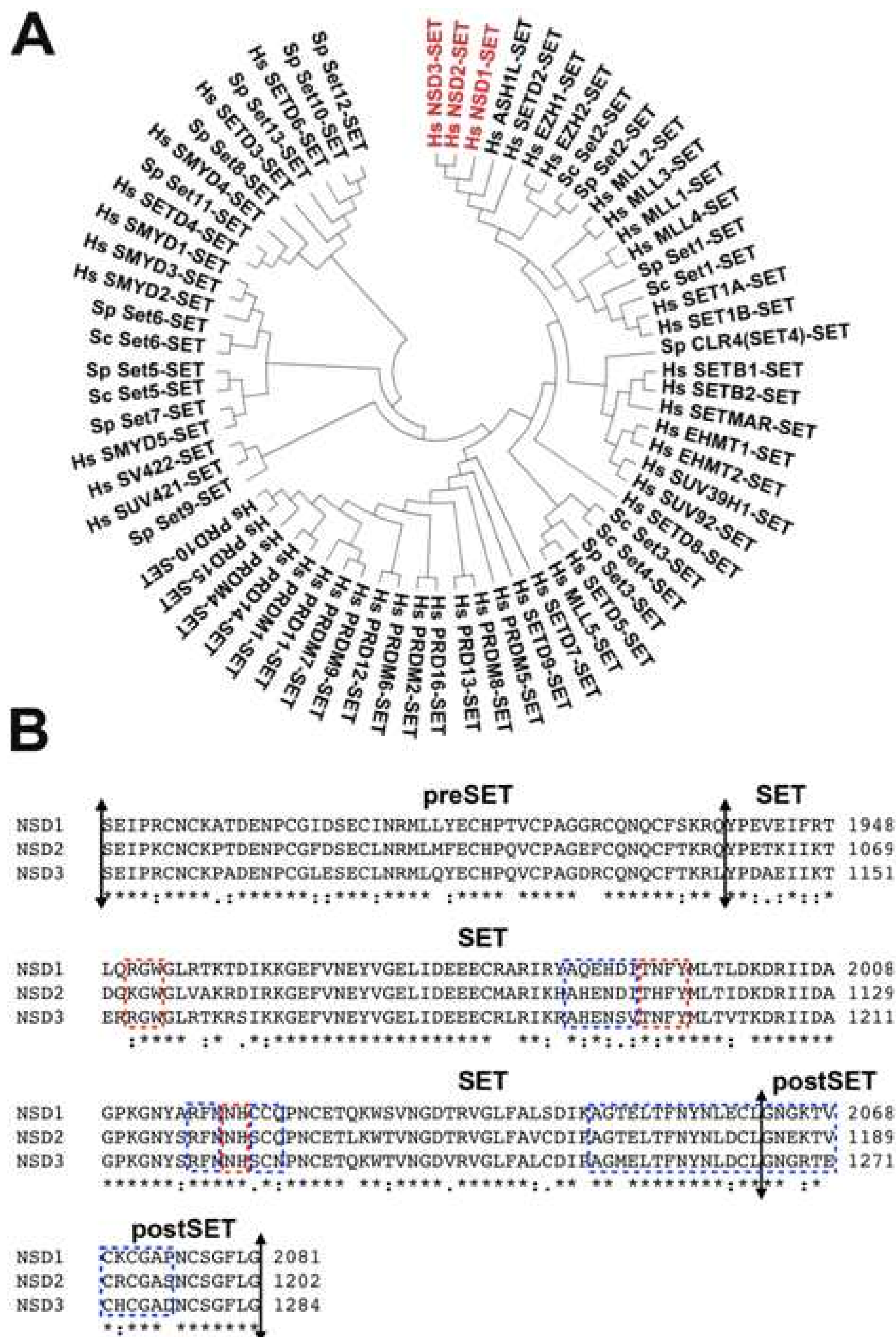

Figure 2. Sequence relationship amongst HMTases and between NSDs for the SET domain.

(A)Unrooted phylogenic tree of the protein sequences for the SET domains of human and yeast HMTases. The NSD family is distinct compared to other known HMTases (in red color). The tree was constructed using the neighbor-joining (NJ) method after a multiple sequence alignment with CLUSTAL 2.1. Hs: *Homo sapiens* Sc: *Saccharomyces cerevisiae* Sp: *Saccharomyces pombe*

(B)Sequences alignment of the preSET, SET and postSET subdomains of NSD1, NSD2 and NSD3. Boxed in blue are the regions involved in histone-tails binding. Boxed in red are the regions responsible for theS-Adenosylmethionine. The multiple sequence alignment was done with CLUSTAL 2.1

incubation in buffer A with 50 mM 2-mercaptoethanol at 4 ℃ for 48 h, eluted in buffer A, concentrated using Amicon Ultra centrifugal filters and then used for methyltransferase assays. A small portion of purified NSD2-SET was resolved on a SDS-PAGE. Coomassie staining gel showed soluble and pure NSD2-SET at around expected molecular weight of 33.2 KDa (Figure 1).

Histone Methyltransferase Assay

Histone Methyltransferase activities of NSD2-SET on H3K36 and H4K20 were measured by colorimetric quantification kits (Epigentek, USA) following the manufacturer's protocol. Briefly, purified recombinant NSDs-CTD (0.2 µg and 2.0 µg) were incubated with a recombinant histone H3 or H4 (Epigentek, USA) and a methyl group donor (Adomet) in the strip wells for 60-90 min at room temperature or 37℃. The specific antibodies attached to the bottom of the strip wells captured methylated substrates. Excess of purified NSDs-CTD, histones, and Adomet was thoroughly washed away and the strip wells attached with antibody-H3/H4me were incubated with the labelled detection antibodies for 60 min at 24℃, swirling at 100 rpm in the dark. The colour development step was conducted after thoroughly washing the wells. The strip wells were analyzed with an ELISA plate reader at 450 nm. The level of methylation is proportional to the intensity of the absorbance. Assays were done in duplicate individual experiments. The results were normalized against the control that does not contain any enzymes (NC in Figure 3).

Results and discussion

Histone methyltransferases are emerging as valuable drug targets for numerous types of cancers where HMTase levels are increased (Morishita and di Luccio, 2011a, b). NSD2/MMSET is key molecular target against multiple myeloma that remains an incurable malignancy in mature (Asangani et al. 2012; Ezponda et al. 2012; Hudlebusch et al. 2011; Morishita and di Luccio 2011a, b; Yang et al. 2012b). However, HMTase inhibitors is limited and only one NSD2 inhibitor, MCTP39, has been advertised (Arul M. Chinnaiyan 2011; Morishita and di Luccio 2011a, b; Upadhyay et al. 2012; Yao et al. 2011; Yuan et al. 2012). The lack of HMTase inhibitors and especially NSD2 inhibitors is primarily caused by the lack structural

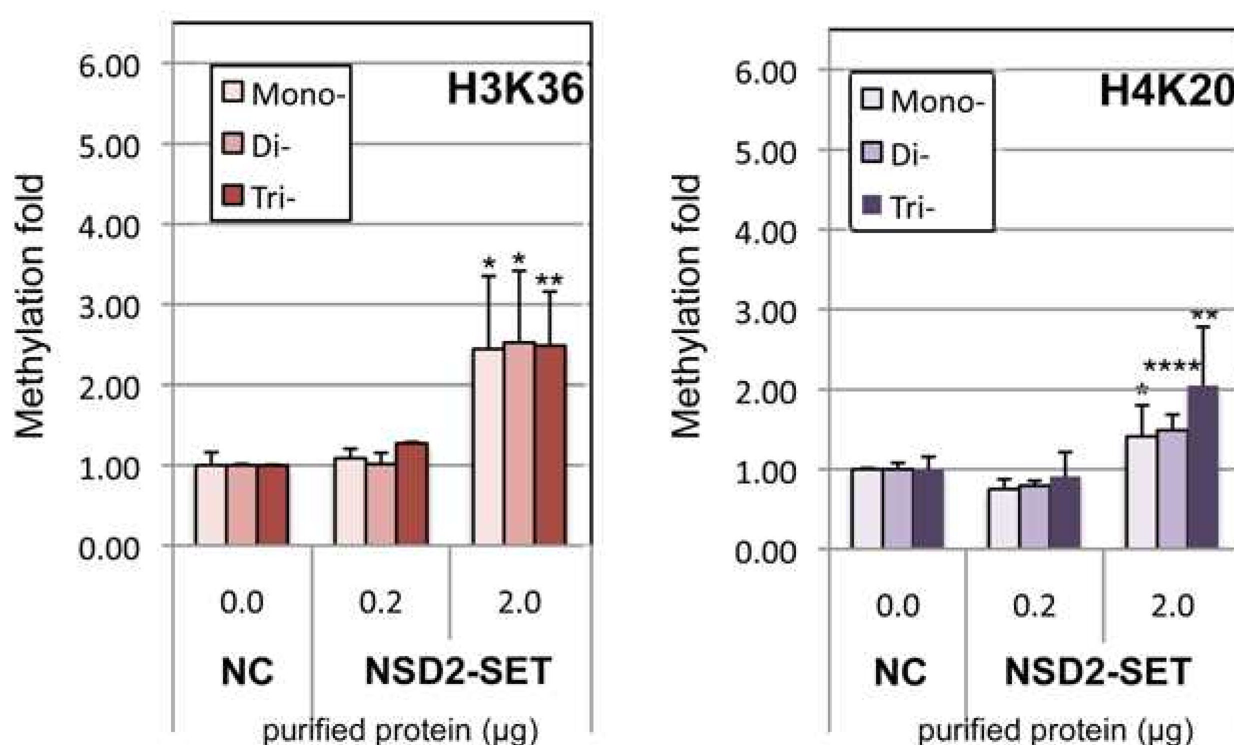

Figure 3. ***in vitro*** **HMTase activities of NSD2-SET on H3 and H4 substrates**

HMTase activities of NSD2-SET on H3K36 abd H4K20 were measured by colorimetric quantification (See Materials and Methods). HMTase assays were done in duplicate individual experiments and all samples were in duplicate. The results were normalized against controls that do not contain any enzymes (0 g) (NC). Asterisks indicate P-values; *p<0.05, **p<0.02, ***p<0.005, ****p<0.001

knowledge on structure-function relationship of HMTase and NSD2 more specifically.

The structure of NSD2 is unknown and the closest crystal structure solved is the apo SET domain of NSD1. Following our previous study on NSD1-SET, we build by homology modelling, a model of NSD2-SET exploiting the crystal structure of NSD1-SET as both share 75.9% sequence identity and 90.1% similarity (Morishita and di Luccio 2011b) (Qiao et al. 2011) (Figure 1). The quality and accuracy of the set of models were assessed by the H-factor, a novel quality metric for homology modelling we recently introduced (di Luccio and Koehl 2011, 2012). Due to the high sequence conservation of the SET domain between NSD1 and NSD2, the modelling of NSD2-SET represents an ideal case.

Previously, we studied the movement and role of the regulatory loop located at the interface between the SET and postSET domain of NSD1-SET (Figure 1) (Morishita and di Luccio, 2011b). In a closed conformation, the binding of H3 or H4 tails is sterically prevented. However, this is not observed in H3K9 and H3K4 family of HMTases (Southall et al. 2009; Wu et al. 2010) (Qiao et al. 2011). We previously identified that the regulatory loop of NSD1-SET underwent significant displacements, with a rotation ~45° and a translation ~6Å at the tip, that open a binding groove largely negatively charged suitable for the docking of H3 or H4 tails (Figure 1) (Morishita and di Luccio 2011b). Since both NSD1- and NSD2-SET are highly related, therefore, it is likely that the opening of NSD2-SET proceeds the same way as for NSD1-SET. In addition, as we described for NSD1-SET, NSD2-SET may have recognition sequence covering at least 7 amino-acids in par with the H4K20 HMTase SET8 (Kudithipudi et al. 2012).

In this study, as a first step before entering a pipeline for protein x-ray crystallography, we cloned, recombinantly expressed and purified the catalytic SET domain of NSD2 (Figure 1). We cloned the SET domain of human NSD2 into an *Escherichia coli* expression vector from the pTYB family where the insert is fused next to an affinity tag, the intein protein. The pTYB family of vectors contains an IPTG-inducible T7 promoter and the protein expression was induced at 15℃ to increase protein solubility and reduce inclusions body. Following a series of test-expression, inducing the protein expression at 15℃ for 4 h was found to be optimum for NSD2-SET. Residual protein chaperones from the *Escherichia coli* expression strain BL21 strain was found around 72Kda following affinity chromatography intein-chitin system. Next, the removal of the chaperones was performed by incubating NSD2-SET-intein on the chitin column, with a solution of ATP- $MgCl_2$. The ATP- $MgCl_2$ induced the release of the ATP-dependent *E. coli* chaperones that were further eluded on the intein-chitin affinity chromatography. In the NEB-intein-chitin system, the intein tag is fused to NSD2 through a cysteine residue. β-mercaptoethanol is added to induce the specific self-cleavage of the intein, which releases the target protein from the chitin-bound intein tag. The resulting recombinant NSD2-SET was concentrated to 10 mg/ml using Amicon Ultra centrifugal filters and then used for methyltransferase assays. Once resolved on an SDS gel, the recombinant NSD2-SET is 95-98% pure. The production yield for NSD2-SET is 0.17 mg of protein per liter of BL21 *E. coli* culture. Noteworthy, the current market value for recombinant NSD2-SET (Reaction Biology Corp. USA) is $550 for 50 μg of similar purity.

Next, we assayed *in vitro* NSD2-SET against human H3K36 and H4K20 substrates (Figure 3) as some HMTases such as SET9 do not retain significant methyltransferase activity *in vitro* (Wang et al. 2009). We quantified *in vitro* the mono (me1), di (me2) and tri (me3) methylation against the biological histone targets of NSD2, H3H36 and H4K20 (Figure 3). Using histone as substrate, recombinant NSD2-SET showed significant HMTase activities, in par with the catalytic efficiencies described *in vivo* (Li et al. 2009). In our hands, recombinant NSD2-SET is proven to be stable and retained catalytic properties for an extended period of time when stored at -80℃. In this study, we achieved the recombinant protein expression of NSD2-SET with a production yield and purity compatible with protein crystallization for further entering a pipeline for protein x-ray crystallography.

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References

Allen SH, Sevin T, Timothy AC (2013) Epigenetic therapy: use of agents targeting deacetylation and methylation in cancer management. OncoTargets and therapy 6.

Angrand PO, Apiou F, Stewart AF, Dutrillaux B, Losson R, Chambon P (2001) NSD3, a new SET domain-containing gene, maps to 8p12 and is amplified in human breast cancer cell lines. *Genomics* 74: 79-88.

Arul M. Chinnaiyan SL, Qi Cao, Irfan Asangani (2011) Compositions and methods for inhibiting mmset, uspto, Ed.

(USA: The Regents of the University of Michigan, Ann Arbor, Mi (US)), pp. 51.

Asangani IA, Ateeq B, Cao Q, Dodson L, Pandhi M, Kunju LP, Mehra R, Lonigro RJ, Siddiqui J, Palanisamy N(2012) Characterization of the EZH2-MMSET Histone Methyltransferase Regulatory Axis in Cancer. *Mol Cell* 49: 80-93.

Basavapathruni A, Jin L, Daigle SR, Majer CR, Therkelsen CA, Wigle TJ, Kuntz KW, Chesworth R, Pollock RM, Scott MP (2012) Conformational adaptation drives potent, selective and durable inhibition of the human protein methyltransferase DOT1L. *Chemical biology & drug design* 80: 971-980.

Brito JL,Walker B, Jenner M, Dickens NJ, Brown NJ, Ross FM, Avramidou A, Irving JA, Gonzalez D, Davies FE. (2009) MMSET deregulation affects cell cycle progression and adhesion regulons in t(4;14) myeloma plasma cells. *Haematologica* 94:78-86.

Brueckner B, Kuck D, Lyko F. (2007) DNA Methyltransferase Inhibitors for Cancer Therapy. *The Cancer J* 13:17-22.

Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. (1998) The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood* 92: 3025-3034.

Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, Allain CJ, Klaus CR, Raimondi A, Scott MP.(2013) Potent inhibition of DOT1L as treatment for MLL-fusion leukemia. *Blood* 122: 1017-1025.

di Luccio E, Koehl P. (2011) A quality metric for homology modeling: the H-factor. *BMC Bioinformatics* 12: 48.

di Luccio E, Koehl P. (2012) The H-factor as a novel quality metric for homology modeling. *J Clin Bioinforma* 2: 18.

Ezponda T, Popovic R, Shah MY, Martinez-Garcia E, Zheng Y, Min DJ, Will C, Neri A, Kelleher NL, Yu J.(2012) The histone methyltransferase MMSET/WHSC1 activates TWIST1 to promote an epithelial-mesenchymal transition and invasive properties of prostate cancer. *Oncogene.*

He C, Li F, Zhang J, Wu J, Shi Y. (2013) The methyltransferase NSD3 has chromatin-binding motifs, PHD5-C5HCH, that are distinct from other NSD (nuclear receptor SET domain) family members in their histone H3 recognition. *J Biol Chem* 288: 4692-4703.

Hudlebusch HR, Santoni-Rugiu E, Simon R, Ralfkiaer E, Rossing HH, Johansen JV, Jorgensen M, Sauter G, Helin K. (2011) The histone methyltransferase and putative oncoprotein MMSET is overexpressed in a large variety of human tumors. *Clin Cancer Res* 17: 2919-2933.

Kassambara A, Klein B, Moreaux J. (2009) MMSET is overexpressed in cancers: Link with tumor aggressiveness. *Biochem Biophysical Res Comm* 379: 840-845.

Kim JY, Kee HJ, Choe NW, Kim SM, Eom GH, Baek HJ, Kook H, Seo SB. (2008) Multiple-myeloma-related WHSC1/MMSET isoform RE-IIBP is a histone methyltransferase with transcriptional repression activity. *Mol Cell Biol* 28: 2023-2034.

Klein B, Kassambara A, Moreaux J. (2009) MMSET is overexpressed in cancers: Link with tumor aggressiveness. *Biochem Biophysical Res Comm* 379: 840-845.

Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, Porter Scott M, Chesworth R, Moyer MP, Copeland RA.(2013) Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc Natl Acad Sci* 110: 7922-7927.

Kristensen LS, Nielsen HM, Hansen LL. (2009) Epigenetics and cancer treatment. *Eur JPharm* 625: 131-142.

Kudithipudi S, Dhayalan A, Kebede AF, Jeltsch A. (2012). The SET8 H4K20 protein lysine methyltransferase has a long recognition sequence covering seven amino acid residues. *Biochimie* 94: 2212-2218.

Lane AA, Chabner BA. (2009) Histone Deacetylase Inhibitors in Cancer Therapy. *J Clinical Oncology* 27:5459-5468.

Li Y, Trojer P, Xu CF, Cheung P, Kuo A, Drury WJ, Qiao Q, Neubert TA, Xu RM, Gozani O. (2009) The target of the NSD family of histone lysine methyltransferases depends on the nature of the substrate. *J Biol Chem* 284: 34283-34295.

Liu F, Chen X, Allali-Hassani A, Quinn AM, Wasney GA, Dong A, Barsyte D, Kozieradzki I, Senisterra G, Chau I. (2009) Discovery of a 2,4-diamino-7-aminoalkoxyquinazoline as a potent and selective inhibitor of histone lysine methyltransferase G9a. *J Med Chem* 52: 7950-7953.

Lucio-Eterovic AK, Singh MM, Gardner JE, Veerappan CS, Rice JC, Carpenter PB (2010) Role for the nuclear receptor-binding SET domain protein 1 (NSD1) methyltransferase in coordinating lysine 36 methylation at histone 3 with RNA polymerase II function. *Proc Natl Acad Sci USA* 107: 16952-16957.

Mack GS. (2010) To selectivity and beyond. *Nature biotechnology* 28:1259-1266.

Malmquist NA, Moss TA, Mecheri S, Scherf A, Fuchter MJ. (2012) Small-molecule histone methyltransferase inhibitors display rapid antimalarial activity against all blood stage

forms in Plasmodium falciparum. *Proc Natl Acad Sci USA* 109 16708-16713.

Marango J, Shimoyama M, Nishio H, Meyer JA, Min DJ, SirulnikA, Martinez-Martinez Y, Chesi M, Bergsagel PL, Zhou MM, (2008) The MMSET protein is a histone methyltransferase with characteristics of a transcriptional corepressor. *Blood* 111, 3145-3154.

Martinez-Garcia E, Popovic R, Min DJ, Sweet SM, Thomas PM, Zamdborg L, Heffner A, Will C, Lamy L, Staudt LM. (2011) The MMSET histone methyl transferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells. *Blood* 117, 211-220.

Min J, Feng Q, Li Z, Zhang Y, Xu RM. (2003). Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* 112: 711-723.

Mirabella F, Wu P, Wardell CP, Kaiser MF, Walker BA, Johnson DC, Morgan GJ. (2013) MMSET is the key molecular target in t(4;14) myeloma. *Blood cancer J* 3:e114.

Morishita M, di Luccio E. (2011a) Cancers and the NSD family of histone lysine methyltransferases. *Biochim Biophys Acta* 1816: 158-163.

Morishita M, di Luccio E. (2011b) Structural insights into the regulation and the recognition of histone marks by the SET domain of NSD1. *Biochem Biophysical Res Comm* 412: 214-219.

Pei H, Zhang L, Luo K, Qin Y, Chesi M, Fei F, Bergsagel PL, Wang L, You Z, Lou Z. (2011). MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. *Nature* 470, 124-128.

Qiao Q, Li Y, Chen Z, Wang, M, Reinberg D, Xu RM. (2011) The structure of NSD1 reveals an autoregulatory mechanism underlying histone H3K36 methylation. *J Biol Chem* 286: 8361-8368.

Rosati R, La Starza R, Veronese A, Aventin A, Schwienbacher C, Vallespi T, Negrini M, Martelli MF, Mecucci, C. (2002) NUP98 is fused to the NSD3 gene in acute myeloid leukemia associated with t(8;11)(p11.2;p15). *Blood* 99: 3857-3860.

Southall SM, Wong PS, Odho Z, Roe SM, Wilson JR. (2009) Structural basis for the requirement of additional factors for MLL1 SET domain activity and recognition of epigenetic marks. *Mol Cell* 33:181-191.

Spannhoff A, Hauser AT, Heinke R, Sippl W, Jung M. (2009a) The Emerging Therapeutic Potential of Histone Methyltransferase and Demethylase Inhibitors. *ChemMedChem* 4, 1568-1582.

Spannhoff A, Hauser AT, Heinke R, Sippl W, Jung M. (2009b) The emerging therapeutic potential of histone methyltransferase and demethylase inhibitors. *ChemMedChem* 4: 1568-1582.

Spannhoff A, Sippl W, Jung M (2009c) Cancer treatment of the future: Inhibitors of histone methyltransferases. *The Inter J Biochem Cell Biol* 41: 4-11.

Strahl BD, Allis CD. (2000) The language of covalent histone modifications. *Nature* 403:41-45.

Upadhyay AK, Rotili D, Han JW, Hu R, ChangY, Labella D, Zhang X, Yoon YS, Mai A, Cheng, X. (2012) An analog of BIX-01294 selectively inhibits a family of histone H3 lysine 9 Jumonji demethylases. *J Mol Biol* 416, 319-327.

Verma SK, Tian X, LaFrance LV, Duquenne C, Suarez DP, Newlander KA, Romeril SP, Burgess JL, Grant SW, Brackley JA.(2012). Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. ACS *Medicinal Chemistry Letters* 3: 1091-1096.

Wagner EJ, Carpenter PB. (2012) Understanding the language of Lys36 methylation at histone H3. *Nature Reviews Molecular Cell Biology* 13:115-126.

Wang Y, Reddy B, Thompson J, Wang H, Noma K, Yates, JR, 3rd, Jia S. (2009) Regulation of Set9-mediated H4K20 methylation by a PWWP domain protein. *Mol Cell* 33: 428-437.

Wu H, Min J, Lunin VV, Antoshenko T, Dombrovski L, Zeng H, Allali-Hassani A, Campagna-Slater V, Vedadi M, Arrowsmith CH, (2010). Structural biology of human H3K9 methyltransferases. *PLoS One* 5, e8570.

Yang P, Guo L, Duan ZJ,Tepper CG, Xue L, Chen X, Kung HJ, Gao A, Zou JX, Chen HW. (2012a) Histone methyltransferase NSD2/MMSET mediates constitutive NF-kappaB signaling for cancer cell proliferation, survival, and tumor growth via a feed-forward loop. *Mol Cell Biol.*

Yang P, GuoL, Duan ZJ, Tepper CG, Xue L, Chen X, Kung HJ, Gao AC, Zou JX, Chen HW (2012b) Histone methyltransferase NSD2/MMSET mediates constitutive NF-kappaB signaling for cancer cell proliferation, survival, and tumor growth via a feed-forward loop. *Mol Cell Biol* 32: 3121-3131.

Yao Y, Chen P, Diao J, Cheng G, Deng L, Anglin JL, Prasad BV, Song Y. (2011) Selective inhibitors of histone methyltransferase DOT1L: design, synthesis, and crystallographic studies. *J Am Chem Soc* 133:16746-16749.

Yuan Y, Wang Q, Paulk J, Kubicek S, Kemp MM, Adams DJ, Shamji AF, Wagner BK, and Schreiber SL.(2012) A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. *ACS Chem Biol* 7: 1152-1157.