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Original Article

Methylation of the JMJD2B epigenetic regulator differentially affects its ability to coactivate the ETV1 and JUN transcription factors

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Abstract: Objectives: Jumonji C domain-containing (JMJD) 2B (JMJD2B) is a transcriptional cofactor and histone demethylase that is involved in prostate cancer formation. However, how its function is regulated by posttranslational modification has remained elusive. Hence, we examined if JMJD2B would be regulated by lysine methylation. Methods: Through in vitro methylation assays and Western blotting with methyl-lysine specific antibodies, we analyzed lysine methylation within JMJD2B. Identified methylated lysine residues were mutated to arginine residues and the respective impact on JMJD2B transcriptional activity measured with a reporter gene assay in human LNCaP prostate cancer cells. Results: We discovered that JMJD2B is methylated on up to six different lysine residues. Further, we identified the suppressor of variegation 3-9/enhancer of zeste/trithorax (SET) domain-containing protein 7/9 (SET7/9) as the methyltransferase being responsible for this posttranslational modification. Mutating the methylation sites in JMJD2B to arginine residues led to diminished coactivation of the Ju-nana (JUN) transcription factor, which is a known oncogenic protein in prostate tumors. In contrast, methylation of JMJD2B had no impact on its ability to coactivate another transcription factor associated with prostate cancer, the DNA-binding protein E26 transformation-specific (ETS) variant 1 (ETV1). Consistent with a potential joint action of JMJD2B, SET7/9 and JUN in prostate cancer, the expression of JMJD2B in human prostate tumors was positively correlated with both SET7/9 and JUN levels. Conclusions: The identified SET7/9-mediated methylation of JMJD2B appears to impact its cooperation with selected interacting transcription factors in prostate cancer cells. Given the implicated roles of JMJD2B beyond prostate tumorigenesis, SET7/9-mediated methylation of JMJD2B possibly also influences the development of other cancers, while its impairment might have relevance for obesity or a global developmental delay that can be elicited by reduced JMJD2B activity.

Keywords: Gene transcription, lysine demethylase 4B (KDM4B), posttranslational modification, prostate cancer, SET domain-containing 7 (SETD7)

Introduction

Jumonji C domain-containing (JMJD) 2B (JMJD2B), also known as lysine demethylase 4B (KDM4B), is an enzyme that targets lysine 9 and lysine 36 on histone H3 as well as lysine 26 on histone H1.4. It can demethylate H3K9me$_2$, H3K36me$_3$, H3K36me$_2$ and H1.4K26me$_3$, but most efficiently removes one methyl group from H3K9me$_3$ [1-4]. Given that H3K9me$_3$ is an eminent epigenetic mark for transcriptionally repressed genes [5], JMJD2B's enzymatic activity is predicted to activate gene promoters.

JMJD2B performs pleiotropic functions and is a potential oncoprotein in various cancers [6-10]. This particularly pertains to prostate cancer, where JMJD2B is overexpressed and its downregulation compromised cell proliferation and sensitized to anti-androgen therapy. Mechanistically, this included the binding of JMJD2B to the androgen receptor, the myelocytomatosis (MYC) oncoprotein or the E26 transformation-specific (ETS) variant 1 (ETV1), thereby stimulating the ability of these DNA-binding proteins to upregulate gene transcription [11-19].

The transcription factor ETV1 can become overexpressed in prostate cancer especially when chromosomal translocations fuse its gene to androgen-inducible regulatory sequences [20-24], yet ETV1 overexpression may also be brought about by fusion-independent mechanisms [25]. Upon ETV1 overexpression in mouse prostates, development of prostatic intraepithelial neoplasia ensued [26-29], indicating that ETV1 overexpression is causally related to prostate cancer initiation. Moreover, ETV1 and JMJD2A, a close homolog of JMJD2B, synergized in promoting prostate tumorigenesis in a genetically engineered mouse model, and ETV1 cooperated with either JMJD2A or JMJD2B in stimulating gene transcription, including of the Yes-associated 1 (*YAP1*) proto-oncogene [14].

In this report, we explored how JMJD2B might be regulated in its activity through posttranslational modification. We discovered that JMJD2B can be methylated by the suppressor of variegation 3-9/enhancer of zeste/trithorax (SET) domain-containing protein 7/9 (SET7/9; also called SET domain-containing 7 (SETD7)), an enzyme that is reportedly overexpressed in human prostate tumors and capable of stimulating prostate cancer cell proliferation [30]. Our examinations suggest that methylation of JMJD2B by SET7/9 can affect its coactivation potential, providing a conceivable mechanism by which this posttranslational modification may promote prostate tumorigenesis.

Materials and methods

Cloning of JMJD2B expression vectors

Human *JMJD2B* cDNA was obtained from Origene (Rockville, MD, USA) and corresponded to the transcript variant X1 (GenBank accession number XM_005259521.4; and the cDNA deposited under GenBank BC144292.1), whose translation product contains 34 additional amino acids (position 373-406) compared to the translation product of the NCBI *JMJD2B* reference sequence NM_015015.3. To express N-terminally Flag-tagged JMJD2B in human cells, its open reading frame was cloned into pEV3S-Flag [31]. To express portions of JMJD2B as fusions with GST in bacteria, fragments of the JMJD2B open reading frame were cloned into a derivative of pGEX-2T [32]. All recombinant plasmids were validated by Sanger DNA sequencing [33]. This was done at the Oklahoma Medical Research Foundation DNA Sequencing Core (Oklahoma City, OK, USA) with sequencing primers specific to pEV3S-Flag (5'-GGGGGATCTTGGTGGCGTG-3') or pGEX-2T (5'-CCAAAATCGGATCTGG-3').

Immunoprecipitation and Western blotting

Human embryonic kidney 293T cells (CRL-3216; American Type Culture Collection, Manassas, VA, USA) were transiently transfected by the calcium phosphate coprecipitation method [34]. Cells were lysed 36 h after transfection as described [35] and immunoprecipitations performed with indicated antibodies [36]. Then, proteins were subjected to SDS polyacrylamide gel electrophoresis [37] and separated proteins blotted onto polyvinylidene difluoride membrane [38]. After incubation with primary and horseradish peroxidase-coupled secondary antibodies [39, 40], detection of proteins was done with enhanced chemiluminescence and exposure to film [41]. The following antibodies were used at a 1:1000 dilution: rabbit polyclonal anti-p53-K372me (ab16033; Abcam, Cambridge, United Kingdom), rabbit polyclonal anti-JMJD2B (TA306832; Origene, Rockville, MD, USA), rabbit polyclonal anti-SET7/9 (07-314; Upstate, Boston, MA, USA), mouse monoclonal anti-Flag (F3165; Sigma, St. Louis, MO, USA), and rabbit polyclonal anti-Actin (A2066; Sigma, St. Louis, MO, USA).

Production of glutathione S-transferase (GST) fusion proteins

GST-JMJD2B and GST-SET7/9 expression plasmids were transformed into *Escherichia coli* BL21-CodonPlus bacteria (Stratagene, San Diego, CA, USA) [42]. These bacteria were grown in Luria broth media at 37°C to an optical density of 0.2-0.6 (measured at 595 nm) and then induced with 0.05-0.1 mM isopropyl β-*D*-thiogalactopyranoside and further grown for 8 h at 18°C. Purification of GST fusion proteins was then done utilizing glutathione agarose as described before [43, 44]. These affinity purified proteins were frozen in liquid nitrogen and stored until usage at -80°C.

Methylation assays

In a buffer consisting of 50 mM Tris-HCl pH 8.5, 5 mM $MgCl_2$, 4 mM dithiothreitol, 1 μM

S-[methyl-^{3}H] adenosyl-*L*-methionine (^{3}H-SAM), approximately 2 μg of the GST-JMJD2B fusion proteins were incubated with GST-SET7/9 for 2 h at 30°C. Then, an equal volume of 2× Laemmli sample buffer was added and the samples incubated for 10 min at 95°C [45]. Denatured proteins were then separated via SDS polyacrylamide gel electrophoresis [46], which was followed by blotting onto polyvinylidene difluoride membrane and Ponceau S protein staining [47]. The dried polyvinylidene difluoride membranes were treated with EN^{3}HANCE (Perkin Elmer, Waltham, MA, USA) four times and then exposed to film without intensifying screen at -80°C [48].

Luciferase assays

Human LNCaP prostate cancer cells (CRL-1740; American Type Culture Collection, Manassas, VA, USA) were seeded in poly-*L*-lysine coated 12-wells and grown in a humidified atmosphere with 5% CO_2 to a confluency of approximately 25% [49, 50]. The growth media consisted of Dulbecco's modified Eagle medium (MT-10013CV; Corning, Somerville, MA, USA) supplemented with 10% fetal bovine serum [51]. Cells were transfected employing 4 μg polyethylenimine [52] and the following amounts of DNA: 500 ng TORU luciferase reporter gene construct, 500 ng pBluescript KS$^+$, indicated amounts of empty vector pEV3S or ETV1 or JUN expression vector, and indicated amounts of pEV3S or Flag-tagged JMJD2B expression vectors [53]. After 8-10 h, cells were washed once with 1 ml phosphate-buffered saline [54] and incubated in growth media for another ~40 h [55]. Then, cells were washed once again with 1 ml phosphate-buffered saline and lysed in 250 μl of 25 mM Tris-HCl pH 7.8, 10% glycerol, 2 mM EDTA, 1% Triton X-100, 2 mM dithiothreitol [56]. After removal of debris [57], luciferase activities were determined with the help of a luminometer (LB 9507; Berthold, Bad Wildbad, Germany) as described [58, 59].

Statistical analysis

Prism 6 for Mac OS X (GraphPad Software, Boston, MA, USA) was used to determine statistical significance of experimental data. Averages (n=4) with standard deviations are displayed in case of luciferase activities, and the statistical significance was assessed through one-way analysis of variance (ANOVA) with post hoc Dunnett's multiple comparison test. No data were excluded as outliers from the analysis. For the analysis of coexpression, the Spearman correlation coefficient was calculated. Statistical significance was assumed if $P<0.05$.

Results

SET7/9 methylates JMJD2B

In our quest to identify potential sites of post-translational modification in JMJD2B, we noted that some of its lysine residues conform to a reported SET7/9 consensus methylation site, (K/R)(S/T/A)K [60]. One of the known targets of SET7/9 is the tumor suppressor p53, whose methylation on lysine 372 can be detected with respective p53-K372me antibodies [61]. Hence, we speculated that such an antibody may also recognize a methylated lysine residue(s) in JMJD2B. Indeed, when JMJD2B was coexpressed with SET7/9, we observed that a p53-K372me antibody recognized immunoprecipitated JMJD2B (**Figure 1A**). No methylation was observed in the absence of coexpressed SET7/9 or in the presence of the catalytically inactive H297A mutant of SET7/9 (**Figure 1A**). Further, we found that endogenous JMJD2B was also methylated in 293T cells (**Figure 1B**).

To identify which region(s) in JMJD2B could be methylated by SET7/9, we divided JMJD2B into four fragments and purified respective GST fusion proteins. These fragments were employed in an in vitro methylation experiment, where radioactively labeled ^{3}H-SAM served as a methyl donor and purified GST-SET7/9 as the methyltransferase. We observed strong methylation of JMJD2B amino acids 352-580, but none with GST alone (**Figure 1C**). Also, JMJD2B amino acids 2-351 and 741-1130 did not serve as SET7/9 substrates, but there was a marginal methylation observable with JMJD2B amino acids 581-740. From these data, we conclude that substantial SET7/9-mediated methylation only occurs within JMJD2B amino acids 352-580. Notably, these amino acids are not part of any of the known functional domains of JMJD2B (see **Figure 1C**, top).

Identification of methylation sites within JMJD2B

To identify methylation sites within JMJD2B amino acids 352-580, we subdivided these

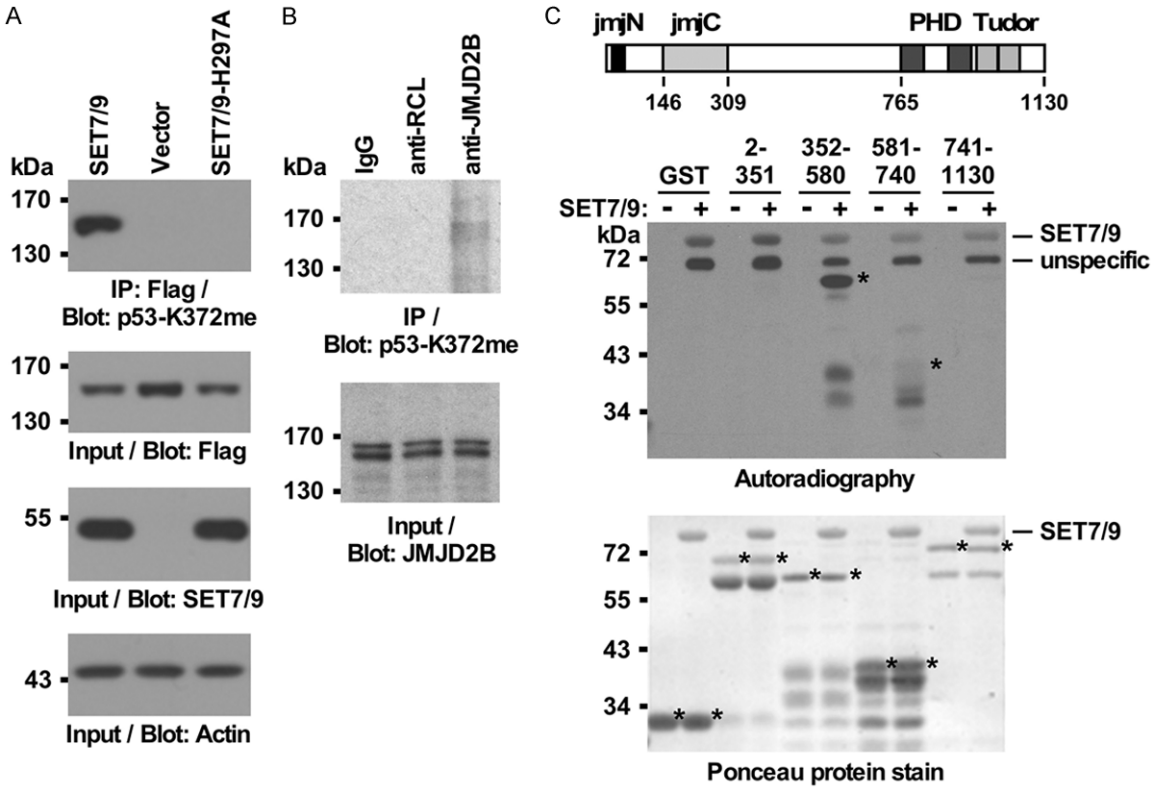

Figure 1. SET7/9 methylates JMJD2B. A. Immunoprecipitation (IP) of Flag-tagged JMJD2B with anti-Flag antibodies from 293T cells upon coexpression with wild-type SET7/9 or its catalytically inactive H297A mutant. Methylation was assessed with p53-K372me antibodies (top), while the bottom three panels show Western blots with the input levels of Flag-JMJD2B, SET7/9 or actin. B. Immunoprecipitation of endogenous JMJD2B from 293T cells; IgG and anti-RCL antibodies served as negative controls. C. In vitro methylation of JMJD2B by GST-SET7/9 utilizing radioactive ^{3}H-SAM. Either GST or fusions of GST to indicated JMJD2B amino acids (asterisks mark the respective full-length proteins, but smaller polypeptides that were likely due to proteolytic degradation are also visible in the Ponceau stains) were used as substrates. The top shows a sketch of JMJD2B with its four known domains: catalytic jmjC, accessory jmjN, double PHD and double Tudor domains.

amino acids into two parts (amino acids 352-430 and 431-580) and observed that both of these parts were substrates for SET7/9 (**Figure 2A**). Therefore, we constructed even smaller fragments representing JMJD2B amino acids 352-387, 388-430, 431-488 and 486-580 and noted that all of them could be methylated by SET7/9 in vitro (**Figure 2B**), indicating that there are at least four different methylation sites in JMJD2B.

Within JMJD2B amino acids 352-387, there are four lysine residues (see **Figure 2C**), but only the one at position 368 matches the SET7/9 consensus methylation site, (K/R)(S/T/A)K [60]. Hence, we mutated K368 to arginine and this R368 mutation abolished SET7/9-mediated methylation of JMJD2B amino acids 352-387 (**Figure 2C**), identifying K368 as the one and only methylation site within JMJD2B amino acids 352-387. Then, we individually mutated all four lysine residues within JMJD2B amino acids 388-408 and found that only mutation of K397 and K401 resulted into reduced methylation; further, joint mutation of these two lysine residues (R397/401 mutant) completely suppressed SET7/9-mediated methylation (**Figure 2C**), identifying K397 and K401 as further methylation sites in JMJD2B. Next, we focused on amino acids 404-430 that harbor five lysine residues. Yet, mutating K415 alone already abolished SET7/9-mediated methylation (**Figure 2C**), indicating that K415 is the only relevant methylation site within amino acids 404-430. Similarly, K485 that matches the (K/R)(S/T/A)K SET7/9 consensus site is the only substrate for SET7/9 in JMJD2B amino acids 431-488 (**Figure 2C**), indicating that

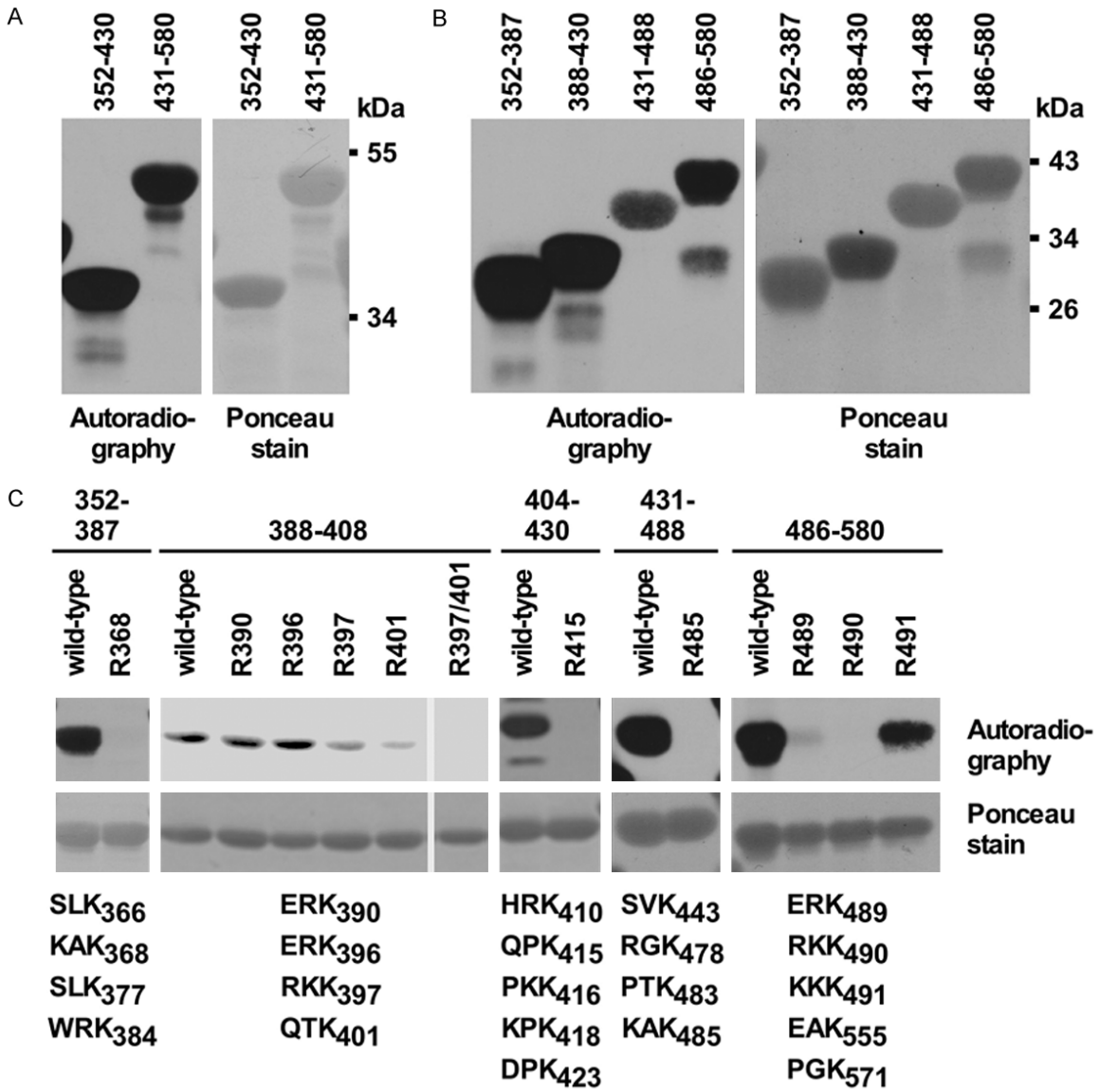

Figure 2. Identification of methylation sites in JMJD2B. A. Indicated JMJD2B amino acids fused to GST were employed as substrates in an in vitro methylation assay with purified SET7/9 and the radioactive methyl donor, ^{3}H-SAM. B. Likewise with smaller JMJD2B fragments. C. Wild-type and indicated point mutants of GST-JMJD2B fusions were subjected to in vitro methylation assays. Below are listed all lysine residues (plus the two preceding amino acids) within the respective JMJD2B fragments. Only K368 and K485 match the reported SET7/9 consensus sequence, (K/R)(S/T/A)K.

K485 is a fifth methylation site in JMJD2B. Lastly, we analyzed JMJD2B amino acids 486-580. Mutation of K490 to arginine completely abolished SET7/9-mediated methylation, while mutation of K489 and K491 led to somewhat reduced methylation (**Figure 2C**). We hypothesize that mutation of the latter two lysine residues alters the affinity of K490 for SET7/9 and thereby indirectly reduced methylation of JMJD2B amino acids 486-580; accordingly, K489 and K491 are not considered SET7/9 methylation sites, while K490 is. Altogether, we have identified six lysine residues (K368, K397, K401, K415, K485 and K490) that can be methylated by SET7/9 in vitro.

We then introduced respective K→R mutations into full-length JMJD2B, expressed these mutants in 293T cells, and purified them by virtue of a N-terminal Flag-tag through immuno-

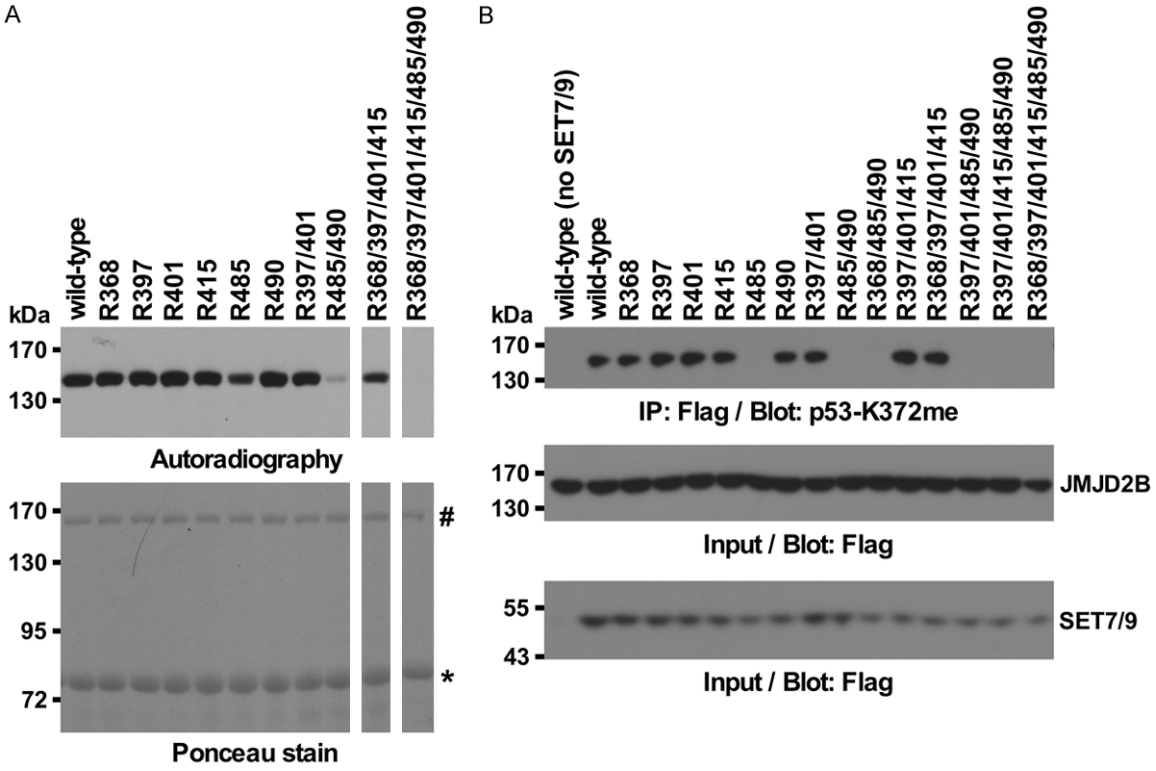

Figure 3. Confirmation of methylation sites in full-length JMJD2B. A. Flag-tagged JMJD2B or indicated mutants thereof (marked by # in Ponceau stain) were immunoprecipitated from 293T cells and then methylated by GST-SET7/9 (marked by * in Ponceau stain) in vitro. B. Likewise, indicated Flag-JMJD2B proteins were coexpressed with Flag-SET7/9 in 293T cells and methylation assessed after anti-Flag immunoprecipitation with p53-K372me antibodies (top). Bottom two panels show input levels for JMJD2B and SET7/9.

precipitation. These proteins were incubated in vitro with SET7/9 and methylation assessed. As shown in **Figure 3A**, mutation of any single lysine residue had no obvious effect except for the R485 mutation. But joint mutation of K485 and K490 greatly reduced in vitro methylation, while the R368/397/401/415 mutant was just slightly affected in its ability to become methylated. Joint mutation of all six lysine residues completely abrogated SET7/9-mediated methylation (**Figure 3A**). Altogether, these data implicate that K485 and K490 are major methylation sites, while K368, K397, K401 and K415 are minor ones. Please note that the fact that the R368/397/401/415/485/490 mutant was no longer methylated strongly suggests that the marginal amount of methylation observed with JMJD2B amino acids 581-740 (see **Figure 1C**) was likely an artefact in this truncation due to non-natural exposure of lysine residue(s) towards SET7/9.

We also expressed the K→R mutants of JMJD2B together with SET7/9 in 293T cells and probed for in vivo methylation with the p53-K372me antibody (**Figure 3B**). Mutation of K485, either alone or in combination with other K→R mutations, led to JMJD2B no longer being recognized by this antibody, indicating that this antibody exclusively recognized JMJD2B when methylated on K485. This substantiated that K485 is an in vivo methylation site for SET7/9. Unfortunately, we were unable to find commercially available anti-methyl-lysine antibodies that would recognize methylation on K368, K397, K401, K415 or K490 in vivo.

JMJD2B methylation modulates its transactivation potential

To test how methylation of JMJD2B would affect its transactivation potential, we utilized a reporter gene construct, in which the firefly luciferase gene is driven by the 12-O-Tetradecanoylphorbol-13-acetate Oncogene Responsive Unit (TORU) derived from the polyomavirus enhancer that can be bound and cooperatively induced by ETS and activator protein-1 (AP1)

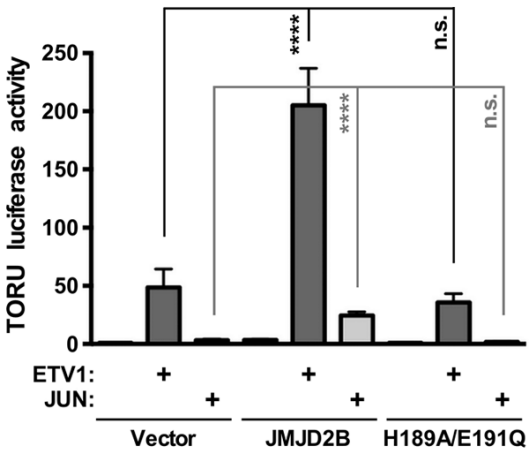

Figure 4. Synergy between JMJD2B and either ETV1 or JUN in human LNCaP prostate cancer cells. 6 ng JMJD2B (wild-type or H189A/E191Q catalytic mutant) expression vector was cotransfected with 15 ng of ETV1 or JUN expression vector. Shown is the activation of the cotransfected TORU luciferase reporter (mean with standard deviation; n=4). ****, P<0.0001; n.s., not significant (one-way ANOVA with Dunnett's multiple comparisons).

proteins [62]. Specifically, the ETS transcription factor ETV1 has previously been shown to activate the TORU-luciferase reporter gene [63-65]. And accordingly, ETV1 stimulated the TORU reporter gene by ~50-fold in human LNCaP prostate cancer cells (**Figure 4**). In contrast, JMJD2B had only a moderate 3-fold effect, probably due to recruitment of JMJD2B to the TORU reporter by endogenous ETS or AP1 proteins. However, when ETV1 and JMJD2B were coexpressed, transcriptional synergy was observed (>200-fold activation). No synergy was observable with a catalytically impaired JMJD2B protein, the H189A/E191Q mutant (**Figure 4**), indicating that catalytic activity of JMJD2B is needed for its ability to coactivate ETV1, similarly as described before [14]. We also tested the ability of the transcription factor JUN, which is one component of AP1 [66, 67] and that is also capable of binding to JMJD2B [68], to cooperate with JMJD2B. Likewise, we found that wild-type JMJD2B, but not its H189A/E191Q catalytic mutant, synergized with JUN in activating the TORU reporter (**Figure 4**). Hence, we have established that the TORU luciferase reporter assay is a suitable means to test the cooperation of JMJD2B with either ETV1 or JUN.

Then, we determined how mutation of its methylation sites would modulate the JMJD2B coactivation potential in LNCaP cells. In the absence of coexpressed ETV1 or JUN, none of the tested methylation site mutants was significantly different from wild-type JMJD2B (**Figure 5**). Likewise, we did not observe any change in synergy with ETV1 upon mutation of JMJD2B methylation sites (**Figure 5**). In contrast, the synergy between JMJD2B and JUN was affected by methylation of JMJD2B. The most severe effects, a downregulation by ~60%, were observed with the R485/490 double mutant and the 6xR mutant, in which all mapped JMJD2B methylation sites were mutated (**Figure 5**); in contrast, joint mutation of the four minor methylation sites (K368, K397, K401 and K415) did not significantly reduce the ability of JMJD2B to coactivate JUN (**Figure 5**). Further, single mutation of K485 or K490 also did not significantly affect the coactivation potential of JMJD2B (not shown). Altogether, these data indicate that SET7/9-mediated methylation of JMJD2B has selective effects: in case of coactivation of ETV1, it appears irrelevant, but in case of modulating JUN, methylation of JMJD2B is required for efficient coactivation.

Discussion

In this report, we have identified a new substrate for the SET7/9 methyltransferase, namely the JMJD2B epigenetic regulator. This further supports the notion that SET7/9 is primarily a non-histone methyltransferase [69-71] rather than - as originally purported - a histone H3 lysine 4 methyltransferase [72, 73]. Two major methylation sites, K485 and K490, were identified in JMJD2B, whose joint mutation reduced the ability of JMJD2B to coactivate JUN, an established oncoprotein [74, 75]. However, mutation of JMJD2B methylation sites had no impact on coactivating the ETV1 transcription factor, indicating that SET7/9-mediated methylation of JMJD2B will only affect a limited cadre of JMJD2B-regulated genes. Given that JMJD2 proteins can not only demethylate lysine residues on histones, but also on non-histone proteins [6, 7, 10], it is conceivable that JMJD2B demethylates repressive methyl marks on histones within nucleosomes as well as on to-be-identified coactivators binding to JUN in order to stimulate JUN-dependent gene transcription (**Figure 6A**). Interestingly, none of the six JMJD2B methylation sites are conserved in its closest homologs, JMJD2A and JMJD2C

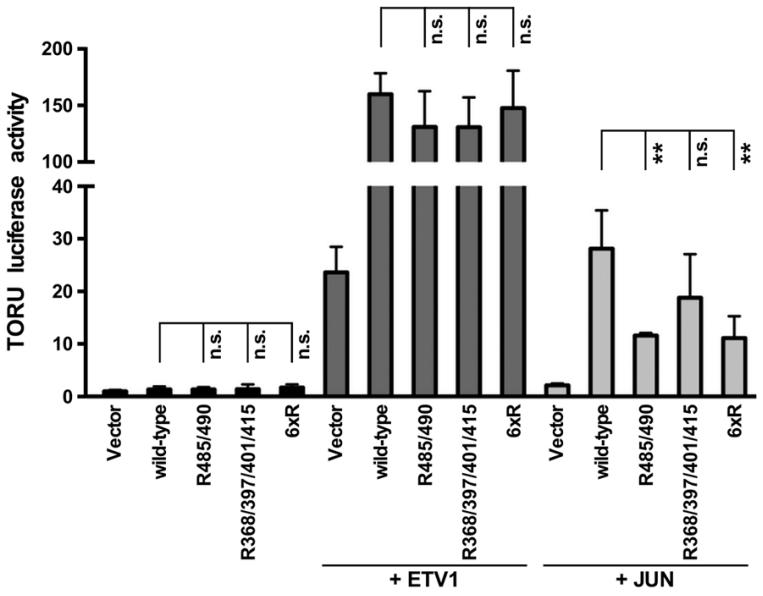

Figure 5. Activity of JMJD2B methylation site mutants. Wild-type or methylation site mutants of JMJD2B (4 ng expression vector) were cotransfected with either ETV1 or JUN (each 10 ng expression vector) into LNCaP cells and resultant TORU luciferase activity measured. Shown are means with standard deviation (n=4). The statistical significance between respective wild-type and methylation site mutants was determined with one-way ANOVA (Dunnett's multiple comparisons). **, P<0.01; n.s., not significant.

(**Figure 6B**), suggesting that this could be one means to differentiate the often overlapping functions of these three JMJD2 proteins [6, 7, 10].

Similar to JMJD2B and SET7/9, JUN is overexpressed in prostate tumors and can promote disease development [76-78]. Hence, these three proteins together may cooperate in the neoplastic transformation of prostate cells. Consistently, these three proteins are likely co-overexpressed in prostate adenocarcinomas, since *JMJD2B* mRNA levels were positively correlated with both *SET7/9* (**Figure 6C**) and *JUN* mRNA levels (**Figure 6D**), the latter possibly reflecting that *JUN* is a known JMJD2B target gene [79]. And JMJD2B's cooperation with SET7/9 and/or JUN may extend to other cancers such as acute myeloid leukemia, clear cell renal carcinoma, breast, gastric, colorectal or liver cancer, where JMJD2B has also been found to be overexpressed [8, 9, 80, 81] and accordingly could be inactivated with small molecule inhibitors targeting its catalytic center [82-84] in order to ameliorate disease severity and outcome. Our data suggest that suppression of SET7/9-mediated methylation of JMJD2B represents another strategy to curtail the oncogenic effects of JMJD2B in prostate and other cancers.

Aside from its role in cancer, JMJD2B performs many functions in normal cells and other diseases. Although two different *Jmjd2b* whole-body knockout mouse models displayed no obvious defects during embryogenesis [85, 86], pathologic changes were observable upon *Jmjd2b* knockout in adult mice. For instance, tissue-specific *Jmjd2b* knockout in mammary glands led to their delayed development [85], while knockout in adipocytes or in the whole body caused metabolic abnormalities and obesity [87, 88] and knockout in neurons led to defective spine maturation, deficits in working memory and hyperactivity in a novel environment [89]. Moreover, mono- and bi-allelic *JMJD2B* pathogenic mutations were found in patients afflicted with global developmental delay or autism, and heterozygous *Jmjd2b*$^{+/-}$ mice showed a reduction in brain volume which may cause the aforementioned phenotypes [90-92]. These latter data indicate that haploinsufficiency of JMJD2B is already enough to elicit serious defects, and accordingly the observed ~60% reduction in coactivation of JUN with the JMJD2B-R485/490 mutant strongly suggests that SET7/9-mediated methylation will profoundly affect JMJD2B-dependent biological processes.

Another role of JMJD2B is in stem cell biology. In part in conjunction with other histone demethylases, JMJD2B was required for embryonic stem cell renewal or reprogramming into pluripotency, but on the other hand also promoted the osteogenic differentiation of mesenchymal stem cells [93-95]. As SET7/9 appears to promote pluripotency in human embryonic stem cells [96], one may speculate whether this also involves SET7/9-mediated methylation of JMJD2B. Lastly, while JMJD2B is a true histone demethylase that acts in the cell nucleus, it has also been found in the cytoplasm

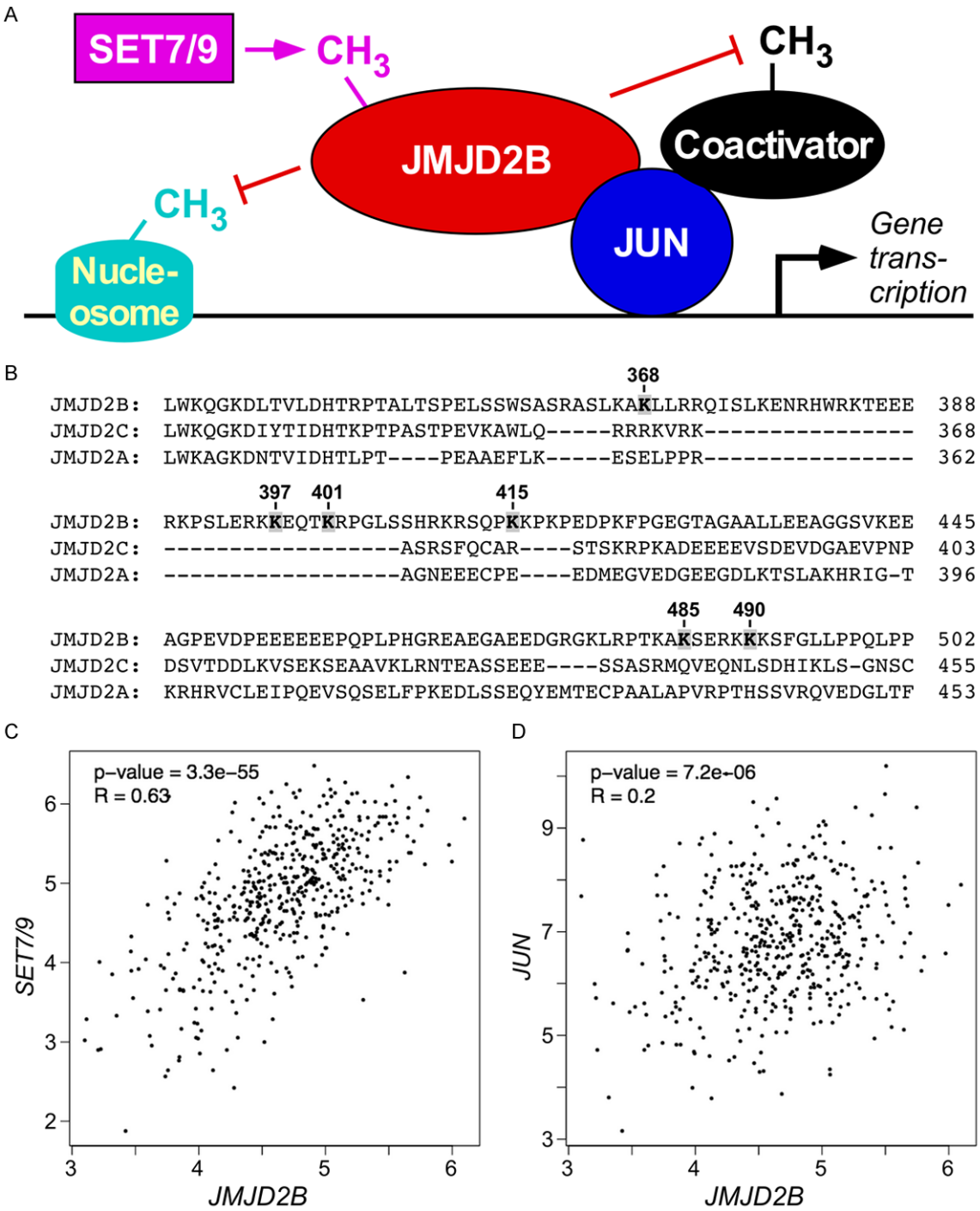

Figure 6. A. Model of how SET7/9 methylates and thereby stimulates the coactivator potential of JMJD2B. Please note that JMJD2B may in part coactivate JUN-dependent transcription through demethylation (indicated by —| in the figure) of methylated histones within nucleosomes and/or of methylated coactivators binding to JUN. B. No conservation of JMJD2B methylation sites in JMJD2A or JMJD2C. Amino acid sequences for JMJD2A (amino acids 331-619), JMJD2B (amino acids 332-670) and JMJD2C (amino acids 333-596) were derived from the NCBI entries NP_055478.2, XP_005259578.2 and NP_055876.2, respectively. Please note that the utilized JMJD2B protein was the X1 isoform, which has a 34 amino acids insert (amino acids 373-406) compared to the frequently used JMJD2B isoform 1 (NP_055830.1), and that isoform 1 also encompasses four of the identified six SET7/9 methylation sites, including the two major ones corresponding to K485 and K490. C and D. Correlation between *JMJD2B* mRNA levels and either *SET7/9* or *JUN* mRNA levels in human prostate adenocarcinomas. Data were derived from The Cancer Genome Atlas (TCGA) through the GEPIA (Gene Expression Profiling Interactive Analysis) webtool. Shown are log2 values of transcripts per million; R = Spearman correlation coefficient.

where its enzymatic activity regulated the unfolded protein response [97]. This suggests that JMJD2B is not only demethylating histones, but potentially also other (nuclear and cytoplasmic) proteins. Given that some JMJD proteins have been shown to hydroxylate various proteins [98-100], one can as well imagine that SET7/9-mediated methylation of JMJD2B not only regulates its demethylation but also hydroxylation activity, thereby providing another mechanism how JMJD2B may pleiotropically affect the proteome and in so doing could modulate cell homeostasis and pathological changes.

Conclusion

The identification of SET7/9-mediated lysine methylation of the JMJD2B protein and how it can selectively stimulate the cooperation with DNA-binding factors such a JUN has provided new insights how posttranslational modification may stimulate JMJD2B transcriptional activity and thereby prostate cancer development. However, some limitations of our study that should be addressed in the future are: (i) the absence of animal models testing how JMJD2B methylation on particularly K485 and K490 would modulate its physiological functions in development, homeostasis and malignancy; (ii) the unanswered question whether methylation of JMJD2B affects its ability to impose epigenetic changes; or (iii) if SET7/9-mediated methylation of JMJD2B leads to enhanced JUN-dependent gene transcription and thereby stimulation of tumor cells not only in prostate carcinomas, but also in the many other cancers where SET7/9, JMJD2B and JUN exert cancer-critical functions [9, 67, 101, 102].

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Disclosure of conflict of interest

None.

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