

Role of the Histone H3 Lysine 4 Methyltransferase, SET7/9, in the Regulation of NF- κ B-dependent Inflammatory Genes

RELEVANCE TO DIABETES AND INFLAMMATION^{*[5]}

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Nuclear factor κ -B (NF- κ B)-regulated inflammatory genes, such as TNF- α (tumor necrosis factor- α), play key roles in the pathogenesis of inflammatory diseases, including diabetes and the metabolic syndrome. However, the nuclear chromatin mechanisms are unclear. We report here that the chromatin histone H3-lysine 4 methyltransferase, SET7/9, is a novel coactivator of NF- κ B. Gene silencing of SET7/9 with small interfering RNAs in monocytes significantly inhibited TNF- α -induced inflammatory genes and histone H3-lysine 4 methylation on these promoters, as well as monocyte adhesion to endothelial or smooth muscle cells. Chromatin immunoprecipitation revealed that SET7/9 small interfering RNA could reduce TNF- α -induced recruitment of NF- κ B p65 to inflammatory gene promoters. Inflammatory gene induction by ligands of the receptor for advanced glycation end products was also attenuated in SET7/9 knockdown monocytes. In addition, we also observed increased inflammatory gene expression and SET7/9 recruitment in macrophages from diabetic mice. Microarray profiling revealed that, in TNF- α -stimulated monocytes, the induction of 25% NF- κ B downstream genes, including the histone H3-lysine 27 demethylase *JMJD3*, was attenuated by SET7/9 depletion. These results demonstrate a novel role for SET7/9 in inflammation and diabetes.

NF- κ B is a pleiotropic transcription factor that plays a pivotal role in regulating multiple biological functions, such as inflammation, immunity, cell proliferation, and apoptosis (1, 2). NF- κ B plays an important role in the regulation of proinflammatory genes, such as TNF- α (tumor necrosis factor α) and MCP-1 (monocyte-chemoattractant protein-1), that are asso-

ciated with several inflammatory diseases, including atherosclerosis, insulin resistance, metabolic syndrome, and diabetes and its complications (3–7). These genes also lead to monocyte activation associated with these inflammatory diseases.

NF- κ B consists of homo- or heterodimers of different subunits, such as p50, p52, p65/RelA, RelB, and c-Rel, with p65/RelA and p50 being the most common and well studied (1, 8). In most unstimulated cells, NF- κ B resides in the cytoplasm in an inactive latent form complexed with its inhibitor subunit, I κ B α . Multiple extracellular stimuli, including inflammatory cytokines, such as TNF- α , and ligands of the receptor for advanced glycation end products (RAGE),² can induce NF- κ B activation by promoting I κ B α phosphorylation and its proteasomal degradation (6, 9). The released p65-p50 dimer then translocates to the nucleus, where it binds to the promoters of NF- κ B-dependent inflammatory genes, such as TNF- α , MCP-1, and IL-6 (interleukin-6), to induce their expression (2). p65 protein is a key transcriptionally active component of NF- κ B whose transactivation potential is enhanced by several coactivators, including CREB-binding protein/p300, p/CAF, and SRC1 (10), which have histone acetyltransferase activity, and CARM1, which has arginine methyltransferase activity (11, 12). Recently, we showed that histone H3 lysine acetylation is enriched at inflammatory gene promoters and cooperates with NF- κ B in monocytes under diabetic conditions (13). These reports suggest that chromatin modifications, such as histone lysine acetylation and arginine methylation, are key regulators of NF- κ B activity. However, it is not known whether histone lysine methylation mediated by specific histone methyltransferases (HMTs) can modulate NF- κ B activity.

Gene transcription and activation are dynamic processes involving the conversion of compact heterochromatin into transcription factor-accessible euchromatin (14). Histone lysine acetylation by histone acetyltransferases usually increases transcriptional activity, whereas histone H3 lysine

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[5] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. 1 and 2 and Table S1.

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² The abbreviations used are: RAGE, receptor for advanced glycation end products; CREB, cAMP-response element-binding protein; HMT, histone methyltransferase; H3-K4, histone H3-lysine 4; H3-K4Me, histone H3-lysine 4 methylation; EMSA, electrophoretic mobility shift assay; shRNA, short hairpin RNA; HUVEC, human umbilical vein endothelial cells; VSMC, vascular smooth muscle cells; HVSVC, human vascular smooth muscle cells; RT, reverse transcription; ChIP, chromatin immunoprecipitation; QPCR, quantitative PCR; PMA, phorbol 12-myristate 13-acetate; Si, SET7/9 shRNA; CON, control.

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methylation, on the other hand, is associated with either gene activation or repression, depending on the number and specific methylation sites (14–16). Histone H3 lysine methylation states also generate specific epigenetic profiles on chromatin together with other histone modifications, such as phosphorylation, acetylation, and ubiquitination, as suggested by the histone code (17). Recently, several HMTs have been identified (18, 19), including the Suv39 and G9a family, that methylate histone H3 at Lys⁹ and facilitate heterochromatin formation and gene silencing (17, 20). On the other hand, methylation of histone H3 at Lys⁴ by SET1/2 family HMTs correlates with active chromatin and gene activation (15).

SET7/9 is a human HMT that can target H3-K4 and regulate gene expression (14, 21–24). It can also methylate nonhistone protein substrates and regulate transcriptional activity through alternate mechanisms (21, 22, 25). It was proposed that SET7/9-mediated histone methylation may compete with histone deacetylases or prevent histone H3 Lys⁹ methylation and thus facilitate transcriptional activation (21, 22). Furthermore, recent evidence shows that H3-K4Me is associated with the transcriptional initiation complex and elongation machinery (15, 26, 27). This suggests a novel role for H3-K4Me in gene activation and has thus triggered a flurry of activity in this area.

However, the role of SET7/9 and associated H3-K4Me in the regulation of inflammatory genes is not yet known. In this report, we examined whether SET7/9 can cooperate with NF- κ B and regulate NF- κ B-dependent genes and chromatin H3-K4Me induced not only by TNF- α but also by S100b, a ligand of the RAGE receptor for advanced glycation end products, relevant to the pathology of diabetes and its complications. Our results show that the down-regulation of SET7/9 in monocytes can attenuate the expression of key NF- κ B-dependent genes induced by these inflammatory stimuli and that SET7/9 is a previously unrecognized regulator of a subset of NF- κ B dependent inflammatory genes. These data for the first time reveal a new biological role for SET7/9 and H3-K4Me in monocytes related to inflammation and diabetes.

EXPERIMENTAL PROCEDURES

Materials—Bovine brain S100b peptide was obtained from Calbiochem, and human TNF- α was from R&D Systems (Minneapolis, MN). The following antibodies were utilized in the co-immunoprecipitation, Western blotting analyses, and chromatin immunoprecipitation assays: anti-p65 (SC-8008) and p300 (SC-585) from (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-I κ B α (9242; Cell Signaling), anti- β -actin (Sigma), histone H3 (ab1791; Abcam), anti-SET7/9 (07-314), anti-K4-mono (07-436), and trimethylation (07-473) (Upstate Biotechnology, Inc.). [γ -³²P]ATP (3000 Ci/mmol) was purchased from PerkinElmer Life Sciences, and the EMSA kit was from Promega (Madison, WI). T4 polynucleotide kinase was from Promega (Madison, WI). Wild type SET7/9 was a generous gift from Dr. Y. Zhang (University of North Carolina, Chapel Hill, NC). The lentiviral vector-based SET7/9 short hairpin RNA (shRNA) construct was from Dr. D. Reinberg (Robert Wood Johnson Medical School) and Dr. Rauscher and Dr. Ivanoff (Wistar Institute). Lentiviral vector-based packaging plasmids, pCHGP-2, pCMV-Rev, and pCMV-G, have been

described earlier (28). SET7/9 catalytically inactive construct was from Dr. D. Reinberg, and wild type p65 was from Dr. B. Forman (Beckman Research Institute of City of Hope).

Cell Culture—Human THP-1 monocytic cells and HEK293 cell lines were from the American Type Culture Collection (Manassas, VA) and cultured as described earlier (6). Human umbilical vein endothelial cells (HUVEC) and human vascular smooth muscle cells (HVSMC) were obtained from Cascade Biologics (Portland, OR) and Cambrex (East Rutherford, NJ), respectively, and cultured as per the supplier's instructions.

RNA Preparation, Relative RT-PCR, and Real Time Quantitative RT-PCR—Total RNA from cells were isolated and the mRNA levels of genes determined by relative RT-PCR or QPCR, as previously described (29). PCR products were fractionated on 2.0% agarose gels and photographed using an AlphaImager 2000 Documentation and Analysis system (Alpha Innotech, San Leandro, CA). The densities of amplified products corresponding to specific genes and 18 S RNA or β -ACTIN were determined with Quantity One software (Bio-Rad). Results are expressed as -fold stimulation over control after normalizing with the levels of an internal standard (18 S RNA). Real time QPCRs were performed using SYBR Green PCR Master Mix and the 7300 real time PCR system (Applied Biosystems, Foster City, CA), as reported earlier (29). All primer sequences used for RT-PCRs and QPCRs are listed in supplemental Table S1.

Transient and Stable Transfections—For transient transfections, HEK293 cells were transfected with plasmids using FuGENE 6 (Roche Applied Science) and used after 72 h. For lentiviral vector-based knockdown, we co-transfected SET7/9 shRNA construct and packaging plasmids pCMV-G, pCMV-Rev, and pCHGP-2 into 293T cells in a 60-mm dish by standard calcium phosphate precipitation. Viral supernatants were collected 24, 36, and 48 h post-transfection, filtered, and used for infecting THP-1 cells in 12-well plates in the presence of 4 μ g/ml Polybrene (Sigma). The infected THP-1 cells were positively selected by GFP sorting to obtain cells stably expressing the shRNA that were further expanded and used for all subsequent experiments.

Chromatin Immunoprecipitation (ChIP) Assays—THP-1 cells were either left untreated or treated with TNF- α (10 ng/ml) or S100b (40 μ g/ml) for the indicated time periods. Cells were fixed with 1% formaldehyde. ChIP assays were then performed using a kit (Upstate Biotechnology, Inc.) according to manufacturer's protocols and also as previously described (13), using the indicated antibodies. An aliquot of the cell lysates was used to isolate total input DNA. Amplifications of the immunoprecipitated DNA were performed by regular PCR or real time QPCRs. All reactions were performed in triplicate in a final volume of 25 μ l. Standard curves were generated using glyceraldehyde-3-phosphate dehydrogenase primers. Dissociation curves were run to detect nonspecific amplification, and we confirmed that single products were amplified in each reaction. All primer sequences used for ChIP-PCRs and ChIP-QPCRs are listed in supplemental Table S1.

EMSA—THP-1 cells were either left untreated or treated with TNF- α (10 ng/ml) for 30 min. Nuclear extracts were prepared as described below. The EMSAs were performed with a

kit from Promega according to the manufacturer's protocols. The oligonucleotides containing NF- κ B binding sites from the *MCP-1* promoter or a consensus NF- κ B binding site (supplemental Table S1) were end-labeled with [γ - 32 P]ATP (3000 Ci/mmol) using T4 polynucleotide kinase. Nuclear extracts were incubated with 32 P-labeled oligonucleotides in a 20- μ l reaction volume for 20 min at room temperature. In some reactions, nuclear extracts were preincubated with cold oligonucleotides (100 \times) to determine the specificity of binding or preincubated with p65 antibody to perform supershift assays. DNA-protein complexes were fractionated on nondenaturing polyacrylamide gels. Gels were dried and exposed to x-ray films to detect DNA-protein complexes.

Co-immunoprecipitation—HEK-293 (2×10^6) cells were grown in Dulbecco's modified Eagle's medium and co-transfected with p65 and SET7/9 expression plasmids. After 48 h, whole cell extracts were prepared with lysis buffer (Upstate Biotechnology) and centrifuged (10 min at 12,000 rpm). The supernatants were incubated with 2 μ g of anti-p65 antibody and 30 μ l of a 50% suspension of protein A-agarose (Upstate Biotechnology) overnight at 4 $^{\circ}$ C, followed by washing four times with phosphate-buffered saline containing 150 mM NaCl. Immunoblotting was performed with antibodies to p65 or SET7/9. Control for specificity was performed with β -actin.

Immunofluorescent Staining—Immunofluorescent staining was performed as described (30). Briefly, serum-depleted vascular smooth muscle cells (VSMC) were treated with or without TNF- α , as indicated. Cells were then washed with phosphate-buffered saline and fixed in 3% formaldehyde for 20 min, washed again, and immunostained with the indicated antibodies, followed by appropriate secondary antibodies conjugated to rhodamine or fluorescein isothiocyanate. Coverslips were mounted on slides and viewed under a confocal microscope LSM510 (Carl Zeiss, Inc.).

Cell Fractionation—Cyttoplasmic and nuclear fractions were prepared using a kit (Pierce) and subjected to Western blot analysis using anti-I κ B or anti-p65.

Monocyte Binding Assays—Binding assays were performed as previously described (6). Briefly, THP-1 cells (10^6 cells/ml) were incubated with (2',7'-Bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorescein acetoxyethyl ester) fluorescent dye (10 μ g/ml) in phosphate-buffered saline for 30 min at 37 $^{\circ}$ C to label the cells and washed twice with serum-free medium. For HUVEC and THP-1 cell binding assays, about 80% confluent HUVEC were prestimulated with TNF- α for 4 h and then incubated with either labeled control THP-1 cells or labeled SET7/9 knockdown cells for 1 h. For HVSMC, control or SET7/9 knockdown THP-1 cells were treated with TNF- α for 4 h and then labeled. Fluorescence-labeled THP-1 cells were then incubated with untreated HVSMC for 1 h. For both HUVEC and HVSMC binding assays, nonspecifically bound THP-1 cells were removed by carefully washing several times with serum-free medium. Specifically bound THP-1 cells were photographed with a fluorescent microscope and quantified by Image Pro5 software.

Isolation and Culture of Mouse Macrophages—All animal studies were performed in accordance with an institutionally approved protocol. Thioglycollate-elicited peritoneal macrophages were isolated from control (normal saline) and strepto-

zotocin-induced (50 mg/kg, 5 consecutive days) diabetic mice as described previously (31). Macrophages were plated in culture dishes in growth medium and 4 h later washed to remove detached cells. Attached macrophages were placed in low serum medium, stimulated the next day with TNF- α , followed by RNA extraction or ChIP assays.

Microarray Experiments and Data Analysis—SET7/9 knockdown (shRNA) and control THP-1 cells were harvested after a 2-h treatment with or without TNF- α (10 ng/ml), and RNAs were then extracted using Qiagen RNeasy minikits following the manufacturer's instructions. Three independent biological replicates were performed for each culture condition. Synthesis and labeling of cRNA targets from the cellular RNA, hybridization of GeneChips, and signal detection were carried out by the Microarray Core Facility at City of Hope. Briefly, biotinylated cRNA was generated using 5 μ g of total RNA using T7 RNA polymerase. The Affymetrix GeneChip Human Genome U133 plus version 2.0 array (Affymetrix, Santa Clara, CA) was used to define gene expression profiles. For microarray hybridization, the GeneChip arrays were hybridized with 20 μ g of fragmented cRNA targets and then washed using a Fluidics Station 450 (Affymetrix). Staining was performed with streptavidin-PE. Array images were scanned at 11 μ m resolution using a high resolution GeneChip Scanner 3000 (Hewlett-Packard).

Quality assessment and statistical analysis of gene expression data were performed using the R/Bioconductor packages. To ensure the high quality of the microarray experiments, quality assessment steps implemented in Bioconductor package "AffyExpress" were applied to the data. Raw intensity measurements of all probe sets were converted into expression measurements using the "RMA" method. The "LIMMA" package was then used to identify the genes differentially expressed between CON and SET7/9 knockdown cells with TNF- α treatment using two-way analysis of variance model. The significant "interaction" genes were selected with a *p* value cut-off of 0.05.

RESULTS

Regulation of a Subset of NF- κ B-dependent Genes Is Impaired in SET7/9-deficient Cells—We first evaluated the effects of SET7/9 loss-of-function on key NF- κ B-dependent genes by using shRNAs. These experiments were first performed in HEK-293 cells transiently transfected with plasmid constructs expressing SET7/9 shRNA (SI) or the scrambled control vector (CON) and stimulated with TNF- α for various time periods. Fig. 1A shows that TNF- α can induce *MCP-1*, *IL-8*, and *TNF- α* in HEK-293 cells transfected with CON. In contrast, the induction of all of these genes was markedly reduced (by 55–80%) in cells transfected with SET7/9 shRNA vector (Fig. 1A, SI). Western blotting demonstrated that the shRNA effectively reduced SET7/9 protein levels by over 50% relative to control (Fig. 1B), whereas the internal control β -actin was not affected.

We next used a lentiviral vector expressing SET7/9 shRNA to stably transfect THP-1 human monocytes, as described under "Experimental Procedures." We could attain over 80% knockdown of SET7/9 protein in these monocytes stably expressing SI (Fig. 1D). Furthermore, TNF- α -induced expression of *MCP-1*, *TNF- α* , and *IL-8* were all markedly reduced in the SI cells relative to CON (Fig. 1C), as determined by relative RT-

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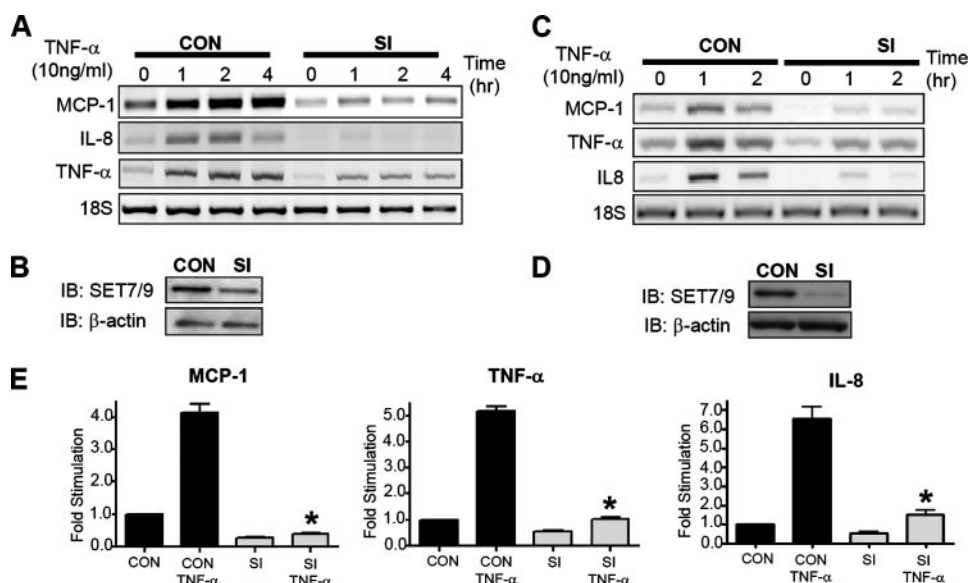


FIGURE 1. SET7/9 knockdown inhibits expression of key NF- κ B-dependent genes. *A*, HEK293 cells were transiently transfected with plasmid vectors expressing SET7/9 shRNA (SI) or scrambled sequence (CON). Inflammatory gene expression was determined by RT-PCR using 18S primers as internal control after cells were stimulated with or without TNF- α (10 ng/ml) for various time periods. *B*, cell lysates were immunoblotted (IB) with SET7/9 and β -actin antibodies to confirm the knockdown of SET7/9 protein. *C*, THP-1 cells were stably transfected with lentiviral vectors expressing SET7/9 shRNA or scrambled sequence, and RT-PCRs were performed to detect MCP-1, TNF- α , and IL-8 expression after TNF- α treatment (10 ng/ml) for various time periods. *D*, Western blot to demonstrate SET7/9 knockdown efficiency by the lentiviral vector in THP-1 cells. *E*, quantification of TNF- α -induced gene expression at the 1 h time point in THP-1 cells. *, $p < 0.001$ SI + TNF- α versus CON + TNF- α , $n = 3$.

PCRs using 18 S as control. Quantification of data from multiple independent experiments at the 1 h time point (*three bar graphs* in Fig. 1*E*) shows that SI significantly blocks TNF- α -induced MCP-1, TNF- α , and IL-8 gene expression relative to CON. These new data show that SET7/9 is important for the efficient activation of key NF- κ B-dependent inflammatory genes.

SET7/9 and p65 May Colocalize in the Same Cellular Complex—In order to explore the mechanisms by which SET7/9 regulates NF- κ B-dependent genes, we first examined whether SET7/9 affects I κ B α degradation or nuclear translocation of NF- κ B p65. Cytoplasmic and nuclear protein extracts from both control and SET7/9 knockdown THP-1 cells stimulated with TNF- α for various time periods were immunoblotted with I κ B α and p65 antibodies. Fig. 2*A* (*top*) shows that, as expected, TNF- α could induce I κ B α degradation by 30 min, followed by resynthesis at 60 min in control cells (CON). However, this was not altered in the SET7/9-deficient cells (SI). Furthermore, TNF- α -induced p65 nuclear translocation was also similar in both CON and SI cells (Fig. 2*A*, *third panel*). α -Tubulin (*second panel*) and histone H3 (*fourth panel*) levels served as loading controls for cytosolic and nuclear proteins, respectively. Thus, SET7/9 modulation of NF- κ B activity does not involve the alteration of events involved in I κ B α degradation or p65 nuclear translocation.

We next tested whether SET7/9 and p65 can interact together in a cellular *in vivo* context. For this, we performed co-immunoprecipitation with lysates from HEK293 cells transfected with SET7/9 and p65 expression vectors followed by immunoblotting with p65 antibody or control β -actin antibody. As shown in Fig. 2*B*, SET7/9 can be seen in the p65 immu-

noprecipitates (*top*). In contrast, β -actin was not detected in the p65 immunoprecipitates. Furthermore, very low amounts of SET7/9 and p65 proteins or none at all were detected in immunoprecipitates obtained with β -actin antibody, indicating the specificity of SET7/9 and p65 interaction (Fig. 2*B*, *left*). Although these results do not fully support a direct interaction, they still suggest that SET7/9 and p65 may be present in the same cellular complex *in vivo*. This is further supported by the immunofluorescence data in Fig. 2*C* showing cytosolic and nuclear co-localization of p65 with SET7/9 especially in response to 2 h of TNF- α (*bottom* of Fig. 2*C*). The expression of SET7/9 itself was not altered by TNF- α treatment (Fig. 2*D*). These data suggest that SET7/9 may act as a novel transcriptional cofactor of NF- κ B p65.

SET7/9 Enzymatic Activity Is Required for Regulating the Expression of Key Inflammatory Genes—

Since SET7/9 is a H3-K4 HMT, we next wanted to determine whether its HMT activity is involved in the regulation of NF- κ B-dependent genes. We compared the effects of TNF- α in cells transfected with wild type SET7/9 plasmid, or a methylation mutant of SET7/9 (32), which has no methyltransferase activity. Fig. 2*E* shows that, in wild type SET7/9 plasmid-overexpressing HEK293 cells, the expression of key NF- κ B-regulated genes MCP-1 and TNF- α is similar to that in control cells. No further increase in gene expression was seen in cells transfected with wild type SET7/9 plasmid, possibly due to the presence of sufficient amounts of endogenous SET7/9. On the contrary, TNF- α -induced MCP-1 and TNF- α expressions were clearly attenuated in cells expressing the methylation mutant of SET7/9, although not as effectively as with SI. *Bar graph* quantification of the data is seen in Fig. 2, *F* and *G*.

Recent reports showed that SET7/9 can activate transcription by methylating nonhistone protein substrates, such as TAF10 and p53 (25, 33, 34). However, we did not detect any methylated p65 in immunoprecipitates obtained using a methyl-lysine-specific antibody (data not shown). Taken together, these data are consistent with and further reinforce the SET7/9 shRNA data and suggest that SET7/9 may regulate NF- κ B-dependent genes through modifications of chromatin histone lysine at these specific gene promoters.

SET7/9 Is Recruited to the Promoters of Inflammatory Genes and Enhances p65 Recruitment to These Gene Promoters—

We next used ChIP assays to further investigate how SET7/9 shRNA may affect chromatin events at the promoters of inflammatory genes. ChIP-enriched DNA obtained with each antibody was PCR-amplified using primers spanning NF- κ B sites on the MCP-1 and TNF- α promoters (Fig. 3 and supple-

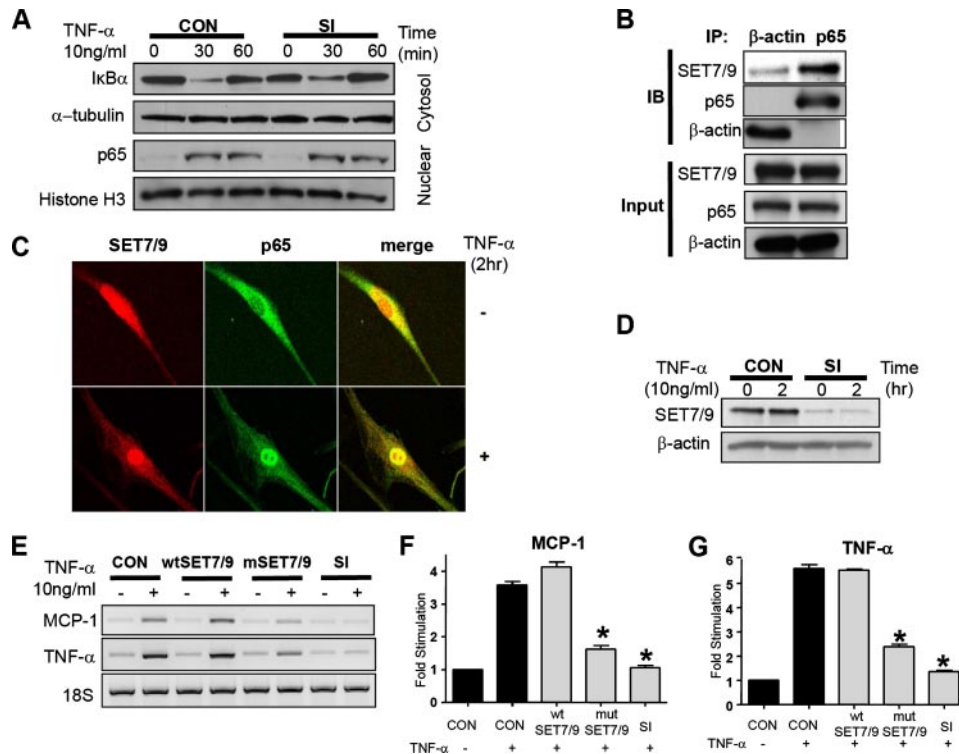


FIGURE 2. Interaction of SET7/9 with NF- κ B p65 and requirement of SET7/9 methyltransferase activity for inflammatory gene regulation. A, SET7/9 does not affect I κ B α degradation or p65 nuclear translocation upon TNF- α (10 ng/ml) treatment. Cytosolic and nuclear fractions prepared from SET7/9 knockdown THP-1 cells (SI) and control (CON) cells after treatment with TNF- α (10 ng/ml) for various time periods were subjected to immunoblotting (IB) with indicated antibodies. B, SET7/9 and p65 colocalize in the same cellular complex. HEK293 cells were co-transfected with SET7/9 and p65 expression vectors, and cell lysates were immunoprecipitated with β -actin antibody (left) or p65 antibody (right). Immunoprecipitates were immunoblotted with the indicated antibodies on the left. Representative of three separate experiments. C, HVSMC grown on coverslips were stimulated with TNF- α for 2 h, and p65 and SET7/9 were detected by immunofluorescent staining with p65 or SET7/9 antibody, followed by confocal microscopy. D, SET7/9 protein levels measured by Western blotting in control or SET7/9 knockdown THP-1 cells before and after TNF- α treatment. E, HEK293 cells were transiently transfected with an empty vector (CON) or vectors expressing wild type SET7/9 (wtSET7/9) or enzymatically inactive SET7/9 (mSET7/9). Basal and TNF- α (10 ng/ml, 2 h)-induced gene expression were determined by RT-PCR. F and G, quantification of data from E (*, $p < 0.001$ methylated mutant of SET7/9 + TNF- α or SI + TNF- α versus CON + TNF- α , $n = 3$).

mental Fig. 1). As shown in Fig. 3A), in response to TNF- α , NF- κ B p65, SET7/9, and p300 (a coactivator histone acetyltransferase associated with active genes) were all recruited simultaneously to the *MCP-1* promoter in control THP-1 cells (CON) in response to TNF- α . These promoter events were associated with increased K4 trimethylation. However, in the SET7/9 knockdown cells (SI), the enrichment of these factors (p65, SET7/9, and p300) as well as K4Me3 levels at these promoters were clearly attenuated. Similar events were also noted at the *TNF- α* promoter (ChIP assays in Fig. 3B) with Lys⁴ methylation also showing marked reduction. Input DNA control is shown in the bottom panel. These ChIP-enriched DNA samples were also analyzed by real time QPCR for *MCP-1* promoter, and the data in the bar graphs in Fig. 3C confirm that the TNF- α -induced enrichments of p65, SET7/9, and histone H3 Lys⁴ trimethylation at the *MCP-1* promoter were significantly attenuated in SET7/9-deficient THP-1 cells (SI). Lys⁴ monomethylation was significantly attenuated by 1 h at the *MCP-1* promoter (Fig. 3C) and at the *TNF- α* promoter (0.8 ± 0.1 SI + TNF- α for 1 h versus 1.7 ± 0.3 CON + TNF- α for 1 h, $n = 3$, $p < 0.05$, not shown), although the stimulation by TNF- α was less impressive than trimethylation. Specificity of the ChIP assays

was demonstrated by showing that promoter enrichments with TNF- α were seen only at regions spanning NF- κ B sites and not adjacent sites (supplemental Fig. 1, A and B). These results show that SET7/9 can be recruited to inflammatory gene promoters and that, in the absence of SET7/9, there is reduced p65 enrichment at these promoters.

SET7/9 Deficiency Does Not Affect p65 DNA Binding as Assessed by EMSAs—To test whether SET7/9 can affect the direct DNA binding properties of NF- κ B p65 to consensus oligonucleotides *in vitro*, we performed EMSAs. As shown in Fig. 4, we tested the interaction of p65 to oligonucleotides containing the NF- κ B binding site in the *MCP-1* promoter (Fig. 4, top) as well as to oligonucleotides containing generic NF- κ B consensus sequence (Fig. 4, bottom). It is seen that clear p65-DNA complexes are formed in THP-1 cells treated with TNF- α (Fig. 4, compare lanes 2 and 3 or lanes 8 and 9). Furthermore, these DNA-protein complexes were not affected by SET7/9 depletion (Fig. 4, compare lane 3 versus lane 5, or lane 9 versus lane 12). The specificities of the labeled DNA-p65 complexes were further confirmed by competition with unlabeled cold DNA (Fig. 4E, lane 3 versus lane 6). The presence of p65 in the complexes was confirmed by supershifting with a p65 antibody (Fig. 4E, lanes 10 and 13). These results differ from the ChIP assays, which showed that SET7/9 deficiency decreases p65 promoter occupancy in the chromatin. These data support the notion that SET7/9 acts as an NF- κ B coactivator in histone-coated nucleosomal DNA through its histone methyltransferase activity and not by directly regulating the binding of NF- κ B to its target cis-elements.

S100b-induced Gene Expression Is Also Decreased by SET7/9 Knockdown—S100b is an inflammatory protein and also a ligand of RAGE. Evidence shows that S100b is increased in diabetes and can mimic diabetic conditions by eliciting similar effects as advanced glycation end products (9). It can induce a variety of proinflammatory events through RAGE and NF- κ B activation in THP-1 and primary human monocytes (6, 9). We next tested whether an inflammatory diabetic stimulus can also function via SET7/9 (*i.e.* whether S100b-induced NF- κ B downstream gene expression is also impaired by SET7/9 knockdown). Fig. 5A shows that S100b increases *MCP-1* and *TNF- α* mRNA expression in a time-dependent manner in control (CON) THP-1 monocytes. However, these effects were significantly reduced in SET7/9 stable knockdown THP-1 cells (SI)

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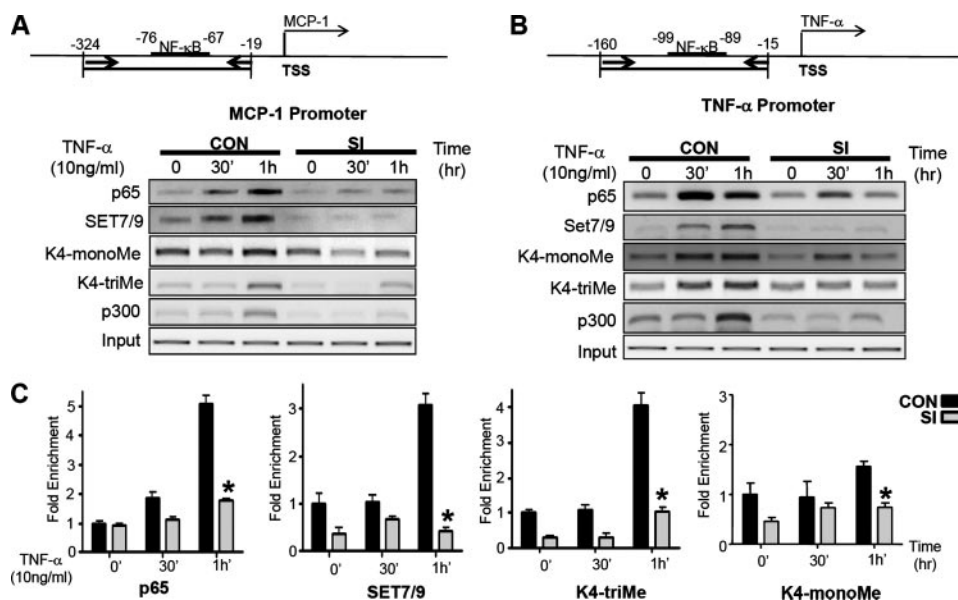


FIGURE 3. Inhibition of TNF- α -induced chromatin events in SET7/9 knockdown THP-1 cells. Wild type (CON) or SET7/9 stable knockdown THP-1 cells (SI) were treated with or without TNF- α for the indicated time periods, and ChIP assays were performed with anti-p65, anti-SET7/9, anti-H3K4-monomethylation, anti-H3K4-trimethylation, or anti-p300 antibodies. ChIP-enriched samples were analyzed by regular PCR using *MCP-1* (A) and *TNF- α* (B) promoter primers. Specific locations of *MCP-1* promoter primers are shown in supplemental Fig. 1. Data are representative of three separate experiments. B, real time QPCRs with ChIP-enriched DNA using *MCP-1* promoter primers showing significant changes and confirmation of data in A and C (*, $p < 0.005$ SI + TNF- α for 1 h versus CON + TNF- α for 1 h, $n = 3$).

(Fig. 5, A and bar graphs in B). These data are consistent with S100b and TNF- α acting through a common transcription factor, namely NF- κ B, and the HMT SET7/9 to activate key downstream genes.

We next used ChIP assays to evaluate whether the RAGE ligand S100b can also promote SET7/9 enrichment *in vivo* at inflammatory gene promoters similar to TNF- α . ChIP experiments in Fig. 5C show that S100b can increase p65 enrichment at the *MCP-1* promoter in THP-1 cells by 30 min. Interestingly, we also observed for the first time that S100b can increase H3-K4Me and the occupancy of the K4 HMT, SET7/9, at the *MCP-1* promoter. We also tested the *in vivo* relevance by performing ChIP assays with macrophages derived from streptozotocin-injected diabetic mice. These diabetic macrophages expressed increased levels of *Mcp-1* compared with mice injected

with normal saline (NS) (Fig. 5D). Furthermore, the ChIP-real time QPCR data in Fig. 5E show a marked increase in SET7/9 occupancy at the *Mcp-1* promoter in response to TNF- α in the diabetic macrophages relative to nondiabetic.

These ChIP data strongly suggest that SET7/9 and H3-K4Me levels positively correlate with the promoter p65 recruitment. These new results show that SET7/9 may be recruited to the chromatin at specific gene promoters in response to inflammatory, diabetic, and proatherogenic stimuli to cooperate with and enhance NF- κ B activity. Thus, SET7/9 may regulate key proinflammatory genes associated with monocyte dysfunction, diabetes, and its complications.

SET7/9 Is Not Involved in Monocyte Differentiation but Affects Monocyte Adhesion—Monocytes play important roles during inflammation, as characterized by their ability to adhere and migrate into subendothelial sites or adipose tissues and differentiate into macrophages, all key steps in the pathogenesis of inflammatory diseases, such as atherosclerosis and diabetes. Since our data showed that SET7/9 can regulate NF- κ B-dependent inflammatory genes, we further examined the functional relevance of SET7/9 to key monocyte functions, such as macrophage differentiation and adhesion. We first treated both CON and SET7/9 shRNA expressing THP-1 monocytes (SI) with the phorbol ester phorbol 12-myristate 13-acetate (PMA) (10 or 20 nM) to induce monocyte differentiation and then examined key macrophage markers by RT-PCR. Results showed that PMA-induced expression of key markers of monocyte-macrophage differentiation, such as scavenger receptor A (SRA), CD36, and macrophage colony-stimulating factor (M-CSF) were not affected by SET7/9 knockdown (Fig. 6A), demonstrating that SET7/9 does not modulate monocyte-macrophage differentiation. These results also indicate that the

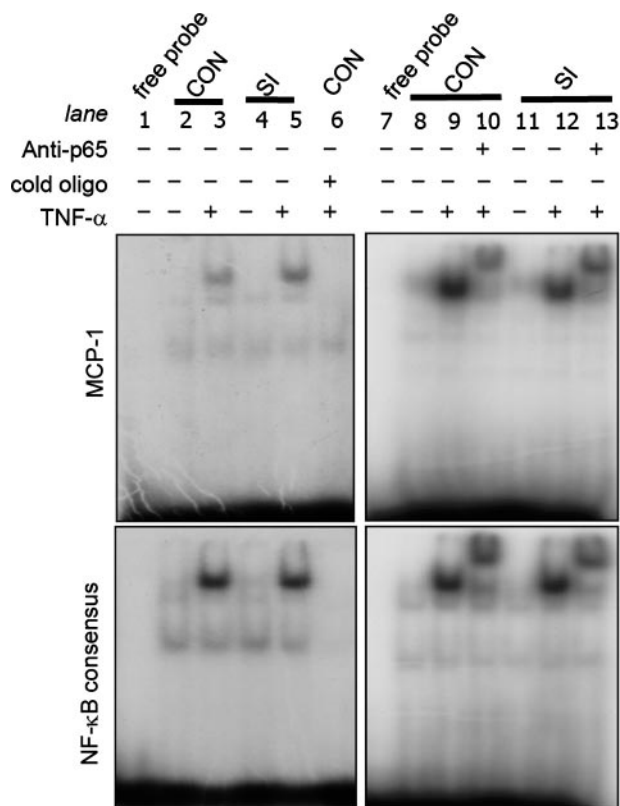


FIGURE 4. SET7/9 does not affect the binding activity of p65 to oligonucleotides containing NF- κ B consensus binding sites: EMSA using 32 P-labeled oligonucleotide probes corresponding to NF- κ B binding site sequence in the *MCP-1* promoter (top) or generic NF- κ B consensus binding site (bottom) with nuclear extract from control (CON) or SET7/9 knockdown (SI) cells with and without TNF- α treatment. Specificity of DNA-protein complexes was confirmed by competition with cold unlabeled DNA oligonucleotides (lanes 4 and 6). The presence of p65 was confirmed by supershifting with p65 antibodies (lanes 10 and 13).

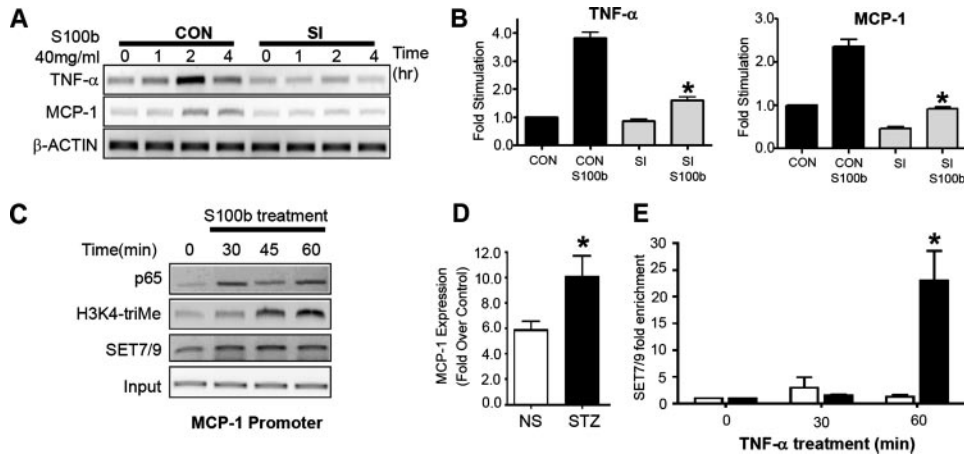


FIGURE 5. Role of SET7/9 in inflammatory gene expression related to diabetes. *A*, regulation of S100b-induced gene expression by SET7/9. THP-1 cells (both CON and SI) were treated with or without the RAGE ligand S100b (40 μ g/ml), and gene expression was analyzed by relative RT-PCR using β -actin as internal control. *B*, quantification of data from three independent experiments (*, $p < 0.001$, SI + S100b versus CON + S100b). *C*, ChIP assays showing recruitment of SET7/9 to the MCP-1 promoter in THP-1 cells stimulated with S100b. Wild type THP-1 cells were treated without or with S100b (40 μ g/ml) for the indicated time periods, and ChIP assays were performed using the indicated antibodies. ChIP enriched samples were analyzed using MCP-1 promoter primers. Results are representative of three experiments. *D*, increased MCP-1 expression in macrophages of diabetic mice. Macrophages derived from control (normal saline; NS) and streptozotocin (STZ)-induced diabetic mice were stimulated with TNF- α (10 ng/ml) for 1 h, and MCP-1 gene expression was analyzed by real time RT-QPCR (*, $p < 0.05$ streptozotocin versus normal saline, $n = 3$). *E*, enhanced TNF- α induced recruitment of SET7/9 in diabetic macrophages. Control (open bars) and diabetic (filled bars) macrophages were treated with or without TNF- α and then subjected to ChIP assays using SET7/9 antibodies followed by QPCR with MCP-1 promoter primers. Results are expressed as -fold over control after normalization with input samples (*, $p < 0.001$ diabetic macrophages with TNF- α for 60 min versus Control macrophages with TNF- α for 60 min, $n = 3$).

effect of SET7/9 is specific to NF- κ B, since PMA primarily activates AP-1 transcription factor.

Monocyte adhesion to endothelial cells and VSMC and their subendothelial recruitment are key steps in the pathogenesis of atherosclerosis and inflammation (35). Several cytokines, chemokines, and cell adhesion molecules can promote monocyte adhesion, including proatherogenic TNF- α and MCP-1 that are produced by all endothelial cells, VSMC, and monocytes (36, 37). *In vitro*, TNF- α pretreatment of these cells can enhance monocyte adhesion, thus indicating the involvement of both autocrine and paracrine TNF- α /NF- κ B signaling. In the next step, we performed functional assays to determine the effect of SET7/9 deficiency on the adhesive properties of THP-1 monocytes to HVSMC or HUVEC.

Monocyte-VSMC or monocyte-HUVEC adhesion assays were performed using fluorescently labeled THP-1 CON and THP-1 SI cells. Interestingly, the binding of untreated or TNF- α -treated THP-1 cells to HVSMC was significantly attenuated in SI cells relative to CON cells (Fig. 6B). Similarly, the adhesion of SET7/9-deficient THP-1 cells to TNF- α -treated HUVEC was also significantly reduced (Fig. 6C). These functional data clearly demonstrate that SET7/9 knockdown can impair TNF- α signaling and the production of key chemokines and that SET7/9 plays an important role in key monocyte functions related to inflammatory diseases.

An Unbiased Screening for SET7/9-regulated NF- κ B Downstream Genes—The results up to now indicate that specific inflammatory genes, such as MCP-1, IL-8, and TNF- α , are regulated by SET7/9. In order to profile other potential genes regulated by both SET7/9 and NF- κ B in a genome-wide scale, we performed an unbiased microarray analysis to identify NF- κ B downstream genes that are also affected by SET7/9 knockdown. mRNA samples were obtained from

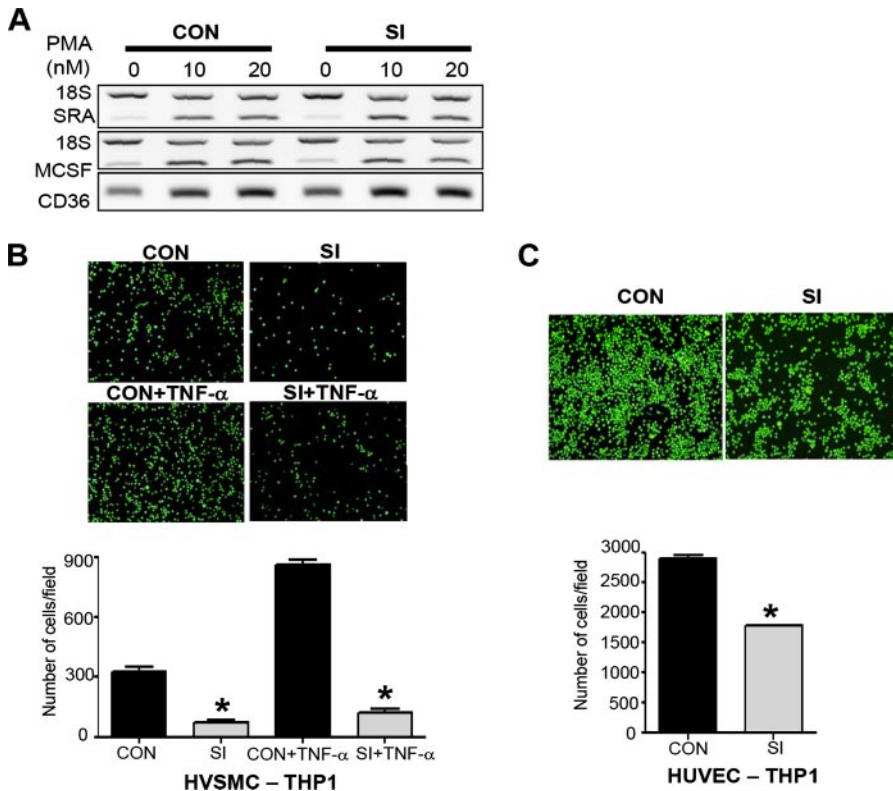


FIGURE 6. SET7/9 regulates monocyte adhesion but not monocyte differentiation. *A*, wild type (CON) or SET7/9 knockdown THP-1 cells (SI) were treated with PMA, followed by RT-PCRs to detect markers of monocyte differentiation, scavenger receptor A (SRA), macrophage colony-stimulating factor (MCSF), and CD36. *B*, adhesion of THP-1 cells (TNF- α -treated or -untreated) to HVSMC monolayers is attenuated in SET7/9 knockdown cells (*, $p < 0.001$ SI versus CON or SI + TNF- α versus CON + TNF- α , $n = 3$). *C*, adhesion of untreated THP-1 cells to TNF- α -treated HUVEC monolayers is also attenuated in SET7/9 knockdown cells (*, $p < 0.001$ SI versus CON, $n = 3$). Binding assays with HUVEC or HVSMC were performed as described under "Experimental Procedures." Bound monocytes were quantified with Quantity One software (Bio-Rad).

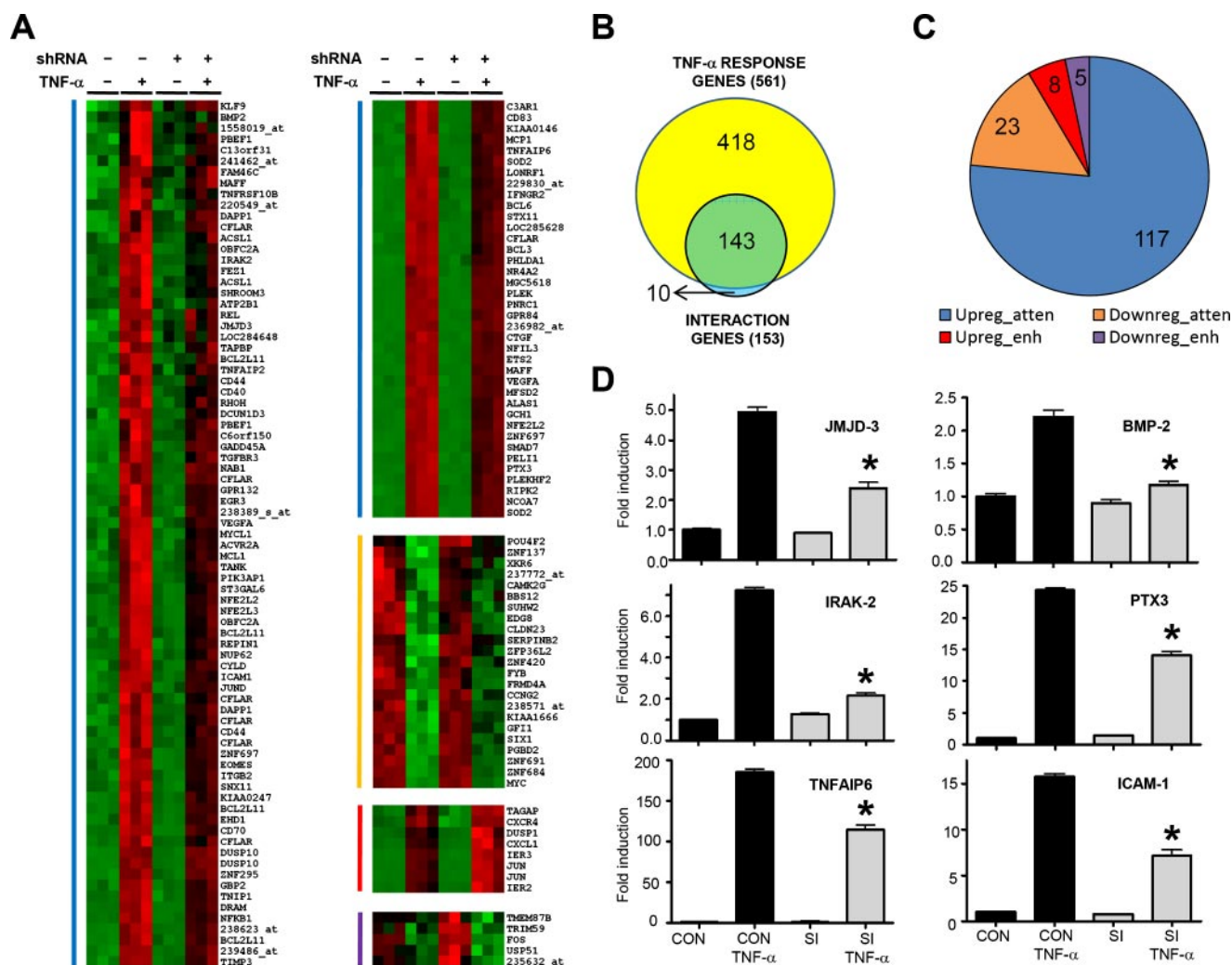


FIGURE 7. SET7/9 regulates a subset of TNF- α -regulated genes. *A*, unbiased microarray screening of SET7/9-regulated TNF- α downstream genes. Expression profiling experiments were performed in triplicate using RNA from both SET7/9 knockdown (shRNA) and control THP-1 cells treated with or without TNF- α (10 ng/ml). Shown are the 153 Affymetrix probe sets divided into four clusters with significant interaction effects ($p < 0.05$) from two-way analysis of variance analysis (see “Experimental Procedures”). Gene cluster with blue label on the left represent genes up-regulated by TNF- α but whose induction is attenuated by SET7/9 shRNA (*Upreg-atten*). The orange cluster is down-regulated-attenuated (*Downreg-atten*); red and purple are clusters up-regulated-enhanced (*Upreg-enh*) and down-regulated-enhanced (*Downreg-enh*), respectively. *B*, Venn diagram showing that a significant fraction of TNF- α downstream genes are affected by SET7/9 shRNA. *C*, pie chart from the data in *A* comparing gene numbers of the four different THP-1 clusters of interaction genes. *D*, validation of microarray results showing expression changes of six “interaction genes” by real time PCR. Black bars, CON THP-1 cells; gray bars, SET7/9 knockdown THP-1 cells (*, $p < 0.01$, SI + TNF- α versus CON + TNF- α , $n = 3$).

both CON and SET7/9 knockdown cells before and after TNF- α treatment. For each condition, we obtained three independent biological replicates and hybridized them to separate Affymetrix microarrays (see “Experimental Procedures”). This experimental design allowed us to perform variation analyses (two-way analysis of variance) to assess the effects of two different factors (TNF- α treatment and SET7/9 knockdown) on gene expression. We performed two-way analysis of variance analysis for every gene on the array and identified the groups of genes whose -fold changes after TNF- α treatment were significantly different ($p < 0.05$) between SET7/9 shRNA cells and control monocytes. The identified genes designated as “interaction genes” would be those regulated by the interaction effect of SET7/9 knockdown and NF- κ B in TNF- α -treated cells.

In total, we identified 153 “interaction” genes ($p < 0.05$), which include over one-fourth of all of the 561 TNF- α -responsive genes (Fig. 7, *A* and *B*). These genes were further classified

into four clusters (Fig. 7, *A* and *C*). In the pie chart in Fig. 7*C*, *Upreg-atten* (up-regulated-attenuated) represents genes whose -fold induction by TNF- α was reduced or attenuated in the SET7/9 knockdown THP-1 cells. *Downreg-atten* (down-regulated-attenuated) represents genes that were inhibited by TNF- α to a lesser extent in SI cells. *Upreg-enh* (up-regulated-enhanced) and *DownReg-Enh* (down-regulated-enhanced) represent the corresponding opposite scenarios. Consistent with the proposed role of SET7/9 as an NF- κ B co-activator, the largest cluster of interaction genes (up-regulated-attenuated, encompassed by a blue bar on the left in Fig. 7*A*) comprised 117 genes (77%) whose -fold induction by TNF- α was clearly attenuated in SET7/9 shRNA-treated cells. Furthermore, *MCP-1* was present in this cluster, thereby supporting our data in Fig. 1.

We also chose six additional genes (*JMJD3*, *BMP-2*, *IRAK-2*, *PTX3*, *TNFAIP6*, and *ICAM-1*) from this cluster for further

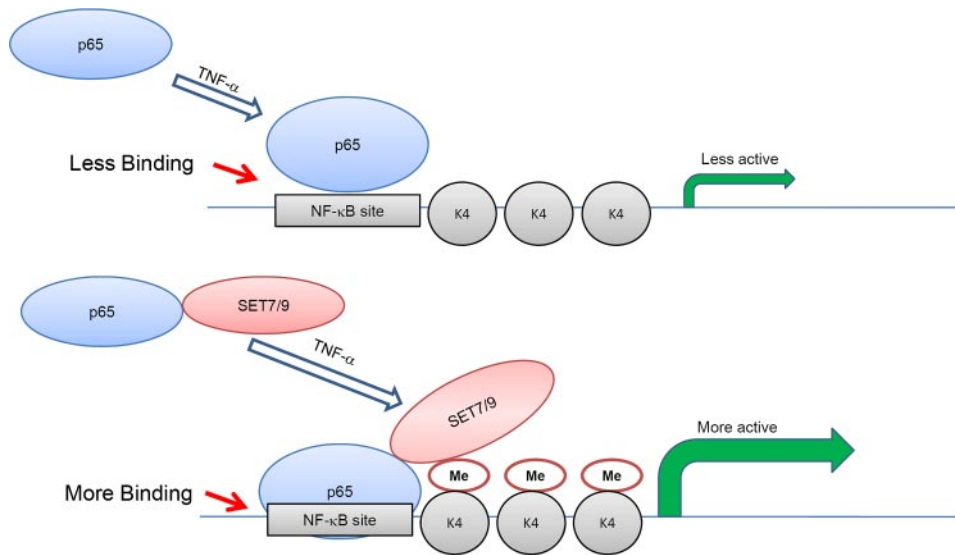


FIGURE 8. Schematic representation of the proposed regulatory function of SET7/9 on NF- κ B and inflammatory gene expression. SET7/9 can stabilize and enhance p65 recruitment to a subset of gene promoters, and this is accompanied by increased H3-K4 methylation and augmented gene expression. Thus, SET7/9 may transform the promoter from a relatively “dormant” to more “active” state and thus allow efficient transactivation by NF- κ B.

validation of the microarray data. We were able to confirm by RT-QPCR that SET7/9 knockdown does indeed attenuate TNF- α -induced expression of these genes in SI cells (Fig. 7D). Interestingly, one of these genes is the histone H3 Lys²⁷ demethylase *JMJD3*, which has recently been shown to be directly regulated by NF- κ B in mouse macrophages, and *JMJD3* activity was required for *BMP-2* gene expression (38). Our current results show that both *JMJD3* and *BMP-2* are among the top candidates in the up-regulated-attenuated cluster. These new results suggest a novel mutual inhibitory mechanism between SET7/9-regulated H3-K4Me and *JMJD3*-mediated histone H3 Lys²⁷ demethylation in the process of activating the NF- κ B trans-regulatory pathway.

The second largest cluster of interaction genes was composed of 23 genes (15%) that are repressed by TNF- α , and this repressive effect was attenuated in SET7/9 knock-down cells (down-regulated-attenuated genes, orange bar and wedge in Fig. 7, A and C, respectively). This cluster, along with the up-regulated-attenuated cluster, collectively represents 92% of all of the interaction genes, thereby strongly supporting the idea that SET7/9 is a key contributor to NF- κ B transactivity. Notably, half (10 of 20) of the known genes in this down-regulated-attenuated cluster are transcription factors, suggesting a complex secondary trans-regulatory network in response to TNF- α . Additional studies are needed to explore the significance of this novel observation. Only eight and five genes showed enhanced induction and repression, respectively, by TNF- α in SET7/9 knockdown cells (Fig. 7, A and C, red and purple).

In order to further demonstrate that SET7/9 affects NF- κ B function as opposed to binding, we evaluated a key NF- κ B target gene (*IL-1 β*) from the microarray that did not belong to the “interaction subset” and was not affected by SET7/9 (supplemental Fig. 2A). We examined whether NF- κ B binding to its promoter (by ChIP assays) is dependent on SET7/9. ChIP-QPCRs to evaluate chromatin events at the *IL-1 β* promoter

showed that *IL-1 β* is regulated by NF- κ B but not SET7/9. Thus, in both CON and SET7/9-depleted cells, p65 was recruited to the *IL-1 β* promoter after TNF- α treatment, but SET7/9 recruitment or Lys⁴ methylation was not observed (supplemental Fig. 2B).

DISCUSSION

NF- κ B-dependent inflammatory gene expression has been extensively studied due to involvement of these target genes in the pathology of several inflammatory diseases, including atherosclerosis, insulin resistance, and diabetes and its complications. In this paper, we investigated how key nuclear chromatin remodeling events, especially histone H3-K4Me and the K4 HMT, SET7/9, modulate NF- κ B downstream genes. Our results show for

the first time that H3-K4Me by SET7/9 can regulate the expression of a subset of key NF- κ B downstream inflammatory genes by interacting with NF- κ B and modulating chromatin remodeling events at their promoters. SET7/9 shRNA blocked the expression of inflammatory genes in 293 cells as well as THP-1 monocytes, suggesting that the HMT enzymatic activity of SET7/9 may be important for these effects. Furthermore, apart from TNF- α , SET7/9 could also regulate S100b-induced inflammatory gene expression. ChIP assays demonstrated that knockdown of SET7/9 impaired TNF- α -induced recruitment of p65 and other chromatin factors associated with active gene expression, such as the coactivator histone acetyltransferase p300, to the promoters of key inflammatory genes. SET7/9 occupancy at the *MCP-1* gene promoter was also enhanced *in vivo* in TNF- α -treated macrophages derived from diabetic mice in parallel with increased expression of *MCP-1*. These results demonstrate that SET7/9 is an important member in the p65 gene activation complex on these promoters and may therefore act as a novel coactivator of inflammatory genes in diseases such as atherosclerosis and diabetes. The proposed scheme in Fig. 8 suggests that SET7/9 and its Lys⁴-methylating activity can help to further open the chromatin for enhanced transcription of a subset of NF- κ B-dependent genes.

Although we observed a reduction in histone H3-K4 methylation at the promoters of a subset of genes, global mono-, di-, or trimethylation levels were not affected in SET7/9 knock-down cells, as revealed by Western blotting (data not shown). This confirms that SET7/9 is not the only HMT that mediates H3-K4 methylation and that the loss of SET7/9 can be compensated by other endogenous H3-K4 HMTs, such as SET1, SMYD3, and MLLs (19). SET7/9 may have an active role in methylating specific promoters as a co-factor for NF- κ B. This specific regulatory mechanism for NF- κ B might not be limited to SET7/9, and other histone modifications, such as histone

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arginine methylation via CARM-1 HMT, may also play a role (11, 12).

In addition, SET7/9 can methylate not only histone H3-K4 but also other transcription factors, such as p53 and TAF10 (22, 25). In our study, although the methyltransferase activity was required for SET7/9 to regulate NF- κ B, no p65 was detected in immunoprecipitates obtained with a general methyl-lysine antibody,³ suggesting that SET7/9 most likely does not co-activate p65 by methylating it. On the other hand, it most likely activates NF- κ B in nucleosomal DNA through its histone methyltransferase activity and not by directly regulating the binding of NF- κ B to its target cis-elements. This is also supported by our observation that the binding of p65 to NF- κ B consensus sequence DNA oligonucleotides *in vitro* (as determined by EMSAs) was not affected by SET7/9 knockdown (Fig. 4).

Using ChIP assays, we demonstrated simultaneous TNF- α -induced recruitment of p65, p300, and SET7/9 at the *MCP-1* and *TNF- α* promoters along with increased H3-K4Me levels (especially trimethylation) and that these promoter events were significantly attenuated in SET7/9 knockdown cells. These results suggest that SET7/9 not only enhances p65 occupancy at specific NF- κ B regulated promoters but might also stabilize the p65 complex at these sites by methylating H3-K4 (Fig. 8). SET7/9-mediated K4 methylation may transform the promoter from a relatively “dormant” to more “active” state and thus allow stronger or more efficient NF- κ B-induced transactivation of at least a subset of key inflammatory gene promoters (Fig. 8). These results are consistent with the emerging concept that active gene promoters are associated with H3K4 trimethylation (39).

Our data also indicate that SET7/9 and p65 may exist in the same cellular complex (Fig. 2, B and C). However, not all NF- κ B downstream genes were regulated by SET7/9, with IL-1 β being one such candidate. Thus, additional factors (such as chromatin environment) may play key roles in determining the involvement of SET7/9, to further fine tune and augment NF- κ B activity only at specific gene promoters.

Our results showing decreased promoter H3-K4 trimethylation by SET7/9 depletion is rather surprising but also interesting. Earlier reports have identified SET7/9 as a histone lysine monomethyltransferase, but most of these were *in vitro* studies (21–23, 40). Further work is needed to determine whether SET7/9 can directly promote Lys⁴ trimethylation and whether this can also enhance transcription initiation *in vivo*. Our data also cannot exclude the possibility that the reduction of H3-K4 trimethylation by SET7/9 knockdown was due to indirect mechanisms, such as altered recruitment of other factors, histone methyltransferases, or demethylases.

In order to profile genes that are regulated by both NF- κ B and SET7/9, we used microarray screening and uncovered a set of TNF- α -regulated genes whose inductions were affected by SET7/9 knockdown in monocytes. A significant fraction of TNF- α -regulated genes (153, over 25%) were affected by SET7/9 knockdown, and over 90% of these showed an attenu-

ated response in the SET7/9 knockdown cells. These results strongly suggest that SET7/9 is a key regulator of NF- κ B function and can enhance the sensitivity of target genes to TNF- α . The largest group of these “interaction” genes (77%) is present in the up-regulated-attenuated cluster, which is consistent with our proposed model of SET7/9 being a co-activator of NF- κ B.

Our microarray data also revealed additional possible exciting mechanisms that may cause impaired TNF- α response during SET7/9 deficiency. It was recently reported that in mouse macrophages, the histone demethylase activity of *JMJD3* is required for the removal of the repressive histone modification histone H3 Lys²⁷ trimethylation on the *BMP-2* promoter and subsequent activation of *BMP-2* via NF- κ B (38). Our current results suggest that this regulatory mechanism is also conserved in human cells, since we noted that TNF- α can induce the expression of *JMJD3* and *BMP-2*, and, more importantly, the inductions of both *JMJD3* and *BMP-2* were attenuated in parallel by SET7/9 shRNA. These data suggest a novel cross-talk mechanism between two mutual counterregulatory histone modifications (H3-K4Me and histone H3 Lys²⁷ methylation) for certain NF- κ B downstream genes (including *BMP-2*), in which the loss of an H3-K4 HMT would impair histone H3 Lys²⁷ demethylases and thereby promote gene repression directly or via subsequent increase in histone H3 Lys²⁷ methylation. Overall, the results suggest that regulation of epigenetic histone modifications may be a potential mechanism for the specificity of SET7/9 in regulating NF- κ B activity.

Importantly, we found that SET7/9 has a functional role in monocytes, since SET7/9 knockdown altered key monocyte functions. Thus, SET7/9 shRNA-expressing THP-1 cells exhibited significantly lower adhesion to HVSMC and HUVEC. Since monocyte binding to endothelial and smooth muscle cells represents key steps in the pathogenesis of inflammation and atherosclerosis (41, 42), these results suggest a key role for SET7/9 in these disease states. This was also supported by our microarray data, which revealed that several proinflammatory and atherosclerotic factors, including *MCP-1*, *ICAM-1*, and *CD40*, were induced by TNF- α treatment, and importantly, the induction of these genes was clearly attenuated in the SET7/9 deficient cells (Fig. 7A). Taken together, our new findings demonstrate that chromatin modification by SET7/9 promotes proinflammatory events in monocytes. SET7/9 may therefore be a novel therapeutic target for inflammatory diseases, including diabetes and related metabolic disorders.

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