Original Article

MKL1 mediates TNF-α induced pro-inflammatory transcription by bridging the crosstalk between BRG1 and WDR5

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Abstract

Tumor necrosis factor alpha (TNF-α) is a cytokine that can potently stimulate the synthesis of a range of pro-inflammatory mediators in macrophages. The underlying epigenetic mechanism, however, is underexplored. Here we report that the transcriptional modulator megakaryocytic leukemia 1 (MKL1) is associated with a histone H3K4 methyltransferase activity. Re-ChIP assay suggests that MKL1 interacts with and recruits WDR5, a component of the COMPASS complex responsible for H3K4 methylation, to the promoter regions of pro-inflammatory genes in macrophages treated with TNF-α. WDR5 enhances the ability of MKL1 to stimulate the promoter activities of pro-inflammatory genes. In contrast, silencing of WDR5 attenuates TNF-α induced production of pro-inflammatory mediators and erases the H3K4 methylation from the gene promoters. Of interest, the chromatin remodeling protein BRG1 also plays an essential role in maintaining H3K4 methylation on MKL1 target promoters by interacting with WDR5. MKL1 knockdown disrupts the interaction between BRG1 and WDR5. Together, our data illustrate a role for MKL1 in moderating the crosstalk between BRG1 and WDR5 to activate TNF-α induced pro-inflammatory transcription in macrophages.

Keywords: MKL1, WDR5, BRG1, macrophage, transcriptional regulation

Introduction

Macrophages constitute a major source of inflammation in both health and disease states[1]. Macrophage-associated inflammation is generally believed to be a double-edged sword serving both protective and pathogenic roles. When orderly programmed, it helps maintain tissue homeostasis. When aberrantly constructed, it disrupts physiologic integrity and contributes to a host of human diseases such as metabolic syndrome[2], atherosclerosis[3], and cancer[4–5]. In macrophages, inflammation-related transcriptional programs are dictated by a handful of conserved transcriptional factors (TF) that include NF-κB, AP-1, and STAT[6]. The activities of these sequence-specific TFs are modulated by various co-factors, which may either

enhance or suppress the inflammatory response. We have previously identified megakaryocytic leukemia 1 (MKL1) as a co-factor for NF-κB[7]. MKL1 interacts with NF-κB and stimulates NF-κB-dependent trans-activation of target genes to promote the pathogenesis of pulmonary hypertension and colitis[8–10]. The mechanism whereby MKL1 mediates NF-κB-dependent pro-inflammatory transcription, however, is not completely defined.

Engagement of the epigenetic machinery represents a paradigm of transcriptional regulation in eukaryotes[11]. Typically, transcriptionally active chromatin is marked by methylated histone H3K4 while transcriptional repression is synonymous with methylated H3K9 and H3K27[12]. In eukaryotes, histone H3K4 methylation is catalyzed by the COMPASS complex (short for complex proteins associated with SET1)[13]. COMPASS operates in a modular mode: whereas catalytic subunits, including MLL1, MLL2, MLL3, MLL4, and SET1 are exchangeable, structural/regulatory subunits, including ASH2, WDR5, WDR82, and RpBP5, are invariable. How individual COMPASS components regulate pro-inflammatory transcription in macrophages remains to be clearly sorted out.

A range of epigenetic factors have been documented to participate in the regulation of pro-inflammatory transcription in macrophages. For instance, brahma-related gene 1, or BRG1, was reported to control temporal production of pro-inflammatory mediators induced by lipopolysaccharide (LPS) and free fatty acids[14–15]. Several histone methyltransferases, including MLL1[16], MLL4[17], and SET7/9[18], have all been implicated in the regulation of macrophage-derived inflammation by modulating NF-κB activity. Here we report that MKL1 regulates NF-κB-dependent pro-inflammatory transcription by moderating the crosstalk between WDR5 and BRG1.

Materials and methods

Cell culture

Human monocytic/macrophage-like cells (THP-1, ATCC) and human embryonic kidney fibroblast cells (HEK293, ATCC) were maintained in DMEM supplemented with 10% FBS. Mouse embryonic fibroblast (MEF) cells were isolated from wild type or MKL1 deficient mice[19]. Procedures involving animals were approved by the intramural Ethics Committee on Humane Treatment of Experimental Animals.

Plasmids, transfection, and reporter assay

Expression constructs for MKL1, WDR5, as well as promoter constructs have been described before[9].

siRNAs for MKL1 and WDR5 were purchased from Dharmacon. Transient transfections were performed with Lipofectamine LTX (Invitrogen). Luciferase activities were assayed 24-48 h after transfection using a luciferase reporter assay system (Promega). All experiments were repeated three times.

ChIP and Re-ChIP assay

ChIP assays were performed essentially as described before[20–21]. Aliquots of lysates containing 200 μg of nuclear protein were used for each immunoprecipitation reaction with anti-MKL1 (Santa Cruz), anti-BRG1 (Abcam), anti-WDR5 (Bethyl Laboratories), anti-dimethylated H3K4 (Millipore/Upstate), and anti-tri-methylated H3K4 (Millipore/Upstate). For re-ChIP, immune complexes were eluted with the elution buffer (1% SDS, 100mmol/L NaCO$_3$), diluted with the re-ChIP buffer (1% Triton X-100, 2 mmol/L EDTA, 150 mmol/L NaCl, 20 mmol/L Tris pH 8.1), and subject to immunoprecipitation with a second antibody of interest. All experiments were repeated three times.

RNA extraction and real-time PCR

RNA was extracted using an RNeasy RNA isolation kit (Qiagen). Reverse transcriptase reactions were performed using a SuperScript First-strand synthesis system (Invitrogen). Real-time PCR reactions were performed on an ABI STEPONE Plus (Life Tech) with primers and Taqman probes purchased from Applied Biosystems. All experiments were repeated three times.

In vitro HMT assay

In vitro histone methyltransferase assay was performed as described before[22]. Briefly, precipitated immune complex was mixed with histone H3 (Millipore), S-adenosyl methionine (SAM, Sigma), BSA, and MAB buffer (50 mmol/L Tris pH 8.5, 20 mmol/L KCl, 10 mmol/L MgCl$_2$, 10 mmol/L β-mercaptoethanol, 250 mmol/L sucrose). After incubation at 37°C overnight, SDS loading buffer was added to stop reactions, and the methylation of histone H3 was determined by Western blotting.

Statistical analysis

One-way ANOVA with post-hoc Scheffe analyses were performed using an SPSS package. *P* values smaller than 0.05 were considered statistically significant (*).

Results

MKL1 is associated with a histone H3K4 methyltransferase activity in macrophages

We have previously shown that MKL1 mediates LPS-

induced inflammatory response by influencing histone H3K4 methylation[9]. We asked whether MKL1 might be in a complex with an catalytically capable H3K4 methyltransferase in macrophages. To this end, we performed *In vitro* histone methyltransferase (HMT) assay. MKL1 was isolated from THP-1 cells by immunoprecipitation and incubated with recombinant histone H3 in the presence of the methyl donor SAM; RbBP5, a known component of the H3K4 methyltransferase complex[13], was included as a positive control whereas pre-immune IgG was included as a negative control. As shown in *Fig. 1A*, MKL1 was co-precipitated with an activity sufficient to catalyze H3K4 methylation. Re-ChIP assay showed that in response to TNF-α stimulation MKL1 was incorporated into a complex with WDR5, a well-defined component of the mammalian H3K4 methyltransferase complex[23], on the promoter regions of several pro-inflammatory mediators (*Fig. 1B*). Indeed, TNF-α treatment enhanced the occupancies of WDR5 on gene promoters in macrophages; MKL1 depletion with siRNA, however, significantly downregulated the binding of WDR5 (*Fig. 1C*), suggesting that MKL1 might play a role in recruiting WDR5 to the promoter regions of pro-inflammatory mediators. Of interest, we also observed that MKL1 was responsible for recruiting BRG1 (*Fig. 1D*), the catalytic subunit of the mammalian chromatin remodeling complex, and, to a lesser extent, BRM (*Fig. 1E*) to the gene promoters. Taken together, we conclude that MKL1 is associated with a histone H3K4 methyltransferase complex that includes WDR5, BRG1, and possibly BRM.

WDR5 cooperates with MKL1 to activate pro-inflammatory transcription

Next, we probed the involvement of WDR5 in TNF-α induced trans-activation of pro-inflammatory mediators in macrophages. As shown in *Fig. 2A*, WDR5 was a relatively weak activator of pro-inflammatory genes in reporter assays. In the presence of MKL1, however, WDR5 markedly enhanced the trans-activation of pro-inflammatory genes by TNF-α. Additionally, WDR5 cooperated with MKL1 to upregulate the activity of a κB reporter constructed by fusing two tandem repeats of the NF-κB response element to the luciferase gene (*Fig. 2B*). On the contrary, WDR5 knockdown by siRNA (*Fig. 2C* for efficiency) attenuated the induction of endogenous pro-inflammatory mediators by TNF-α (*Fig. 2D*). Collectively, these data suggest that WDR5 might play a role in mediating the activation of pro-inflammatory genes in response to TNF-α treatment in macrophages.

WDR5 and BRG1 regulate histone H3K4 methylation surrounding gene promoters

Next, we tackled the potential mechanism whereby WDR5 contributes to the regulation of pro-inflammatory transcription. Knockdown of WDR5 almost completely erased histone H3K4 di-methylation (*Fig. 3A*) and tri-methylation (*Fig. 3B*), two prominent markers for transcriptional activation[24], surrounding the proximal promoter regions of pro-inflammatory genes. Of note, BRG1 also appeared to play a role in the regulation of histone H3K4 methylation surrounding gene promoters because BRG1 knockdown achieved similar effects as WDR5 knockdown (*Fig. 3C–3D*), suggesting of a possible interplay between WDR5 and BRG1 in the regulation of pro-inflammatory transcription. Indeed, Re-ChIP assays showed that WDR5 formed a complex with BRG1 on the proximal promoter regions of pro-inflammatory genes in macrophages treated with TNF-α (*Fig. 4A*). Therefore, WDR5 might regulate TNF-α induced pro-inflammatory transcription by interacting with BRG1 to promote histone H3K4 methylation.

MKL1 mediates the crosstalk between WDR5 and BRG1

Finally, we asked whether MKL1 could broker the communication between WDR5 and BRG1 to regulate TNF-α induced pro-inflammatory transcription. Depletion of MKL1 with siRNA significantly weakened the interaction between WDR5 an BRG1 on the proximal promoter regions of pro-inflammatory genes (*Fig. 4B*). Similarly, the interaction between WDR5 and BRG1 on the proximal promoter regions of pro-inflammatory genes was much stronger in bone-marrow derived macrophages (BMDM) isolated from wild type (WT) mice than from MKL1 knockout (KO) mice (*Fig. 4C*). Finally, we found that co-expression of WDR5 and BRG1 enhanced the 2xκB reporter activity in WT mouse embryonic fibroblast (MEF) cells but not in KO MEF cells; re-introduction of ectopic MKL1 restored the cooperation between WDR5 and BRG1 (*Fig. 4D*). In summary, we propose that MKL1 could moderate the crosstalk between different epigenetic factors to stimulate TNF-α induced pro-inflammatory transcription.

Discussion

Epigenetic regulation of gene expression during the pathogenesis of human diseases remains an intriguing and everlasting area of research[25–27]. We present evidence here that MKL1 plays an essential role in
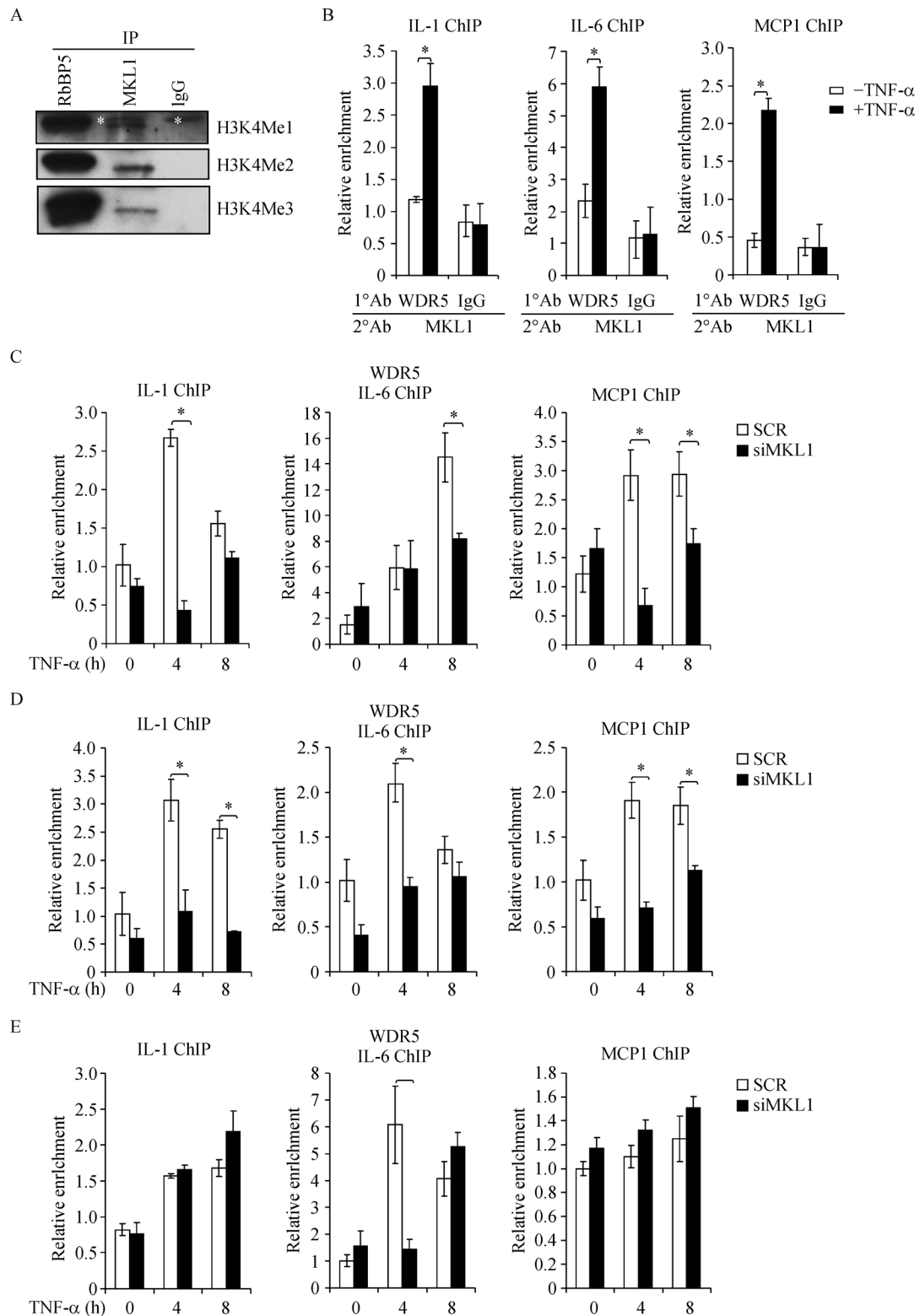
Fig. 1 **MKL1 is associated with histone H3K4 methyltransferase activity in macrophages.** A: Whole cell lysates were extracted from THP-1 cells. HMT assay was performed as described in Methods. White asterisk, non-specific band. B: THP-1 cells were treated with or without TNF-α for 12 hours. Re-ChIP assay was performed with indicated antibodies. C–D: THP-1 cells were transfected with siRNA targeting MKL1 or scrambled siRNA (SCR) followed by treatment with TNF-α for 12 hours. ChIP assay was performed with anti-WDR5 (C), anti-BRG1 (D), or anti-Brm (E).
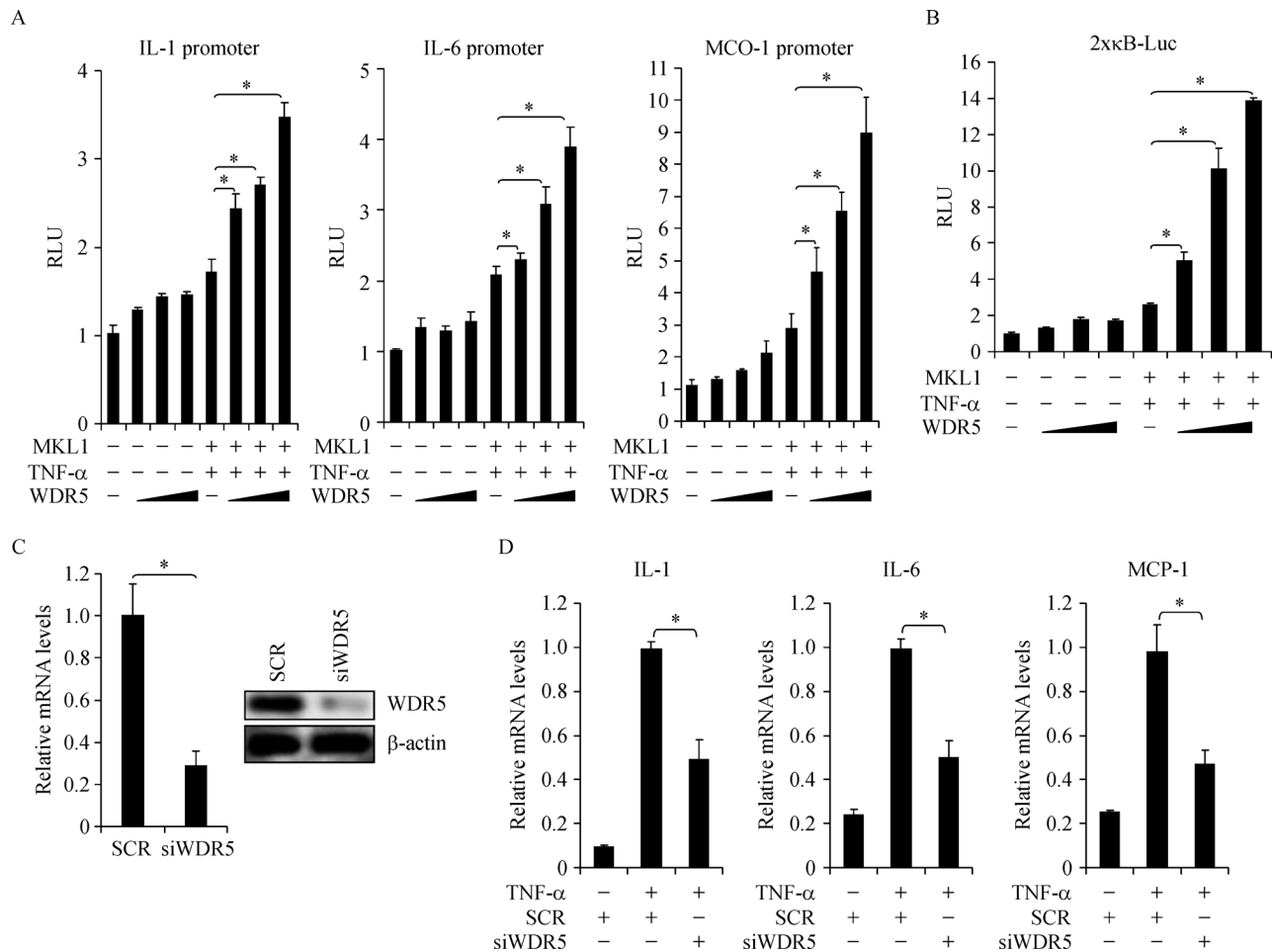
Fig. 2 WDR5 cooperates with MKL1 to activate pro-inflammatory transcription. A: Different promoter-luciferase constructs were transfected into HEK293 cells with MKL1 and/or WDR5 followed by treatment with TNF-α for 12 hours. Luciferase activities were normalized by both protein concentration and GFP fluorescence. B: A κB reporter was transfected into HEK293 cells with MKL1 and/or WDR5 followed by treatment with TNF-α for 12 hours. Luciferase activities were normalized by both protein concentration and GFP fluorescence. C: THP-1 cells were transfected with siRNA targeting WDR5 or scrambled siRNA (SCR). Knockdown efficiency was examined by qPCR and Western. D: THP-1 cells were transfected with siRNA targeting WDR5 or scrambled siRNA (SCR) followed by treatment with TNF-α for 12 hours. Expression of pro-inflammatory mediators was examined by qPCR.

bridging the dialog between BRG1, a chromatin remodeling protein, and WDR5, a subunit of the H3K4 methyltransferase complex, to stimulate the transcription of NF-κB dependent pro-inflammatory transcription. Several independent investigations have implicated BRG1 in the regulation of H3K4 methylation. For instance, Zager and Johnson have shown that during renal ischemia-reperfusion injury, enhanced binding of BRG1 to gene promoters is paralleled by progressive elevations of H3K4 methylation although it remains undetermined whether there is a cause-effect relationship[28]. Davis *et al* have reported that treatment of the BRG1 null SW-13 cells with HDAC inhibitors restores BRG1 expression and at the same time stimulates H3K4 hypermethylation[29].We have previously shown that BRG1 is essential in maintaining H3K4 methylation on the endothelin (ET-1) gene promoter, likely through interacting with WDR5, in

response to angiotensin II stimulation[22]. These data clearly establish a role for BRG1 in regulating locus-specific H3K4 methylation. It is not known at this point how genomewide H3K4 methylation patterns would be influenced by BRG1. In human embryonic stem cells, BRG1 has been found to co-occupy with monomethylated H3K4, catalyzed by the MLL3/4-containing COMPASS complex[30], on early developmental enhancers[31]. In cancer cells, loss-of-function mutation of BRG1 appears to influence genomewide distribution of H3K4 trimethylation although the mechanism is not clear[32]. Future studies should make an effort in deciphering the connection between BRG1 and H3K4 methylation in a cell- and context-dependent manner.

We show that WDR5 cooperates with BRG1 to stimulate NF-κB dependent pro-inflammatory transcription. Of note, WDR5 has been reported to form extensive communications with other epigenetic factors
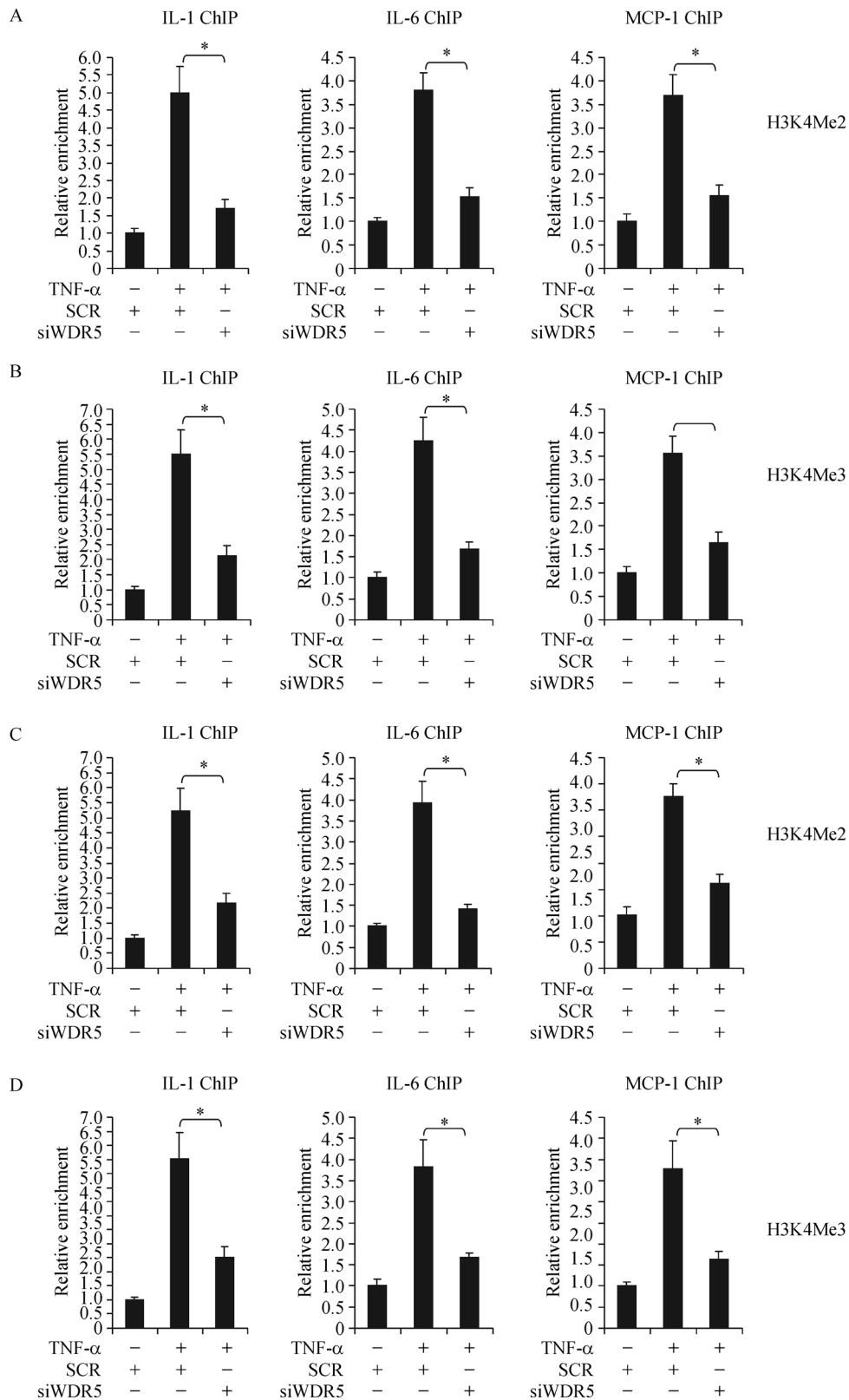
Fig. 3 **WDR5 and BRG1 regulate histone H3K4 methylation surrounding gene promoters.** A–B: THP-1 cells were transfected with siRNA targeting WDR5 or scrambled siRNA (SCR) followed by treatment with TNF-α for 12 hours. ChIP assay was performed with anti-H3K4Me2 (A) or anti-H3K4Me3 (B). C–D: THP-1 cells were transfected with siRNA targeting BRG1 or scrambled siRNA (SCR) followed by treatment with TNF-α for 12 hours. ChIP assay was performed with anti-H3K4Me2 (C) or anti-H3K4Me3 (D).
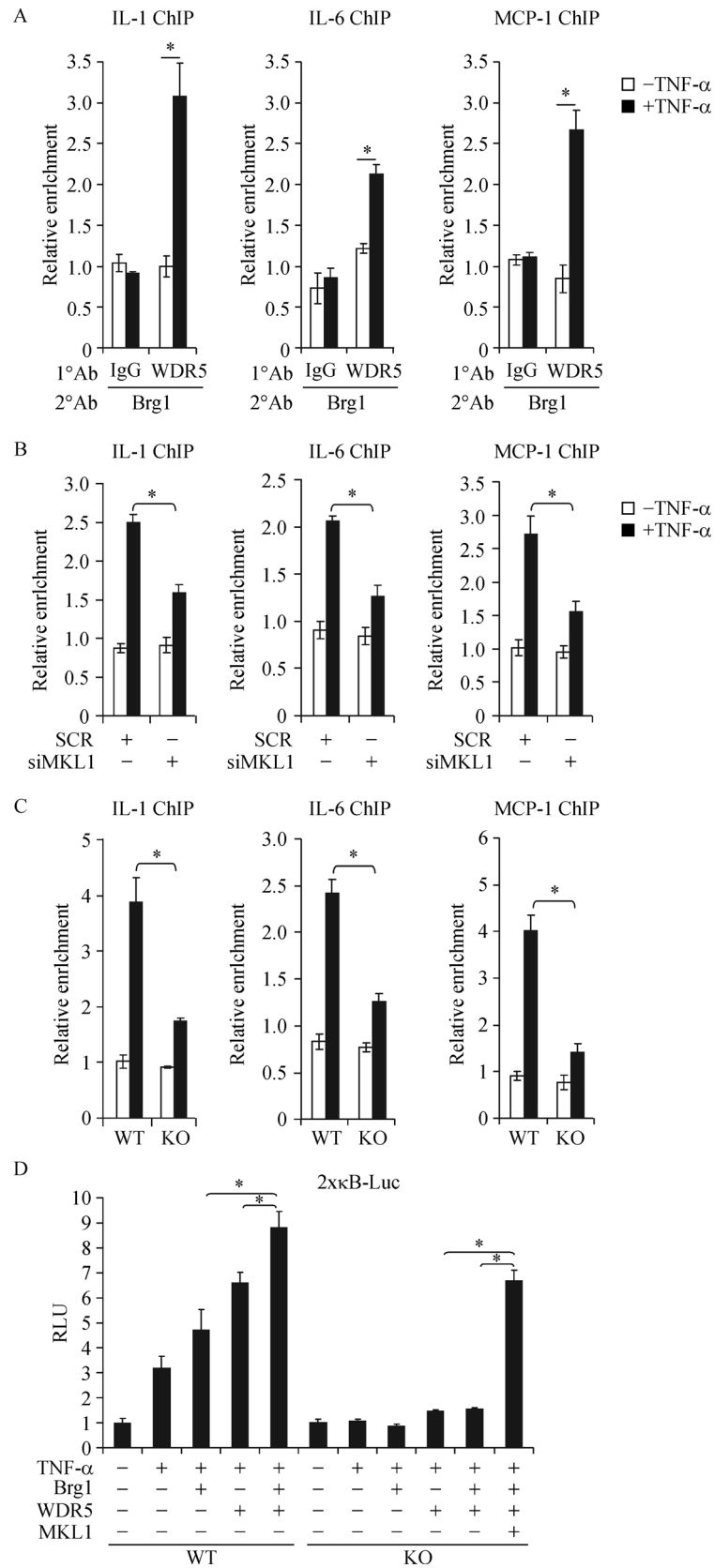
Fig. 4 **MKL1 mediates the crosstalk between WDR5 and BRG1.** A: THP-1 cells were treated with or without TNF-α for 12 hours. Re-ChIP assay was performed with indicated antibodies. B: THP-1 cells were transfected with siRNA targeting MKL1 or scrambled siRNA (SCR) followed by treatment with TNF-α for 12 hours. Re-ChIP assay was performed with indicated antibodies. C: MEF cells isolated from wild type (WT) or *MKL1* knockout (KO) mice were treated with or without TNF-α for 12 hours. Re-ChIP assay was performed with indicated antibodies. D: A κB reporter was transfected into WT or KO MEF cells with MKL1, BRG1, and/or WDR5 followed by treatment with TNF-α for 12 hours. Luciferase activities were normalized by both protein concentration and GFP fluorescence.

to regulate transcription. For instance, WDR5 can interact with the acetyltransferase p300 in cancer cells and renal epithelial cells to regulate p53-dependent[33] and SMAD-dependent[34] transcription, respectively. WDR5 can also collaborate with PRMT5, a histone arginine methyltransferase, to activate the transcription of class II major histocompatibility complex (MHC II) genes[35]. Of interest, the interaction between WDR5 and GClnc1, a long non-coding RNA (lncRNA), ensures its recruitment to a subset of pro-metastasis genes to activate transcription in gastric cancer cells[36], suggesting that non-protein factors could also contribute to the tethering of WDR5 to selective promoters although it is not clear whether these processes are independent of each other or act in concert. The dynamic interplay between WDR5 and other epigenetic factors likely reflect the extricate nature of transcriptional regulation, which deserves further investigation.

Our data suggest that MKL1 contributes to NF-κB dependent pro-inflammatory transcription by moderating the crosstalk between WDR5 and BRG1. Mounting evidence suggests that MKL1 relies heavily on the epigenetic machinery to regulate transcription[8–10,21–22,34,37–41]. In keeping with our finding, Yu et al. have demonstrated that MKL1 is responsible for the dissemination of H3K4 trimethylation on the promoter regions of genes involved in inflammation across the genome[42]. It remains unanswered whether this function of MKL1 relies on WDR5 and/or BRG1. ChIP-seq analysis of MKL1 in macrophages has found that in addition to NF-κB, MKL1 also preferentially binds to the target promoters of SRF, STAT, and IRF1, all of which are key regulators of inflammatory response[43]. It would be interesting to determine whether MKL1 exploits a similar strategy to stimulate the transcription of those target genes.

In summary, we present evidence to show that MKL1 mediates TNF-α induced pro-inflammatory transcription by bridging the crosstalk between BRG1 and WDR5. A recent study has found that small peptides that disrupt the interaction between WDR5 and MLL1/SET1 specifically inhibits the activity of MLL1/SET1-containing COMPASS[44]. It is possible to employ this strategy to interfere with the MKL1-WDR5-BRG1 interaction in order to control cellular inflammatory response in an attempt to treat inflammation-related diseases.

Acknowledgements

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