BBA - Molecular Basis of Disease 1865 (2019) 2551–2561

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

BRG1 regulates endothelial-derived IL-33 to promote ischemia-reperfusion induced renal injury and fibrosis in mice

Li Liu[a,1], Lei Mao[a,1], Xiaoyan Wu[b,1], Teng Wu[a,c,1], Wenjin Liu[d], Yuyu Yang[e], Tao Zhang[f], Yong Xu[a,c,*]

[a] Key Laboratory of Targeted Intervention of Cardiovascular Disease and Collaborative Innovation Center for Cardiovascular Translational Medicine, Department of Pathophysiology, Nanjing Medical University, Nanjing, China
[b] The Laboratory Center for Basic Medical Sciences, Nanjing Medical University, Nanjing, China
[c] Institute of Biomedical Research, Liaocheng University, Liaocheng, China
[d] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China
[e] Jiangsu Key Laboratory for Molecular and Medical Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing, China
[f] Department of Geriatric Nephrology, Jiangsu Province Hospital, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

ARTICLE INFO

Keywords:
Acute kidney injury
Brahma related gene 1
Epigenetics
Renal fibrosis
Transcriptional regulation

ABSTRACT

Endothelial-derived factors regulate a wide range of pathophysiological events. It has been reported previously that IL-33 promotes acute kidney injury (AKI) in mice although the underlying epigenetic mechanism is unclear. In the present study we investigated the role of BRG1, a chromatin remodeling protein, in AKI with a focus on its regulation of IL-33 expression in endothelial cells. *Smarca4*-flox mice were crossbred with *Cdh5*-Cre mice to achieve endothelial-specific deletion of BRG1. AKI was induced by unilateral renal ischemia followed by reperfusion. Compared to wild type (WT) littermates, endothelial conditional BRG1 knockout (CKO) mice were protected from ischemia-reperfusion induced AKI as evidenced by decreased plasma creatinine levels, attenuated caste and tubular necrosis, and diminished immune infiltrates. CKO mice also developed less severe renal fibrosis as indicated by expression levels of extracellular matrix proteins, picrosirius red staining of collagenous tissues, and quantification of renal hydroxylproline levels. Of interest, renal expression of IL-33 was down-regulated as result of endothelial BRG1 deficiency. In cultured endothelial cells, BRG1 directly bound to the IL-33 promoter to activate transcription. Endothelial cell-derived conditioned media promoted the synthesis of pro-fibrogenic proteins in renal tubular epithelial cells. Knockdown of either BRG1 or IL-33 in endothelial cells blunted the pro-fibrogenic response in renal tubular epithelial cells. In conclusion, we propose that BRG1 may contribute to ischemia-reperfusion induced renal injury and fibrosis by promoting IL-33 transcription in endothelial cells.

1. Introduction

The vascular endothelial cells play versatile roles in a myriad of physiological and pathophysiological processes. Typically, the endothelium acts as a physical barrier separating the basal laminae from the circulation preventing inadvertent activation of the clotting pathway and thrombosis. The endothelial function extends beyond being a static architecture and is encapsulated by an array of bioactive substances produced and secreted by endothelial cells. For instance, rhythmic contraction and relaxation of the vessels rely on endothelial-derived vasoconstrictive and vasodilative factors such as NO and endothelin. Accordingly, mice with a deficiency in eNOS, the enzyme responsible for NO synthesis, develop spontaneous hypertension [1]. The notion that the endothelium may function as an endocrine (or more precisely angiocrine) organ is further exemplified by a string of recent findings that show the regulatory role endothelial cell-derived factors exert on tissue injury, regeneration, and fibrosis [2].

Interleukin 33 (IL-33) is immunomodulatory cytokine primarily expressed in endothelial cells [3]. IL-33 mediated cellular responses, *via* its trans-membrane receptor ST2R, are implicated in the pathogenesis of a host of human diseases [4]. Despite extensive research, it remains controversial whether IL-33 confers protection against or exacerbates renal injuries. For instance, it has been shown that IL-33 administration aggravates while IL-33 neutralization attenuates cisplatin induced acute kidney injury (AKI) in mice [5]. Contradictorily, genetic deletion of IL-33 in mice offers no protection against cisplatin induced AKI [6]. Serum

* Corresponding authors at: Jiangsu Province Hospital, 300 Guangzhou Rd, Nanjing 210009, China.
E-mail addresses: zht779100@njmu.edu.cn (T. Zhang), yjxu@njmu.edu.cn (Y. Xu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbadis.2019.06.015
Received 2 February 2019; Received in revised form 30 May 2019; Accepted 17 June 2019
Available online 19 June 2019
0925-4439/

IL-33 levels correlate with the development of several distinctive renal pathologies in humans and in animal models, including renal carcinoma [7], diabetic nephritis [8], systemic lupus erythematosus [9], and AKI [10–13]. In most cases, IL-33 message levels have been found to be similarly up-regulated during renal injuries although the underlying mechanism is not fully appreciated.

Brahma related gene 1, or BRG1, is the catalytic component of the mammalian chromatin modeling complex. BRG1 is essential for vascular development possibly by epigenetically regulating the Wnt/β-catenin pathway [14]. It has been demonstrated that BRG1 is not essential for postnatal endothelial survival under physiological conditions although accumulating evidence suggests that BRG1 plays indispensable roles in endothelial dysfunction contributing to the pathogenesis of cardiac hypertrophy [15], cardiac ischemia-reperfusion injury [16], and abdominal aortic aneurysm [17]. Of interest, the ability of BRG1 to regulate endothelial dysfunction appears to be mediated by endothelial-derived humoral factors such as endothelin (ET-1) [15] and colony stimulating factor (CSF1) [17]. Here we report that endothelial-specific deletion of BRG1 in mice are protected from renal ischemia-reperfusion injury. BRG1 contributes to acute kidney injury possibly by interacting with HIF-1α to epigenetically activating the transcription of IL-33 in endothelial cells.

2. Results

2.1. Endothelial BRG1 deficiency attenuates AKI in mice

To induce acute kidney injury (AKI), endothelial conditional BRG1 knockout (CKO) mice and wild type (WT) littermates were subjected to unilateral renal ischemia for 30 min followed by reperfusion (IR). The mice were sacrificed 48 h after the surgical procedure to evaluate renal injuries. Immunofluorescence staining confirmed that BRG1 levels were significantly down-regulated in endothelial cells in the CKO kidneys compared to the WT kidneys (Fig. S1). Plasma creatinine levels were significantly elevated following IR in mice, but the increase was less prominent in CKO mice than in WT mice (Fig. 1A). There was a similar down-regulation of plasma BUN levels in CKO mice compared to WT mice (Fig. 1B). H&E staining showed that IR procedure provoked severe renal injuries including extensive tubular necrosis and caste; the injuries were much milder in CKO mice than in WT mice (Fig. 1C). We measured the expression of HAVCR (Fig. 1D) and LCR2 (Fig. 1E), two biomarkers of renal injury, in both WT and CKO mice; BRG1 deficiency alleviated the induction of HAVCR and LCR2 consistent with the decrease of AKI. TUNEL assay confirmed that renal tubular apoptosis was alleviated in mice as a result of endothelial BRG1 deletion (Fig. 1F). Finally, renal reactive oxygen species (ROS) levels were measured by DHE staining (Fig. 1G) and DCFH-DA staining (Fig. 1H). Compared to WT mice, CKO mice displayed lower ROS levels in the kidneys. In accordance, expression levels of $p47^{phox}$ and $p67^{phox}$, two members of the NADPH oxidase complex, were down-regulated in the CKO kidneys; Nrf2, an antioxidant protein, was not significantly altered (Fig. 1I). Of note, BRG1 expression was up-regulated in endothelial cells as early as 12 h after the renal ischemia-reperfusion injury compared to the control group; up-regulation of BRG1 expression in endothelial cells persisted at 24 h and 48 h after the procedure (Fig. S2). Combined, these data demonstrate that endothelial BRG1 deficiency attenuates AKI in mice.

2.2. Endothelial BRG1 deficiency ameliorates renal inflammation

Inflammation is both a consequence and a promoter of AKI. We next examined the effect of endothelial BRG1 deficiency on renal inflammation. Immunohistochemical stainings showed that there were significantly higher numbers of immune infiltrates in the kidneys in WT mice following the IR procedure, including $CD3^+$ T lymphocytes (Fig. 2A), $CD68^+$ macrophages (Fig. 2B), and $CD45^+$ leukocytes (Fig. 2C), suggesting a heightened inflammatory response; the increase in immune infiltrates was collectively suppressed by endothelial BRG1 deficiency. In addition, quantitative PCR analyses revealed that AKI induced by IR procedure up-regulated a panel of pro-inflammatory mediators, including IFN-γ (Fig. 2D), IL-1β (Fig. 2E), IL-6 (Fig. 2F), and TNF-α (Fig. 2G), in the kidneys in WT mice. The elevation of renal pro-inflammatory mediators was less robust in CKO mice compared to WT mice. The decrease in immune cell infiltration and consequently production of pro-inflammatory mediators in the CKO kidneys, we suspect, might be due to the down-regulation of adhesion molecules, including ICAM-1 (Fig. 2H), VCAM-1 (Fig. 2I), and E-selectin (Fig. 2J), which is consistent with our previously published data showing that BRG1 is essential for the induction of adhesion molecules in endothelial cells in response to pro-inflammatory stimuli [18,19].

2.3. Endothelial BRG1 deficiency dampens renal fibrosis following AKI

Next, we gauged the long-term effect of endothelial BRG1 deficiency on AKI. There was extensive renal fibrosis 2wk after the IR procedure as evidenced by increased expression levels of several pro-fibrogenic molecules including collagen type I (*Col1a1*, Fig. 3A), collagen type III (*Col1a3*, Fig. 3B), and smooth muscle cell actin (*Acta2*, Fig. 3C) in the kidneys. Endothelial deletion of BRG1 weakened the induction of pro-fibrogenic gene expression. Further, picrosirius staining (Fig. 3D) and Masson's trichrome staining (Fig. 3E) confirmed that renal fibrosis was not as widespread in CKO mice as in WT mice, indicative of a diminished fibrogenic response. Additionally, quantification of renal hydroxylproline levels revealed that there were fewer collagenous tissues in CKO kidneys than in WT kidneys (Fig. 3F).

2.4. BRG1 activates IL-33 transcription in vascular endothelial cells

IL-33 is a pleiotropic cytokine with both detrimental and protective roles. Recent studies suggest that IL-33 is predominantly expressed in endothelial cells and that IL-33 promotes AKI in mice by regulating inflammatory response and fibrosis [5,20]. Now that we observed a decrease of inflammation and fibrosis in the kidneys in CKO mice compared to WT mice (Fig. 2A), we hypothesized that BRG1 might be necessary for IL-33 transcription in endothelial cells. Quantitative PCR analyses confirmed that IL-33 expression was indeed down-regulated in the kidneys in CKO mice at both 48 h (Fig. 4A) and 2wk (Fig. 4B) after the onset of AKI. In cultured endothelial cells, hypoxia-reoxygenation (HR) potentiated the production of IL-33; over-expression of wild type (WT) BRG1 but not enzyme deficient (ED) BRG1 further enhanced IL-33 induction (Fig. 4C). On the contrary, BRG1 knockdown by two separate pairs of siRNAs attenuated IL-33 induction by HR (Fig. 4D). We then transfected an IL-33 promoter-luciferase construct into endothelial cells. HR stimulation strongly activated the IL-33 promoter activity, which was further enhanced by over-expression of WT BRG1 but not ED BRG1 (Fig. 4E), suggesting that regulation of IL-33 by BRG1 likely occurred at the transcriptional level.

Previously it has been shown that hypoxia inducible factor 1 (HIF-1α) regulates IL-33 transcription by binding to its proximal promoter [21]. Immunoprecipitation assay showed that HIF-1α formed a complex with BRG1 (Fig. 4F). Co-expression of HIF-1α and BRG1 synergistically activated the IL-33 promoter activity in endothelial cells (Fig. 4G). More importantly, HR promoted the binding of BRG1 to the HIF site on the IL-33 promoter, which was abrogated by HIF-1α knockdown (Fig. 4H, I). Together, these data suggest that BRG1 might contribute to IL-33 trans-activation by interacting with HIF-1α.

2.5. BRG1 contributes to IL-33 transcription by modulating histone modifications

We next explored the potential epigenetic mechanism(s) whereby BRG1 might regulate IL-33 transcription. In response to hypoxia-reoxygenation, much higher levels of histone H3 acetylation (AcH3) were

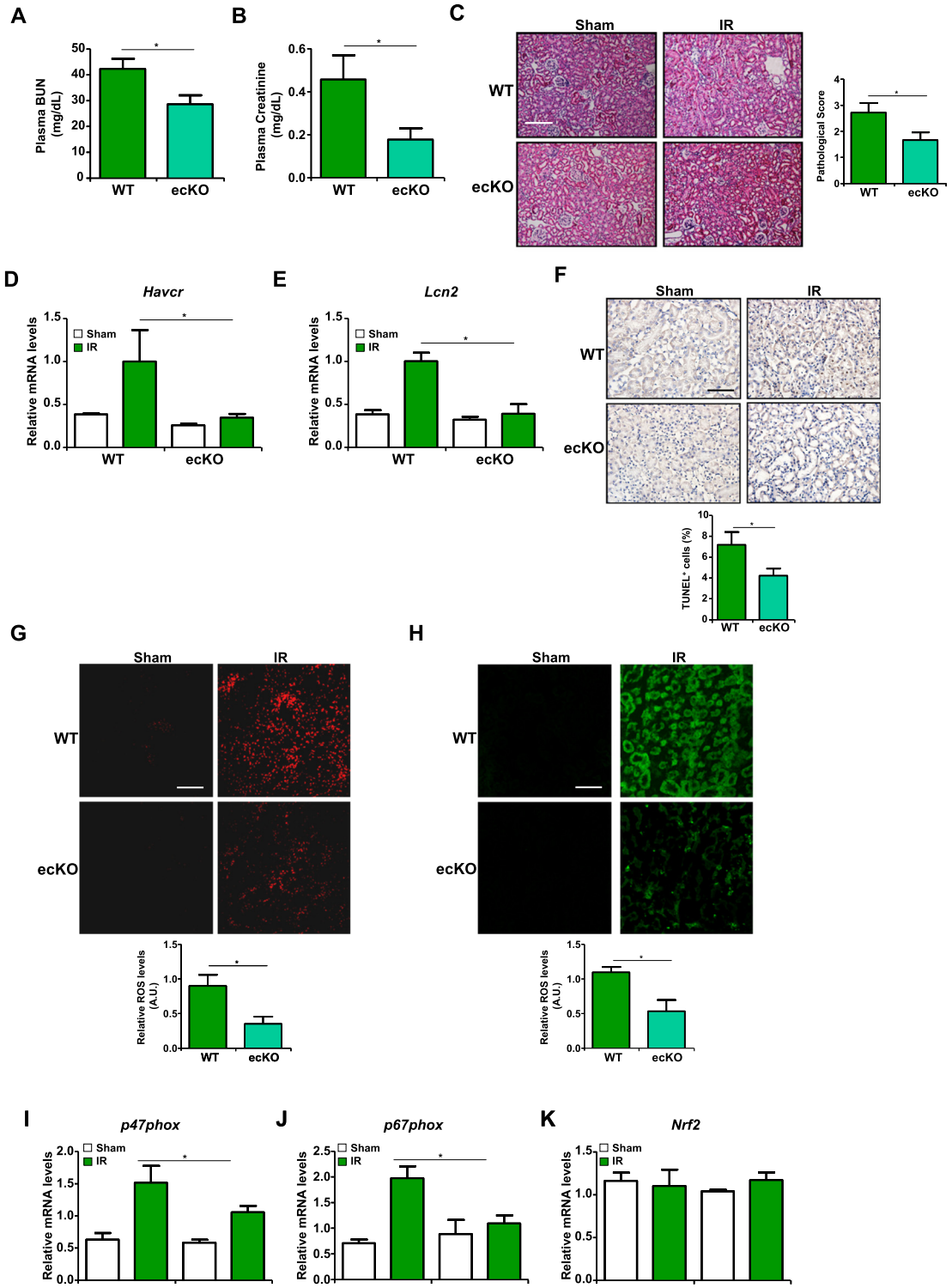
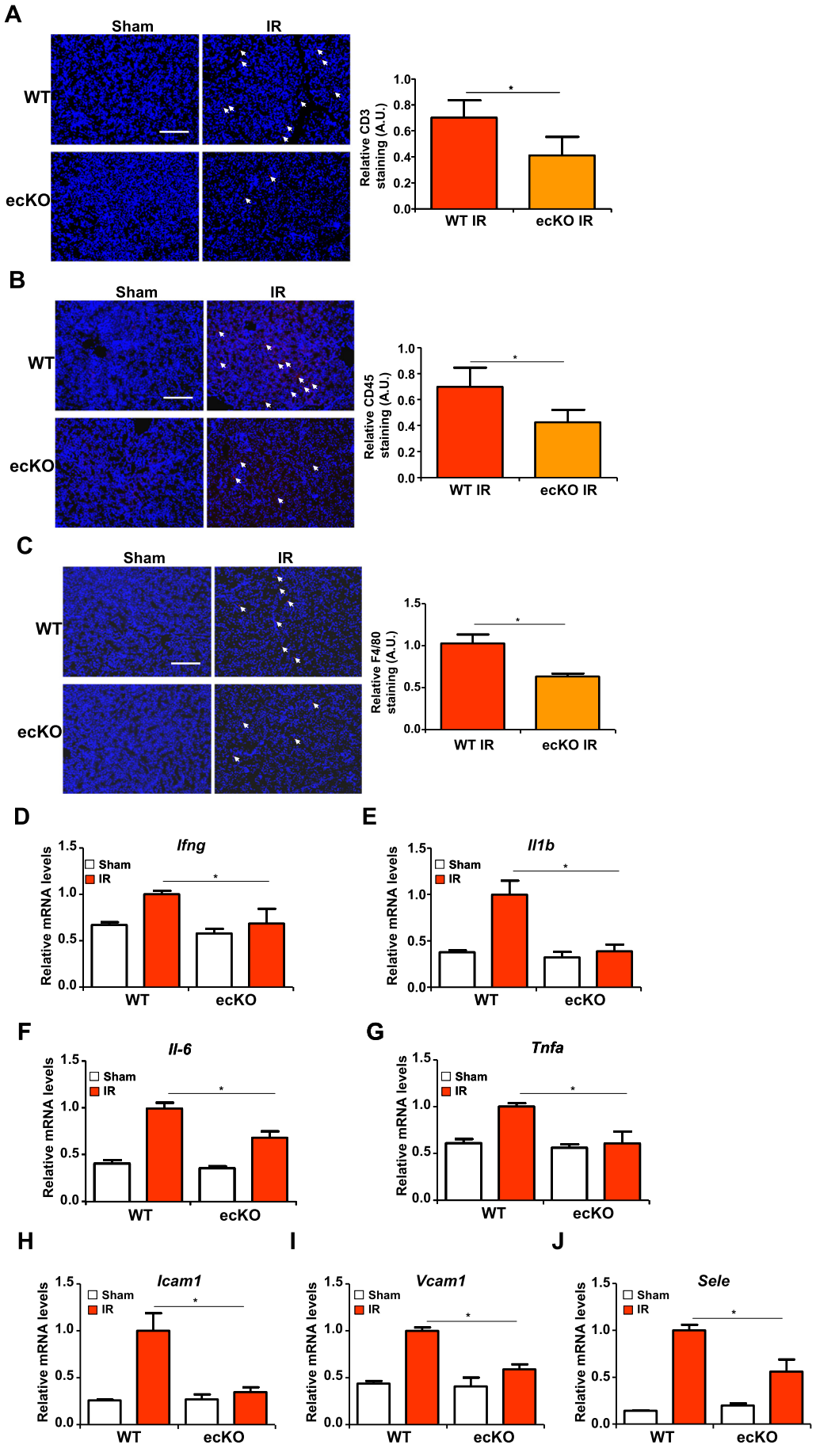


Fig. 1. Endothelial BRG1 deficiency attenuates AKI in mice. (A–K) CKO mice and WT mice were subjected to the IR procedure as described in Methods. The mice were sacrificed at 48 h following the surgery. Plasma BUN (A) and creatinine (B) levels. (C) H&E staining of paraffin sections. Pathological grading was performed as described in Methods. Expression levels of Havcr1 (D) and Lcn2 (E) were examined by qPCR. (F) Renal tubular apoptosis was examined by TUNEL. (G, H) ROS levels were measured by DHE and DCFH-DA staining and quantified with Image J. Expression levels of p47 (I), p67 (J), and Nrf2 (K) were examined by qPCR. N = 6 mice for each group.

detected on the IL-33 promoter region compared to the normoxia condition indicative of active transcription; BRG1 depletion, however, significantly dampened the accumulation of AcH3 on the IL-33 promoter but not the GAPDH promoter (Fig. 5A). Likewise, BRG1 appeared to be essential for the enrichment of trimethylated H3K4 (H3K4Me3), another marker for actively transcribed chromatin, on the IL-33 promoter (Fig. 5B). On the contrary, the levels of dimethylated H3K9 (H3K9Me2), typically associated with repressive chromatin, were observed to decrease on the IL-33 promoter in HR-treated cells compared to normoxia-treated cells and BRG1 knockdown restored H3K9Me2



(caption on next page)

Fig. 2. Endothelial BRG1 deficiency ameliorates renal inflammation. (A-J) CKO mice and WT mice were subjected to the IR procedure as described in Methods. The mice were sacrificed at 48 h following the surgery. Paraffin embedded sections were stained with anti-CD3 (A), anti-CD45, or anti-F4/80 (C) and quantified by Image Pro. Expression levels of IFN-γ (D), IL-1β (E), IL-6 (F), TNF-α (G), ICAM-1 (H), VCAM-1 (I), and E-selectin (J) were examined by qPCR. N = 4–5 mice for each group.

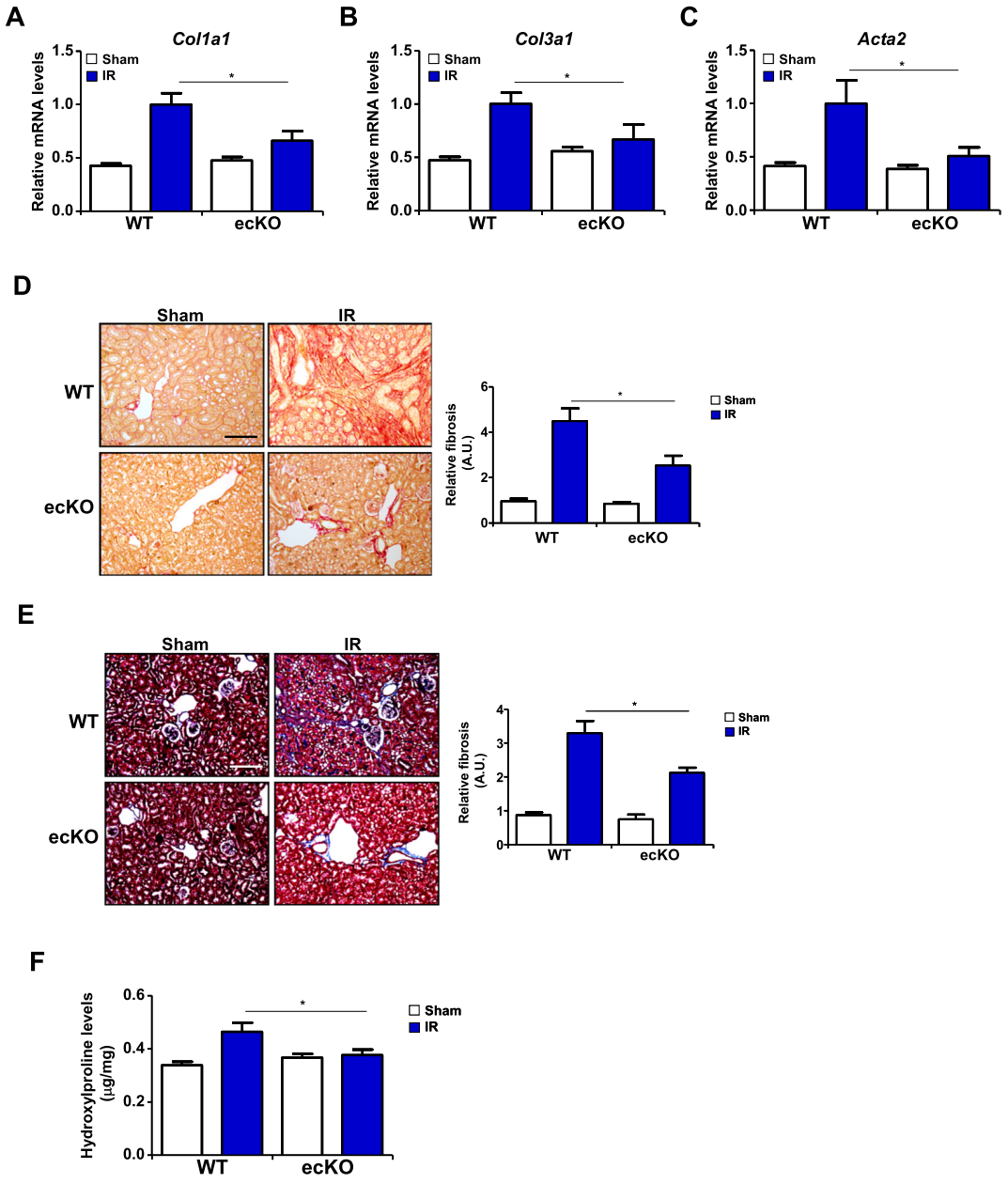


Fig. 3. Endothelial BRG1 deficiency dampens renal fibrosis following AKI. (A–F) CKO mice and WT mice were subjected to the IR procedure as described in Methods. The mice were sacrificed at 2w following the surgery. Expression levels of collagen type I (A), collagen type III (B), and α-SMA (C) were examined by qPCR. Paraffin sections were stain with picrosirius red (D) and Massons' trichrome (E) and quantified with Image Pro. (F) Renal hydroxylproline levels. N = 6 mice for each group.

levels (Fig. 5C). In accordance, occupancies of p300, an H3 acetyltransferase (Fig. 5D), ASH2, a regulatory subunit of the H3K4 methyltransferase complex (Fig. 5E), and KDM3A, an H3K9 demethylase (Fig. 5F), were found to be dependent on BRG1 as BRG1 silencing down-regulated the recruitment of all three histone modifying proteins to the IL-33 promoter. Together, these data suggest that BRG1 may contribute to IL-33 transcription by modulating histone modifications.

2.6. BRG1 mediates a crosstalk between endothelial cells and tubular epithelial cells

Finally we tackled the question as to whether endothelial-derived, BRG1-dependent production of IL-33 may elicit a pro-fibrogenic response in renal tubular epithelial cells (HK-2) *via* inter-cellular crosstalk. To this end, vascular endothelial cells were subjected to normoxia or hypoxia-reoxygenation and the conditioned media (CM) were collected to treat HK-2 cells. CM collected from endothelial cells exposed to hypoxia-reoxygenation indeed induced a pro-fibrogenic response as indicated by augmented expression of α-SMA (*ACTA2*) and collagen type I (*COL1A1*) in HK-2 cells (Fig. 6A). MTT assay showed that there was an increase in cell death when HK-2 cells were exposed to the hypoxia-reoxygenation CM (Fig. 6B). Over-expression of BRG1 in endothelial cells strongly enhanced the pro-fibrogenic and pro-death capacity of the hypoxia-reoxygenation CM. BRG1 knockdown in

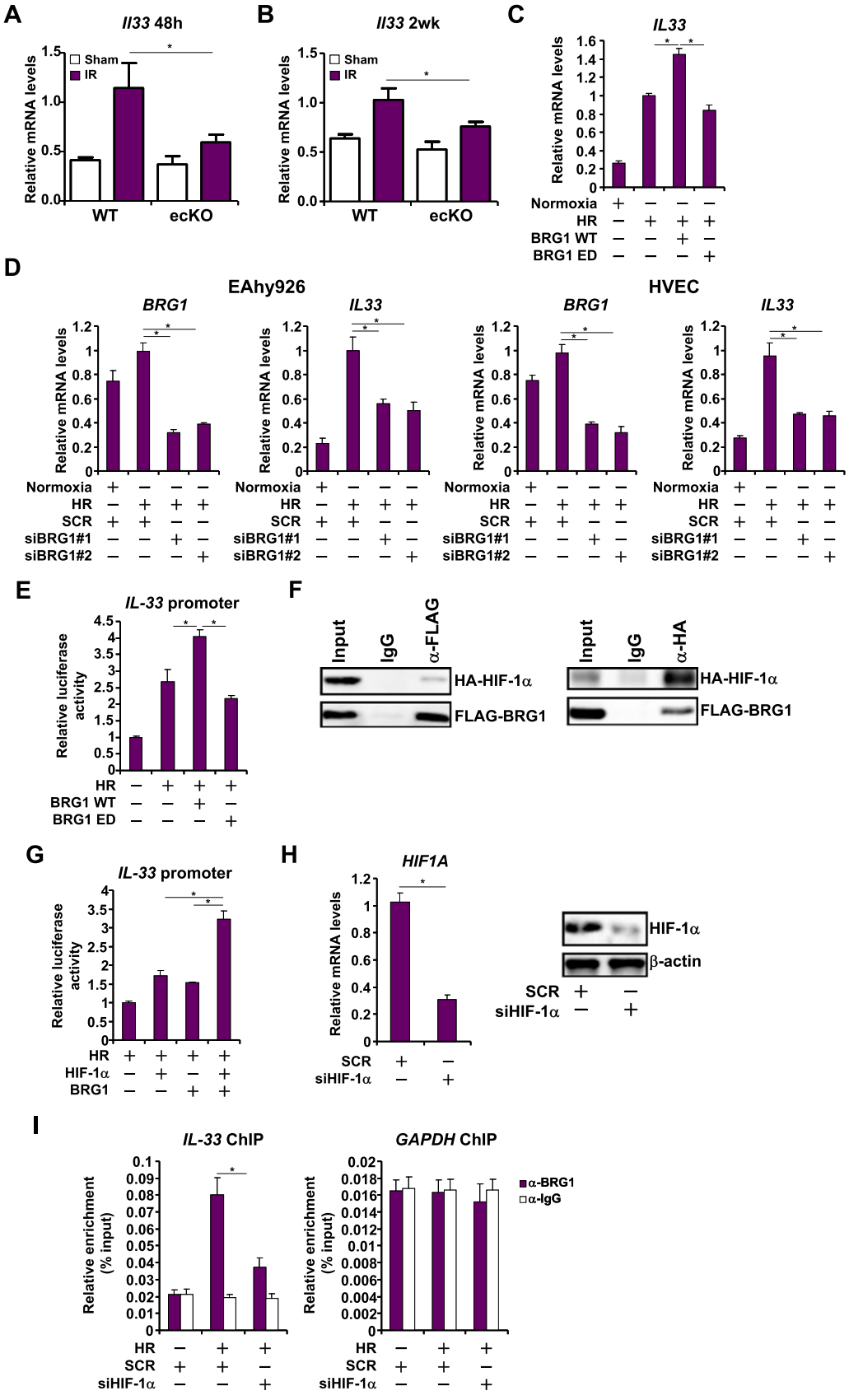


Fig. 4. BRG1 activates IL-33 transcription in vascular endothelial cells. (A, B) CKO mice and WT mice were subjected to the IR procedure as described in Methods. The mice were sacrificed at 48 h (A) and 2w (B) following the surgery. Renal expression of IL-33 was examined by qPCR. (C) Wild type (WT) or enzyme deficient (ED) BRG1 was transfected into EAhy926 cells followed by hypoxia-reoxygenation. Gene expression levels were examined by qPCR. (D) EAhy926 cells and HVECs were transfected with small interfering RNA targeting BRG1 or scrambled siRNA (SCR) followed by hypoxia-reoxygenation. Gene expression levels were examined by qPCR. (E) An IL-33 promoter-luciferase construct was transfected into EAhy926 cells with WT BRG1 or ED BRG1 followed by hypoxia-reoxygenation. Luciferase activities were normalized by both protein concentration and GFP fluorescence. (F) HA-tagged HIF-1α and FLAG-tagged BRG1 were transfected into HEK293 cells. Immunoprecipitation assays were performed with indicated antibodies. (G) An IL-33 promoter-luciferase construct was transfected into EAhy926 cells with indicated expression constructs followed by hypoxia-reoxygenation. Luciferase activities were normalized by both protein concentration and GFP fluorescence. (H, I) EAhy926 cells were transfected with small interfering RNA targeting HIF-1α or SCR followed by hypoxia-reoxygenation. Knockdown efficiency was examined by Western. ChIP assays performed with indicated antibodies.

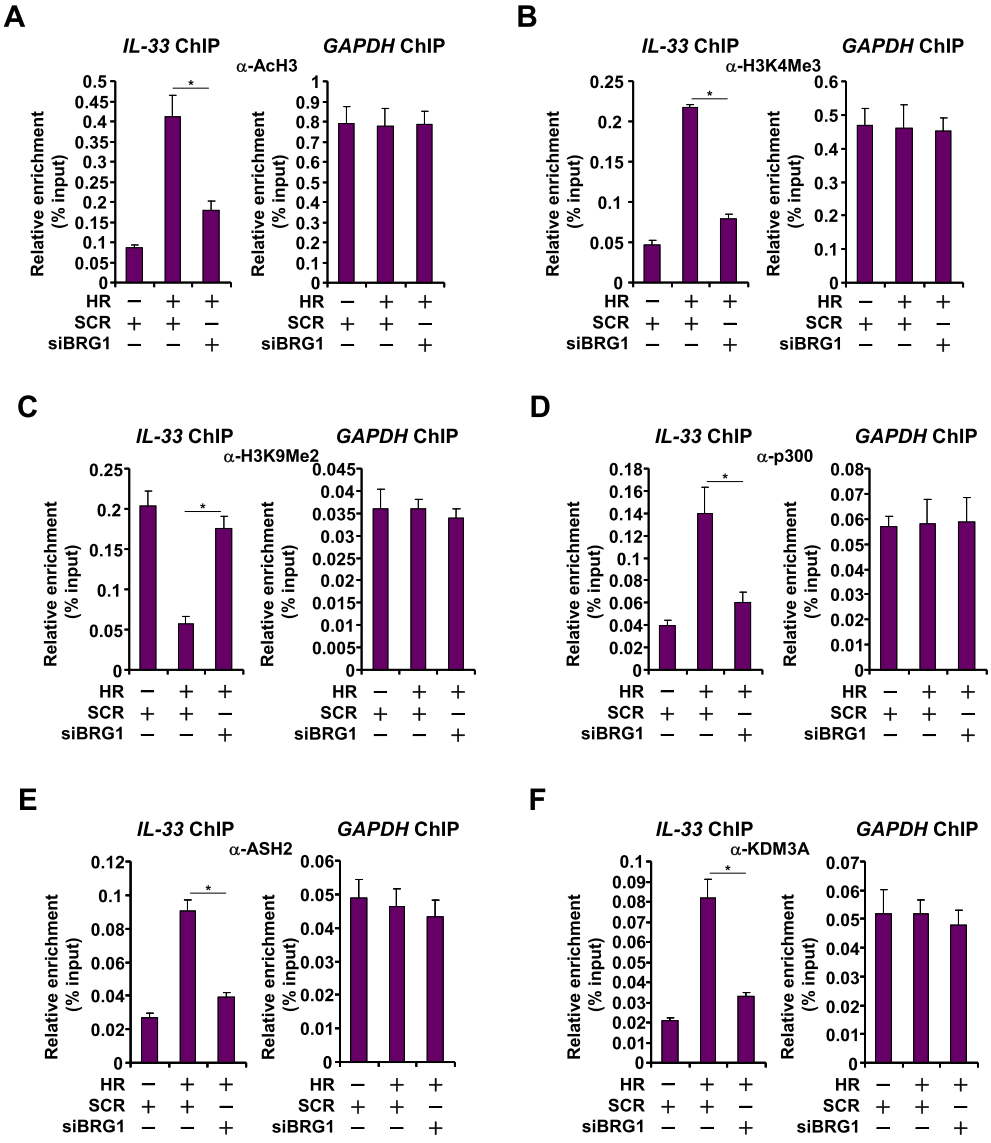


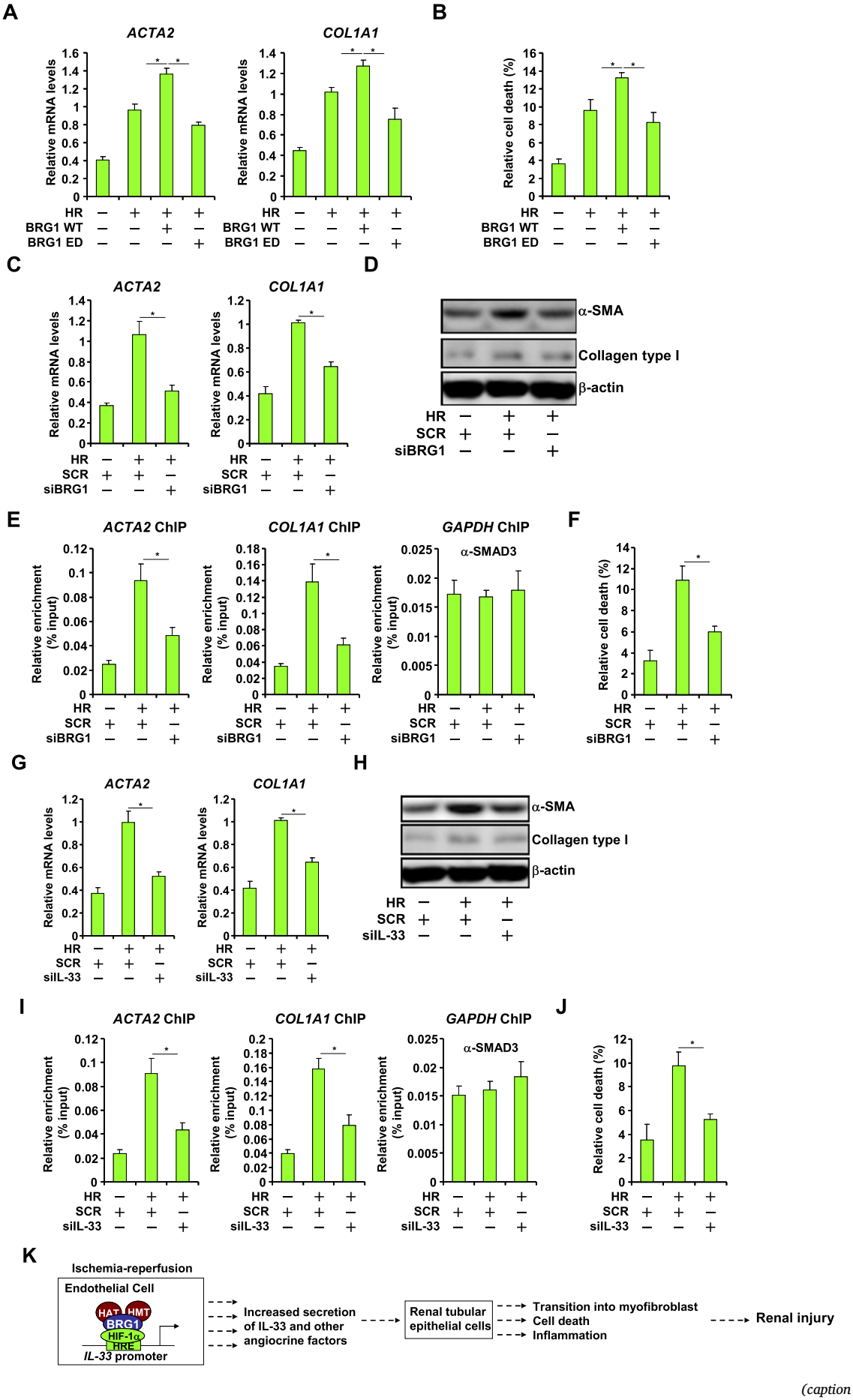
Fig. 5. BRG1 contributes to IL-33 transcription by modulating histone modifications. (A–F) EAhy926 cells were transfected with small interfering RNA targeting BRG1 or SCR followed by hypoxia-reoxygenation. ChIP assays were performed with acetyl H3 (A), trimethyl H3K4 (B), dimethyl H3K9 (C), p300 (D), ASH2 (E), and KDM3A (F).

endothelial cells, on the other hand, significantly weakened the expression of pro-fibrogenic genes in HK-2 cells (Fig. 6C, D). Of interest, HK-2 treated with the hypoxia-reoxygenation CM displayed stronger binding of SMAD3, a well-established pro-fibrogenic transcription factor, to the *ACTA2* promoter and the *COL1A1* promoter, which was blunted by BRG1 silencing (Fig. 6E). CM collected from BRG1-depleted endothelial cells provoked less cell death in HK-2 cells (Fig. 6F). Likewise, IL-33 depletion negated the evocation of a pro-fibrogenic response by endothelial-derived CM in HK-2 cells, as demonstrated by the reduction of α-SMA and collagen type I expression (Fig. 6G, H), dampened SMAD3 binding to target promoters (Fig. 6I), and amelioration of cell death (Fig. 6J). Collectively, these data suggest that BRG1 may broker a crosstalk between endothelial cells and renal tubular epithelial cells, possibly *via* IL-33, to promote renal fibrosis.

3. Discussion

Endothelial-derived cues are instrumental in regulating tissue injury and repair. These signals, in the form of secreted and diffusive polypeptides free of coating, are often termed angiocrine signals to reflect their endothelial origin [2]. IL-33 is an endothelial-derived cytokine that has been implicated in renal inflammation and fibrosis [5,20]. We have previously shown that the chromatin remodeling protein BRG1 possesses the unique ability to regulate the transcription of an array of angiocrine factors thereby contributing to the pathogenesis of pathological hypertrophy [15] and abdominal aortic aneurysm [17]. Here we present evidence to demonstrate that BRG1 links IL-33 trans-activation in endothelial cells to renal ischemia-reperfusion injury (Fig. 6K).

We show that endothelial BRG1 deficiency alleviates renal inflammation following ischemia-reperfusion in mice with a concomitant reduction in IL-33 levels. This observation is consistent with previous reports demonstrating that IL-33 administration augmented while IL-33 deficiency attenuated renal inflammation in mice subjected to AKI [5,22]. Of intrigue, it has been observed that IL-33 treatment suppresses the production of pro-inflammatory cytokines in cultured macrophages *in vitro* [23]. In addition, deletion of the IL-33 receptor ST2 in mice dampens the polarization of alternatively activated macrophages (AAM) [24] suggesting that IL-33 may be a multifaceted factor capable of both promoting and antagonizing pro-inflammatory response in a context-dependent manner. The uncertainty regarding the role of IL-33



(caption on next page)

Fig. 6. BRG1 mediates a crosstalk between endothelial cells and tubular epithelial cells. (A, B) WT BRG1 or ED BRG1 was transfected into EAhy926 cells followed by hypoxia-reoxygenation. Conditioned media were collected to treat renal epithelial cells. Conditioned media were collected to treat renal epithelial cells. Gene expression levels were examined by qPCR. Cell viability was evaluated by MTT assay. (C–F) EAhy926 cells were transfected with small interfering RNA targeting BRG1 or SCR followed by hypoxia-reoxygenation. Conditioned media were collected to treat renal epithelial cells. Gene expression levels were examined by qPCR and Western. ChIP assay was performed with anti-SMAD3. Cell viability was evaluated by MTT assay. (G–J) EAhy926 cells were transfected with small interfering RNA targeting IL-33 or SCR followed by hypoxia-reoxygenation. Conditioned media were collected to treat renal epithelial cells. Gene expression levels were examined by qPCR and Western. ChIP assay was performed with anti-SMAD3. Cell viability was evaluated by MTT assay. (K) A schematic model. In response to ischemia-reperfusion, HIF-1α recruits BRG1 to the IL-33 promoter along with histone acetyltransferases (HAT) and histone methyltransferases (HMT) to activate IL-33 transcription. Production and secretion of IL-33 (and possibly other angiocrine factors) from endothelial cells consequently creates a pro-injury microenvironment that leads to increased immune infiltrates and inflammation, renal tubular cell apoptosis, and transition of epithelial cell into myofibroblast, which collectively contribute to renal injury.

in cellular inflammation is mirrored by its regulation of renal injuries because there remain significant controversies as to whether IL-33 protects against or exacerbates the loss of renal functions following various injurious stimuli. It should be noted that BRG1 may contribute to the regulation of AKI in an IL-33-independent manner. For instance, BRG1 is an activator of intercellular adhesion molecule 1 (ICAM-1) in endothelial cells [18,19], whose deficiency protects the mice from endotoxin induced acute renal failure [25]. It is thus mostly likely that a panel of BRG1-dependent factors collectively mediate its regulation of AKI *in vivo*. Further investigations are clearly merited to establish a causal relationship between BRG1 deficiency and alteration of endothelial transcriptome underlying AKI.

BRG1 is an epigenetic co-factor that relies on sequence-specific transcription factors to be recruited to regulate locus-specific transcription events. We show here that HIF-1α is essential for BRG1 to bind to the IL-33 promoter and activate IL-33 transcription in endothelial cells. It should be pointed out that two separate studies have found that endothelial-deletion of HIF-1α exerts marginal effects on renal ischemia-reperfusion injury in mice although it remains undetermined whether IL-33 is altered or not in the kidneys [26,27]. One possible explanation could be that HIF-1α is redundant for BRG1 recruitment to the IL-33 promoter *in vivo*. Alternatively, HIF-1α dependent BRG1 recruitment and the ensuing induction of IL-33 transcription may not be the rate-limiting step in AKI.

We provide evidence to show that a BRG1-dependent pro-fibrogenic cue emitted from endothelial cells, likely IL-33, could potentially promote the synthesis of ECM proteins in cultured tubular epithelial cells. This observation may partially explain the attenuation of renal fibrosis following ischemia-reperfusion as a result of BRG1 deficiency. Further, this pro-fibrogenic response may be mediated by SMAD3 in tubular epithelial cells because SMAD3 binding to the *ACTA2* and the *COL1A1* promoters is significantly enhanced. This is consistent with a previous report that suggests IL-33 may regulate the TGF-β signaling [28]. It is noteworthy that alternative scenarios exist that may account for the inter-cellular crosstalk resulting in renal fibrosis. For instance, we have previously shown that BRG1 plays an essential role regulating endothelial derived endothelin (ET-1) to promote cardiac fibrosis [15]. Clearly, this lingering issue merits further investigation.

An emerging theme regarding transcriptional regulation by BRG1 is its ability to engage other branches of the epigenetic machinery especially histone modifying enzymes. We show here that several histone modifying proteins, including p300, ASH2, and KDM3A, are involved in IL-33 transcription although a direct role for any one of these factors remains to be determined. Recent investigations suggest that p300, ASH2, and KDM3A are all intimately involved in the regulation of inflammation [29–31] and fibrogenesis [32,33], two key processes associated with ischemia-reperfusion induced kidney injury. Of interest, inhibition of p300 by curcumin, a small-molecule compound derived from *Curcuma longa* (turmeric), attenuates kidney injury in mice [34]. Therefore, our data provide rationale for screening for inhibitors of histone modifying enzymes to alleviate AKI.

In summary, our data support a link between activation of IL-33 transcription by BRG1 in endothelial cells and renal ischemia-reperfusion injury. Future studies exploiting additional cell and animal models would hopefully pave the way for the development of novel therapeutic solutions against renal injury.

4. Materials and methods

4.1. Animals

All animal experiments were review and approved by the intramural Committee on Ethical Conduct of Animal Experiments. To delete BRG1 in endothelial cells, *Smarca4*$^{f/f}$ mice [35] were crossed to the *Cdh5*-ERT2-Cre mice [17]. To induce renal ischemia-reperfusion injury, 6–8 week-old male mice were anesthetized with ketamine. The renal pedicle was clamped with nontraumatic microaneurysm clamps (Roboz Surgical Instrument Co.). Clamps were removed after 45 min. Body temperature was controlled at 37 °C throughout the procedure. The mice were sacrificed 48 h or 2wk after the surgery.

4.2. Histology

Histological analyses were performed essentially as described [36]. Paraffin sections were stained with hematoxylin and eosin (Sigma), picrosirius red (Sigma), or Masson's trichrome (Sigma) according to standard procedures. Parallel sections were stained for cell surface markers. Briefly, sections were blocked with 10% normal goat serum for 1 h at room temperature and then incubated with anti-CD68 (Abcam), anti-F4/80 (Biorad), or anti-CD3 (BD Bioscicences) antibodies followed by incubation with donkey secondary antibodies (Jackson ImmunoResearch). The nuclei were counterstained with DAPI (Sigma). Pictures were taken using an Olympus IX-70 microscope. Quantifications were performed with Image Pro.

4.3. Cell culture, plasmids, transient transfection, and reporter assay

Immortalized human endothelial cells (EAhy926, ATCC), immortalized human renal tubular epithelial cells (HK-2, ATCC), and HEK293 cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS, Hyclone). Human primary aortic endothelial cells (HAECs) were purchased from Lonza and maintained as previously described [37]; three different batches of primary cells were used in this study. BRG1 expression plasmids [38], HIF-1α expression constructs [39], and IL-33 promoter-lucferase constructs [21] have been previously described. Small interfering RNAs were purchased from Dhamarcon. Transient transfections were performed with Lipofectamine 2000 (Invitrogen). Cells were harvested 48 h after transfection and reporter activity was measured using a luciferase reporter assay system (Promega) as previously described [40].

4.4. RNA isolation and real-time PCR

RNA was extracted with the 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 Prism 7500 system with the following primers: *Ifng*, 5′-GTTACTGCCACGGCACAGTCATTG-3′ and 5′-ACCATCCT

TTTGCCAGTTCCTCCAG-3′; *Il-1b*, 5′-AATCTGTACCTGTCCTGCGT GTT-3′ and 5′-TGGGTAATTTTTGGGATCTACACTCT-3′; *Il-6*, 5′-GAGGA TACCACTCCCAACAGACC-3′ and 5′-AAGTGCATCATCGTTGTTCAT ACA-3′; *Tnfa*, 5′-AAAGACCAGGTGGAGTGGAAGAAC-3′ and 5′-CTCAG TGCCGATGGAGTCCGAGTA-3′; Havcr1, 5′-CTGGAATGGCACTGTGAC ATCC-3′ and 5′-GCAGATGCCAACATAGAAGCCC-3′; *Lcn2*, 5′-GAAATA TGCACAGGTATCCTC-3′ and 5′-GTAATTTTGAAGTATTGCTTGTTT-3′; *Col1a1*, 5′-GAAGCACGTCTGGTTTGGA-3′ and 5′-ACTCGAACGGGAAT CCATC-3′; *Col1a2*, 5′-CCAACAAGCATGTCTGGTTAG GA-3′ and 5′-TCAAACTGGCTGCCACCA-3′; *Acta2*, 5′-ACTGGGACGACATGGAA AAG-3′ and 5′-GTTCAGTGGTGCCTCTGTCA-3′; *Il-33*, 5′-TGAGACTCCG TTCTGGCCTC-3′ and 5′-CTCTTCATGCTTGGTACCCGAT-3′; *Nrf2*, 5′-TCTCCTCGCTGGAAAAAGAA-3′ and 5′-AATGTGCTGGCTGTGCT TTA-3′; $p47^{phox}$, 5′-CCACACCTCTTGAACTTC-3′ and 5′-GCCATCTAGG AGCTTATG-3′; $p67^{phox}$, 5′-CCACTCGAGGATTTGCTTCA-3′ and 5′-ATC TTGGAATGCCTGGGCTC-3′; *Icam1*, 5′-TTCACACTGAATGCCAGCTC-3′ and 5′-GTCTGCTGAGACCCCTCTTG-3′; Vcam1, 5′-CCCAGGTGGAGGT CTACTCA-3′ and 5′-CAGGATTTTGGGAGCTGGTA-3′; *Sele*, 5′-AGCTAC CCATGGAACACGAC-3′ and 5′-ACGCAAGTTCTCCAGCTGTT-3′.

4.5. Protein extraction and Western blot

Whole cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100) with freshly added protease inhibitor (Roche) as previously described [41]. Specific antibodies or pre-immune IgGs (P.I.I.) were added to and incubated with cell lysates overnight before being absorbed by Protein A/G-plus Agarose beads (Santa Cruz). Precipitated immune complex was released by boiling with 1× SDS electrophoresis sample buffer. Western blot analyses were performed with anti-HIF-1α (Santa Cruz, sc-10790), anti-FLAG (Sigma, F3165), anti-HA (Sigma, H3663), anti-α-SMA (Sigma, A5228), and anti-β-actin (Sigma, A2228) antibodies.

4.6. Chromatin immunoprecipitation (ChIP)

Chromatin Immunoprecipitation (ChIP) assays were performed essentially as described before [42–48]. In brief, chromatin in control and treated cells were cross-linked with 1% formaldehyde. Cells were incubated in lysis buffer (150 mM NaCl, 25 mM Tris pH 7.5, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate) supplemented with protease inhibitor tablet and PMSF. DNA was fragmented into ~200 bp pieces using a Branson 250 sonicator. Aliquots of lysates containing 200 μg of protein were used for each immunoprecipitation reaction with anti-BRG1 (Abcam, ab110641), anti-p300 (Santa Cruz, sc-584), anti-KDM3A (Bethyl Laboratories, A301-538A), anti-ASH2 (Bethyl Laboratories, A300-489A), anti-trimethyl H3K4 (Millipore, 07-449), anti-dimethyl H3K9 (Millipore, 07-441), anti-acetyl H3 (Millipore, 06-599), anti-SMAD3 (Abcam, ab28379), or pre-immune IgG. Precipitated DNA was amplified with the following primers: *IL-33* promoter; 5′-ATTCTATCC TTCAACATGC-3′ and 5′-AAGGAAGACTGCTGCTGTAC-3′; *GAPDH* promoter, 5′-GGGTTCCTATAAATACGGACTGC-3′ and 5′-CTGGCACTGCA CAAGAAGA-3′.

4.7. MTT assay

Cell viability was measured using an MTT kit (Abcam) per vendor's recommendation. Data were expressed as % viability compared to control arbitrarily set as 1.

4.8. Statistical analysis

One-way ANOVA with post-hoc Scheffé analyses were performed by SPSS software (IBM SPSS v18.0, Chicago, IL, USA). Unless otherwise specified, values of $p < 0.05$ were considered statistically significant.

Abbreviations

BRG1	Brahma related gene 1
HAVCR	hepatitis A virus cellular receptor
LCN2	lipocalin 2
DHE	dihydroethidium
DCFH-DA	2′-7′dichlorofluorescin diacetate
HIF-1α	hypoxia inducible factor 1 alpha
KDM3A	lysine demethylase 3A
IL-33	interleukin 33
AKI	acute kidney injury
IRI	ischemia reperfusion injury

Transparency document

The Transparency document associated with this article can be found, in online version.

Declaration of Competing Interest

None.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81670618, 81800426), Education Commission of Jiangsu Province (18KJB310009), and Nanjing Medical University (2017NJMU012).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2019.06.015.

References

[1] A. Godecke, U.K. Decking, Z. Ding, J. Hirchenhain, H.J. Bidmon, S. Godecke, J. Schrader, Coronary hemodynamics in endothelial NO synthase knockout mice, Circ. Res. 82 (1998) 186–194.
[2] S. Rafii, J.M. Butler, B.S. Ding, Angiocrine functions of organ-specific endothelial cells, Nature 529 (2016) 316–325.
[3] E.S. Baekkevold, M. Roussigne, T. Yamanaka, F.E. Johansen, F.L. Jahnsen, F. Amalric, P. Brandtzaeg, M. Erard, G. Haraldsen, J.P. Girard, Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules, Am. J. Pathol. 163 (2003) 69–79.
[4] M. De la Fuente, T.T. MacDonald, M.A. Hermoso, The IL-33/ST2 axis: role in health and disease, Cytokine Growth Factor Rev. 26 (2015) 615–623.
[5] A. Akcay, Q. Nguyen, Z. He, K. Turkmen, D. Won Lee, A.A. Hernando, C. Altmann, A. Toker, A. Pacic, D.G. Ljubanovic, A. Jani, S. Faubel, C.L. Edelstein, IL-33 exacerbates acute kidney injury, J Am Soc Nephrol 22 (2011) 2057–2067.
[6] K. Ravichandran, S. Holditch, C.N. Brown, Q. Wang, A. Ozkok, M.C. Weiser-Evans, R. Nemenoff, M. Miyazaki, H. Thiessen-Philbrook, C.R. Parikh, D. Ljubanovic, C.L. Edelstein, IL-33 deficiency slows cancer growth but does not protect against cisplatin-induced AKI in mice with cancer, Am. J. Physiol. Ren. Physiol. 314 (2018) F356–F366.
[7] C.W. Wu, Y.G. Wu, C. Cheng, Z.D. Hong, Z.M. Shi, S.Q. Lin, J. Li, X.Y. He, A.Y. Zhu, Interleukin-33 predicts poor prognosis and promotes renal cell carcinoma cell growth through its receptor ST2 and the JNK signaling pathway, Cell. Physiol. Biochem. 47 (2018) 191–200.
[8] N.K. Chen, T.W. Chong, H.L. Loh, K.H. Lim, V.H. Gan, M. Wang, O.L. Kon, Negative regulatory responses to metabolically triggered inflammation impair renal epithelial immunity in diabetes mellitus, J. Mol. Med. (Berl) 91 (2013) 587–598.
[9] Z. Yang, Y. Liang, W. Xi, C. Li, R. Zhong, Association of increased serum IL-33 levels with clinical and laboratory characteristics of systemic lupus erythematosus in Chinese population, Clin. Exp. Med. 11 (2011) 75–80.
[10] Q. Huang, Z. Niu, J. Tan, J. Yang, Y. Liu, H. Ma, V.W. Lee, S. Sun, X. Song, M. Guo, Y. Wang, Q. Cao, IL-25 elicits innate lymphoid cells and multipotent progenitor type 2 cells that reduce renal ischemic/reperfusion injury, J Am Soc Nephrol 26 (2015) 2199–2211.
[11] G.H. Park, H.K. Shinn, J.H. Kang, W.J. Na, Y.H. Kim, C.S. Park, Anti-interleukin-33 reduces ovalbumin-induced nephrotoxicity and expression of kidney injury Molecule-1, Int. Neurourol. J. 20 (2016) 114–121.
[12] F.M. Kandemir, S. Kucukler, E. Eldutar, C. Caglayan, I. Gulcin, Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: a multi-biomarker approach, Sci. Pharm. 85 (2017).

[13] D. Martin-Sanchez, O. Ruiz-Andres, J. Poveda, S. Carrasco, P. Cannata-Ortiz, M.D. Sanchez-Nino, M. Ruiz Ortega, J. Egido, A. Linkermann, A. Ortiz, A.B. Sanz, Ferroptosis, but not necroptosis, is important in nephrotoxic folic acid-induced AKI, J Am Soc Nephrol 28 (2017) 218–229.
[14] C.T. Griffin, C.D. Curtis, R.B. Davis, V. Muthukumar, T. Magnuson, The chromatin-remodeling enzyme BRG1 modulates vascular Wnt signaling at two levels, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 2282–2287.
[15] X. Weng, L. Yu, P. Liang, L. Li, X. Dai, B. Zhou, X. Wu, H. Xu, M. Fang, Q. Chen, Y. Xu, A crosstalk between chromatin remodeling and histone H3K4 methyltransferase complexes in endothelial cells regulates angiotensin II-induced cardiac hypertrophy, J. Mol. Cell. Cardiol. 82 (2015) 48–58.
[16] Z. Li, X. Zhang, S. Liu, S. Zeng, L. Yu, G. Yang, J. Guo, Y. Xu, BRG1 regulates NOX gene transcription in endothelial cells and contributes to cardiac ischemia-reperfusion injury, Biochim. Biophys. Acta Mol. basis Dis. 1864 (2018) 3477–3486.
[17] X. Zhang, S. Liu, X. Weng, T. Wu, L. Yu, Y. Xu, J. Guo, Brg1 trans-activates endothelium-derived colony stimulating factor to promote calcium chloride induced abdominal aortic aneurysm in mice, J. Mol. Cell. Cardiol. 125 (2018) 6–17.
[18] D. Chen, F. Fang, Y. Yang, J. Chen, G. Xu, Y. Xu, Y. Gao, Brahma-related gene 1 (Brg1) epigenetically regulates CAM activation during hypoxic pulmonary hypertension, Cardiovasc. Res. 100 (2013) 363–373.
[19] F. Fang, D. Chen, L. Yu, X. Dai, Y. Yang, W. Tian, X. Cheng, H. Xu, X. Weng, M. Fang, J. Zhou, Y. Gao, Q. Chen, Y. Xu, Proinflammatory stimuli engage brahma related gene 1 and brahma in endothelial injury, Circ. Res. 113 (2013) 986–996.
[20] H. Liang, F. Xu, X.J. Wen, H.Z. Liu, H.B. Wang, J.Y. Zhong, C.X. Yang, B. Zhang, Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion, Eur. J. Pharmacol. 812 (2017) 18–27.
[21] M. Sun, C. He, W. Wu, G. Zhou, F. Liu, Y. Cong, Z. Liu, Hypoxia inducible factor-1alpha-induced interleukin-33 expression in intestinal epithelia contributes to mucosal homeostasis in inflammatory bowel disease, Clin. Exp. Immunol. 187 (2017) 428–440.
[22] M. Ferhat, A. Robin, S. Giraud, S. Sena, J.M. Goujon, G. Touchard, T. Hauet, J.P. Girard, J.M. Gombert, A. Herbelin, A. Thierry, Endogenous IL-33 contributes to kidney ischemia-reperfusion injury as an Alarmin, J Am Soc Nephrol 29 (2018) 1272–1288.
[23] L.D. Hazlett, S.A. McClellan, R.P. Barrett, X. Huang, Y. Zhang, M. Wu, N. van Rooijen, E. Szliter, IL-33 shifts macrophage polarization, promoting resistance against Pseudomonas aeruginosa keratitis, Invest. Ophthalmol. Vis. Sci. 51 (2010) 1524–1532.
[24] M. Kurowska-Stolarska, B. Stolarski, P. Kewin, G. Murphy, C.J. Corrigan, S. Ying, N. Pitman, A. Mirchandani, B. Rana, N. van Rooijen, M. Shepherd, C. McSharry, I.B. McInnes, D. Xu, F.Y. Liew, IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation, J. Immunol. 183 (2009) 6469–6477.
[25] X. Wu, R. Guo, Y. Wang, P.N. Cunningham, The role of ICAM-1 in endotoxin-induced acute renal failure, Am. J. Physiol. Ren. Physiol. 293 (2007) F1262–F1271.
[26] J. Kalucka, G. Schley, A. Georgescu, B. Klanke, S. Rossler, J. Baumgartl, J. Velden, K. Amann, C. Willam, R.S. Johnson, K.U. Eckardt, A. Weidemann, Kidney injury is independent of endothelial HIF-1alpha, J. Mol. Med. (Berl) 93 (2015) 891–904.
[27] P.P. Kapitsinou, H. Sano, M. Michael, H. Kobayashi, O. Davidoff, A. Bian, B. Yao, M.Z. Zhang, R.C. Harris, K.J. Duffy, C.L. Erickson-Miller, T.A. Sutton, V.H. Haase, Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury, J. Clin. Invest. 124 (2014) 2396–2409.
[28] Q.Y. Tan, Z.S. Cheng, TGFbeta1-Smad signaling pathway participates in interleukin-33 induced epithelial-to-mesenchymal transition of A549 cells, Cell. Physiol. Biochem. 50 (2018) 757–767.
[29] L. Yu, X. Weng, P. Liang, X. Dai, X. Wu, H. Xu, M. Fang, F. Fang, Y. Xu, MRTF-A mediates LPS-induced pro-inflammatory transcription by interacting with the COMPASS complex, J. Cell Sci. 127 (2014) 4645–4657.
[30] L. Yu, F. Fang, X. Dai, H. Xu, X. Qi, M. Fang, Y. Xu, MKL1 defines the H3K4Me3 landscape for NF-kappaB dependent inflammatory response, Sci. Rep. 7 (2017) 191.
[31] Z. Fan, Z. Li, Y. Yang, S. Liu, J. Guo, Y. Xu, HIF-1alpha coordinates epigenetic activation of SIAH1 in hepatocytes in response to nutritional stress, Biochim. Biophys. Acta, Gene Regul. Mech. 1860 (2017) 1037–1046.
[32] Q.J. Zhang, T.A.T. Tran, M. Wang, M.J. Ranek, K.M. Kokkonen-Simon, J. Gao, X. Luo, W. Tan, V. Kyrychenko, L. Liao, J. Xu, J.A. Hill, E.N. Olson, D.A. Kass, E.D. Martinez, Z.P. Liu, Histone lysine dimethyl-demethylase KDM3A controls pathological cardiac hypertrophy and fibrosis, Nat. Commun. 9 (2018) 5230.
[33] Z. Fan, C. Hao, M. Li, X. Dai, H. Qin, J. Li, H. Xu, X. Wu, L. Zhang, M. Fang, B. Zhou, W. Tian, Y. Xu, MKL1 is an epigenetic modulator of TGF-beta induced fibrogenesis, Biochim. Biophys. Acta, Gene Regul. Mech. 1849 (2015) 1219–1228.
[34] Y. Fan, H. Chen, H. Peng, F. Huang, J. Zhong, J. Zhou, Molecular mechanisms of curcumin renoprotection in experimental acute renal injury, Front. Pharmacol. 8 (2017) 912.
[35] N. Li, M. Li, W. Hong, J. Shao, H. Xu, H. Shimano, J. Lu, Y. Xu, Brg1 regulates prolipogenic transcription by modulating SREBP activity in hepatocytes, Biochim. Biophys. Acta Mol. basis Dis. 1864 (2018) 2881–2889.
[36] X. Zhang, S. Liu, X. Weng, S. Zeng, L. Yu, J. Guo, Y. Xu, Brg1 deficiency in vascular endothelial cells blocks neutrophil recruitment and ameliorates cardiac ischemia-reperfusion injury in mice, Int. J. Cardiol. 269 (2018) 250–258.
[37] X. Weng, L. Yu, P. Liang, D. Chen, X. Cheng, Y. Yang, L. Li, T. Zhang, B. Zhou, X. Wu, H. Xu, M. Fang, Y. Gao, Q. Chen, Y. Xu, Endothelial MRTF-A mediates angiotensin II induced cardiac hypertrophy, J. Mol. Cell. Cardiol. 80 (2015) 23–33.
[38] N. Li, M. Kong, S. Zeng, Z. Xu, M. Li, W. Hong, X. Chu, X. Sun, M. Zhu, Y. Xu, The chromatin remodeling protein BRG1 regulates APAP-induced liver injury by modulating CYP3A11 transcription in hepatocyte, Biochim. Biophys. Acta Mol. basis Dis. 1864 (2018) 3487–3495.
[39] J.S. Isaacs, Y.J. Jung, E.G. Mimnaugh, A. Martinez, F. Cuttitta, L.M. Neckers, Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway, J. Biol. Chem. 277 (2002) 29936–29944.
[40] L. Liu, W. Hong, M. Li, H. Ren, J. Wang, H. Xu, X. Shi, Y. Xu, A cross talk between BRG1 and males absent on the first contributes to reactive oxygen species production in a mouse model of nonalcoholic steatohepatitis, Antioxid. Redox Signal. (2018).
[41] L. Liu, X. Wu, H. Xu, L. Yu, X. Zhang, L. Li, J. Jin, T. Zhang, Y. Xu, Myocardin-related transcription factor A (MRTF-A) contributes to acute kidney injury by regulating macrophage ROS production, Biochim. Biophys. Acta Mol. basis Dis. 1864 (2018) 3109–3121.
[42] S. Zeng, X. Wu, X. Chen, H. Xu, T. Zhang, Y. Xu, Hypermethylated in cancer 1 (HIC1) mediates high glucose induced ROS accumulation in renal tubular epithelial cells by epigenetically repressing SIRT1 transcription, Biochim. Biophys. Acta, Gene Regul. Mech. 1861 (2018) 917–927.
[43] J. Shao, X. Weng, L. Zhuo, L. Yu, Z. Li, K. Shen, W. Xu, M. Fang, Y. Xu, Angiotensin II induced CSF1 transcription is mediated by a crosstalk between different epigenetic factors in vascular endothelial cells, Biochim. Biophys. Acta, Gene Regul. Mech. 1862 (2019) 1–11.
[44] Z. Li, B. Chen, X. Weng, L. Yu, M. Song, M. Fang, J. Guo, Y. Xu, The histone methyltransferase SETD1A regulates thrombomodulin transcription in vascular endothelial cells, Biochim. Biophys. Acta, Gene Regul. Mech. 1861 (2018) 752–761.
[45] Z. Li, B. Chen, W. Dong, W. Xu, M. Song, M. Fang, J. Guo, Y. Xu, Epigenetic activation of PERP transcription by MKL1 contributes to ROS-induced apoptosis in skeletal muscle cells, Biochim. Biophys. Acta, Gene Regul. Mech. 1861 (2018) 905–915.
[46] X. Weng, Y. Zhang, Z. Li, L. Yu, F. Xu, M. Fang, L. Hou, J. Ge, Y. Xu, Class II transactivator (CIITA) mediates IFN-gamma induced eNOS repression by enlisting SUV39H1, Biochim. Biophys. Acta, Gene Regul. Mech. 1862 (2019) 163–172.
[47] Y. Yang, L. Liu, M. Fang, H. Bai, Y. Xu, The chromatin remodeling protein BRM regulates the transcription of tight junction proteins: implication in breast cancer metastasis, Biochim. Biophys. Acta, Gene Regul. Mech. 1862 (2019) 547–556.
[48] Z. Li, P. Li, Y. Lu, D. Sun, X. Zhang, Y. Xu, A non-autonomous role of MKL1 in the activation of hepatic stellate cells, Biochim. Biophys. Acta, Gene Regul. Mech. 1862 (2019) 609–618.