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VE-cadherin regulates migration inhibitory factor synthesis and release

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Abstract

Objective Vascular endothelial (VE)-cadherin-mediated adherens junction is critical to maintain endothelial integrity. Besides its role of homophilic intercellular adhesion, VE-cadherin also has a role of outside-in signaling with functional consequences for vascular physiology. However, the nature of these signals remains not completely understood.

Materials and methods Human umbilical vein endothelial cells (HUVECs) were used in cell culture experiments. Confluent HUVECs were treated with VE-cadherin function-blocking antibodies BV9 (50 μg/ml) or IgG control. Antibody array was used to screen for cytokine/chemokine in supernatant. For VE-cadherin knockdown, siRNA transfection was used. ELISA, Western blot, and qRT-PCR were used to confirm the expression of screened cytokine/chemokine. To explore the possible mechanisms, Scr phosphorylation was detected and Scr inhibitor PP2 (1 μM) was used. To investigate in vivo relevance of the findings, BV9 and the indicated neutralizing antibodies were injected into mice and then lung vascular leak and inflammation were examined by Evans blue assay and lung tissue H&E, respectively.

Results Using a non-biased, high-throughout human cytokine/chemokine antibody array, we first found that disruption of VE-cadherin-mediated adhesion by function-blocking antibody BV9 triggered the release of migration inhibitory factor (MIF). This VE-cadherin-mediated release of MIF further confirmed by ELISA with both VE-cadherin blocking antibody and siRNA technique was due to enhanced expression of MIF mRNA, which was mediated by Src kinase activation. In addition, in vivo lung vascular leak induced by VE-cadherin function-blocking antibody was partly alleviated by neutralizing MIF.

Conclusions VE-cadherin regulates MIF synthesis and release via Src kinase. Our data provide additional evidence to the concept that VE-cadherin transfers intracellular signals to coordinate the state of cell–cell adhesion with gene expression.

Keywords Endothelial cells · VE-cadherin · Outside-in signaling · Migration inhibitory factor · Src

Introduction

VE-cadherin-mediated adherens junction is critical to maintain endothelial integrity. VE-cadherin contains multiple domains able to modulate its adhesive and clustering properties. The extracellular region of VE-cadherin is composed of five homologous domains. The intracellular domain of VE-cadherin interacts with cytoplasmic proteins including α-catenin, β-catenin, plakoglobin, and p120 and others [1]. Disruption of the VE-cadherin-mediated adherens junction is underlined in many pathologies and diseases including atherosclerosis, vascular malformations, hemorrhagic stroke, sepsis and others [1].

Besides its role of homophilic intercellular adhesion, VE-cadherin also participates directly and indirectly in intracellular signaling pathways that control cell behaviors. Several studies demonstrate that VE-cadherin has a crucial role in

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early vascular organization. Mice lacking endothelial VE-cadherin die early due to altered vascular development [2, 3]. Even an incomplete reduction of VE-cadherin expression also prevents the formation of a stable vasculature [4]. In the normal vasculature, there is an important inhibitory mechanism to avoid uncontrolled sprouting and vascular branching due to connection of tip cells and successful anastomosis. However, in the absence of VE-cadherin, the tips of endothelial cells fail to sense the cell-to-cell contact and keep searching for other connections [5]. It is also known that inhibition of endothelial cell proliferation by the establishment of cell-to-cell contacts requires both the extracellular adhesive domain and the intracellular catenin-binding region of VE-cadherin [6]. These observations underline the ability of VE-cadherin not only to maintain vascular barrier function but also to affect vascular homeostasis via transferring intracellular signals. However, the nature of these signals remains not completely understood.

Here, using a non-biased, high-throughout human cytokine antibody array, we first identified that disruption of VE-cadherin-mediated adhesion triggers the release of MIF. This VE-cadherin-mediated release of MIF further confirmed by both VE-cadherin blocking antibody and siRNA technique was due to enhanced expression of MIF mRNA, which was mediated by Src kinase activation. In addition, we showed that loss of vascular barrier function in vivo and lung inflammation induced by VE-cadherin function-blocking antibody was partly alleviated by neutralizing MIF.

Materials and methods

Antibodies and reagents

The following reagents and antibodies were used: anti-MIF antibody (Santa Cruz), neutralizing anti-MIF antibody (Millipore), IgG control (Santa Cruz), anti-VE-cadherin antibody (BV9, Santa Cruz), anti-VE-cadherin antibody (C-19, Santa Cruz), c-Src antibody (Santa Cruz), phospho-Src Family (Tyr416) antibody (Cell Signaling), Alexa Fluor 488-conjugated secondary antibody (Thermo Scientific), Human Cytokine Antibody Array (ARY005), Human MIF ELISA (RayBiotech), Mouse MIF ELISA (Proteintech), Evans blue dye (Sigma).

Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza and grown in Medium 200 supplemented with Low Serum Growth Supplement (LSGS) (Cascade Biologics). HUVECs were used at a low passage number (2–6) for in vitro assays. Cells were cultured in a humidified incubator at 37 °C and 5% CO_2. In cell culture experiments, treatments with antibodies (BV9 or IgG control) were carried out with a concentration of 50 μg/ml. PP2 (1 μM) was added 30 min before antibody treatment or 48 h after siRNA transfection. For Scr phosphorylation studies, cells were serum-starved overnight in M200 with 1% FBS and without growth factors.

RNA interference

VE-cadherin siRNA and scrambled siRNA were synthesized by GenePharma Co., Ltd. (GenePharma, Shanghai, China). The previously validated siRNA sequence targeting VE-cadherin is shown below [7]: 5′-GGAACCAGA UGCACAUUGAUU-3′. The scrambled siRNA control is a nontargeting siRNA from GenePharma. For the transfection of siRNA, HUVECs were seeded for 24 h at about 80% confluence, and then transfection of siRNA was performed with HiPerfect transfection reagent (Qiagen) according to the manufacturer's protocol.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

To measure MIF expression, total RNA was isolated using the Trizol reagent according to the manufacturer's instructions (Invitrogen). One microgram of each total RNA was reverse transcribed to complementary DNA, using the Reverse Transcription System (Promega). qRT-PCR analysis was conducted using SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers. The primer primers for MIF were used as described previously [8]:

forward, 5′-CTCTCCGAGCTCACCCAGCAG-3;
reverse, 5′-CGCGTTCATGTCGTAATAGTT-3′.

MIF gene expression levels were normalized to the expression of the housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase). The MIF mRNA levels relative to GAPDH were calculated by $2^{-\Delta CT}$ values and averaged per group. The fold changes of gene expression relative to the control were calculated by $2^{-\Delta\Delta CT}$.

Measurement of cytokines/chemokines in culture supernatant by antibody array

After reaching confluence, the cells were changed to M200 with 1% FBS. BV9 (50 μg/ml) or IgG (50 μg/ml) control was added to culture medium for another 24 h. Then the culture medium was collected. For cytokine/chemokine measurement, supernatants were analyzed by Human Cytokine Antibody Array in accordance with the manufacturer's instructions.

Measurement of MIF concentration in culture supernatant or plasma in mice

After the indicated treatment, supernatants in culture medium or plasma in mice were collected. MIF levels were then assessed using human or mouse MIF ELISA kit, respectively, in accordance with the manufacturer's instructions.

Immunofluorescence staining

After the indicated treatment, cells cultured on eight-well chamber slides were washed with PBS, fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, and incubated with 1% BSA for blocking. After that, the cells were probed with goat primary antibody against VE-cadherin (C-19) for 2 h at room temperature. Secondary fluorescence-conjugated IgG-Alexa Fluor 488 antibody was incubated for 1 h at room temperature. Images of localization of VE-cadherin were taken with an inverted fluorescence microscope (Olympus).

Western blot

After indicated time of treatment, cells were washed 1 × with cold phosphate-buffered saline, cells were lysed by RIPA buffer (Pierce) supplemented with protease and phosphatase cocktails. Lysates were incubated on ice for 15 min and spun at 12 000 r.p.m. at 4 °C in a microcentrifuge. The supernatant was collected for further analysis. Equal amounts of proteins were loaded on gels, separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with primary and HRP-linked secondary antibodies; specific binding was detected by a chemiluminescence system.

Lung permeability and H&E

Animal experiments were approved the Animal Ethics Committee of Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine. Male C57BL/6 mice (8–10 weeks old) were used. Lung vascular leak was assessed by measuring the pulmonary concentration of Evans blue. Antibodies (BV9 or IgG control, 100 μg/mouse) were injected into the tail vein. For MIF-neutralizing experiments, mice were injected with 100 μg of mouse monoclonal antibody against MIF (anti-MIF) simultaneously with BV9 or IgG control. These injections were followed at different times by a second i.v. injection of Evans blue (100 μl/mouse, 1% solution) into the lateral tail vein. Ten minutes after Evans blue injection, animals were killed, their lungs were perfused free of blood with ice-cold PBS. Evans blue was extracted from tissues by homogenizing the lungs in formamide, incubating for 24 h at 60 °C. Then supernatant were collected and quantified by measuring optical density at 620 nm. Evans blue content was always referred to the dry weight of the lung tissue. For histopathology, lung tissues were harvested and fixed using 4% formaldehyde. Tissues were then embedded, sectioned, and stained with H&E. After images were taken with a Nikon Eclipse E800 microscope with a 20 × objective, the lung inflammation was evaluated by semi-quantitative analysis of histopathologic lung injury scores as previously described [9]. Images were analyzed by an investigator who was blinded to the identity of the slides. The selection of concentration for BV9 and MIF-neutralizing antibodies was based on previous publications. Neutralizing antibody against MIF had been previously shown to neutralize MIF bioactivity [10].

Statistical analysis

The Student's *t* test was used to determine statistical significance. Data are expressed as the mean ± standard deviation (SD). The significance level was set at $p < 0.05$.

Results

Cytokine antibody array identifies that the disruption of VE-cadherin-mediated adhesion triggers MIF release

VE-cadherin-blocking monoclonal antibodies (mAbs) directed to the human VE-cadherin extracellular region can be useful tools to study the role of endothelial cell junctions in permeability control and other endothelial functions [11]. One of these mAbs, clone BV9, binds to EC3-EC4 and influences VE-cadherin homophilic adhesion. Since higher concentrations do not produce a more marked effect, a saturating concentration of the BV9 (50 μg/mL) was used in all our in vitro experiments [11]. As shown in Fig. 1b, BV9 reduced localization of VE-cadherin at intercellular junctions (Fig. 1a). Previous work shows that VE-cadherin disappearance from junctions is due to the diffusion of the molecule on the cell membrane and not due to its internalization [12]. Consistent with this finding, our subsequent western blot analysis also showed that VE-cadherin protein level remained unchanged (Fig. 1b).

To identify whether and which inflammatory cytokines/chemokines will be affected after the disruption of VE-cadherin-mediated adhesion, we performed a non-biased, high-throughout approach using human cytokine antibody array containing 36 cytokine capture antibodies immobilized on the membrane (Fig. 1c). As shown in Fig. 1d and e, among 36 cytokines on the array BV9 markedly induced the release of MIF into supernatant. The data indicate that disruption of VE-cadherin homophilic interactions by BV9 triggers the

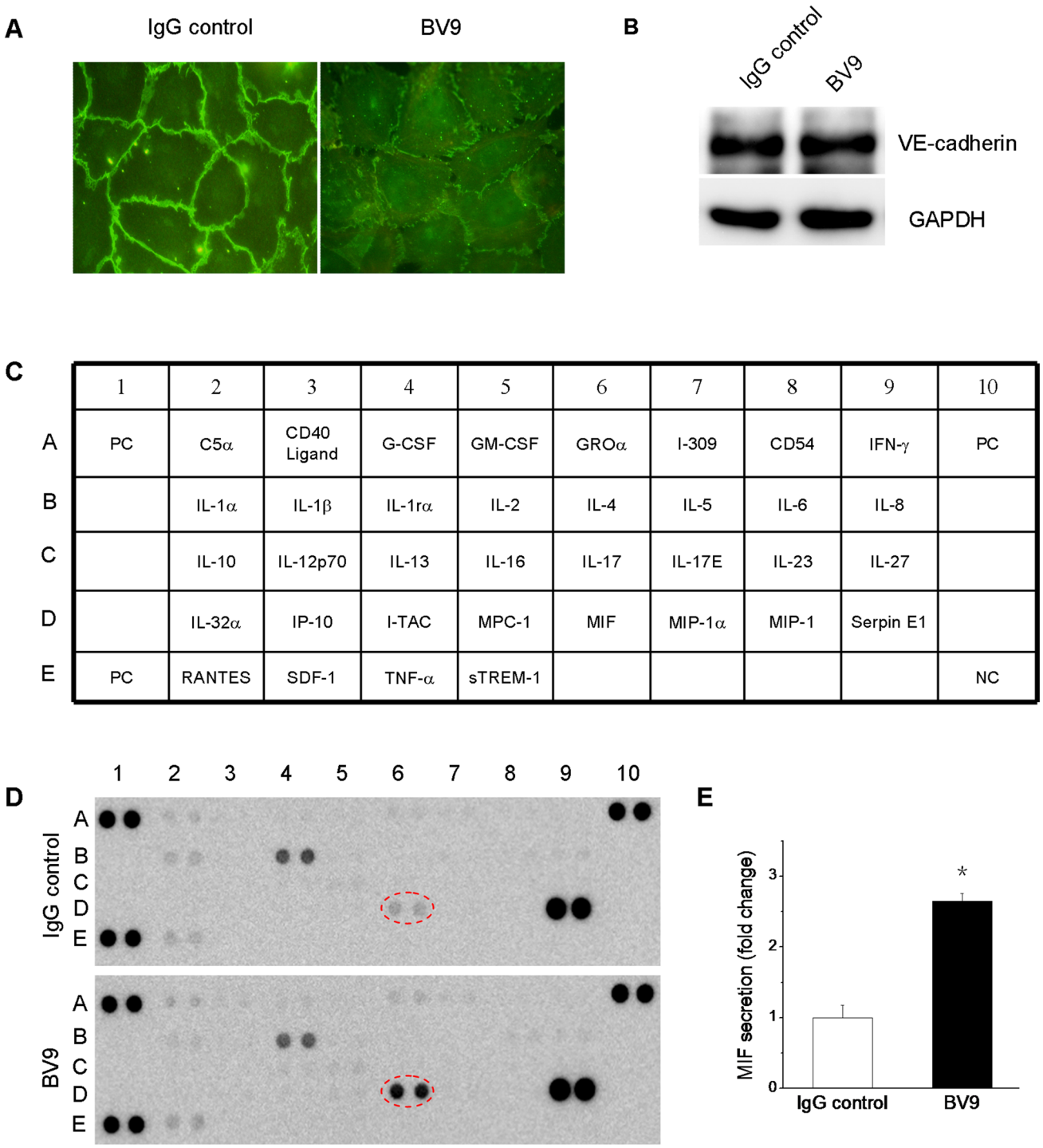


Fig. 1 Cytokine antibody array identifies that VE-cadherin blocking antibody triggers MIF release. After HUVECs reach confluence, cells were replaced with Medium 200 with 1% FBS and treated with IgG control or VE-cadherin blocking antibody BV9 (50 μg/ml) for another 6 or 24 h. **a** Immunofluorescence staining of VE-cadherin following IgG control or BV9 treatment for 6 h. **b** Western blot analysis of VE-cadherin protein following IgG control or BV9 treatment for 6 h. **c** Map of the cytokine antibody array. **d** Cytokine antibody array of supernatant from HUVECs cultured in the presence of IgG control or BV9 for 24 h. *PC* positive control, *NC* negative control. Representative immunoblot of two experiments is shown. **e** Relative expression of MIF in cytokine antibody array. *$p < 0.05$, compared with IgG control

release of MIF, which may be mediated by the outside-in signaling role of VE-cadherin.

VE-cadherin knockdown increases endothelial MIF expression and release

To further evaluate the role of VE-cadherin in regulation of MIF, genetic approach using small interference RNA (siRNA) was used to knockdown VE-cadherin. While VE-cadherin protein was reduced by VE-cadherin siRNA, MIF expression was dramatically increased (Fig. 2a). At the same time, the secretion of MIF into supernatant evaluated by ELISA was also significant increased in VE-cadherin siRNA group (Fig. 2b). To determine the change of MIF mRNA expression upon VE-cadherin gene silencing, we measured MIF mRNA level after VE-cadherin siRNA transfection and observed that VE-cadherin knockdown increased MIF mRNA expression significantly (Fig. 2c and Supplemental Table 1). These results suggest that reducing the VE-cadherin level by siRNA technique can also stimulate the expression and secretion of MIF.

VE-cadherin blocking enhances MIF expression

To determine whether blocking VE-cadherin by BV9 regulates MIF at the transcriptional level, mRNA expression of MIF was examined by qRT-PCR. BV9 treatment induced mRNA expression of MIF in a time-dependent manner (Fig. 3a and Supplemental Table 2). The increased expression of MIF was also confirmed at the protein level by Western blot analysis of cell lysates (Fig. 3b). The data suggest that BV9 upregulates MIF expression at both transcriptional and post-transcriptional levels.

VE-cadherin regulation of MIF is mediated by Src kinase

VE-cadherin forms homophilic complexes that recruit cytoplasmic regulators, including catenins, c-Src tyrosine kinase, and several protein phosphatases [1]. The assembly/disassembly of cadherin-based junctional complexes thus couples cell–cell adhesiveness to intracellular signaling and gene expression. Previous study observes that loss of VE-cadherin-mediated adhesion induces the activation of Src kinase [13]. Consistently, we also detected Src activation following BV9 treatment, which was prevented by the Src kinase inhibitor PP2, as evidenced by phosphorylation of Tyrosine-416 (Fig. 4a).

To address whether Src kinase is involved in the regulation of MIF by VE-cadherin, the Src kinase inhibitor PP2 was added for 30 min before BV9 to allow for complete blockade. Control treated with inhibitor alone was also included to determine its effect on MIF. Cells were

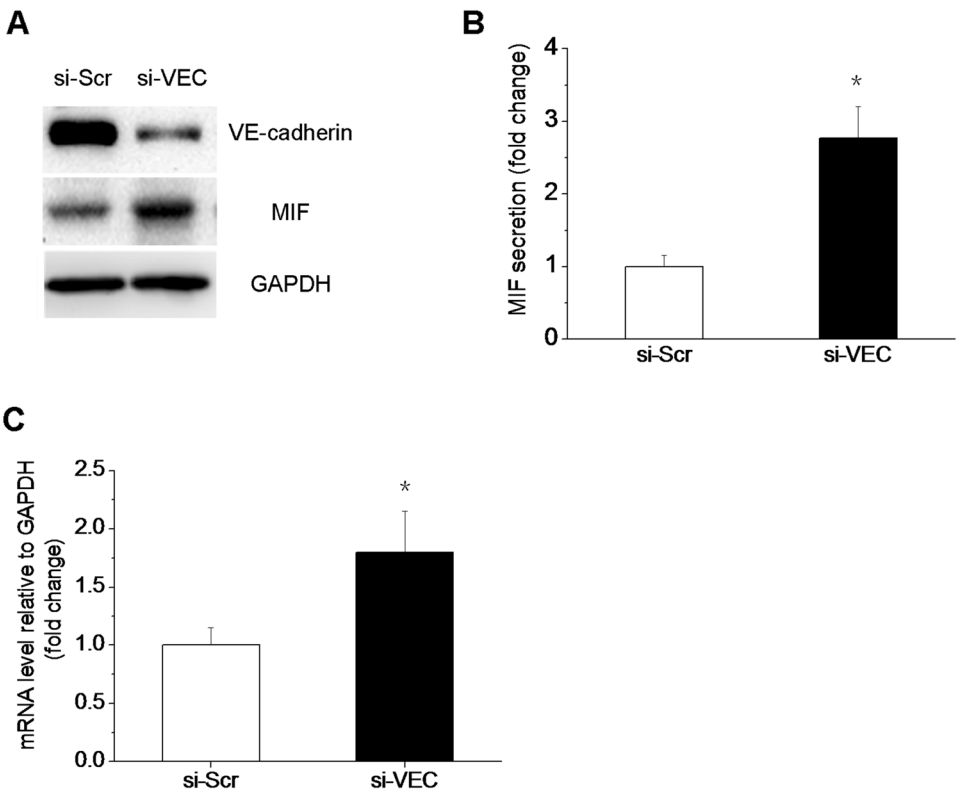


Fig. 2 VE-cadherin knockdown increases endothelial MIF expression and release. HUVECs were transfected with siRNAs in full medium for 48 h, then cells were replaced with Medium 200 containing 1% FBS for another 24 h. After that, cell lysates or supernatant were collected for Western blot, ELISA, or qRT-PCR, respectively. **a** VE-cadherin knockdown increased endothelial MIF expression detected by Western blot. **b** VE-cadherin knockdown increased endothelial MIF release examined by ELISA. Results are presented by relative changes of MIF concentration in supernatant. **c** VE-cadherin knockdown increased MIF mRNA expression. si-Scr, si-Scramble; si-VEC, si-VE-cadherin. *$p < 0.05$, compared with si-Scr

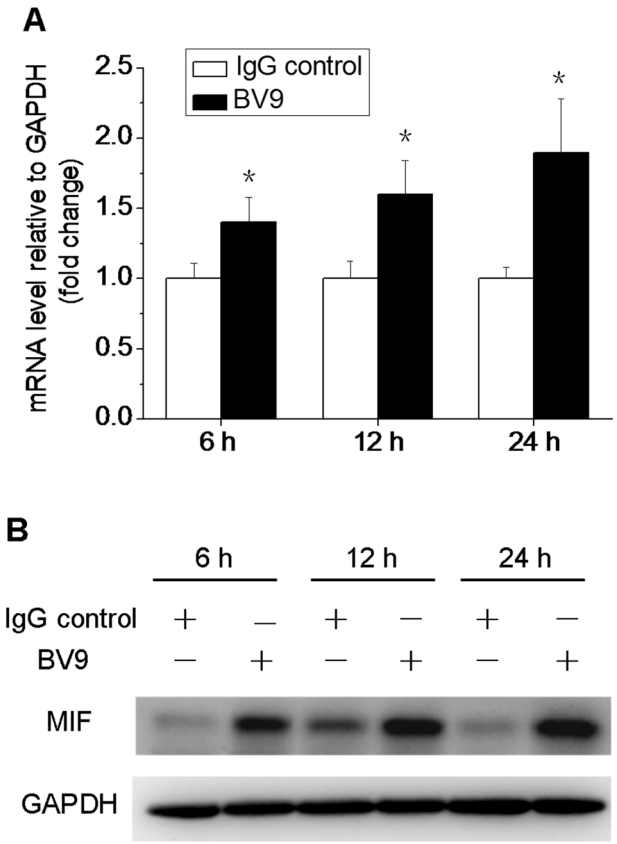


Fig. 3 VE-cadherin blocking antibody enhances MIF mRNA and protein expression. After HUVECs reach confluence, cells were replaced with Medium 200 containing 1% FBS and treated with IgG control or BV9 for indicated time points. Cell lysates were then used for qRT-PCR or Western blot, respectively. **a** BV9 treatment increased MIF mRNA expression determined by qRT-PCR. Fold change of MIF mRNA levels normalized to GAPDH are shown. **b** BV9 treatment increased MIF protein expression. Representative immunoblot of three experiments is shown. *$p < 0.05$, compared with IgG control at each corresponding time points

allowed to incubate for another 24 h. BV9-induced MIF mRNA levels were significantly reduced by PP2 (Fig. 4b and Supplemental Table 3). In addition, VE-cadherin knockdown-induced increase of MIF mRNA expression was also inhibited by PP2 (Fig. 4c and Supplemental Table 4).

To further test whether Src inhibition by PP2 can inhibit the secretion of MIF, MIF concentration in supernatant was examined by ELISA. Pharmacological inhibition of Src kinase by PP2 significantly reduced the amount of MIF in supernatant induced by both BV9 treatment and VE-cadherin knockdown (Fig. 4d, e). The data indicate that Src mediates VE-cadherin regulation of MIF.

Fig. 4 VE-cadherin regulation of MIF is mediated by Src. **a** BV9 treatment triggered Src activation as evidenced by Src (Tyr-416) phosphorylation (p-Tyr-416) compared to IgG control, which was prevented by Src inhibitor PP2 (1 μM) pretreatment. In the inhibitor experiment, IgG control or BV9 treatment period is 10 min. **b** PP2 inhibited BV9-induced increased expression of MIF mRNA (24 h) as determined by qRT-PCR. **c** PP2 prevented VE-cadherin knockdown-induced increased expression of MIF mRNA (72 h after transfection) determined by qRT-PCR. HUVECs were transfected with siRNAs in full medium for 48 h, then cells were replaced with Medium 200 containing 1% FBS for another 24 h in the presence of PP2. Fold change of MIF mRNA levels normalized to GAPDH are shown. **d** PP2 inhibited BV9-induced secretion of MIF (24 h) as assayed by ELISA. **e** PP2 inhibited VE-cadherin knockdown-induced secretion of MIF (24 h) as assayed by ELISA. HUVECs were transfected with siRNA in full medium for 48 h, then cells were replaced with Medium 200 containing 1% FBS for another 24 h in the presence of PP2. The supernatant was then collected for analysis. Results in **d** and **e** are presented by relative fold change of MIF concentration in supernatant. *$p < 0.05$

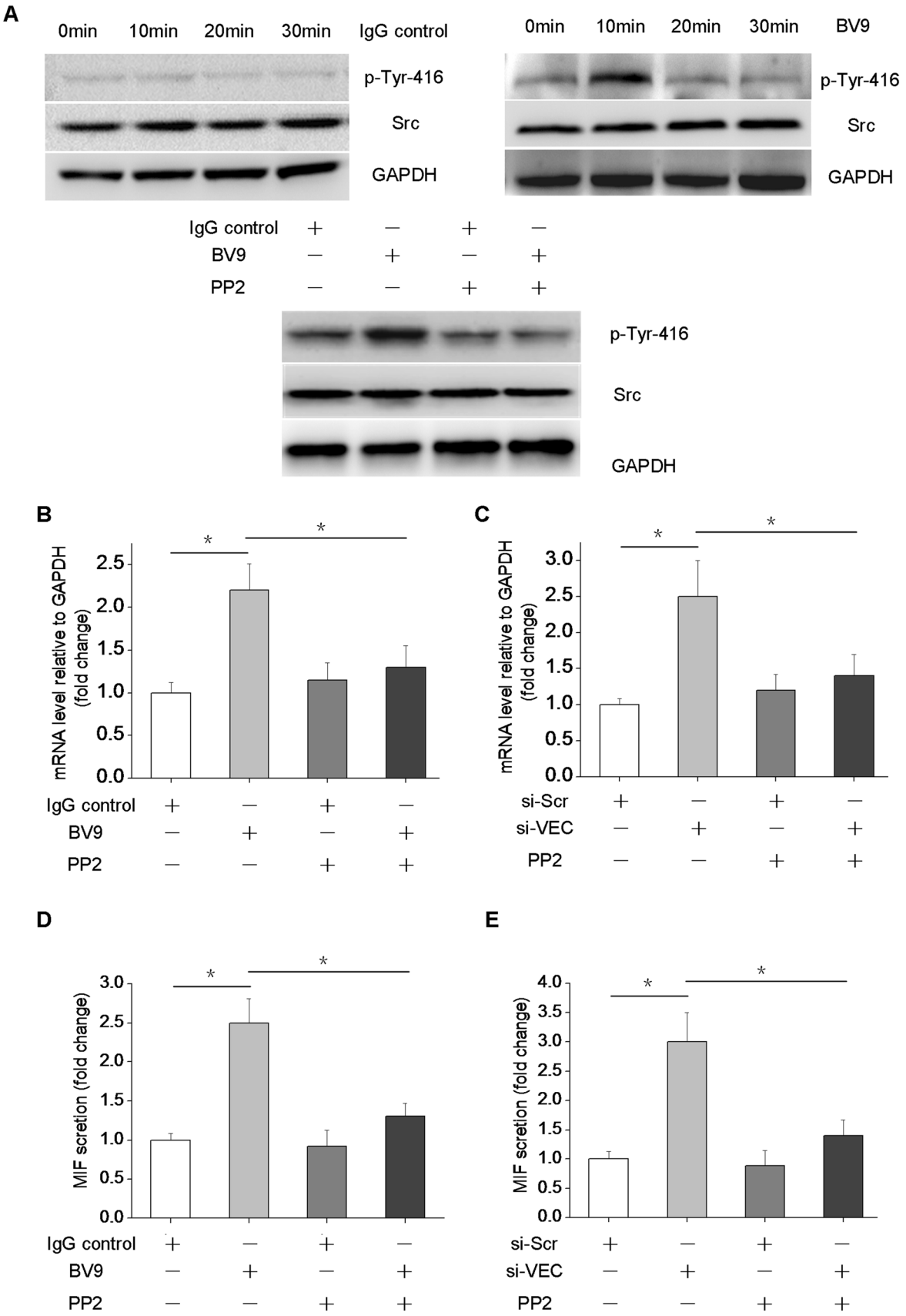
BV9 induces MIF release in vivo and neutralizing MIF ameliorates BV9-induced lung vascular leak and inflammation

To further investigate the in vivo relevance of VE-cadherin regulation of MIF, BV9 (100 μg/mouse) was administered to mice. Administration of BV9 induced a marked time-dependent increase of lung permeability (Fig. 5a). BV9 treatment (6 h) also triggered MIF release into circulation (Fig. 5b).

Based on our preliminary experiments, to explore the role of VE-cadherin-induced increase of MIF and to examine if MIF acts as a mediator sustaining the endothelial barrier disruption induced by VE-cadherin interruption, we used anti-MIF strategy to see if it can alleviate lung vascular leak and inflammation. Neutralizing anti-MIF antibody (100 μg/mouse) previously shown being capable of neutralizing MIF bioactivity was injected simultaneously with BV9 [10]. Neutralizing anti-MIF administration ameliorated BV9-induced lung vascular leak (Fig. 5c) and lung inflammation (6 h) as evidenced by light microscopic histopathology of lung and lung injury score (Fig. 5d, e). The data show that MIF contributed partly to sustained lung pathology triggered by disruption of VE-cadherin-mediated adhesion.

Discussion

In addition to mediating homophilic adhesions, VE-cadherin has some intracellular binding partners. Thus, VE-cadherin also directly or indirectly affects numerous signaling pathways enabling cell–cell contacts to touch upon multiple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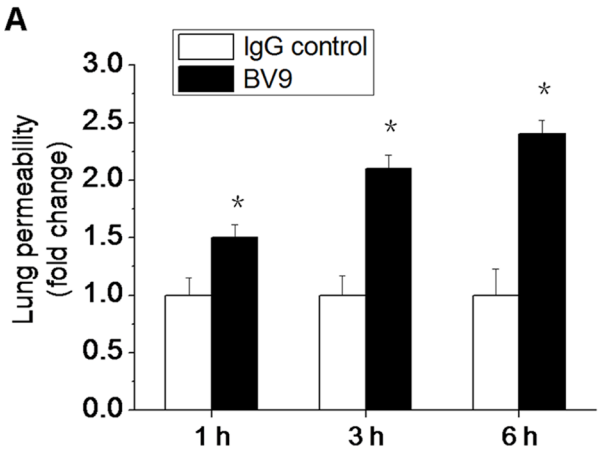
A
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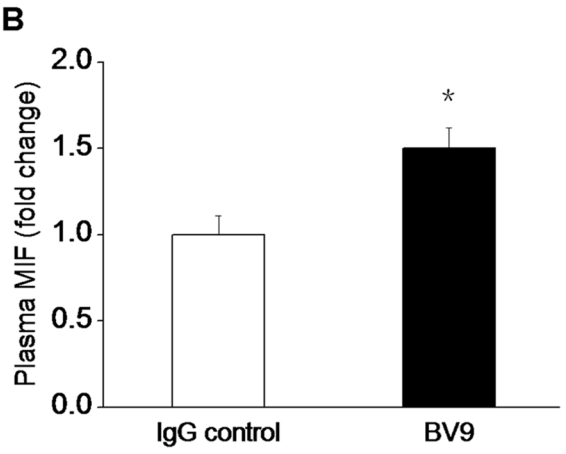
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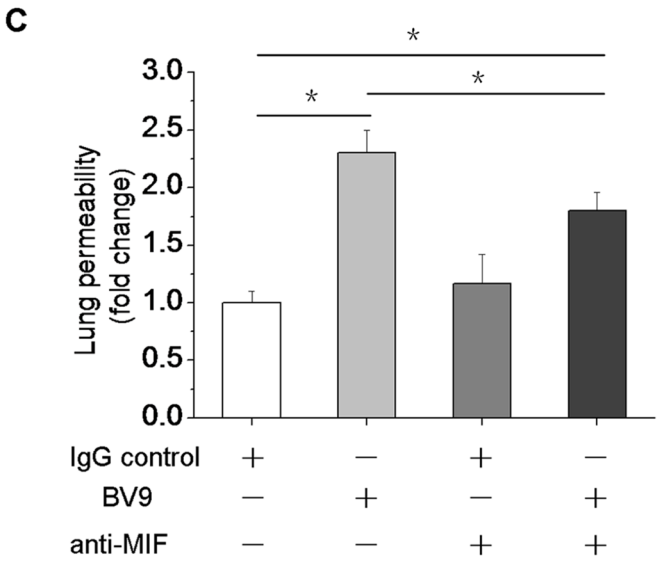
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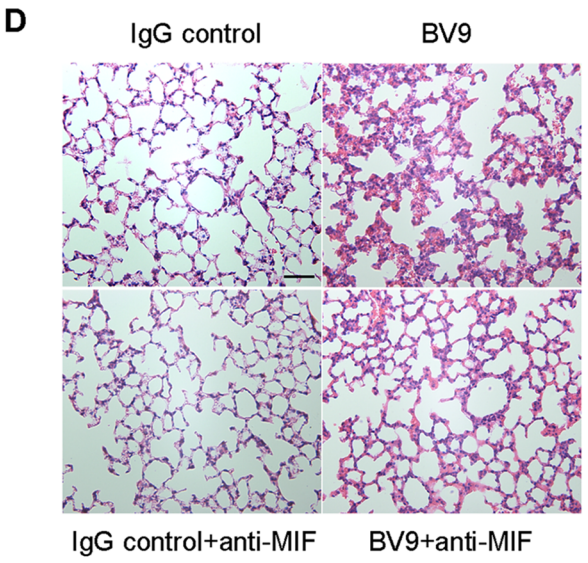
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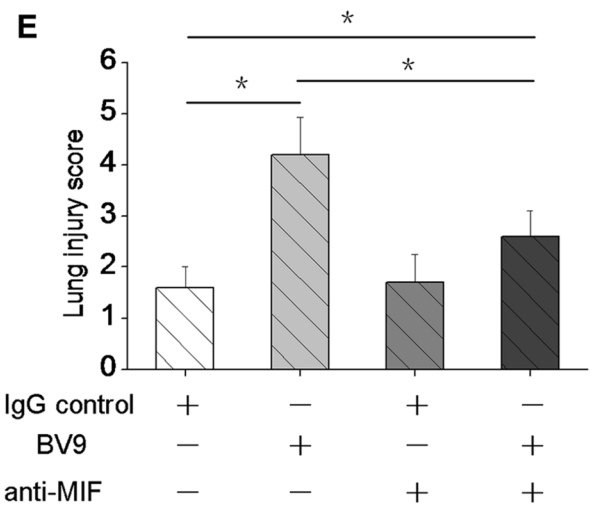
BV9+anti-MIF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◂**Fig. 5** BV9 induces MIF release in vivo and neutralizing MIF ameliorates BV9-induced lung vascular leak and inflammation. **a** BV9-induced lung vascular leak. Data were expressed as fold increase in lung permeability as evaluated by Evans blue in comparison to animals treated with the IgG control. *$p < 0.05$, compared with IgG control at each corresponding time points. **b** BV9 treatment (6 h) triggered MIF release into circulation. *$p < 0.05$, compared with IgG control. **c** Neutralizing MIF using anti-MIF antibody ameliorated BV9-induced lung vascular leak (6 h). *$p < 0.05$. **d** Neutralizing MIF improved BV9-induced lung inflammation (6 h) as evidenced by lung H&E. Representative images are shown. Magnification, 20×; scale bar, 100 μm. **e** Lung injury score was determined. Data in **a**–**c** and **e** show the mean ± SD results from four mice per group. *$p < 0.05$

biological outcomes in embryonic development and tissue homeostasis [1]. In the present study, we demonstrated that, while in resting confluent endothelial cells, VE-cadherin homophilic adhesions suppress the activation of Src to maintain endothelial homeostasis, disassembly of VE-cadherin-mediated adhesion increases expression of MIF through activation of Src. Our findings added evidence to the concept that VE-cadherin homophilic interaction has an outside-in signaling role.

Using a non-biased cytokine profiling, we first identified that disruption of VE-cadherin-mediated adhesion triggers the release of MIF. Subsequently, using VE-cadherin function-blocking antibody and siRNA techniques, we confirmed that VE-cadherin regulated MIF synthesis and release. MIF is an important cytokine in the modulation of inflammatory and immune responses. An increase in serum MIF patients with systemic inflammation is observed, and correlates with disease severity and a higher risk of early mortality [14–19]. The anti-MIF antibody or small molecule inhibitor has therapeutic potential for the treatment of inflammatory diseases [14–17, 20]. Consistent with our in vitro results, we showed that BV9 stimulated MIF release in vivo. Furthermore, neutralizing MIF using anti-MIF antibody partly alleviates BV9-induced lung vascular permeability and inflammation.

Accumulating evidence suggests a role of VE-cadherin in influencing endothelial cell behavior by various outside-in signaling processes. Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5, providing a connection between adherens junctions and tight junctions [21]. VE-cadherin functions by stimulating the phosphorylation of FoxO1 through Akt activation and by limiting the translocation of β-catenin to the nucleus. Thus, cadherins may serve as stoichiometric competitors of nuclear signals in bound state. However, decreased binding of catenin to cadherin cytoplasmic tails facilitates their engagement in gene control. In epithelial cells, there are findings that E-cadherins can initiate outside-in signal transducing pathways through the engagement of tyrosine kinase receptors, which was independent of β-catenin binding or p120-catenin binding [22, 23]. In endothelial cells specifically, group IV phospholipase A(2) α (cPLA2α), one of phospholipase A(2) enzymes which hydrolyze phospholipids to liberate arachidonic acid for the biosynthesis of prostaglandins and leukotrienes, translocates from the cytoplasm to the Golgi complex in response to cell confluence. It is demonstrated that VE-cadherin controls cPLA2α localization and enzymatic activity, with functional consequences for vascular physiology [24, 25]. Similar to E-cadherin homophilic ligation directly signaling through Rac [26], another study showed that alteration of VE-cadherin extracellular interactions using VE-cadherin blocking antibody BV9 modulates Rac activation and endothelial barrier function, suggesting that VE-cadherin outside-in signaling controls locally Rac activity [27].

The cytoplasmic domain of VE-cadherin contains nine tyrosine residues that represent potential target sites for tyrosine kinases, which may participate in recruiting the signaling proteins that associate with VE-cadherin [28, 29]. Indeed, in vascular endothelial cells, VE-cadherin is basally associated with c-Src and Csk (C-terminal Src kinase), a negative regulator of Src activation [7, 28, 29]. In accordance with this, similar to a previous study using EDTA to disrupt VE-cadherin extracellular interactions [13], we showed that in endothelial cells blocking VE-cadherin adhesion using function-blocking antibody also activated Src. Furthermore, we found that this Src activation is essential for VE-cadherin regulation of MIF. In line with previous studies using another VE-cadherin mAb BV13 [12], when added to cultured endothelial cells, BV9 induces a redistribution of VE-cadherin from intercellular junctions; intravenous administration of BV9 induced a significant increase in vascular permeability and inflammation in lungs. Our in vitro finding of MIF regulation by VE-cadherin is in vivo relevance, by showing that BV9 stimulated MIF release in vivo and partly alleviation of BV9-induced lung vascular permeability and inflammation by neutralizing MIF.

While being well established that Src recruitment initiates the activation of multiple pathways, it remains possible that Src phosphorylation by BV9 stimulation may recruit additional unknown proteins, which in turn might be necessary for its interaction with MIF and its export out of the cell. Previous reports revealed that activation of Golgi-localized Src kinases can regulate protein trafficking activity in secretory pathways and the Golgi-associated protein p115 is a critical intermediary component in the mediation of MIF unconventional secretion from monocytes/macrophages [30, 31]. Whether such a mechanism is involved in the export of MIF upon BV9 stimulation in endothelial cells remains to be answered. In addition, Src activation can be linked to the nuclear transcription factors including NF-κB, which is involved in MIF gene activation [32]. Future experiments to investigate the possible signaling pathway and transcription factors involved may provide more details of the regulatory mechanism. Together, our data provide additional evidence

to the concept that VE-cadherin uses signaling mediators to coordinate the state of cell–cell adhesion with gene expression by showing that VE-cadherin regulates MIF synthesis and release via Src.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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