Interleukin-17 Induces Angiogenesis in Human Choroidal Endothelial Cells In Vitro

Ying Chen, Murui Zhong, Liang Liang, Fengjuan Gu, and Hui Peng

Department of Ophthalmology, the First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology, Chongqing Eye Institute, Chongqing, China

Correspondence: Hui Peng, Department of Ophthalmology, the First Affiliated Hospital of Chongqing Medical University, 1 You Yi Road, Yu Zhong District, Chongqing 400016, PR China; penghui20140614@126.com.

YC and MZ contributed equally the work presented here and should therefore be regarded as equivalent authors.

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Purpose. The proinflammatory cytokine interleukin-17 (IL-17) has recently been shown to promote angiogenesis. In addition, a receptor for IL-17, IL-17 receptor C (IL-17RC), is enriched in patients with wet, age-related macular degeneration (AMD), a disease characterized by the formation of choroidal neovascularization. However, the role of IL-17 in choroidal endothelial cells (CECs) angiogenesis has not been defined. This study was conducted to determine the effect of IL-17 on proliferation, migration, and tube formation of human CECs.

Methods. Expression patterns of IL-17 receptor A (IL-17RA) and IL-17RC on isolated human CECs were analyzed by flow cytometry and immunofluorescent staining. Proangiogenic effects of IL-17 on CECs was determined by proliferation assays with a water-soluble tetrazolium cell proliferation reagent kit, wound healing migration assays, and tube formation assays using basement membrane matrix. Cytoskeletal changes were observed by F-actin immunofluorescent staining. Activated Rac1 and RhoA levels were analyzed by pull-down assays.

Results. Interleukin-17RA and IL-17RC were present on human CECs. Interleukin-17 enhanced migration and tube formation but did not affect proliferation. Moreover, IL-17 induced rearrangement of the actin cytoskeleton and upregulated activated Rac1 and RhoA in CECs. The PI3K inhibitor wortmannin suppressed CEC migration, cytoskeleton rearrangement, and upregulation of activated Rac1 and RhoA induced by IL-17.

Conclusions. Interleukin-17 elicits a proangiogenesis effect on human CECs in vitro by promoting migration and tube formation. The promoted migration effect was dependent on PI3K-Rac1 and RhoA-mediated actin cytoskeleton remodeling.

Keywords: choroidal endothelial cell, neovascularization, PI3K, Rac1, RhoA, F-actin

Wet age-related macular degeneration (AMD) is the common cause of irreversible central blindness in older populations worldwide.[1] It is characterized by choroidal neovascularization (CNV), which involves pathologic angiogenic sprouting from the choroidal vessels, penetrating Bruch's membrane, and growing into the subretinal space or subretinal pigment epithelium (RPE). The new blood vessels are often leaky and bleed into the subretinal space, resulting in vision loss.[2] Treatments for CNV in AMD include injections of humanized anti-vascular endothelial growth factor (VEGF) antibody fragments to inhibit neovascularization.[3,4] However, patients are subjected to numerous intravitreal injections and office visits. Additionally, not all patients respond to such monotherapy, suggesting VEGF inhibition alone is insufficient to effectively control progression of the disease.[5] More research is needed to fully understand AMD pathophysiology, to identify and target other proangiogenic molecules that may play a role in AMD.

A growing body of evidence indicates that inflammation and the immune system may play key roles in the development of AMD.[6] In addition to invasion by vascular cells, histopathologic evaluation of CNV shows tissue invasion by extravascular cells including inflammatory cells such as macrophages, lymphocytes, and granulocytes.[7] These inflammatory cells in situ are capable of producing a variety of cytokines that could affect the rate of CNV. Recent reports have demonstrated involvement of the inflammatory cytokine interleukin-17 (IL-17; R&D Systems, Minneapolis, MN, USA) in the pathogenic inflammation of AMD.[8–10] Interleukin-17 is the signature cytokine of T-helper 17 (Th17) cells and plays a pivotal role in inducing expression of proinflammatory cytokines and chemokines in the pathogenesis of autoimmune and inflammatory diseases.[11] In addition to its powerful proinflammatory effect, IL-17 has the potential to upregulate VEGF from rheumatoid arthritis (RA) synoviocytes and to promote development of microvessel structures in RA and tumor growth.[12,13] Furthermore, a recent study using laser-induced CNV mice reported that IL-17 had a strong potential for promoting neovascularization in a VEGF-independent manner.[10] Importantly, RNA transcript levels for IL-17 were shown to be elevated in sera and macular tissues of AMD patients.[9,14] All these reports suggest IL-17 could be involved in the pathogenesis of wet AMD by promoting neovascularization.[15]

It has been well established that IL-17 cytokines exert their effect through an interaction with their cognate receptors. Interleukin-17 receptor A (IL-17RA) is ubiquitously expressed with levels that are higher in hemopoietic cells than in other cell types. In contrast, IL-17 receptor C (IL-17RC) is expressed

primarily by nonhemopoietic cells in tissues such as adrenal glands, prostates, liver, and thyroid.[16] Interleukin-17RC has been shown to be associated primarily with IL-17-induced human microvascular endothelial cell chemotaxis and tube formation in RA and to have elevated messenger RNA and protein expression levels in the affected retina and choroid in wet AMD.[12,14] This evidence supports the possibility that IL-17 directly affects the formation of CNV locally.

Increased expression of IL-17 and IL-17RC in retina and choroid in patients with CNV as well as the proangiogenic effect of IL-17 could indicate that IL-17 is capable of regulating pathological choroidal angiogenesis; however, the role of IL-17 has not been studied extensively in the context of wet AMD with CNV. In this study, we investigated the role of IL-17 in regulating choroidal endothelial cell (CEC) proliferation, migration, and tube formation, all of which are considered fundamental steps in the development of CNV. Additionally, we identified a molecular mechanism by which IL-17 activates CEC migration.

Materials and Methods

Isolation and Culture of Human CECs

Human ocular posterior segments free of any known ocular disease were obtained from Chongqing Eye Bank (Chongqing, China). The retina was torn from RPE with forceps, and the RPE was rubbed off of the choroid with a cotton swab. Choroids were torn from sclera, and endothelial cells were isolated from 2 choroids of 1 donor by treatment with 0.2% type II collagenase in minimum essential medium (Gibco, Grand Island, NY, USA) at 37°C and 5% CO_2 until the tissue was visibly digested. The cell suspension was filtered through sterile 70-mm and 40-mm filters (BD Biosciences, Franklin Lakes, NJ, USA). The filtered suspension was centrifuged at 300*g* and resuspended in isolation medium containing anti-CD31 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to product instructions. The isolated cells were resuspended in endothelial growth medium-2 (EGM-2 MV; Lonza, Basel, Switzerland) with 5% fetal bovine serum (FBS) and seeded onto 6-well plates (Corning, Tewksbury, MA, USA) coated with fibronectin (BD Biosciences). Choroidal endothelial cells were incubated at 37°C and 5% CO_2 in a humidified atmosphere. Medium was changed every 4 days. Cells were passaged with 0.025% trypsin and 0.01% EDTA in sterile phosphate-buffered saline (PBS). Choroidal endothelial cells between passages 3 and 5 were used for experiments.

Proliferation Assay

Cellular proliferation was measured using a water-soluble tetrazolium (WST-1) cell proliferation reagent kit (product no. C0035; Beyotime, Shanghai, China) according to product instructions. Choroidal endothelial cells were seeded at 2×10^3 cells per well of 96-well plates. Cells were starved in EGM-2 MV basal medium containing 0.1% FBS for 24 h, followed by treatment with a range of IL-17 dosages (0.1, 1, 10, 20, or 100 ng/mL) in medium or treatment with medium alone (control) for 72 h. Cells were then incubated in WST-1 solution for 2 h at 37°C, and 450-nm absorbance was measured by spectrophotometer (SpectraMax; Molecular Devices, Sunnyvale, CA, USA).

Transwell Migration Assay

Following 24-h incubation in EGM-2 MV basal medium with 0.1% FBS, CECs (1×10^5cells/200 μL EGM-2 MV basal medium) were seeded in the upper wells of a 24-well transwell chamber (Corning) with fibronectin-coated polycarbonate membranes (8-μm pore size). Varying concentrations of IL-17 (0.1–100 ng/mL) were added to the bottom wells to a final volume of 600 μL. Cultures were incubated for 6 h at 37°C and then fixed with 4% paraformaldehyde in PBS and stained with 4,6-diamidino-2-phenylindole (DAPI) for 3 minutes at room temperature. Cells that had not migrated were removed with a cotton swab, and the membrane was analyzed by epifluorescence microscopy. The average number of cells migrating through the membrane was calculated by counting cell numbers per field of view (FOV) for 10 nonoverlapping FOV at ×10 magnification, in 3 biological replicates (average of 3 × 10 FOV/well, using single wells per biological replicate). Specificity of IL-17-induced CEC migration was tested. Choroidal endothelial cell chemotaxis was examined with IL-17-neutralizing antibody (20 μg/mL for 1 h at 37°C; R&D Systems).

Monolayer Wound Healing Assay

Choroidal endothelial cells were grown to confluence in 6-well plates in EGM-2 MV with 5% FBS and then starved in EGM-2 MV with 0.1% FBS for 24 h. The monolayer was wounded with a sterile pipette tip and washed with PBS to remove debris. The wounded monolayer was pretreated with wortmannin (100 nM), or not, for 20 minutes and then incubated with IL-17A (20 ng/mL), or without. Images were taken at the indicated time points, and the wound closure was calculated using Image Pro Plus 6.0 software (Media Cybernetics, Warrendale, PA, USA).

Tube-Forming Assay

Ice-cold growth factor-reduced basement membrane matrix (Matrigel; BD Biosciences) was added at 300 μL per well to precooled 24-well plates and allowed to polymerize at 37°C for 30 minutes. Choroidal endothelial cells (1×10^5) in 300 μL of EGM-2 MV basal medium with or without 20 ng/mL IL-17A were plated onto the gel surface and incubated at 37°C for 6 h. Cell rearrangement and tube formation were visualized by phase-contrast microscopy. Images of five random fields at ×5 magnification per well were taken, and endothelial tube length was quantified using Image Pro Plus 6.0 software.

Immunofluorescence Staining

Isolated CECs were seeded onto 1% fibronectin-coated glass coverslips and fixed with 4% PBS for 20 minutes at room temperature. Fixed cells were blocked in PBS containing 10% goat serum for 30 minutes and incubated with rabbit anti-von Willebrand factor (vWF; catalog no. F3520; Sigma-Aldrich Corp., St. Louis, MO, USA) overnight at 4°C. Rabbit immunoglobulin G (IgG; R&D Systems) was used as isotype control. Cells were washed three times in PBS and then incubated with fluorescent dye-conjugated secondary antibody (Dylight 488; Jackson Immunoresearch Laboratories, West Grove, PA, USA) for 30 minutes at room temperature, followed by 2 minutes of nuclear staining with DAPI. Choroidal endothelial cells stained for F-actin, a rinse in PBS, fixation with 4% paraformaldehyde in PBS for 20 minutes, and permeabilization with 0.1% Triton X-100 for 3 minutes. Permeabilized cells were stained with Alexa Fluor 488-phalloidin for 40 minutes at room temperature (Molecular Probes, Eugene, OR, USA). All fluorescent images were taken with a fluorescence microscope (model DM6000; Leica, Wetzlar, Germany).

Flow Cytometry (FCM) Analysis

Monoclonal antibodies (mAb) targeting CD31 (Miltenyi Biotec), IL-17RA (ebioscience, San Diego, CA, USA), and IL-17RC (R&D Systems) were used for flow cytometry. Cells were

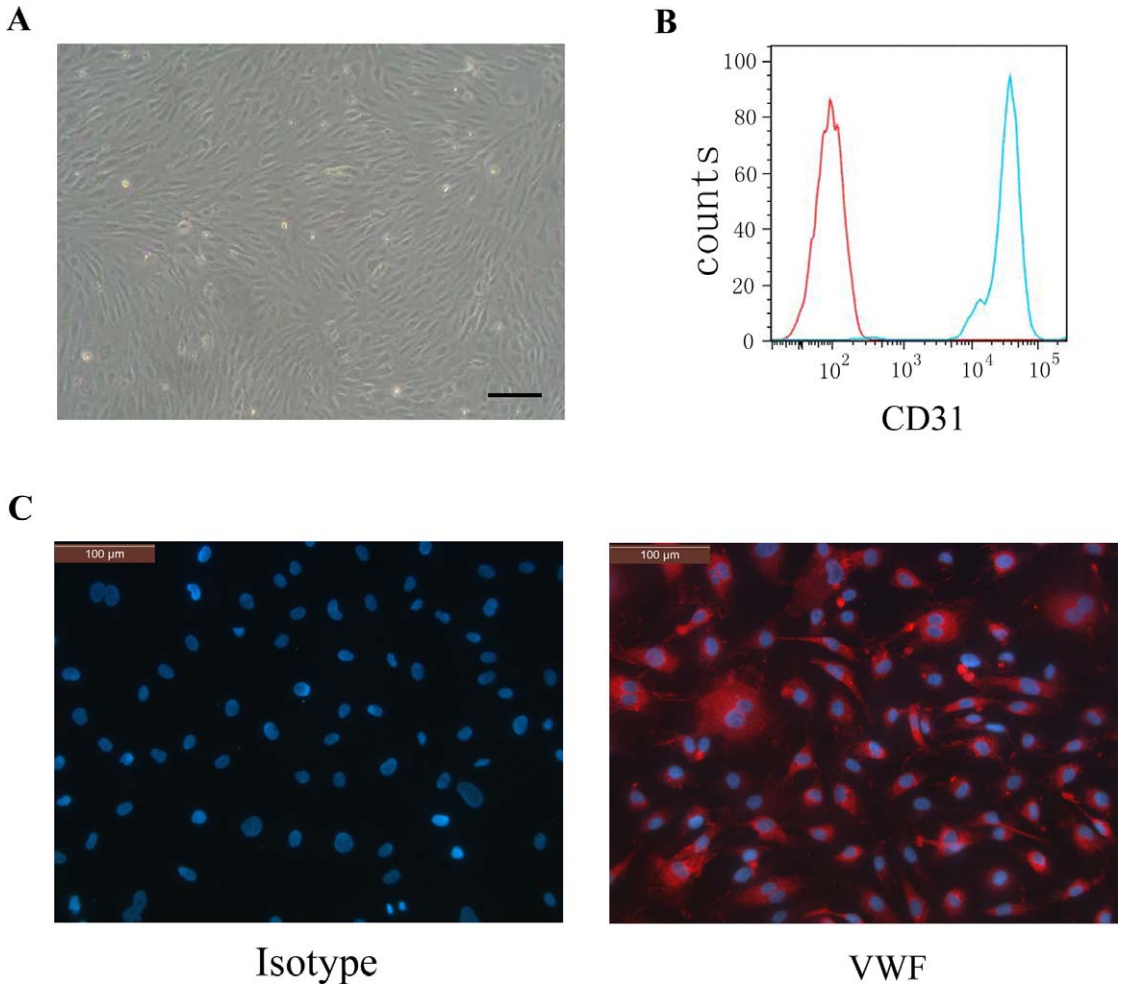

FIGURE 1. Identification of cultured CECs. (**A**) Phase-contrast photomicrograph of confluent CECs (passage 2) demonstrating a cobblestone appearance. (**B**) Expression of CD31 on CECs was tested by flow cytometry. CD31 expression is shown by the shift in fluorescence intensity of the specific antibody (*blue line*) over the isotype control (*red line*). (**C**) Immunofluorescence photomicrograph of CECs stained for vWF. (**A**, **C**) *Scale bar*: 100 µm.

detached from plates by trypsin digestion and rinsed with PBS. Aliquots of 1×10^6 cells were incubated with allophycocyanin cell–conjugated mAb against human CD31 and IL-17RA and phycoerythrin-conjugated mAb against human IL-17RC according to the product instructions. Data collection and analysis were performed using a flow cytometer (FACSCalibur; BD PharMingen, San Jose, CA, USA) and appropriate software (CellQuest; BD PharMingen).

Rac1 and RhoA Pull-Down assay

Activation of Rac1 and RhoA was tested using the available Rac1 and RhoA activation assay kit (Cytoskeleton, Denver, CO, USA) according to the recommendations of the manufacturer. Briefly, CECs were cultured under conditions identical to those described for the immunofluorescence staining assay. At the indicated points, the cells were washed with ice-cold PBS and lysed in ice-cold lysis buffer, with the addition of a 1:100 final dilution of a protease inhibitor cocktail. Lysed cells were harvested with a cell scraper and clarified by centrifugation at 4°C. Samples were divided into aliquots, snap frozen with liquid nitrogen, and stored at −70°C until used. Equal volumes of supernatants were incubated for 1 h at 4°C in the presence of glutathione *S*-transferase (GST)-fused p21 activated kinase 1 (PAK)-p21 binding domain (PBD) or Rhotekin-Rho binding domain (RBD) bound to glutathione-Sepharose beads for adsorption of GTP-Rac1 or GTP-Rho, respectively, followed by three washes in the supplied wash buffer. Bound proteins were eluted in sample buffer and analyzed by Western blotting, using antibodies against Rac1 and RhoA.

Statistical Analyses

All results are expressed as means ± SD with n values indicated. The Student t-test or one-way ANOVA test was used for statistical comparison between groups. A P value less than 0.05 was considered statistically significant.

RESULTS

Human CECs Constitutively Express IL-17RC and Weakly Express IL-17RA

Human CECs isolated from all donors showed cobblestone morphology typical of endothelial cells (Fig. 1A). Choroidal endothelial cells purity was above 95% as analyzed by FCM for CD31 (Fig. 1B) and immunofluorescent staining for vWF (Fig. 1C). Constitutive expression of IL-17RC by human CECs was confirmed by immunofluorescent staining and FCM (Figs. 2A,

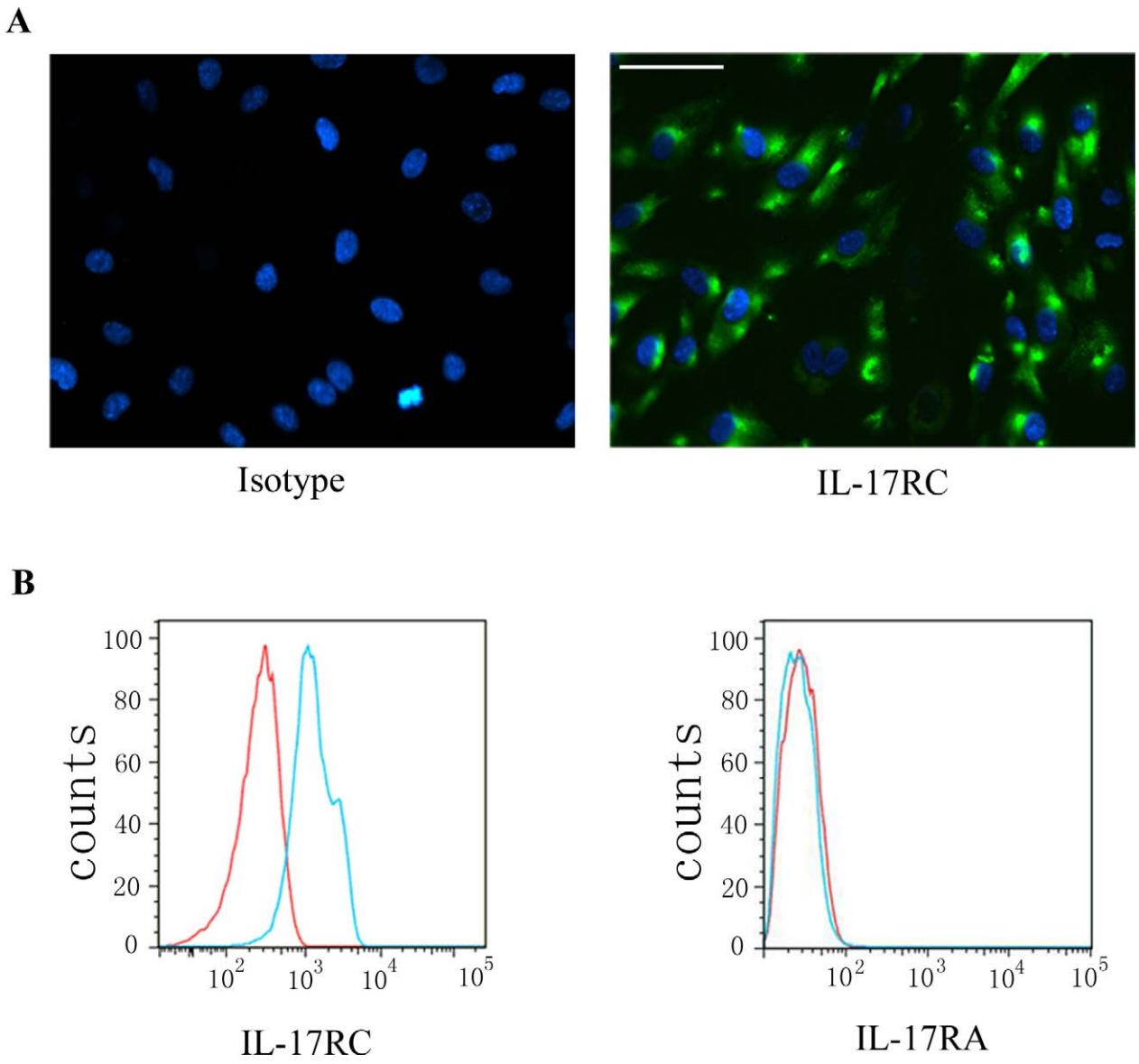

FIGURE 2. Expression of IL-17RC and IL-17RA on CECs. (**A**) Immunofluorescence photomicrograph of CECs stained for IL-17RC. *Scale bar*: 50 μm. (**B**) Flow cytometry analysis of IL-17RC and IL-17RA expression on CECs. Interleukin-17RC and IL-17RA expression on cells is shown by the shift in fluorescence intensity of the specific antibody (*blue line*) over the isotype control (*red line*).

2B); however, expression of IL-17RA by these cells was detected at a low level by using FCM (Fig. 2C) and was undetectable by immunofluorescent staining.

IL-17 Enhances Migration and Tube Formation but Not Proliferation of CECs

Treatment of CECs with a wide range of doses of human IL-17 (0.1–100 ng/mL) had no direct effect on proliferation (Fig. 3). However, IL-17 enhanced CEC transwell migration with concentrations $\geq$1 ng/mL, with the maximal migration effect observed with approximately 20 ng/mL (Fig. 4). The effect of IL-17 on wound repair of CECs was assayed by determining the migration distance across a wound by direct visualization with light microscopy. Migration distance increased over time for both IL-17–stimulated and control groups. However, IL-17 induced a longer migration distance than that in the control group at 12 h ($P = 0.007$) (Fig. 5). Furthermore, we found 20 μg/mL IL-17 significantly promoted capillary-like structure

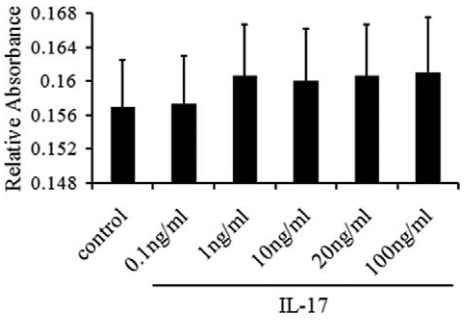

FIGURE 3. Effect of IL-17 (0.1–100 ng/mL) on the proliferation of CECs. Cell proliferation was measured with WST-1 at 24 h. Data are means ± SD of results from at least three independent experiments. *$P < 0.05$ vs. control.

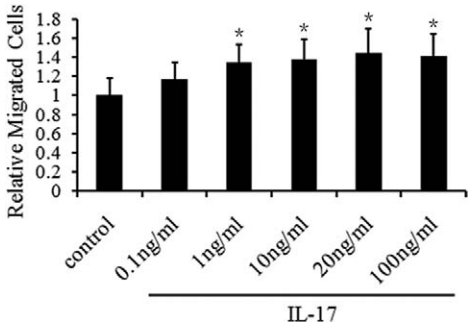

FIGURE 4. Interleukin-17 chemotactic effects on CECs. Chemotactic activity was estimated based on the number of cells that had migrated through the filter of the transwell chamber. Data are means ± SD of results from at least three independent experiments. Control was set as 1. $^{*}P < 0.05$ vs. control.

formation of CECs cultured on growth factor-reduced basement membrane matrix and increased tube length by 26% ($P = 0.001$; Fig. 6).

IL-17 Modulates Actin Cytoskeleton Reorganization in CECs

We examined the cytoskeletal changes following IL-17 treatment by staining for F-actin. Figure 7 shows representative images of actin cytoskeleton reorganization induced by IL-17. In unstimulated serum-starved cells, actin filaments localized mainly in the cortical region of the cell (Fig. 7A). Treatment with 20 ng/mL IL-17 resulted in F-actin filament disassembly. Furthermore, we observed the cells forming filopodia and membrane ruffling, phenotypes associated with cell motility at the cell periphery (Fig. 7B). Following prolonged IL-17 treatment, the cells displayed prominent stress fibers related to cell contraction across the cell body (Fig. 7C).

Upregulation of Activated Rac1 and RhoA in CECs Stimulated by IL-17

Rac1 and RhoA belong to the small GTPases of the Rho family and play a vital role in controlling actin dynamics. Cell migration is driven by the dynamic reorganization of the actin cytoskeleton into filopodia, lamellipodia, and stress fibers. Activation of Rac1 and RhoA is required for lamellipodia and stress fibers formation, respectively. Expression of active Rac1 and RhoA in CECs stimulated by 20 ng/mL IL-17 were detected by GST-PAK pull-down assay and Western blotting (Fig. 8). Results showed that expression of activated Rac1 increased rapidly in IL-17–stimulated CECs (at 5 and 10 minutes) and was distinct from that of the control group, suggesting a potential underlying mechanism involving Rac1-dependent IL-17–mediated actin cytoskeletal rearrangement (Fig. 8). In comparison with Rac1, expression of activated RhoA changed relatively slowly. Specifically, there was no significant change in RhoA expression compared to that in the control group from 0 to 5 minutes, but RhoA expression increased significantly at 15 minutes (Fig. 8). These results indicated that IL-17 could activate Rac1 rapidly but RhoA relatively slowly.

Inhibition of PI3K Reduces Cell Migration, Cytoskeleton Changes, and Upregulation of Activated Rac1 and RhoA Induced by IL-17 in CECs

To examine whether PI3K was involved in IL-17–induced migration, cytoskeleton changes, and upregulation of activated Rac1 and RhoA, we stimulated the cells with IL-17 followed by treatment with wortmannin (a PI3K inhibitor) to observe potential effects as well as expression of activated Rac1 and RhoA. Wortmannin reduced IL-17–stimulated CEC migration at 12 h in wound repair experiments ($P = 0.013$) (Fig. 5) and led to reduced membrane ruffling and actin stress fiber production (Fig. 7). Wortmannin treatment did not have a significant influence on the formation of filopodia in IL-17–stimulated CECs (Fig. 7). Activated Rac1 ($P = 0.007$ at 5 minutes, $P = 0.001$ at 15 minutes) and RhoA ($P = 0.031$) expression induced by IL-17 was notably inhibited by wortmannin, suggesting PI3K is involved in IL-17-mediated activation of Rac1 and RhoA (Fig. 8).

DISCUSSION

In this study, we successfully cultured human CECs to ≥95% purity, allowing for direct experimentation for assessing the role of IL-17 on CEC physiology associated with CNV in wet AMD. A number of studies have shown that IL-17 plays a role through interaction with its receptors IL-17RA and IL-17RC.[17–19] We first confirmed expression of IL-17RA and IL-17RC on the purified, passaged CECs to determine IL-17 responsiveness. A recent report also revealed that expression of IL-17RC increased in peripheral blood cells as well as in the affected retina and choroid in wet AMD patients as a result of IL-17RC promoter hypomethylation.[14] Together, these findings,

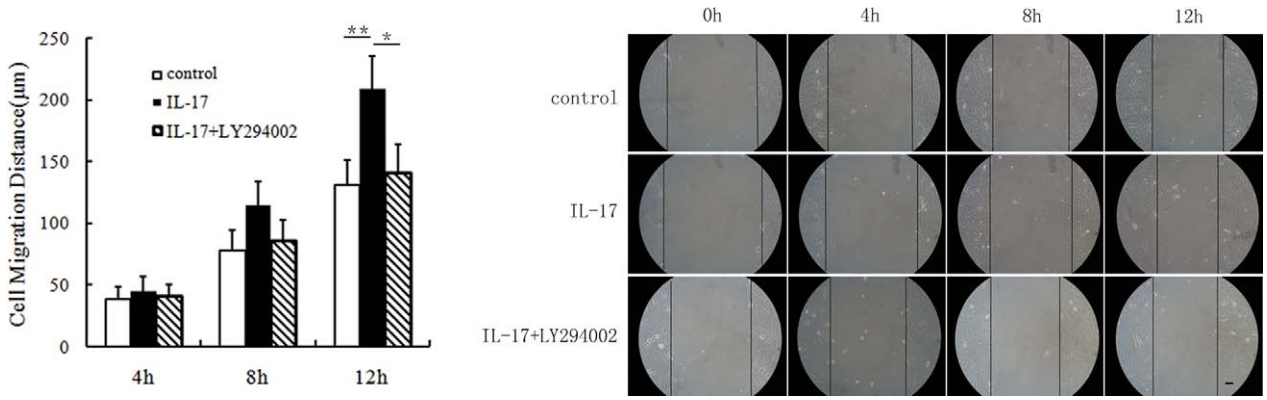

FIGURE 5. Interleukin-17 promoted wound repair in CECs via the PI3K pathway. Choroidal endothelial cells were grown to confluence and scratched in a 6-well plate. Wound healing distance was recorded by phase-contrast microscopy over a 12-h time course following perturbation. *Scale bar*: 100 μm. Data are means ± SD of results from at least three independent experiments. Control was set as 100%. $^{*}P < 0.05$, $^{**}P < 0.01$.

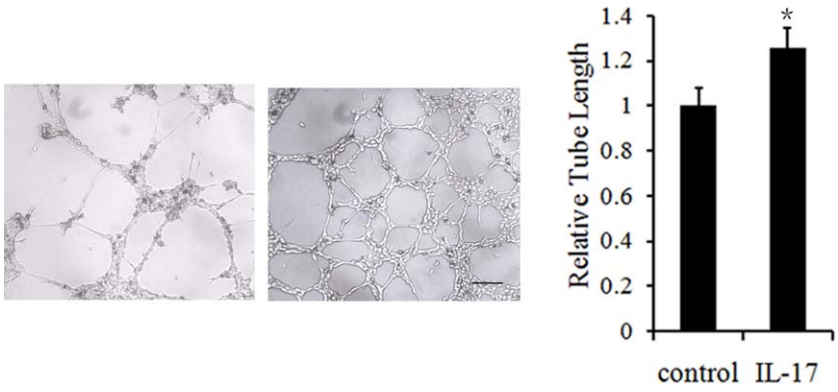

Figure 6. Interleukin-17 induced tube formation in CECs. Cells were seeded onto plates coated with growth factor-reduced basement membrane matrix and exposed to IL-17 for 16 h. Endothelial tube formation was imaged by microscopy, and the tube length was analyzed. *Scale bar*: 100 μm. Data are means ± SD of results from at least four independent experiments. Control was set to 1. $^*P < 0.05$ vs. the control.

in addition to our observations, support the hypothesis that IL-17 can regulate development of CNV in wet AMD through interaction with IL-17RA and IL-17RC. A recent study showed that IL-17 retinotoxicity can be prevented by inhibiting IL-17RC.[20] It is worthwhile to note that our data also showed that the extent of expression of IL-17RC is much higher than that of IL-17RA in CECs, implying that IL-17RC may play a more important role in mediating the function of IL-17 in wet AMD.

Next, we evaluated the direct angiogenic effect of IL-17 on CECs, using a proliferation assay, revealing no change in CEC proliferation. It is known that cytokines, which can stimulate angiogenesis in vivo and fail to induce mitogenic activity for vascular endothelial cells in vitro, have been grouped with indirect angiogenic factors. Therefore, the inability of IL-17 to stimulate proliferation suggests that it belongs to the indirect class of angiogenic stimulators. Based on this result, we directed our efforts to understanding potential indirect angiogenic effects of IL-17 on CECs such as migration and tube formation. Our results demonstrate that IL-17 markedly enhances the migration of CECs, which was shown by the transwell migration assay and wound repair assay, as well as the tube formation assay. In order to explore the mechanisms by

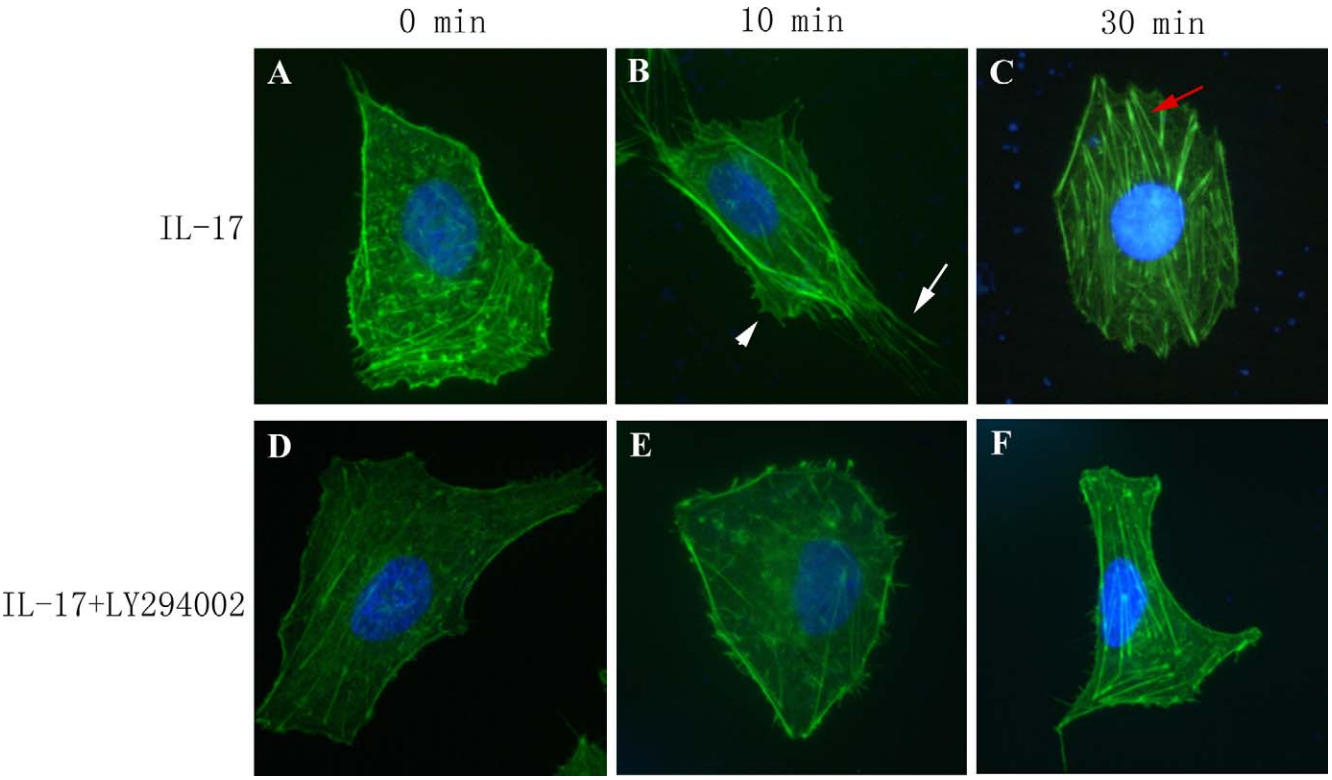

Figure 7. Interleukin-17 induced actin cytoskeletal reorganization in CECs via the PI3K pathway. Choroidal endothelial cells were fixed with methanol and immunostained with Alexa Fluor 488 phalloidin (*green*) for F-actin and DAPI for nuclei (*blue*). Fluorescence images were captured, and microscopic actin filaments were observed mainly in the cortical region of the cell at 0 minutes. After 20 minutes of stimulation with 20 ng/mL IL-17A, the cells formed filopodia (*white arrow*) and membrane ruffling (*arrowhead*, 0 minutes). After 30 minutes of IL-17A treatment, the cells displayed prominent stress fibers (*red arrow*). Wortmannin reduced the formation of filopodia, membrane ruffling, and stress fibers.

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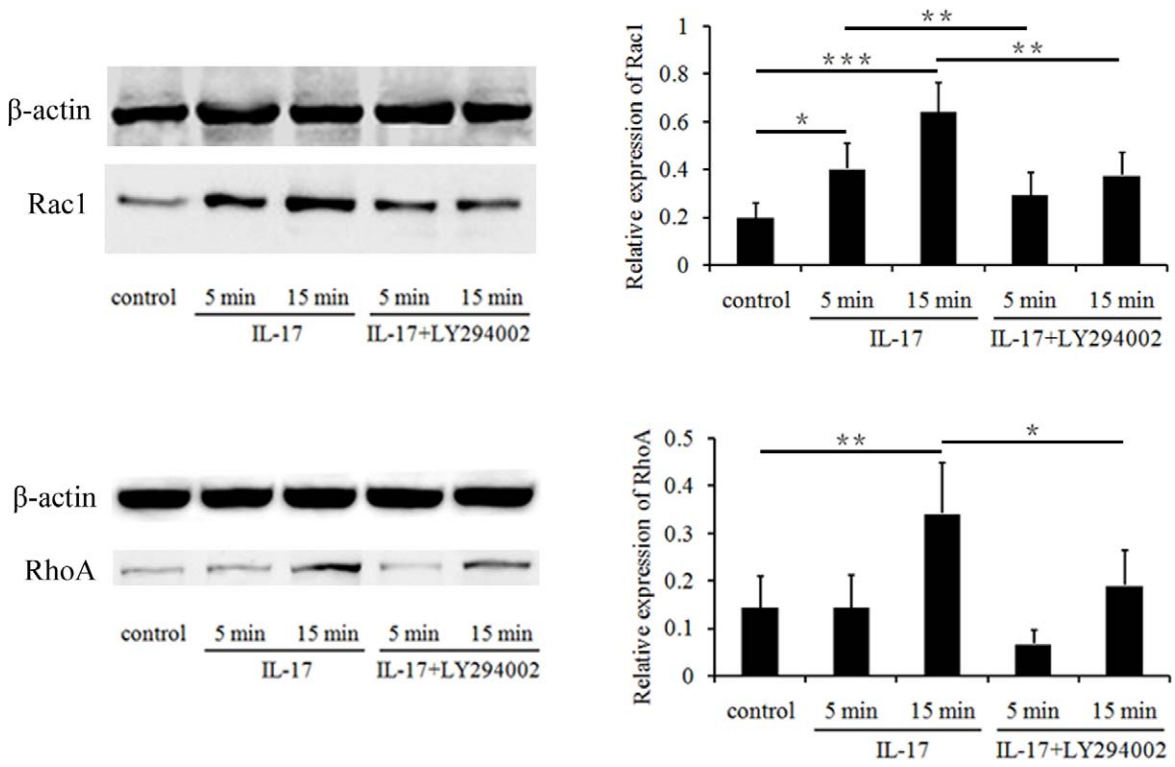

FIGURE 8. Interleukin-17 activated Rac1 and RhoA via the PI3K pathway. Choroidal endothelial cells were subjected to pull-down and Western blot analyses using specific antibodies. β-Actin served as loading control. Activated Rac1 expression levels at 5 and 15 minutes were significantly increased compared with those of control and could be inhibited by wortmannin treatment (*$P < 0.05$). However, activated Rac1 expression at 5 minutes was not different from that of control After 15 minutes, Rac1 expression was significantly increased compared to that of control, and the effect was inhibited by wortmannin (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Data are means ± SD of results from at least three independent experiments.

which IL-17 mediates migration, we examined whether IL-17 can influence F-actin rearrangement. It is well known that cell migration is closely related to actin remodeling, which is a major cytoskeletal component of endothelial cells. Actin-based endothelial cell motility is dependent upon the constant remodeling of the actin cytoskeleton into filopodia, lamellipodia, and stress fibers.[21,22] In the present study, we found that IL-17 treatment not only induced the filopodia and lamellipodia extensions but also increased actin stress fibers in CECs. Cells use filopodia to sense guidance cues, lamellipodia to assure the swimming movement, and stress fibers to mediate cell body contraction, all of which facilitate forward progression. Our data indicate that IL-17–promoted migration in CECs is associated with intracellular actin remodeling.

There is a growing body of evidence showing that of all the signaling molecules that are involved in cell migration, it is the small GTPases of the Rho family that play a vital role in controlling actin dynamics, in particular Rac1, RhoA, and Cdc42.[23] Rac1 can stimulate the formation of protruding membrane ruffles and lamellipodia at the leading edge of migrating cells, whereas RhoA mediates stress fiber formation and cell contractility.[24,25] In our investigations of the possible intracellular pathway associated with IL-17–induced actin remodeling, we found that IL-17–induced pronounced augmentation of both Rac1 and RhoA activity. Rac1 is activated earlier than RhoA in CECs. This event corresponds to the process of actin remodeling and is characterized by formation of membrane ruffles, which appear before stress fibers. Thus, IL-17–induced actin cytoskeletal reorganization may be associated with an increase of both activated Rac1 and RhoA in CECs.

Several studies have reported Rac1 activation dependence on PI3K activity.[26–28] Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration.[29] PI3K has been shown to activate Rac1 at the leading edge by interaction with either receptor tyrosine kinases or G protein-coupled receptors.[30] Additionally, PI3K was found to activate RhoA in Wnt5a-treated human gastric cancer cells.[31] In our study, the PI3K inhibitor wortmannin could reduce activation of Rac1 by IL-17, and inhibit the lamellipodia formation associated with activated Rac1. Similar results were obtained for RhoA activation and stress fiber formation, both of which were also repressed by wortmannin. Therefore, it is possible that PI3K is upstream of Rac1 and RhoA activation in CECs stimulated by IL-17 and that IL-17–triggered actin remodeling functions at least partly via the PI3K-Rac1 and RhoA pathways. In addition, we observed that the effects of IL-17 on wound repair were blunted by wortmannin. These data provide evidence that the promigration effect of IL-17 on human CECs was dependent on PI3K-Rac1 and RhoA-mediated actin cytoskeletal remodeling.

In conclusion, this study revealed the presence of IL-17RA and IL-17RC on isolated human CECs and showed that IL-17 can promote migration and tube formation independent of CEC proliferation. The promotion of IL-17-induced cell migration was shown to be associated with the activation of both Rac1 and RhoA, events which led to cytoskeletal alteration. Pretreatment of CECs with the PI3K inhibitor wortmannin was found to reduce membrane ruffling as well as expression of both activated Rac1 and RhoA. Collectively, these data suggest that PI3K is downstream of IL-17 and that PI3K functions as an upstream activator of Rac1 and RhoA,

subsequently affecting the cytoskeletal arrangement and inducing migration of IL-17-stimulated CECs. These findings suggest that molecular targeting of IL-17 may be a feasible therapeutic approach for treating wet AMD.

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