Involvement of IL-37 in the Pathogenesis of Proliferative Diabetic Retinopathy

Mengmeng Zhao,[1] Yongguang Hu,[1] Ying Yu,[2] Qing Lin,[1,3] Jianhua Yang,[1] Shao Bo Su,[1] Guo-Tong Xu,[4] and Tianshu Yang[1]

[1]Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China
[2]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China
[3]Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
[4]Department of Ophthalmology, Shanghai Tenth People Hospital, and Tongji Eye Institute, Tongji University School of Medicine, Shanghai, China

Correspondence: Tianshu Yang, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200092, China; tianshuy@tongji.edu.cn.
Guo-Tong Xu, Department of Ophthalmology, Shanghai Tenth People Hospital and Tongji Eye Institute and Department of Regenerative Medicine, Tongji University School of Medicine, 1239 Siping Road, Shanghai 200092, China; gtxu@tongji.edu.cn.

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

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Purpose. Interleukin-37 is suggested as a novel proangiogenic factor in our previous study. In this study, the role of IL-37 was investigated in proliferative diabetic retinopathy (PDR).

Methods. Vitreous fluids from 10 patients with PDR and 8 controls were collected. The levels of IL-37 were determined by ELISA and the relationship between IL-37 and VEGF-A/Ang-2 was analyzed. The effects of IL-37 on chorioretinal endothelial cell (RF/6A) proliferation, migration, and tube formation were determined by BrdU incorporation assay, Boyden chamber assay, scratch-wound assay and tube formation assay.

Results. The concentration of IL-37 in the PDR group was 95.09 ± 5.22 pg/mL and 34.91 ± 5.61 pg/mL in control group ($P = 0.001$). The level of IL-37 was highly related to the level of Ang-2 ($P = 0.009$, $r = 0.772$) and VEGF-A ($P = 0.003$, $r = 0.827$) in the PDR group, and VEGF expression in RF/6A cell was upregulated by IL-37 at low concentration. Interleukin-37 remarkably promoted RF/6A cell proliferation and migration. Interleukin-37 (1 ng/mL) remarkably stimulated tube formation with an increase of 85.3% for total tubule length and 74.1% for branching points compared with PBS control.

Conclusions. The level of IL-37 is elevated in vitreous fluids of patients with PDR and correlates with the level of VEGF-A and Ang-2. Interleukin-37 stimulates proangiogenic response of retinal endothelial cells in vitro, suggesting the involvement of IL-37 in the pathogenesis of PDR.

Keywords: IL-37, proliferative diabetic retinopathy, angiogenensis, VEGF

Proliferative diabetic retinopathy (PDR), a serious complication of diabetes mellitus, is the leading cause of severe vision loss and blindness.[1] Proliferative diabetic retinopathy mainly occurs when the microvascular function of retina is destroyed, followed by neovascularization induced by retinal ischemia and production of proangiogenic factors.[2] These vessels, however, mostly are dysregulated, which results in vitreous hemorrhage, tractional retinal detachment, neovascular glaucoma, and even visual loss.[3] Previous studies suggest that proangiogenic cytokines, such as VEGF, Ang-2, TGF-β, and CCN1, are probably involved in the pathogenesis of PDR. Among these cytokines, the proangiogenic effect of VEGF in PDR has been established by many clinical studies.[4–8]

Interleukin-37, a newly reported member of the IL-1 family, was recognized as an anti-inflammatory cytokine in numerous inflammatory diseases,[9] such as systemic lupus erythematosus, rheumatoid arthritis (RA), and ankylosing spondylitis.[10–12] Interleukin-37 executes its function by binding to the IL-18 receptor α (IL-18Rα) and SIGIRR/IL-1R8 in allergic airway inflammation in mice.[13,14] Notably, some members of the IL-1 family are potent angiogenic factors involved in ocular neovascularization. For example, IL-1β, which angiogenic activity has been demonstrated in vivo and in the model of cornea neovascularization,[15–17] is significantly increased in the vitreous fluid of PDR patients.[18] Another IL-1 family member, IL-18, was reported to act as either an angiogenic or an angiostatic factor. Studies in mice showed that IL-18 knockout mice exhibit abnormal retinal vascular development.[19]

Remarkably, IL-37 is involved in some pathological process in which angiogenesis plays a critical role. For example, IL-37 increases in synovial tissue from RA patients[11] and is detected in foam cells of atherosclerosis patients.[9,20] However, the role of IL-37 in retinal angiogenesis in PDR remains unclear.

In this research, we found that IL-37 played an important role in retinal angiogenesis, and may be a relevant factor in the pathogenesis of PDR. Interleukin-37 was upregulated in the vitreous humor from PDR patients, and the upregulation of IL-37 was positively interrelated with vitreous levels of VEGF-A and Ang-2. Furthermore, stimulation of chorioretinal endothelial cells (RF/6A) with IL-37 triggered endothelial cell proliferation, migration, and vascular-like structure formation.

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Materials and Methods

Sample Collection

The vitreous samples of 10 patients diagnosed with PDR and 8 control subjects with nondiabetic ocular diseases were collected from the Affiliated Hospital of Nantong University. All patients gave informed consent before enrollment. This study strictly followed the tenets of the Declaration of Helsinki and all experimental procedures were granted by the institutional review board of Tongji University. To ensure reliability, cytokine levels in vitreous fluids were determined in triplicate or quadruplicate by ELISA.

Cell Culture and Reagents

Monkey chorioretinal vessel endothelial cells (RF/6A) were purchased from the American Type Culture Collection (Manassas, VA, USA and Rockville, MD, USA), and the cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 ng/mL streptomycin, and 100 U/mL penicillin (GIBCO, Grand Island, NY, USA). The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere. Recombinant human IL-37 and Anti-IL-37 antibodies were purchased from R&D Systems (Minneapolis, MN, USA).

Cell Proliferation Assay

A total of 1.2×10^4 RF/6A cells were seeded in 96-well plates in 200 μL DMEM containing 2% FBS. Cells were treated with different concentrations of IL-37 for 24 hours. Cell proliferation was measured by BrdU ELISA kit (Abcam, Cambridge, MA, USA) according to standard protocols. Briefly, incorporated BrdU in proliferating cells was detected by horseradish peroxidase (HRP)-conjugated antibody. The absorbance (450–550 nm) of the HRP substrate is proportional to the quantity of BrdU-incorporated cells, which is a direct indication of cell proliferation.

Endothelial Wound Healing Assay

Monkey chorioretinal vessel endothelial cells (RF/6A) were starved overnight in DMEM without FBS. A scratch-wound was generated on the monolayer of RF/6A cells with a 200 μL pipette tip. The cells were stimulated with IL-37 at indicated concentrations, and cell proliferation was inhibited with 2.5-μg/mL cytosine arabinoside (Sigma-Aldrich Corp., St. Louis, MO, USA). Endothelial cell migration was recorded with microscope (Nikon Instruments, Inc., Melville, NY, USA) 6, 12, and 24 hours after the wound scratch. The width of the wound was measured by Photoshop (Adobe Systems, Inc., San Jose, CA, USA).

Modified Boyden Chamber Migration Assay

Monkey chorioretinal vessel endothelial cells (RF/6A) were starved in serum-free medium overnight. A total of 2.5×10^4 RF/6A cells in 100 μL DMEM was added to the upper chamber. The lower chambers were filled with 500 μL DMEM with indicated concentration of IL-37. Nonmigrating cells were removed with a cotton swab and migrated cells were fixed, stained with 1% crystal violet and counted in three different fields under microscope.

Matrigel Tube Formation Assay

Monkey chorioretinal vessel endothelial cells (RF/6A) (10^4 in 100 μL DMEM) were seeded on growth factor–reduced Matrigel (BD Biosciences, Bedford, MA, USA) in 96-well plates in complete medium supplemented with indicated concentrations of IL-37. After 6 hours, the capillary-like structures were recorded with microscopy and tube length and branching points were measured with Photoshop (Adobe Systems, Inc.).

Enzyme-Linked Immunosorbent Assay

The concentration of IL-37, VEGF-A, and Ang-2 in vitreous fluids was determined by ELISA kit (R&D Systems) according to the supplier's instructions. In brief, the plates were incubated with capture antibodies overnight at 4°C, and then covered with blocking buffer for 60 minutes at room temperature, followed by incubation with 100 μL standards, controls, and vitreous samples. After 2 hours, the plates were washed and the detection antibody was added. After further incubation, the plate was washed again and the substrate added. The reaction was stopped after color emerged, and the plates were read at 450 and 540 nm.

Messenger RNA Isolation and Quantitative PCR

Cells were lysed with TRIzol (Sigma-Aldrich Corp.), and mRNA was extracted and reverse transcribed according to the manufacturer's directions (Qiagen, Valencia, CA, USA). Probes for monkey VEGF-A (NCBI: NM_001278384.1) were synthesized. Gene expression was normalized to β-actin: VEGF sense, 5′-CCCACTGAGGAGTCCAACA-3′, and antisense, 5′-CAAATGC TTTCTCCGCTCT-3′; β-actin sense, 5′-CCAGGGCGTTATGG TAGGCA-3′, and antisense, 5′-TTCCATATCGTCCCAGTTGGT-3′.

Data and Statistical Analyses

Aggregate data are presented as means ± SEM. GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analyses. Significant differences between samples were analyzed with the two-tailed Student's *t*-test. The Spearman rank correlation was adapted to analyze the correlation between IL-37 and other cytokines. A P value of less than 0.05 was considered significant for all analyses.

Results

The Baseline Characteristics of Both Groups in This Study

A total of 18 patients with PDR (cases, $n = 10$) and without PDR (controls, $n = 8$) were included in this study. The controls were age-matched nondiabetic patients with idiopathic macular diseases, including two patients with macular hole and six patients with epiretinal membrane (ERM). In the PDR group, one patient received laser panretinal photocoagulation (PRP) 1 year ago, and two patients were treated with retinal photocoagulation three or five times a year. The other three patients with PDR were treated with iodized lecithin and no patient was given anti-VEGF treatment in both groups. All the patients in the PDR group were being treated with insulin or oral hypoglycemic drugs.

The baseline features of the population are listed in Table 1. The mean ± SD age was 61.1 ± 10.89 years in the controls and 58.1 ± 9.47 years in the PDR group, with no significant difference between the groups ($P = 0.414$). The male-to-female ratio was 4:4 in the controls and 4:6 in the PDR group. The percentage of patients with vitreous hemorrhage and anterior chamber neovascularization is significantly higher in the PDR group than that in controls (100% vs. 0%, 80% vs. 0%, respectively).There was no statistical significance in sex ($P = 0.414$) or age ($P = 0.641$), but a statistical significance in

Table 1. Characteristics of the Patients in the Study

Characteristics	PDR	Control	*P*
Number	10	8	–
Sex, M/F	4/6	4/4	0.414*
Age, mean ± SD, y	58.1 ± 9.47	61.1 ± 10.89	0.641†
Hypertension, *n* (%)	6 (60)	0 (0)	0.000*
Vitreous hemorrhage, *n* (%)	10 (100)	0 (0)	0.000*
Anterior chamber neovascularization, *n* (%)	8 (80)	0 (0)	0.000*
Cataract, *n* (%)	9 (90)	7 (87.5)	0.534*
Prior treatments			
PC,‡ *n* (%)	3 (30)	0 (0)	–
Anti-VEGF	0 (0)	0 (0)	–
Iodized lecithin	3 (30)	1 (12.5)	–

* Pearson χ^2 test.
† Independent Student's *t*-test.
‡ Retinal photocoagulation.

vitreous hemorrhage ($P = 0.000$), hypertension ($P = 0.000$), and anterior chamber neovascularization ($P = 0.000$) analyzed by Pearson χ^2 test or independent Student's *t*-test.

Interleukin-37 Is Increased in the Vitreous Humor From Patients With PDR

To investigate the role of IL-37 in PDR, the levels of IL-37 in the vitreous humor from both the PDR group and control group were measured by ELISA and Western blot. Although IL-37 was undetectable by Western blot analysis, IL-37 was detected in all of the vitreous samples by ELISA. In the PDR and control groups, the concentration of IL-37 was 95.09 ± 5.22 pg/mL and 34.91 ± 5.61 pg/mL, respectively ($P = 0.001$) (Fig. 1). Overall, IL-37 levels were significantly higher in the vitreous of PDR patients compared with control subjects.

The Level of IL-37 Correlates With Proangiogenic Cytokines in PDR

To reveal the role of IL-37 in the angiogenesis of PDR, we determined the levels of proangiogenic factors in the vitreous humor. Results of cytokines tested are shown in Table 2. Among the six cytokines tested, Ang-1, Ang-2, VEGF-A, and IL-6 were significantly elevated in the PDR group. The levels of BFGF were below the limit of detection. To exclude the confounding factors caused by vitreous hemorrhage, we compared the levels of total vitreous protein between PDR and control groups and found no significant difference (Supplementary Fig. S1A). Furthermore, no correlation was found between total vitreous protein levels and vitreous IL-37 levels ($r^2 = 0.045$, $P = 0.397$), VEGF levels ($r^2 = 0.0002$, $P = 0.951$), and Ang-2 levels ($r^2 = 0.036$, $P = 0.453$), which indicates that the difference between PDR and control groups was not due to presence of blood in the vitreous (Supplementary Figs. S1B–D).

As the VEGF and Ang-2 were reported as key factors responsible for angiogenesis in PDR and have been shown to be upregulated in PDR eyes,[4,5] we analyzed the relationship between VEGF/Ang-2 and IL-37. The levels of both VEGF-A (1762.7 ± 772.7 vs. 49.5 ± 16.5 pg/mL, $P = 0.05$) and Ang-2 (1009.6 ± 436.8 vs. 89.4 ± 22.7 pg/mL, $P = 0.05$) in the vitreous fluids from PDR patients were significantly increased compared with controls (Figs. 2A, 2B). When analyzed by Spearman correlation coefficient, the level of IL-37 significantly correlates with the level of Ang-2 ($P = 0.009$, $r = 0.772$) and VEGF-A ($P = 0.003$, $r = 0.827$) in the PDR group and no correlation was observed in the control group (Figs. 2C, 2D). Notably, except VEGF-A and Ang-2, no significant correlation was observed between the level of IL-37 and the level of other cytokines as analyzed by the Spearman correlation coefficient. To further reveal the relationship between IL-37 and VEGF-A, we detected the expression of VEGF-A in the RF/6A cells treated with recombinant mature IL-37. The level of VEGF-A mRNA was examined by RT-PCR and the level of VEGF-A protein was examined by ELISA. The result showed that the mRNA level of VEGF-A was upregulated by IL-37 at the concentration of 0.01 ng/mL and 0.1 ng/mL (Fig. 2E). Consistently, VEGF expression at the protein level in RF/6A cells was upregulated by IL-37, ranging from 0.05 ng/mL to 1 ng/mL (Fig. 2F). Furthermore, the concentration of VEGF in the supernatant was significantly increased when cells were stimulated by 0.5 ng/mL IL-37 (Fig. 2F).

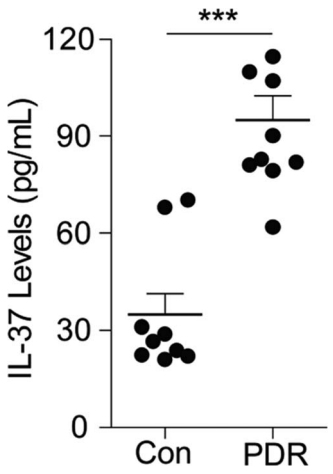

Figure 1. Interleukin-37 is increased in the vitreous fluids from patients with PDR and without PDR. The concentration of IL-37 in the vitreous of patients with PDR ($n = 10$) and control subjects (Con, $n = 8$) was determined by ELISA. Data are presented as mean ± SEM. Statistical comparison was performed with Student's *t*-test. $^{***}P < 0.01$.

Interleukin-37 Promotes RF/6A Cell Proliferation

The activation of endothelial cells, including cell proliferation and migration, is the hallmarker of initiation of angiogenesis. To determine the role of IL-37 in retinal neovascularization in PDR, the impact of IL-37 on chorioretinal vessel endothelial cell (RF/6A) proliferation was determined by BrdU incorporation. Serum-starved RF/6A cells were treated with different concentrations of IL-37 for 48 hours. The result showed that recombinant hIL-37, when added to the cell culture at the concentration ranging from 0.5 ng/mL to 1 ng/mL, remarkably promoted RF/6A cell proliferation, whereas the proliferative response to IL-37 decreases at high concentration (Fig. 3).

Interleukin-37 Enhanced RF/6A Cell Migration

Parallel with its function on proliferation, IL-37 stimulated the RF/6A cell migration in modified Boyden chamber assay, as quantified by the number of cells migrated to the lower chamber. Chorioretinal vessel endothelial cells (RF/6A) were starved overnight and then cultured with varying concentrations of IL-37 for 24 hours. The migrated cells were counted by three blinded readings. The mean cell migration stimulated by 1 ng/mL and 5 ng/mL IL-37 was 706 and 497 per well, respectively, whereas the control was 77 per well (Figs. 4A, 4B). In the scratch-wound assay, IL-37 promoted wound-induced cell migration at 6, 12, and 24 hours after wound

Table 2. Cytokine Levels in Vitreous Samples

Cytokine	PDR, pg/mL, Mean ± SD	Control, pg/mL, Mean ± SD	*P**	*r* (*P* Value)†
Ang-2	1009.6 ± 436.8	89.4 ± 22.7	0.05	0.772 (0.009)
BFGF	–	–	–	–
HGF	4017.82 ± 858.25	2597.3 ± 381.23	0.17	0.469 (0.171)
Ang-1	63.70 ± 14.54	–	0.01	–
VEGF-A	1762.7 ± 772.7	49.5 ± 16.5	0.05	0.827 (0.003)
IL-6	41.28 ± 5.58	21.5 ± 2.87	0.01	0.272 (0.446)

–, The concentration is beyond the detection limit of ELISA.
* Independent Student's *t*-test.
† Spearman correlation coefficient between levels of cytokine and IL-37 in PDR group.

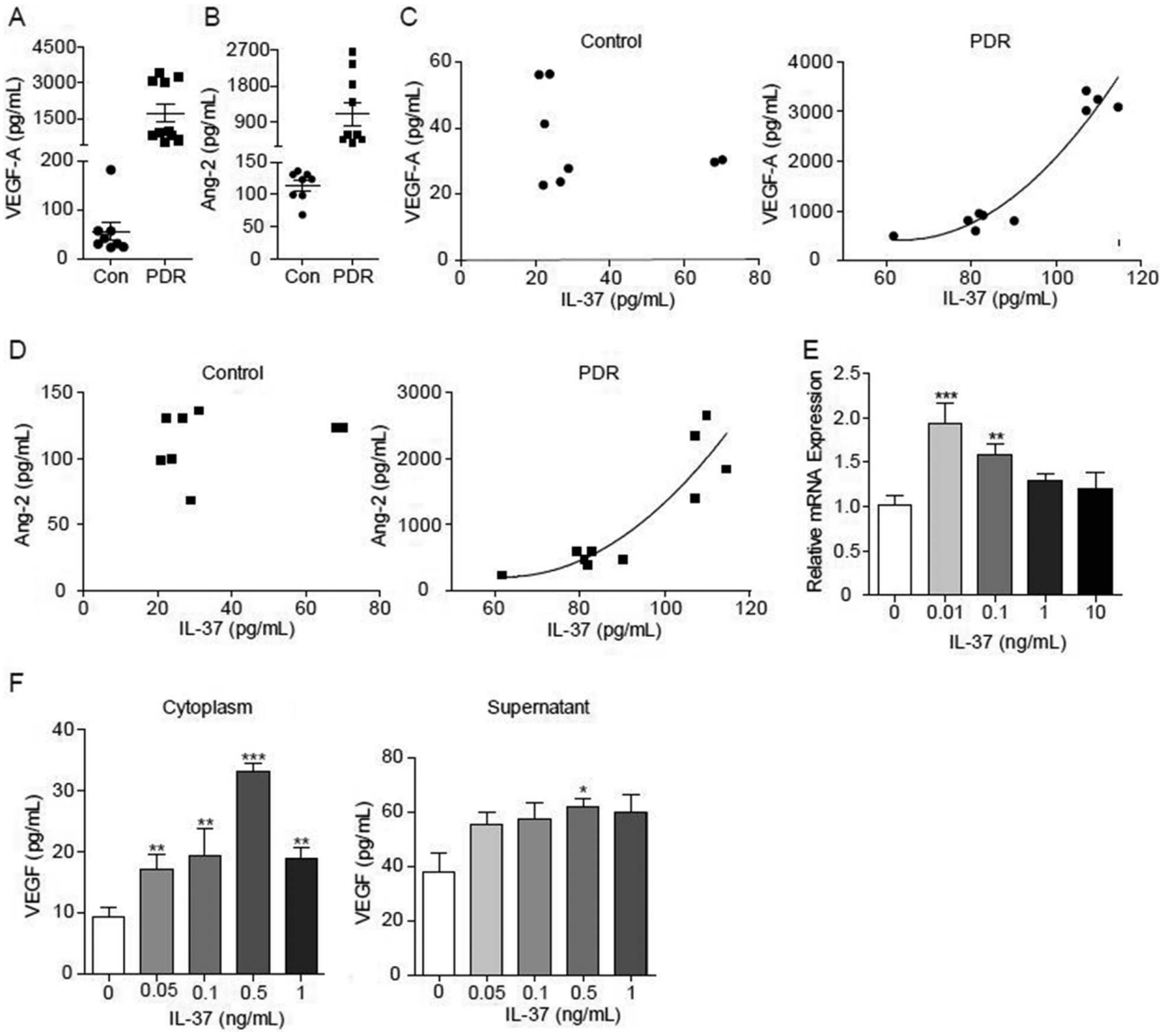

Figure 2. The levels of IL-37 are positively related with levels of Ang-2 and VEGF in PDR vitreous fluid. (**A**, **B**) The concentrations of VEGF-A (**A**) and Ang-2 (**B**) were significantly increased in PDR eyes ($P < 0.01$). (**C**, **D**) Scatter plot of IL-37 and Ang2 in control (Con) and PDR eyes. *Best-fit* line represents quadratic regression. (**E, F**) Both protein and mRNA level of VEGF-A were induced by IL-37 in RF/6A cells. Data are presented as mean ± SEM. Statistical comparison was performed with Student's *t*-test and Spearman correlation coefficient. $^{*}P < 0.05$; $^{***}P < 0.01$.

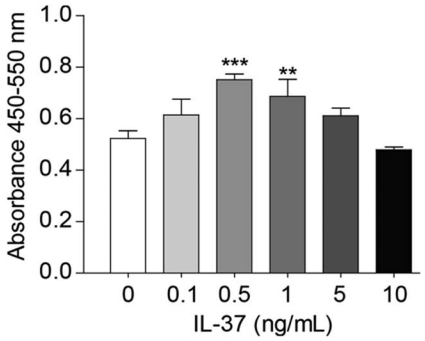

Figure 3. Interleukin-37 promotes RF/6A cell proliferation. Chorioretinal endothelial cells (RF/6A) were stimulated with indicated concentrations of IL-37 (0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, and 10 ng/mL) for 12 hours, and cell proliferation was measured using BrdU ELISA assay. Data are presented as mean ± SEM. Statistical comparison was performed with Student's *t*-test. $^{**}P < 0.05$; $^{***}P < 0.01$.

formation (Figs. 4C, 4D). Approximately 502-μm width of the wound surface was covered by migrating RF/6A cells treated with 1 ng/mL IL-37, whereas 337-μm width of the scratched area was covered by RF/6A cells treated with vehicle 12 hours after scratching. These results together suggest that IL-37 promotes RA/6A cell migration.

Interleukin-37 Enhanced the Formation of Vascular-Like Structures

To gain deeper insight in the involvement of IL-37 in retinal neovascularization, we determined the effect of IL-37 on endothelial tube formation. The RF/6A cells were cultured on Matrigel with IL-37 for 6 hours, and the total tubule length and branching points were analyzed (Fig. 5A). The results showed that IL-37 (1 ng/mL) remarkably stimulated tube formation with an increase of 85.3% for total tubule length (Fig. 5C) and 74.1% for branching points (Fig. 5B) compared with PBS control. The specificity of IL-37 was validated by abolishing the effect of IL-37 with IL-37–neutralizing antibodies (Figs. 5D, 5E).

Discussion

In our previous study, we provided evidence that IL-37 is a novel regulator of developmental and pathological angiogenesis.[21] Interleukin-37 promoted retinal neovascularization in a mouse model of retinal vascular development and oxygen-induced retinopathy (OIR). However, the function of IL-37 in PDR has not been investigated. In this study, we first reported that IL-37 was a potential regulator of PDR. The levels of IL-37 were elevated by 172% in the vitreous samples of PDR patients compared with control subjects measured by ELISA. Interleukin-37 was undetectable in the vitreous humor of the PDR patients by Western blot, which is probably because the average concentration of IL-37 in the vitreous samples was beyond the detection limit of Western blot. The positive correlation of IL-37 with VEGF and Ang-2 in the vitreous was revealed. We also presented evidence that IL-37 promoted RF/6A cell proliferation and migration at the concentration ranging from 0.5 ng/mL to 1 ng/mL in vitro. In addition, IL-37 enhances capillary-like structure formation of RF/6A cells. The stimulatory effect of IL-37 on tube formation and migration of RF/6A cells at the concentration measured in the vitreous (0.03 ng/mL and 0.1 ng/mL) was not significant (Supplementary Figs. S2A–C). This is probably because the vitreous concentration of IL-37 did not reach the optimal concentration in the in vitro experiments, which were performed in a very short period of time. Together, these results suggest the potential role of IL-37 in the pathogenesis of PDR. To our knowledge, our study is the first to analyze the level of IL-37 in the vitreous of humans with PDR, which may provide considerable basic research for the role of IL-37 in the pathogenesis of PDR.

Retinal angiogenesis is a pathogenic characteristic of PDR, in which multiple cytokines have been revealed to act as mediators in PDR through promoting ocular neovascularization. For example, previous studies have demonstrated the principal contribution of VEGF to ocular neovascularization in PDR.[4,22] Intravitreal treatment of PDR patients with bevacizumab, a specific VEGF antibody, results in rapid regression of retinal and iris neovascularization.[23] In our previous study, we have shown that retinas from IL-37–treated mice exhibited significantly increased disordered neovascular region in a mouse model of OIR.[24] Because the murine homologue of IL-37 has not been found so far, it is intriguing to investigate the pathological relevance of IL-37 in human ocular diseases. The analysis of proangiogenic cytokines in the vitreous fluid revealed significant increase of IL-37 in eyes with PDR and the increase positively correlated with the level of vitreous VEGF-A. Our in vitro study further shows that IL-37 induces VEGF-A expression in cultured RF/6A cells at low concentration, suggesting that IL-37 not only acts directly on endothelial cells to promote angiogenesis but also stimulates VEGF-A production to further enhance angiogenesis. In addition, upregulation of Ang-2 in the vitreous fluid of PDR patients has been reported, and the concentration of Ang-2 significantly correlated with the level of VEGF in diabetic eyes.[5] We have analyzed the relationship between IL-37 and Ang-2 in PDR, and found that the upregulation of IL-37 in vitreous fluids remarkably correlated with increased level of Ang-2. The relationship between IL-37 and VEGF/Ang-2 indicates that IL-37 is a potential regulator of retinal angiogenesis.

In diabetic retinopathy, hypoxia is considered as the primary inducer for angiogenesis by enhancing the production of proangiogenic factors and breaking down the balance between the stimulators and inhibitors of angiogenesis. Hypoxia-inducible factor-1α, an upstream regulator of VEGF and Ang-2, is reported as a crucial mediator of neovascularization under hypoxic conditions, which induced IL-37 expression and secretion as shown by our previous study.[24–26] It is thus speculated that specific patho-physiological stimuli in PDR, such as hypoxia, can lead to upregulation and release of IL-37, which subsequently induces VEGF expression and stimulates endothelial cell activation.

Our study suggests that, as an anti-inflammatory cytokine, IL-37 was involved in PDR pathogenesis and directly contributed to angiogenesis by inducing endothelial cell activation. It is recognized that inflammation is implicated in pathological angiogenesis, although the underlying mechanistic link between inflammation and angiogenesis is still being investigated.[25] Interleukin-37 has been reported to be a powerful anti-inflammatory cytokine that suppresses inflammation. The IL-37 transgenic mice exhibited reduced inflammatory response in the model of hepatitis, colitis, and psoriasis.[26–28] Indeed, many proinflammatory cytokines released during inflammation are potent activators of endothelial cells, such as TNF-α, IL-6, and IFN-γ,[29] which argues against the possibility that IL-37 regulates endothelial cell function through modulating inflammatory responses. More importantly, in the experiments using chorioretinal endothelial RF/6A cells, IL-37 induced endothelial cell proliferation and migration at low concentration ranging from 0.5 ng/mL to 1 ng/mL. Moreover, IL-37 significantly enhanced tube formation of RF/6A cell in Matrigel matrix in vitro. These results suggest that IL-37 directly induces

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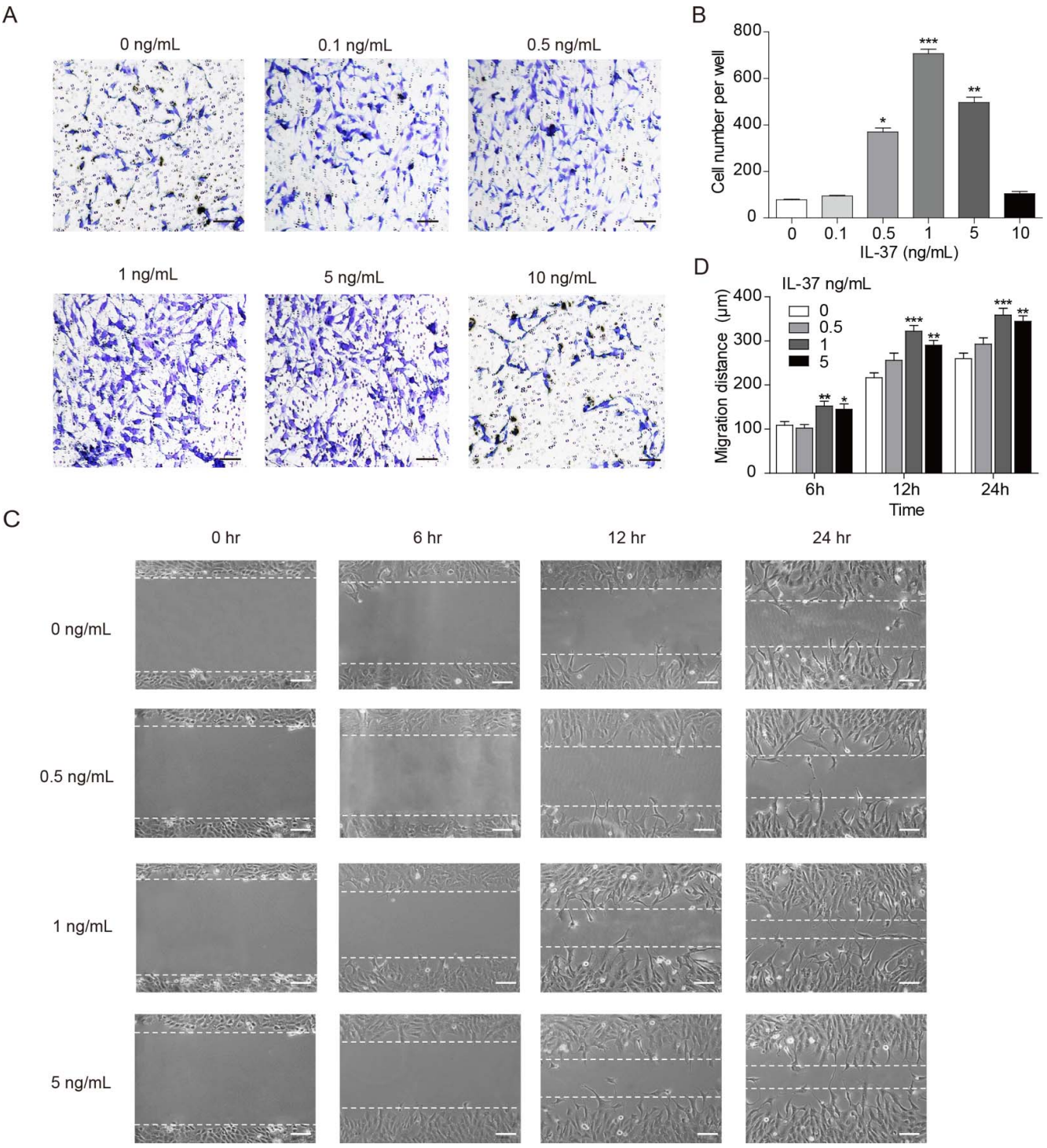

Figure 4. Interleukin-37 enhances RF/6A cell migration. (**A**) In modified Boyden Chamber migration assay, RF/6A cells were induced to migrate from the upper chamber to the lower chamber in the presence of different concentrations of IL-37 for 12 hours. Representative images of migrated cells stained with crystal violet are shown. *Scale bar*: 100 μm. (**B**) Quantification of migrated cells in Boyden Chamber migration assay. $n = 3$ per group. (**C**) In scratch-wound assay, after a scratch was created on the confluent monolayer of RF/6A cells, cells were stimulated with indicated concentrations of IL-37 and representative images after 0, 6, 12, and 24 hours after scratch wounding are shown. *Scale bars*: 200 μm. (**D**) Quantification of migration distance in scratch-wound assay. $n = 9$ per group. Data are presented as mean ± SEM. Statistical comparison was performed with Student's *t*-test. $^{*}P < 0.05$; $^{***}P < 0.01$.

activation of chorioretinal endothelial cells. The receptors and intracellular signaling pathways by which IL-37 exerts its proangiogenic effect and regulates ocular angiogenesis are still under further investigation.

In conclusion, our results demonstrated that IL-37 was increased in the vitreous fluids of PDR patients and revealed the positive correlation between IL-37 and VEGF-A/Ang-2. In addition, IL-37 is a powerful activator of chorioretinal vessel

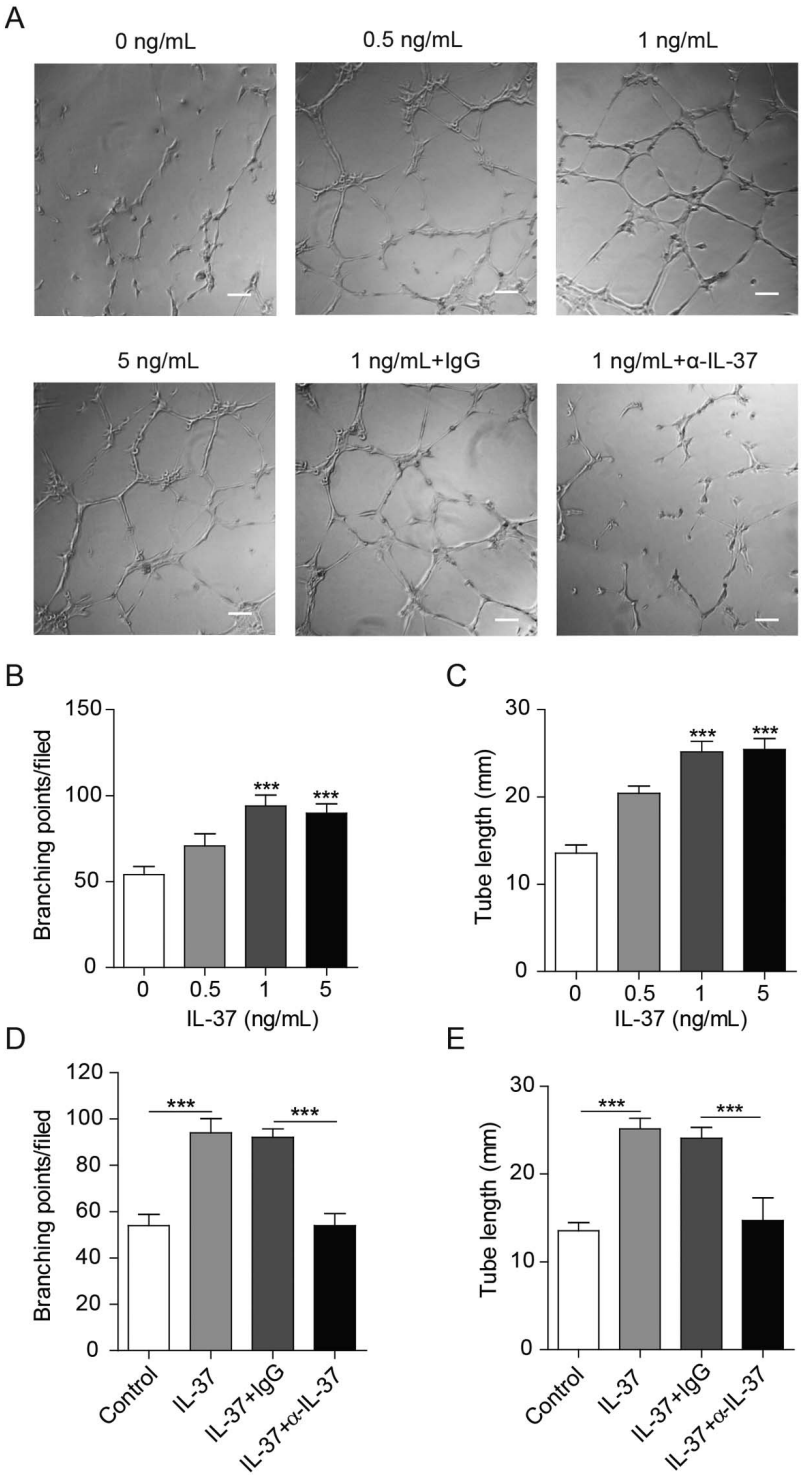

Figure 5. Interleukin-37 promotes tube formation of RF/6A cells. (**A**) Chorioretinal endothelial cells (RF/6A) were stimulated with indicated concentrations of IL-37 with or without IL-37–specific antibodies for 6 hours. Representative images of tube structure are shown. *Scale bars*: 100 μm. (**B**, **C**) Quantification of branching points (**B**) and tube length (**C**) by Photoshop. (**D**, **E**) Induced tube formation of endothelial cells by IL-37 was abolished by IL-37–neutralizing antibodies. $n = 4$ per gruoup. Data are presented as mean ± SEM. Statistical comparison was performed with Student's *t*-test. $^{*}P < 0.05$; $^{***}P < 0.01$.

endothelial cells. Although further studies are needed to uncover the IL-37 signal pathway involved in the angiogenesis, IL-37 may be a potentially new therapeutic target in PDR pathological angiogenesis.

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