Abstract. The purpose of this study is to address the effect and mechanism of stromal cell-derived factor-1 (SDF-1) α/chemokine (C-X-C motif) receptor 4 (CXCR4) signaling on capillary tube formation of human retinal vascular endothelial cells (HRECs). The expression of CXCR4 in HRECs was quantified by reverse transcription (RT-PCR) and western blotting. The effects of SDF-1α/CXCR4 signaling in capillary tube formation and migration of HRECs was examined using three-dimensional Matrigel assay and wound scratching assay respectively in vitro. Cell proliferation of HRECs was examined using cell counting kit (CCK)-8 assay in the presence of different concentrations of SDF-1α protein. The effect of SDF-1α/CXCR4 signaling in HREC expression of VEGF, basic fibroblast growth factor (bFGF), IL-8 and intercellular cell adhesion molecule (ICAM)-1 was examined using RT-PCR and western blotting. RT-PCR and western blot analysis revealed CXCR4 was expressed in HRECs. The number of intact capillary tubes formed by HRECs in the presence of SDF-1α was markedly more compared with a PBS treated control group. However, it was reduced with treatment with an CXCR4 antagonist. Wound scratching assay showed a significant increase in the number of migrated HRECs under SDF-1α stimulation and the number was reduced with treatment with an CXCR4 antagonist. RT-PCR and western blotting showed that SDF-1α significantly promoted VEGF, bFGF, IL-8 and ICAM-1 expression in HRECs. The proliferation of HRECs in the presence of SDF-1α was promoted in a dosage-dependent manner. SDF-1α/CXCR4 signaling can increase HREC capillary tube formation through promoting HREC migration, proliferation and expression of VEGF, bFGF, IL-8 and ICAM-1.

Introduction

Retinal pathological neovascularization is a primary characteristic of neovascular age-related macular degeneration, retinopathy of prematurity, retinal vein occlusion and diabetic retinopathy and it is the main cause of refractory blindness worldwide (1,2). Typical choices for treatment of retinal neovascularization involve vitrectomy, retinal laser photo-coagulation and cryotherapy; however, they are invasive and the functional results are typically suboptimal (3-6). Vascular endothelial growth factor (VEGF) and its receptors have been shown to be pivotal for the production and advancement of neovascular eye diseases; they have therefore become the ideal targets for anti-angiogenesis therapy (7). However, anti-VEGF agents can also induce local and systemic side effects (8). Therefore, to create novel therapeutic targets, it is necessary to obtain fuller knowledge of the mechanisms of ocular neovascularization (6).

The pathogenesis for ocular vascular diseases is associated with hypoxia, chronic inflammation and high level of angiogenic factors such as VEGF, platelet-derived growth factor-B (PDGF-B) and stromal cell-derived factor-1 (SDF-1)α (9). VEGF is pivotal in retinal neovascularization prompted by hypoxia-induced retinal injury. Hypoxia in the retina causes compensatory alterations in blood flow, the overexpression of cytokines and angiogenesis (10,11). Currently, the lack of efficacious anti-VEGF treatments could be due to the impacts of this treatment on the HIF pathway-mediated expression of other pro-angiogenic factors, including PDGF-B, insulin-like growth factor 1, erythropoietin and SDF-1 (12,13).

SDF is part of the CXC subfamily of chemokines and it was first cloned from murine bone marrow and described as a pre-B cell growth stimulating factor (14). The chemokine receptor, chemokine (C-X-C motif) receptor 4 (CXCR4),

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was first cloned as an orphan chemokine receptor and it was
determined to be expressed on numerous cell types, including
monocytes, lymphocytes and hematopoietic and endothelial
progenitor cells (15-18). CXCR4 regulates numerous
activities, including chemotaxis, adhesion, proliferation and
survival (19). Moreover, CXCR4 is also detected in endothelial
cells, which suggests a possible role for SDF-1α/CXCR4 cell
signaling in angiogenesis (20). Considerable evidence suggests
that SDF-1α/CXCR4 signaling is involved in the process of
pathological neovascularization (21-29). Jin et al (21) report
SDF-1α is implicated in revascularization of ischemic hind limbs through recruitment of CXCR4+ hemangiocytes. Our
previous studies found that CXCR4 expressed on leukocytes,
such as monocytes, stimulates monocytes chemotaxis,
resulting in recruitment of leukocytes to inflammatory sites;
SDF-1α-treated mice exhibited enhanced alkali-induced corneal neovascularization through enhanced intracorneal
progenitor cells infiltration and increased macrophage VEGF
expression (22,23).
CXCR4/SDF-1α signaling is pivotal in the progression of a
few types of ocular neovascularization, such as corneal
neovascularization, diabetic retinopathy and oxygen-induced
ischemic retinopathy (22-29), but the precise mechanism of
its effects in ocular neovascularization still needs further
exploration. In the present study, HREC bio-functions were
examined in SDF-1α recombinant protein or CXCR4 antagon¬
ist treated groups and was compared with control group in vitro.
The expression of angiogenic factors and transcription
factors in HRECs were detected and compared. The present
study provided the definitive evidence of critical role of
SDF-1α/CXCR4 signaling in HREC behavior of tube forma-
tion, proliferation and migration.

Materials and methods

Reagents and antibodies. CXCR4 antagonist (AM3100 octa-
hydrochloride, cat. no. 3299/50) was purchased from Tocris Bioscience. Recombinant human SDF-1α (CXCL12) protein
(cat. no. 350-NS-050) was purchased from R&D Systems.
CCK-8 kit was purchased from Dojindo Laboratories, Inc. Trypsin-EDTA was purchased from MilliporeSigma.
Rabbit anti-bFGF, VEGF, IL-8 and ICAM-1 antibodies were purchased from Santa Cruz Biotechnology, Inc. Primers
were synthesized by GeneScript. Total RNA extraction kit and reverse transcription kit were purchased from Qiagen Sciences,
Inc. D2000 DNA Ladder (cat. no. M1060) was purchased from Solarbio. Gelred nucleic acid stain (cat. no. orb14998, 1:5000) was purchased from Yaji Biological Technology
ltd. Human retinal vascular endothelial cells (HRECs; cat.
no. YS0884) were purchased from Yaji Biological Technology
Co., Ltd.

Cell culture and treatment of HRECs. The HRECs were cul-
tivated with DMEM (HyClone; Cytiva) containing 10% (v/v)
FBS, 100 µg/ml streptomycin and 100 U/ml penicillin
(HyClone; Cytiva) and incubated in an incubator under humid,
5% CO₂ and 37°C conditions (30). The HRECs were exposed
to PBS treated control group, SDF-1α groups, in which
SDF-1α protein (200 ng/ml) combined with CXCR4 antagonist
were added (1 nmol/ml). The HRECs were passaged by
trypsinization at ~90% confluence and subcultured in either
6-well or 96-well plates with the SDF-1α protein and/or the
CXCR4 antagonist for either 12 h or 24 h depending on the
assay conditions. The cells cultured were all at 37°C.

Cell migration assay. Cell horizontal migration ability was
detected by wound healing assay for assessment of the effects of
SDF-1α/CXCR4 signaling on the migration of HRECs, as
described in detail previously (31). The cells were seeded in a
6-well plate and scratched with a 100 µl pipette tip to obtain
scratches of a constant width when cells reached ~80% conflu-
ence. After scratching, the well was gently washed twice with
PBS to remove the detached cells. Fresh serum-free medium
(DMEM) was then added into each well. The cells were then
seeded with human recombinant SDF-1α protein or SDF-1α protein plus CXCR4 antagonist in the experimental wells,
whereas the control wells were treated with PBS. Images were
captured of the cells invading the wound line at 0, 12 and
24 h with Olympus TMS inverted phase contrast microscope
(Olympus Corporation) and measured distances traveled by
the cells from the wound edge to the cell-free space to calculate
the migration rate.

Cell proliferation assay. HREC proliferation was analyzed
using Cell Counting Kit-8 (Dojindo Molecular Technologies,
Inc.) (32). The cells were diluted and seeded in a 96-well plate
at a density of 8,000 cells/well in 100 µl of DMEM with
10% FBS and different concentrations of SDF-1α protein or
SDF-1α protein plus CXCR4 antagonist. After incubation at
37°C for 24 h, 10 µl CCK-8 was added to each well and incu-
bated at 37°C for 2 h in an incubator. Subsequently, absorbance
was measured at a wavelength of 450 nm using a microplate
reader (Thermo Multiskan EX plate reader; Thermo Fisher
Scientific, Inc.).

Tube formation assay. A tube formation assay was performed
to assess the effect of SDF-1α/CXCR4 signaling on HRECs. In
brief, Matrigel (50 µl/well; Becton, Dickinson and Company)
was applied to a 96-well plate and plate was put into a 37°C
incubator for 30 min. Then, HRECs were seeded onto the
gel and kept for 6 h at 37°C condition. Image-Pro Plus 6.0
(Media Cybernetics, Inc.) was used for imaging, followed by statistical analyses on tube number. Each experiment was conducted in triplicate.

Reverse transcription-quantitative (RT-q)PCR. RT-qPCR was used to analyze the transcript levels of CXCR4, VEGF, bFGF, IL-1β, IL-6, IL-8, ICAM-1, IL-18, TNF-α, monocyte chemotactic protein 1, zonula occludens-1 and VE-cadherin (33). The total RNA was extracted from Hrecs (2x10^5 cells) using Trizol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The RNA concentration and the absorbance values on a 260 and a280 nm were measured by nanodrop nd-1000 spectrophotometer (Invitrogen; Thermo Fisher Scientific, Inc.) and the OD 260/OD280 ratio of RNA was between 1.8 and 2.0, which could be used as a template for reverse transcription. Thereafter, complementary (c)DNA was generated via RT reaction by using a PrimeScript first strand cDNA synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The RNA concentration and the absorbance values on A260 and A280 nm were measured by Nanodrop Nd-1000 spectrophotometer (Invitrogen; Thermo Fisher Scientific, Inc.) and the OD260/OD280 ratio of RNA was between 1.8 and 2.0, which could be used as a template for reverse transcription. Thereafter, complementary (c)DNA was generated via RT reaction by using a PrimeScript first strand cDNA synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Subsequent qPCR was performed using SYBR green reagent (Takara Biotechnology Co., Ltd.) on an ABI 7000 PCR instrument (Thermo Fisher Scientific, Inc.). The 20 μl PCR reaction mixture consisted of 10 μl SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.), 2 μl cDNA template, 0.8 μl Primer (0.4 μl each forward and reverse) and 7.2 μl dH₂O. The primer pairs used are listed in Table I. All primers used were purchased from Genescript. PCR was performed by initial denaturation at 95°C for 10 min, followed by 40 cycles of 10 sec at 95°C and 20 sec at 50°C and a final extension of 25 sec at 72°C. The relative mRNA levels were measured by quantification cycle values using the 2^(-ΔΔCq) method (34). Data display the average of triplicate experiments.

Western blot analysis. Immunoblotting analysis was adopted for the detection of HRECs expression levels of VEGF, bFGF, IL-8, ICAM and ERK1/2 as well as associated phosphorylated signaling proteins of ERK1/2 and PI3K. 6-well plates were used to culture the HRECs (2.5x10^5 cells) in DMEM containing 10% FBS. The cell medium was replaced with the media without serum for another 24 h once 95% cell confluence was achieved. Then, the treated cells were washed twice using chilled PBS. Then, protein lysate (Beyotime institute of Biotechnology) was added to each well, prior to collecting the proteins on ice. Lysate protein concentrations were evaluated using the BCA method (Beyotime Institute of Biotechnology). SDS-PAGE was performed using in-house produced 10% gels. Equal

| Table I. Sequences of primers used for reverse transcription PCR. |
|---------------------|---------------------|---------------------|---------------------|
| **Primers** | **Sequence (5’→3’)** | **Product Size (bp)** | **Annealing temperature (˚C)** | **PCR Cycle (sec)** |
| CXCR4 | F: TGTCCATTCTTTGGCTCTGGTT | 1,020 | 57 | 37 |
| | R: GTCACCTCGTTTTCTCTTTGG | | | |
| VEGF | F: CTTGTGCTCTACCTCCACCC | 118 | 60 | 40 |
| | R: GCAGTAGGTGCGGTGATAGA | | | |
| bFGF | F: CAGCGGCCGTGATGCTGAAA | 100 | 60 | 40 |
| | R: TACCTGGATGAGAGGTGTCG | | | |
| IL-1β | F: GCAGAAGTACTGTGAGCTGCC | 109 | 60 | 40 |
| | R: CTTGGAGAAGGACACTTACT | | | |
| IL-6 | F: CAATAACCACCCCTGACCCCA | 106 | 60 | 40 |
| | R: AAAGCTGCGCAGAATGAGATG | | | |
| IL-8 | F: GTGTCAGTTTTTGGCAAGAG | 117 | 60 | 40 |
| | R: GTGTGGTCACACTCTCAAATCACT | | | |
| ICAM-1 | F: CCAGGAGACACTGCAGACAG | 100 | 60 | 40 |
| | R: CTTGACTGTCACCTCGGCTCC | | | |
| IL-18 | F: TGACCAAGGAATCCGGCTCC | 117 | 60 | 40 |
| | R: GCCTACCTTCTGGCTGCTGCT | | | |
| TNF-α | F: GCTGCATTTGGAGTGATCG | 119 | 60 | 40 |
| | R: CTACAGGCTTGTCATCGCGG | | | |
| MCP-1 | F: GATCTCATGCGAGGCCTCG | 105 | 60 | 40 |
| | R: TCAACGAGATCTCCCTGGCG | | | |
| ZO-1 | F: TCAAGGGAAGCCTCTCTGA | 108 | 60 | 40 |
| | R: ATACTGGCAAGGCAATGAGG | | | |
| VE-cadherin | F: CTTCAACCAGACCAATCACA | 113 | 60 | 40 |
| | R: ACTTGTCATCCGGTTCTGG | | | |
| GAPDH | F: CAAATTCATGGCACCAGTCA | 108 | 60 | 40 |
| | R: GCATCGCCCCACTTGGTT | | | |

F, forward primer; R, reverse primer.
amounts of protein (50 µg) were loaded per lane. The separated proteins were transferred onto PVDF membranes (0.45 µm) purchased from MilliporeSigma and were blocked with 5% skimmed milk dissolved in 1X TBS containing 0.3% Tween-20 for 1.5 h at room temperature to inhibit endogenous reactions. The membranes were then incubated with the blocking buffer-diluted primary antibodies overnight at 4°C. After rinsed the following day using a Tris-HCl (pH 7.4) buffer (20 mM) as well as Tween-20 (0.1%), membranes were incubated again at room temperature with the corresponding secondary antibodies bound to horseradish peroxidase for 1 h. The protein blots were promptly visualized using a 1 Tanon-5200 Multi-imaging System after treatment using an enhanced chemiluminescence (ECL) kit obtained from Tanon Science and Technology. The relative protein levels were quantified by ImageJ (version 1.5, National Institutes of Health). The experiments were performed in triplicate.

**Flow cytometry.** Flow cytometry was performed to identify the feature of Hrecs. The Hrecs were seeded in 60 mm wells (1x10^6 cells) cultured with DMEM containing 10% FBS. When 95% cell confluence was achieved, the cells were harvested by trypsinization for staining. After washing twice using chilled PBS, the cells (1x10^6 per 100 µl) were co-stained with 10 µl a PC-conjugated mouse anti-human CD106 and alexa Fluor 700-conjugated mouse anti-human CD54 antibodies or IgG isotype as control for 30 min at 4°C. After washing with PBS, the cells were analyzed with Beckman Coulter FC500 Flow Cytometer. Data were analyzed using FlowJo 7.6 (Tree Star). The experiments were performed in triplicate.

**Statistical analysis.** SPSS 20.0 (IBM Corp.) was employed to perform all data analyses. All data were depicted as mean ± standard error (number of observations). Comparisons between variables were carried out by a two-tailed unpaired Student’s t test. Comparisons among multiple datasets were performed by one-way analysis of variance (ANOVA) and Bonferroni correction was used to adjust P-values for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**The expression of CXCR4 in HRECs.** First, the expression of CXCR4 in HRECs was examined. CXCR4 mRNA and protein expression were detected in HRECs. The expression of CXCR4 in HRECs suggested the possible involvement of the SDF-1α/CXCR4 interactions in the biological function of HRECs. Additionally, the expression of CD106 and CD54 in HRECs was examined using flow cytometry to identify the characteristic of HRECs (Fig. 1).

**Effects of SDF-1α/CXCR4 signaling on cell proliferation.** To determine the effects of SDF-1α/CXCR4 signaling on vascular endothelial cell bio-function, the effect of SDF-1α/CXCR4 signaling in cell proliferation of HRECs in vitro was assessed. After incubation with SDF-1α or SDF-1α plus CXCR4 antagonist for 24 h, cell viability was evaluated. HRECs incubated with SDF-1α showed a significant increasing in cell proliferation compared with the control, while HRECs incubated with CXCR4 antagonist after precondition with SDF-1α showed a significant reduction in cell proliferation compared with 100 ng/ml or 200 ng/ml SDF-1α groups (Fig. 2).
SDF-1α/CXCR4 signaling was capable of promoting cell proliferation. These data indicated that an enhancement in proliferation of Hrecs after SdF-1α stimulation was responsible for the promotion effect of SdF-1α/CXCR4 signaling on tube formation of Hrecs in vitro. In addition, the elevating of cell proliferation of Hrecs peaked in the 200 ng/ml SdF-1α group when compared with other groups including 10, 50 and 100 ng/ml SdF-1α groups as well as 500 ng/ml SdF-1α group or other higher concentration groups, so the dose of 200 ng/ml SdF-1α was chosen as the intervention concentration to treat Hrecs in subsequent protein examining experiment and three-dimensional Matrigel vascular tube formation assay.

Effects of SDF-1α/CXCR4 signaling on cell migration. The effects of SDF-1α/CXCR4 signaling on HREC migration have yet to be reported, to the best of the authors’ knowledge. To evaluate whether SDF-1α/CXCR4 signaling affects the process of the migration of Hrecs, a scratch wound assay was performed in vitro to measure the migration property of Hrecs in different concentration of SDF-1α or SDF-1α combined with CXCR4 antagonist. As shown in Fig. 3, compared with control group, a significantly accelerated wound closure was shown in the group treated with SDF-1α and the wound almost closed at 24 h after injury. However, the wound area was still wide in HRECs with CXCR4 antagonist treatment after precondition with SDF-1α at 24 h. The quantitative data of the migration distance were shown in Fig. 3B and C. Those data showed that SDF-1α/CXCR4 signaling had the potential of promoting the migration property of HRECs.

Effects of SDF-1α/CXCR4 signaling on tube formation of HRECs. To determine whether SDF-1α/CXCR4 signaling plays a role in the process of tube formation of HRECs, HREC cell line was seeded in Matrigel-coated 96-well plates. After incubation for 12 h, the cells were able to form tubes. HRECs incubated with SDF-1α showed a significant increasing in tube formation compared with control cells (Fig. 4). After incubated with SDF-1α and CXCR4 antagonist, the number of tubes formed was significantly reduced. Tube formation quantification and statistical analysis demonstrated SDF-1α/CXCR4 signaling was able to promote HREC tube formation.

Enhanced angiogenic factors, p-ERK1/2 and p-PI3K expression in SDF-1α treated HRECs. The balance between angiogenic and anti-angiogenic elements establishes the results of angiogenesis operations in different situations (35). Therefore, the mRNA and protein expression of angiogenic factors in HRECs were next determined. Among the angiogenic associated factors, such as VEGF, bFGF, IL-8 and ICAM-1, which were detected, the mRNA expression of VEGF, bFGF, IL-8 and ICAM-1 were increased in SDF-1α treated cells compared with control groups (Fig. 5). VEGF, bFGF, IL-8 and ICAM-1 protein expression also revealed that VEGF, bFGF, IL-8 and ICAM-1 were increased by the treatment with SDF-1α compared with PBS-treatment (Fig. 6). These analyses indicated that the SDF-1α treatment elevated the expression of the angiogenic factors, VEGF, bFGF, IL-8 and ICAM-1 and as a result, overturned the balance to encourage angiogenesis.

The present study also examined p-ERK1/2 and p-PI3K expression in HRECs. PI3K and ERK1/2 activation are
integral components of pro-angiogenic signaling pathway and promotes endothelial migration and proliferation. The present study sought to determine whether SdF-1α/cXcr4 signaling had effects on cell migration and proliferation through activation of ERK1/2 and PI3K in Hrecs. It was found that p-ERK1/2 and p-PI3K expression were markedly increased in SdF-1α treated Hrecs (Fig. 7). These results suggested that SdF-1α induces ERK1/2 and PI3K activation and therefore promoted angiogenesis.

Discussion

The authors have previously documented that SdF-1α-treated mice showed improved alkali-induced corneal neovascularization via amplified intracorneal progenitor cell infiltration and elevated VEGF expression by macrophages, while SdF-1α neutralizing antibody- or cXcr4 antagonist-treated mice demonstrated impeded experimental alkali-induced corneal neovascularization via downregulated VEGF and C-Kit expression (22,23). The results provided evidence that SDF-1α/CXCR4 signaling is implicated in corneal neovascularization and its potential of pro-angiogenesis may be through indirect effects of promoting VEGF secreting by intracorneal macrophages and C-Kit positive progenitor cell migration. In addition, various evidence indicate that SdF-1α/CXCR4 signaling may have direct effects on vascular endothelial biofunction (36-39). However, further exploration on the mechanism of these direct effects is required. In order to delineate the direct effects of SDF-1α/CXCR4 signaling on vascular endothelial function of proliferation, migration and tube formation, the present study performed an in vitro study using HREC to evaluate SDF-1α/CXCR4 signaling directed pro-angiogenesis efficacy.

The present study showed that SDF-1α/CXCR4 signaling has the ability to increase tube formation of HREC by promoting HREC proliferation and migration and VEGF, bFGF, IL-8 and ICAM-1 production of HREC. These results indicated that SDF-1α/CXCR4 signaling has pro-angiogenesis property not only through activating cell types of monocytes/macrophages but also through activating vascular endothelial migration, proliferation and pro-angiogenic cytokine secretion. Thus, the data verified the hypothesis that SDF-1α/CXCR4 signaling has an important role in angiogenesis through indirect and direct pathways, which had not been confirmed in our previous study (22,23).

Endothelial migration and proliferation are initial steps for angiogenesis (40). Any effects on these two steps may subsequently have an impact on vascular tube formation (40). Various studies indicate that VEGF, bFGF, IL-8 and ICAM-1 are involved in vascular endothelial migration and proliferation. VEGF and bFGF as well as other pro-angiogenic cytokines promote the process of vascular endothelial migration and proliferation while ADAMTS-1 and TSP-1 inhibit these processes (41,42). SDF-1/CXCR4 signaling promotes angiogenesis through multiple pathways, including recruiting macrophages, c-Kit positive cells and stroma cells and elevating expression level of pro-angiogenic factors by macrophages and stroma cells (43). The present study also examined the effects of SDF-1/CXCR4 signaling on HREC migration and proliferation (22,23). Consistent with the hypothesis of the present study, in SDF-1α stimulating groups, both HREC migration width and proliferation rate were greater compared with those in the PBS treated group, while in the CXCR4 antagonist-treated groups, HREC migration width
7 and proliferation rate were reduced more compared with the SdF-1α treated groups. This indicated that directly promoting endothelial migration and proliferation would be another crucial pathway for SdF-1/cXcr4 signaling implicated in the process of angiogenesis.

The process of angiogenesis is precisely modulated by a series of pro- and anti-angiogenic molecules under physiologic condition, while under pathologic condition, the expression balance upset, serious consequences, such as neovascularization, may occur (44). Angiogenic factors, such as VEGF and bFGF have strong efficacy in stimulating blood vessel formation (45). These factors are expressed by various cells, including fibroblasts, macrophages, neutrophils and also by vascular endothelial cells themselves (46). The present study detected the mRNA and protein expression of VEGF, bFGF, IL-8, ICAM-1 and other cytokines in HRECs and the results showed that the expression of VEGF, bFGF, IL-8 and ICAM-1 in SDF-1α treated cells were significantly higher compared with control cells. It indicated that SDF-1/CXCR4 signaling is implicated in the process of angiogenesis by altering pro-angiogenic milieu and thereby causing neovascularization (47,48). These results are consistent with other reports (49-51), which report that SDF-1α promotes pro-angiogenic cytokine expression in endothelial cells. The previous reports and the results of the present study imply that SDF-1/CXCR4 signaling would be a candidate for treating vascularization diseased by blocking or silencing the signaling.

To explore the mechanisms of how SDF-1/CXCR4 signaling mediated HREC capillary tube formation, the present study also evaluated the influence of SDF-1/CXCR4 signaling on signal expression of PI3K/Akt and ERK1/2.
Several signaling pathways are involved in the process of angiogenesis. Activation of PI3K/Akt and ERK1/2 in endothelial cells is a crucial intracellular signaling step for angiogenesis (52,53). Barbero et al (54) report that SDF-1/cXcr4 axis are capable of activating various signaling pathways, including PI3K/Akt and ERK1/2, in the process of tumor development and promote tumor vascular growth through these activated signaling pathways (55). Lin et al (56) report that SDF-1/cXcr4 signaling can promote tumor cell proliferation and migration by activating PI3K/Akt signaling. Based on these studies, the present study examined whether SDF-1/cXcr4 signaling promoted HREC proliferation, migration or capillary tube formation through PI3K/Akt and ERK1/2. The present study found that SDF-1/cXcr4 signaling promoted the expression of active p-PI3K and p-ERK1/2, suggesting that SDF-1/cXcr4 signaling had pro-angiogenesis property via activating PI3K/Akt and ERK1/2 signaling.

In conclusion, the findings of the present study illustrated a novel mechanism of SDF-1/cXCR4 signaling effects on the process of neovascularization. It promoted HREC capillary tube formation by promoting cell proliferation and cell migration. The effects may work by enhancing cytokine expression, such as VEGF and bFGF, and promoting these functions of HRECs via activating PI3K/Akt and ERK1/2 signaling. These results could provide a theoretical basis for the possibility of suppressing ocular neovascularization by inhibiting SDF-1/cXCR4 signaling using anti-SDF-1 antibody or anti-CXCR4 antagonist or other blocking agents.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

GL, HW, XL and XY designed the study, led the experiments, prepared figures and wrote the manuscript. XY, XL, HW, YX, LC and ZC analyzed the data and prepared the figures. GL and PL conceived, designed and coordinated the study as well as drafted the manuscript. GL and PL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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