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Original Article

Silencing of transient receptor potential canonical channel 4 inhibits endothelial progenitor cell angiogenesis by suppressing VEGF and SDF-1

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Abstract: Objectives: Endothelial progenitor cells (EPCs) play a crucial role in acquired angiogenesis and endothelial injury repair. Transient receptor potential canonical channel 4 (TRPC4), a key component of store-operated calcium channels, is essential for EPC function. While the role of TRPCs has been clarified in vascular diseases, the relationship between TRPC4 and EPC function, along with the underlying molecular mechanisms, remains unclear and requires further elucidation. Methods: EPCs were isolated from canine bone marrow and identified by morphology and flow cytometry. TRPC4 was transfected into EPCs using lentivirus or negative control, and its expression was assessed using real-time polymerase chain reaction (RT-PCR). Proliferation, migration, and tube formation were evaluated using Cell Counting Kit-8 (CCK-8), Transwell, and Matrigel assays, respectively. Levels of vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1) were measured using enzyme-linked immunosorbent assay (ELISA). Results: TRPC4 mRNA expression was significantly reduced in TRPC4-short hairpin RNA (shRNA) transfected EPCs compared to the normal control (NC)-shRNA groups. Migration and tube formation were significantly decreased after TRPC4 silencing, while proliferation showed no difference. Additionally, levels of SDF-1 and VEGF in EPCs were markedly reduced following TRPC4 silencing. Conclusion: TRPC4 plays a crucial role in regulating angiogenesis in EPCs. Silencing of TRPC4 can lead to decreased angiogenesis by inhibiting VEGF and SDF-1 expression, suggesting that TRPC4 knockdown might be a novel therapeutic strategy for vascular diseases.

Keywords: Endothelial progenitor cells, angiogenesis, transient receptor potential canonical channel 4

Introduction

Rheumatoid arthritis (RA) is a chronic joint inflammation accompanied by systemic inflammation, affecting approximately 1% of the global population with an estimated 50 million patients worldwide [1]. RA is characterized by the formation of pannus, leading to a refractory autoimmune disease that results in bone and joint destruction [2, 3]. The disability rate of RA is the highest among all joint diseases, significantly impacting the health and quality of life of patients. Currently, there are no specific drugs for RA, and commonly used medications such as non-steroidal anti-inflammatory drugs carry risks of side effects like liver and kidney damage, necessitating cautious for long-term use [4]. Given that the main pathology of RA involves synovial pannus formation, which is driven by angiogenesis [5], targeting angiogenesis may offer a novel therapeutic approach for RA.

Endothelial progenitor cells (EPCs) are precursors of endothelial cells (ECs), possessing high self-renewing and proliferative capacities. They can attach to the extracellular matrix and differentiate into mature ECs. Circulating EPCs primarily originate from bone marrow [6]. Under physiological conditions, ECs injury triggers adjacent vascular ECs to migrate, proliferate, and differentiate for repair. However, when the systemic EC damage exceeds the capacity for physiological repair bone marrow-derived EPCs are mobilized into the bloodstream to participate in systemic endothelial repair [7]. In a study of rabbit with aneurysms, Aronson et al. [8] found that EPC engraftment contributed to endothelial layer formation within the lumen of

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the aneurysm and participated in the self-repair. Activation of EPCs through erythropoietin administration can effectively reduce the risk of intracranial aneurysm formation and rupture [9]. Additionally, EPCs play a role in repairing damaged intima and slowing down the progression of atherosclerosis [10, 11]. Understanding the mechanisms of angiogenesis is especially important in the treatment of vascular diseases, with key signaling pathways such as hypoxia-inducible factor 1α (HIF1α)/vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR) [12], Ang/Tie2 [13], Delta like canonical notch ligand 4 (DLL4)/Notch [14], and transient receptor potential canonical channel (TRPC) [15] implicated in this process. Thereinto, TRPC is the key to induce extracellular calcium influx to promote angiogenesis [16]. By measuring the mRNA and protein levels, it was found that various TRPC superfamily members were expressed in cultured EPCs [17]. The TRPC channels (TRPC1-7) are Ca^{2+} permeable non-selective cation channels, distributed in the human body [18, 19]. Current investigations have revealed that the TRPC superfamily, including TRPC4, is strongly connected with the initiation and progression of cardiovascular diseases [20-24]. A previous study has shown that TRPC4 is involved in angiogenesis, vascular permeability regulation, vascular tone regulation, oxidative stress responses, and hypoxia-induced vascular remodeling [25]. Another study suggested that VEGF-induced neovascularization in retina was restrained by the suppression of TRPC4 [26]. However, the effect of TRPC4 silencing on EPCs is still unclear.

Therefore, this study aimed to investigate the impact of TRPC4 silencing on EPC function and elucidate the underlying molecular mechanisms. Our findings may contribute to the development of new and effective treatments for RA.

Material and methods

Expression of TRPC4, VEGF, and SDF1 in EPCs

The expression profiling by array (GSE20283, https://www.ncbi.nlm.nih.gov/geo/) on EPCs was obtained from the Gene Expression Omnibus (GEO) database. This dataset contains genome-wide expression data for three EPCs and three outgrowth endothelial cells (OECs), all derived from humans. Differential expression analysis between the two groups was performed using "GEO2R", with selection criteria set as $P < 0.05$ and $|log2FC| > 1.5$. Functional enrichment analysis of the differentially expressed genes was conducted using the David database (https://david.ncifcrf.gov/). Furthermore, the expression levels of TRPC4, VEGF, and SDF1 genes in EPCs and OECs were compared using a t-test.

Isolation and culture of bone marrow-derived EPCs

This study was ethically approved by the Animal Care and Use Committee of Guangxi Medical University. Bone marrow samples were collected from 2-week-old male dogs, and monocytes were isolated using density gradient centrifugation. The isolated monocytes were then seeded in cell culture flasks coated with fibronectin (Sigma, USA) and allowed to incubate overnight at 37°C in 5% CO_2. Subsequently, endothelial growth medium 2 (EGM-2; Lonza, USA) was added, and the cells were cultured in a humidified incubator at 37°C with 5% CO_2 for induction. Upon reaching approximately 80% confluence, the cells were passaged following digestion with 0.25% trypsin. Periodic observation of cell morphology using an inverted microscope confirmed the presence of cells displaying endothelial morphology. Surface antigen markers of endothelial progenitor cells were identified using flow cytometry.

Flow cytometry

The surface phenotypes of these putative canine EPCs were assessed using flow cytometry (FACSAria™ III, BD Biosciences, USA) after two passages. Briefly, when the cells reached approximately 80% confluence, they were fixed in 4% paraformaldehyde for 0.5 h. Subsequently, they were incubated for 0.5 h with PE-labeled flow cytometry antibodies CD133 (eBioscience, USA) and CD34 (eBioscience, USA), as well as the negative control IgG (eBioscience, USA) at 25°C in the dark. Afterward, the cells were evaluated by flow cytometry to determine the positive rate of CD133 and CD34 expression.

TRPC4 knockdown in EPCs

Lentiviruses carrying short hairpin RNAs (shRNAs) targeting TRPC4 were employed to knockdown this gene, while control shRNAs (**Table 1**)

Table 1. The sequence of shRNA

Lentivirus	Sequence
NC-shRNA	TTCTCCGAACGTGTCACGT
TRPC4-shRNA1	ACGCTCCAGACTCAACATCTA
TRPC4-shRNA2	CAGCATGAATTCACAGAGTTT
TRPC4-shRNA3	ATGCAAAGGAAGAGGACTCTA

Notes: NC = normal control; TRPC4 = transient receptor potential canonical channel 4.

were used as three candidate shRNAs in the subsequent analyses. Cells were transfected with the lentiviruses mentioned above, and the knockdown of TRPC4 was confirmed by RT-PCR following this procedure. The shRNA exhibiting the highest silencing efficiency was selected for subsequent experiments.

Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA expression levels of TRPC4 in EPCs transfected with TRPC4-shRNA1, TRPC4-shRNA2, TRPC4-shRNA3, or normal control (NC)-shRNA for 48 h were analyzed using RT-PCR. Cellular RNA was isolated from EPCs using Trizo (Invitrogen, CA, USA), followed by cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's instructions. RT-PCR was performed on a QuantStudio-5 system (Applied Biosystems) following the provided protocols. The relative expression of TRPC4 mRNA levels was determined using the 2-ΔΔCt method, with Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) serving as the internal control. The primer sequences are listed in **Table 2**.

Cell proliferation assay

The impact of TRPC4 silencing on the proliferation of EPCs was evaluated using the Cell Counting Kit-8 (CCK-8, Dojindo, Japan). EPCs were seeded in 96-well plates at a density of 5×10^3 cells per well. At 24, 48, 72, and 96 hours post-transfection, 10 μl of CCK-8 solution was added to each well. Subsequently, the absorbance at 450 nm was measured using a microplate reader.

Cell migration assay

To assess the migratory function of cells, Transwell Boyden chambers (8.0 μm, Corning, NY, USA) were utilized to quantify the effects of TRPC4 silencing on EPC migration. Transfected cells with TRPC4-shRNA or NC-shRNA at a concentration of 5×10^4 cells per well were seeded into the upper chamber of 24-well Transwell plates. Following a 72 h incubation period at 37°C, the cells were detached using 0.25% trypsin and adjusted to a density of 2×10^5 cells/ml. Subsequently, 200 μl of the cell suspension was added to the upper chamber, while the lower chamber was filled with 800 μl of EBM-2 containing 20% serum. After 24 h of migration at 37°C, non-migrated cells were removed from the upper surface, and the migrating cells were manually counted in four randomly selected fields at ×100 magnification.

Tube formation assay

The ability of EPCs transfected with TRPC4-shRNA or NC-shRNA to form tubes in Matrigel was assessed using the following protocol: Matrigel (Corning Co. Ltd., USA) was thawed overnight at 4°C, and then 300 μl of Matrigel was added to each well of a 24-well plate and allowed to solidify at 4°C for 30 min. The plates were then transferred to a 37°C incubator for an additional 30 minutes to ensure complete gelation. EPCs were starved in M199 medium (Gibco, USA) for 12 h without cytokines and fetal bovine serum to promote endothelial growth arrest. Following starvation, cells transfected with TRPC4-shRNA or NC-shRNA were seeded onto the Matrigel-coated wells and cultured in a 5% CO_2 humidified incubator at 37°C for 6 h. Tubular structures formed by the cells were visualized and imaged using an inverted microscope at 100× and 200× magnifications. Five micrographs were taken at different positions for each sample. The capability of EPCs to form tubular structures was evaluated by counting the number of tube-like structures present in each image.

Enzyme-linked immunosorbent assay (ELISA)

Canine VEGF ELISA kit, canine SDF-1 ELISA kit, canine Angiopoietin-1 (Ang-1) ELISA kit, and canine Matrix metalloproteinase-2 (MMP-2) ELISA kit (Jianglai Bio, China) were used to quantify the protein content of VEGF, SDF-1, Ang-1, and MMP-2 in the supernatant of incubated EPCs (cells transfected with TRPC4-shRNA or NC-shRNA). The quantification was

Table 2. Primers sequences

Gene	Forward (5'-3')	Reverse (5'-3')
TRPC4	GGCGGGCTGCTGATAATTTG	TTCGAAGCCTGAGCTGACTG
GAPDH	GCACCACCAACTGCTTGGCT	GGCCGTCACGCCACATCTT

Notes: TRPC4 = transient receptor potential canonical channel 4; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

performed following the manufacturer's instructions provided with each kit.

Statistical analysis

All experimental data were presented as mean ± standard deviation (MEAN ± SD) and analyzed using SPSS23.0. The paired t-test was employed for comparisons within the two groups, while one-way analysis of variance (ANOVA) was used to compare multiple groups at the same time point. Post hoc pairwise comparison was conducted using the LSD-t test. Repeated measures analysis of variance was employed to compare differences among groups at multiple time points. Statistical significance was set at $P < 0.05$. To ensure reproducibility, each experiment was repeated a minimum of three times.

Results

Expression of TRPC4, VEGF, and SDF1 in EPCs

We retrieved EPCs-related expression spectral data from the GEO database and analyzed the genetic variations between EPCs and OECs. As illustrated in **Figure 1A**, we identified 2060 differentially expressed genes ($P < 0.05$ & $|log2FoldChange| > 1.5$) between these cell types. Functional analysis of these differentially expressed genes revealed their involvement in various cellular components, molecular functions, and biological processes. Specifically, they were associated with lysosomes, focal adhesions, lysosomal lumens, cell surfaces, and other cellular components, as well as protein binding, extracellular matrix structural constituents, signaling receptor activity, integrin binding, actin filament binding, receptor binding, actin binding, and other molecular functions. Moreover, these genes mediated biological processes such as extracellular region inflammatory responses, angiogenesis, positive regulation of cell migration, immune response, cell adhesion, positive regulation of interleukin-6 production, cell migration and others. Additionally, they affected the signaling pathway of osteoclast differentiation, as depicted in **Figure 1B**, **1C**. Further investigation into TRPC4, VEGF, and SDF1 genes showed no significant difference in TRPC4 expression between EPCs and OECs ($P < 0.05$, **Figure 1D**). However, the expression levels of VEGF and C-X-C Motif Chemokine Ligand 12 (SDF1) in EPCs were notably higher than those in OECs ($P < 0.05$, **Figure 1E**, **1F**).

EPC isolation, cultivation, and identification

Density gradient centrifugation was used to isolate EPCs from canine bone marrow. After 3 days of incubation (**Figure 2A**), cells adhered to the wall. By the 7th day, the colonies gradually increased and integrated (**Figure 2B**). By day 10, the cells showed a typical pebble-like structure (**Figure 2C**) and progressively expanded outward. Tubular structures within the matrix were also observed during the incubation period (**Figure 2D**), indicative of EPC functionality. The cell count was 101.67 ± 11.06 on the 3rd day, 338.33 ± 48.34 on the 7th day, and 1436.67 ± 99.16 on the 10th day (**Figure 2E**). Moreover, surface antigen CD133 and CD34 (**Figure 3A-C**) expression on progenitor cells were examined by flow cytometry, revealing CD133-positive cells at 77.06 ± 4.63% and CD34-positive cells at 88.53 ± 2.93% (**Figure 3D**). These results were consistent with previous studies on these cells [27, 28]. Taken together, these characteristics allowed for the identification of EPCs.

mRNA expression of TRPC4 following transfection

Lentivirus-mediated TRPC4-shRNA was employed in EPCs to investigate the function of TRPC4 in angiogenesis. The mRNA expression of TRPC4 was assessed via RT-qPCR. The results revealed that compared to the vehicle group (0.71 ± 0.27), each of the TRPC4-shRNA groups (TRPC4-shRNA1 (0.09 ± 0.03), TRPC4-shRNA2 (0.10 ± 0.04), and TRPC4-shRNA3 (0.02 ± 0.01)) effectively silenced TRPC4, with the TRPC4-shRNA3 group demonstrating the most significant silencing effect ($P < 0.05$), as depicted in **Figure 4**. Consequently, TRPC4-shRNA3 was selected for use in subsequent experiments.

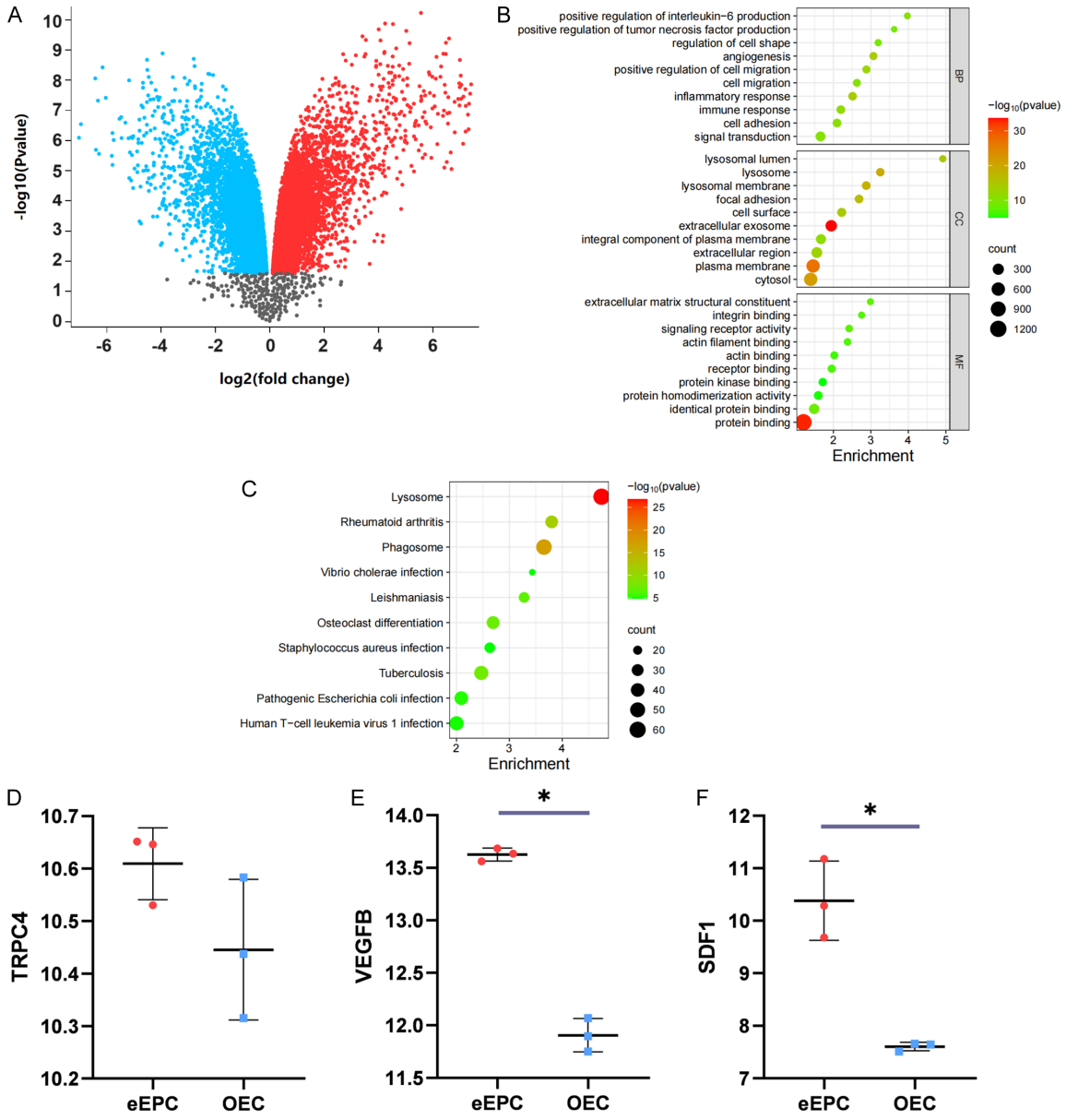

Figure 1. Expression of TRPC4, VEGF, and SDF1 in EPCs. A: Volcano plot of GSE20283 differential gene analysis; B: GO enrichment analysis of the differential genes; C: KEGG pathway enrichment analysis of the differential genes; D: TRPC4 expression levels in eEPCs and OECs; E: VEGFB expression levels in eEPCs and OECs; F: SDF1 expression levels in eEPCs and OECs. *P < 0.05. BP = biological processes; CC = cell components; MF = molecular function; KEGG = Kyoto Encyclopedia of Genes and Genomes; eEPCs = early endothelial progenitor cells; OECs = outgrowth endothelial cells; TRPC4 = transient receptor potential canonical channel 4; VEGF = vascular endothelial growth factor; SDF-1 = C-X-C Motif Chemokine Ligand 12.

TRPC4 knockdown inhibited proliferation of EPCs

Cell proliferation was assessed using the CCK-8 assay. Repeated measures analysis of variance indicated a significant main effect of group ($F = 4.308$, $P = 0.039$) and a significant main effect of time ($F = 80.715$, $P < 0.001$). However, the interaction effect of time and group was not significant ($F = 0.606$, $P = 0.732$). Specifically, at the 72-hour and 96-hour time points, the optical density (OD) value of the TRPC4-shRNA3 group was significantly lower than that of the NC-shRNA (vehicle group, $P < 0.05$), indicating that silencing TRPC4 had a noticeable effect on EPCs proliferation (**Figure 5**).

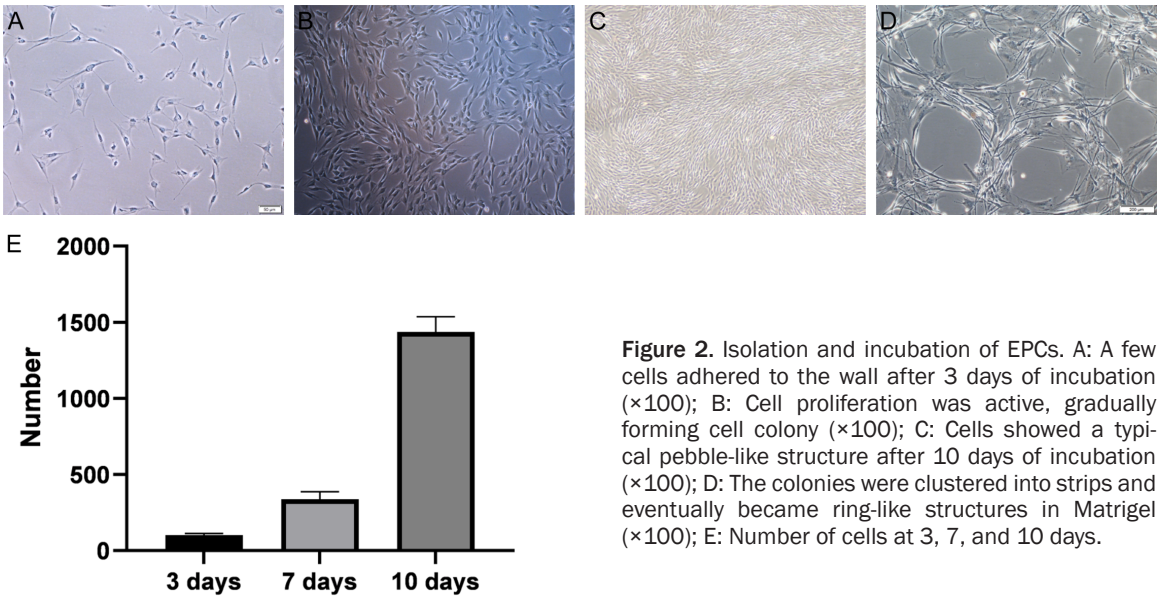

Figure 2. Isolation and incubation of EPCs. A: A few cells adhered to the wall after 3 days of incubation (×100); B: Cell proliferation was active, gradually forming cell colony (×100); C: Cells showed a typical pebble-like structure after 10 days of incubation (×100); D: The colonies were clustered into strips and eventually became ring-like structures in Matrigel (×100); E: Number of cells at 3, 7, and 10 days.

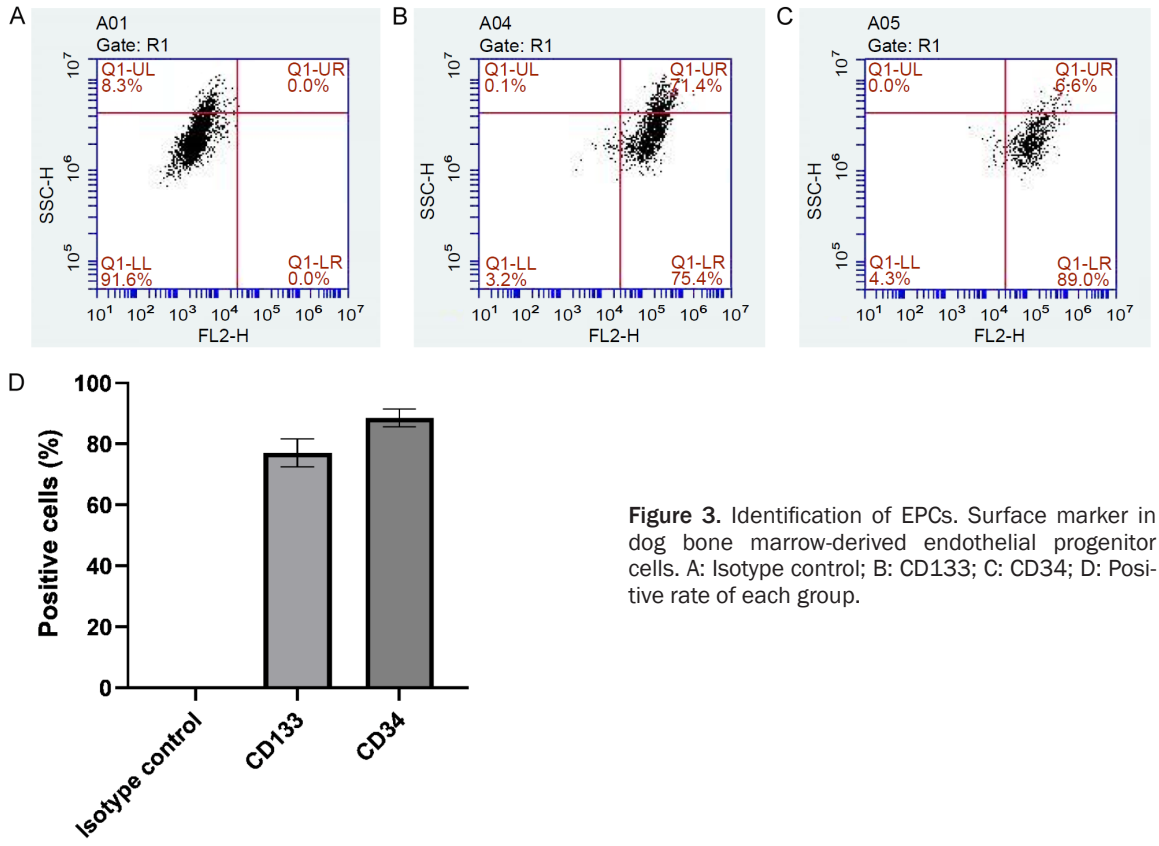

Figure 3. Identification of EPCs. Surface marker in dog bone marrow-derived endothelial progenitor cells. A: Isotype control; B: CD133; C: CD34; D: Positive rate of each group.

TRPC4 knockdown inhibited migration of EPCs

The trans-endothelial migration ability of EPCs was investigated to assess their capacity to reach injured sites for neovascularization during vascular repair. Transwell chamber assays were conducted to compare the number of cells crossing the endothelial monolayer between the TRPC4-shRNA3 group and the vehicle group and to investigate the impact of TRPC4 silencing on the trans-endothelial migration ability of EPCs. The results demonstrated a sig-

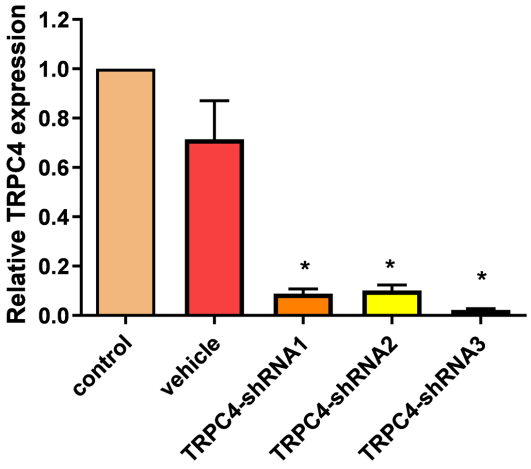

Figure 4. Selection of optimal silent target. The relative mRNA expression of TRPC4 in EPCs after transfection with lentivirus was compared. TRPC4-shRNA3 was the most effective target. TRPC4 = transient receptor potential canonical channel 4; shRNA = short hairpin RNA; *P < 0.05 vs. NC group.

nificant reduction in trans-endothelial cells and capacity in the TRPC4-shRNA3 group (P < 0.05), indicating that silencing TRPC4 inhibited EPC migration (**Figure 6**).

TRPC4 knockdown inhibited tube formation of EPCs

In vitro, the formation of tube-like structures is a crucial indicator of EPCs' angiogenic potential. To assess whether silencing TRPC4 affects the angiogenic capacity of EPCs, the tube formation ability of EPCs in Matrigel was compared between the TRPC4-shRNA3 and vehicle groups. The results showed that the TRPC4-shRNA3 group exhibited significantly fewer tubules in EPCs, with almost no tubule formation observed (P < 0.05), indicating that silencing TRPC4 inhibited EPCs' ability to form tubes (**Figure 7**).

TRPC4 knockdown inhibited the expression of VEGF and SDF-1

The molecular mechanism underlying the effect of TRPC4 knockdown on EPCs was elucidated by examining the expression of VEGF and SDF-1. ELISA results revealed that the levels of VEGF protein (14.05 ± 1.26) and SDF-1 protein (4.52 ± 0.86) in the TRPC4-shRNA3 group were significantly lower than those in the vehicle group (31.51 ± 1.72 and 22.54 ± 1.35, P < 0.05), indicating that silencing TRPC4 suppressed the activation of VEGF and SDF-1 (**Figure 8A**, **8B**). In addition, we assessed the levels of Ang-1 and MMP-2, which showed that the levels of Ang-1 (0.45 ± 0.08) and MMP-2 (6.52 ± 0.94) in the TRPC4-shRNA3 group were significantly lower than those in the vehicle group (1.25 ± 0.04 and 10.21 ± 0.81) (P < 0.05, **Figure 8C**, **8D**).

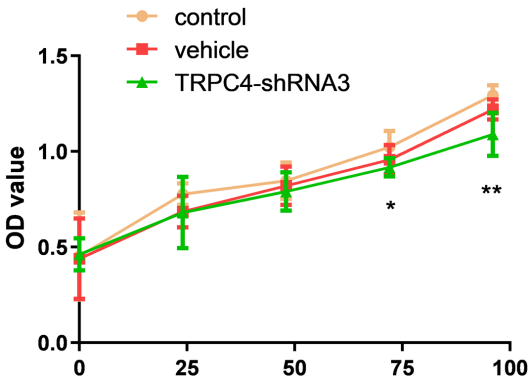

Figure 5. Proliferation of EPCs after silencing of TRPC4. *P < 0.05 vs. the vehicle group; **P < 0.01 vs. the vehicle group. Notes: TRPC4 = transient receptor potential canonical channel 4; OD = optical density.

Discussion

This study investigated the impact of TRPC4 knockdown on the function of EPCs and the potential molecular mechanisms involved. Our findings suggest that TRPC4 knockdown significantly reduced angiogenesis in canine EPCs by inhibiting migration and tube formation. This inhibition of angiogenesis may be mediated through the regulation of VEGF and SDF-1 expression. These results hint at a potential novel approach for treating neovascularization in RA.

Angiogenesis accompanied by hyperplasia and inflammation is fundamental to pannus formation and joint destruction in RA. Targeting synovial angiogenesis has thus emerged as an important therapeutic strategy for RA. TRPCs, as the main Ca^{2+} intracellular channels, play pivotal roles in various cellular functions and are widely distributed in the cardiovascular system [29, 30]. TRPC channels can modulate angiogenesis through various pathways. Pro-angiogenic cytokines such as VEGF and bFGF can activate TRPC channels, leading to increased calcium ions in ECs and subsequent angiogenesis [25, 31]. Abnormal function and

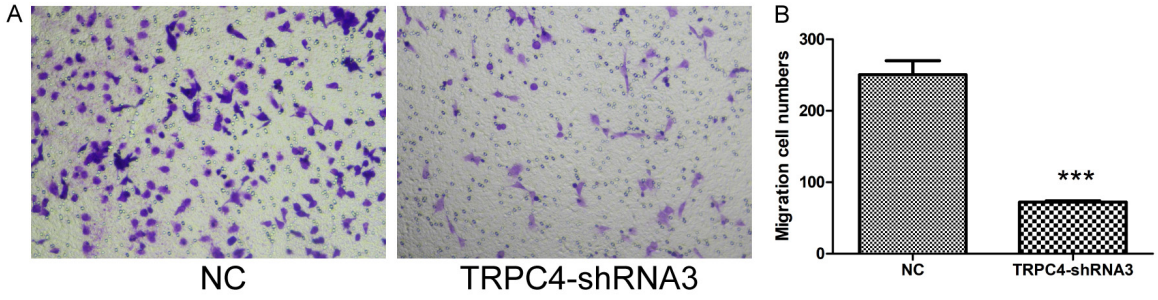

Figure 6. Migration of EPCs after the silence of TRPC4. The migrating cells were counted with four random fields at ×100 magnification. Significant differences were observed between the two groups. NC = vehicle group; TRPC4 = transient receptor potential canonical channel 4; shRNA = short hairpin RNA. ***P < 0.001 vs. the vehicle group.

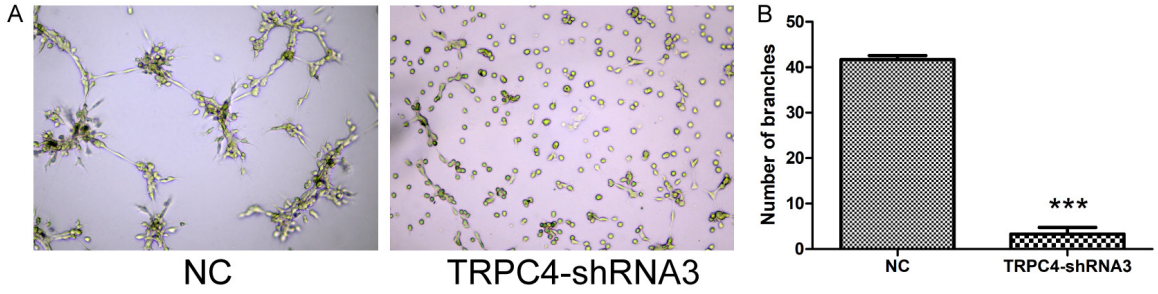

Figure 7. Tube formation of EPCs after the silence of TRPC4. The tube structure was observed at ×100 magnification. The number of branches in the TRPC4 shRNA group was significantly lower than that in the NC group. NC = vehicle group; TRPC4 = transient receptor potential canonical channel 4; shRNA = short hairpin RNA. ***P < 0.001 vs. the vehicle group.

expression of TRPCs can cause a variety of cardiovascular diseases, like atherosclerosis and hypertension [32]. EPCs derived from TRPC1-knockout mice showed significantly lower migration and angiogenic activity by blocking the Calmodulin/eNOS pathway, suggesting that TRPC1 plays an essential role for angiogenesis [33]. Study of Andrikopoulos et al. revealed that endothelial tube formation was significantly inhibited by TRPC3 knockdown, which is an indicator of angiogenesis [34]. The contribution of TRPC3 in pro-angiogenesis has been demonstrated in EPCs [35]. TRPC6 is also crucial for angiogenesis through inhibiting the migration and proliferation of ECs by TRPC6 dominant-negative mutants [36]. Although the roles of TRPC1, TRPC3, and TRPC6 in angiogenesis have been extensively studied, TRPC4, as an important member of non-selective Ca^{2+} channels, has garnered increasing attention. It is involved in angiogenesis, vascular permeability regulation, vascular tone modulation, oxidative stress-induced responses, and hypoxia-induced vascular remodeling [25]. Song et al. found that inhibition of TRPC4 had a significant effect on retinal neovascularization by injecting TRPC4-shRNA into the intravitreal retina of mice [26]. Although numerous studies have explored the relationship between TRPCs and angiogenesis, the role of TRPC4 in EPCs is still unknown.

To study the character of TRPC4 in angiogenesis in RA, lentivirus-mediated shRNA was employed to knockdown TRPC4 in EPCs. The effects of TRPC4 knockdown on cell proliferation, migration, and tube formation were also investigated. Our results showed that migration and tube formation were markedly decreased in the TRPC4-shRNA group compared to the NC-shRNA, while no significant change was observed in proliferation. These findings suggest that silencing TRPC4 may be a method to inhibit angiogenesis. Numerous studies have found that VEGF can significantly promote the proliferation, migration, and chemotaxis of vascular endothelial cells in various tissues and organs, such as bone, brain, and tumor [37-39] and is the main regulator of angiogenesis under physiological and pathological conditions [40].

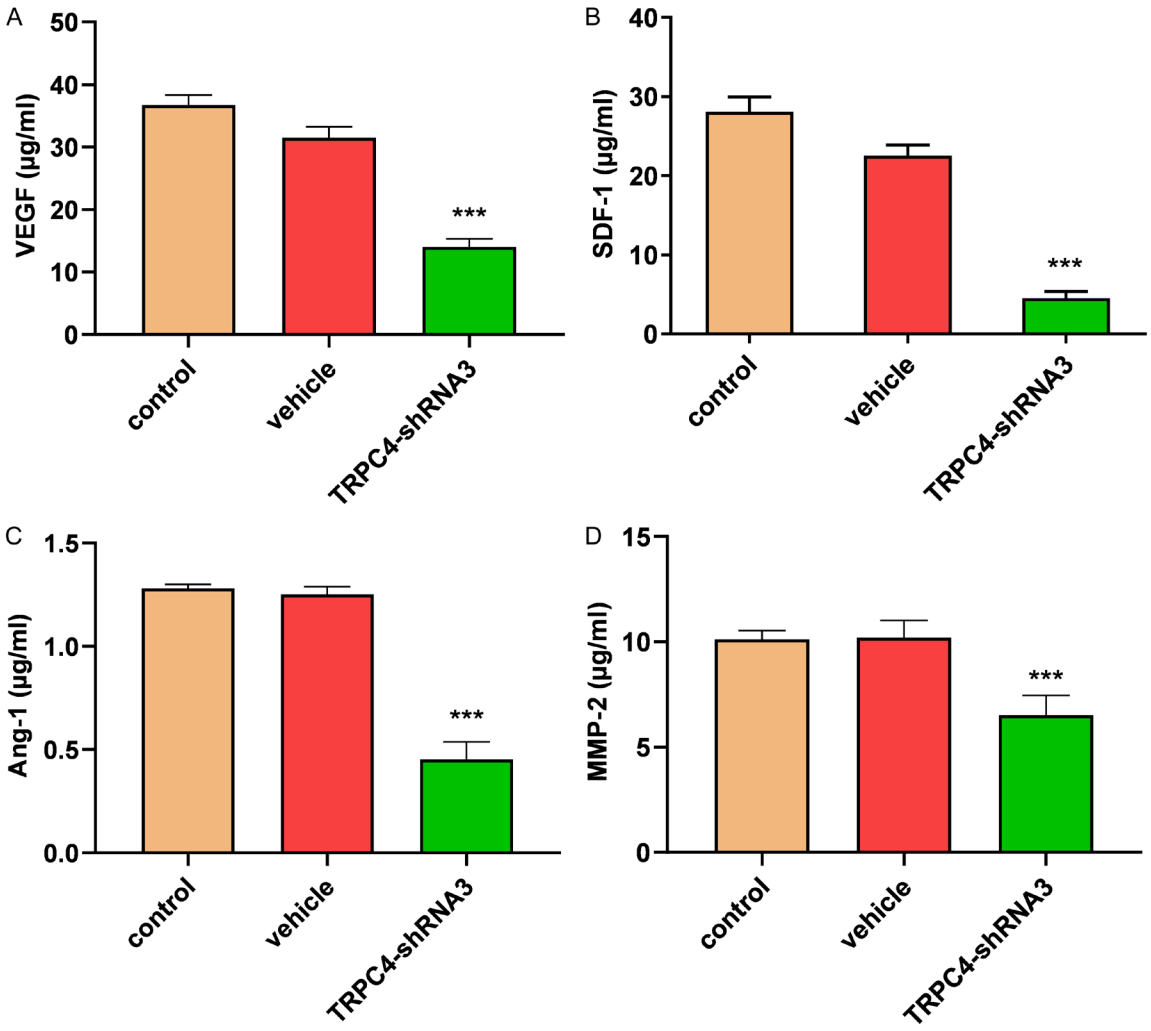

Figure 8. Concentration of VEGF, SDF-1, Ang-1 and MMP-2 in EPCs after the silence of TRPC4. The VEGF, SDF-1, MMP-2, and Ang-1 levels in the TRPC4-shRNA3 group were significantly lower than those in the control group. TRPC4 = transient receptor potential canonical channel 4; shRNA = short hairpin RNA; VEGF = vascular endothelial growth factor. ***P < 0.001 vs. the vehicle group.

In the present research, we discovered that VEGF expression was distinctly decreased after TRPC4 silencing, indicating that TRPC knockdown inhibited the angiogenesis in EPCs partly due to the decrease of VEGF expression. SDF-1 is a crucial chemokine that can mobilize hematopoietic stem cells and EPCs, and recruit bone marrow-derived circulating cells to participate in angiogenesis by binding with its receptors CXCR4 and CXCR7 [41]. SDF-1 has been suggested to have an influential effect during RA angiogenesis, synovitis, bone, and cartilage injury [42]. Furthermore, the decrease of SDF-1 level can play a therapeutic role in RA [43]. Our results revealed that SDF-1 expression decreased significantly when TRPC4 was silenced. Of note, SDF-1 and VEGF are critical synergists for the involvement of EPCs in angiogenesis [44]. Since VEGF indirectly modulates SDF-1 expression levels [45], we speculated that silencing of TRPC4 might inhibit angiogenesis by restraining the expression of VEGF in EPCs, possibly by regulating the expression of SDF-1. As a basic protein with strong angiogenic activity, Ang-1 is mainly present in normal plasma and solid tumor tissues, and is an important regulator of EC angiogenesis [46]. MMP-2 is a gelatinase secreted into the extracellular matrix, and up-regulation of MMP-2 can promote microvascular network formation [47]. Inhibiting the secretion of VEGF, SDF-1, Ang-1, and MMP-2 by EPCs is one of the ways to inhi-

bit angiogenesis. Consequently, the underlying effects of TRPC4-related molecular mechanisms of EPC angiogenesis in RA remain to be further investigated. However, the environment of in vitro culture is not exactly the same as that in vivo, so the experimental results may be different from those of in vivo experiments. This study also has limitations such as lack of functional recovery experiments, animal experiments, and drug target exploration. In the future, further animal experiments and clinical trials are needed to explore which drugs can act on TRPC4 and whether its mechanism inhibits or promotes EPC angiogenesis, so as to provide a novel and effective treatment for RA.

Conclusion

In conclusion, silencing of TRPC4 effectively suppressed angiogenesis in EPCs. This effect appears to be mediated by the downregulation of SDF-1 expression, which is regulated by VEGF. Consequently, targeting TRPC4 through knockdown strategies holds promise as a potential therapeutic approach for managing RA.

Acknowledgements

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Disclosure of conflict of interest

None.

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