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Tianjiao Ren April 7, 2022

Role of Satellite Cell Secreted Vascular Endothelial Growth Factor in Promoting Angiogenesis

by

Tianjiao Ren

Laura Hansen, PhD
Adviser

Department of Biology

Laura Hansen, PhD

Adviser

Robert Taylor, MD/PhD

Committee Member

William Kelly, PhD

Committee Member

2021

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By

Tianjiao Ren

Laura Hansen, PhD

Adviser

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Abstract

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Peripheral Artery Disease (PAD) is prevalent in the United States. The estimated lifetime risk of PAD in 2021 was estimated to be 19%, 22%, and 30% in White, Hispanic, and Black Americans, respectively (1). Symptoms include difficulty walking and claudication. Prolonged and untreated PAD can progress to critical limb ischemia (2). Patients may be candidates for angioplasty to reopen arteries or bypass surgery to revascularize around the affected area (3) (4) (5). Current non-invasive treatment to promote revascularization via exercise therapy is insufficient, as individual response varies. Satellite cells (SCs) are muscle progenitor cells localized near capillaries beneath the basal lamina of muscle fibers. SCs play a role in skeletal muscle repair and adaptation to exercise (6). In addition to their role in myofiber differentiation, SCs also play a role in promoting angiogenesis tube formation and increasing vascular flow (7). The mechanism of this is largely unknown. Previous studies have shown increased and similar angiogenesis in response to conditioned media from SCs and SC co-culture, suggesting paracrine signaling (8). Preliminary data has shown that encapsulated SCs delivered near the ischemic injury site significantly improve vascular growth and perfusion in hind limb ischemic mouse models (9). In the literature, vascular endothelial growth factor (VEGF) is commonly believed to be the key factor responsible for this angiogenesis (10) (11). However, there may be additional factors that play a role in SC promoted collateral vessel growth. SCs with the receptor for advanced glycation end products (RAGE) knocked out had equal expression of VEGF as wildtype SCs, but only the wildtype cells improved vascular response (9). This led to the hypothesis that VEGF is not the only paracrine factor responsible for inducing angiogenesis. To

study the role of SC secreted VEGF on angiogenesis, VEGF expression was knocked down in SCs using siRNA silencer. Migration assays using mouse aortic smooth muscle cells (MASMs) and human aortic endothelial cells (HAECs) allowed for isolated study of the effect of VEGF knockdown SCs on smooth muscle and endothelial cell migration. MASMs showed no significant differential migration towards the stimulus of VEGF knockdown SCs versus control SCs, suggesting additional SC secreted factors promote smooth muscle cell chemotaxis.

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Introduction

Peripheral artery disease (PAD) is prevalent in the United States, affecting 5.8% to 10.7% of individuals greater than 40 years old. The prevalence of this major cardiovascular health problem increases with age, approximately doubling every decade (1). PAD results from atherosclerotic vascular disease. Patients may present with difficulty walking and intermittent claudication, or pain as a result of arterial blockage. If undiagnosed and untreated, PAD can progress to critical limb ischemia (2). The treatment of PAD varies with severity. The most common modalities are supervised exercise therapy, endovascular revascularization, and surgical revascularization (3) (4) (5). Clinical studies have found supervised exercise therapy to be as effective as endovascular revascularization at improving PAD patient functionality (12) (13). Long-term studies have found exercise therapy to also decrease re-intervention rates, as more patients who utilized other revascularization methods had a second intervention within two years (5). Although supervised exercise therapy has proven to be effective in some patients, the mechanism is largely unknown. Studies have found increased collateral vessel growth following exercise therapy (14). This collateral vessel development is associated with increased functional performance and better PAD patient outcomes (15). The specific mechanism of exercise-induced collateral vessel growth is unknown, but we have reason to believe satellite cells play a role.

Satellite cells (SCs) are skeletal muscle progenitor cells responsible for muscle repair and regeneration. They are found beneath the basal lamina of muscle fibers, mostly localized near capillaries, as 88% of SCs are within 20 mm of the vasculature running through the muscle fiber (16). The number of SCs positively correlates with capillarization of the myofiber, as SCs help coordinate myogenesis and revascularization. At rest, SCs are in the G0 state in adult skeletal muscles and are activated upon exercise or injury. Activated SCs exit their quiescent state to proliferate, differentiate, and self-renew (17). Their role in skeletal muscle injury repair and exercise adaptation is well characterized as they differentiate into myofibers (6). The regenerated skeletal muscle must revascularize, but the mechanism of this vessel growth is largely unknown. SCs play a role in promoting angiogenesis tube formation, accelerating endothelial cell chemotaxis, and increasing vascular flow (7). Previous studies have found that alginate capsule delivery of activated SCs to sites near ischemic injury improves ischemic limb perfusion more than empty capsules (9). This suggests that activated SCs stimulate angiogenesis, or the

formation of new collateral vessels. The specific mechanism of this revascularization is not yet known.

Preliminary studies have shown that SCs promote endothelial and smooth muscle cell migration via paracrine signaling. A modified Boyden Chamber assay was run with mouse aortic smooth muscle cells (MASMs) and human umbilical vein endothelial cells (HUVECs) on the top and control media and co-cultured SCs in control media as the lower chamber stimulus. There was a significant increase in the number of migrated MASMs and HUVECs with the stimulus of co-cultured SCs. The same assay was run with HUVECs and an additional stimulus condition of media from SC culture. No significant difference in HUVEC migration was observed between the co-cultured SCs and the SC culture media experimental conditions, indicating that satellite cells signal through secreted paracrine factors that are not dependent on cellular cross-talk (18). Identification of the paracrine factor(s) that drive migratory response of vascular smooth muscle and endothelial cells in angiogenesis could be used in drug development and optimization of PAD treatment for those patients who do not respond positively to exercise therapy.

Ischemic injury and exercise induce SC proliferation and activation, which promotes the transcription and translation of proteins that serve as paracrine signaling molecules which stimulate collateral vessel growth (9). The identity of the secreted paracrine signal(s) responsible for promoting angiogenesis by inducing migration of nearby smooth muscle and endothelial cells is unknown. Current literature suggests vascular endothelial growth factor (VEGF) is responsible for inducing angiogenesis (10) (11). We have reason to believe otherwise. When SCs with the receptor for advanced glycation end products (RAGE) knocked out and wildtype SCs were delivered to sites of vascular injury in induced critical limb ischemia mouse models, only the wildtype SCs improved vascular response. Although there was no differential expression of VEGF, RAGE knockout SCs had no significant pro-angiogenic effect while wildtype SCs significantly improved hindlimb perfusion (9). A comparison of multiplex immunoassay of growth factors and cytokines from ischemic and nonischemic wildtype mice found the secretion of three proteins to be significantly upregulated in the ischemia-activated wildtype SCs: monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α) , and interleukin 6 (IL-6). VEGF was not significantly upregulated.

This study focused on the role of SC secreted VEGF on angiogenesis. If the hypothesis that VEGF is not the sole chemoattractant responsible for promoting collateral vessel growth holds true, it is expected that the knockdown of VEGF in SCs will not cause a significant difference in its angiogenic effects.

Methods

In vitro studies were used to isolate the effects of SC secreted growth factors on endothelial and smooth muscle cell migration.

Cell Culture

SCs were isolated from the adductor and gastrocnemius muscle from mice. After mechanical and enzymatic digestion, cells were filtered and purified. The resulting viable cells were cultured on collagen coated plates in Hams/F-10 media (Hyclone) with 20% fetal bovine serum (Sigma), penicillin/streptomycin (Hyclone), and HEPES (Hyclone). Mouse aortic smooth muscle primary cells were harvested from mouse aortas and cultured in Dulbecco's Modified Eagle's Medium – high glucose with 10% fetal bovine serum (Sigma), penicillin/streptomycin (Hyclone), and L-glutamine. Human aortic endothelial cells were purchased from LONZA and cultured in EGM-2 Endothelial Cell Growth Medium (LONZA).

Transfection

VEGF was knocked down in SCs with silencer siRNA using the Lipofectamine RNAiMAX Transfection Protocol (Life Technologies). On Day 0, SCs were seeded at 150,000 cells per well on a 24-well plate or 250,000 cells per well on a 6-well plate. Duplicate wells were used for each condition: scramble siRNA, VEGF siRNA, and wildtype SCs. After 20 hours, cell media was changed for all conditions and transfection reagent was introduced for scramble siRNA and VEGF siRNA conditions. Lipofectamine was diluted with Opti-MEM Reduced Serum Medium and combined with diluted select siRNA silencer. After combining and incubating to allow for formation of RNA-lipid complexes, 25 pmol of siRNA and 7.5 uL of Lipofectamine RNAiMAX was added per well on 6-well plates and 5 pmol of siRNA and 1.5 uL of Lipofectamine RNAiMAX was added per well on 24-well plates. SCs were incubated for 48 hours to allow for successful knockdown of VEGF RNA expression and protein secretion.

VEGF knockdown was verified through quantification of VEGF RNA and protein secreted into the cultured media on 6-well plates.

Reverse Transcription Quantitative Polymerase Chain Reaction

VEGF gene expression was quantified using reverse transcription-polymerase chain reaction analysis. RNA was harvested and purified from transfected 6-well plates using RNeasy (Qiagen). Using Superscript III reverse transcription enzyme (Qiagen), RNA extracted from SCs of each experimental condition produced cDNA. After cDNA purification with QiaQuick (Qiagen), VEGF and Glyceraldehype-3-Phosphate Dehydrogenase (GAPDH) expression was quantified on an Applied Biosystems StepOnePlus Real-Time PCR system in triplicate. VEGF and GAPDH were Quantitect Primer Assays from Qiagen.

Enzyme-linked Immunosorbent Assay

VEGF protein expression was quantified using enzyme-linked immunosorbent assay (ELISA) on the supernatant from each experimental condition on the same 6-well plate used in RNA knockdown verification. Debris was spun down and VEGF protein secreted from SCs into the culture media was quantified in duplicate using Quantikine ELISA for Mouse VEGF (R&D Systems).

Boyden Chamber Migration Assay

After verification of successful VEGF RNA and protein secretion knockdown, modified Boyden chamber migration assays were performed in duplicate to study MASM and HAEC migration in response to different stimulating environments. Costar Transwell plates with 6.5 mm inserts and polycarbonate membranes with 8- μm pores were coated in collagen, then washed with serum free media. Experimental migratory conditions were prepared.

For MASM migrations, media and transfection reagents from scramble siRNA SCs, VEGF siRNA SCs, and wildtype SCs were washed off with serum free media. Additional duplicate wells with serum free media were created. The positive control condition for MASMs was serum free media with added platelet-derived growth factor (PDGF) protein. An additional

condition of serum free media with added VEGF protein was also included to investigate the effect of VEGF protein on MASM migration.

For HAEC migrations, media and transfection reagents from scramble siRNA SCs, VEGF siRNA SCs, and wildtype SCs were washed off with serum free media. Additional duplicate wells with serum free media were created. The positive control condition for HAECs was serum free media with added VEGF protein.

Collagen coated membranes were placed over each migratory condition and equal number of MASMs or HAECs were added above the membrane of each well. The migration of seeded cells was monitored as the seeded MASMs or HAECs passed down through the semipermeable membrane towards the migratory condition in the bottom chamber. After allowing the MASMs and HAECs 4 hours to migrate, non-migrated cells were removed from the top side of the membranes. Membranes were fixed in chilled methanol, washed in phosphate buffered saline, and mounted on slides with Vectashield mounting media with 4,6-diamidino-2-phenylindole (Vector Labs). Migrated cells were visualized using a Zeiss Axioskope 2 with Axiocam Camera with a 20x Plan-Neo 0.5 numerical aperture objective with 2 random fields per membrane. Each experimental condition migration was performed in duplicate, resulting in 4 random fields per experimental condition. Example pictures are shown in Supplemental Material 2 and 3. Migrated cells were counted using Image J (National Institutes of Health).

Statistical Analysis

Data was collected and quantified before statistical analysis. For all studies, GraphPad Prism 8.3 was used to visualize data and run one-way ANOVA with multiple comparisons. A *p*-value under 0.05 indicated significant difference.

Results

VEGF was significantly knocked down in SCs using the transfection protocol.

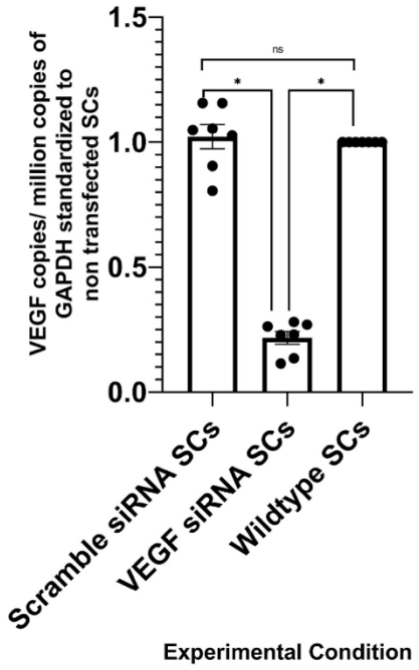


Figure 1: VEGF RNA expression was

Quantitative polymerase chain reaction (RT-qPCR) was used to verify VEGF RNA knockdown. Results were standardized to RNA expression in wildtype SCs. VEGF RNA expression in SCs transfected with scramble siRNA resulted in no significant change. One-way ANOVA analysis resulted in a F-value of 193 and a *p*-value <0.0001, indicating significant differences in VEGF RNA expression between the three experimental conditions (n=7). Multiple comparisons between groups found a significant decrease in VEGF expression from VEGF siRNA SCs as compared to scramble siRNA SCs, indicating successful VEGF RNA knockdown (Figure 1). The transfection protocol did not result in a significant change in VEGF RNA expression as no significant difference was found between VEGF RNA in scramble siRNA SCs and wildtype SCs.

Next, protein knockdown was ensured using enzyme-linked immunosorbent assay (ELISA). Results were standardized to VEGF protein expression from wildtype SCs. One-way ANOVA analysis resulted in a F-value of 25.17 and a *p*-value of 0.0012, indicating significant differences in VEGF protein secretion between the three experimental conditions (n=3). Multiple comparisons between groups found VEGF to be significantly knocked down in VEGF siRNA SCs compared to scramble siRNA SCs, indicating significant VEGF protein secretion knockdown (Figure 2). The transfection protocol did

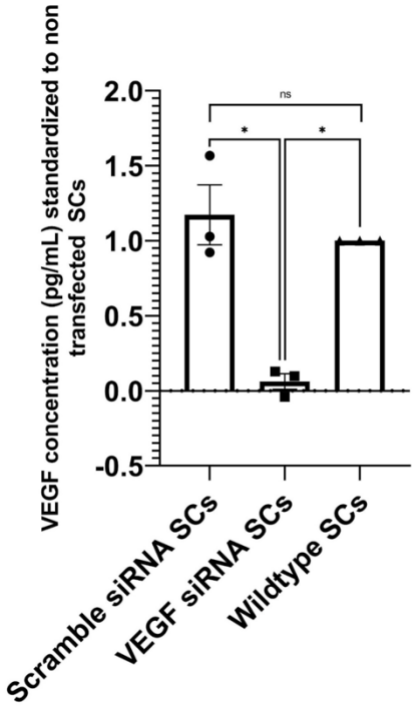


Figure 2: VEGF protein secretion was

not result in significant changes to protein expression, as no significant difference was found between VEGF protein secretion from scramble siRNA and wildtype SCs. Note the negative VEGF protein concentration point in VEGF siRNA SCs may be a result of reagent interference (Supplemental Material 1).

After verification of successful VEGF knockdown, MASM and HAEC migration assays were run to study the effect of VEGF knockdown SCs on promoting angiogenesis.

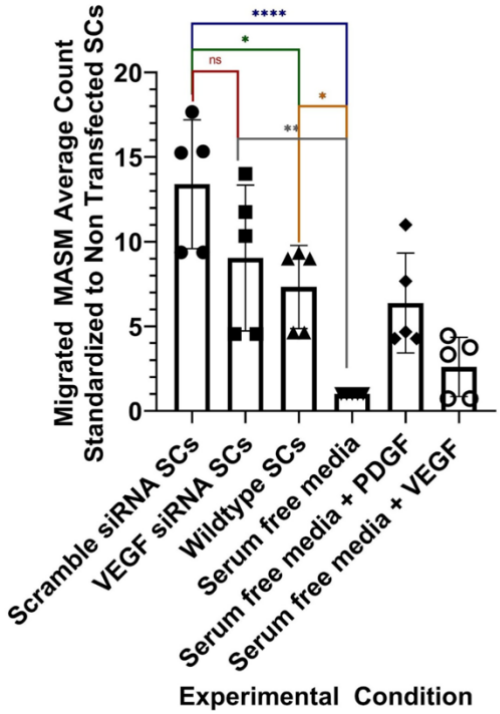


Figure 3: Migration assays studied the effect of VEGF knockdown on promoting MASM

Modified Boyden chamber migration assays were performed to study the effect of VEGF knockdown on mouse aortic smooth muscle cell (MASM) migration (n=4). Results were standardized to the migrated MASM count towards serum free media. One-way ANOVA analysis resulted in a F-value of 11.82 and a *p*-value <0.0001 indicating significant differences in MASM migration in response to the six migratory conditions. Multiple comparisons between experimental groups showed no significant difference in MASMs migrated towards scramble siRNA SCs compared to VEGF siRNA SCs (adjusted *p*-value = 0.2065), shown in red in Figure 3. A significant increase was observed between wildtype SCs and serum free media (adjusted *p*-value 0.0233). A significant increase was also observed between scramble siRNA SCs and wildtype SCs (adjusted *p*-value = 0.0316), possibly indicating a significant stimulatory effect of scramble siRNA or the transfection protocol on MASM migration (Figure 3). Significant differences were also found in response to serum free media versus scramble siRNA (adjusted *p*-value <0.0001) and serum free media versus VEGF siRNA SCs (adjusted *p*-value 0.0025).

This modified Boyden chamber migration assay was repeated with HAECs to study the effect of VEGF knockdown on endothelial cell migration. Again, data was standardized to the migrated HAEC count towards serum free media. One-way ANOVA analysis resulted in a F- value of 1.479 and a *p*-value of 0.2799, indicating no significant difference in HAEC migration in response to the five migratory conditions (n=3). Multiple comparisons showed no significant difference between HAEC migration towards the various migratory conditions which may be due to the small number of experiments and high variability (Figure 4).

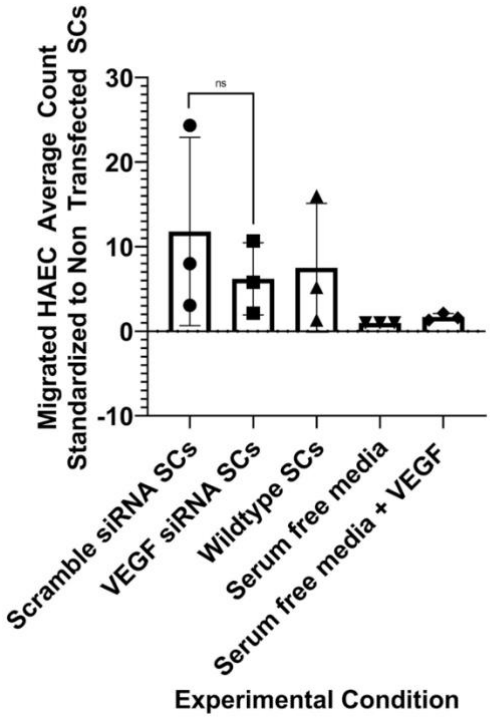


Figure 4: Migration assays studied the effect of VEGF knockdown on promoting HAEC

Discussion

The purpose of this study was to determine the paracrine signaling mechanism by which satellite cells regulate vascular growth. If the hypothesis that VEGF is not the sole chemoattractant responsible for inducing endothelial and smooth muscle migration in collateral vessel growth holds true, we expect to observe no significant difference in the number of MASMs and HAECs that migrate towards the environment with VEGF knockout SCs compared to the migration towards scramble siRNA SCs. If VEGF knockdown does not induce a significant decrease in MASM and HAEC migration, VEGF is not the primary SC secreted paracrine factor responsible for inducing angiogenesis.

VEGF knockdown was successful as both VEGF RNA expression and protein secretion were significantly decreased. VEGF siRNA SCs had significantly decreased VEGF protein and RNA secretion levels compared to scramble siRNA SCs (Figure 1 & 2). VEGF protein knockdown verification using Western Blotting was attempted multiple times and repeatedly failed to show significant protein knockdown. Western blots were run using cell lysate from the transfected SC cell cultures. After numerous troubleshooting attempts, no VEGF protein knockdown was observed. Since we wanted to study paracrine signaling from the transfected cells, ELISA could be used on the supernatant from cell culture to study the VEGF protein levels secreted from the transfected SCs. This allowed for successful verification of decreased VEGF protein secretion from VEGF siRNA SCs. In addition to being released in response to stimulation, VEGF has also been shown to be stored intracellularly in mast cells (19). It is unknown whether VEGF is stored in SCs as well. The storage of VEGF in vesicles of SCs is a plausible explanation for the failure to visualize VEGF protein knockdown from cell lysate, but ability to verify VEGF knockdown from SC secretion into the culture media.

MASM migration assays verify the role of SCs in inducing angiogenesis. A significant increase in smooth muscle migration was observed in response to wildtype SCs stimulation when compared to serum free media stimulation. The role of the factor of interest VEGF on MASM migration showed no significant change in MASM chemotaxis towards SCs expressing VEGF and SCs with VEGF knockdown. Scramble siRNA SCs and VEGF siRNA SCs were compared to account for MASM migratory effects from the transfection protocol. Since the knockdown of VEGF resulted in no significant decrease in MASM migration, this suggests that VEGF is not

the only angiogenic factor secreted by SCs that induces MASM migration in collateral vessel growth. A significant increase in scramble siRNA SCs and wildtype SCs indicates an effect from the transfection protocol. Further studies can include an additional control group with SCs transfected without siRNA to determine the MASM migratory effects of the transfection protocol.

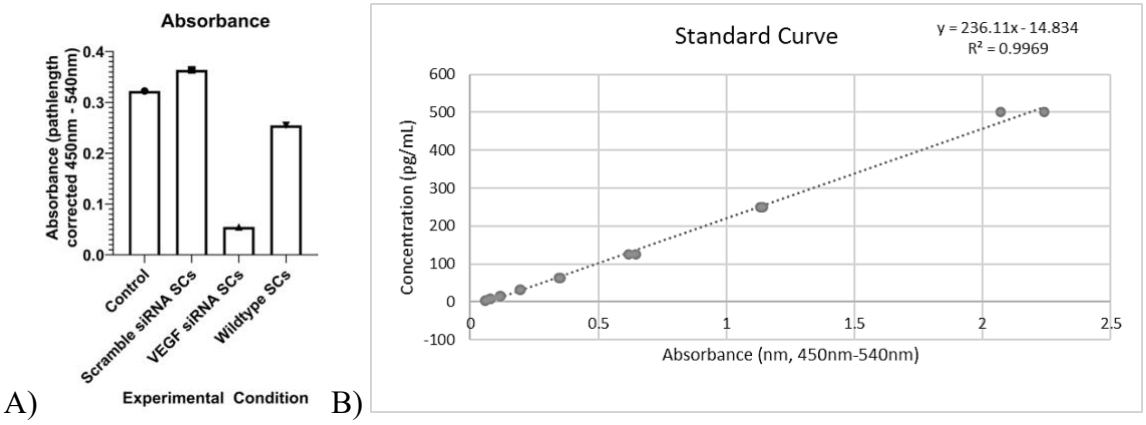
No significant differences were found in any of the 5 experimental conditions for HAECs. No conclusive results can be made from the HAEC migration assays. This may be due to high variability in results between the 3 experiments. Future replication experiments are needed to decrease variability and establish a relationship between VEGF knockdown and HAEC migration.

In conclusion, the results suggest that VEGF may not be the sole factor for inducing smooth muscle cell migration in SC promoted collateral vessel growth. Repeat HAEC migration assays are needed to provide conclusive results on the importance of VEGF on endothelial cell migration. For future experiments, an additional migratory condition will include SCs transfected using the same transfection protocol without siRNA since significantly increased MASM migration was observed towards the migratory condition of scramble siRNA compared to wildtype SCs.

Since angiogenesis is mainly a result of smooth muscle and endothelial cell migration, both HAEC and MASM migration must be studied before determining the role of VEGF on angiogenesis. MASM migration assays suggest additional factors in SC induced angiogenesis. Further HAEC studies are required to reach a valid conclusion on the role of VEGF on endothelial cells. If VEGF is not the only cytokine factor responsible for inducing collateral vessel growth, future experiments can aim to identify other factors that play an important role. Studies can begin with targeting the factors that were found to be differentially expressed in ischemic versus nonischemic models: myocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (9). The role of these factors can be studied using in vitro assays with knockout satellite cells using the same siRNA silencer method described for VEGF. After determining which factor(s) lead to differential smooth muscle and endothelial cell migration, an in vivo add back study can be used to study the factor in an animal model.

Supplemental Material

Figure S1



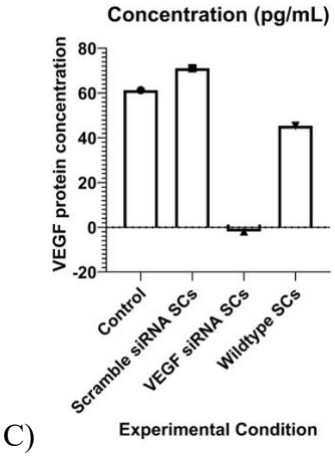


Figure S1: A) Absorbance readings from the ELISA assay showed significant decrease in absorbance for VEGF siRNA SCs. B) Standards were used to produce a standard curve with a R squared on 0.9969. C) The equation from the standard curve allowed for calculation of VEGF protein concentrations from absorbances. The concentration of VEGF protein from the siRNA knockdown was found to be negative. The standard curve resulted in a R-squared of 0.9969 and the control sample fell within the expected protein concentration range, indicating validity of the assay. The negative VEGF protein concentration in the VEGF knockdown condition may be a result of reagent interference.

Figure S2: MASM migration visualization

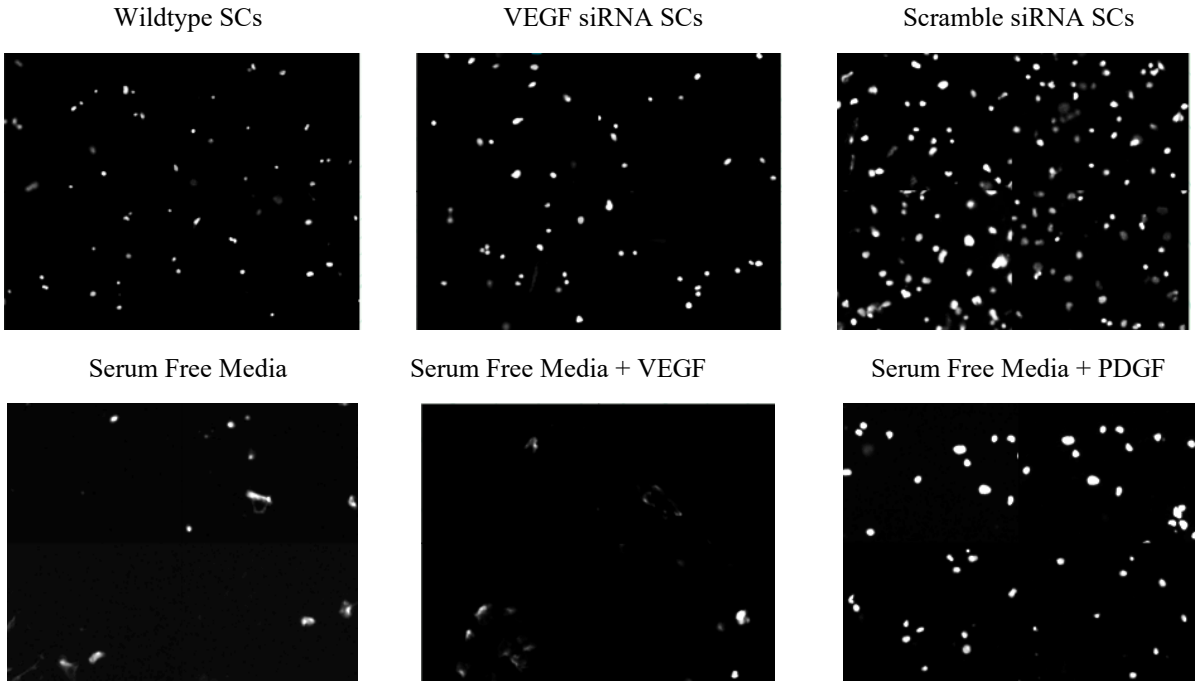
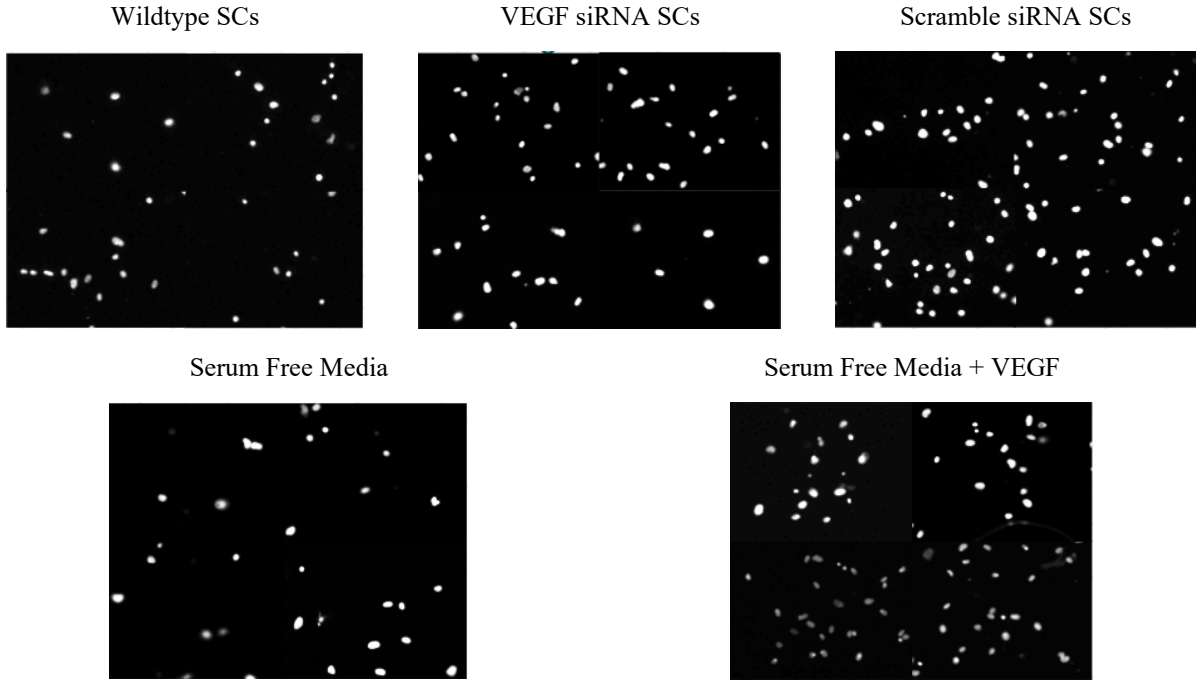


Figure S3: HAEC migration visualization



References

1. Virani, Salim S., et al. “Heart Disease and Stroke Statistics—2021 Update.” *Circulation*, vol. 143, no. 8, 2021, doi:10.1161/cir.0000000000000950. *The American Jornal of Medicine*, pp. 1133-1141.

2. Campia, Umberto, et al. “Peripheral Artery Disease: Past, Present, and Future.” T*he American Journal of Medicine*, vol. 132, no. 10, 2019, pp. 1133–1141., doi:10.1016/j.amjmed.2019.04.043. *Circulation*.

3. Conte, Michael S., et al. “Global Vascular Guidelines on the Management of Chronic Limb-Threatening Ischemia.” *Journal of Vascular Surgery*, vol. 69, no. 6, 2019, doi:10.1016/j.jvs.2019.02.016.

4. Thukkani, Arun K., and Scott Kinlay. “Endovascular Intervention for Peripheral Artery Disease.” *Circulation Research*, vol. 116, no. 9, 2015, pp. 1599–1613., doi:10.1161/circresaha.116.303503.

5. Bouwens, Elke, et al. “Supervised Exercise Therapy and Revascularization: Single-Center Experience of Intermittent Claudication Management.” *Vascular Medicine*, vol. 24, no. 3, 2019, pp. 208–215., doi:10.1177/1358863x18821175.

6. De Lisio, Michael, and Jean Farup. “The role of satellite cells in activity-induced adaptations: breathing new life into the debate.” *The Journal of physiology* vol. 595,19 (2017): 6225-6226. doi:10.1113/JP274969.

7. Rahbarghazi, Reza et al. “Juxtacrine and paracrine interactions of rat marrow-derived mesenchymal stem cells, muscle-derived satellite cells, and neonatal cardiomyocytes with endothelial cells in angiogenesis dynamics.” *Stem cells and development* vol. 22,.

8. Rhoads, R P et al. “Satellite cells isolated from aged or dystrophic muscle exhibit a reduced capacity to promote angiogenesis in vitro.” *Biochemical and biophysical research communications* vol. 440,3 (2013): 399-404. doi:10.1016/j.bbrc.2013.09.085.

9. Hansen, Laura, et al. “Satellite Cell Expression of RAGE (Receptor for Advanced Glycation End Products) Is Important for Collateral Vessel Formation.” J*ournal of the American Heart Association*, 2021, doi:10.1161/jaha.120.022127.

10. Dai, J., and A.b.m. Rabie. “VEGF: an Essential Mediator of Both Angiogenesis and Endochondral Ossification.” *Journal of Dental Research*, vol. 86, no. 10, 2007, pp. 937–950., doi:10.1177/154405910708601006.

11. Melincovici, Carmen Stanca et al. “Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis.” *Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie* vol. 59,2 (2018): 455-467.

12. Pandey, Ambarish, et al. “Comparative Efficacy of Endovascular Revascularization Versus Supervised Exercise Training in Patients With Intermittent Claudication.” *JACC: Cardiovascular Interventions*, vol. 10, no. 7, 2017, pp. 712–724., doi:10.1016/j.jcin.20.

13. Fakhry, Farzin, et al. “Endovascular Revascularisation versus Conservative Management for Intermittent Claudication.” *Cochrane Database of Systematic Reviews*, vol. 2018, no. 3, 2018, doi:10.1002/14651858.cd010512.pub2.

14. Roth, D M, et al. “Effect of Long-Term Exercise on Regional Myocardial Function and Coronary Collateral Development after Gradual Coronary Artery Occlusion in Pigs.” *Circulation*, vol. 82, no. 5, 1990, pp. 1778–1789., doi:10.1161/01.cir.82.5.1778.

15. Mcdermott, Mary M., et al. “Proximal Superficial Femoral Artery Occlusion, Collateral Vessels, and Walking Performance in Peripheral Artery Disease.” *JACC: Cardiovascular Imaging*, vol. 6, no. 6, 2013, pp. 687–694., doi:10.1016/j.jcmg.2012.10.024.

16. Mounier, Rémi, et al. “Blood Vessels and the Satellite Cell Niche.” *Current Topics in Developmental Biology Myogenesis*, 2011, pp. 121–138., doi:10.1016/b978-0-12-385940-2.00005-x.

17. Dhawan, Jyotsna, and Thomas A. Rando. “Stem Cells in Postnatal Myogenesis: Molecular Mechanisms of Satellite Cell Quiescence, Activation and Replenishment.” *Trends in Cell Biology*, vol. 15, no. 12, 2005, pp. 666–673., doi:10.1016/j.tcb.2005.10.007.

18. Hansen, L., Liu, W., Joseph, G., Weiss, D. and Taylor, W.R. (2017), The Paracrine Effects of Satellite Cells on Collateral Vessel Formation. *The FASEB Journal*, 31: lb668-lb668. https://doi.org/10.1096/fasebj.31.1_supplement.lb668.

19. Grützkau, A et al. “Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF206.” *Molecular biology of the cell* vol. 9,4 (1998):.