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Transient Repetitive Exposure To Low Level Light Therapy Enhances Collateral Blood Vessel Growth In The Ischemic Hindlimb Of The Tight Skin Mouse

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Abstract

The tight skin mouse (Tsk^{-/+}) is a model of scleroderma characterized by impaired vasoreactivity, increased oxidative stress, attenuated angiogenic response to VEGF, and production of the angiogenesis inhibitor angiostatin. Low level light therapy (LLLT) stimulates angiogenesis in myocardial infarction and chemotherapy-induced mucositis. We hypothesize repetitive LLLT restores vessel growth in the ischemic hindlimb of Tsk^{-/+} mice by attenuating angiostatin and enhancing angiomin effects *in vivo*.

C57Bl/6J and Tsk^{-/+} mice underwent ligation of the femoral artery. Relative blood flow to the foot was measured using a laser Doppler imager. Tsk^{-/+} mice received LLLT (670 nm, 50 mW cm², 30 J/cm²) for 10 min/day for 14 days. Vascular density was determined using lycopersicom lectin staining. Immunofluorescent labeling, western blot analysis, and immunoprecipitation were used to determine angiostatin and angiomin expression.

Recovery of blood flow to the ischemic limb was reduced in Tsk^{-/+} compared with C57Bl/6 mice two weeks after surgery. LLLT treatment of Tsk^{-/+} mice restored blood flow to levels observed in C57Bl/6 mice. Vascular density was decreased, angiostatin expression was enhanced and angiomin depressed in the ischemic hindlimb of Tsk^{-/+} mice. LLLT treatment reversed these abnormalities.

LLLT stimulates angiogenesis by increasing angiomin and decreasing angiostatin expression in the ischemic hindlimb of Tsk^{-/+} mice.

INTRODUCTION

The use of low level light therapy (LLLT) to stimulate new blood vessel growth has recently received considerable attention. Wavelengths in the red and near infrared spectrum

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(600~1300 nm) generate an optical window to produce effects on biological systems *in vivo* and *in vitro*. This optical window is favorable to biological discovery because at these wavelengths there is enhanced tissue penetration to light secondary to reduced dermal melanin absorption. In addition, the limited chromophore absorption (e.g. melanin, water, fatty acids) allied to LLLT in this particular wave length range results in a minimal rate of heat production (1–3). Far red and near infrared radiation reduced experimental myocardial infarct size in a model of prolonged coronary artery occlusion and reperfusion (4). Red and infrared lasers also enhanced tissue healing and cardioprotection by stimulating angiogenesis in various animal models of ischemia (5, 6). The mechanisms by which LLLT exerts these protective effects remain incompletely studied, but stimulated release of nitric oxide (NO) appears to play a central role in this process. Photolytic dissociation of bound NO to cytochrome c oxidase (a photoreceptor) was previously documented (7). More recently, Lohr *et al* demonstrated that LLLT in the far red and near infrared spectrum stimulates NO release from nitrosyl hemoglobin and myoglobin (4). Notably, collateral blood vessel growth is dependent on sufficient quantities of NO. The tight skin mouse (Tsk^{-/+}) is an experimental model of systemic scleroderma characterized by impaired vasoreactivity, increased oxidative stress, attenuated angiogenic response to vascular endothelial growth factor, and excessive production of the angiogenesis inhibitor angiostatin (8, 9). These features are associated with impaired blood vessel development in response to chronic ischemic stress. We tested the hypothesis that transient, repetitive LLLT exposure restores collateral blood vessel growth in the ischemic hindlimb of Tsk^{-/+} mice by attenuating the influence of angiostatin and enhancing the salutary effects of angiotensin *in vivo*.

MATERIALS AND METHODS

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Experimental Preparation

Male Tsk^{-/+} and C57Bl/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice underwent unilateral ligation of the femoral artery distal to the origin of the arteria profunda femoris. Relative blood flow to the foot was measured under similar conditions (controlled room temperature; 1% isoflurane anesthesia) using laser Doppler imaging (LDI, Moor Instruments, Wilmington, DE) immediately after surgery and on postoperative days 3, 7 and 14. The ratio of blood flow in the ischemic to normal (non-operated) leg was calculated for each mouse. All mice were euthanized by cervical dislocation in the presence of 5% isoflurane anesthesia 14 days after hindlimb ischemia.

Irradiation Parameters

Tsk^{-/+} mice were randomly assigned to receive LLLT. Briefly, mice were placed in a box with a transparent bottom on top of a light emitting diode array (4 cm x 10 cm) with a peak wavelength of 670 nm (NIR Technologies, Waukesha, WI) immediately after surgery and once per day for 10 min with an intensity of 50 mW/cm², corresponding to a total energy density of 30J/cm² for 14 consecutive days or placebo (n=10 per group). Power output was determined at the surface of transparent bottomed box by a light meter (X97 Irradiance, GigaHertz-Optik). On days of blood flow measurements LLLT was delivered after the LDI scans.

Vascular Density

Ten μm frozen sections of gastrocnemius muscle from ischemic and normal legs obtained from C57Bl/6J mice and from $\text{Tsk}^{-/+}$ mice with and without LLLT treatment were incubated with biotinylated lycopersicum lectin, a murine endothelial cell marker, (Vector Laboratories, Burlingame, CA) in a $1\text{ }\mu\text{g}/\mu\text{L}$ dilution for 30 minutes at 37°C , followed by a alkaline phosphatase staining and labeling with red alkaline phosphatase substrate for visualization. Images of the sections were captured with a TM Nikon microscope and analyzed with the Nikon Element software (Nikon Instruments Inc, Melville, NY).

Western Blot Analysis

The cellular fraction of the gastrocnemius muscle was probed for angiostatin from ischemic and normal legs of C57Bl/6J mice and from $\text{Tsk}^{-/+}$ mice with and without NIR treatment using Western blot analysis. Briefly, gastrocnemius muscle homogenates were separated on SDS-PAGE (4–20%). Nitrocellulose membranes containing the separated proteins were blocked in 10% nonfat dry milk solution of Tris-buffered saline (TBS). The blot was then incubated overnight using angiostatin polyclonal antibody (1:1,000; Abcam, Cambridge, MA) in TBS containing nonfat dry milk. After washing four times, the blot was incubated with the horseradish peroxidase-conjugated donkey anti-rabbit IgG (Santa Cruz Biotechnologies, Santa Cruz, CA). Bands were visualized with ECL plus reagent (GE Healthcare, Piscataway, NJ). Determination of relative band densities was performed using image acquisition and analysis software (Image J, Bethesda, MD).

Immunoprecipitation

Immunoprecipitation was performed by incubating homogenized gastrocnemius muscle (500 μg) with angiostatin polyclonal antibody (5 $\mu\text{g}/\text{mg}$ of total cell protein; Abcam) for 16 h at 4°C , followed by 2 h incubation with protein A agarose beads. After boiling for 5 min, samples were resolved using precast 4–15% Tris-HCl gels (Criterion; Bio-Rad, Hercules, CA), and protein was transferred to a nitrocellulose membrane. Immunoblots were performed with rabbit polyclonal anti-angiomotin (1:1000 for tissue; Abcam) and were incubated overnight at 4°C . Membranes were incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:5,000 for the tissue; Jackson ImmunoResearch Laboratories, West Grove, PA) and developed using ECL plus Western blot chemiluminescence detection reagent (GE Healthcare). Densitometric analysis was performed using image acquisition and analysis software (Image J).

Immunofluorescent Labeling

Gastrocnemius muscles were mounted in convenient specimen matrix for cryo-sectioning (TissueTek OCT, Sakura, Torrance, CA) and $10\text{-}\mu\text{m}$ sections were cut. Subsequently, these sections were fixed for 10 minutes with 1% paraformaldehyde in phosphate buffered saline (PBS), followed by permeabilization with 0.5% Triton-X in PBS for 5 min at room temperature. The primary antibody for angiostatin (1:200, Abcam) or angiomotin (1:50, Santa Cruz Biotechnologies) was applied after a single wash with PBS (5 min). The slides were incubated for 30 min at 37°C . The appropriate secondary antibody conjugated with Alexa 488 (Invitrogen, Carlsbad, CA) was applied after washing with PBS. The secondary antibodies were incubated for 30 min at 37°C . After two washes with PBS, slides were incubated with To-Pro for 3 min at room temperature ($1\mu\text{g}/\text{mL}$, Invitrogen). The slides were sealed with an aqueous mounting media and stored at -20°C until analysis using confocal microscopy.

Statistical Analysis

Statistical analysis of data within and between groups was performed with analysis of variance for repeated measures followed by Bonferroni's modification of Student's t test. The null hypothesis was rejected when $P < 0.05$. All data are expressed as mean $\pm$ standard error mean (SEM).

RESULTS

All mice showed comparable decreases in blood flow to the ischemic limb immediately after surgery (figure 1). Blood flow gradually recovered in the ischemic limb in a time-dependent manner in all three experimental groups (data not shown for days 3 and 7). Two weeks after surgery, blood flow to the ischemic limb had recovered substantially in all groups, but blood flow was significantly ($P < 0.05$) less in $Tsk^{-/+}$ compared with C57BL/6J mice. $Tsk^{-/+}$ mice receiving LLLT had recovery of ischemic limb blood flow that was similar to that observed in C57BL/6J mice and was significantly greater than blood flow in $Tsk^{-/+}$ mice alone. Vascular density was also reduced in $Tsk^{-/+}$ mice without LLLT two weeks after hindlimb ischemia compared with C57BL/6 mice (figure 2). Notably, vascular density in $Tsk^{-/+}$ mice exposed to LLLT was significantly greater than that observed in C57BL/6 or $Tsk^{-/+}$ mice without NIR.

Angiostatin and angiomin ratio was unchanged in the ischemic compared with the normal hindlimb in C57BL/6 mice two weeks after femoral artery ligation (figure 3) as demonstrated using immunohistochemistry. In contrast, angiostatin/angiomin ratio was increased in the ischemic hindlimb of $Tsk^{-/+}$ mice compared with C57BL/6 mice because angiostatin expression was augmented and angiomin expression was markedly depressed. Notably, daily exposure to LLLT restored the angiostatin/angiomin ratio in $Tsk^{-/+}$ mice to values similar to those observed in C57BL/6 mice because angiostatin expression was reduced and angiomin expression was enhanced in the presence of this intervention. Western blot analysis confirmed that angiomin was markedly depressed in the ischemic hindlimb of $Tsk^{-/+}$ mice two weeks after femoral artery ligation, and this effect was reversed by LLLT treatment (figure 4). Immunoprecipitation for angiostatin and immunoblotting for angiomin on protein lysates from gastrocnemius muscles demonstrated that less angiomin was bound to angiostatin in the ischemic hindlimb of $Tsk^{-/+}$ mice alone compared with C57BL/6 mice or $Tsk^{-/+}$ mice treated with LLLT (figure 5).

DISCUSSION

The current results demonstrate that temporal restoration of blood flow and collateral blood vessel growth after femoral artery ligation are inhibited in the $Tsk^{-/+}$ mouse model of systemic scleroderma. This inhibition is concomitant with increases in angiostatin and reductions in angiomin expression, which suggests there is a fundamental imbalance between these essential pro- and anti-angiogenic proteins in $Tsk^{-/+}$ mice in the presence of severe hindlimb ischemia. Previous observations indicate that $Tsk^{-/+}$ mice have an impaired ability to produce new blood vessels in response to ischemia, due to chronically elevated oxidative stress (8,9). Stimulation of collateral development is well suited for LLLT, as these vessels are often located superficially, thereby allowing effective penetration. The current results further demonstrate that transient, repetitive exposure to LLLT for two weeks after femoral artery ligation restores normal compensatory vascular development in $Tsk^{-/+}$ mice. This finding occurred, at least in part, to shifting the balance toward a pro-angiogenic environment through the attenuated expression of angiostatin concomitant with enhanced expression of angiomin.

Previous studies demonstrated that NO plays a central role in the beneficial effects of LLLT on vascular development (10). Nitric oxide synthase (NOS) produces the majority of NO *in vivo*, but, smaller quantities of NO may also be generated through reduction of nitrite, especially during hypoxic conditions when NOS activity is limited (11). Deoxyhemoglobin (12), deoxymyoglobin (13), and cytochrome c oxidase (14–16) have been shown to reduce nitrite to NO. NO generated through this mechanism may bind to vacant deoxygenated heme moieties, resulting in the formation of iron-nitrosyl hemoglobin (HbNO) or myoglobin (MbNO). Lohr *et al* demonstrated that LLLT releases NO that is specifically bound in HbNO and MbNO (4). We did not specifically examine NO metabolism in the current investigation, but it appears likely that release of NO bound to hemoglobin or myoglobin by LLLT exposure in ischemic hindlimb of Tsk^{-/+} mice may have been involved in new blood vessel development. Notably, Namba *et al* (17) reported that endothelial NOS (eNOS) overexpression increased VEGF production (a key angiogenic protein), and other investigators noted that eNOS upregulation facilitates increased NO production as a result (18, 19). Thus, a paracrine loop exists between the endothelial cells (where NO is formed) and vascular smooth muscles cells (where VEGF is produced).

Angiostatin is a well described and potent inhibitor of angiogenesis. Koshida *et al* demonstrated that angiostatin directly impairs endothelium-dependent vasodilation, thereby providing at least one explanation why angiostatin is anti-angiogenic (20). Troyanovsky *et al* demonstrated that angiostatin is inhibited in the presence of angiostatin (21). Angiostatin binds to the angiostatin-binding domain of angiostatin (22), whereas angiostatin exerts its effects through its PDZ-binding domain located at the C-terminal. Minor deletions in this region have been shown to disable angiostatin. Binding of angiostatin to the angiostatin-binding domain may inhibit the PDZ-binding domain of angiostatin (23). Angiostatin is essential for endothelial cell migration (24) and regulates cell-cell junctions (22). These functions are important for new blood vessel development and growth. The current results demonstrated that Tsk^{-/+} mice exhibit increased angiostatin and reduced angiostatin levels in the ischemic hindlimb model that was associated with impaired development of collateral vessels. LLLT treatment increased angiostatin expression and reduced angiostatin expression in the ischemic limb of Tsk^{-/+} mice. This shift in the balance between pro- and anti-angiogenic proteins by LLLT exposure most likely encouraged the development of new collateral blood vessels observed in our experiments.

The current results must be interpreted within the constraints of several potential limitations. We did not specifically measure the direct effects of LLLT on oxidative stress, but we previously demonstrated that Tsk^{-/+} mouse model has increased levels of oxidative stress under baseline conditions (8). Fitzgerald *et al* established that exposure to far red and near infrared light decreases damage caused by oxidative stress *in vivo* (25). Thus, it is reasonable to assume that repetitive LLLT treatment also reduced oxidative stress in Tsk^{-/+} mice. Far red and near infrared light exposure has been shown to increase NO (4, 10) and decrease ROS production (26), but we did not measure NO and ROS concentrations or the activities of enzymes that produce these molecules in the current investigation. The precise mechanism by which LLLT affects angiostatin expression is unknown. Angiostatin has been shown to facilitate pathological angiogenesis. Arigoni (27) and Levchenko (28) demonstrated the use of anti-angiostatin antibodies to reduce the spread of invasive cancers. Whether LLLT-induced release of NO affects angiostatin expression or activity to favorably enhance vascular development remains to be determined; further investigation will be necessary to define the therapeutic threshold and mechanism of action of LLLT on angiostatin in models of ischemic injury. Finally, we did not examine the effects of LLLT on blood vessel development and angiostatin/angiostatin expression in normal (C57BL/6) mice. We were interested in the potential actions of LLLT on vascular development to

ischemic stress in a known model of reduced vascularity, and focused our attention on the Tsk^{-/+} mouse model of scleroderma as a result.

In summary, the current results indicate that transient, repetitive exposure to LLLT for two weeks after femoral artery ligation is capable of restoring normal compensatory vascular development after an irreversible ischemic insult in a murine model of systemic scleroderma. These findings occurred, at least in part, as a consequence of attenuated production of the anti-angiogenic protein angiostatin concomitant with enhanced production of angiominin *in vivo*.

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References

1. Bozkurt A, Onaral B. Safety assessment of near infrared light emitting diodes for diffuse optical measurements. *Biomed Eng Online*. 2004; 3(1):9. [PubMed: 15035670]
2. Stadler I, Lanzafame RJ, Oskoui P, Zhang RY, Coleman J, Whittaker M. Alteration of skin temperature during low-level laser irradiation at 830 nm in a mouse model. *Photomed Laser Surg*. 2004; 22(3):227–31. [PubMed: 15315730]
3. Hamblin MR, Demidova TN. Mechanisms of low level of light therapy. *Biomedical Optics*. 2006; 2006:614001–12.
4. Lohr NL, Keszler A, Pratt P, Bienengraber M, Warltier DC, Hogg N. Enhancement of nitric oxide release from nitrosyl hemoglobin and nitrosyl myoglobin by red/near infrared radiation: potential role in cardioprotection. *J Mol Cell Cardiol*. 2009; 47(2):256–63. [PubMed: 19328206]
5. Bibikova A, Belkin V, Oron U. Enhancement of angiogenesis in regenerating gastrocnemius muscle of the toad (*Bufo viridis*) by low-energy laser irradiation. *Anat Embryol (Berl)*. 1994; 190(6):597–602. [PubMed: 7534455]
6. Ad N, Oron U. Impact of low level laser irradiation on infarct size in the rat following myocardial infarction. *Int J Cardiol*. 2001; 80(2–3):109–16. [PubMed: 11578700]
7. Borutaite V, Budriunaite A, Brown GC. Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols. *Biochim Biophys Acta – Bioenergetics*. 2000; 1459(2–3):405–412.
8. Weihrauch D, Xu H, Shi Y, Wang J, Brien J, Jones DW, Kaul S, Komorowski RA, Csuka ME, Oldham KT, Pritchard KA. Effects of D-4F on vasodilation, oxidative stress, angiostatin, myocardial inflammation, and angiogenic potential in tight-skin mice. *Am J Physiol Heart Circ Physiol*. 2007; 293(3):H1432–41. [PubMed: 17496220]
9. Xu H, Zaidi M, Struve J, Jones DW, Krolikowski JG, Nandedkar S, Lohr NL, Gadicherla A, Pagel PS, Csuka ME, Pritchard KA, Weihrauch D. Abnormal fibrillin-1 expression and chronic oxidative stress mediate endothelial mesenchymal transition in a murine model of systemic sclerosis. *Am J Physiol Cell Physiol*. 2011; 300(3):C550–6. [PubMed: 21160034]
10. Zhang R, Mio Y, Pratt PF, Lohr NL, Warltier DC, Whelan HT, Zhu D, Jacobs ER, Medhora M, Bienengraeber M. Near infrared light protects cardiomyocytes from hypoxia and reoxygenation injury by a nitric oxide dependent mechanism. *J Mol Cell Cardiol*. 2008; 46:4–14. [PubMed: 18930064]
11. Rengasamy A, Johns RA. Determination of Km for oxygen of nitric oxide synthase isoforms. *J Pharmacol Exp Ther*. 1996; 276(1):30–3. [PubMed: 8558447]
12. Nagababu E, Ramasamy S, Abernethy DR, Rifkin JM. Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. *Biol Chem*. 2003; 278(47):46349–56.
13. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Wacławski MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, Gladwin

- MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med*. 2003; 9(12):1498–505. [PubMed: 14595407]
14. Castello PR, David PS, McClure T, Crook Z, Poyton RO. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab*. 2006; 3(4):277–87. [PubMed: 16581005]
 15. Ball KA, Castello PR, Poyton RO. Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: Implications for phototherapy. *Journal of Photochemistry and Photobiology B: Biology*. 2011; 102(3):182–91.
 16. Poyton RO, Ball KA. Therapeutic photobiomodulation: Nitric oxide and a novel function of mitochondrial cytochrome c oxidase. *Discov Med*. 2011 Feb; 11(57):154–9. [PubMed: 21356170]
 17. Namba T, Koike H, Murakami K, Aoki M, Makino H, Hashiya N, Ogihara T, Kaneda Y, Kohno M, Morishita R. Angiogenesis induced by endothelial nitric oxide synthase gene through vascular endothelial growth factor expression in a rat hindlimb ischemia model. *Circulation*. 2003; 108(18):2250–7. [PubMed: 14568906]
 18. Jozkowicz A, Cooke JP, Guevara I, Huk I, Funovics P, Pachinger O, Weidinger F, Dulak J. Genetic augmentation of nitric oxide synthase increases the vascular generation of VEGF. *Cardiovasc Res*. 2001; 51(4):773–83. [PubMed: 11530111]
 19. Ankoma-Sey V, Wang Y, Dai Z. Hypoxic stimulation of vascular endothelial growth factor expression in activated rat hepatic stellate cells. *Hepatology*. 2000; 31(1):141–8. [PubMed: 10613739]
 20. Koshida R, Ou J, Matsunaga T, Chilian WM, Oldham KT, Ackerman AW, Pritchard KA. Angiostatin: a negative regulator of endothelial-dependent vasodilation. *Circulation*. 2003; 107:803–6. [PubMed: 12591747]
 21. Troyanovsky B, Levchenko T, Mansson G, Matvienko O, Holmgren L. Angiomotin: an angiostatin binding protein that regulates endothelial cell migration and tube formation. *Cell Biol*. 2001; 152(6):1247–54.
 22. Bratt A, Birot O, Sinha I, Veitonmäki N, Aase K, Ernkvist M, Holmgren L. Angiomotin regulates endothelial cell-cell junctions and cell motility. *J Biol Chem*. 2005; 280(41):34859–69. [PubMed: 16043488]
 23. Levchenko T, Aase K, Troyanovsky B, Bratt A, Holmgren L. Loss of responsiveness to chemotactic factors by deletion of the C-terminal protein interaction site of angiomotin. *J Cell Sci*. 2003; 116:3803–10. [PubMed: 12902404]
 24. Ernkvist M, Luna Persson N, Audebert S, Lecine P, Sinha I, Liu M, Schlueter M, Horowitz A, Aase K, Weide T, Borg JP, Majumdar A, Holmgren L. The Amot/Patj/Syx signaling complex spatially controls RhoA GTPase activity in migrating endothelial cells. *Blood*. 2009; 113(1):244–53. [PubMed: 18824598]
 25. Fitzgerald M, Bartlett CA, Payne SC, Hart NS, Rodgers J, Harvey AR, Dunlop SA. Near Infrared Light Reduces Oxidative Stress and Preserves function in CNS Tissue Vulnerable to Secondary Degeneration following Partial Transfection of the Optic Nerve. *J Neurotrauma*. 2010; 27(11):2107–19. [PubMed: 20822460]
 26. Liang HL, Whelan HT, Eells JT, Wong-Riley MTT. Near-infrared light via light-emitting diode treatment is therapeutic against rotenone- and 1-methyl-4-phenylpyridium ion-induced neurotoxicity. *Neuroscience*. 2008; 153:963–974. [PubMed: 18440709]
 27. Arigoni M, Barutello G, Lanzardo S, Longo D, Aime S, Curcio C, Iezzi M, Zheng Y, Barkefors I, Holmgren L, Cavallo F. A vaccine targeting angiomotin induces an antibody response which alters tumor vessel permeability and hampers the growth of established tumors. *Angiogenesis*. 2012; 15(2):305–16. [PubMed: 22426512]
 28. Levchenko T, Veitonmäki N, Lundkvist A, Gerhardt H, Ming Y, Berggren K, Kvanta A, Carlsson R, Holmgren L. Therapeutic antibodies targeting angiomotin inhibit angiogenesis in vivo. *FASEB J*. 2008; 22:880–9. [PubMed: 17984175]

Figure 1.

A) Histograms illustrating blood flow in the ischemic hindlimb of C57Bl/6J mice and Tsk^{-/+} mice with and without NIR exposure measured using Laser Doppler imaging. Blue depicts low or no blood flow, red depicts high blood flow. B) Collateral growth was robust in C57Bl/6J and Tsk^{-/+} mice treated with NIR after 14 days, but collateral development was less pronounced in Tsk^{-/+} mice in the absence of NIR.

Figure 2.

Histograms depicting vascular density measured using lycopersicon lectin staining 14 days after femoral artery ligation in C57Bl/6J mice and Tsk^{-/+} in the absence and presence NIR exposure. *Significantly ($P < 0.05$) different from C57Bl/6J mice; †significantly ($P < 0.05$) different from Tsk^{-/+} mice without NIR treatment.

Figure 3.

Representative immunofluorescence examples of angiostatin and angiomotin expression in the ischemic (ischemic limb; IL) and normal (control limb; CL) hindlimb of C57Bl/6J mice and Tsk^{-/+} mice with and without NIR exposure (top panels). Histograms illustrating the ratio of angiostatin to angiomotin appear in the bottom panel. Note that the ratio of angiostatin to angiomotin is greater in Tsk^{-/+} mice without NIR treatment compared with C57Bl/6J mice and Tsk^{-/+} mice with NIR treatment.

Figure 4. Representative western blots (top panel) on protein lysates from gastrocnemius muscle and histograms (bottom panel) illustrating that angiostatin expression is enhanced ($P<0.05$) in Tsk^{-/-} mice without NIR treatment compared with C57Bl/6J mice and Tsk^{-/-} mice with NIR treatment.

Figure 5. Immunoprecipitation for angiotensin and immunoblotting for angiotensinogen on protein lysates from gastrocnemius muscle demonstrating that less angiotensin was bound to angiotensin in Tsk^{-/-} mice without NIR treatment compared with C57Bl/6J mice and Tsk^{-/-} mice with NIR treatment.