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# Impaired arteriogenesis in syndecan-1$^{-/-}$ mice

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ABSTRACT

*Background:* Collateral artery development (arteriogenesis) is an important compensatory response to arterial occlusion caused by atherosclerosis. The heparan sulfate proteoglycan syndecan-1 (sdc1) has previously been shown to affect the response to arterial injury but has yet been studied in arteriogenesis. We tested the hypothesis that sdc1 knockout (sdc1$^{-/-}$) mice would revascularize more poorly than wild type (wt) mice, and then used bone marrow transplantation experiments to determine whether sdc1's effect on arteriogenesis was due to its presence in the local tissue environment or in bone marrow derived cells.

*Materials and methods:* Hindlimb ischemia was induced by femoral artery ligation in wt and sdc1$^{-/-}$ female mice as well as in wt and sdc1$^{-/-}$ female mice transplanted with wt bone marrow or in wt mice transplanted with sdc1$^{-/-}$ bone marrow. Blood flow recovery was assessed by laser Doppler perfusion imaging. Arteriogenesis was assessed by measuring the diameter of the dominant collateral pathway after pressure perfusion fixation and intra-aortic contrast injection at 28 d. Immunohistochemistry was used to assess angiogenesis and peri-collateral macrophage infiltration at 7 d, postoperatively.

*Results:* Sdc1$^{-/-}$ mice had impaired blood flow recovery in response to hindlimb ischemia. This impaired recovery was not secondary to a defect in capillary angiogenesis nor was it due to decreased peri-collateral macrophage infiltration. Wt bone marrow did not rescue the impaired recovery of sdc1$^{-/-}$ mice.

*Conclusions:* Sdc1 affects arteriogenesis in response to hindlimb ischemia and is required in the local tissue environment for normal arteriogenesis.

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## 1. Introduction

Collateral artery development (arteriogenesis) is the normal physiologic adaptation to occlusion of main highway arteries. Arteriogenesis plays an important role in the recovery from ischemic insults such as coronary artery occlusion, and in patients with peripheral arterial disease. Unfortunately, even well-developed collateral arteries generally only carry approximately 30% of the flow originally carried by the occluded artery [1,2]. Gaining a better understanding of the biological factors that influence arteriogenesis will help design new treatments for patients with peripheral arterial disease and chronic arterial occlusions and stenoses.

Syndecans are transmembrane heparan sulfate proteoglycans that serve as co-receptors for growth factors by binding extracellular growth factors and facilitating interaction with their cognate receptors [3]. Heparan sulfate proteoglycans, furthermore, interact with the extracellular

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matrix and integrins to promote focal adhesion formation, as well as to allow the integrins to interact with key extracellular matrix molecules such as fibronectin and collagen [4,5]. There are four mammalian syndecans, syndecan-1, 2, 3, and 4, which are differentially expressed in various tissues in a cell-type and developmentally specific manner [6].

Syndecan 1 (sdc1) is of particular interest for arterial remodeling, as it has previously been demonstrated to have a role in angiogenesis [3] and in the response to arterial injury. Fukai *et al.* demonstrated upregulation of sdc1 within ligated carotid arteries and increased intimal hyperplasia in ligated carotid arteries of sdc1$^{-/-}$ mice, suggesting a role for sdc1 in suppressing smooth muscle cell proliferation [7]. Sdc1 is also the dominant syndecan expressed in monocytes [8,9], which plays an important role in stimulating arteriogenesis [10–13]. Macrophages expressing sdc1 accumulate in both human aortic aneurysms and a mouse model of angiotensin-induced aortic aneurysm [14]. Sdc1 has also been shown to be a co-receptor for fibroblast growth factor (FGF), a growth factor critical for arteriogenesis [15–17].

We wanted to test the hypothesis that sdc1 is required for arteriogenesis, and furthermore whether its role in arteriogenesis was due to its effects on monocytes derived from the bone marrow or its effects on the local tissue environment, possibly through expression in vascular smooth muscle cells. To do this, we measured blood flow recovery and collateral artery development in response to hindlimb ischemia in female mice lacking sdc1 and compared them to wild type (wt) mice. We then performed bone marrow transplantation to isolate the role of sdc1 in circulating blood cells *versus* vascular wall cells.

## 2. Methods

### 2.1. Animals

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committees of the University of Washington and the VA Puget Sound Health Care System. Age-matched female progeny, 3–5 mo old (wt, sdc1$^{+/+}$) and sdc1 knockout (sdc1$^{-/-}$), from homozygotes derived from sdc1$^{\pm}$breeding pairs (previously backcrossed nine generations to C57BL/6 and obtained from Dr Alexander Clowes) were used for all experiments except the bone marrow transplantation experiments. For bone marrow transplantation experiments, C57BL/6 mice from a colony derived from Jackson Laboratories (Bar Harbor, ME) were used. All mice were maintained under standard conditions 12 hour-light–dark cycles, with access to chow and water *ad libitum*. Only female mice were used as they collateralize less well than male mice in response to hindlimb ischemia [18].

### 2.2. Hindlimb ischemia

Unilateral femoral artery ligation was performed by isolating the left femoral artery away from the femoral vein and nerve under isoflurane anesthesia. Mice were premedicated with 0.05 mg/kg of buprenorphine for analgesia. The artery was ligated with 6-0 silk suture just distal to the superficial epigastric artery. Each mouse, therefore, had an ischemic (left) hindlimb, and a control (right) unoperated hindlimb.

### 2.3. Laser Doppler perfusion imaging

Laser Doppler perfusion imaging was carried out immediately postoperatively, and on postoperative days 7, 14, 21, and 28. Mice were allowed to equilibrate to 37°C while lying prone on a homeothermic warming blanket under 1.5% isoflurane anesthesia and 1 L/min $O_2$. The footpads of both hindlimbs were scanned using a MoorLDI2 Laser Doppler perfusion imager (Moor Instruments, Wilmington, DE). Values are expressed as a ratio of the ischemic (left) footpad divided by the control (right).

### 2.4. Pressure perfusion fixation

A laparotomy incision was made under 2% isoflurane anesthesia with 1 L/min $O_2$. A 22-gauge silicon catheter was inserted in the infrarenal aorta. After a nick was made in the inferior vena cava to allow outflow, 10 mL of vasodilator solution (1 mM nitroprusside, 10 mM adenosine, and 2.5 U/mL heparin in saline) was infused through the infrarenal aorta. The mouse was then pressure perfusion fixed using methyl Carnoy solution at 100 mm Hg pressure. After fixation, contrast (60% barium sulfate, liquid EZ-paque or Microfil) was infused until the hindlimb arteries were filled with contrast. The hindlimb was then removed. Gross vessel diameters were documented, and then the hindlimbs were immersion fixed in methyl Carnoy overnight, dehydrated in EtOH, and submitted for decalcification and paraffin processing.

### 2.5. Gross vessel diameters

As the gracilis collateral pathway is one of the dominant collateral pathways following femoral artery ligation and is immediately visible after removing the skin [19], we chose to measure gracilis collateral diameters after pressure perfusion fixation to assess collateral enlargement in reaction to hindlimb ischemia. Digital pictures were taken of the gracilis collaterals with a millimeter ruler included within the picture. Vessel diameters of the two largest gracilis collaterals in each hindlimb were measured using Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA) using the internal ruler for calibration, and then averaged to provide a gracilis collateral diameter for the control and ischemic hindlimbs. The values for both legs were averaged together for the unoperated mice.

### 2.6. Immunohistochemistry

To identify capillaries, wheat germ agglutinin (WGA) lectin staining was carried out on methyl Carnoy fixed 10 μm paraffin sections of muscle harvested from the thigh (adductor and quadriceps) and the calf (tibialis anterior and gastrocnemius) of sdc1$^{+/+}$ and sdc1$^{-/-}$ mice 7 d after induction of hindlimb ischemia. Slides were counterstained with eosin to identify muscle fibers. To identify macrophages, we performed anti-Mac2 staining (primary antibody anti-Mac2 1:100, generously provided from Elaine Raines) with a biotinylated secondary antibody followed by ABC amplification (Vector Laboratories, Burlingame, CA) followed by 3,3′-diaminobenzidine staining.

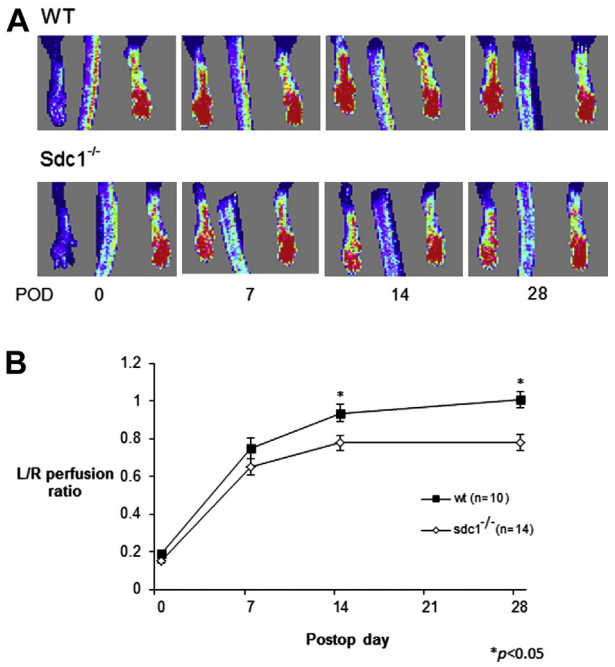


**Fig. 1 – In response to hindlimb ischemia induced by left femoral artery ligation, sdc1 knockout (sdc1$^{-/-}$) mice had poorer recovery of blood flow perfusion to the footpad than wt mice as measured by laser Doppler perfusion imaging over 28 d after ligation. (A) Representative laser Doppler perfusion images over 28 d. POD, postoperative day. (B) Graphical representation of averaged ratios. Values are expressed as a ratio of the left (ischemic) over the right (control) footpad flux. (Color version of the figure is available online.)**

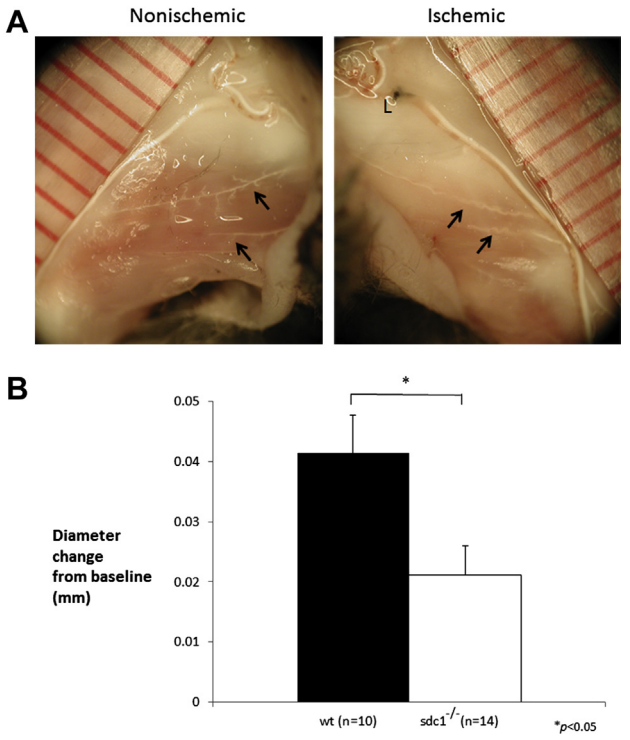


**Fig. 2 – Sdc1$^{-/-}$ mice did not increase their gracilis collateral diameter as much as wt mice. Gracilis collateral diameter was measured after pressure perfusion fixation and administration of intra-arterial contrast using a catheter in the infrarenal aorta. (A) Representative photomicrographs from the right (nonischemic hindlimb) and left (ischemic hindlimb) showing the contrast within the gracilis collaterals. The right hindlimb had better penetration of contrast into the branches. The left hindlimbs collaterals show the expected increase in diameter and tortuosity after femoral artery ligation. Arrows indicated the measured collaterals. L marks the location of the silk ligature. (B) Graphical representation of the change in diameter from baseline after 28 d. (Color version of the figure is available online.)**

### 2.7. *Capillary and/or muscle fiber ratios*

Digital pictures at ×40 magnification were captured of five random sections stained with WGA lectin and/or eosin: control and ischemic tibialis anterior, gastrocnemius, adductor, and quadriceps of sdc1$^{-/-}$ and sdc1$^{+/+}$ mice. Muscle fibers and capillaries were quantified by three blinded observers. The averaged ratios were compared between groups.

### 2.8. *Bone marrow transplantation*

Bone marrow cells were collected from the tibia and femur of sdc1$^{+/+}$ and sdc1$^{-/-}$ mice. Recipient mice were pretreated with antibiotic for 2 d (enrofloxacin) and then sublethally irradiated with 950 rad. They were then given $1 \times 10^6$ cells intravenously through the tail vein and allowed to engraft over 4 wk while the antibiotics were continued. Hindlimb ischemia was induced 4–6 wk after bone marrow transplantation. Only mice that had successful engraftment of the donor bone marrow survived and were thus studied.

### 2.9. *Statistics*

All values are expressed as mean ± standard error of the mean. The Student *t*-test was used to test the difference between the two groups for the laser Doppler perfusion imaging. Analysis of variance was used to test the difference between multiple groups for the capillary and/or muscle fiber ratio, gross artery diameters, and peri-collateral macrophage counts. *Post hoc* analysis was carried out using Newman–Keuls. GraphPad Prism was used for all statistical analysis. $P < 0.05$ was considered significant.

## 3. Results

Figure 1 shows that mice lacking in sdc1 (sdc1$^{-/-}$, $n = 14$) showed impaired perfusion recovery compared with wt mice ($n = 10$) at 28 d after femoral artery ligation as measured by laser Doppler perfusion imaging (L/R ratio 0.80 ± 0.043 *versus* 0.97 ± 0.054, $P = 0.013$). The difference in perfusion recovery could not be attributed to capillary angiogenesis. There was no difference between the capillary and/or muscle fiber ratios

**Table – Perivascular macrophage counts 1 wk after hindlimb ischemia.**

| Mouse genotype | Thigh | Mid-collateral | Calf |
|---|---|---|---|
| $Sdc1^{+/+}$ | 2.0 ± 0.4 | 6.1 ± 1.3 | 5.8 ± 0.9 |
| $Sdc1^{-/-}$ | 1.4 ± 0.4 | 6.5 ± 1.3 | 5.3 ± 1.0 |

of the $sdc1^{+/+}$ ($n = 4$) and the $sdc1^{-/-}$ mice ($n = 3$) in either the thigh (quadriceps and adductor) or calf muscles (tibialis anterior and gastrocnemius muscles) at day 7 ($sdc1^{+/+}$ 1.45 ± 0.06, 1.3 ± 0.05, 1.6 ± 0.2, and 1.3 ± 0.05 *versus* $sdc1^{-/-}$ 1.6 ± 0.06, 1.25 ± 0.03, 1.8 ± 0.2, and 1.3 ± 0.1 for quadriceps, adductor, tibialis anterior, and gastrocnemius, respectively, all $P > 0.05$).

Recovery from hindlimb ischemia depends in part on the size and number of baseline collaterals, which varies according to the mouse strain [20]. To confirm that $sdc1^{-/-}$ mice had the same number of baseline gracilis collaterals as $sdc1^{+/+}$ mice, we also pressure perfusion fixed and injected arterial contrast into five mice of each genotype that had not undergone hindlimb ischemia. The number of baseline gracilis collaterals was not different between $sdc1^{-/-}$ mice and $sdc1^{+/+}$ (data not shown). However, the baseline gracilis collateral diameter was larger for the $sdc1^{-/-}$ mice than the $sdc1^{+/+}$ mice (0.065 ± 0.0039 mm *versus* 0.051 ± 0.0050 mm, $P = 0.007$). Although the $sdc1^{-/-}$ mice began at baseline with larger gracilis collaterals than the $sdc1^{+/+}$ mice, at 28 d after femoral artery ligation, this difference was gone. In fact, the increase in the size of collaterals was significantly larger for the $sdc1^{+/+}$ mice than the $sdc1^{-/-}$ mice, as shown in Figure 2.

Macrophages begin to accumulate in the perivascular space starting at 3 d after hindlimb ischemia and remain elevated out to 28 d [21]. Because sdc1 is the predominant syndecan expressed in monocytes, and the loss of sdc1 may affect the mobility or migration of monocytes into the periphery from the circulation, we assessed peri-collateral macrophages of the five largest collaterals at three different levels (thigh, mid-collateral, and calf) in $sdc1^{+/+}$ ($n = 4$) and $sdc1^{-/-}$ ($n = 3$) mice at 7 d after induction of hindlimb ischemia. Peri-collateral macrophage counts were not different between $sdc1^{+/+}$ and $sdc1^{-/-}$ mice at any level (Table).

After arterial injury, others have observed sdc1 expression in vascular smooth muscle cells [22]. We also detected sdc1 staining in arterial smooth muscle cells in some collateral arteries at 7 d after induction of hindlimb ischemia in the $sdc1^{+/+}$ mice (Fig. 3A). Sdc1 is not normally detectable in the arterial wall, as confirmed by a lack of staining in baseline mice and in the control hindlimb (Fig. 3B).

Mice lacking sdc1 showed impaired recovery of perfusion, less collateral enlargement, but the same number of macrophages in the peri-collateral space. To more precisely define the respective roles of circulating cells *versus* the local vascular tissues in collateral artery development, we performed bone marrow transplantation of $sdc1^{-/-}$ bone marrow into C57BL/6 mice and C57BL/6 bone marrow into $sdc1^{-/-}$ mice. The reciprocal transplantation of $sdc1^{+/+}$ bone marrow into C57BL/6 mice and into $sdc1^{-/-}$ mice was also performed. Wt bone marrow did not rescue the deficit in revascularization seen in $sdc1^{-/-}$ mice. $Sdc1^{-/-}$ bone marrow did not cause C57BL/6 mice to reperfuse more poorly than wt bone marrow. Figure 4 shows that at 14 d after femoral artery ligation, wt mice transplanted with wt bone marrow had the highest perfusion ratios, and $sdc^{-/-}$ mice transplanted with wt bone marrow had the poorest perfusion.

## 4. Discussion

Mice deficient in sdc1 revascularize more poorly than wt mice. This deficit is apparently due to poor collateral artery development (arteriogenesis), as $sdc1^{-/-}$ mice did not enlarge their collateral arteries as much as wt mice in response to ischemia. It is not a deficit in capillary development (angiogenesis) as the capillary muscle fiber ratios after ischemia did not differ between wt and $sdc1^{-/-}$ mice. Furthermore, sdc1 appears to be required in the local tissue environment to enhance arteriogenesis rather than being required in cells derived from the bone marrow.

In the quiescent, healthy state of the artery, sdc1 stabilizes or inhibits vascular smooth muscle cell (VSMC) proliferation. However, on injury, sdc1 is upregulated and may contribute to growth factor binding and VSMC proliferation [19,20]. Confirming this, we found positive VSMC staining for sdc1 in the

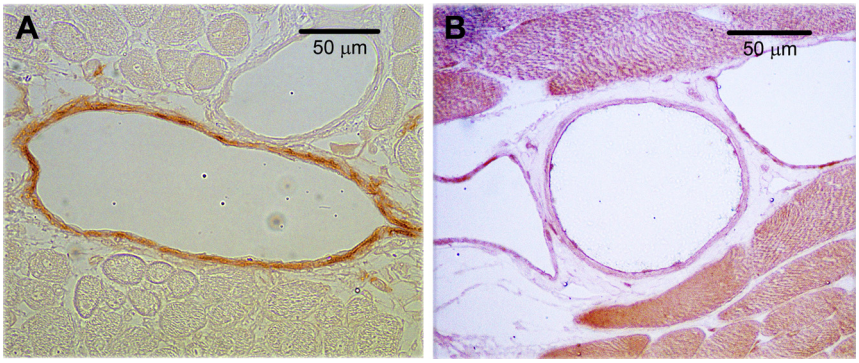


**Fig. 3 – (A) Collateral artery stained with anti-sdc1 shows staining in the medial layer of the collateral artery. (B) Control section from the right (nonischemic leg) shows no anti-sdc1 staining in the central artery or paired accompanying veins. (Color version of the figure is available online.)**

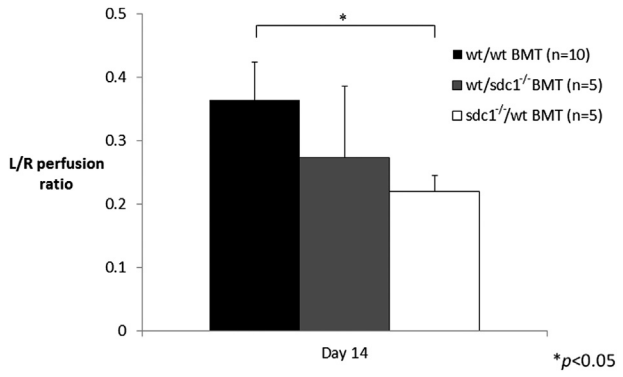


**Fig. 4 – Sdc1$^{-/-}$ mice transplanted with wt bone marrow (sdc1$^{-/-}$/wt bone marrow transplantation) had significantly worse perfusion at 14 days after femoral artery ligation than wt mice transplanted with wt bone marrow (wt/wt bone marrow transplantation). Wt mice transplanted with sdc1$^{-/-}$ bone marrow (wt/sdc1$^{-/-}$ bone marrow transplantation) had intermediate perfusion, which was not significantly different from the wt/wt bone marrow transplantation group.**

injured wt mice. The sdc1 knockouts showed impaired collateral artery development, supporting the idea that upregulation of sdc1 is important for collateral enlargement.

The mechanisms of sdc1's promotion of collateral development are likely multiple, including the promotion of cell-growth factor interaction, binding cell adhesion receptors, and facilitating cell-matrix interactions. Sdc1 is a co-receptor for FGF [13,23]. FGF is a known stimulant of collateral artery development and is upregulated after hindlimb ischemia [16,17]. However, FGF is not the whole story, because FGF2$^{-/-}$ mice do not show impaired blood flow recovery after induction of hindlimb ischemia [24]. And despite sdc1's known role in angiogenesis through its interactions with the $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins [25], we did not observe a deficit in capillary angiogenesis in response to hindlimb ischemia in sdc1$^{-/-}$ mice, suggesting that sdc-1 is not required for capillary angiogenesis in response to hindlimb ischemia.

Although sdc1 is the primary syndecan expressed in macrophages and monocytes [8,9], we did not observe a difference in recruitment of peri-collateral macrophages between sdc1 knockout and wt mice, and transplantation of wild-type circulating monocytes did not restore the normal reperfusion phenotype. This suggests that sdc1 is not required for monocytes and/or macrophages to home the peri-collateral space during collateral development. This is contrary to prior experiments, which showed that sdc1$^{-/-}$ mice had increased inflammatory cell-endothelial cell interaction [26]. Our findings are also supported by the bone marrow transplantation experiments, which suggest that sdc1 is required in the local vascular tissue environment rather than in the bone marrow for normal collateral development after hindlimb ischemia.

Having demonstrated the importance of this key heparin sulfate proteoglycan in the recovery from ischemia, more work will be needed to discriminate its individual effects via growth factor binding, cell signaling, and adhesion receptors. Although the bone marrow transplantation experiments demonstrate that monocytes from the bone marrow are not required for sdc1's effect on arteriogenesis, this does not rule out a contribution by local resident macrophages. The number of peri-collateral macrophages was not different between sdc1$^{-/-}$ and wt mice, but we did not assess whether there was a difference in the macrophage subtypes between the two groups. Macrophage subtypes likely affect arteriogenesis differently with the M2 resident macrophages likely to be proarteriogenic [27,28].

## 5. Conclusions

Sdc1 has a role in arteriogenesis, which is most likely mediated through induction of its expression in vascular smooth muscle in response to hindlimb ischemia.

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## Disclosure

The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans Affairs or the United States Government.

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## REFERENCES

[1] Schaper W, Flameng W, Winkler B, et al. Quantification of collateral resistance in acute and chronic experimental coronary occlusion in the dog. Circ Res 1976;39:371.
[2] Helisch A, Wagner S, Khan N, et al. Impact of mouse strain differences in innate hindlimb collateral vasculature. Arterioscler Thromb Vasc Biol 2006;26:520.
[3] Beauvais DM, Ell BJ, McWhorter AR, Rapraeger AC. Syndecan-1 regulates alphavbeta3 and alphavbeta5 integrin activation during angiogenesis and is blocked by synstatin, a novel peptide inhibitor. J Exp Med 2009;206:691.
[4] Stepp MA, Daley WP, Bernstein AM, et al. Syndecan-1 regulates cell migration and fibronectin fibril assembly. Exp Cell Res 2010;316:2322.
[5] Vuoriluoto K, Jokinen J, Kallio K, Salmivirta M, Heino J, Ivaska J. Syndecan-1 supports integrin alpha2beta1-mediated adhesion to collagen. Exp Cell Res 2008;314:3369.
[6] Tkachenko E, Rhodes JM, Simons M. Syndecans: new kids on the signaling block. Circ Res 2005;96:488.
[7] Collateral circulation: heart, brain, kidney, limbs. Boston: Kluwer Academic Publishers; 1993.

[8] Wang W, Haller CA, Wen J, Wang P, Chaikof EL. Decoupled syndecan 1 mRNA and protein expression is differentially regulated by angiotensin II in macrophages. J Cell Physiol 2008;214:750.
[9] Yeaman C, Rapraeger AC. Post-transcriptional regulation of syndecan-1 expression by camp in peritoneal macrophages. J Cell Biol 1993;122:941.
[10] Bergmann CE, Hoefer IE, Meder B, et al. Arteriogenesis depends on circulating monocytes and macrophage accumulation and is severely depressed in op/op mice. J Leukoc Biol 2006;80:59.
[11] Capoccia BJ, Gregory AD, Link DC. Recruitment of the inflammatory subset of monocytes to sites of ischemia induces angiogenesis in a monocyte chemoattractant protein-1-dependent fashion. J Leukoc Biol 2008; 84:760.
[12] Schaper W, Scholz D. Factors regulating arteriogenesis. Arterioscler Thromb Vasc Biol 2003;23:1143.
[13] Heil M, Ziegelhoeffer T, Pipp F, et al. Blood monocyte concentration is critical for enhancement of collateral artery growth. Am J Physiol Heart Circ Physiol 2002;283: H2411.
[14] Wen J, Wang P, Smith SV, Haller CA, Chaikof EL. Syndecans are differentially expressed during the course of aortic aneurysm formation. J Vasc Surg 2007;46:1014.
[15] Filla MS, Dam P, Rapraeger AC. The cell surface proteoglycan syndecan-1 mediates fibroblast growth factor-2 binding and activity. J Cell Physiol 1998;174:310.
[16] Baffour R, Berman J, Garb JL, Rhee SW, Kaufman J, Friedmann P. Enhanced angiogenesis and growth of collaterals by in vivo administration of recombinant basic fibroblast growth factor in a rabbit model of acute lower limb ischemia: dose-response effect of basic fibroblast growth factor. J Vasc Surg 1992;16:181.
[17] Unger EF, Banai S, Shou M, et al. Basic fibroblast growth factor enhances myocardial collateral flow in a canine model. Am J Physiol 1994;266:H1588.
[18] Peng X, Wang J, Lassance-Soares RM, et al. Gender differences affect blood flow recovery in a mouse model of hindlimb ischemia. Am J Physiol Heart circulatory Physiol 2011;300:H2027.
[19] Unthank JL, Sheridan KM, Dalsing MC. Collateral growth in the peripheral circulation: a review. Vasc Endovascular Surg 2004;38:291.
[20] Chalothorn D, Faber JE. Strain-dependent variation in collateral circulatory function in mouse hindlimb. Physiol Genomics 2010;42:469.
[21] Troidl C, Jung G, Troidl K, et al. The temporal and spatial distribution of macrophage subpopulations during arteriogenesis. Curr Vasc Pharmacol 2013;11:5.
[22] Fukai N, Kenagy RD, Chen L, Gao L, Daum G, Clowes AW. Syndecan-1: an inhibitor of arterial smooth muscle cell growth and intimal hyperplasia. Arterioscler Thromb Vasc Biol 2009;29:1356.
[23] Zong F, Fthenou E, Wolmer N, et al. Syndecan-1 and FGF-2, but not FGF receptor-1, share a common transport route and co-localize with heparanase in the nuclei of mesenchymal tumor cells. PLoS One 2009;4:e7346.
[24] Sullivan CJ, Doetschman T, Hoying JB. Targeted disruption of the FGF2 gene does not affect vascular growth in the mouse ischemic hindlimb. J Appl Physiol 2002;93:2009.
[25] Rapraeger AC. Synstatin: a selective inhibitor of the syndecan-1-coupled IGF1R-αvβ3 integrin complex in tumorigenesis and angiogenesis. FEBS J 2013;280:2207.
[26] Gotte M, Bernfield M, Joussen AM. Increased leukocyte-endothelial interactions in syndecan-1-deficient mice involve heparan sulfate-dependent and -independent steps. Curr Eye Res 2005;30:417.
[27] Takeda Y, Costa S, Delamarre E, et al. Macrophage skewing by phd2 haplodeficiency prevents ischaemia by inducing arteriogenesis. Nature 2011;479:122.
[28] Jetten N, Donners MM, Wagenaar A, et al. Local delivery of polarized macrophages improves reperfusion recovery in a mouse hind limb ischemia model. PLoS One 2013;8:e68811.