**Adipose-derived stem cell-derived microvesicle-released miR-210 promoted angiogenesis of endothelial cells by regulating RUNX3**

**Running title: MVs-released miR-210 modulates angiogenesis via RUNX3**

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**Abstract**

Objective：To explore the potential mechanism of miRNA released from adipose-derived stem cell (ADSC)-derived micro vesicle (MV) on the modulation of angiogenesis.

Materials and methods: miR-210 level was detected by qT-PCR. Alix, VEGF and RUNX3 expressions were detected by Western blot. The proliferation, migration and invasion of human umbilical vein endothelial cells (HUVECs) were observed by MTT assay and Transwell assay. Luciferase reporter gene assay was conducted to validate the targeting activity of MVs-released miR-210 on RUNX3.

Results: Hypoxia significantly increased the expression of MVs-released miR-210. MVs released from ADSCs in hypoxic group significantly promoted the proliferation, migration and invasion of HUVECs. Overexpression of miR-210 significantly upregulated VEGF expression, and promoted the proliferation, migration and invasion of HUVECs. Besides, RUNX3 was identified as the direct of miR-210 in HUVECs. Overexpression of miR-210 decreased RUNX3 expression and promoted the proliferation, migration and invasion of HUVECs, while overexpression of RUNX3 inhibited these promotion effects. In vivo experiment showed that MVs derived from ADSCs under hypoxia increased miR-210 level and capillary density, and inhibition of miR-210 decreased capillary density. We also found MVs downregulated RUNX3 expression, and inhibition of miR-210 upregulated RUNX3 expression.

Conclusion: miR-210 released from ADSCs-derived MVs promoted angiogenesis by targeting RUNX3, which revealed one of the mechanisms of ADSCs-derived MVs on the promotion of angiogenesis.

**Keywords**: adipose-derived stem cells; microvesicle; miR-210; RUNX3; angiogenesis

**Introduction**

Critical limb ischemia is a severe condition lacking of sufficient supply of oxygen and nutrients to extremities, which is secondary to peripheral vascular disease [[1](#_ENREF_1)]. It affects 200 million people worldwide, and can lead to high morbidities and mortalities [[2](#_ENREF_2)]. The treatments, such as angioplasty and stent implantation, have improved the macrovascular function. However, there are still many cases that can not be relieved or cured by surgery, and they can only survive by amputation. Adipose-derived stem cells (ADSCs) are adult multipotent stem cells that have multilineage differentiation potential, which can promote angiogenesis to increase local blood supply [[3](#_ENREF_3)]. Recently, more and more researchers focus on the effect of ADSCs on the treatment of damaged capillary in lower-limb ischemia, and show ADSCs transplantation accelerates the regeneration of lower-limb by secretion of growth factor [[4](#_ENREF_4),[5](#_ENREF_5)]. However, the mechanism of ADSCs on the promotion of angiogenesis is still not clear.

Microvesicles (MVs) are one of extracellular vesicles origined from plasma membrane of a variety of cells, which function as an important intercellular communication [[6](#_ENREF_6)]. MVs can target recipient cells and affect the functions of recipient cells by delivering their contents of RNAs, lipids and proteins [[6](#_ENREF_6)]. Reports about MVs on the promotion of angiogenesis in ischemic diseases are increasing in recent years. For example, Zhao et al found that MVs secreted by ADSCs had role in angiogenesis in the treatment of ischemic diseases [[7](#_ENREF_7)]. Jung et al proved that MVs secreted from induced pluripotent stem cells-derived cardiomyocytes could exert protective role in cardiovascular disease by regulating angiogenesis [[8](#_ENREF_8)]. In addition, reports showed that MVs contained various small noncoding RNAs, such as miRNAs [[9](#_ENREF_9)]. Our previous report observed that ADSCs-derived MVs delivered miR-31 to modulate the function of human umbilical vein endothelial cells (HUVECs) thus to promote angiogenesis [[10](#_ENREF_10)]. Other MVs-contained miRNAs still need to be explored to reveal the mechanism of the proangiogenesis.

MicroRNAs (miRNA) are a kind of small non-coding RNAs that can be contained in MVs and transfer to neighboring or distant cells, thus to modulate the function of recipient cells [[11](#_ENREF_11)]. It has been reported that miR-210 was overexpressed in ischaemic vascular disease and could enhance angiogenesis [[12](#_ENREF_12)]. Our previous report found that miR-210 released from ADSCs-derived MVs was remarkably increased under hypoxic condition [[10](#_ENREF_10)]. Researchers also observed that miR-210 released from human leukemia cell-derived MVs was remarkably increased under hypoxic (1%) condition, and MVs enhanced the migration of HUVECs and tube formation [[13](#_ENREF_13)]. Runt-related transcription factor-3 (RUNX3) belongs to the Runt family that plays a role in angiogenesis in hypoxic responses [[14](#_ENREF_14)]. Researchers have found that RUNX3 supressed cell migration, invasion and angiogenesis in human renal cell carcinoma, prostate cancer and gastric cancer [[15-17](#_ENREF_15)]. Lee et al found that miRNA can modulate angiogenesis by directly targeting RUNX3 [[15](#_ENREF_15)]. Moreover, bioinformatic software predicted the combination sites between miR-210 and RUNX3. Thus, we assumed that miR-210 released from ADSCs-derived MVs promoted angiogenesis of HUVECs by regulating RUNX3.

In this study, we proved that miR-210 released from ADSCs-derived MVs promoted angiogenesis by targeting RUNX3, which revealed one of the mechanisms of ADSCs-derived MVs on the promotion of angiogenesis.

**Materials and methods**

*Cell culture*

hADSCs and HUVECs were purchased from American Type Culture Collection (ATCC). ADSCs were maintained in α-modified minimum essential medium (α-MEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 2 mM glutamine (Sinopharm Chemical Reagent Co.,Ltd, China), 0.2 mM ascorbic acid (Sinopharm Chemical Reagent Co.,Ltd, China), and 1% antibiotic/antimycotic solution (Gibco, USA) in 5% CO2 incubator under 37 °C. HUVECs were maintained in RPMI-1640 medium (Gibco, USA) supplemented with 10% FBS (Gibco, USA) in 5% CO2 incubator under 37℃. ADSCs cultured in hypoxic condition for 24 h (an incubator chamber flushed with 1% O2, 5% CO2, and 94% N2) were collected for the following experiments.

*Transfection of lentiviral vectors encoding shRNA*

Lentiviral expression vector was constructed by ViraPower™ II Lentiviral Gateway™ Expression System (Invitrogen, USA) as previously reported [[18](#_ENREF_18)]. ADSCs were transfected with miR-210 mimic, miR-210 inhibitor lentiviral vectors with polybrene (8 μg/mL; Sigma, USA), and scramble sequence was set as negative control (NC). Twenty-four hours later, cells were cultured in medium containing 1 μg/mL puromycin (Sinopharm Chemical Reagent Co., China) for selection.

*Preparation of MVs*

MVs were isolated from the ADSCs under nonhypoxic or hypoxic condition. According to our previous report, ADSCs were centrifuged at 500 g, 12,000 g, and 100,000 g for 10 min, 30 min, 60 min at 4℃, respectively [[19](#_ENREF_19)]. Pelleted MVs were washed with PBS for one time and centrifuged at 100,000 g for 60 min at 4℃ to remove residual soluble factors. MVs was washed and resuspended in PBS. Protein concentration of MVs was detected by NanoDropTM 8000 spectrophotometer (Thermo Fisher Scientific, USA).

*Transwell assay*

HUVECs migration was conducted by a 24-well Transwell system (Corning, USA) which allowed cells to migrate through the 5-μm-pore sized polycarbonate membrane. 200 μL (1×105 cells) of HUVECs suspensions were added to the upper chamber of the Transwells. The lower chamber was added with 600 μL RPMI-1640 medium containing 1% FBS. Twenty-four hours later, HUVECs were fixed in absolute ethanol for 10 min, then stained with 0.5% crystal violet for 10 min. HUVECs that migrated to the lower side of the inserts were observed under microscope (Olympus, Japan; magnification: ×200). The method of HUVECs invasion was similar, and the medium in upper chamber was serum-free medium.

*MTT assay*

HUVECs (3×104 cells) at logarithmic growth phase were suspended in 100μl medium, and seeded into a 96-well plate at a density of 3×104. HUVECs were treated with MVs for 24 h, then HUVECs were treated with 10 μl of MTT reagent at 37 ℃ for 4 h. Then, HUVECs were treated with 100 μl of solubilization solution at 37 ℃ for 12 h. The optical density (OD) value was determined at a wavelength of 490 nm by a microplate reader (Bio-Rad, USA). Cell viability=(OD value of treated groups)/(OD value of control group).

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis* Isolation of total RNA from MVs and HUVECs was conducted using Rneasy Mini Kit (QIAGEN, USA). miRNA reverse transcribed to complementary DNAs (cDNAs) were prepared using iScript™ cDNA Synthesis Kit (QIAGEN, USA). RT-qPCR was conducted using QIAGEN Fast Cycling PCR Kit (QIAGEN, USA) for 35 cycles in a T100 PCR machine (Bio-Rad, USA). U6 was used as the control gene. A 2-step cycle protocol was used to measure mRNA expression, and the data were analyzed using CFX Manager™ software (Bio-Rad, USA). The relative miR-210 level was calculated by the comparative 2-ΔΔCq method. Primers of miR-210 forward 5’-ACACTCCAGCTGGGCTGTGCGTGACAGCGG-3’, RUNX3 forward 5’-AGGCATTGCGCAGCTCAGCGGAGTA-3’, and reverse 5’- TCTGCTCCGTGCTGCCCTCGCACTG-3’, U6 forward 5’-CTCGCTTCGGCAGCACA-3’, and reverse 5’-ACGCTTCACGAATTTGCGT-3’.

*Western blot analysis*

The proteins from HUVECs were extracted using lysis buffer (Beyotime Biotechnology, China), centrifuged at 12,000 g for 10 min at 4℃, and supernatants were collected. Concentrations of proteins were determined using a BCA Protein Assay kit (Pierce, USA). Protein samples that contained equal amounts of proteins (50μg) were separated on a 12% SDS-PAGE gel. Then, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Thermo Scientific, USA) which blocked with 5% skim milk for 90 min at room temperature, and incubated with the following primary antibodies at 4℃ overnight: anti-Alix (Invitrogen, USA, cat. no. PA5-52873), anti-VEGF (Merckmillipore, USA, cat. no.07-1420), anti-RUNX3 (Invitrogen, USA, cat. no. PA1-318), and anti-β-actin (Invitrogen, USA, cat. no. PA1-46296). The membranes were washed and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (Pierce, USA, cat. no. 31491B) at room temperature for 1 h. Protein was detected by the ExperionTM automatic electrophoretic workstation (Bio-Rad, USA) and analyzed by Image Lab 4.1 software (Bio-Rad, USA).

*Luciferase reporter assays*

The pIS0 plasmid containing wide type (WT) or mutant (MUT) miR-210 recognized sites, miR-210 mimic or miR-210 inhibitor, and renilla luciferase vector were co-transfected into HUVECs by Lipofectamine 2000 reagent (Invitrogen, USA). Forty-eight hours later, HUVECs were collected and luciferase activities were measured using Dual-Luciferase Reporter Assay (Promega, USA).

*Establishment of Lower-limb ischemia (LI) nude mice model*

This animal experiment was approved by the Ethics Committee of Nanchang University. Twenty-five C57Bl/6 mice were purchased from laboratory animal center of Nanchang University, and kept in homeothermal room under a 12 h of light/dark cycle with no limitation to food and water. All mice were anesthetized with 3% isoflurane, and burprenorphine was used in early postoperative period. Lower-limb ischemia was conducted by ligation of the femoral artery at the inguinal ligament and popliteal fossa, then the artery and all branches were excised as previous report [[1](#_ENREF_1)]. Negative control (NC) and miR-210 inhibitor (50μmol/L) were transfected into ADSCs under hypoxic condition, and MVs were isolated from ADSCs. 100 mg/ml of MVs were diluted by 0.9% saline and subcutaneously injected into the flank of LI mice every two days. Twenty-eight days after induction of ischemia, lower-limb muscle tissues were collected, and capillary density was measured.

*Statistical analysis*

All experiments were independently repeated for three times. SPSS 17.0 software was used to analyze the data, and the data of the quantitative studies were presented as mean±standard deviation (SD). Comparisons between two groups or among multiple groups were analyzed by Student’s t test or one-way analysis of variance (ANOVA), with P <0.05 considered statistically significant.

**Results**

*miR-210 expression in ADSCs-derived MVs under hypoxia*

To figure out whether miR-210 was abnormally expressed in ADSCs-derived MVs under hypoxia, ADSCs were cultured under nonhypoxic (20% O2) and hypoxic condition (1% O2) for 24 h. We found protein level of Alix (MV marker) was significantly upregulated in MVs in hypoxic group (Figure 1A). And miR-210 expression was significantly upregulated in ADSCs-derived MVs under hypoxic condition (Figure 1B).

*MVs from hypoxic group promoted proliferation and migration of HUVECs*

To study the effect of MVs release on the proliferation and migration of HUVECs,

MVs that isolated from ADSCs under nonhypoxic and hypoxic condition were co-cultured with HUVECs. We found that MVs in hypoxic group significantly promoted the proliferation (Figure 2A), migration (Figure 2B) and invasion (Figure 2C) of HUVECs.

*Effects of miR-210 on the proliferation and migration of HUVECs*

To explore the effect of miR-210 on the proliferation and migration of HUVECs, miR-210 mimic or inhibitor was transfected into ADSCs under nonhypoxic or hypoxic condition. Then, ADSCs-derived MVs were co-cultured with HUVEC. As shown in Figure 3A and 3B, miR-210 expression and VEGF expression in miR-210 mimic group were upregulated than pre-NC group. Meanwhile, miR-210 mimic promoted proliferation (Figure 3C), migration and invasion (Figure 3D) of HUVECs. According to Figure 3E and 3F, miR-210 expression and VEGF expression in miR-210 inhibitor group were downregulated than NC group. And miR-210 inhibitor supressed proliferation (Figure 3G), migration and invasion (Figure 3H) of HUVECs.

*RUNX3 was a direct target of MV-released miR-210 in HUVECs*

Bioinformatics software mircoRNA.org predicted that there were combination sites between miR-210 and 3’ UTR of RUNX3 (Figure 4A). RUNX3 mRNA (WT) and RUNX3 mRNA (MUT) were constructed. WT RUNX3-UTR-pIS0 and miR-210 inhibitor were co-transfected into HUVECs, and luciferase activity in miR-210 inhibitor group was higher than NC group, while there was no significant change in luciferase activity after co-transfected with Mu-RUNX3-UTR-pIS0 and miR-210 inhibitor (Figure 4B). After the transfection of miR-210 inhibitor to HUVECs, mRNA and protein levels of RUNX3 in miR-210 inhibitor group were significantly upregulated than NC group (Figure 4B). WT RUNX3-UTR-pIS0 and miR-210 mimic were co-transfected into HUVECs, and luciferase activity in miR-210 mimic group was lower than pre-NC group, while there was no significant change in luciferase activity after co-transfected with Mu-RUNX3-UTR-pIS0 and miR-210 mimic (Figure 4C). After the transfection of miR-210 mimic to HUVECs, mRNA and protein levels of RUNX3 in miR-210 mimic group were significantly lower than pre-NC group (Figure 4C). These results suggested that RUNX3 was negatively regulated by miR-210.

*miR-210 regulated the proliferation and migration of HUVECs via RUNX3*

To show the evidence that miR-210 regulated the proliferation and migration of HUVECs via RUNX3, miR-210 mimic or inhibitor was transfected into ADSCs under nonhypoxic or hypoxic condition, and MVs were isolated from ADSCs. After the transfection of pcDNA-RUNX3 to HUVECs, MVs were co-cultured with HUVECs. As shown in Figure 5A, miR-210 mimic inhibited the expression of RUNX3, and pcDNA-RUNX3 reversed this inhibition effect. miR-210 mimic promoted the expression of VEGF, and pcDNA-RUNX3 reversed this promotion effect (Figure 5B). We also observed that miR-210 mimic promoted the proliferation (Figure 5C), migration and invasion (Figure 5D) of HUVECs, and pcDNA-RUNX3 reversed these effects. After the transfection of si-RUNX3 to HUVEC, MVs were co-cultured with HUVECs. As shown in Figure 5E, miR-210 inhibitor promoted the expression of RUNX3, and si-RUNX3 reversed this promotion effect. miR-210 inhibitor supressed the expression of VEGF, and si-RUNX3 reversed this inhibition effect (Figure 5F). MTT and Transwell assay showed that miR-210 inhibitor supressed the proliferation (Figure 5G), migration and invasion (Figure 5H) of HUVECs, and si-RUNX3 reversed these effects.

*MVs-released miR-210 promoted angiogenesis in vivo*

To confirm MVs-released miR-210 promoted angiogenesis via RUNX3, we established lower-limb ischemia (LI) nude mice model. We found that capillary density in LI mice was decreased than sham mice, MVs increased capillary density, and miR-210 inhibitor decreased capillary density again (Figure 6A). We also found miR-210 level was downregulated in LI mice, MVs upregulated miR-210 level, and miR-210 inhibitor downregulated miR-210 level (Figure 6B). RUNX3 expression was upregulated in LI mice, MVs supressed RUNX3 expression, and miR-210 inhibitor increased RUNX3 expression (Figure 6C). These findings indicated that MVs-released miR-210 downregulated the expression of RUNX3 to promote angiogenesis.

**Discussion**

In the present study, we proved miR-210 released from ADSCs-derived MVs played a protective effect in LI mice to promote angiogenesis of endothelial cells by the remarkable decrease of RUNX3 level and increase of capillary density.

ADSCs are multipotent cells derived from adipose tissue that have a wide differentiation potential and have the ability to differentiate into endothelial cells, which can be considered as forefront threapy for the treatment of ischemic diseases [[20](#_ENREF_20)]. Borlongan et al made his point that stem cell-based therapy promoted angiogenesis and was effective in the treatment of ischemic diseases [[21](#_ENREF_21)]. Besides the wide differentiation potential of ADSCs, paracrine secretion of ADSCs is another main approach to achieve the therapeutic effect by secreting TNF-α, IFN-γ, TGF-β, and vascular endothelial growth factor (VEGF) to promote angiogenesis [[7](#_ENREF_7)]. In this study, miR-210 and Alix levels were measured under hypoxia because ADSCs were exposed under hypoxic condition in LI. Deveza et al have shown that hypoxia enhanced the paracrine secretion of angiogenic factors from ADSCs and improved the survival of endothelial cells [[22](#_ENREF_22)]. Our result showed that hypoxia increased the levels of Alix and miR-210, and the viability, migration and invasion of HUVECs, which were consistent with previou report [[22](#_ENREF_22)].

MVs derived from stem cells have been found as new mechanism of paracrine secretion of stem cells, and exploited as new therapeutic approach of stem cells to ischemic diseases [[23](#_ENREF_23)]. Ratajczak et al further concluded that paracrine factors and MVs were riched in stem cells, and MVs could act as a potent pro-angiopoietic factor [[24](#_ENREF_24)]. Chen et al reported that MVs secreted from mesenchymal stem cells could produce various factors, such as angiogenin, VEGF, and monocyte chemotactic protein-1 (MCP-1) to promote angiogenesis [[25](#_ENREF_25)]. Bian et al found that mesenchymal stem cells-derived extracellular vesicles could promote the formation of blood vessels in ischemic heart rats [[26](#_ENREF_26)]. These reports all suggested that stem cells-derived MVs can promote angiogenesis by secreting contents to target cells. MVs contain proteins, lipids, mRNA, miRNA, etc [[23](#_ENREF_23)], and recent researches have reported that miRNA delivered by MVs played vital roles in regulating the targeted cells [[27-29](#_ENREF_27)]. In this study, we found miR-210 delivered by MVs remarkably promoted proliferation and migration of HUVECs, suggesting miR-210 playing a positive role in proangiogenesis.

miRNA directly targeted RUNX3 to supress angiogenesis in gastric cancer [[15](#_ENREF_15)]. Our study confirmed RUNX3 was a direct target of miR-210, and negatively regulated by miR-210. We also observed that miR-210 increased the level of proangiogenic factor VEGF, and overexpression of RUNX3 decreased VEGF level. Besides, miR-210 promoted the viability, migration and invasion of HUVECs, and overexpression of RUNX3 inhibited these promotion effects. In vivo experiment showed that MVs derived from ADSCs under hypoxia increased miR-210 level and capillary density of LI mice, and inhibition of miR-210 decreased miR-210 level and capillary density. In vivo experiment also found MVs downregulated RUNX3 expression and inhibition of miR-210 upregulated RUNX3 expression.

In conclusion, our findings determined hypoxia promoted the release of MVs, and miR-210 was overexpressed in MVs derived from ADSCs under hypoxia. Importantly, we found miR-210 released from ADSCs-derived MVs promoted angiogenesis by targeting RUNX3 in vivo and in vitro.

**Conflict of interest**

All authors declare that there is no conflict of interest.

**Figure legends**

Figure 1. miR-210 expression in ADSCs-derived microvesicles under hypoxia. ADSCs-derived MVs were divided into two groups: nonhypoxic and hypoxic groups. ADSCs of Hypoxic group were treated with 1% O2 for 24 h, and ADSCs of nonhypoxic group were treated with 20% O2. A. MV marker (Alix) was measured by western blot. B. miR-210 expression in ADSCs-derived MVs was measured by qRT-PCR. \*P<0.05, compare with nonhypoxic group.

Figure 2. MVs from hypoxic group promoted proliferation and migration of HUVECs. MVs were isolated from ADSCs treated with or without hypoxia, and co-cultured with human umbilical vein endothelial cells (HUVEC). A. Cell proliferation was detected by MTT assay. B. Cell migration and invasion were detected by Transwell assay. \*P<0.05, compare with nonhypoxic group.

Figure 3. Effects of miR-210 on the proliferation and migration of HUVECs. miR-210 mimic was transfected into ADSCs under nonhypoxic condition. ADSCs-derived MVs were co-cultured with HUVEC. A. qRT-PCR showed that miR-210 expression in miR-210 mimic group was upregulated than pre-NC group. B. Western blot showed that VEGF expression in miR-210 mimic group was upregulated than pre-NC group. C. MTT assay showed miR-210 mimic promoted proliferation of HUVECs. D. Transwell assay showed miR-210 mimic promoted migration and invasion of HUVECs. \*P<0.05, compare with pre-NC group.

miR-210 inhibitor was transfected into ADSCs under hypoxic condition. ADSCs-derived MVs were co-cultured with HUVEC. E. qRT-PCR showed that miR-210 expression in miR-210 inhibitor group was downregulated than NC group. F. Western blot showed that VEGF expression in miR-210 inhibitor group was downregulated than NC group. G. MTT assay showed miR-210 inhibitor supressed proliferation of HUVECs. H. Transwell assay showed miR-210 inhibitor supressed migration and invasion of HUVECs. \*P<0.05, compare with NC group.

Figure 4. RUNX3 was a direct target of miR-210. A. Bioinformatics software mircoRNA.org was used to predict microRNAs that bind to RUNX3, and found there were combination sites between miR-210 and 3’ UTR of RUNX3. B. Wide type (WT) and Mutation (MUT) RUNX3 3’ UTR were inserted into plasmid pIS0 to construct RUNX3 mRNA (WT) and RUNX3 mRNA (MUT). WT RUNX3-UTR-pIS0 and miR-210 inhibitor were co-transfected into HUVEC. Luciferase activity was higher than NC group, while there was no significant change in luciferase activity after co-transfected with Mu-RUNX3-UTR-pIS0 and miR-210 inhibitor. miR-210 inhibitor was transfected into HUVEC. RUNX3 levels in miR-210 inhibitor group were significantly higher than NC group. C. WT RUNX3-UTR-pIS0 and miR-210 mimic were co-transfected into HUVEC. Luciferase activity was lower than pre-NC group, while there was no significant change in luciferase activity after co-transfected with Mu-RUNX3-UTR-pIS0 and miR-210 mimic. miR-210 mimic was transfected into HUVEC. RUNX3 levels in miR-210 mimic group were significantly lower than pre-NC group. \*P<0.05, compare with pre-NC or NC group.

Figure 5. miR-210 regulated the proliferation and migration of HUVECs via RUNX3. miR-210 mimic was transfected into ADSCs under nonhypoxic condition, and MVs were isolated. pcDNA-RUNX3 was transfected into HUVEC, then MVs were co-cultured with HUVECs. This experiment included 4 groups: pre-NC, miR-210 mimic, miR-210 mimic+pcDNA, and miR-210 mimic+pcDNA-RUNX3. A. Western blot showed that miR-210 mimic inhibited the expression of RUNX3, and pcDNA-RUNX3 reversed this effect. B. Western blot showed that miR-210 mimic promoted the expression of VEGF, and pcDNA-RUNX3 reversed this effect. C. MTT assay showed that miR-210 mimic promoted the proliferation of HUVECs, and pcDNA-RUNX3 reversed this effect. D. Transwell assay showed that miR-210 mimic promoted the migration of HUVECs, and pcDNA-RUNX3 reversed this effect.

miR-210 inhibitor was transfected into ADSCs under hypoxic condition, and MVs were isolated. si-RUNX3 was transfected into HUVEC, then MVs were co-cultured with HUVECs. This experiment included 4 groups: NC, miR-210 inhibitor, miR-210 inhibitor+si-control, and miR-210 inhibitor+si-RUNX3. E. Western blot showed that miR-210 inhibitor promoted the expression of RUNX3, and si-RUNX3 reversed this effect. F. Western blot showed that miR-210 inhibitor supressed the expression of VEGF, and si-RUNX3 reversed this effect. G. MTT assay showed that miR-210 inhibitor supressed the proliferation of HUVECs, and si-RUNX3 reversed this effect. H. Transwell assay showed that miR-210 inhibitor supressed the migration of HUVECs, and si-RUNX3 reversed this effect. \*P<0.05, compare with pre-NC or NC group. #p<0.05, compared with miR-210 mimic+pcDNA or miR-210 inhibitor+si-control group.

Figure 6. MVs-released miR-210 promoted angiogenesis in vivo. Lower-limb ischemia (LI) nude mice model was established. Twenty-five C57Bl/6 mice were divided into five groups: Sham, LI, LI+MVs (non-transfection), LI+MVs+NC, and LI+MVs+miR-210 inhibitor groups. A. Hindlimb muscle tissues were collected 28 days after induction of ischemia, and capillary density was measured. B. miR-210 expression was detected by qRT-PCR. C. RUNX3 expression was detected by western blot. \*P<0.05, compare with sham group. #p<0.05, compared with LI group. &p<0.05, compared with LI+MVs+NC group.

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