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Adipose-derived regenerative cells as a promising therapy for cardiovascular diseases: an overview

Shukuro Yamaguchi[1], Yuki Shimizu[1], Toyoaki Murohara[1] and Rei Shibata[2]

[1]*Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[2]*Department of Advanced Cardiovascular Therapeutics, Nagoya University Graduate School of Medicine, Nagoya, Japan*

ABSTRACT

The number of patients with ischemic cardiovascular diseases is significantly increasing as populations age. Therapeutic angiogenesis has been developed as a new treatment strategy for such patients. In recent years, the presence of mesenchymal stem cells in adipose tissues was reported, and regenerative medicine using these cells has attracted attention worldwide. In this review, we describe how the transplantation of adipose-derived regenerative cells enhances angiogenesis and tissue regeneration because of their multilineage potential and cytokine secretion. Then, the current status of therapeutic angiogenesis using adipose-derived regenerative cells in the field of cardiovascular medicine was also described. These cells present great advantages over bone marrow mononuclear cells, as these need easier, shorter, and less invasive preparations as well as less ethical concerns and immunological problems. The efficacy of adipose-derived regenerative cell transplantation in the treatment of various diseases was examined in several clinical trials with favorable results. Currently, a multicenter study of therapeutic angiogenesis using these cells is being conducted in patients with critical limb ischemia. In conclusion, we expect that this method will soon be established as a treatment for cardiovascular diseases that have been refractory to conventional treatments.

Keywords: ADRCs, angiogenesis, critical limb ischemia, myocardial infarction

Abbreviations:
ADRC: adipose-derived regenerative cell
BM-MSC: bone marrow-derived mesenchymal stem cell
EC: endothelial cell
VEGF: vascular endothelial growth factor

INTRODUCTION

The number of patients with ischemic diseases (eg, ischemic heart disease, cerebrovascular disease, and peripheral arterial occlusive disease) is significantly increasing as populations age

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Corresponding Author: Rei Shibata, MD, PhD
Department of Advanced Cardiovascular Therapeutics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Tel: +81-52-744-2837, Fax: +81-52-744-2138, E-mail: rshibata@med.nagoya-u.ac.jp

worldwide.[1,2] Therapeutic angiogenesis has been developed as a new treatment strategy for such patients. Gene therapy research for therapeutic angiogenesis using vascular endothelial growth factor (VEGF) started in the 1990s.[3] Then, the knowledge on vascular endothelial progenitor cells was reported.[4,5] Vascular endothelial progenitor cells were shown to be mobilized from the bone marrow. Based on this, the transplantation of autologous bone marrow mononuclear cells was attempted, and it led to the induction of angiogenesis in ischemic tissues.[6,7] Vascular regeneration therapy using bone marrow mononuclear cell transplantation was developed for patients with critical limb ischemia who do not respond to conventional treatment and require amputation of the affected limb (no-option critical limb ischemia). Although its efficacy and safety were reported (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial),[6] some patients were refractory to the therapy, such as those with diabetes, renal failure, and smoking.[6,7] Therefore, new methods to improve the treatment efficacy and new sources of transplant cells for therapeutic angiogenesis are urgently needed. Among the candidates, adipose-derived regenerative cells (ADRCs) have drawn attention as a new source of transplant cells.[8-10] Therapeutic validation and clinical trials in Japan and overseas evaluated the efficacy of ADRCs in the treatment of various diseases (eg, Crohn's disease-associated fistulas, breast reconstruction after partial mastectomy, osteogenesis for bone defects, and stress urinary incontinence).[11-13] In this review, we showed our recent findings and the current status of therapeutic angiogenesis using ADRCs in the field of cardiovascular medicine. Specifically, the characteristics of ADRCs that make them a suitable therapeutic agent were described. Then, their angiogenesis-inducing capabilities were described. We also focused on the paracrine effects that the factors released by ADRCs have on angiogenesis and the capacity of these cells to differentiate into cardiovascular cells. Lastly, we reviewed the clinical trials on ADRC treatment for cardiovascular diseases.

DEFINITIONS OF ADRCs

Pittenger et al demonstrated the presence of bone marrow-derived mesenchymal stem cells (BM-MSCs) in human bone marrow.[14] BM-MSCs can differentiate into mesoderm-derived cells (eg, osteoblast cells, adipose cells, and chondrocytes).[14] Zuk et al reported the presence of stem cells in human subcutaneous adipose tissue with characteristics similar to those of BM-MSCs.[15,16] These cells have attracted attention as a new source of cell therapy.

Adipose tissue mainly comprises two classes of cells—mature adipocytes and stromal cells. The latter constitute the stromal vascular fraction, which is obtained by enzymatic treatment and centrifugation of adipose tissue.[15,16] It includes blood components derived from peripheral blood, adipose stromal cells, vascular endothelial cells, and vascular wall cells.[15]

Regarding the definitions of stem cells in adipose tissue or cell population, including the stem cells, there are several terms that overlap in meaning.

1) Processed lipoaspirate (PLA): This is a concept proposed by Zuk et al, as noted above. PLA refers to a cell population, including stem cells, in adipose tissue. PLA cells are adherent in culture and are considered to be a cell population similar to adipose-derived stromal/stem cells (ASC).
2) Stromal vascular fraction (SVF): SVF is obtained by the collagenase digestion of adipose tissue and centrifugation. SVF is a heterogeneous cell population, consisting of stem cells, endothelial cells (ECs), pericytes, and hematopoietic lineage cells. It refers to an uncultured cell population.
3) ASC: ASCs are obtained by culturing SVF. They have cell surface markers similar to bone-marrow-derived MSCs and have the potential to differentiate into fat, cartilage, and bone.

4) ADRC: Although ADRC often refers to an uncultured cell population as SVF does, proliferative cells after multiple passages (ie, cultured cells) are also included in its definition in some publications.

In this review, we use the term ADRC in a broad sense to include stem cells or cell group irrespective of cultures and described their characteristics.

FEATURES OF ADRCs

ADRCs can be cultured without specific liquid media, and large numbers can be obtained after several passages.[15] Approximately ≥90% of ADRC surface antigens are homologous to those of BM-MSCs, and human ADRCs are CD34-positive in early passage cultures.[16,17] The most advantageous feature of ADRCs is that large counts of autologous cells can be obtained in a relatively easy manner. In humans, subcutaneous adipose tissue can be obtained by established methods, such as liposuction under local or general anesthesia,[15] and the procedure is less invasive than the collection of bone marrow mononuclear cells.[15] In addition, BM-MSCs constitute only approximately 0.01%–0.001% of mononuclear cells obtained from bone marrow aspirates, whereas ADRCs constitute relatively large proportions of the stromal vascular fraction obtained from liposuction aspirates.[14-16] Therefore, sufficient ADRC numbers can be isolated from a small amount of tissue and cultured for treatment. Furthermore, a large amount of subcutaneous adipose tissue can be harvested, so many cells can be collected. In addition, since autologous cell transplantation is available, there are less ethical concerns and immunological problems, such as allergy and rejection, which are both great advantages.

ADRCs AND ANGIOGENESIS

Previous studies reported that ADRCs can induce angiogenesis and tissue regeneration.[18,19] A study using a hindlimb ischemia mouse model showed that ADRC transplantation promotes angiogenesis after hindlimb ischemia.[8,9,20] In addition, the expression and production of stromal cell-derived factor-1 and VEGF in ischemic tissue and peripheral blood increased after ADRC transplantation.[20] Moreover, ADRC transplantation led to the improvement of cardiac function and survival after myocardial infarction in mouse and rat models of myocardial infarction.[21-23] Increases in angiogenic factors (eg, VEGF and hepatocyte growth factor) and microvessels in ischemic areas were also observed.[21,22] Another study using mice reported that the local administration of ADRCs did not affect remote tumor growth or metastasis,[24] suggesting that this procedure is safe. In conclusion, these experimental studies clearly showed that ADRC transplantation has a strong angiogenesis-inducing capability and protective effect against cardiovascular diseases, and that the procedure is safe.

PARACRINE EFFECT OF ADRCs ON ANGIOGENESIS

The protective effects of ADRCs on cardiovascular diseases and their mechanism of action rely, at least in part, on their angiogenesis-inducing capacity. The paracrine factors released by ADRCs play a vital role in the improvement of ischemic diseases.

ADRC transplantation into mouse with infarcted hearts revealed that only 9% of the capillaries induced by ADRCs consist of ECs differentiated from ADRCs, and the other 91% was assumed to originate from the paracrine action of ADRCs.[25] In the hindlimb ischemia mouse model of our

study, the differentiation of ADRCs into vascular ECs could not be confirmed,[20] suggesting that ADRCs may rather differentiate into stromal cells, such as perivascular cells. Several researchers showed that ADRC-conditioned medium increases the survival, migration, and tube formation of ECs in vitro.[9,26] These data indicate that the angiogenesis induced by ADRC transplantation is mainly due to the paracrine effect of ADRCs.

The quantitative analysis of cytokine expression in an ADRC-conditioned medium revealed that ADRCs secrete multiple angiogenic factors, including VEGF, hepatocyte growth factor, transforming growth factor beta, basic fibroblast growth factor, placental growth factor, angiopoietin 1 and 2, and stromal cell-derived factor 1.[22,26,27] These cytokines promote cell viability, proliferation, migration, and vascular formation.[9,27] Of those, VEGF and hepatocyte growth factor seem to largely contribute to the angiogenic effects of ADRCs, as the inhibition of either cytokines diminishes the EC viability and migration competency.[9] In a murine model of hindlimb ischemia, stromal cell-derived factor 1 was found to act as a key regulator of neovascularization by recruiting endothelial progenitor cells from the bone marrow to ischemic tissue.[20]

In addition, other types of secreting agents have drawn attention from researchers. Microvesicles collected from culture supernatants of ADRCs were found to have angiogenic properties.[27] A conditioned medium from ADRCs has a plenty of microvesicles, which have microRNAs, such as miR-31 and miR-126.[28,29] Recent research showed that miR-31 is significantly increased in microvesicles from ADRCs, and that it is a key substance contributing to vascular-like tube formation and migration in human umbilical vein ECs and microvessel outgrowth in mouse aortic rings.[28] In addition, it showed that factor-inhibiting hypoxia-inducible factor 1 is the target of miR-31, indicating its function in mediating the angiogenic effects of miR-31.[28] In another study, miR-126 was reported to be a key component of extracellular vesicles from ADRCs, promoting EC migration and angiogenesis along with VEGF.[29] miR-126 stimulates EC mobilization via the Erk1/2 MAPK signaling pathway by suppressing Spred1 expression.[29] In conclusion, ADRCs secrete various paracrine factors that contribute to their strong angiogenic effects observed after transplantation.

DIFFERENTIATION TOWARD CARDIOVASCULAR CELLS

The capability of ADRCs to differentiate into ECs and cardiomyocytes remains controversial. Several in vivo studies reported that ADRCs engrafted into infarcted myocardium were retained after several weeks, but it showed few or no evidence of differentiation toward ECs and cardiomyocytes.[21,25] In contrast, a certain subset of ADRCs was capable of differentiating into ECs when incubated on a medium containing growth factors.[8,30-33] CD34-positive/CD31-negative ADRCs showed a high expression of EC markers when cultured in an endothelial growth medium supplemented with insulin-like growth factor and VEGF.[30] Other cell subsets of CD34-negative/CD31-negative ADRCs can also have similar cell marker characteristics as human umbilical vein ECs cultured on a medium with basic fibroblast growth factor and VEGF.[32] In addition, another study emphasized the promoting effect of basic fibroblast growth factor on the differentiation of ADRCs into ECs.[33] Furthermore, ADRCs exhibit the capacity to differentiate into cardiomyocytes.[31,34] Various reagents were applied to ADRCs to promote their differentiation into cardiomyocytes: 5-azacytidine (5-Aza), transforming growth factor, and angiotensin II.[31] ADRCs were transformed into cardiomyocytes by incubating them with 5-Aza-containing medium.[35] These cells were able to beat spontaneously in a culture and expressed myosin heavy chain, α-actin, and troponin-I, as confirmed by immunostaining.[35] The directional differentiation of ADRCs by angiotensin II during incubation also resulted in the expression of myosin heavy chain and

troponin-I in differentiated cells, but the cells did not beat on their own.[36] ADRCs cultured with transforming growth factor beta 1 also stained positively for cardiac myosin heavy chain and α-actin. They showed the mRNA expression of cardiac-specific genes.[37] In conclusion, the ability of ADRCs to differentiate into cardiovascular cells remains controversial; therefore, further studies are needed. Nevertheless, ADRCs may exert protective cardiovascular effects.

CLINICAL TRIALS ON ADRC TREATMENT FOR CARDIOVASCULAR DISEASES

Several clinical trials were conducted in the cardiovascular field.[38-40] The APOLLO and ADVANCE trials as well as the PRECISE trial were conducted in Europe in patients with acute myocardial infarction and chronic myocardial ischemia, respectively. Moreover, the Athena trial was conducted in the United States in patients with chronic heart failure.

The APOLLO trial was conducted to evaluate the safety and feasibility of ADRC treatment in patients with ST-elevation acute myocardial infarction who underwent successful percutaneous coronary intervention.[38] It proved that the entire process from the liposuction of adipose tissue to the intracoronary injection of ADRCs is safe and feasible, and no severe adverse events related to ADRC treatment were reported.[38] It also demonstrated that ADRC therapy improves the cardiac function and alleviates the perfusion defect and scar formation in the myocardium.[38] This study was followed by ADVANCE, a phase IIb/III clinical trial initiated in 2011 that was conducted in patients with ST-elevation acute myocardial infarction.[38] The results of this trial are yet to be released. In the PRECISE trial, ADRCs were injected into the myocardium under the guidance of the NOGA® XP Cardiac Navigation System in patients with ischemic cardiomyopathy who were deemed unsuitable for revascularization.[39] The ADRC treatment significantly preserved the maximal oxygen consumption and increased the left ventricular total mass.[39] Moreover, the ADRC treatment increased the global wall motion score index and alleviated the myocardial ischemia.[39] The safety and effectiveness of autologous ADRCs against chronic myocardial ischemia were also assessed in the Athena trial, which suggested the symptomatic benefits of ADRC implantation.[40]

Several clinical studies also showed the safety and efficacy of ADRCs for peripheral artery diseases.[11-13] Two clinical studies on ADRC therapy reported the safety and efficacy of culture-expanded ADRC implantation in patients with critical limb ischemia.[11,12] Lee et al showed that multiple intramuscular injections of culture-expanded ADRC significantly improved the ulcer healing, pain rating scale, and claudication walking distance of 15 patients.[11] Bura et al also injected culture-expanded ADRCs into the gastrocnemius and anterior compartment of the ischemic leg of seven patients, resulting in critical limb ischemia grade improvement and increased TcPO2.[12]

In Japan, our group demonstrated the clinical efficacy of the autologous ADRC transplantation in patients with no-option critical limb ischemia.[13] Approximately 300 g of autologous subcutaneous adipose tissue was obtained by liposuction under anesthesia. ADRCs were isolated from the liposuction aspirate fluid using a centrifugal cell separator; then, these were transplanted into ischemic limbs by intramuscular injection. As of this writing, therapeutic angiogenesis was performed using ADRCs in five patients.[13] Six months after transplantation, all patients showed improvement in pain, reduction in size or healing of the ulcer, improvement in 6-min walking distance, and avoided the amputation of affected limbs.[13] Based on these results, a multicenter prospective study involving eight institutions in Japan (Nagoya University holds the secretariat role) is being conducted.[41] Currently, cell therapy using ADRCs is progressing in various forms in the field of cardiovascular medicine.

CONCLUSION

ADRCs have angiogenesis-inducing and tissue-regenerating abilities due to the action of secreted paracrine factors (Figure). However, the ability of ADRCs to differentiate into cardiovascular cells remains controversial and requires further investigation. ADRCs can be prepared more easily and less invasively than bone marrow mononuclear cells, and these present less ethical concerns and immunological problems. Moreover, the procedure takes a relatively shorter time, and its safety has been confirmed. Several studies evaluated the efficacy of ADRC transplantation in the treatment of various diseases, reporting favorable results. Currently, we are conducting a multicenter study on therapeutic angiogenesis using ADRCs in patients with critical limb ischemia. In the near future, we expect that therapeutic angiogenesis using ADRCs will be established as a treatment for cardiovascular diseases that have been refractory to conventional treatments (Figure).

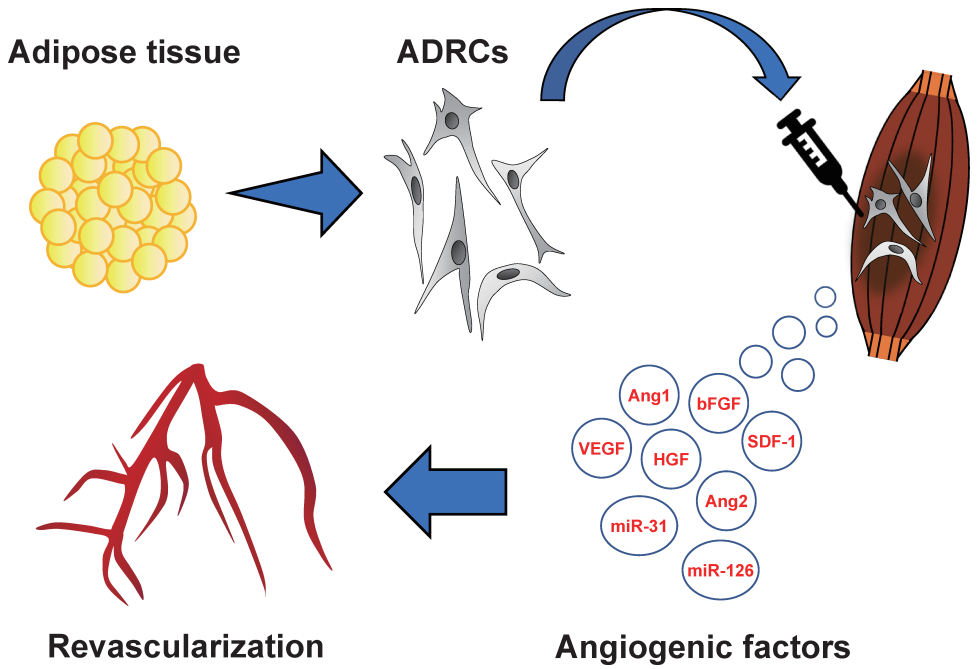

Figure Therapeutic angiogenesis using ADRCs
Subcutaneous adipose tissue was isolated from patients. ADRCs were isolated from the adipose tissues and directly implanted into the muscles. The implantation of ADRCs released angiogenic cytokines/chemokines and extracellular microvesicles containing miRNAs that stimulate local angiogenic responses.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

REFERENCES

1 Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation*. 2020;141(9):e139–e596. doi:10.1161/CIR.0000000000000757.
2 Nowbar AN, Howard JP, Finegold JA, Asaria P, Francis DP. 2014 global geographic analysis of mortality from ischaemic heart disease by country, age and income: statistics from World Health Organization and United Nations. *Int J Cardiol*. 2014;174(2):293–298. doi:10.1016/j.ijcard.2014.04.096.
3 Giacca M, Zacchigna S. VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. *Gene Ther*. 2012;19(6):622–629. doi:10.1038/gt.2012.17.
4 Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275(5302):964–967. doi:10.1126/science.275.5302.964.
5 Murohara T. Angiogenesis and vasculogenesis for therapeutic neovascularization. *Nagoya J Med Sci*. 2003;66(1–2):1–7.
6 Matoba S, Tatsumi T, Murohara T, et al. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. *Am Heart J*. 2008;156(5):1010–1018. doi:10.1016/j.ahj.2008.06.025.
7 Murohara T. Therapeutic Angiogenesis with somatic stem cell transplantation. *Korean Circ J*. 2020;50(1):12–21. doi:10.4070/kcj.2019.0288.
8 Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation*. 2004;109(5):656–663. doi:10.1161/01.CIR.0000114522.38265.61.
9 Nakagami H, Maeda K, Morishita R, et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler Thromb Vasc Biol*. 2005;25(12):2542–2547. doi:10.1161/01.ATV.0000190701.92007.6d.
10 Moon MH, Kim SY, Kim YJ, et al. Human adipose tissue–derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cell Physiol Biochem*. 2006;17(5–6):279–290. doi:10.1159/000094140.
11 Lee HC, An SG, Lee HW, et al. Safety and effect of adipose tissue-derived stem cell implantation in patients with critical limb ischemia: a pilot study. *Circ J*. 2012;76(7):1750–1760. doi:10.1253/circj.cj-11-1135.
12 Bura A, Planat-Benard V, Bourin P, et al. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. *Cytotherapy*. 2014;16(2):245–257. doi:10.1016/j.jcyt.2013.11.011.
13 Katagiri T, Kondo K, Shibata R, et al. Therapeutic angiogenesis using autologous adipose-derived regenerative cells in patients with critical limb ischaemia in Japan: a clinical pilot study. *Sci Rep*. 2020;10(1):12. doi:10.1038/s41598-020-73096-y.
14 Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143–147. doi:10.1126/science.284.5411.143.
15 Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211–228. doi:10.1089/107632701300062859.
16 Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13(12):4279–4295. doi:10.1091/mbc.e02-02-0105.
17 Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15(6):641–648. doi:10.1016/j.jcyt.2013.02.006.
18 Bruun K, Schermer E, Sivendra A, et al. Therapeutic applications of adipose-derived stem cells in cardiovascular disease. *Am J Stem Cells*. 2018;7(4):94–103.
19 Hutchings G, Janowicz K, Moncrieff L, et al. The proliferation and differentiation of adipose-derived stem cells in neovascularization and angiogenesis. *Int J Mol Sci.* 2020;21(11). doi:10.3390/ijms21113790.
20 Kondo K, Shintani S, Shibata R, et al. Implantation of adipose-derived regenerative cells enhances ischemia-induced angiogenesis. *Arterioscler Thromb Vasc Biol*. 2009;29(1):61–66. doi:10.1161/ATVBAHA.108.166496.
21 Cai L, Johnstone BH, Cook TG, et al. IFATS collection: Human adipose tissue-derived stem cells induce

angiogenesis and nerve sprouting following myocardial infarction, in conjunction with potent preservation of cardiac function. *Stem Cells*. 2009;27(1):230–237. doi:10.1634/stemcells.2008-0273.

22 Wang L, Deng J, Tian W, et al. Adipose-derived stem cells are an effective cell candidate for treatment of heart failure: an MR imaging study of rat hearts. *Am J Physiol Heart Circ Physiol*. 2009;297(3):H1020–1031. doi:10.1152/ajpheart.01082.2008.

23 Ishii M, Shibata R, Shimizu Y, et al. Multilayered adipose-derived regenerative cell sheets created by a novel magnetite tissue engineering method for myocardial infarction. *Int J Cardiol*. 2014;175(3):545–553. doi:10.1016/j.ijcard.2014.06.034.

24 Suzuki J, Shimizu Y, Tsuzuki K, et al. No influence on tumor growth by intramuscular injection of adipose-derived regenerative cells: safety evaluation of therapeutic angiogenesis with cell therapy. *Am J Physiol Heart Circ Physiol*. 2021;320(1):H447–H457. doi:10.1152/ajpheart.00564.2020.

25 Yang D, Wang W, Li L, et al. The relative contribution of paracine effect versus direct differentiation on adipose-derived stem cell transplantation mediated cardiac repair. *PLoS One*. 2013;8(3):e59020. doi:10.1371/journal.pone.0059020.

26 Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004;109(10):1292–1298. doi:10.1161/01.CIR.0000121425.42966.F1.

27 Li X, Ma T, Sun J, et al. Harnessing the secretome of adipose-derived stem cells in the treatment of ischemic heart diseases. *Stem Cell Res Ther*. 2019;10(1):196. doi:10.1186/s13287-019-1289-7.

28 Kang T, Jones TM, Naddell C, et al. Adipose-derived stem cells induce angiogenesis via microvesicle transport of miRNA-31. *Stem Cells Transl Med*. 2016;5(4):440–450. doi:10.5966/sctm.2015-0177.

29 Togliatto G, Dentelli P, Gili M, et al. Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications. *Int J Obes (Lond)*. 2016;40(1):102–111. doi:10.1038/ijo.2015.123.

30 Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation*. 2004;110(3):349–355. doi:10.1161/01.CIR.0000135466.16823.D0.

31 Ma T, Sun J, Zhao Z, et al. A brief review: adipose-derived stem cells and their therapeutic potential in cardiovascular diseases. *Stem Cell Res Ther*. 2017;8(1):124. doi:10.1186/s13287-017-0585-3.

32 Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochem Biophys Res Commun*. 2005;332(2):370–379. doi:10.1016/j.bbrc.2005.04.135.

33 Ning H, Liu G, Lin G, Yang R, Lue TF, Lin CS. Fibroblast growth factor 2 promotes endothelial differentiation of adipose tissue-derived stem cells. *J Sex Med*. 2009;6(4):967–979. doi:10.1111/j.1743-6109.2008.01172.x.

34 Léobon B, Roncalli J, Joffre C, et al. Adipose-derived cardiomyogenic cells: in vitro expansion and functional improvement in a mouse model of myocardial infarction. *Cardiovasc Res*. 2009;83(4):757–767. doi:10.1093/cvr/cvp167.

35 Rangappa S, Fen C, Lee EH, Bongso A, Sim EK, Wei EK. Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg*. 2003;75(3):775–779. doi:10.1016/s0003-4975(02)04568-x.

36 Song K, Wang Z, Li W, Zhang C, Lim M, Liu T. In vitro culture, determination, and directed differentiation of adult adipose-derived stem cells towards cardiomyocyte-like cells induced by angiotensin II. *Appl Biochem Biotechnol*. 2013;170(2):459–470. doi:10.1007/s12010-013-0210-6.

37 Gwak SJ, Bhang SH, Yang HS, et al. In vitro cardiomyogenic differentiation of adipose-derived stromal cells using transforming growth factor-beta1. *Cell Biochem Funct*. 2009;27(3):148–154. doi:10.1002/cbf.1547.

38 Houtgraaf JH, den Dekker WK, van Dalen BM, et al. First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2012;59(5):539–540. doi:10.1016/j.jacc.2011.09.065.

39 Perin EC, Sanz-Ruiz R, Sánchez PL, et al. Adipose-derived regenerative cells in patients with ischemic cardiomyopathy: The PRECISE Trial. *Am Heart J*. 2014;168(1):88–95.e82. doi:10.1016/j.ahj.2014.03.022.

40 Henry TD, Pepine CJ, Lambert CR, et al. The Athena trials: autologous adipose-derived regenerative cells for refractory chronic myocardial ischemia with left ventricular dysfunction. *Catheter Cardiovasc Interv*. 2017;89(2):169–177. doi:10.1002/ccd.26601.

41 Shimizu Y, Kondo K, Fukumoto Y, et al. Rationale and design of therapeutic angiogenesis by cell transplantation using adipose-derived regenerative cells in patients with critical limb ischemia - TACT-ADRC multicenter trial -. *Circulation Reports*. 2020;2(9):531–535. doi:10.1253/circrep.CR-20-0055.