Integrins $\alpha 4\beta 1$ and $\alpha V\beta 3$ are Reduced in Endothelial Progenitor Cells from Diabetic Dyslipidemic Mice and May Represent New Targets for Therapy in Aortic Valve Disease

Alexandru Filippi$^1$, Alina Constantin$^1$, Nicoleta Alexandru$^1$, Geanina Voicu$^1$, Cristina Ana Constantinescu$^1$, Daniela Rebleanu$^1$, Madalina Fenyo$^1$, Dan Simionescu$^2$, Agneta Simionescu$^{1,2}$, Ileana Manduteanu$^1$, and Adriana Georgescu$^1$

Abstract

Diabetes reduces the number and induces dysfunction in circulating endothelial progenitor cells (EPCs) by mechanisms that are still uncovered. This study aims to evaluate the number, viability, phenotype, and function of EPCs in dyslipidemic mice with early diabetes mellitus and EPC infiltration in the aortic valve in order to identify possible therapeutic targets in diabetes-associated cardiovascular disease. A streptozotocin-induced diabetic apolipoprotein E knock-out (ApoE$^{-/-}$) mouse model was used to identify the early and progressive changes, at 4 or 7 days on an atherogenic diet after the last streptozotocin or citrate buffer injection. Blood and aortic valves from diabetic or nondiabetic ApoE$^{-/-}$ animals were collected. EPCs were identified as CD34 and vascular endothelial growth factor receptor 2 positive monocytes, and the expression levels of $\alpha 4\beta 1$, $\alpha V\beta 3$, $\alpha V\beta 5$, $\beta 1$, $\alpha L\beta 2$, $\alpha 5$ integrins, and C-X-C chemokine receptor type 4 chemokine receptor on EPC surface were assessed by flow cytometry. The number of CD34 positive cells in the aortic valve, previously found to be recruited progenitor cells, was measured by fluorescence microscopy. Our results show that aortic valves from mice fed 7 days with an atherogenic diet presented a significantly higher number of CD34 positive cells compared with mice fed only 4 days with the same diet, and diabetes reversed this finding. We also show a reduction of circulatory EPC numbers in diabetic mice caused by cell senescence and lower mobilization. Dyslipidemia induced EPC death through apoptosis regardless of the presence of diabetes, as shown by the higher percent of propidium iodide positive cells and higher cleaved caspase-3 levels. EPCs from diabetic mice expressed $\alpha 4\beta 1$ and $\alpha V\beta 3$ integrins at a lower level, while the rest of the integrins tested were unaffected by diabetes or diet. In conclusion, reduced EPC number and expression of $\alpha 4\beta 1$ and $\alpha V\beta 3$ integrins on EPCs at 4 and 7 days after diabetes induction in atherosclerosis-prone mice have resulted in lower recruitment of EPCs in the aortic valve.

Keywords

EPC, endothelial progenitor cells, integrins, recruitment, aortic valve

Introduction

Endothelial progenitor cells (EPCs) are a small fraction of circulating monocytes involved in vascular repair and angiogenesis. According to the protocol by which they are separated, their number in healthy subjects was shown to vary between 0.1% and 0.01% of all monocytes$^1$. When a cardiovascular event occurs, EPC peripheral numbers are transiently increased by granulocyte-macrophage colony-stimulating factor.
stimulating factor, stromal cell-derived factor 1 (SDF-1), vascular endothelial growth factor (VEGF), and erythropoietin-mediated bone marrow mobilization. On the contrary, EPC counts are reduced in diabetes mellitus patients with cardiovascular events, and this reduction is thought to be caused by reduced mobilization due to lower nitric oxide availability, reduced proliferation and differentiation of bone marrow cells into EPCs, and also lower SDF-1, VEGF, and erythropoietin levels which not only reduce EPC availability but also their homing. SDF-1 is a chemokine whose actions are exerted through the C-X-C chemokine receptor type 4 (CXCR4) receptor expressed on all hematopoietic stem cells and involved in their homing and mobilization.

Various integrins are of importance in EPC mobilization from bone marrow, adhesion to activated endothelial cells (ECs), platelets, and extracellular matrix. It is already well known that integrins are cell adhesion receptors that mainly bind to extracellular matrix ligands and cell-surface ligands. In humans, the integrin family is made of 24 transmembrane αβ heterodimers, formed from 18α and 8β subunits, binding targets such as the fibronectin RDG motif, vitronectin, fibronectin, the epitope GFOGER of collagen, laminin, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule (ICAM)-1,-2,-3,-5, and others. Integrins play roles not only in cell to cell and cell to extracellular matrix adhesion but also in cell shape change, migration, differentiation, proliferation, and apoptosis through inside-out and outside-in signaling.

Integrins are involved in EPC homing and recruitment of circulatory cells as well as in interaction with the extracellular matrix. The dimeric α4β1 integrin (also called very late antigen-4, VLA-4) is a known ligand of VCAM-1 and fibronectin expressed on monocytes, lymphocytes, natural killer cells, eosinophils, neutrophils, and also on progenitor cells. This integrin dimer was involved in EPC recruitment by the activated endothelium as well as in EPC retention in the bone marrow. In diabetes, protein kinase A (PKA)-mediated phosphorylation of α4β1 reduces EPC mobilization from the bone marrow.

There is data supporting that the αvβ3 and αvβ5 integrins, promiscuous ligands for vitronectin, fibrinectin, fibronogen, osteopontin, and von Willebrand factor, are involved in EPC adherence to denuded vessels as the inhibition of αvβ3 and αvβ5 integrins blocks the re-endothelialization of denuded arteries. Another integrin shown to be involved in the adhesion is the β3 integrin binding the receptor for advanced glycation end products at the surface of ECs, while the αvβ3 integrin dimer binds ICAM-1 on activated ECs.

Integrin levels are altered in pathology both with roles in healing mechanisms and as collateral targets. In models of shear stress, similar to that in atherosclerosis or valve stenosis, upregulation of the β1 and β3 integrins, facilitating EPC adhesion to the lesion site, and EPC differentiation associated with lower CD34 and CD133 stem cell marker mRNA levels were observed in cultured human glomerular epithelial cells, the presence of high (25 mM) glucose upregulated α5 and αvβ3 integrins and downregulated α1, α2, and α3 integrins, leading to an overall lower collagen IV binding.

However, due to the scarce circulatory presence of EPCs, their integrin expression profile was, to our knowledge only, evaluated after culturing, proliferation, and differentiation. In this study, we aimed to evaluate circulatory EPC number, viability, and integrin expression profile in the early stages of diabetes using a streptozotocin (STZ)-induced diabetic model on mice prone to atherosclerotic lesions. Moreover, since there are reports which suggest that in patients with aortic stenosis, valvular EC regeneration is impaired not only by increased senescence of valvular ECs but also by a reduced number and function of circulating EPCs, we also aimed to evaluate the aortic valve homing and recruitment ability of EPCs in the context of progressive diabetic valvulopathy.

### Materials and Methods

#### Animals

Apolipoprotein E knock-out (ApoE/−) mice from the breeding colony of Taconi were bred in our facility at the Institute of Cellular Biology and Pathology (ICBP) “Nicolea Simionescu”, kept under a 12 h light:12 h dark cycle, with food and water ad libitum. All experimental protocols were approved by the Ethics Committee of ICBP “Nicolea Simionescu” and by the national authority in charge, ANSVSA.

Male, 12-weeks-old, ApoE/− mice were injected intraperitoneally (i.p.) for five consecutive days with 55 mg/kg of body weight of STZ (Sigma-Aldrich, St. Louis, MO, USA) in citrate buffer (final citrate concentration 20.7 mM), pH 4.5, or with an equivalent volume of citrate buffer (CIT, pH 4.5) as recently described by Tucureanu et al. After the last i.p. injection, the diet was switched from standard chow to atherogenic diet (standard chow supplemented with 1% cholesterol and 15% butter) for 4 days (STZ4 and CIT4 groups, 8 and 7 animals, respectively) or 7 days (STZ7 and CIT7 groups, 8 animals each) when the animals were sacrificed. Consequently, four experimental groups of mice were established: STZ4, STZ7, and appropriate controls such as CIT4 and CIT7. After profound surgical anesthesia was induced with a ketamine and xylazine mixture (100 mg/10 mg/kg body weight) via i.p. injection, blood was collected on 5 mM EDTA through a ventricular puncture, mice were perfused with phosphate-buffered saline (PBS; pH 7.2), and the aortic valves were collected from each animal by dissection.

The biochemical and echocardiographic parameters, as well as the aortic valve histology of these animals, were presented at length in our recent article. This model of STZ-induced diabetes on a background of diet-induced atherosclerosis recapitulates the major aspects of valve pathology within 7 days of atherogenic diet after the last STZ injection.
**Flow Cytometry Analysis of Circulating Endothelial Progenitor Cells**

The mononuclear cell fractions were obtained from the whole blood of each mouse by density gradient centrifugation using Histopaque-1077 (density 1.077 g/ml; Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol. The remaining erythrocytes were lysed by a 5-min incubation with ammonium-chloride-potassium (ACK) lysing buffer (Life Technologies, Waltham, MA, USA), and unspécific binding was blocked by washing with 2% fetal bovine serum (FBS) in PBS. Cells were numbered and aliquoted at 2 × 10^7/sample. All samples, except the autofluorescence and isotype control samples, were incubated with both anti-CD34-AlexaFluor488 (FAB65181G; R&D Systems, MN, USA) and anti-VEGFR2 allophycocyanín (APC) (FAB4432A; R&D Systems, MN, USA) antibodies for the identification of EPCs as previously described by Georgescu et al. In addition, each sample was also incubated with anti-CD133-phycocerythrin (PE) (BZ-141204; Biolegend, San Diego, CA, USA), anti-CXCRIPE (FAB21651P-100; R&D Systems, MN, USA), anti-α2β1 integrin (unconjugated ab13219; Abcam, Cambridge, UK), anti-α2 integrin-PE (103905; Biolegend, San Diego, CA, USA), anti-β1-PE (FAB2405P; R&D Systems, MN, USA), anti-α4β1 (unconjugated BZ-103705; Biolegend, San Diego, CA, USA), anti-α5β3-PE (sc7312; Santa Cruz, Dallas, TX, USA), anti-caspase3 (unconjugated 9664; Cell Signaling Technology, Leiden, The Netherlands), anti-TRF2 (unconjugated MA141001; Thermo Fisher Scientific, Waltham, MA, USA), anti-α2β3 (unconjugated sc81632; Santa Cruz, Dallas, TX, USA) antibodies and propidium iodide. Where needed conjugated secondary antibodies such as goat anti-mouse-PE (ab7002; Abcam), goat anti-rat-PE (A10545; Invitrogen Waltham, MA, USA), and goat anti-rabbit-PE (F0110; R&D Systems, MN, USA) were also added after a wash. Samples were measured using a Gallios Beckman Coulter flow cytometer (ex: 488 nm, em: 525 nm BP for Alexa Fluor 488; em: 575 nm BP for PE; and ex: 635 nm, em: 660 nm BP for APC), and data were analyzed using Flowing Software 2 (Turku University, Finland). For the gating strategy see Supplemental Fig. 1.

**Immunohistological Examination of Aortic Valve Leaflets**

The heart specimens were cryoprotected in solutions containing increasing concentrations of glycerol (5%, 10%, 20%, and 50%), washed in 3% sucrose, snap-frozen in liquid nitrogen, and mounted in OCT compound (NEG-50, Thermo Scientific, Waltham, MA, USA). Serial cryostat sections, 5-µm thick (Leica CM1850, IL, USA), containing the three aortic valvular leaflets were collected on poly-L-lysine-treated slides. The sections were fixed in cold acetone for 20 min at −20 °C, washed, and incubated in 0.1% Sudan Black B in ethanol for 1 min for autofluorescence reduction. After blocking in 3% bovine serum albumin (BSA) for 30 min, the sections were incubated overnight at 4 °C with anti-CD34-AlexaFluor488 (FAB65181G; R&D Systems, MN, USA). The next day, the sections were incubated for 5 min with 0.4 µg/ml 4′,6-diamidino-2-phenylindole (DAPI); coverslips were mounted using ProLong Gold Antifade Mountant (Thermo Fisher Scientific, Waltham, MA, USA) and after curing the mountant for 24 h at 4 °C, the coverslips were sealed with acrylic nail polish. Bright-field images, as well as DAPI and CD34-AlexaFluor488 fluorescence images, were acquired with a fluorescence microscope (Olympus IX81, Shinjuku, Tokyo, Japan), using the same exposure for all sections. All cells, as indicated by the DAPI-stained nuclei and the CD34 positive cells were counted automatically using an ImageJ 1.48v Macro requiring the user to select the leaflet area from bright-field images. The CD34 positive cells (EPCs) were defined as regions of more than 500 contiguous pixels each having a fluorescence intensity of more than the mean intensity + 2 standard deviations of the pixels’ grayscale intensity in that image.

**Results**

**ApoE−/− Mice with Diabetes and High-Fat Diet, a Model for Diabetic Dyslipidemia**

In this experimental model, mean glycemia values for CIT control groups were 144.3 ± 5.2 mg/dl for CIT4 and 144.3 ± 5.2 mg/dl for CIT7, while for STZ groups, these were 219.1 ± 18.2 mg/dl for STZ4 and 271.8 ± 25.8 mg/dl for STZ7 as previously published21. Also, the duration of the atherogenic diet of 4 or 7 days significantly interacted with diabetes to increase plasma cholesterol and triglyceride concentration and induce early molecular and functional changes in aortic heart valves21.

**Number and Viability of Circulating Endothelial Progenitor Cells Decline in ApoE−/− Mice with Diabetes and High-Fat Diet**

EPCs phenotype was tested by flow cytometry, where EPCs were identified as CD34/VEGFR double-positive monocyte-derived cells. Another marker for circulating EPCs (early EPCs) described in the literature is the stem-cell marker CD133 and, as expected, the majority (more than 94%) of the cell death as shown by the higher cleaved Caspase 3 levels in EPCs from the 7-day groups, regardless of diabetes (two-way ANOVA, **P < 0.01).**
no source of variation from diet’s length, reduced the number of circulatory EPCs to about two-thirds of the ones observed in citrate controls, even at 4 days after the last STZ injection (0.16 and 0.15% EPC cells in CIT groups vs 0.10 and 0.11% in STZ groups at 4 and 7 days, respectively) (two-way ANOVA, \(^{*}P < 0.05\), Fig. 2B). This is probably caused by a drop in EPC’s mobilization from bone marrow as suggested by a possible reduction in CXCR4 expression in cells...
from diabetic animals (our results showed a reduction by 42.1\% at 4 days and by 50.8\% at 7 days; however, these results were not statistically significant) (two-way ANOVA, \( P = 0.08 \); Fig. 2C). We also examined TRF2 levels, as indicative of cell senescence, and not only EPCs in all groups showed a higher level of this marker compared with other monocytes, but EPCs from diabetic animals had double TRF2 levels compared with the nondiabetic controls; however, no statistically significant modifications were seen between the treatment groups (see Fig. 2D).

Expression of \( \alpha 4\beta 1 \) and \( \alpha V\beta 3 \) Integrins on Circulating Endothelial Progenitor Cells Diminishes in ApoE\(^{-/-}\) Mice with Diabetes and High-Fat Diet

In our flow cytometry experiments, the \( \alpha 4\beta 1 \) integrin expression level was significantly decreased in EPCs from diabetic animals (STZ4 and STZ7 groups compared with CIT4 and CIT7 groups) (two-way ANOVA, \( \#\#\# P < 0.001 \), Fig. 3A). In EPCs of diabetic origin, we also found a decrease in \( \alpha V\beta 3 \) integrin expression level (two-way ANOVA, \( \#\# P < 0.01 \), Fig. 3D). Also, by applying flow cytometry analysis, we found no significant changes in the other integrins tested, \( \alpha 5 \) and \( \beta 1 \) monomers and \( \alpha V\beta 5 \) and \( \alpha L\beta 2 \) integrins (Fig. 3B, C, E, F).

Recruitment of CD34 Positive Cells in Aortic Valve Leaflets Decreases in ApoE\(^{-/-}\) Mice with Diabetes and High-Fat Diet

To evaluate whether the altered integrin expression observed was associated with altered EPC recruitment in the aortic valve (Fig. 4A, C-F), we assayed the CD34 positive cell counts between investigated experimental groups (STZ4, CIT4 and STZ7, CIT7). We observed that the 7-
day atherogenic diet led to a higher CD34 positive cell number in the control group (CIT7), while diabetes (STZ7 group) significantly reduced that number to values observed at earlier time points (CIT4 and STZ4 groups; one-way ANOVA, Bonferroni post-test, *P* < 0.05; see Fig. 4B).

**Discussions and Conclusions**

Circulating progenitor cells are more affected in diabetes associated with atherosclerosis, but the mechanisms involved still need to be uncovered, especially in early diabetes. The aim of the present study was to investigate the mechanisms involved in the dysfunction of circulating EPCs in early diabetes associated with severe atherosclerosis compared with severe atherosclerosis alone. Moreover, we evaluated the consequences of EPC dysfunction in their recruitment in aortic valve lesions. To this end, we used ApoE<sup>−/−</sup> mice with a high-fat diet to mimic severe atherosclerosis (CIT group) or ApoE<sup>−/−</sup> mice with high-fat diet and diabetes (STZ group), to mimic diabetes combined with atherosclerosis. The EPCs were identified and quantified by CD34 and VEGFR2 staining, and the expression levels of α4β1, α5β1, αvβ3, β1, α4β2, α5 integrins, and CXCR4 chemokine receptor on EPC surface were assessed at 4 or 7 days after the last STZ or CIT injection when the diet was switched from standard chow to atherogenic diet. Also, EPC recruitment in the aortic valves was
measured by identifying CD34 positive cells in valve sections from the same four established experimental groups (STZ4, STZ7, CIT4, and CIT7), as CD34 positive valve cells were previously found to be recruited progenitor cells.

Our data showed that the number and function of EPCs declined in ApoE−/− mice with diabetes and a high-fat diet. Also, the present results support the claims on the importance of a hypocaloric, polysaturated fat-rich diet, seeing how the 7 days atherogenic diet alone was able to reduce EPCs’ viability to two-thirds through apoptosis induction.

Regarding the function of EPCs, it was previously shown that EPCs from diabetic patients have normal adhesion to fibronectin and collagen but altered adhesion to activated human umbilical vein ECs. Our results provide possible clues toward the mechanism involved in this previous finding: α4β1 integrin involved in adhesion to activated ECs is reduced in EPC from diabetic animals (STZ4 and STZ7 groups), but not RGD-binding integrin (α5β1) which bind fibronectin. Also, at both time points, EPCs from diabetic mice expressed αβ3 integrin at a lower level, the rest of integrins being seemingly unaffected by diabetes superimposed on atherosclerosis or atherosclerosis alone.

The observed trend of diminution of CXCR4 expression levels in our study is in agreement with published data showing a 44% reduction in CXCR4 positive EPC cells in diabetic patients compared with healthy controls. Moreover, our data show that this modification occurs early in the progression of diabetes, CXCR4 levels being already reduced at 4 days after the last STZ injection (STZ4 group).

To the best of our knowledge, there have been no studies investigating the EPC phenotype and their contributions to aortic valve disease in diabetes associated with atherosclerosis or in atherosclerosis alone. In our experiments, aortic valves from mice fed 7 days with a high-fat diet showed an increased number of CD34 positive cells compared with animals on shorter diet lengths and their diabetic counterparts. In a previous article, we showed that at this time point, VCAM-1 and P-selectin are significantly increased in the aortic valve, a hallmark of endothelium activation. This probably leads to EPC recruitment from the circulation in dyslipidemic controls (CIT4 and CIT7 groups) but, due to their lower number and lower VCAM-1 ligand, α4β1, EPCs from diabetic animals are recruited less efficiently.

Further studies are needed to elucidate the mechanisms leading to the decrease of EPC numbers in diabetes associated with severe atherosclerosis and to investigate the role of EPCs in repairing early lesions associated with aortic valve disease.

Recently, Abplanalp et al. showed that PKA-mediated phosphorylation of α4β1 induced by high glucose plays a role in bone marrow retention of EPCs. Thus, the reduced α4β1 integrin levels observed in circulatory EPCs may be caused by cells expressing lower levels of α4β1 being able to escape into the bloodstream while those expressing higher levels being retained in the bone marrow. These results point to potential therapeutic avenues: one route would be that of bone marrow PKA inhibition, which would help EPC mobilization, and another could be the i.v. administration of autologous or allogeneic EPCs modified to express higher α4β1 and α5β3 levels to improve their adhesion at sites of vascular and valvular lesions. This latter route is currently under investigation in our group.

In conclusion, we show here that early stage diabetes superimposed on atherosclerosis induced alterations in EPC number, phenotype, and homing. These data indicate that functional disruption of α4β1 and α5β3 integrins on EPCs may represent a potential therapeutic target for aortic valve disease by potentially influencing the reparative capacity of valvular ECs in severe atherosclerosis-associated diabetes mellitus.

**Ethical Approval**

All experimental protocols were approved by the Ethics Committee of ICBP “Nicolae Simionescu” and by the national authority in charge, ANSVSA (authorization 304/10.10.2016).

**Statement of Human and Animal Rights**

All experimental procedures involving animals were conducted in accordance with national, European, and international legislation on the use of experimental animals in biomedical research and approved by the Ethics Committees from IBPC “Nicolae Simionescu” (accredited by the Order No. 789 from February 21, 2008, according to the national Law No. 206 from May 27, 2004).

**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: this work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

In addition, the authors declare that the research was conducted in the absence of any either commercial or financial relationships that could be construed as a potential conflict of interest.

**ORCID iD**

Alexandru Filippi https://orcid.org/0000-0002-4948-5454

**Supplemental Material**

Supplemental material for this article is available online.

**References**

1. Bogoslovsky T, Maric D, Gong Y, Qu B, Yang K, Spatz M, Hallenbeck J, Diaz-Arrastia R. Preservation and enumeration of endothelial progenitor and endothelial cells from peripheral blood for clinical trials. Biomark Med. 2015;9(7):625–637.
2. Wils J, Favre J, Bellien J. Modulating putative endothelial progenitor cells for the treatment of endothelial dysfunction and cardiovascular complications in diabetes. Pharmacol Ther. 2017;170:98–115.

3. Antonio N, Fernandes R, Soares A, Soares F, Lopes A, Carvalheiro T, Paiva A, Pego GM, Providencia LA, Goncalves L, Ribeiro CF. Reduced levels of circulating endothelial progenitor cells in acute myocardial infarction patients with diabetes or pre-diabetes: accompanying the glycemic continuum. Cardiovasc Diabetol. 2014;13:101.

4. Thum T, Fracarollo D, Schultheiss M, Froese S, Galuppo P, Widder JD, Tsikas D, Ertl G, Bauersachs J. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. Diabetes. 2007;56(3):666–674.

5. Tsukada S, Masuda H, Jung SY, Yun J, Kang S, Kim DY, Park JH, Ji ST, Kwon SM, Asahara T. Impaired development and dysfunction of endothelial progenitor cells in type 2 diabetic mice. Diabetes Metab. 2017;43(2):154–162.

6. Sainz J, Sata M. CXCR4, a key modulator of vascular progenitor cells. Arterioscler Thromb Vasc Biol. 2007;27(2):263–265.

7. Takada Y, Ye X, Simon S. The integrins. Genome Biol. 2007;8(5):215.

8. Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res. 2010;339(1):269–280.

9. Miranti CK, Brugge JS. Sensing the environment: a historical perspective on integrin signal transduction. Nat Cell Biol. 2002;4(4):E83–E90.

10. Chan BM, Elices MJ, Murphy E, Hemler ME. Adhesion to vascular cell adhesion molecule 1 and fibronectin. Comparison of alpha 4 beta 1 (VLA-4) and alpha 4 beta 7 on the human B cell line JY. J Biol Chem. 1992;267(12):8366–8370.

11. Qin G, Li M, Silver M, Wecker A, Bord E, Ma H, Gavin M, Goukassian DA, Yoon YS, Papayannopoulou T, Asahara T, et al. Functional disruption of alpha4 integrin mobilizes bone marrow-derived endothelial progenitors and augments ischemic neovascularization. J Exp Med. 2006;203(1):153–163.

12. Abplanalp WT, Conklin DJ, Cantor JM, Ginsberg MH, Wysoczynski M, Bhatnagar A, O’Toole TE. Enhanced integrin alpha4beta1-mediated adhesion contributes to a mobilization defect of endothelial progenitor cells in diabetes. Diabetes. 2016;65(11):3505–3515.

13. Caiado F, Dias S. Endothelial progenitor cells and integrins: adhesive needs. Fibrogenesis Tissue Repair. 2012;5:4.

14. Kokubo T, Uchida H, Choi ET. Integrin alpha(4)beta(3) as a target in the prevention of neointimal hyperplasia. J Vasc Surg. 2007;45(Suppl A):A33–A38.

15. Hayakawa K, Pham LD, Arai K, Lo EH. Reactive astrocytes promote adhesive interactions between brain endothelium and endothelial progenitor cells via HMGB1 and beta-2 integrin signaling. Stem Cell Res. 2014;12(2):531–538.

16. Verloop RE, Koolwijk P, van Zonneveld AJ, van Hinsbergh VW. Proteases and receptors in the recruitment of endothelial progenitor cells in neovascularization. Eur Cytokine Netw. 2009;20(4):207–219.

17. Cui X, Zhang X, Guan X, Li H, Li X, Lu H, Cheng M. Shear stress augments the endothelial cell differentiation marker expression in late EPCs by upregulating integrins. Biochem Biophys Res Commun. 2012;425(2):419–425.

18. Suzuki Y, Yamamoto K, Ando J, Matsumoto K, Matsuda T. Arterial shear stress augments the differentiation of endothelial progenitor cells adhered to VEGF-bound surfaces. Biochem Biophys Res Commun. 2012;423(1):91–97.

19. Kitsiou PV, Tzinia AK, Stetler-Stevenson WG, Michael AF, Fan WW, Zhou B, Tsiliyri EC. Glucose-induced changes in integrins and matrix-related functions in cultured human glomerular epithelial cells. Am J Physiol Renal Physiol. 2003;284(4):F671–F679.

20. Matsumoto Y, Adams V, Walther C, Kleinecke C, Brugger P, Linke A, Walther T, Mohr FW, Schuler G. Reduced number and function of endothelial progenitor cells in patients with aortic valve stenosis: a novel concept for valvular endothelial cell repair. Eur Heart J. 2009;30(3):346–355.

21. Tucureanu MM, Filippi A, Alexandru N, Ana Constantinescu C, Ciortan L, Macarie R, Vadana M, Voicu G, Frunza S, Nistor D, Simionescu A, et al. Diabetes-induced early molecular and functional changes in aortic heart valves in a murine model of atherosclerosis. Diab Vasc Dis Res. 2019;16(6):562–576.

22. Georgescu A, Alexandru N, Andrei E, Tiorencu I, Dragan E, Tarzicu C, Ghiorghe S, Badila E, Bartos D, Popov D. Circulating microparticles and endothelial progenitor cells in atherosclerosis: pharmacological effects of irbesartan. J Thromb Haemost. 2012;10(4):680–691.

23. Georgescu A, Alexandru N, Andrei E, Dragan E, Cochar D, Dias S. Effects of transplanted circulating endothelial progenitor cells and platelet microparticles in atherosclerosis development. Biol Cell. 2016;108(8):219–243.

24. Georgescu A, Alexandru N, Nemeicz M, Tiorencu I, Popov D. Irbesartan administration therapeutically influences circulating endothelial progenitor cell and microparticle mobilization by involvement of pro-inflammatory cytokines. Eur J Pharmacol. 2013;711(1–3):27–35.

25. Dadini GP, Avogaro A. Potential manipulation of endothelial progenitor cells in diabetes and its complications. Diabetes Obes Metab. 2010;12(7):570–583.

26. Gossel M, Khosla S, Zhang X, Higano N, Jordan KL, Loeffler D, Enriquez-Sarano M, Lennon RJ, McGregor U, Lerman LO, Lerman A, et al. Role of circulating osteogenic progenitor cells in calcific aortic stenosis. J Am Coll Cardiol. 2012;60(19):1945–1953.

27. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation. 2002;106(22):2781–2786.