Glycation of Apolipoprotein A-IV Impairs Angiogenesis Through The Orphan Nuclear Receptor Nur77

Hongjuan Liu (liuerfenfei@126.com)
Tongji University Tenth People's Hospital: Shanghai Tenth People's Hospital https://orcid.org/0000-0002-3087-4951

Yang Dai
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Yawei Xu
Tongji University Tenth People's Hospital: Shanghai Tenth People's Hospital

Lin Lu
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Original investigation

Keywords: glycated apo A-Ⅳ, angiogenesis, limb, endothelial dysfunction, Nur77

Posted Date: May 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-468317/v1

Abstract

Background

Apo A-Ⅰ played an key role in Cardiovascular diseases, but the effect and mechanism of glycated apo A-Ⅰ on angiogenesis remains unclear.

Methods

In this study, we demonstrated that oral administration of glycated apoA-Ⅰ impaired blood perfusion recovery in a mouse hind-limb ischemia model. A reduction in blood perfusion recovery at day 21 was observed in the ischemic tissue of apoA-Ⅰ and glycated apoA-Ⅰ-treated mice.

Results

In this study, we demonstrated that glycated apo A-Ⅰ impaired blood perfusion recovery in a mouse hind-limb ischemia model. And in vitro study also showed that glycated apo A-Ⅰ inhibited the migration, proliferation, and tube-formation abilities of endothelial cells. Further research revealed that glycated apo A-Ⅰ regulated angiogenesis partly by interrupting Nur77. In addition, CML levels were increased in patients with Lower limb circulation (n = 30) compared with those with no limb circulation(n = 50).

Conclusions

We found impaired angiogenesis induced by glycated apo A-Ⅰ might contribute to poor coronary collateral growth by inhibit the expression of Nur77.

Introduction

T2DM and Angiogenesis

Type 2 diabetes mellitus (T2DM) are established risk factors for cardiovascular disease (CVD) [1], and the incidence of diabetes is increasing day by day, expecting to exceed half a billion by 2030 year[2]. The different physiology, mechanisms and changes at the microvascular level in T2DM, would have significant implications to future CV risk[1], so it’s necessary to explore it’s mechanism to find new Strategy for microvascular disease.

Angiogenesis is one of the most important mechanisms of microvascular development., which can also provide an alternative route for flow, collateral circulation in the heart to improve myocardial function[3, 4]. Angiogenesis involves several important steps, including activation, migration, and proliferation of differentiated endothelial cells and key protein regulation, such as VEGFA/VEGFR2,PI3K/AKT[5, 6],

VEGF/VEGFR2 is a well-known pro-angiogenic factor for promoting neovascularization[7, 8]. PI3K/AKT pathway can also increase VEGF secretion and modulates the expression of other angiogenic factors such as nitric oxide and angiopoietins[9]. Many factors can affect angiogenesis [10]. Previous studies have shown Apolipoprotein A-Ⅰ, Apolipoprotein A-Ⅱ enhances proliferation of human endothelial progenitor cells and promotes angiogenesis[11]. Apo A-IV, the largest member of the exchangeable apo-lipoproteins family at 44 k Da, is a major component of HDL[12],which is a lipid emulsifying protein linked to a range of protective roles in obesity, diabetes, and cardiovascular disease[13].Apo A-IV presents anti-inflammatory, anti-atherogenic, and anti-oxidative properties[14–16]. More recent studies have implied it's potentially important role in altering the glucose sensitivity of pancreatic islets to alter insulin secretion [17]. But the mechanistic details for these functions remain unclear. Apolipoprotein could happen CML glycation with diabetes mills[18]. CML, a well-characterized and major AGE structure, has been proved to playing important role in the diabetic vascular complications specifically ischemic heart disease[19, 20]. but it's role in angiogenesis is not clear.

In this study, we need to explore if glycated apo A-IV relate with Collateral Circulation Disorder with Diabetes, and it's role and mechanism in angiogenesis and microvascular circulation.

Materials And Methods

Study Population

We enrolled a total of 300 patients with T2DM who were referred for diagnostic coronary angiography between January 2014 and November 2017. The diagnoses of T2DM and dyslipidemia were according to the published guidelines from the American Diabetes Association. We grouped the patients with T2DM into Group I (n=80, control group: subjects without evidence of cardiovascular diseases or diabetes, who received annual physical checkups.), Group II: subjects with no coronary artery stenosis or stenosis. Group III: subjects with coronary artery stenosis or stenosis. This study complied with the Declaration of Helsinki. The study protocol was approved by the local hospital ethics committee, and we obtained written informed consent from all the participants.

Isolation of HDL, and quantification of CML.

To circumvent the interference and masking effects of albumin and immunoglobulins in plasma, we obtained apo A-IV by immunoprecipitating it from isolated HDL. We analyzed apo A-IV glycation (CML) through Western blotting. HDL was isolated from 50 ml of fresh plasma by ultracentrifugation, as previously described (17). The HDL solution was then incubated with an anti–apo A-IV and CML antibody (1:1000, Santa Cruz, California). Western blotting was repeated 3 times for each HDL sample. The absolute intensity of apo A-IV glycation and the content of apo A-IV were calculated as density values, which was calculated as the ratio of the normalized absolute intensity of apo A-IV glycation to the normalized content of apo A-IV ([apo A-IV sample glycation/apo A-IV standard glycation]/[apo A-IV sample/apo A-IV standard]).The relative intensity of apo A-IV glycation was determined in all participants.

Preparation of the glycated apo A-IV recombinant protein.

We performed apo A-IV glycation by incubating apo A-IV in a solution containing 0.01% ethylene diamenete tracetic acid, 0.01% sodium azide and 20 mmol/l glyoxal for 7 days, after which we dialyzed the solution.

Ischemic Hind-Limb Model and Blood Flow Monitoring

(i) The animal studies were approved by the animal care committee of Shanghai Jiao Tong University. And (ii) all procedures must conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes or the NIH Guide for the Care and Use of Laboratory Animals. Overall, 40 C57BL/6 male mice aged 5 weeks were purchased from the Model Animal Center of Nanjing University and housed in a pathogen-free isolation facility under a 12/12-hour light–dark cycle with free access to water and food. Streptozotocin (STZ)(obtained from Sigma Aldrich Milan Italy) was dissolved in 0.01 M sodium citrate buffer (pH 4.5) and administrated i.p. at a dose of 40 mg/kg body weight (bw) , continuous 4 days , and by feeding the animals (n = 24) with a high fat diet (HFD: 59% fat, 15% protein, 20% carbohydrates),to induce Diabetes[21]. The diabetes mice were divided into 4 groups: the control group (n=10), the saline group (n=10)(intraperitoneal injection), the ApoA4 group (n=10)(i.p.) (20μg, as cilinical)and the G-APOA4 group (n=10) (i.p.) (20μg). Drug was injected 4 times per week. After 2 weeks of administration, unilateral hind-limb ischemia was surgically performed by left femoral vessel (artery and vein) removal and excision of femoral bifurcation with all branches, details were described previous (19). Hind-limb blood perfusion was measured with laser Doppler perfusion imaging. The results were expressed as the ratio of perfusion in the ischemic (left) versus nonischemic (right) hind limb.

Tissue Preparation and Immunochemistry

1%(m/V, or 5mg/kg) Pentobarbital was I.P to anesthesia mice, Depth of anesthesia was determined by the pedal reflex test, the animals were euthanized by decapitation t, and the Gastrocnemius tissue was removed from the ischemic hind limb at21 days. For mouse capillary density identification, the tissues were stained with monoclonal CD31 antibodies. For quantification, the capillaries were counted in 5 randomly selected microscopic fields. For downstream signal, the tissues were stained at 80°C and detected with Western Blot. The experimental protocol was approved by the Committee on Animal Resources from Shanghai Jiao Tong University.

Cell Culture

We obtained Human Umbilical Vein Endothelial Cells (HUVECs) from company (Portland, OR). The cells were maintained in Medium 200 supplemented with growth factors and cultured in a humidified atmosphere of 5% CO_2 at 37°C.

Glycation treatment

Medium Cell culture medium mix with different concentrations of glycate APOA-IV and APOA-IV (10, 50, and 100 mmol/ L) for 24 hours at 37°C.

Overexpression plasmid and GFP of Nur77 was obtain from Shanghai Jima Pharmaceutical Technology Co., Ltd, which (Final concentration 50nM) was transferred into HUVECs, which was verified in 48h by qPCR.

Wound-Healing Assay

HUVECs were plated on 6-well plates and incubated with glycated APOA-IV and APOA-IV (10, 50, and 100 mmol/L) or serum media (control) for 24 hours. The HUVECs were then wounded with a sterile pipette tip, and the width of each wound line was photographed (Olympus) at 0 and 24 hours.

Cell Migration

Assay Cell migration assays were performed using a Boyden chamber (Millipore). The HUVECs were either incubated with glycate APOA-IV and APOA-IV medium or serum. Cells ($0.5*10^6$ cells per mL) were then harvested and placed in the upper chamber, with the lower chamber filled with 500uL of cell culture medium containing 10% FBS. After 8 hours of incubation, the non-migrated HUVECs on the upper side of the membrane surface were removed by wiping with a cotton swab. The migratory cells were then fixed and stained with crystal violet solution and quantified by optical density (560 nm) measurement.

Tube Formation Assay

Matrigel (BD Bioscience) was added to 96-well plates, with 50uL in each well. After treatment with glycated apo A-IV or apo A-IV for 24 hours, HUVECs were added to the 96-well plates. Cells plated on Growth Factor-Reduced Matrigel served as the negative control. Cells were examined in 4 random microscopic fields, and total length of the capillary structures and branch point numbers were measured by Image-Pro Plus version 6.2 (Media Cybernetics).

Western Blot

Proteins from cell lysates at equal loadings were subjected to 7.5% to 12.5% SDS-PAGE and then transferred to polyvinylidene difluoride membranes. Membranes were further blocked in 5% nonfat milk, followed by incubation with the corresponding primary antibodies overnight at 4°C and with horseradish peroxidase–conjugated secondary antibodies for 1 hour at room temperature. The PI3K antibody (abcam,1:1000), Akt antibody (abcam,1:1000), VEGFR2 antibody (abcam,1:1000), VEGFA antibody (abcam,1:1000), Blots were detected using an electrochemiluminescence system (GE Healthcare Biosciences) and qualified with Quantity One (Bio-Rad) software. We also detected β-actin as the protein loading control.

Statistical Analysis

Statistical analyses were performed with SPSS software (version 20.0) and GraphPad Prism 5. Continuous variables were expressed as mean SD. For normally distributed continuous variables, we use unpaired Student t tests to assess differences. One-way ANOVA was used for multiple comparisons. P value of <0.05 was considered statistically significant.

Data and Resource Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in the published article (and its online supplementary files).

Results

1.Correlation between glycated apo A IV in patients' plasma

The relative intensity of apo A-IV glycation (CML expression) was markedly increased in T2DM patients with CAD (Group III) compared with T2DM patients without CAD (Group II $p < 0.001$) and glomerular filtration(Group I), indicted that apo A-IV glycation Positively correlated with Collateral circulation(Figures 1).

2.G-apo-A-IV inhibited Angiogenesis and the VEGFR2 Pathway in vitro

Endothelial cells were incubated with glycated apo A- or apo A-, compared with the serum medium, glycated apo A- significantly inhibited endothelial migration ability in a modification-dependent manner, as observed from the transwell and wound-healing results (Figure 2C, E). Furthermore, endothelial cells stimulated with glycated apo A- medium showed a reduced capillary capacity, with tube lengths and branch point numbers both decreased (Figure 2A).

To explore the molecular mechanism of glycated apo A- of inhibiting endothelial angiogenesis, we examined the protein expression of VEGFA VEGFR2 and its downstream signal mediators(such as AKT/PI3K/mTOR/e-NOS) by Western blot analysis. The glycated APOA-IV significantly decreased VEGFR2 and downstream PI3K expression as well as Akt/mTOR/e-NOS phosphorylation in a modification-dependent manner(details show as Figure 3).

3.G-apoA- Suppressed Angiogenesis and the VEGFR2 Pathway in STZ-induced hyperglycemic mice

Because the in vitro studies could not determine explicitly whether glycated apo A-IV or apo A-IV inhibit the endothelial angiogenesis process, in vivo studies were used STZ induced diabetes model.

We examined whether oral administration of g-apo A-IV impaired blood perfusion recovery in a mouse hind-limb ischemia model. After surgery (day 0), the laser Doppler perfusion imaging result showed that surgical induction of hind-limb ischemia reduced blood perfusion equally in both groups. Blood flow

recovery, however, was significantly attenuated in mice treated with g-apo A-IV compared with control mice at day 21 (0.690.07 versus 0.890.04, P<0.05) after surgery (Figure 4A/B).

Staining with CD31 and α-SMA is generally used to indicate the presence of vessels. We can see that there were same CD31 and α-SMA -stained vessels in apo A-IV treated hyperglycemic mice with normal control mice, whereas G-apo A-IV (10mg/kg) reduced the increased retinal vessels in STZ-induced hyperglycemic mice (Fig.4C). And also VEGFA content in vitreous cavity was the same with the apo A-IV group, however G-apo A-IV (10 mg/kg) reduced the VEGFA/VEGFR2 Pathway) (Fig. 5).

4.G-apo A-Ⅳ inhibit Nur77 expression to Suppressed Angiogenesis in vitro and vivo

Overexpression plasmid and GFP of Nur77 was transfer in HUVECs, verified in 48h by qPCR. And then we fund the HUVEC Proliferation and migration ability were restored, and tube formation of HUVEC after G-apo A-IV treated was rescued. And also the important protein like VEGFA、VEGFR2、PI3K was up-regulated(details as Figure 6).

And the same, Adeno-associated virus of overexpress Nur77 was Intraperitoneal injection to STZ-mice. And then we fund the Lower limb ischemia were restored(Doppler Blood flow and CD31), and also the important protein like VEGFA、VEGFR2、PI3K was up-regulated(Figure 7).

Discussion

Firstly, in our clinical research, we found diabetes patients with Microvascular circulatory disorders showed a 3.5-fold increased level of apo A-Ⅳ glycation (CML), but it's role and mechanism in angiogenesis was not unclear. Moreover, we found Nur77 protein play an important role in this process from mRNA chip. So in the last experiments, we tested the hypothesis that glycation of apo A-Ⅳ could impair angiogenesis. And the potential mechanism tests were carried out in cellular. To the best of our knowledge, this report is the first demonstrating G-apo A-Ⅳ can inhibit endothelial migration, proliferation, and tube formation partly by interrupting the VEGFR2/PI3K/Akt pathway in cellular and a mouse hind-limb ischemia model, might contribute to diabetes.

Coronary collateral development Belongs to microvascular circulatory disorders ,which is a supplying perfusion to ischemic tissue distal to an occluded segment [22, 23]. An imbalance between pro- and antiangiogenic factors might lead to poor collateral growth [24, 25]. Consequently, previous study had indicate that increasing CML levels serve as a risk factor for poor collateral growth[26, 27], but it’s underlying mechanism remains uncharacterized. In this study, we reported for the first time that the concentration of g-apo A-Ⅳ (CML) was increased in patients with Collateral circulation disorder compared with those without subjects.

First, in vivo Lower limb ischemia mouse model, we found a reduction in blood flow recuperation at both the macrovascular and microvascular levels at day 21 was observed in the G-apo A-Ⅳ treated mice compared with that of the apo A-Ⅳ group. And the impaired tissue perfusion induced by G-apo A-Ⅳ ,such as

the content of CD31 and α-SMA were reduced, which might be mediated by the attenuation of endothelial angiogenic function. Similarly, recent studies have demonstrated endothelial dysfunction and reduced endothelial nitric oxide synthase in G-apo A-Ⅰ treated mice[28]. Our results combined with others suggest that exposure to G-apo A-Ⅰ in vivo could impair endothelial function.

We also evaluated the antiangiogenic activity of G-apo A-Ⅰ on HUVECs in vitro. The effect of glycated proteins on endothelial cells has not been studied. G-apo A-Ⅰ has been proved can mediate protein alteration, which can yield both structural and functional changes in different pathophysiological conditions(32410861). Clinical and basic studies has illustrated that G-apo A-Ⅰ lipoproteins, like glycated low- and high density lipoproteins, harbor atherogenic properties[29, 30]. And in the present research, we incubated HUVECs with glycated apo A-Ⅰ cell culture medium to mimic the overall circulating G-apo A-Ⅰ state in vivo. Our results proved that the functional of endothelial cells are impaired following G-apo A-Ⅰ treatment: a significant reduction in migration, impairment of both proliferation and tube formation were observed in cells. And VEGF is the most potent regulator of angiogenesis. The angiogenic response to VEGF is mainly mediated through activation of VEGFR2 and initiation of its downstream signaling pathway to promote angiogenesis[31, 32]. Several lines of investigation suggest that inhibition of VEGFR2 induces impaired angiogenesis[33]. PI3K/AKT/mTOR pathway can also increase VEGF secretion and modulates angiogenesis[9, 34]. And our in vitro study indicated that the observed decrease in angiogenesis ability in G-apo A-Ⅰ treated endothelial cells could be attributed to the attenuation of VEGFR2 expression rather than phosphorylation. Our in vivo and in vitro studies together indicated that G-apo A-Ⅰ limits physiological angiogenesis.

And we found a new target protein Nur77, who play an important role in G- apo A-Ⅰ induced anti-angiogenesis. Nur77(NR4A1) is one of the NR4A subfamily, which consists of 3 well conserved members, Nur77, Nurr1 (NR4A2) and NOR-1 (NR4A3), respective[35]. NR4A receptors are immediate-early genes that are regulated by many physiological stimuli including growth factors, hormones, and inflammatory signals and are involved in a wide array of important biological process, including cell apoptosis, brain development, glucose metabolism, and vascular remodeling[36]. NR4A receptors are classified as orphan receptors because of it's structure, which consisting of an N-terminal transactivation domain, a central 2-zinc-finger DNA binding domain, and a C-terminal ligand-binding domain[37]. And previous study had shown that Nur77 overexpression has been shown to increase cell survival and angiogenesis in ECs[38]. But the function of endogenous Nur77 in endothelial activation has not been previously studied, and it’s role in glycated induced anti-angiogenesis also unclear. In our study we found G-apo A-Ⅰ down-regulate Nur77, and when knock down Nur77, the G-apo A-Ⅰ anti-angiogenesis role is weakened.

Conclusion

In summary, this report is the first describing G-apo A-Ⅰ regulate the expression of Nur77 and as a negative regulator of angiogenesis and thus a contributor to poor collateral growth in vivo and in vitro. This study could provide a reference for novel risk assessment and therapeutic strategy for patients with diabetes and microcirculation disorders.

Declarations

Ethics approval and consent to participate

Our study uses blood samples from patients and has obtained an exemption from informed consent.

Consent for publication

all authors Consent for publication.

Availability of data and materials

data and materials are availability, no fraud.

Competing interests

there is no conflict of interest for each author.

Funding

No related founding.

Authors' contributions

Dr. Yawei Xu is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

The authors would like to thank Yang Dai researched data. Yawei Xu reviewed/edited the manuscript. Lin Lu contributed to discussion and reviewed/edited the manuscript. And thank for Ruijin hospital and the tenth hospital.

Authors' information (optional)

Hongjuan Liu, 1986-, PHD of Cardiology department of the tenth hospital.

References

1. Strain, W.D. and P.M. Paldánius, *Diabetes, cardiovascular disease and the microcirculation.* Cardiovasc Diabetol, 2018. **17**(1): p. 57.
2. Cole, J.B. and J.C. Florez, *Genetics of diabetes mellitus and diabetes complications.* Nat Rev Nephrol, 2020. **16**(7): p. 377-390.
3. Jiang, L., et al., *Bach1 Represses Wnt/β-Catenin Signaling and Angiogenesis.* Circ Res, 2015. **117**(4): p. 364-375.

4. Wang, N., et al., *Hyaluronic Acid Oligosaccharides Improve Myocardial Function Reconstruction and Angiogenesis against Myocardial Infarction by Regulation of Macrophages.* Theranostics, 2019. **9**(7): p. 1980-1992.
5. Viallard, C. and B. Larrivée, *Tumor angiogenesis and vascular normalization: alternative therapeutic targets.* Angiogenesis, 2017. **20**(4): p. 409-426.
6. Chrifi, I. and L. Louzao-Martinez, *CMTM4 regulates angiogenesis by promoting cell surface recycling of VE-cadherin to endothelial adherens junctions.* 2019. **22**(1): p. 75-93.
7. Porta, M. and E. Striglia, *Intravitreal anti-VEGF agents and cardiovascular risk.* Intern Emerg Med, 2020. **15**(2): p. 199-210.
8. Sellami, N., et al., *Association of VEGFA variants with altered VEGF secretion and type 2 diabetes: A case-control study.* Cytokine, 2018. **106**: p. 29-34.
9. Karar, J. and A. Maity, *PI3K/AKT/mTOR Pathway in Angiogenesis.* Front Mol Neurosci, 2011. **4**: p. 51.
10. Saito, Y., *The role of the PlGF/Flt-1 signaling pathway in the cardiorenal connection.* J Mol Cell Cardiol, 2021. **151**: p. 106-112.
11. González-Pecchi, V., et al., *Apolipoprotein A-I enhances proliferation of human endothelial progenitor cells and promotes angiogenesis through the cell surface ATP synthase.* Microvasc Res, 2015. **98**: p. 9-15.
12. Kim, S.Y. and M. Yu, *High-Density Lipoprotein in Lupus: Disease Biomarkers and Potential Therapeutic Strategy.* 2020. **72**(1): p. 20-30.
13. Deng, X., et al., *Role of Conserved Proline Residues in Human Apolipoprotein A-IV Structure and Function.* J Biol Chem, 2015. **290**(17): p. 10689-702.
14. Cazzo, E., et al., *Biliopancreatic Diversion Decreases Postprandial Apolipoprotein A-IV Levels in Mildly Obese Individuals with Type 2 Diabetes Mellitus: a Prospective Study.* Obes Surg, 2017. **27**(4): p. 1008-1012.
15. Qin, X. and P. Tso, *The role of apolipoprotein AIV on the control of food intake.* Curr Drug Targets, 2005. **6**(2): p. 145-51.
16. Broedl, U.C., et al., *Apolipoprotein A-IV is an independent predictor of disease activity in patients with inflammatory bowel disease.* Inflamm Bowel Dis, 2007. **13**(4): p. 391-7.
17. Li, X., et al., *Apolipoprotein A-IV reduces hepatic gluconeogenesis through nuclear receptor NR1D1.* J Biol Chem, 2014. **289**(4): p. 2396-404.
18. Shuvaev, V.V., et al., *Glycation of apolipoprotein E impairs its binding to heparin: identification of the major glycation site.* Biochim Biophys Acta, 1999. **1454**(3): p. 296-308.
19. Hellwig, M., et al., *Metabolization of the Advanced Glycation End Product N-ε-Carboxymethyllysine (CML) by Different Probiotic E. coli Strains.* 2019. **67**(7): p. 1963-1972.
20. K, A.A., S. Muniandy, and S.I. I, *Role of N-(carboxymethyl)lysine in the development of ischemic heart disease in type 2 diabetes mellitus.* J Clin Biochem Nutr, 2007. **41**(2): p. 97-105.

21. Cassano, V., et al., *Metabolic and Cognitive Effects of Ranolazine in Type 2 Diabetes Mellitus: Data from an in vivo Model.* Nutrients, 2020. **12**(2).
22. Hausenloy, D.J., et al., *The coronary circulation in acute myocardial ischaemia/reperfusion injury: a target for cardioprotection.* Cardiovasc Res, 2019. **115**(7): p. 1143-1155.
23. Krackhardt, F., et al., *Insights into coronary collateral formation from a novel porcine semiacute infarction model.* Coron Artery Dis, 2018. **29**(2): p. 127-137.
24. Ozen, G. and X. Norel, *Prostanoids in the pathophysiology of human coronary artery.* Prostaglandins Other Lipid Mediat, 2017. **133**: p. 20-28.
25. Ozkaramanli Gur, D., et al., *The role of novel cytokines in inflammation: Defining peripheral artery disease among patients with coronary artery disease.* Vasc Med, 2018. **23**(5): p. 428-436.
26. Rabbani, N. and P.J. Thornalley, *Advanced glycation end products in the pathogenesis of chronic kidney disease.* Kidney Int, 2018. **93**(4): p. 803-813.
27. Eckhardt, B.A., et al., *Accelerated osteocyte senescence and skeletal fragility in mice with type 2 diabetes.* JCI Insight, 2020. **5**(9).
28. Csiszar, A., et al., *Role of endothelial NAD(+) deficiency in age-related vascular dysfunction.* Am J Physiol Heart Circ Physiol, 2019. **316**(6): p. H1253-h1266.
29. Ozsavci, D., et al., *Native High-Density Lipoprotein and Melatonin Improve Platelet Response Induced by Glycated Lipoproteins.* Folia Biol (Praha), 2018. **64**(4): p. 144-152.
30. Li, B.Q., Y.C. Zhong, and X. Wang, *Plasma oxidized high-density lipoprotein and glycated apolipoprotein A-I concentrations in ST-segment elevation myocardial infarction patients with stress hyperglycaemia or high thrombus burden.* 2018. **123**(3): p. 158-166.
31. Sun, J., et al., *Gαi1 and Gαi3mediate VEGF-induced VEGFR2 endocytosis, signaling and angiogenesis.* Theranostics, 2018. **8**(17): p. 4695-4709.
32. Zhong, M., et al., *TIPE regulates VEGFR2 expression and promotes angiogenesis in colorectal cancer.* Int J Biol Sci, 2020. **16**(2): p. 272-283.
33. Oladipupo, S.S., et al., *Impaired tumor growth and angiogenesis in mice heterozygous for Vegfr2 (Flk1).* Sci Rep, 2018. **8**(1): p. 14724.
34. Bruning, U., et al., *Impairment of Angiogenesis by Fatty Acid Synthase Inhibition Involves mTOR Malonylation.* Cell Metab, 2018. **28**(6): p. 866-880.e15.
35. Wu, L. and L. Chen, *Characteristics of Nur77 and its ligands as potential anticancer compounds (Review).* Mol Med Rep, 2018. **18**(6): p. 4793-4801.
36. Ye, T., et al., *Orphan nuclear receptor TR3/Nur77 differentially regulates the expression of integrins in angiogenesis.* Microvasc Res, 2019. **122**: p. 22-33.
37. Chen, C., et al., *Orphan nuclear receptor TR3/Nur77 biologics inhibit tumor growth by targeting angiogenesis and tumor cells.* Microvasc Res, 2020. **128**: p. 103934.
38. Zeng, H., et al., *Orphan nuclear receptor TR3/Nur77 regulates VEGF-A-induced angiogenesis through its transcriptional activity.* J Exp Med, 2006. **203**(3): p. 719-29.

Figures

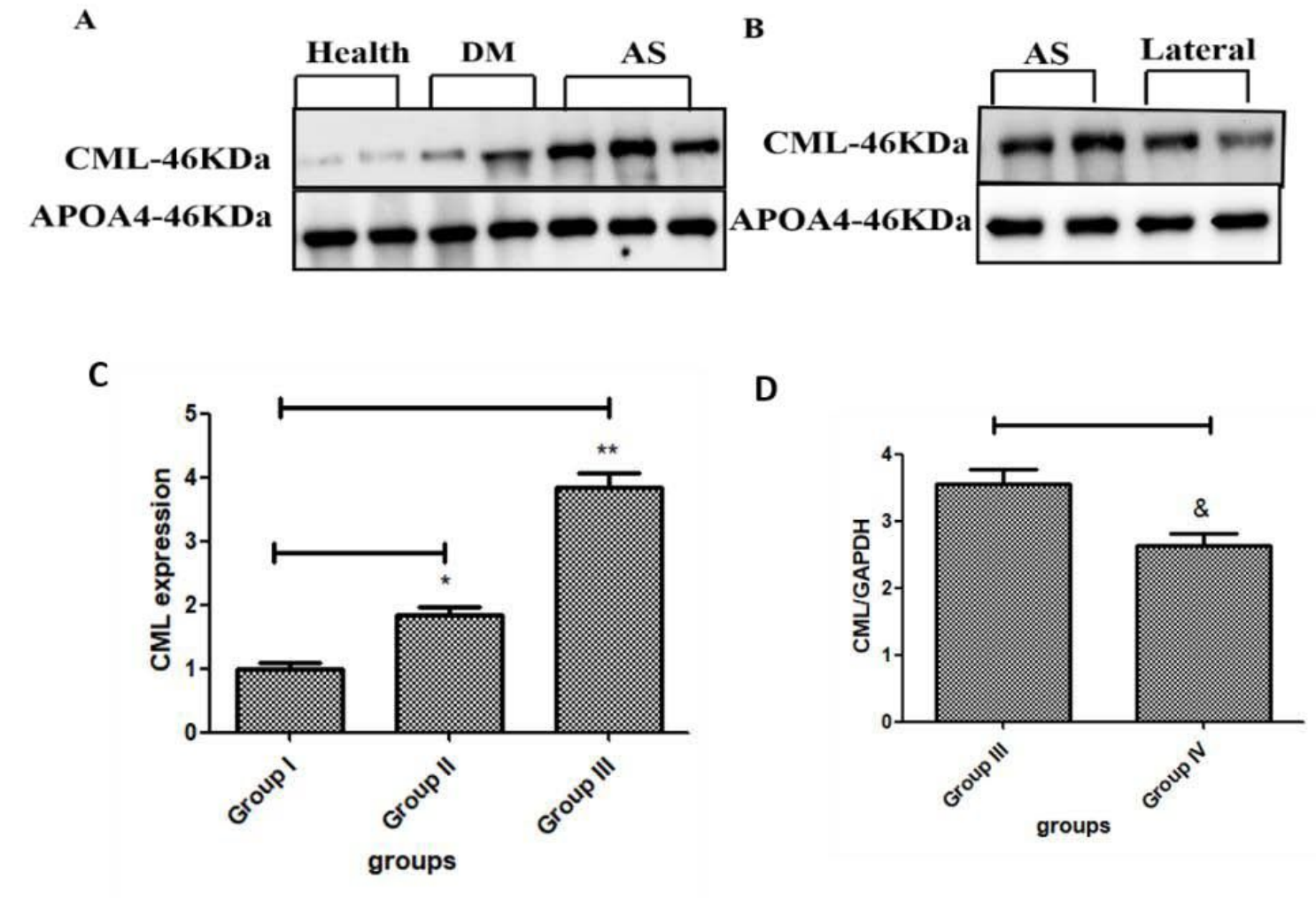

Figure 1

g-apo-A-IV is related to the patient's collateral neoplasia, Health: subjects without evidence of cardiovascular diseases or diabetes served as control subjects, Group I: Healthy volunteers,n=60; Group II: subjects with diabetes,n=80; Group III: subjects with Collateral circulation disorder.n=25;Group IV: subjects with no Collateral circulation disorder. n=55. The CCD group compared with Health and DM group. p□0.05 is statistically significant.

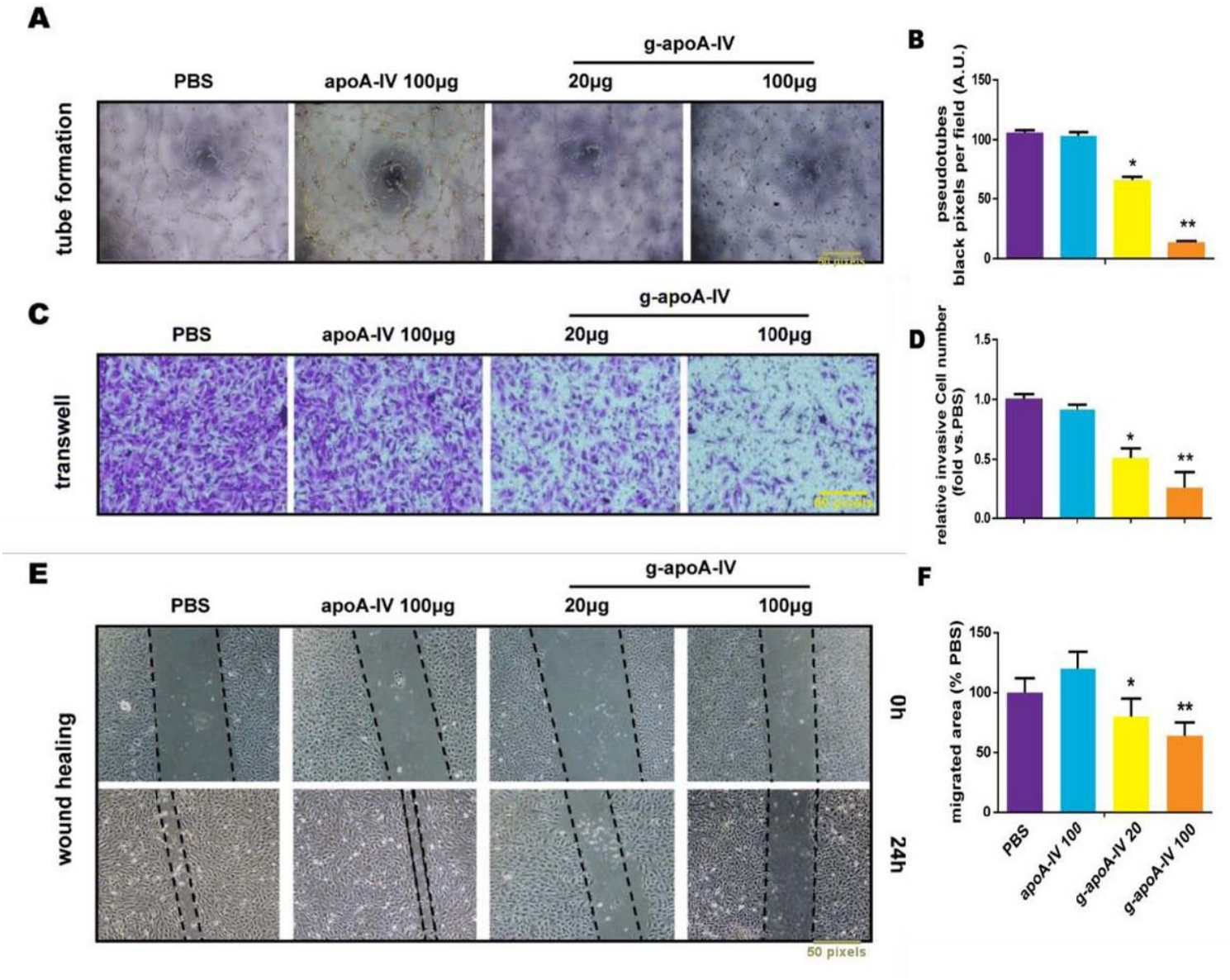

Figure 2

G-apo A-Ⅳ inhibit HUVEC Migration, proliferation and tube formation, PBS group: treated with PBS; apo A-IV group: treated with 100ug/mL apo A-IV; g-apo A-IV group: treated with 20～100ug/mL g-apo A-IV. All groups compared with PBS group and g- apo A-IV group compared with apo A-IV group. p＜0.05 is statistically significant. Experiment repeat more than 3 times.

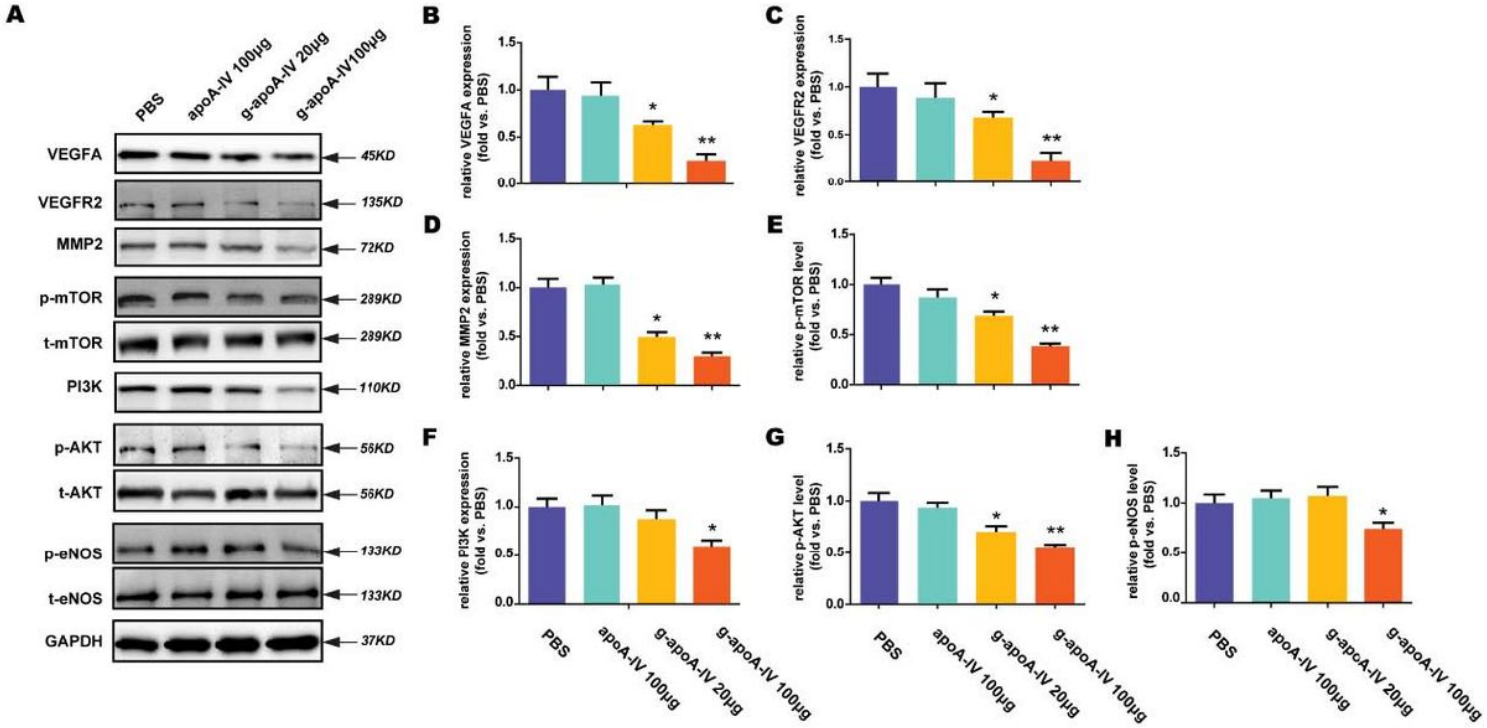

Figure 3

G-apo A-Ⅳ reduced VEGFR2/PI3K expression, PBS group: treated with PBS; apo A-IV group: treated with 100ug/mL apo A-IV; g-apo A-IV group: treated with 20～100ug/mL g-apo A-IV. All groups compared with PBS group and g- apo A-IV group compared with apo A-IV group. p＜0.05 is statistically significant. Experiment repeat more than 3 times.

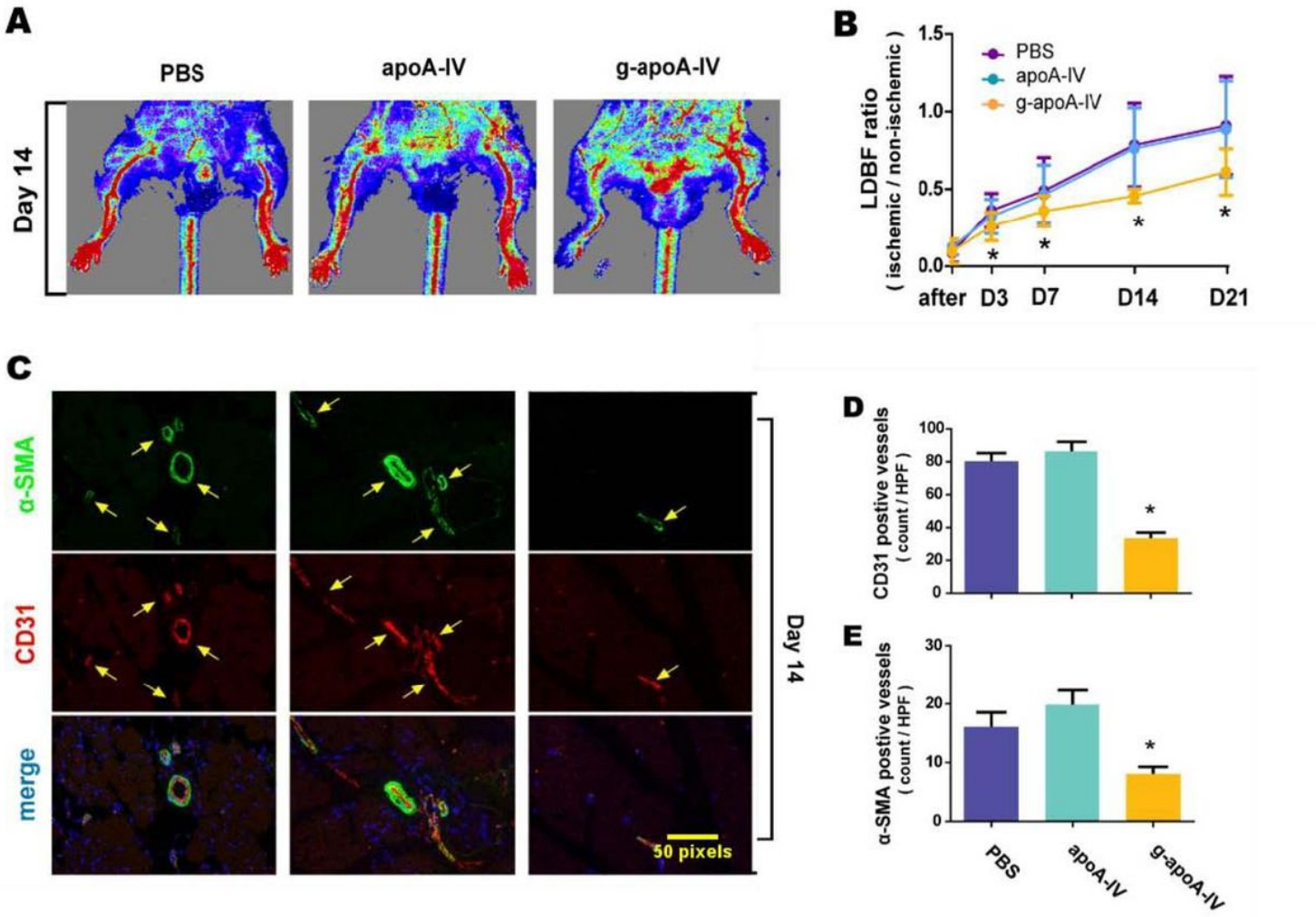

Figure 4

G-apo A-Ⅳ inhibit angiogenesis in Lower limb ischemia mice model, PBS group: treated with PBS; apo A-IV group: treated with 100ug apo A-IV; g-apo A-IV group: treated with 100ug g-apo A-IV. All groups compared with PBS group and g- apo A-IV group compared with apo A-IV group. p＜0.05 is statistically significant. n=10.

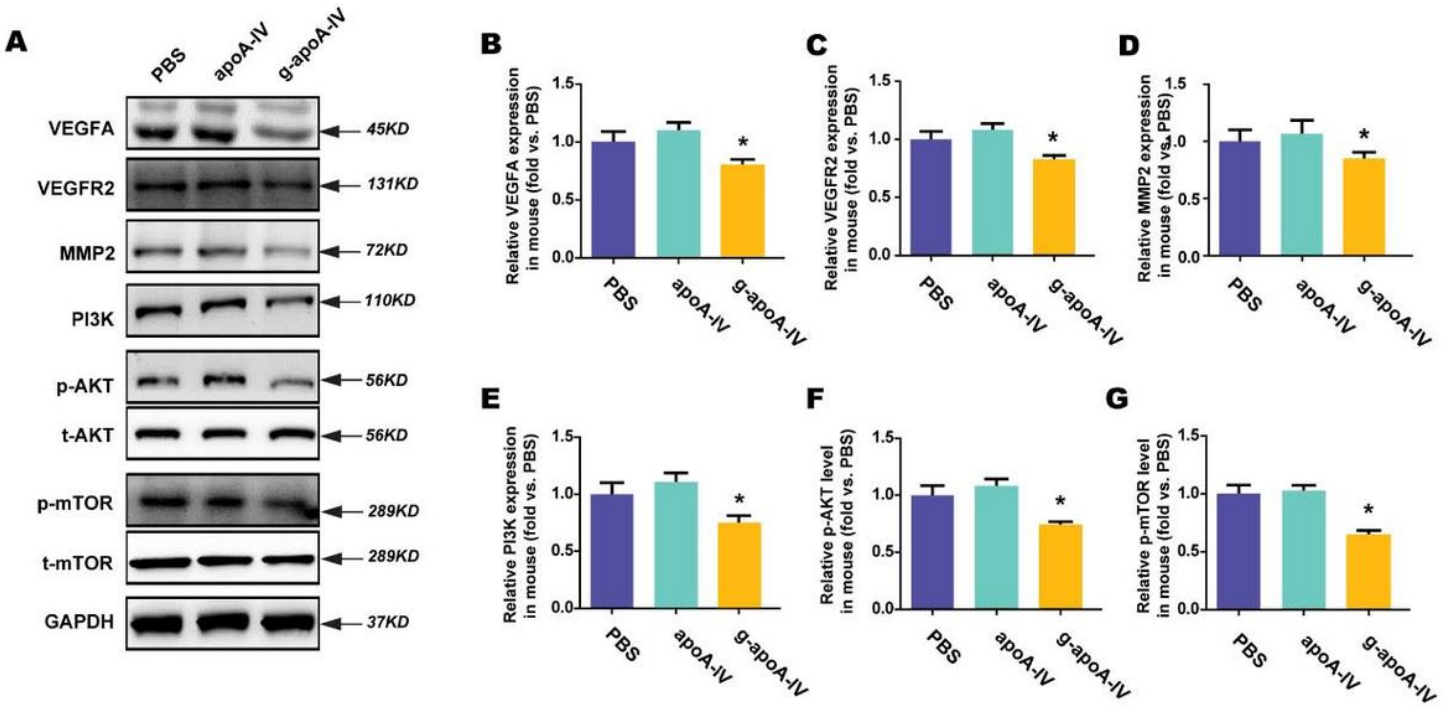

Figure 5

G-apo A-Ⅳ reduced VEGFR2/PI3K expression, PBS group: treated with PBS; apo A-IV group: treated with 100ug apo A-IV; g-apo A-IV group: treated with 100ug g-apo A-IV. All groups compared with PBS group and g- apo A-IV group compared with apo A-IV group. p＜0.05 is statistically significant. n=10.

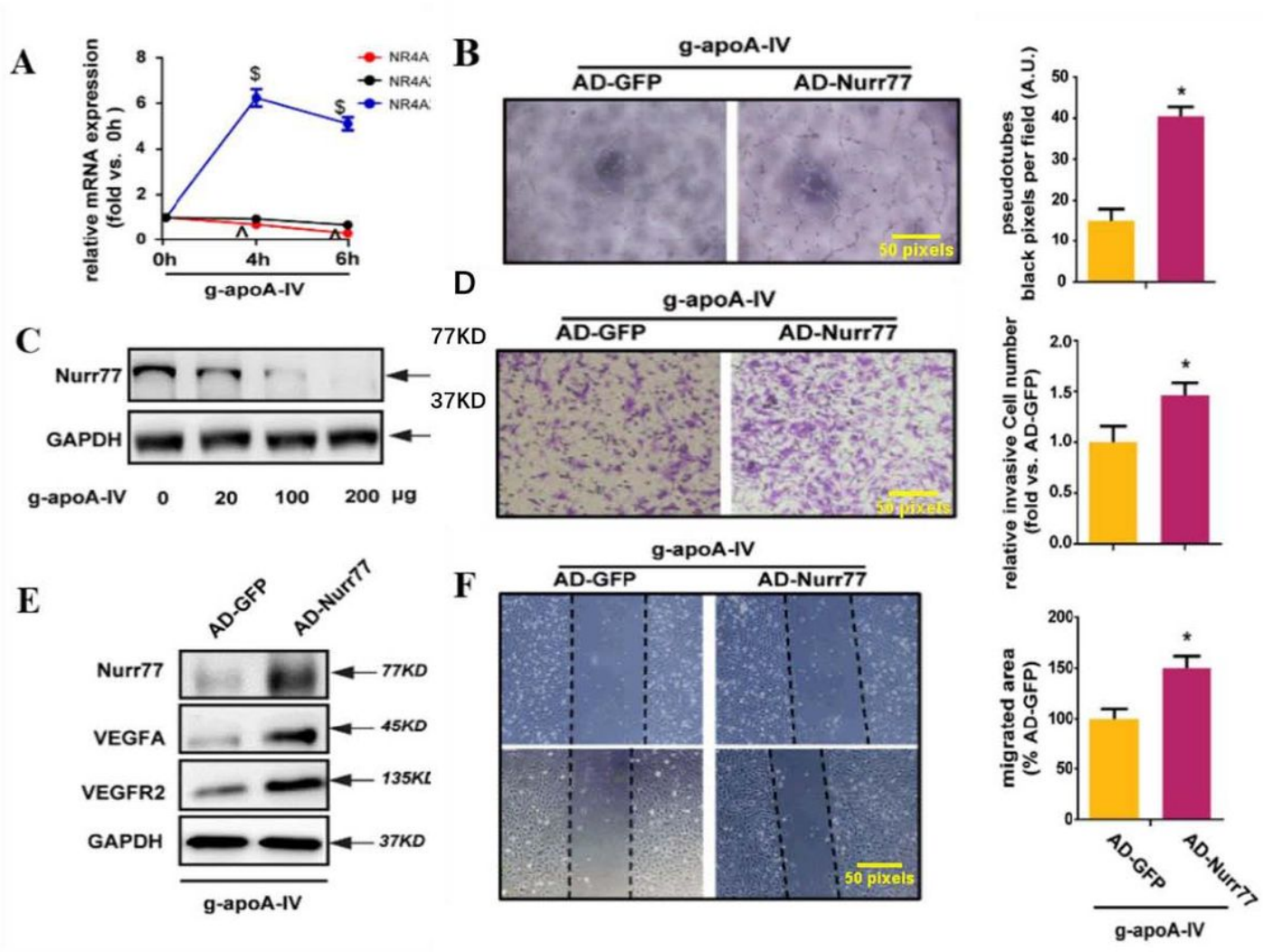

Figure 6

g-apo-A-IV inhibits endothelial tube formation through Nur77 in vitro, apo A-IV group: treated with 100ug/mL apo A-IV; g-apo A-IV group: treated with 100ug/mL g-apo A-IV. AD-Nur77: overexpression by Plasmid transfer; AD-GFP as control group. All groups compared with AD-GFP group, G-apo A-IV group compared with apo A-IV group. p□0.05 is statistically significant. Experiment repeat more than 3 times.

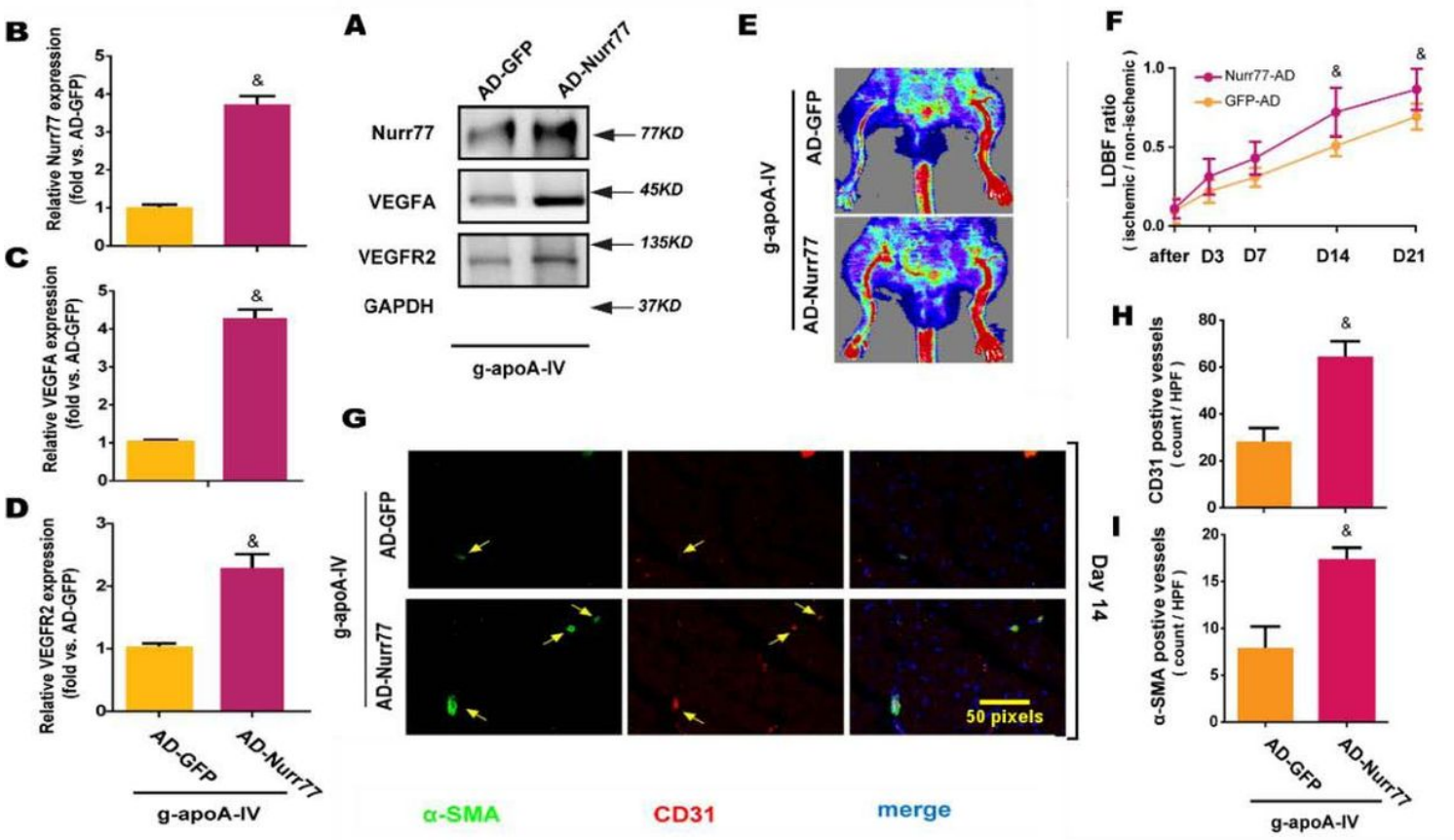

Figure 7

g-apo-A-IV inhibits endothelial tube formation through Nur77 in vivo, apo A-IV group: treated with 100ug apo A-IV; g-apo A-IV group: treated with 100ug g-apo A-IV. AD-Nur77: overexpression by Plasmid transfer; AD-GFP as control group. All groups compared with AD-GFP group, G-apo A-IV group compared with apo A-IV group. p□0.05 is statistically significant. n=10.