Biochemical and Biophysical Research Communications 423 (2012) 79–84

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of Lp(a)-induced functional impairment of endothelial cells and endothelial progenitor cells by hepatocyte growth factor

Masaaki Iwabayashi [a], Yoshiaki Taniyama [a,b,*], Fumihiro Sanada [a], Junya Azuma [a,b], Kazuma Iekushi [a,b], Keita Okayama [a], Amarnath Chatterjee [a], Hiromi Rakugi [b], Ryuichi Morishita [a,*]

[a] Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan
[b] Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 2 May 2012
Available online 23 May 2012

Keywords:
Lp(a)
HGF
PAD
Senescence
EPC

ABSTRACT

Background: Lipoprotein (a) (Lp(a)) is one of the risk factors for peripheral artery disease (PAD). Our previous report demonstrated that hepatocyte growth factor (HGF) gene therapy attenuated the impairment of collateral formation in Lp(a) transgenic mice. Since risk factors for atherosclerosis accelerate endothelial senescence and impair angiogenesis, we examined the role of Lp(a) in dysfunction and senescence of endothelial progenitor cells (EPC) and endothelial cells.

Methods: In vitro and in vivo incorporation assays were performed using ex-vivo expanded DiI-labeled human EPC. Senescence of cultured endothelial cells, production of oxidative stress and angiogenesis function were evaluated by SA-β-galactosidase staining, dihydroethidium (DHE) staining and Matrigel assay, respectively.

Results: EPC transplantation significantly stimulated recovery of ischemic limb perfusion, while EPC pretreated with Lp(a) did not increase ischemic limb perfusion. Impairment of angiogenesis by EPC with Lp(a) was associated with a significant decrease in CD31-positive capillaries and DiI-labeled EPC. Importantly, Lp(a) significantly accelerated the onset of senescence and production of reactive oxygen species (ROS) in human aortic endothelial cells, accompanied by a significant increase in the protein expression of p53 and p21. On the other hand, HGF significantly attenuated EPC dysfunction, senescence, ROS production, and p53 and p21 expression induced by Lp(a).

Conclusion: Lp(a) might affect atherosclerosis via acceleration of senescence, ROS production, and functional impairment of the endothelial cell lineage. HGF might have inhibitory effects on these atherogenic actions of Lp(a).

1. Introduction

Lipoprotein (a) (Lp(a)) is a lipid particle with a structure consisting of apolipoprotein (a) (apo(a)), which is linked covalently to apolipoprotein B-100 (apoB-100) of low-density lipoprotein (LDL) through disulfide bonds [1]. As apo(a), a component of Lp(a) has a high degree of homology with plasminogen [1,2], a high circulating Lp(a) level may inhibit thrombolysis [3] and fibrin clearance, leading to the development of atherosclerosis. We previously showed that serum Lp(a) concentration was negatively correlated with blood flow after creating hindlimb ischemia in a mouse Lp(a) transgenic model [4]. In addition, Lp(a) transgenic mice exhibited lower capillary density after hindlimb ischemia in comparison with wild type mice. Indeed, Lp(a) is considered to be an independent risk factor for peripheral artery disease (PAD) [5], ischemic heart disease and stroke [6,7]. Nevertheless, the underlying mechanisms by which Lp(a) impairs angiogenic function is not fully elucidated.

In this study, we focused on the effect of Lp(a) on the function, especially angiogenesis, of endothelial progenitor cells (EPC) and endothelial cells (EC), since previous reports suggest that risk factors for atherosclerosis impairs angiogenic function of EC and EPC [8]. Atherogenic lipoproteins, such as oxidized LDL [9,10] and remnant-like particles [11], are known to trigger the onset of senescence in EC and EPC via induction of reactive oxygen species (ROS) production. Although Lp(a) has been thought to be an atherogenic lipoprotein, no study has reported the effects of Lp(a) on senescence of EPC and EC. Thus, in the present study, we investigated Lp(a)-induced senescence and impaired angiogenesis of EPC and EC. In addition, we further explored the prevention of Lp(a)-induced dysfunction. Since transfection of HGF plasmid DNA into the adductor muscle of the ischemic limb significantly

* Corresponding authors. Address: Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, 2–2 Yamada-oka, Suita, Osaka 565-0871, Japan. Fax: +81 6 6879 3409 (R. Morishita).
E-mail addresses: taniyama@cgt.med.osaka-u.ac.jp (Y. Taniyama), morishit@cgt.med.osaka-u.ac.jp (R. Morishita).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.086

increased blood flow and capillary density in Lp(a) transgenic mice [4], we hypothesized that HGF might attenuate Lp(a)-induced dysfunction of EPC and EC.

2. Materials and methods

2.1. Ex-vivo expanded EPC transplantation in hindlimb ischemia nude mouse model

All procedures were performed in accordance with the Institutional Animal Committee of Osaka University School of Medicine. To elucidate the effect of Lp(a) on EPC function in vivo, an EPC transplantation model was employed. Male athymic nude mice aged 8–10 weeks were purchased from Charles River Japan. Mice were anesthetized with pentobarbitone for operative resection of one femoral artery, as described previously [12]. One day after operation, 1×10^5 culture-expanded EPC were intravenously injected via the jugular vein. To evaluate EPC incorporation into the vasculature in ischemic muscle, EPC were labeled with DiI. At 1 and 7 days after the induction of ischemia, ischemic/non-ischemic limb blood flow ratio was measured with a laser Doppler imager (Moor LDI-Mark 2, Moor Instruments). Tissue sections from the adductor muscles of ischemic limbs were harvested on day 7. To assess EPC incorporation, tissues were embedded in OCT compound (Sakura Finetek, Tokyo, Japan) and snap-frozen, and sections were stained with anti-mouse CD31 antibody (BD Pharmingen San Diego, CA). The numbers of DiI-labeled EPC was counted, and CD31-positive capillary density was determined in 5 random fields under a fluorescence microscope.

2.2. Cell culture

Human aortic EC (LONZA, Portsmouth, NH) were cultured in endothelial basal medium-2 (EBM-2) (Clonetics, Walkersville, Maryland, USA) supplemented with EGM and 5% fetal bovine serum (FBS). After 24-h starvation with EBM-2 with 0.5% FBS, EC were pretreated with HGF (PeproTech EC, London, UK) (20 or 50 ng/ml) for 3 h before Lp(a) stimulation.

EPC were isolated from fresh human peripheral blood using density gradient centrifugation with Histopaque 1077 (Sigma Chemical Co., St. Louis, MO), as previously described [13]. Isolated EPC from a healthy volunteer were seeded on fibronectin (Sigma)-coated (20 μg/ml) dish. After 4 days of culture with EBM-2 containing EGM and 5% FBS, non-adherent cells were discarded by washing with PBS twice, fresh medium was applied, and EPC were cultured through day 7. On day 7, EPC phenotype was determined using direct fluorescent staining to detect dual binding of FITC-labeled Ulex europaeus agglutinin (UEA)-1 (Sigma) and 1,1′-dioctadecyl-3,3,3′,3′,-tetramethylindocarbocyanine perchlorate (DiI)-labeled acetylated low-density lipoprotein (acLDL; Biomedical Technologies, Stoughton, MA) [13], and the expression of CD31 (Millipore), VE-Cadherin (Cell Signaling Technology) and von Willebland Factor (vWF) (Sigma) as endothelial cell marker were checked as previously described [14–16] (Fig. S1). After 24 h of starvation with EBM-2 with 0.1% FBS, EPC were pretreated with HGF (50 ng/ml) for 3 h before Lp(a) stimulation. Lp(a) was purified from plasma of donors, as previously described [17].

2.3. SA-β-galactosidase activity assay

Senescence associated (SA)-β-galactosidase activity was detected using a Senescence beta-Galactosidase Staining Kit (Cell Signaling Technology, Beverly, MA), as previously described [18]. Briefly, after incubation for 24 h with Lp(a), cells were fixed, followed by application of x-gal and incubation overnight at 37 °C. The number of SA-β-gal-positive cells was manually counted in 10 random fields per dish under bright field illumination.

2.4. Superoxide (O_2^-) detection assay

Superoxide detection in vitro using dihydroethidium (DHE) staining was described previously [19]. After 30 min of incubation with Lp(a), cells were fixed with acetone, permeabilized with 0.1% TritonX-PBS, and stained with 10 μM DHE (Invitrogen, Carlsbad, CA) for 30 min. Fluorescent activity was determined in 10 random fields per dish under a fluorescence microscope.

2.5. Tubule formation assay

First 1×10^5 human aortic EC were seeded on a Matrigel (Growth Factor Reduced Phenol Red Free, Becton–Dickinson, Franklin Lakes, USA)-coated 24-well dish, as previously described [20]. Lp(a) stimulation with or without HGF (50 ng/ml) was performed at the same time. After stimulation with Lp(a), tubule formation in five random fields was evaluated under a phase contrast light microscope at the indicated times. A tubular formation was defined as a structure whose length was at least four times its width [17].

2.6. Incorporation assay

EPC function was determined by EC-EPC incorporation assay, as previously described [21]. EPC stimulated with Lp(a) with or without HGF (50 ng/ml) were labeled with 2.5 μg/ml DiI (Molecular Probes) in PBS for 5 min at 37 °C and 15 min at 4 °C [22]. DiI-labeled EPC (2×10^4) were co-cultured with human aortic EC (8×10^4) on a Matrigel-coated 24-well dish for 24 h. Incorporation ability was determined in 10 random fields per well under a fluorescence microscope.

2.7. Western blotting

The cells were lysed using lysis buffer (50 mmol/L Tris–HCl, 2.5 mmol/L EGTA, 1 mmol/L EDTA, 10 mmol/L NaF, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mmol/L PMSF, 1 mmol/L Na3VO4). Protein extract (10 μg) was fractionated on SDS-PAGE gels and transferred to a PVDF membrane (Millipore, Bedford, MA) as described elsewhere. Antibodies to p53 and α-tubulin were purchased from Santa Cruz Biotechnology. Antibody to $p21^{Waf/Cip1}$ was purchased from Cell Signaling Technology.

2.8. Statistical analysis

All values are expressed as mean ± SD. One way ANOVA followed by Tukey-Kramer's adjustment was used to determine the significance of differences in multiple comparisons. Statistical analysis was performed by one-way analysis of variance (ANOVA) using JMP9.0 (SAS Institute, Inc., Cary, NC USA). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. HGF attenuated impairment of angiogenic activity of EPC induced by Lp(a)

First, we examined the effects of Lp(a) on the angiogenic activity of EPC. To determine angiogenic activity, DiI-labeled EPC were co-cultured with human aortic EC on Matrigel. As shown in Fig. 1, pre-treatment with Lp(a) significantly impaired the ability of EPC to be incorporated into the tube-like structure of EC.

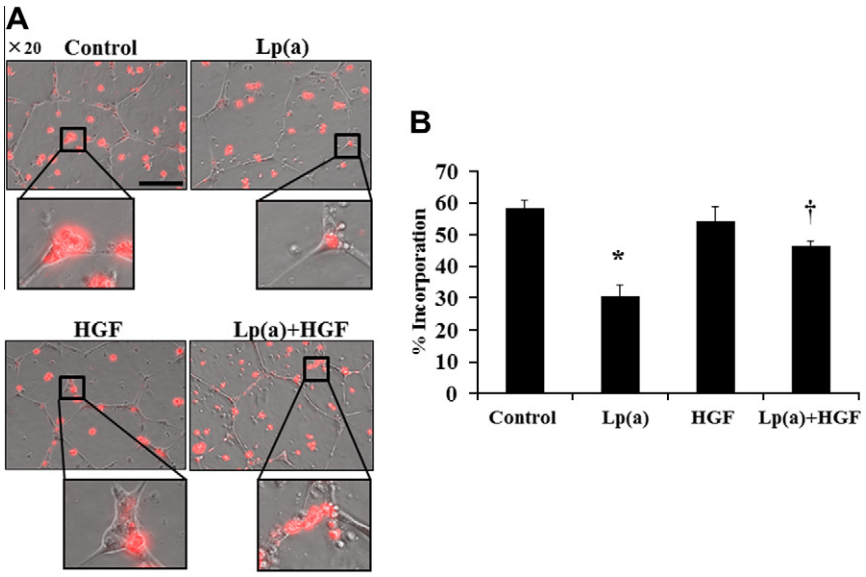

Fig. 1. Effect of Lp(a) on EPC function in vitro. (A) Representative image of tubule of human aortic EC and DiI-labeled EPC (red color). (B) Quantitative analysis of percentage of incorporation of EPC into tubule, determined in 10 random fields per well. Scale bar = 200 μm, $n = 3$, $^{*}P < 0.01$ vs. control, †$P < 0.01$ vs. Lp(a). Control: vehicle, Lp(a): EPC pre-treated with Lp(a) (5 μg/ml), HGF: EPC pre-treated with HGF (50 ng/ml), Lp(a) + HGF: EPC pre-treated with Lp(a) (5 μg/ml) and HGF (50 ng/ml).

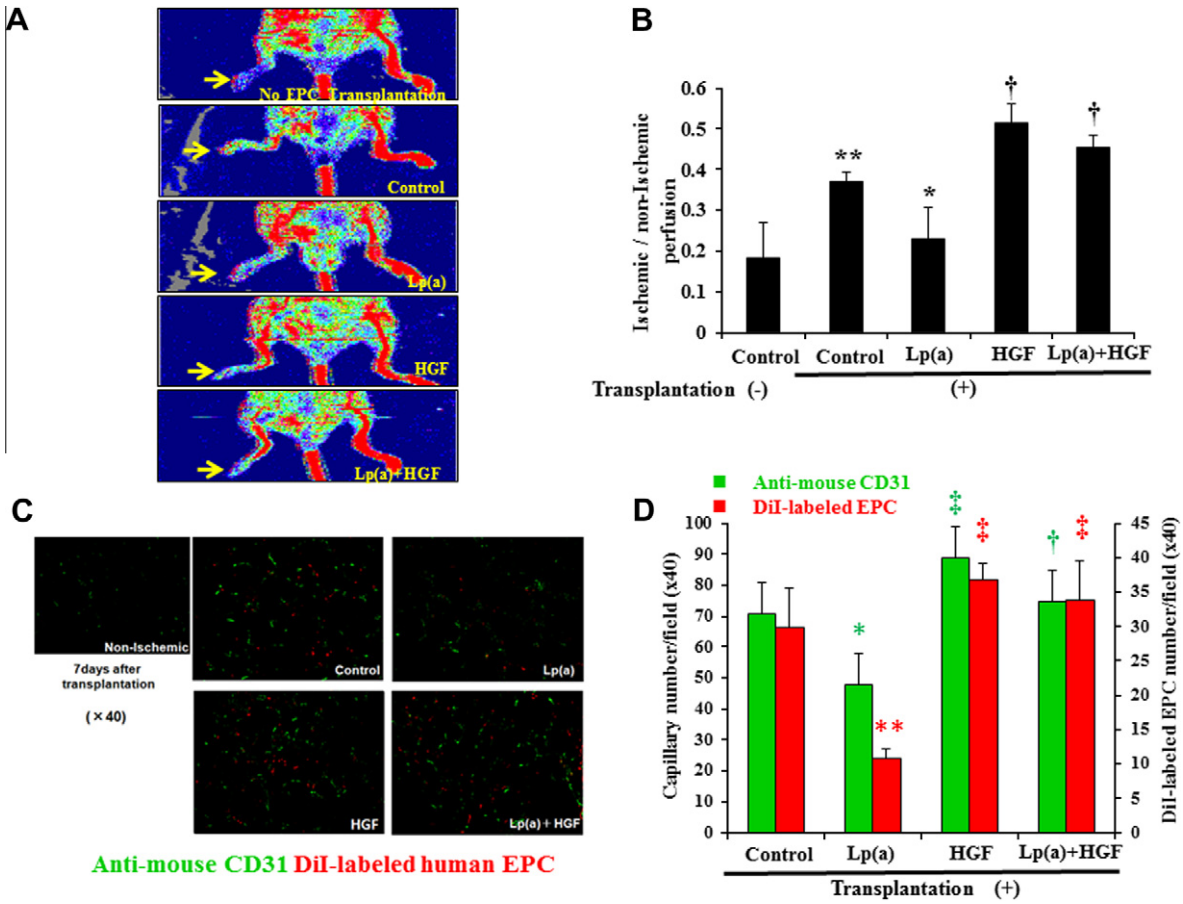

Fig. 2. Effect of Lp(a) on EPC function in vivo. (A) Representative LDI images of limb perfusion on day 7 after transplantation of EPC. Yellow arrows indicate ischemic foot. Red shows blood perfusion. (B) Quantitative analysis of ischemic/non-ischemic limb perfusion ratio by LDI. $n = 3$–4, $^{**}P < 0.01$ vs. EPC (−), $^{*}P < 0.05$ vs. control EPC, †$P < 0.01$ vs. Lp(a). EPC (−) = no EPC transplantation, control = EPC transplantation alone, Lp(a) = transplantation of EPC treated with Lp(a) (5 μg/ml), Lp(a)+HGF = EPC treated with Lp(a) (5 μg/ml) + HGF (50 ng/ml) (C) Representative photograph of double fluorescence in ischemic limb on day 7. CD31-positive cell are identified as green and DiI-labeled EPC are red. (D) Quantitative analysis of DiI-labeled EPC (red) and host CD31-positive endothelial cells (green) in tissue sections of ischemic adductor muscles. $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control, †$P < 0.05$, ‡$P < 0.01$ vs. Lp(a). Control = EPC transplantation alone, Lp(a) = transplantation of EPC treated with Lp(a) (5 μg/ml), Lp(a) + HGF = EPC treated with Lp(a) (5 μg/ml) + HGF (50 ng/ml).

However, co-treatment with HGF significantly attenuated the decrease in incorporation of EPC induced by Lp(a). To further investigate the function of EPC in neovascularization in ischemic tissue, EPC were transplanted into an athymic nude mouse hindlimb ischemia model as an in vivo model. At 7 days after transplantation of EPC, EPC transplantation significantly stimulated the recovery of ischemic limb perfusion as compared to the no treatment group (Fig. 2A and B). However, transplantation of EPC pretreated with Lp(a) did not increase ischemic limb perfusion. In contrast, pretreatment with HGF prevented the impairment of recovery of ischemic limb perfusion. As shown in Fig. 2C and D, pretreatment with Lp(a) resulted in a significant decrease in CD31-positive capillaries in ischemic tissue, associated with a decrease in the number of DiI-labeled EPC as compared to control. In contrast, co-treatment with HGF attenuated the decrease in CD31-positive capillaries and DiI-labeled EPC induced by Lp(a) treatment in ischemic tissue.

3.2. Molecular mechanisms of impairment of EC function by Lp(a)

Since an in vivo study demonstrated that Lp(a) impaired the angiogenic activity of EPC, we further explored its molecular mechanisms. Impairment of angiogenic activity by Lp(a) was confirmed by an in vitro Matrigel tubule formation assay. As shown in Fig. 3A and B, Lp(a) treatment significantly inhibited tubule formation as compared to control in a time and dose-dependent manner. However, addition of HGF ameliorated Lp(a)-induced tubule collapse, as shown in Fig. 3C and D.

Therefore, we next examined whether Lp(a) would influence cellular senescence by measuring SA-β-gal activity in human aortic EC. As shown in Fig. 4A and B, Lp(a) significantly accelerated senescence in human aortic EC. In contrast, pretreatment with HGF significantly attenuated senescence induced by Lp(a). As excessive production of ROS is known to promote cellular senescence, the production of superoxide was measured by dihydroethidium staining. As shown in Fig. 4A and B, Lp(a) stimulation resulted in a significant increase in the production of superoxide in human aortic EC, while pretreatment with HGF significantly decreased the production of superoxide induced by Lp(a) in human aortic EC (Representative photographs are shown in Fig. S2A and B). As cells with DNA damage (e.g. by ROS) have induced activation of the anti-oncogene, p53, and its downstream cyclin dependent kinase inhibitor, p21, leading to cell cycle arrest and induction of premature cell senescence, we finally measured the protein expression of p53 and p21 in human aortic EC. It was observed that incubation of human aortic EC with Lp(a) for 24 h significantly increased p53 expression in a time and dose-dependent manner (Fig. S3A and B). In contrast, treatment with HGF significantly decreased the expression of Lp(a)-induced p53 and its downstream p21 (Fig. 4C and D).

4. Discussion

It is well known that risk factors for atherosclerosis such as angiotensin II (Ang II), high glucose and oxidized low density lipoprotein (LDL) impair the function, such as tubular formation and proliferation, of EC and EPC. Similarly, in clinical practice, the Framingham risk score was negatively correlated with the number and functional activity of circulating EPC [23]. Although EPC number is reported to be decreased in PAD patients with risk factors such as hypertension, diabetes mellitus, and high LDL-cholesterol level, the relationship between Lp(a), a well-known independent

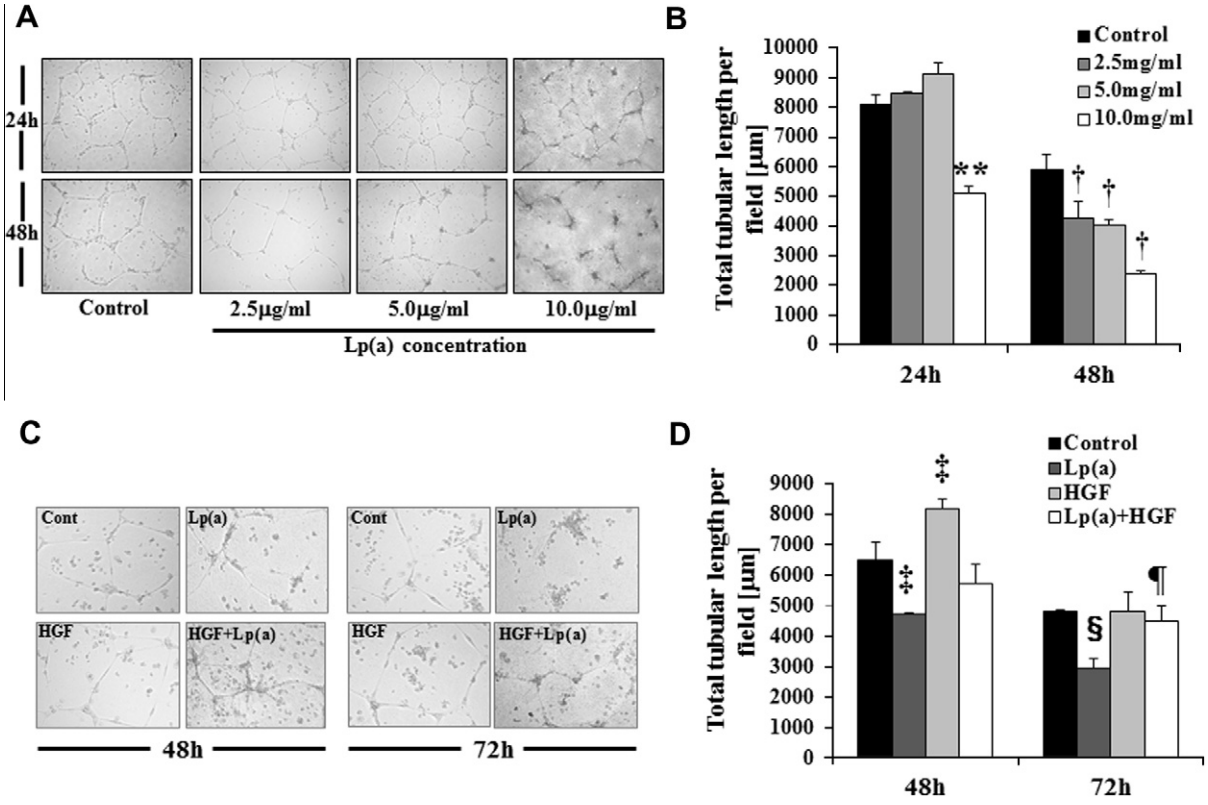

Fig. 3. Effect of Lp(a) on tubule formation of EC. (A) Representative photographs of time-dependent tubule formation of human aortic EC. (B) Quantitative analysis of tubule length. Tubule length was determined in five random fields per well. $n = 3$, $^{**}P < 0.01$ vs. 24 h control, †$P < 0.01$ vs. 48 h control. (C) Representative photographs of formed tubule of HAEC. HAEC were pretreated with HGF (20 ng/ml) for 3 h before stimulation with Lp(a) (5 μg/ml). (D) Quantitative analysis of tubular length. Tubular length were determined in five random fields per well. $n = 3$, ‡$P < 0.05$ vs. 48 h control, §$P < 0.01$ vs. 72 h control, ¶$P < 0.05$ vs. 72 h Lp(a).

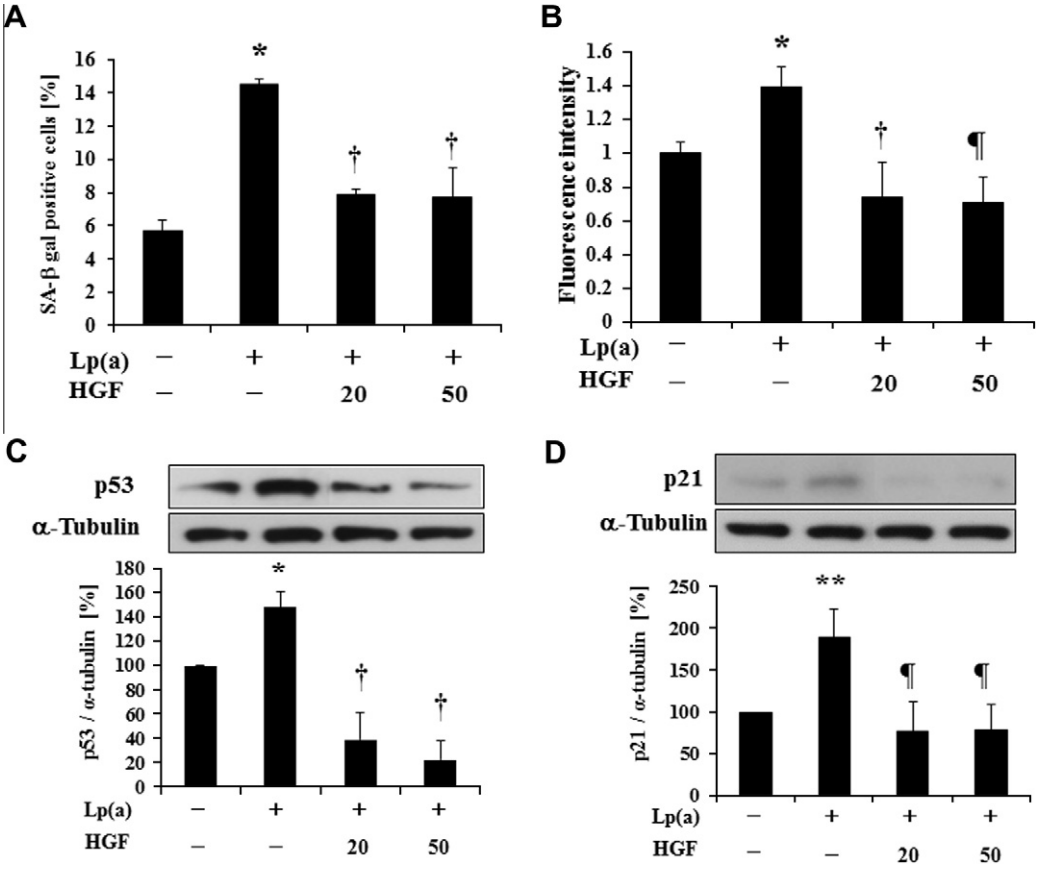

Fig. 4. Inhibitory effects of HGF on Lp(a)-induced endothelial cell senescence, superoxide production, and protein expression of 53 and p21 in EC. (A) Quantitative analysis of SA-β-gal-positive cells. SA-β-gal-positive cells were manually counted in 10 random fields per dish under bright field illumination. $n = 3$, $^{*}P < 0.01$ vs. control, †$P < 0.01$ vs. Lp(a). (B) Quantitative analysis of fluorescence intensity of DHE staining. Fluorescent activity was determined in 10 random fields per dish under a fluorescence microscope. $n = 3$, $^{*}P < 0.05$ vs. control, †$P < 0.05$ vs. Lp(a), ¶$P < 0.01$ vs. Lp(a). (C and D) Effects of Lp(a) (5 μg/ml) and HGF (20 or 50 ng/ml) on p53 (C) and p21 (D) protein expression in human aortic EC. Prior to stimulation of EC with Lp(a) (5 μg/ml) for 24 h, they were treated with HGF (20 or 50 ng/ml) was treated for 3 h. Upper panel: Representative photograph of western blotting. Lower panel: Quantitative analysis of expression of p53. $n = 4$, $^{*}P < 0.05$ vs. control, †$P < 0.01$ vs. Lp(a), ¶$P < 0.05$ vs. Lp(a).

cardiovascular risk factor, and EPC function has not been addressed. Notably, our previous study demonstrated that angiogenic function was significantly diminished in Lp(a) transgenic mice, whereas transfection of HGF plasmid improved the impaired angiogenesis [4]. In addition, we also showed that HGF inhibited Ang II-induced EPC senescence and functional impairment [20]. Therefore, in this study, we first investigated the role of Lp(a) on EPC function in vivo as well as in vitro.

As expected, Lp(a) treatment significantly diminished the incorporation of EPC into the tubule-like structure of EC. Consistently, transplantation of ex-vivo expanded EPC treated with Lp(a) significantly inhibited the recovery of ischemic limb perfusion, associated with a significant decrease in numbers of DiI-labeled EPC and CD31-positive cells in the ischemic hindlimb. EPC is thought to participate in angiogenic processes via various mechanisms [24]. A direct contribution of EPC are believed to be differentiation into EC and induction of vasculogenesis [25]. In addition, an indirect mechanism is thought to be secretion of a variety of cytokines and growth factors, leading to enhancement of proliferation and migration of host EC [26]. Indeed, previous study reported that transplanted EPC is mainly localized in perivascular region in ischemic myocardium [27]. These dual mechanisms may contribute to restoration of ischemic tissue blood flow by EPC transplantation, however, it has not been clarified yet which is the main mechanism of EPC-mediated increase in capillaries in ischemic tissue since it is difficult to dissect in vivo [26]. In the present study, it was observed that DiI-labeled EPC and host CD31 positive cell was not co-localized in ischemic muscle. Thus, it was suggested that transplanted-EPC contributes increase in capillary density by indirect mechanism rather than direct neovascularization, at least in this study.

These data demonstrated that Lp(a) is also a risk factor that influence PAD and critical limb ischemia (CLI), suggesting that EPC or bone marrow transplantation into PAD patients might be less effective in those with a high Lp(a) level. In contrast, we showed that HGF significantly improved post-transplantation ischemic limb perfusion impaired by Lp(a).

Gene therapy using HGF plasmid DNA has been shown to improve ischemic ulcer via an increase in peripheral blood flow in several clinical studies [28,29]. Improvement of clinical symptoms by HGF gene therapy might be partially due to the improvement of EPC dysfunction.

Impairment of EPC function might be due to senescence, since senescent cells lose their angiogenic function [30] because of loss of proliferative activity. In the present study, Lp(a) accelerated the onset of senescence in EC, probably due to excessive O_2^- production, increase in protein expression of p53 and p21, and a significant increase in tubule collapse. ROS is one of the most important player in induction of premature cell senescence, and it was reported that Lp(a) induced excessive O_2^- production in human umbilical vein EC [31] and glomerular cells [32]. Thus, Lp(a) could cause to damage DNA and arrest cell cycle through ROS production. In contrast, HGF significantly inhibited Lp(a)-induced O_2^- production, cell senescence and tubule collapse in EC, consistent with previous reports that HGF has multiple actions such as anti-oxidative [20] and anti-inflammatory effects [33] in vascular cells.

Overall, the present study demonstrated that Lp(a) induced the functional impairment such as impaired incorporation of EPC into blood vessels, progression of senescence, and ROS production of EC, leading to impairment of angiogenic function in endothelial lineage cells. These data indicate that EPC dysfunction and endothelial cell senescence might contribute to the pathogenesis of Lp(a)-induced atherosclerosis. As HGF attenuated Lp(a)-induced dysfunction of EPC and EC, HGF gene therapy might be effective to treat critical limb ischemia in PAD patients with high Lp(a) level. Further clarification of Lp(a)-induced atherosclerosis might provide new therapeutic options to treat the patients with high Lp(a) concentration.

Conflict of interest

None.

Acknowledgments

This work was partially supported by a Grant-in-Aid from the Organization for Pharmaceutical Safety and Research, a Grant-in-Aid from the Ministry of Public Health and Welfare, a Grant-in-Aid from Japan Promotion of Science, and through special coordination funds of the Ministry of Education, Culture, Sports, Science and Technology, of the Japanese Government.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.086.

References

[1] J. McLean, J. Tomlinson, W. Kuang, D. Eaton, E. Chen, G. Fless, A. Scanu, R. Lawn, CDNA sequence of human apolipoprotein (a) is homologous to plasminogen, Nature 330 (1987) 132–137.
[2] D.L. Eaton, G.M. Fless, W.J. Kohr, J.W. McLean, Q.T. Xu, C.G. Miller, R.M. Lawn, A.M. Scanu, Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen, Proceedings of the National Academy of Sciences of the United States of America 84 (1987) 3224–3228.
[3] B.J. Biemond, P.W. Friederich, M.L. Koschinsky, M. Levi, W. Sangrar, J. Xia, H.R. Buller, J.W. ten Cate, Apolipoprotein(a) attenuates endogenous fibrinolysis in the rabbit jugular vein thrombosis model in vivo, Circulation 96 (1997) 1612–1615.
[4] R. Morishita, M. Sakaki, K. Yamamoto, S. Iguchi, M. Aoki, K. Yamasaki, K. Matsumoto, T. Nakamura, R. Lawn, T. Ogihara, Impairment of collateral formation in lipoprotein (a) transgenic mice. therapeutic angiogenesis induced by human hepatocyte growth factor gene, Circulation 105 (2002) 1491.
[5] B. Dieplinger, A. Lingenhel, N. Baumgartner, W. Poelz, H. Dieplinger, M. Haltmayer, F. Kronenberg, T. Mueller, Increased serum lipoprotein (a) concentrations and low molecular weight phenotypes of apolipoprotein (a) are associated with symptomatic peripheral arterial disease, Clinical Chemistry 53 (2007) 1298.
[6] A.G. Bostom, L.A. Cupples, J.L. Jenner, J.M. Ordovas, L.J. Seman, P.W. Wilson, E.J. Schaefer, W.P. Castelli, Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study, JAMA: Journal of the American Medical Association 276 (1996) 544–548.
[7] G. Jurgens, W.C. Taddei-Peters, P. Koltringer, W. Petek, Q. Chen, J. Greilberger, P.F. Macomber, B.T. Butman, A.G. Stead, J.H. Ransom, Lipoprotein(a) serum concentration and apolipoprotein(a) phenotype correlate with severity and presence of ischemic cerebrovascular disease, Stroke: A Journal of Cerebral Circulation 26 (1995) 1841–1848.
[8] T. Minamino, I. Komuro, Vascular cell senescence. contribution to atherosclerosis, Circulation Research 100 (2007) 15.
[9] T. Imanishi, T. Hano, T. Sawamura, I. Nishio, Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction, Clinical and Experimental Pharmacology and Physiology 31 (2004) 407–413.
[10] J. Maier, L. Barenghi, S. Bradamante, F. Pagani, Induction of human endothelial cell growth by mildly oxidized low density lipoprotein, Atherosclerosis 123 (1996) 115–121.
[11] L. Liu, T. Wen, X.Y. Zheng, D.G. Yang, S.P. Zhao, D.Y. Xu, G.H. Lü, Remnant-like particles accelerate endothelial progenitor cells senescence and induce cellular dysfunction via an oxidative mechanism, Atherosclerosis 202 (2009) 405–414.
[12] Y. Taniyama, R. Morishita, K. Hiraoka, M. Aoki, H. Nakagami, K. Yamasaki, K. Matsumoto, T. Nakamura, Y. Kaneda, T. Ogihara, Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes, Circulation 104 (2001) 2344.
[13] C. Kalka, H. Masuda, T. Takahashi, W. Kalka-Moll, M. Silver, M. Kearney, T. Li, J. Isner, T. Asahara, Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 3422.
[14] Y. Fan, J. Ye, F. Shen, Y. Zhu, Y. Yeghiazarians, W. Zhu, Y. Chen, M.T. Lawton, W.L. Young, G.Y. Yang, Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis in vitro, Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism 28 (2008) 90–98.
[15] Y. Wu, J.E. Ip, J. Huang, L. Zhang, K. Matsushita, C.C. Liew, R.E. Pratt, V.J. Dzau, Essential role of ICAM-1/CD18 in mediating EPC recruitment, angiogenesis, and repair to the infarcted myocardium, Circulation Research 99 (2006) 315–322.
[16] S. Dimmeler, A. Aicher, M. Vasa, C. Mildner-Rihm, K. Adler, M. Tiemann, H. Rutten, S. Fichtlscherer, H. Martin, A.M. Zeiher, HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway, The Journal of Clinical Investigation 108 (2001) 391–397.
[17] F. Nakagami, H. Nakagami, M.K. Osako, M. Iwabayashi, Y. Taniyama, T. Doi, H. Shimizu, M. Shimamura, H. Rakugi, R. Morishita, Estrogen attenuates vascular remodeling in Lp(a) transgenic mice, Atherosclerosis 211 (2010) 41–47.
[18] T. Yokoi, K. Fukuo, O. Yasuda, M. Hotta, J. Miyazaki, Y. Takemura, H. Kawamoto, H. Ichijo, T. Ogihara, Apoptosis signal-regulating kinase 1 mediates cellular senescence induced by high glucose in endothelial cells, Diabetes 55 (2006) 1660.
[19] K. Szocs, B. Lassegue, D. Sorescu, L. Hilenski, L. Valppu, T. Couse, J. Wilcox, M. Quinn, J. Lambeth, K. Griendling, Upregulation of Nox-based NAD (P) H oxidases in restenosis after carotid injury, Arteriosclerosis, Thrombosis, and Vascular Biology 22 (2002) 21.
[20] F. Sanada, Y. Taniyama, J. Azuma, K. Iekushi, N. Dosaka, T. Yokoi, N. Koibuchi, H. Kusunoki, Y. Aizawa, R. Morishita, Hepatocyte growth factor, but not vascular endothelial growth factor, attenuates angiotensin II-induced endothelial progenitor cell senescence, Hypertension 53 (2009) 77–82.
[21] O. Tepper, R. Galiano, J. Capla, C. Kalka, P. Gagne, G. Jacobowitz, J. Levine, G. Gurtner, Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures, Circulation 106 (2002) 2781.
[22] H. Iwaguro, J. Yamaguchi, C. Kalka, S. Murasawa, H. Masuda, S. Hayashi, M. Silver, T. Li, J. Isner, T. Asahara, Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration, Circulation 105 (2002) 732.
[23] J. Hill, G. Zalos, J. Halcox, W. Schenke, M. Waclawiw, A. Quyyumi, T. Finkel, Circulating endothelial progenitor cells, vascular function, and cardiovascular risk, New England Journal of Medicine 348 (2003) 593.
[24] T. Asahara, A. Kawamoto, H. Masuda, Concise review: Circulating endothelial progenitor cells for vascular medicine, Stem Cells 29 (2011) 1650–1655.
[25] T. Asahara, H. Masuda, T. Takahashi, C. Kalka, C. Pastore, M. Silver, M. Kearne, M. Magner, J.M. Isner, Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization, Circulation Research 85 (1999) 221–228.
[26] C. Urbich, A. Aicher, C. Heeschen, E. Dernbach, W.K. Hofmann, A.M. Zeiher, S. Dimmeler, Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells, Journal of Molecular and Cellular Cardiology 39 (2005) 733–742.
[27] M. Ii, H. Nishimura, A. Iwakura, A. Wecker, E. Eaton, T. Asahara, D.W. Losordo, Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity, Circulation 111 (2005) 1114–1120.
[28] H. Shigematsu, K. Yasuda, T. Iwai, T. Sasajima, S. Ishimaru, Y. Ohashi, T. Yamaguchi, T. Ogihara, R. Morishita, Randomized, double-blind, placebo-controlled clinical trial of hepatocyte growth factor plasmid for critical limb ischemia, Gene Therapy 17 (2010) 1152–1161.
[29] R. Morishita, H. Makino, M. Aoki, N. Hashiya, K. Yamasaki, J. Azuma, Y. Taniyama, Y. Sawa, Y. Kaneda, T. Ogihara, Phase I/IIa clinical trial of therapeutic angiogenesis using hepatocyte growth factor gene transfer to treat critical limb ischemia, Arteriosclerosis, Thrombosis, and Vascular Biology 31 (2011) 713–720.
[30] H. Miyauchi, T. Minamino, K. Tateno, T. Kunieda, H. Toko, I. Komuro, Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway, The EMBO Journal 23 (2004) 212.
[31] J. Galle, R. Schneider, A. Heinloth, C. Wanner, P. Galle, E. Conzelmann, S. Dimmeler, K. Heermeier, Lp (a) and LDL induce apoptosis in human endothelial cells and in rabbit aorta: role of oxidative stress, Kidney International 55 (1999) 1450–1461.
[32] S. Greiber, M. Kreusel, H. Pavenstadt, P. Schollmeyer, C. Wanner, Lipoprotein (a) induces glomerular superoxide anion production, Nephrology Dialysis Transplantation 12 (1997) 1330.
[33] F. Sanada, Y. Taniyama, K. Iekushi, J. Azuma, K. Okayama, H. Kusunoki, N. Koibuchi, T. Doi, Y. Aizawa, R. Morishita, Negative action of hepatocyte growth factor/c-Met system on angiotensin II signaling via ligand-dependent epithelial growth factor receptor degradation mechanism in vascular smooth muscle cells, Circulation Research 105 (2009) 667–675.