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Deficiency of Receptor-associated Protein Attenuates Angiotensin II-induced Atherosclerosis in Hypercholesterolemic Mice Without Influencing Abdominal Aortic Aneurysms

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Abstract

Objective—Receptor-associated protein (RAP) was initially described as a regulator of low density lipoprotein receptor-related protein 1 (LRP1), but is now known to regulate many proteins. Since the direct effects of RAP on vascular pathologies have not been studied, this study determined whether RAP deficiency influenced angiotensin II (AngII)-induced atherosclerosis and abdominal aortic aneurysms (AAAs) in hypercholesterolemic mice.

Methods and results—Male LDL receptor -/- mice that were either RAP +/+ or -/- were infused with AngII (500 ng/kg/min) for 4 weeks while consuming a saturated fat-enriched diet. RAP deficiency had no effects on body weight or AngII-induced increases of systolic blood pressure. Despite increased plasma cholesterol concentrations, RAP deficiency reduced atherosclerotic lesion size in aortic arches, while having no effect on AngII-induced AAAs. RAP deficiency profoundly reduced LRP1 protein abundance in macrophages, but did not change its abundance in aortic smooth muscle cells. Also, RAP deficiency had no effects on mRNA abundance of LRP1 or lipoprotein lipase in macrophages. To determine whether RAP deficiency in leukocytes influenced AngII-induced atherosclerosis, irradiated male LDL receptor -/- mice were repopulated with bone marrow-derived cells from either RAP +/+ or -/- male mice. The chimeric mice were infused with AngII (500 ng/kg/min) for 4 weeks while fed the saturated fat-enriched diet. RAP deficiency in bone marrow-derived cells did not influence either plasma cholesterol concentrations or atherosclerotic lesion size.

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Disclosures
The authors have nothing to disclose.

Conclusions—Whole body RAP deficiency attenuated atherosclerosis without influencing AAAs in hypercholesterolemic mice infused with AngII. The anti-atherogenic effect was not attributable to RAP deficiency in bone marrow-derived cells.

Keywords

receptor-associated protein; low density lipoprotein receptor-related protein; angiotensin II; atherosclerosis; aneurysms

1. Introduction

Receptor-associated protein (RAP) was identified as a companion of low density lipoprotein (LDL) receptor-related protein 1 (LRP1). RAP was initially defined as an inhibitor of LRP1 ligands, but subsequently demonstrated to act as a chaperone that was linked to the abundance and distribution of LRP1 protein [1-3]. A functional interdependence of RAP on LRP1 regulation was demonstrated by RAP -/- mice that had a diminished abundance of LRP1 protein in liver and brain [4]. However, LRP1 hypomorphism in RAP -/- mice displays cell-specificity, with variable degrees of reductions in the abundance of LRP1 protein in selected tissues and cell types from RAP -/- mice [5,6]. The cell-specific LRP1 hypomorphism is also inferred by the normal breeding features of RAP -/- mice, while deficiency of LRP1 results in embryonic lethality [4]. Therefore, RAP has both LRP1-related and LRP1-independent roles. In addition to LRP1, RAP interacts with many other proteins, including most members of the LDL receptor family, lipoprotein lipase (LPL), and amyloid beta peptide [7,8].

Despite the substantial role of RAP in the regulation of many proteins in the LDL receptor family, no study has determined effects of RAP on the development of atherosclerosis. In contrast, effects of LRP1 in atherosclerosis have been extensively studied in mouse models. There is compelling evidence that cell-specific LRP1 deficiency in hepatocytes, macrophages, or smooth muscle cells (SMCs) augments atherosclerosis in hypercholesterolemic mice [9-13].

Abdominal aortic aneurysms (AAAs) and atherosclerosis are two distinct pathologies that are evoked by different mechanisms. However, in addition to the contribution to atherosclerosis, SMC-specific deficiency of LRP1 in hypercholesterolemic mice also leads to AAAs [12] that resemble the AngII-induced aortic pathology [14]. The similarity between AAAs induced by AngII infusion and LRP1 deficiency implies a commonality of mechanisms in developing this aortic pathology.

Given the functional connection between RAP and LRP1, we hypothesized that RAP deficiency would augment atherosclerosis and induce AAAs in hypercholesterolemic mice infused with AngII. To test this hypothesis, we generated male LDL receptor -/- mice that were either RAP +/+ or -/-. These mice were infused with AngII (500 ng/kg/min) subcutaneously for 4 weeks while consuming a saturated fat-enriched diet. Unexpectedly, whole body deficiency of RAP reduced atherosclerosis but had no effect on AAA formation in hypercholesterolemic mice infused with AngII. Repopulation of irradiated hypercholesterolemic mice with RAP +/+ or -/- bone marrow-derived cells demonstrated that the anti-atherosclerotic effect was not attributable to a mechanism based on RAP in leukocytes.

2. Material and methods

2.1. Mice and diet

LDL receptor -/- (stock # 002207), RAP -/- (stock # 002987) and C57BL/6 (stock # 000664) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). To generate study mice in an LDL receptor -/- background, RAP -/- males were mated to LDL receptor -/- females, and their offspring were bred to generate RAP +/- males and females in the LDL receptor -/- background. Subsequent breeding pairs generated littermate controls of RAP +/+ × LDL receptor -/- and RAP -/- × LDL receptor -/- mice. Age-matched male littermates (8-14 weeks old) were used for the present study. Mice were maintained in a barrier facility and fed a normal mouse laboratory diet. To induce hypercholesterolemia, mice were fed a saturated fat-enriched diet (21% wt/wt milk fat and 0.2% wt/wt cholesterol, Diet # TD. 88137, Harlan Teklad, Madison, WI) for 5 weeks beginning 1 week prior to pump implantation. All study procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2. Genotyping of RAP and LDL receptor

DNA was isolated from tail snips or bone marrow using a DNEasy tissue kit (Cat# 69506, Qiagen, Valencia, CA). RAP and LDL receptor genotypes were determined by PCR. RAP genotyping was performed using the following primers: 5'-GCTAGTGCTGTTGTTGCTGC-3', 5'-TTCTCTCGCGAGTACTTGCC-3', 5'-TGATTGGTACCATCTCTGGGCTGG-3', and 5'-GATTGGGAAGACAATAGCA GGCATGC-3'. PCR of wild-type and disrupted alleles generated amplicons of 75 bp and 130 bp, respectively. LDL receptor genotyping was performed as described previously [15].

2.3. AngII infusion and systolic blood pressure measurement

AngII (500 ng/kg/min) was infused subcutaneously via Alzet osmotic minipumps (Model 2004, Durect Corp, Cupertino, CA) for 28 days as described previously [14]. Systolic blood pressure (SBP) was measured daily for 5 consecutive days before pump implantation and during the last week of AngII infusion on conscious mice using a computerized tail-cuff method (Coda 8, Kent Scientific, Torrington, CT) [16].

2.4. Bone marrow transplantation

Bone marrow transplantation was performed as described previously [17]. Male LDL receptor -/- mice (6 to 8 weeks old) were irradiated with a total of 900 rads divided into two doses delivered 3 hours apart (450 rads × 2) from a cesium γ source. Immediately after the second irradiation, these mice were injected with bone marrow-derived stem cells (1×10^7 cells/recipient mouse) harvested from either RAP +/+ or -/- male mice. Four weeks after the transplantation, mice were fed the saturated fat-enriched diet and osmotic minipumps were implanted 1 week later as described previously [17].

2.5. Plasma measurements

Plasma cholesterol concentrations were measured using an enzymatic based kit (Cholesterol E, Cat# 439-17501, Wako Chemicals, Richmond, VA). Lipoprotein distribution of cholesterol in plasma was determined using size exclusion gel chromatography followed by the enzymatic measurement as described previously [18]. Lipoprotein-cholesterol concentrations were analyzed with a non linear curve fitting software (PeakFit software, SeaSolve Software Inc).

2.6. Quantification of atherosclerotic lesions and AAAs

Atherosclerotic lesion areas in aortic arches were determined using an en face technique as described previously [19,20]. Lesion areas were measured using Image-Pro Plus software (Media Cybernetics, Bethesda, MD) by direct visualization of lesions under a dissecting microscope. Aortic arch region consisted of ascending aorta, arch, and part of descending aorta that was defined from aortic orifice of the left subclavian artery to 3 mm below.

AAAs were quantified and compared by measuring ex vivo diameter of suprarenal aortas using Image-Pro Plus software as described previously [17].

2.7. Immunostaining

Immunostaining of CD68 was performed using a rat anti-mouse CD68 monoclonal antibody (Clone FA-11; Cat# MCA1957, AbD Serotec, Raleigh, NC) in atherosclerotic lesions as described previously [21]. Immunostaining of LRP1 protein was determined in sections of fresh frozen liver, spleen, and aortas from RAP +/+ or -/- mice in an LDL receptor -/- background using a rabbit anti-LRP1 antibody (a kind gift from Dr. Strickland at the University of Maryland).

2.8. Isolation and culture of macrophages and aortic smooth muscle cells

Resident peritoneal macrophages were obtained from male RAP +/+ or -/- mice in an LDL receptor -/- background via lavage of the peritoneal cavity with sterile saline. Cells were counted and incubated in DMEM supplemented with fetal bovine serum (FBS; 10% v/v) and antibiotics (penicillin G 100 IU/ml and streptomycin 100 μg/ml) for 3 hours in a 6-well plate. Cells were washed with serum-free DMEM to remove non-adherent cells, and monolayers were incubated in DMEM with FBS (10% v/v) overnight and subsequently incubated in serum free DMEM.

Aortic SMCs were isolated from male RAP +/+ or -/- mice in an LDL receptor -/- background as described previously [22,23]. After perfusion of saline to remove blood through the left ventricle, aortas were harvested and placed in chilled DMEM, and adventitias were removed immediately. Aortas were then cut open longitudinally and endothelium was denuded. Each segment of the aorta (aortic arch, thoracic, and abdominal aortas) was minced and incubated with DMEM containing type I collagenase (1 mg/ml) and type III elastase (0.125 mg/ml) at 37°C for 2 hours with occasional agitation. Subsequently, cells were centrifuged (3,000 rpm × 10 min at 4°C) and incubated in DMEM with FBS (20% v/v) and antibiotics (penicillin G 100 IU/ml and streptomycin 100 μg/ml). Medium was changed every 4-5 days. Experiments were performed when cells were > 80% confluent.

2.9. Western blot analyses

Cell lysates were prepared in lysis buffer (Cat# 9803, Cell Signaling, Beverly, MA) in the presence of protease inhibitor cocktail (1% v/v; Cat# P8340, Sigma-Aldrich, St. Louis, MO). Protein concentrations were measured using a Bradford protein assay kit (Cat# 500-0116, Bio-Rad Laboratories, Hercules, CA). Western blotting was performed as described previously [15]. Equal amounts of proteins were separated using SDS-PAGE (10% wt/v) and transferred electrophoretically to PVDF membranes. Membranes containing transferred proteins were probed with antibodies against RAP (Cat# 20R-RR002, Fitzgerald, Acton, MA), LRP1 (Cat# 10R-L107b, Fitzgerald), or β-actin (Cat# A5441, Sigma-Aldrich), and subsequently incubated with appropriate horseradish peroxidase conjugated secondary antibodies (HRP-anti rabbit IgG, PI-1000, Vector Laboratory, Burlingame, CA; HRP-anti mouse IgG, A2554, Sigma-Aldrich, St. Louis, MO). Immunoreactivity was visualized by an

enhanced chemiluminescence detection kit (Cat# 34080, Pierce, Rockford, IL) using a Kodak Imager (Model 4000R Pro).

2.10. Real-time PCR of LPL and LRP1

Total RNA of peritoneal macrophages from RAP +/+ (n = 7) and -/- mice (n = 5) were extracted using RNeasy Mini Kit (Cat# 74104, Qiagen, Valencia, CA). Reverse transcription was performed using iScript cDNA Synthesis kit (Cat# 170-8891, Bio-Rad, Hercules, CA), and real-time PCR of LRP1, LPL, and 18S rRNA was processed using iQ™ SYBR Green Supermix kit (Cat 170-8882, Bio-Rad) on a Bio-Rad iCycler. The primers used to detect LRP1 and LPL mRNA are listed in Online Table I. ΔΔCt method was used to calculate the data [24].

2.11. Statistic analyses

SigmaPlot version 11 and SAS version 9.2 were used for the statistical analyses. Two-group comparisons on quantitative variables at a single time point were performed parametrically (Student's t test) or nonparametrically (Mann-Whitney Rank Sum test) according to the results of preliminary tests for within-group normality and equality of variances. Systolic blood pressure data were analyzed by fitting a linear mixed model with explanatory variables of genotype (RAP +/+ and RAP -/-) and time (before and during AngII infusion) along with random effects for individual mice. Quantitative variables are summarized as mean ± standard error of mean (SEM) when assessed parametrically and as median with interquartile range when assessed nonparametrically. A P value < 0.05 is considered statistically significant.

3. Results

3.1. RAP deficiency attenuated atherosclerosis but not AAAs in AngII-infused hypercholesterolemic mice

To determine the contribution of RAP to AngII-induced atherosclerosis and AAA formation, male LDL receptor -/- mice that were either RAP +/+ or -/- were fed a saturated fat-enriched diet and infused with AngII (500 ng/kg/min) for 28 days. RAP deficiency had no significant effect on body weight (Online Table II). Systolic blood pressure was similar between RAP +/+ and -/- mice before the AngII infusion. Chronic AngII infusion increased systolic blood pressure in all study mice and there was no significant difference in the increase of systolic blood pressure between the two genotypes (Online Table II).

The saturated fat-enriched diet profoundly increased plasma cholesterol concentrations in mice of both genotypes. However, mice with RAP deficiency had higher plasma cholesterol concentrations than did their wild type littermates (Online Table II). Plasma lipoproteins were resolved by size-exclusion chromatography (Figure 1A). RAP deficiency significantly increased chylomicron-, VLDL-, and LDL-cholesterol concentrations (Figure 1B). Furthermore, RAP deficient mice had modest reductions of HDL-cholesterol concentrations.

Atherosclerotic lesion areas were measured on intimal surfaces of aortic arches. Despite significant increases in plasma cholesterol concentrations, RAP deficiency significantly reduced atherosclerotic lesion size as compared by percent lesion area and absolute areas of lesions in aortic arches (P < 0.05; Figure 2). Irrespective of the difference in lesion size, immunostaining of CD68 demonstrated that macrophages were the predominant infiltrating cell type in atherosclerotic lesions of both RAP +/+ and -/- mice.

RAP deficiency had no effect on AngII-induced AAA formation as defined by the ex vivo diameter of suprarenal aortas (Online Figure I). In addition, RAP deficiency had no effect on AngII-induced aortic rupture (data not shown).

3.2. RAP deficiency diminished LRP1 protein abundance in macrophages but not in aortic SMCs

RAP deficient mice have reduced LRP1 protein abundance in liver and brain [4]. In the present study, a pronounced reduction of LRP1 protein abundance in liver and spleen was also observed by immunostaining in RAP deficient mice (Online Figure II). Since macrophages are the predominant infiltrating cell type in atherosclerotic lesions and SMCs are the major resident cell type in the aortic wall, we measured LRP1 protein abundance with Western blot analyses in these two cell types using cells isolated from LDL receptor -/- mice that were either RAP +/+ or -/-. RAP deficiency profoundly reduced LRP1 protein abundance in macrophages (Figure 3A). However, it did not change LRP1 protein abundance in primary cultured aortic SMCs harvested from any of the 3 regions (aortic arch, thoracic, and abdominal aorta; Figure 3B). Also, LRP1 protein abundance was not changed in macrophages or aortic SMCs isolated from LDL receptor -/- mice that were incubated with AngII (1 μM) for 24 hours (data not shown). Consistent with the findings from Western blotting analysis, RAP deficiency did not lead to any apparent change of LRP1 protein in the media layers of ascending aortic sections as determined by immunostaining.

Despite the pronounced reduction of LRP1 protein abundance in macrophages, RAP deficiency did not lead to any change of LRP1 mRNA abundance in macrophages (Online Figure IIIA). LPL interacts with RAP and regulates lipoprotein metabolism. However, RAP deficiency did not change mRNA abundance of LPL in macrophages of hypercholesterolemic mice (Online Figure IIIB).

3.3. RAP deficiency in bone marrow-derived cells had no effect on AngII-induced atherosclerosis

Whole body deficiency of RAP reduced AngII-induced atherosclerosis. Macrophages are the major inflammatory cell type in atherosclerotic lesions, and as shown above, RAP deficiency markedly reduced LRP1 protein in macrophages. These findings provided a mechanism-based rationale to determine whether RAP in macrophages contributed to AngII-induced atherosclerosis using bone marrow transplantation. Successful repopulation was verified by PCR of DNA isolated from bone marrow of recipient mice that confirmed the donor RAP genotypes in chimeric mice (Figure 4A).

RAP deficiency in bone marrow-derived cells did not affect body weight (Online Table III). Chronic AngII infusion equivalently increased systolic blood pressures in both groups of chimeric mice (Online Table III). In contrast to whole body deficiency of RAP, deficiency of this protein in bone marrow-derived cells had no effect on plasma cholesterol concentrations or lipoprotein distributions. Furthermore, RAP deficiency in bone marrow-derived cells had no effect on AngII-induced atherosclerotic lesions in chimeric mice fed the saturated fat-enriched diet (Figure 4B).

4. Discussion

The present study unexpectedly demonstrated that whole body RAP deficiency reduced atherosclerosis in hypercholesterolemic mice that were infused with AngII, despite increased plasma cholesterol concentrations. Furthermore, RAP deficiency led to a manifest reduction of LRP1 protein abundance in macrophages, but the absence of RAP in bone marrow-

derived cells had no effect on the development of atherosclerosis. Also unexpected was the lack of effect of whole body RAP deficiency on AngII-induced AAA formation.

Much of the interest in RAP stems from its role in inhibiting the effects of all known LRP1 ligands and its function as a chaperone for this molecule [5]. As a consequence, a mechanistic interpretation of the effects of RAP deficiency on atherosclerosis needs to consider the contribution of LRP1. No studies have been performed on the effects of LRP1 deficiency in atherosclerosis, since whole body depletion of LRP1 results in embryonic death [25]. Also, there are no reports of LRP1 floxed mice being used with Cre expression that is ubiquitously expressed in an inducible manner in mature mice. However, atherosclerotic development has been determined in cell-specific LRP1 depletion in macrophages, SMCs, and hepatocytes. Each of these cell-specific deficiencies of LRP1 increased lesion size in hypercholesterolemic mice [9-13]. Despite the many associations of RAP with LRP1 expression, the effects of RAP deficiency on atherosclerosis contrast those of LRP1 deficiency.

In LDL receptor -/- mice that were infused with AngII and fed the saturated fat-enriched diet, the most abundant cell type in atherosclerotic lesions was lipid-laden macrophages [14], whereas the presence of smooth muscle cells in lesions was not readily distinguishable. LRP1 protein abundance was equivalent in aortic media between mice of the two RAP genotypes. In addition, we did not detect any difference of LRP1 protein abundance in cultured aortic SMCs from RAP +/+ and -/- mice that were incubated with AngII. The inability of RAP deficiency to mimic the effects of SMC-specific LRP1 deficiency might be explained by the findings in the present study that deficiency of RAP does not lead to a LRP1 hypomorphic phenotype in SMCs. In contrast, RAP deficiency promoted a pronounced reduction of LRP1 protein abundance in macrophages. Since macrophage-specific deletion of LRP1 increases atherosclerosis [10,11], the lack of effect of bone marrow cell-derived RAP deficiency appears to contradict the findings in these studies. However, it should be noted that RAP has regulatory functions that extend to many molecules beyond LRP1. It is possible that RAP deficiency leads to compensatory increases or contemporary defects of other members in the LDL receptor family or other classes of proteins that are associated with RAP. Of the molecules that interact with RAP, LPL deficiency in macrophages has been demonstrated to reduce atherosclerosis in mice [26]. RAP deficiency did not change mRNA abundance of LPL in macrophages in the present study. Since RAP regulates many proteins [27], it is likely that reduction of atherosclerosis in RAP -/- mice is mediated through a complex mechanism involving multiple proteins.

Whole body RAP deficiency increased plasma cholesterol concentrations in LDL receptor -/- mice. This has been demonstrated previously and was attributed to inhibition of chylomicron uptake in liver [4,28]. RAP deficiency diminishes protein abundance of LRP1 in mouse liver [4]. As a consequence, in mice with double deficiencies of RAP and LDL receptor, clearance of large size lipoproteins in plasma such as chylomicron, VLDL and LDL were impaired, thereby resulting in increased plasma cholesterol concentrations of these lipoproteins [4]. It is unclear whether chylomicron-sized lipoprotein particles play a role in the development of atherosclerosis [29].

Chronic AngII infusion augments atherosclerosis and promotes AAAs in hypercholesterolemic mice. Although both are influenced by AngII, these two diseases are distinct in both pathologies and mechanisms. Previously, we demonstrated region-specific effects of AngII on aortic contractile responses and morphological heterogeneity [22,23]. Chronic AngII infusion promotes hyperplasia of SMCs in ascending aortas but hypertrophy of SMCs in thoracic and abdominal aortas [23]. In contrast, AngII produces hardly discernable contraction of ascending, descending, and suprarenal aortas, but striking

contraction of infrarenal aortas [22,23]. Although the mechanism is not clear, the phenotypic diversity of aortic SMCs may contribute to the differential effects of RAP deficiency on AngII-induced atherosclerosis and AAAs.

SMC-specific LRP1 deficiency led to development of severe medial thickening and aneurysmal formation in hypercholesterolemic mice [12]. We considered whether the similar phenotype of SMC-specific LRP1 deficiency and AngII-induced AAAs were ascribed to AngII decreasing LRP1 abundance in SMCs. We were unable to determine any change of LRP1 abundance in SMCs isolated from RAP -/- mice. In addition, unlike a study that has reported increased LRP1 protein abundance in human vascular SMCs by AngII [30], we did not identify any contribution of AngII in cultured mouse aortic SMCs to the protein abundance of LRP1. Furthermore, whole body RAP deficiency had no effect on AngII-induced AAA formation. Therefore, the lack of effect of RAP deficiency on AngII-induced AAA formation is consistent with the lack of a hypomorphic LRP1 phenotype in SMCs of RAP -/- mice.

In conclusion, whole body RAP deficiency reduced atherosclerosis, but had no effect on AAA formation in LDL receptor -/- mice infused with AngII. The effects on atherosclerosis were not seen in mice with RAP deficiency in bone marrow-derived cells, despite a profound reduction of LRP1 in macrophages. This study provides strong evidence that RAP contributes to atherosclerosis in an LRP1-independent manner in AngII-infused mice that is not attributable to its absence in bone marrow-derived cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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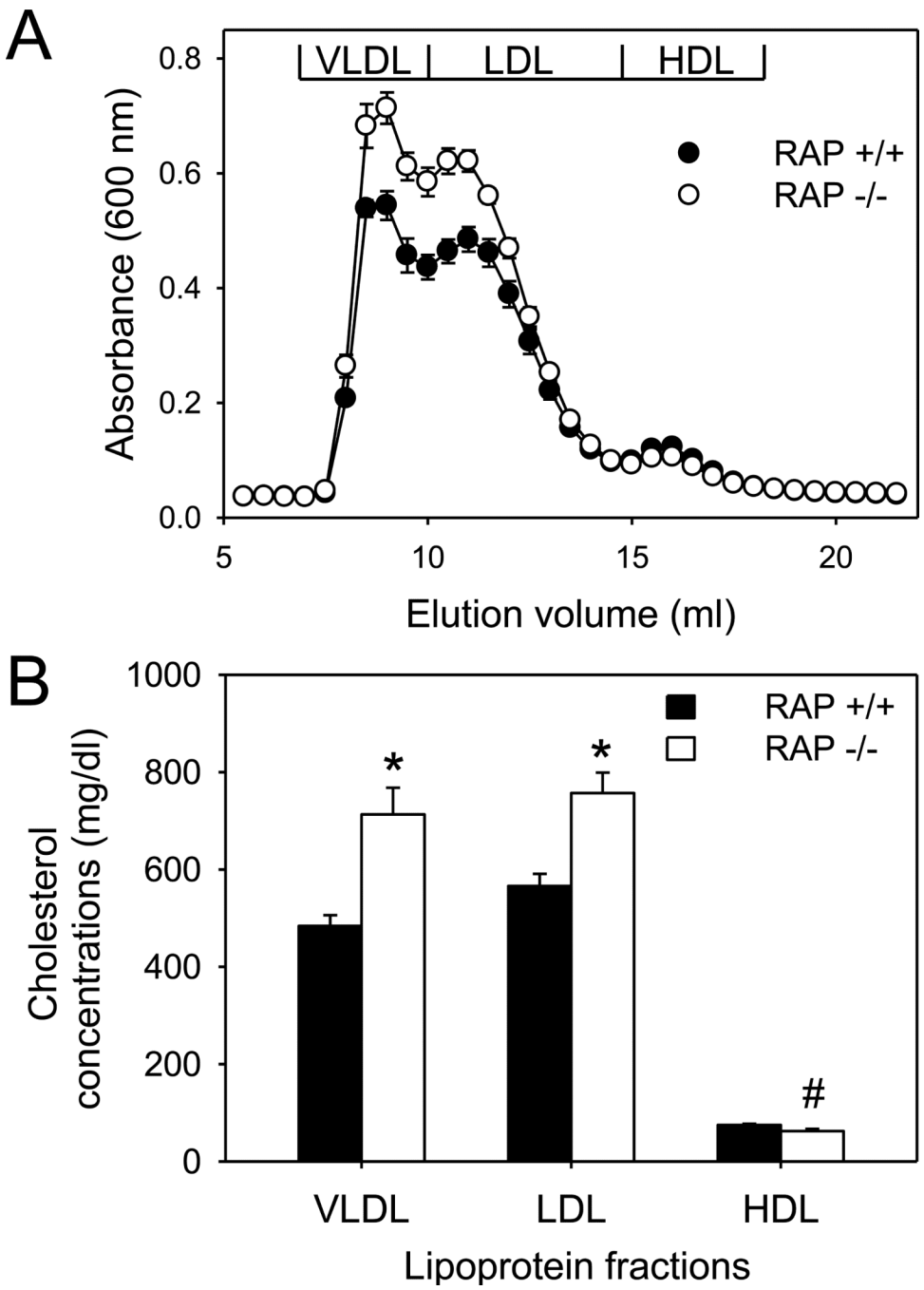

Figure 1. RAP deficiency changed plasma lipoprotein distributions in hypercholesterolemic mice infused with AngII

(**A**) Lipoproteins were resolved by size exclusion chromatography of plasma from individual mice and cholesterol was measured using enzymatic kits in RAP +/+ (n = 10) or -/- (n = 12). Circles and error bars represent mean ± SEM. (**B**) Plasma concentrations of cholesterol in major lipoprotein fractions. Concentrations were calculated using plasma total cholesterol concentrations and non linear curve fitting of size exclusion profiles. Histobars represent means and bars represent SEM. * denotes $P < 0.001$ and # denotes $P = 0.03$, respectively.

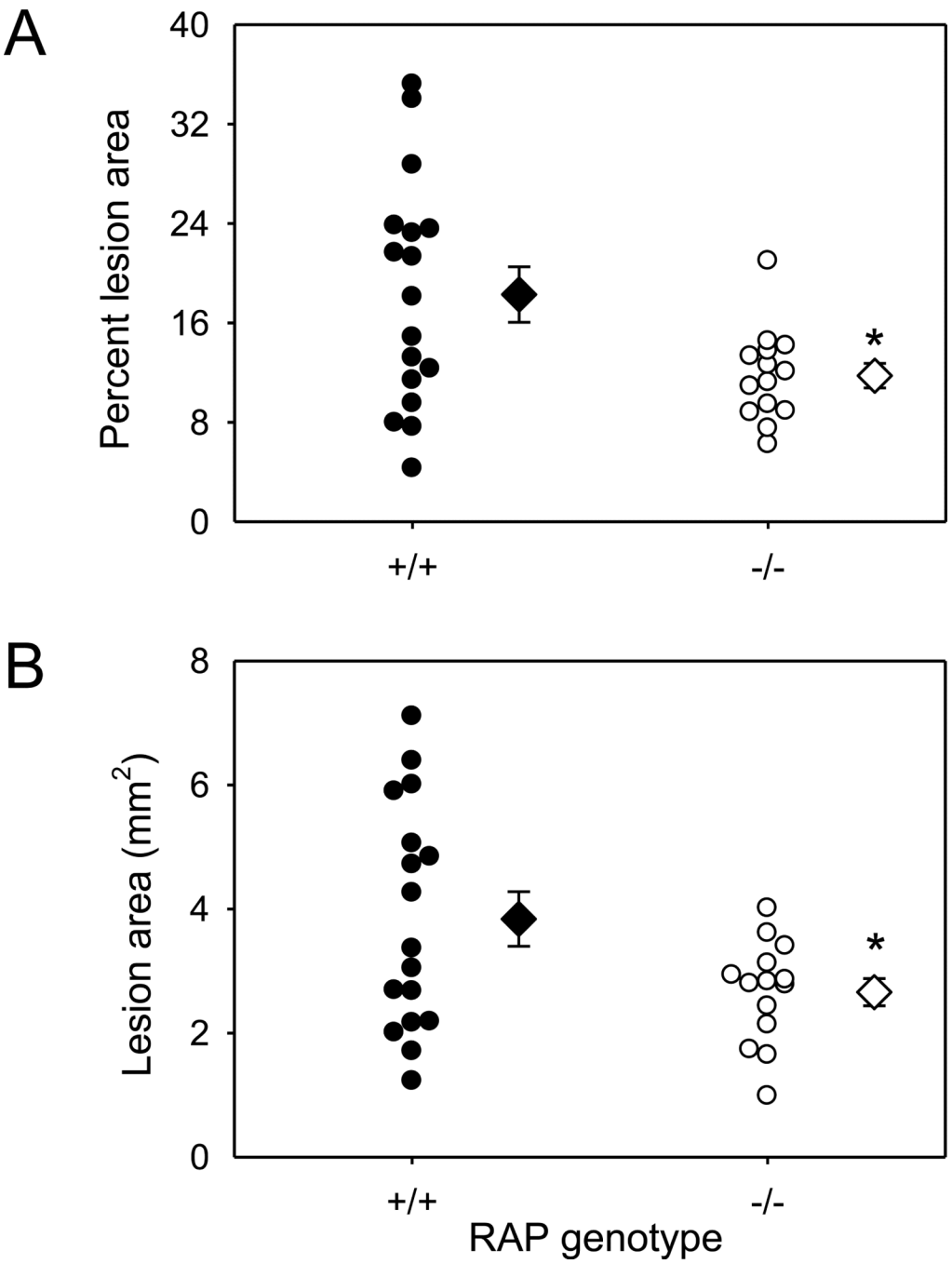

Figure 2. RAP deficiency reduced atherosclerotic lesions in aortic arches in hypercholesterolemic mice infused with AngII

(**A**) Percent lesion areas and (**B**) Lesion areas in aortic arches were measured by an en face technique. * denotes $P < 0.05$ by a two-tailed Student's t test. Circles represent individual hypercholesterolemic mice that were either RAP +/+ ($n = 17$) or -/- ($n = 14$). Diamonds and error bars represent mean ± SEM.

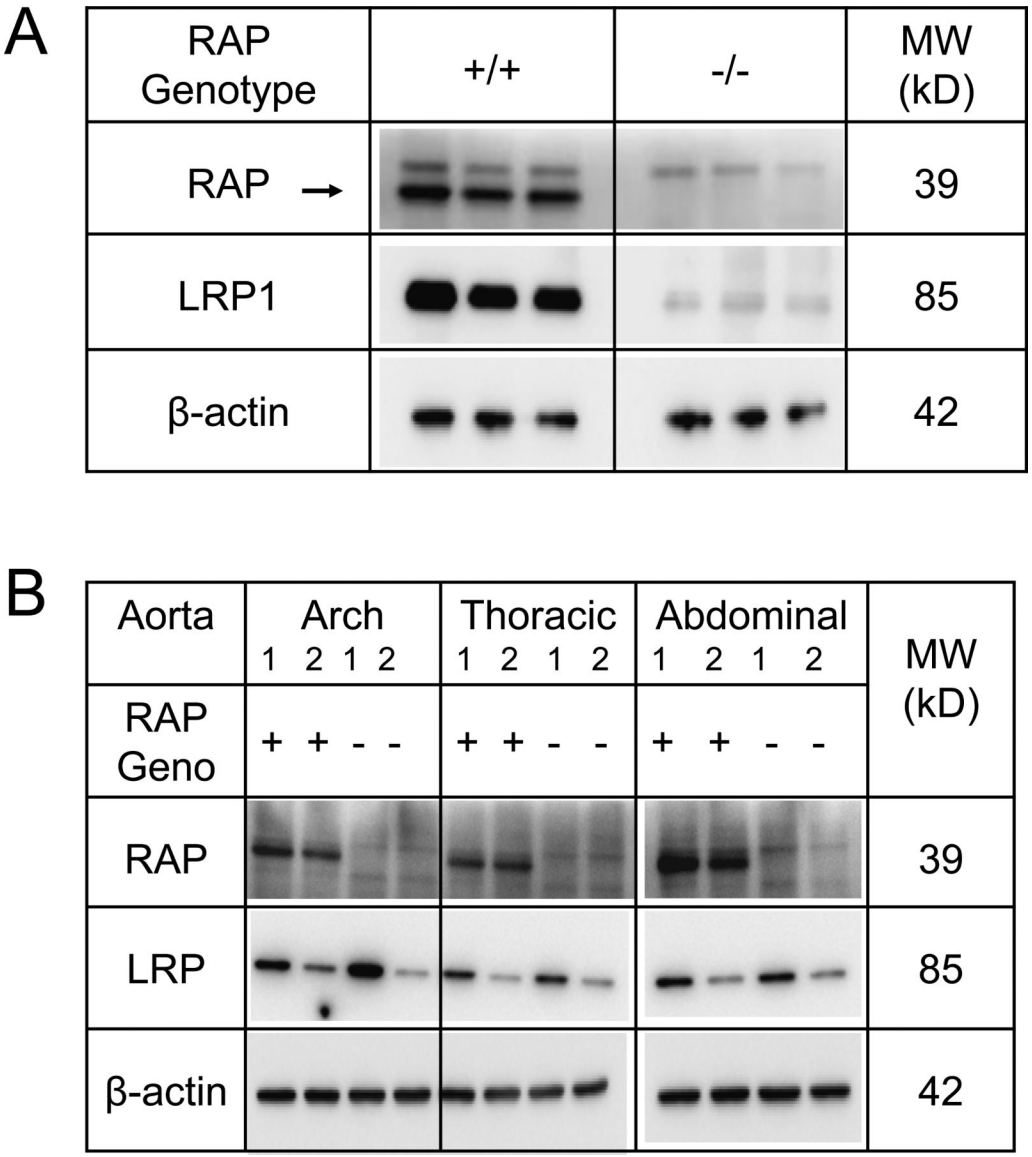

Figure 3. RAP deficiency profoundly reduced the protein abundance of LRP1 in macrophages but not in SMCs

Western blots of RAP, LRP1, and β-actin were performed in (**A**) peritoneal macrophages and **(B**) SMCs isolated from aortic arches, and thoracic and abdominal aortas of RAP +/+ or -/- mice in an LDL receptor -/- background. Lanes 1 and 2 represent protein extracts from cultured SMCs of two independent experiments. In neither experiment did RAP deficiency affect LRP1 abundance.

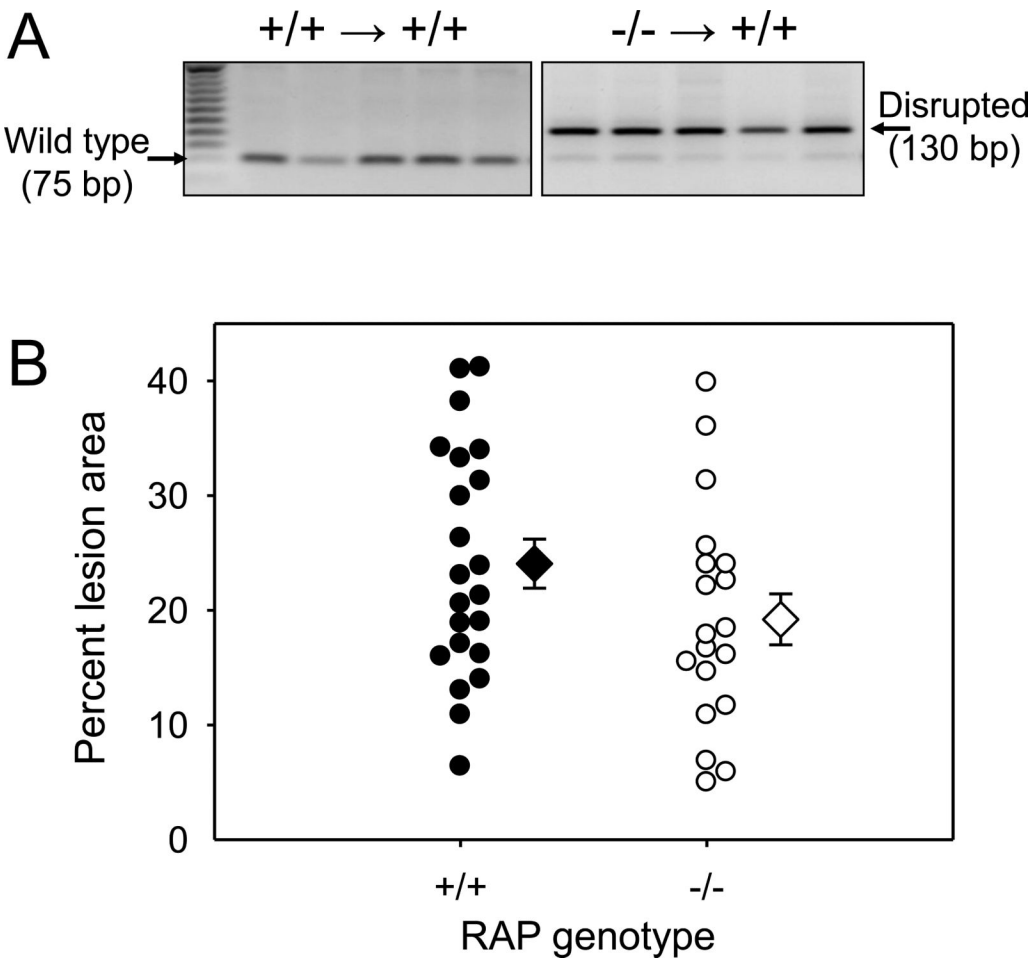

Figure 4. RAP deficiency in bone marrow-derived cells had no effect on atherosclerosis in hypercholesterolemic mice infused with AngII

(**A**) RAP genotypes in bone marrow-derived cells of the recipient mice were verified by PCR. Amplicons of 75 bp and 130 bp represent wild-type allele and the disrupted allele of RAP, respectively. (**B**) Percent lesion areas in aortic arches were determined by the en face measurements. Circles represent individual recipient mice that were repopulated with bone marrow-derived cells from donor mice (RAP +/+, *n* = 22 and RAP -/-, *n* = 19). Diamonds and error bars represent mean ± SEM.