Ablation of Interferon Regulatory Factor 3 Protects Against Atherosclerosis in Apolipoprotein E–Deficient Mice

Hui Liu,* Wen-Lin Cheng,* Xi Jiang,* Pi-Xiao Wang, Chun Fang, Xue-Yong Zhu, Zan Huang, Zhi-Gang She, Hongliang Li

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Abstract—The secretion of adhesion molecules by endothelial cells, as well as the subsequent infiltration of macrophages, determines the initiation and progression of atherosclerosis. Accumulating evidence suggests that IRF3 (interferon regulatory factor 3) is required for the induction of proinflammatory cytokines and for endothelial cell proliferation. However, the effect and underlying mechanism of IRF3 on atherogenesis remain unknown. Our results demonstrated a moderate-to-strong immunoreactivity effect associated with IRF3 in the endothelium and macrophages of the atherosclerotic plaques in patients with coronary heart disease and in hyperlipidemic mice. IRF3−/−ApoE−/− mice showed significantly decreased atherosclerotic lesions in the whole aorta, aortic sinus, and brachiocephalic arteries. The bone marrow transplantation further suggested that the amelioration of atherosclerosis might be attributed to the effects of IRF3 deficiency mainly in endothelial cells, as well as in macrophages. The enhanced stability of atherosclerotic plaques in IRF3−/−ApoE−/− mice was characterized by the reduction of necrotic core size, macrophage infiltration, and lipids, which was accompanied by increased collagen and smooth muscle cell content. Furthermore, multiple proinflammatory cytokines showed a marked decrease in IRF3−/−ApoE−/− mice. Mechanistically, IRF3 deficiency suppresses the secretion of VCAM-1 (vascular cell adhesion molecule 1) and the expression of ICAM-1 (intercellular adhesion molecule 1) by directly binding to the ICAM-1 promoter, which subsequently attenuates macrophage infiltration. Thus, our study suggests that IRF3 might be a potential target for the treatment of atherosclerosis development. (Hypertension. 2017;69:510-520. DOI: 10.1161/HYPERTENSIONAHA.116.08395.) • Online Data Supplement

Key Words: atherosclerosis ■ atherosclerotic plaque ■ coronary artery disease ■ endothelial cells ■ inflammation

Atherosclerosis is a chronic inflammatory disease of large and medium arteries,1,2 which depends on innate and adaptive immune responses.3,4 It is a critical pathological process underlying clinical cardiovascular events that are the predominant cause of mortality and morbidity in industrialized countries worldwide.5 An intact endothelium is required for maintaining normal physiological vascular function, including barrier function, thromboresistance, and anti-inflammatory effects.6 However, after exposure to modified low-density lipoprotein (LDL), endothelial dysfunction produces not only structural but also functional alterations near curvatures, arterial branch ostia, and bifurcations, where disrupted flow with large oscillation occurs.7 The increased permeability of endothelial cells caused by apoptosis and the secretion of ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1) facilitate the infiltration of plasma-modulated lipoproteins and monocyte adhesion/aggregation at the subendothelium.8 Subsequently, the recruited monocyte-macrophages transform into lipid-laden foam cells by uptaking modified lipoproteins. Foam cells in turn secrete more proinflammatory cytokines and extracellular matrix components and promote the migration and proliferation of vascular smooth muscle cells (SMCs), which disrupts the stability of atherosclerotic plaques.9 The enlargement of the necrotic core caused by ineffective clearance ultimately leads to atherothrombotic vascular diseases,10,11 such as myocardial infarction, sudden cardiac death, and stroke.12

The interferon regulatory factor (IRF) family comprises transcriptional regulators of the interferon-induced signaling pathway.13 In mammals, the IRF family includes 9 members (IRF1-9), which are involved in various biological functions,
including innate immune responses, hematopoietic development, and cell survival.14–16 In contrast with other IRFs, IRF3 is found in an inactive form in the cytoplasm of various cell types. Once phosphorylated in response to various stimuli, IRF3 translocates into the nucleus and leads to the induction of type I interferon and interferon-induced gene transcription.17–19 Previous studies have demonstrated that the LPS-MyD88 independent kinase complex phosphorylates IRF3 and results in the production of cytokines involved in innate immunity.20 In addition, IRF3 induces proatherosclerotic gene expression, including that of RANTES, Cc15, and Cxcl10,21–23 and affects endothelial cell proliferation.24 Recently, our group has clarified that IRF3 contributes to cerebral ischemic injury,25 neointima formation,26 hepatic steatosis,27 and cardiac hypertrophy.28 However, the specific role of IRF3 in the development of atherosclerosis is unclear. In this study, after our discovery that IRF3 is upregulated and localized in both endothelial cells and macrophages in patients with coronary heart disease (CHD) and in hyperlipidemic mice, we found that IRF3−/−ApoE−/− mice were resistant to high-fat diet (HFD)–induced atherogenesis. Furthermore, experiments in cross-group bone marrow transplantation indicated that IRF3 deficiency in endothelial cells was more critical for the inhibition of plaque development than its deficiency in bone marrow–derived macrophages. The effect of IRF3 deficiency in endothelial cells was contributed by the reduction of ICAM-1 and VCAM-1 secretion and macrophage infiltration. Our results demonstrated that IRF3 plays a proatherogenic role by increasing inflammation and the instability of atherosclerotic plaques.

Methods and Materials

Human Specimens
Human atheromatous plaques were collected from the coronary arteries of patients with CHD who were undergoing heart transplantation. The control samples were obtained from normal heart donors who were not suitable for transplantation for nonvascular reasons. All the procedures involving human samples were performed according to the principles outlined in the Declaration of Helsinki and were approved by the Renmin Hospital of Wuhan University Institutional Review Board in Wuhan, China.

Animals and Treatment
IRF3 global knockout mice (IRF3−/− on a C57BL/6 background) were generously provided by T. Taniguchi.29 To obtain the IRF3−/−ApoE−/− mice, we generally crossbred the IRF3−/− with ApoE−/− to get IRF3+/− mice. Bone marrow cells from the donor IRF3−/−ApoE−/− or ApoE−/− mice were isolated and injected into ApoE−/− and IRF3−/−ApoE−/− recipient mice through retro-orbital plexus. After 4 weeks of recovery, mice in all 4 groups were fed an HFD for an additional 16 weeks and then subjected to atherosclerotic lesion analysis. The DNA from a blood treatment using a TIANamp Genomic DNA Kit was applied to confirm bone marrow reconstitution via a polymerase chain reaction (PCR) analysis.

Bone Marrow Transplantation Experiment
Eight-week-old male recipient mice were lethally irradiated via total 11 Gy (5.5 Gy on 2 occasions separated by 4 hours) on the day of transplantation. Euthanasia was performed by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Bone marrow cells from the donor IRF3−/−ApoE−/− or ApoE−/− mice were isolated and injected into ApoE−/− and IRF3−/−ApoE−/− recipient mice through retro-orbital plexus. After 4 weeks of recovery, mice in all 4 groups were fed an HFD for an additional 16 weeks and then subjected to atherosclerotic lesion analysis. The DNA from a blood treatment using a TIANamp Genomic DNA Kit was applied to confirm bone marrow reconstitution via a polymerase chain reaction (PCR) analysis.

RNA Isolation and Quantitative Real-Time PCR
After being extracted using a TRizol reagent, the total mRNA from the whole aorta was collected and depurated using DNase. cDNAs were then obtained using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Indianapolis, IN). Inflammatory cytokines were identified by using quantitative real-time PCR with LightCycler 480 SYBR Green 1 Master Mix (Roche) and a LightCycler 480 QPCR System (Roche) in accordance with the manufacturer’s instructions. The quantified transcripts from the samples were normalized against GAPDH gene expression.

Western Blotting Analysis
Five micrograms of protein, extracted from the aorta and homogenized in a lysis buffer as described,32 was processed and then incubated with primary antibodies overnight at 4°C. After incubation with secondary antibodies, relative expression was visualized using a FluorChem E Imager (ProteinSimple, FluorChem E), and membranes were treated with enhanced chemiluminescence reagents (170-5061; Bio-Rad). The specific protein expression was normalized against GAPDH expression.

Immunofluorescence Staining
The mouse tissue sections were performed according to the protocols described earlier.33 After incubating with primary antibodies, the slice was washed in phosphate-buffered solution and incubated with the relevant secondary antibody for another hour. Images were obtained using a fluorescence microscope (OLYMPUS DX51) and DP2-BSW software (version 2.2), and the images were analyzed using Image-Pro Plus 6.0.

Adenovirus Infection and Cell Migration Assay
The AdshIRF3 was constructed as described34 and AdshRNA as a control group. For the migratory macrophage analysis, modified Boyden chambers with Costar Transwell inserts (Corning) were used. Raw 264.7 cells (5×10⁴) were added to the top well for 6 hours. The cells that migrated to the bottom were fixed and stained with crystal violet/20% methanol and counted.

Statistical Analysis
All data are expressed as the means±SD. Comparisons between groups were evaluated using a 2-tailed Student t test or 1-way ANOVAs. All statistical analyses were performed using SPSS, version 16.0. P values <0.05 were considered to be statistically significant.

Results
Upregulation of IRF3 Expression in the Atheromatous Lesions of Human and Mouse
To explore the potential role that IRF3 plays in the development of atherosclerosis, we first collected pathological coronary
arteries from patients with CHD and from ApoE (apolipoprotein E)–deficient mice, a commonly used atherosclerotic animal model, to observe whether the IRF3 expression levels were changed. Western blot analysis revealed markedly increased expression of IRF3 in patients with CHD compared with healthy individuals (Figure 1A). Significantly increased IRF3 levels were also found in atherosclerotic plaques of hyperlipidemic ApoE-deficient mice that received an HFD for 28 weeks compared with those fed normal chow (Figure 1B). The right coronary arteries of patients and the aortic roots of mice, where atherosclerotic plaques accumulate because of oscillatory shear stress, were examined via immunofluorescence staining. Double immunofluorescence staining revealed a strong immunoreactivity of IRF3 expression in the endothelial cell layer (CD31-positive cells) of atherosclerotic arteries compared with healthy arteries (Figure 1C). Similar results

Figure 1. IRF3 (interferon regulatory factor 3) is upregulated in the endothelium and macrophages of atheromatous lesions in humans and mice. A and B. Western blotting analysis of IRF3 protein levels in the right coronary arteries of humans (A) or the aortas of mice (B). The expression levels were normalized against GAPDH and quantified (n=4). C and D. Costaining of the endothelium in human coronary arteries (C) or cross sections of the aortic sinus from ApoE−/− mice (D) for IRF3 (red) and CD31 (green). Scale bar: 50 μm, n=3. E. Costaining of macrophages in patients with coronary heart disease (CHD) or cross sections of the aortic sinus in hyperlipidemic ApoE−/− mice for IRF3 (red) and MAC3 (green) Scale bar: 20 μm, n=3. F. The IRF3 expression in HUVECs or BMDMs upon oxidant LDL (Ox-LDL) treatment. *P<0.05. BMDMs indicates bone marrow–derived macrophages; HFD, high-fat diet; HUVECs, human umbilical vein endothelial cells; LDL, low-density lipoprotein; MAC3, macrophage-3 antigen; NC, normal chow; and PBS, phosphate-buffered solution.
were obtained via immunofluorescence costaining in advanced atherosclerotic lesions from the aortic roots of ApoE−/− mice in the experimental group versus the control group (Figure 1D). Moreover, IRF3 was also found to be expressed in macrophages of atheromatous plaques in patients with CHD and in hyperlipidemic ApoE−/− mice (Figure 1E). In addition, a remarkable increase in IRF3 expression was obtained in the HUVECs on oxidant LDL stimulation, whereas such increase was slight in bone marrow–derived macrophages (Figure 1F). Collectively, these data indicate that IRF3 upregulation is

**Figure 2.** IRF3 (interferon regulatory factor 3) deficiency inhibits atherosclerosis in ApoE−/− mice. A, Western blotting analysis of the IRF3 protein levels in double knockout (IRF3−/−ApoE−/−) mice and their ApoE−/− littermates (n=4). B, En face Oil Red O staining of the aortas of IRF3−/−ApoE−/− and control mice fed with normal chow (NC) or a high-fat diet (HFD). The quantitative analysis is shown in the right (n=10). C, Cross section of aortic sinus stained with hematoxylin and eosin (H&E). Scale bar: 500 μm, n=6. D, Brachiocephalic artery sections stained with H&E. Scale bar: 100 μm, n=5. E and F, Triglycerides (TGs), total cholesterol (TC), and lipoprotein profiles from IRF3−/−ApoE−/− and ApoE−/− mice on an HFD (n=20). *P<0.05 compared with ApoE−/− littermates. HDL indicates high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; NS, not significant; and VLDL, very low-density lipoprotein.
concomitant with activation of endothelial cells and macrophages during atherosclerosis.

**Depletion of IRF3 Decreases the Development of Atherosclerosis**

To determine whether the upregulation of IRF3 expression in atherosclerotic plaques contributes to atherosclerosis, we used a double knockout strategy by crossing IRF3-deleted mice with ApoE−/− mice to assess the effects of IRF3 on atherogenesis in vivo. Western blotting verified the successful generation of the experimental mice (Figure 2A). Eight-week-old male IRF3−/−ApoE−/− mice and their ApoE−/− littermates were fed a normal chow or HFD diet for 28 weeks to stimulate atherosclerotic lesion development. An en face analysis of the whole aortas showed a significant reduction (3.42% versus 9.20%) of plaque development in IRF3−/−ApoE−/− mice compared with ApoE−/− mice when fed with normal chow (Figure 2B). In HFD group, atherosclerotic lesion area increased to 41.81% in the ApoE−/− littermates but was inhibited to 21.06% in IRF3-ablated mice (Figure 2B).

The plaque volume at aortic sinus and brachiocephalic arteries were also measured to assess the effect of IRF3 on atherogenesis in mice. A quantitative analysis showed a significant decrease in lesion area in the IRF3−/−ApoE−/− mice compared with the ApoE−/− mice, both in the aortic root (Figure 2C) and in the brachiocephalic arteries (Figure 2D). We did not observe significant differences in the plasma triglycerides, plasma cholesterol levels, or lipoprotein profiles (very low-density lipoprotein, intermediate-density lipoprotein, LDL, and high-density lipoprotein) between the genotypes fed with an HFD (Figure 2E and 2F).

**IRF3 Deficiency Improved Plaque Stability**

The content of vulnerable plaques is more responsible for the outcome of clinical cardiovascular complications than plaque size. IRF3−/−ApoE−/− mice showed smaller necrotic cores in their aortic sinuses and brachiocephalic arteries than did ApoE−/−, and the fibrous cap cover was thicker (Figure 3A). We next investigated other surrogate properties contributing to plaque stability, including collagen deposition, smooth muscle cells, macrophages, and lipid burden. First, the percentage of collagen in IRF3−/−ApoE−/− mice increased by 33% relative to the percentage in ApoE−/− littermates (Figure 3B and 3F).

Second, the SMC content of the atherosclerotic lesions showed a coordinated effect with a significant 2-fold increase in IRF3−/−ApoE−/− mice compared with the ApoE−/− mice, both in the aortic root (Figure 2C) and in the brachiocephalic arteries (Figure 2D). We did not observe significant differences in the plasma triglycerides, plasma cholesterol levels, or lipoprotein profiles (very low-density lipoprotein, intermediate-density lipoprotein, LDL, and high-density lipoprotein) between the genotypes fed with an HFD (Figure 2E and 2F).

**IRF3 Deficiency Decreases Plaque Inflammation**

Given that inflammation plays a vital role in atherogenesis, we examined the effect of IRF3 deficiency on the expression of inflammatory mediators. We first detected whether IRF3 deletion affects the number or subset of circulating classical monocytes. The flow cytometry analysis showed no significant difference in the numbers of monocyte between the ApoE−/− and IRF3−/−ApoE−/− group marked by CD11b+CD115+. However, the IRF3−/−ApoE−/− mice showed a significant decrease in Ly6Chigh monocytes subset, whereas an increase in Ly6Clow monocytes subset (Figure 5A). The mRNA levels of proinflammatory markers, such as TNF-α, MCP-1, IL-6 (interleukin 6), and IL-1β (interleukin-1β), were decreased in the atherosclerotic lesions of IRF3−/−ApoE−/− mice compared with those of ApoE−/− mice (Figure 5B). Similarly, the IL-6, IL-1β, TNF-α, and inducible nitric oxide synthase levels in the serum were all significantly lower in IRF3−/−ApoE−/− mice than in controls (Figure 5C).

Furthermore, immunofluorescent staining analyses of IL-6 and IL-10 revealed less abundance in the lesions of IRF3−/−ApoE−/− mice than in lesions of ApoE−/− mice littermates (Figure 5D).

**Deletion of IRF3 Attenuates Adhesion Molecule Secretion and Subsequent Macrophage Infiltration**

During early atherogenesis, ICAM-1 and VCAM-1 secreted from dysfunctional endothelial cells mediate monocyte attraction and adhesion to the endothelium. The immunostaining of the aortic root cross sections with ICAM-1–specific antibodies revealed markedly decreased expression of ICAM-1 in the endothelial cell layer of IRF3−/−ApoE−/− mice compared with ApoE−/− mice (Figure 6A). In addition, a moderate decrease in expression of VCAM-1 in the endothelium was also observed in IRF3−/−ApoE−/− mice (Figure 6A). Concomitantly, IRF3 ablation significantly reduced the expression of VCAM-1 and ICAM-1 in IRF3−/−ApoE−/− mice in protein level (Figure 6B). More importantly, we found that IRF3 deletion significantly reduced the abundance of monocytes in the plaque indicated...
by Ly6C immunochemical staining (Figure 6C), suggesting the significant reduction of monocyte recruitment. To figure out whether the reduction of ICAM-1 and VCAM-1 expression after IRF3 ablation is because of direct suppression of gene transcription through an interaction with the promoter, we performed bioinformatics analysis and identified a series of putative interferon stimulated response element–binding sites in ICAM-1 promoter (designated P1–P4). To verify the potential binding sites, we performed chromatin immunoprecipitation of Myc-IRF3 in HUVECs followed by quantitative PCR of the ICAM-1 and VCAM-1 promoters. Chromatin immunoprecipitation (ChIP) assays demonstrated that the binding site for IRF3 was enriched in the P1 region but not in any of the other regions, thus indicating that P1 in ICAM-1 contains the primary site for IRF3 binding (Figure 6D), whereas no significant IRF3 binding site was observed in the VCAM-1 promoter. When treated with TNF-α, IRF3-knocking down HUVECs (Figure S1A in the online-only Data Supplement) expressed
drastically reduced ICAM-1 and VCAM-1 expression in both mRNA (Figure S1B) and protein (Figure 6E) levels compared with HUVECs infected by the control virus. The Transwell migration of macrophages induced by TNF-\(\alpha\)-stimulated HUVECs was also significantly inhibited by AdshIRF3 transfection (Figure 6F). In addition, similar results and decreased proinflammatory cytokines were obtained when these cell were stimulated by oxidant LDL (Figure S1C, S1D, S1E). These results indicated that the effect of IRF3 on atherogenesis partially depended on direct and positive regulation of ICAM-1 expression by IRF3 at transcription level.

**Discussion**

Our study is the first to demonstrate that IRF3 is responsible for accelerated atherogenesis in both endothelial cells and macrophages, and this effect is largely mediated by the production of adhesion molecules and inflammation, thus leading to increased necrotic cores and vulnerable plaques. From a mechanistic point of view, the ablation of IRF3 suppresses VCAM-1 expression and (especially) ICAM-1 expression by directly binding to the ICAM-1 promoter, which subsequently attenuates macrophage migration and infiltration.

Endothelial cells play a critical role in maintaining physiological vascular homeostasis and functions, including inhibition of thrombosis, barrier function, and anti-inflammatory responses. Atherosclerosis preferentially develops in curvatures, arterial branch ostia, and bifurcations, where the endothelium is exposed to turbulent blood flow and oscillatory shear stress. The stress plays an important proatherogenic role and disablers the stability of atherosclerotic plaques. Endothelial dysfunction is a part of the response to the injury caused by hypercholesterolemia exposure. It is characterized by impaired endothelial repair or increased permeability, increased leukocyte adhesion, lipoprotein infiltration, and inflammation, which
initiate the process of atherosclerosis. Understanding the mechanisms underlying the regulation of endothelial function is critical for developing effective treatment for atherosclerosis.

The present study showed that IRF3 is more abundant in the coronary arteries of patients with CHD and in the aortic roots of hyperlipidemic mice, particularly in the endothelial cells, suggesting that IRF3 may be important for endothelial homeostasis and development of atherosclerosis. Indeed, ablation of IRF3 significantly restricted the atherogenesis in HFD-fed ApoE⁻/⁻ mice. In addition, the bone marrow transplantation experiment demonstrated that the IRF3 deficiency in endothelial cells but not hematopoietic cells largely accounted for the antiatherosclerotic effect. The disruption of endothelial integrity caused by the increased apoptosis of endothelial cells and the secretion of adhesion molecules, including the upregulated expression of VCAM-1 and ICAM-1 via activated

Figure 5. The reduction of inflammatory cytokine production by IRF3 (interferon regulatory factor 3) ablation. A, Top, Representative flow plot of mean percentage of CD11b⁺CD115⁺ monocyte in the blood of IRF3⁻/⁻ApoE⁻/⁻ mice and ApoE⁻/⁻ mice. Bottom, Ly6C⁺ and Ly6C⁻ monocyte populations in the blood of IRF3⁻/⁻ApoE⁻/⁻ mice and ApoE⁻/⁻ mice. B, Serum TNF-α (tumor necrosis factor-α), MCP-1 (monocyte chemoattractant protein-1), IL-6 (interleukin 6), and IL-1β (interleukin 1β) levels were measured in ApoE⁻/⁻ and IRF3⁻/⁻ApoE⁻/⁻ mice via ELISA (n=6). *P<0.05 compared with their ApoE⁻/⁻ littermates. C, Quantitative real-time polymerase chain reaction of the mRNAs that encode inflammation in the aorta (n=3). *P<0.05 compared with their ApoE⁻/⁻ littermates. D, The immunofluorescent staining of inflammatory gene expression (both in red color) on sections from the aortic sinus in ApoE⁻/⁻ and IRF3⁻/⁻ApoE⁻/⁻ mice (n=3). iNOS indicates inducible nitric oxide synthase.
endothelial cells, facilitate the aggregation of circulating monocytes/macrophages into subendothelial spaces, aggravate the development of atherosclerosis, and destabilize atherosclerotic plaques. Importantly, we noticed that both the mRNA and the protein level of VCAM-1 and ICAM-1 were decreased in the aortic roots of IRF3-deficient mice and in HUVECs after knocking down IRF3 expression. The effect of IRF3 on ICAM-1 expression was mediated by directly binding to the promoter of ICAM-1, whereas not for VCAM-1. Thus, we speculated that IRF3 directly increased the ICAM-1 expression and promoted the recruitment of monocyte-macrophage infiltration, subsequently producing cytokines and chemokines to accelerate the endothelial cell injury, which can induce VCAM-1 expression. Exposure to hypercholesterolemia and the oscillatory shear stress mechanotransduction are converted into specific biochemical signals that activate intracellular signaling pathways. These pathways ultimately activate numerous endothelial genes, such as ICAM-1 and VCAM-1, that have
potentially proatherogenic properties and contain shear stress response elements in their promoter regions, which depend on or are independent of transcription factor–mediated regulation. Accordingly, we propose that the inhibition of the IRF3-ICAM-1/VCAM-1 axis should be considered to be a crucial target for the prevention of aggravated atherosclerosis.

Homing of macrophages to atherosclerotic plaques critically regulates initiation, extension, inflammation, and rupture of plaques. Our data showed that IRF3 disruption restricted oxidant LDL–induced macrophage migration by decreasing ICAM-1 and VCAM-1 expression. Published data have demonstrated that IRF3 plays a key role in the production of the cytokines implicated in innate immunity and proatherosclerotic gene expression. Our work demonstrated a dramatic decrease in inflammatory mediators in terms of mRNA, as well as serum, and protein levels in IRF3-deficient mice. In patients with clinical cardiovascular diseases, the development of atherosclerosis, especially the alteration of plaque morphology, is a more significant predictor of plaque rupture and the occurrence of acute clinical events than plaque size. Necrotic core formation is a key determinant of vulnerable plaques. Our study demonstrated that IRF3 deficiency significantly decreased the size of necrotic cores both in the aortic sinus and in the brachiocephalic arteries. Furthermore, the fibrous cap cover was thicker. In addition, an analysis of the other features that contributed to vulnerable plaques in IRF3-deficient mice showed markedly increased collagen deposition and SMC content, whereas macrophage infiltration and lipid accumulation showed a significant reduction. Our results demonstrated that IRF3 is a possible candidate for the treatment of atherogenesis, particularly for maintaining plaque stability.

Most of the current literature has described IRF3 function in relation to interferon-mediated immunity. Therefore, the immunity-independent roles of IRF3 are poorly understood. Our recent research has unveiled a multifunctional role of IRF3 on different pathological stimuli, beyond its immune regulatory roles. In fact, IRF3 exerts distinct functions in different tissues and cell types via specific mechanisms. For example, IRF3 deficiency in cases of overnutrition aggravates hepatic steatosis and insulin resistance through the IKK-β/NF-κB (IKK-beta/nuclear factor-kappa B) signaling pathway in hepatocytes, whereas IRF3 associated with endothelial cells and macrophages inhibits the development of atherogenesis and enhances plaque stability by contributing to the inhibition of adhesion molecule secretion and vascular inflammation. Our work highlights the important role of neutralizing IRF3 as a potential pharmacological therapeutic approach to prevent atherosclerosis and stabilize atherosclerotic plaques.

Perspectives
The in vivo and vitro experiments suggest that IRF3, a member of IRF transcription factor family, functions as a positive regulator of the atherosclerosis partially through directly binding with the ICAM-1 promoter, and thereby accumulate macrophage infiltration, which subsequently promote the inflammation response. These observations indicate that blockade of IRF3 expression/activity would be beneficial for the prevention of atherosclerosis development and coronary artery diseases.

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Disclosures
None.

References


Novelty and Significance

**What is New?**

- IRF3 (interferon regulatory factor 3) is first identified to be relevant to both human and mouse atherosclerotic plaque.

- IRF3 is a key modulator responsible for the development of atherosclerosis by increasing the adhesion molecules secretion partially by directly binding to the ICAM-1 (intercellular adhesion molecule 1) promoter.

- The current study advances our understanding about the role of IRF3 in the atherogenesis and provides a novel target for the intervention of atherosclerosis and coronary diseases.

**What is Relevant?**

- The important functions of IRF3 beyond immune response have been identified in hepatic steatosis, cardiac hypertrophy, intimal hyperplasia, and cerebral ischemic injury, whereas its effect on the development of atherosclerosis and its underlying mechanisms are unknown.

Summary

This study demonstrates that IRF3 is responsible for exaggerated atherosclerotic plaques, which is largely mediated by the production of adhesion molecules and inflammation, by directly binding to the ICAM-1 promoter.
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Ablation of Interferon Regulatory Factor 3 protects against atherosclerosis in apolipoprotein E-deficient mice

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**Figure S1** The reduction of ICAM-1, VCAM-1 secretion and infiltrated macrophage in the absence of IRF3.  

A. The IRF3 expression in the HUVECs transfected with AdshRNA and AdshIRF3.  

B. mRNA expression level of ICAM-1 and VCAM-1 in HUVECs upon TNF-α stimulation.  

C. Western blot analysis of VCAM-1 and ICAM-1 in HUVECs treated with Ox-LDL.  

D. The number of migrated macrophages induced by the HUVECs of AdshRNA or AdshIRF3 treated with oxLDL for 24 hours.  

E. mRNA expression level of proinflammatory cytokines in HUVECs upon Ox-LDL stimulation.* P<0.05 versus AdshRNA group.