Emerging roles of Protease-Activated Receptors in Cardiometabolic Disorders

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Summary

Cardiometabolic disorders, including obesity-related insulin resistance and atherosclerosis, share sterile chronic inflammation as a major cause; however, the precise underlying mechanisms of chronic inflammation in cardiometabolic disorders are not fully understood. Accumulating evidence suggests that several coagulation proteases, including thrombin and activated factor X (FXa), play an important role not only in the coagulation cascade but also in the proinflammatory responses through protease-activated receptors (PARs) in many cell types. Four members of the PAR family have been cloned (PAR 1–4). For instance, thrombin activates PAR-1, PAR-3, and PAR-4. FXa activates both PAR-1 and PAR-2, while it has no effect on PAR-3 or PAR-4. Previous studies demonstrated that PAR-1 and PAR-2 activated by thrombin or FXa promote gene expression of inflammatory molecules mainly via the NF-κB and ERK1/2 pathways. In obese adipose tissue and atherosclerotic vascular tissue, various stresses increase the expression of tissue factor and procoagulant activity. Recent studies indicated that the activation of PARs in adipocytes and vascular cells by coagulation proteases promotes inflammation in these tissues, which leads to the development of cardiometabolic diseases. This review briefly summarizes the role of PARs and coagulation proteases in the pathogenesis of inflammatory diseases and describes recent

findings (including ours) on the potential participation of this system in the development of cardiometabolic disorders. New insights into PARs may ensure a better understanding of cardiometabolic disorders and suggest new therapeutic options for these major health threats.

Keywords: Cardiometabolic disorders; Coagulation system; Inflammation; Protease-activated receptors; Activated factor X; Thrombin

Introduction

Sterile chronic inflammation in the vasculature and metabolic organs causes cardiometabolic disorders; however, the mechanisms involved are not fully understood [1-3]. Many cellular and molecular pathways are associated with inflammation in the adipose tissue, liver, pancreatic islets, and vasculature [4-6]. Previous reports have suggested a link between the blood coagulation system and inflammatory diseases [7,8]. Several coagulation proteases and protease-activated receptor (PAR) pathways participate in this machinery [9,10]. Accumulating evidence suggests that several coagulation proteases, including thrombin and activated factor X (FXa), play an important role not only in the coagulation cascade but also in the proinflammatory responses via PARs in many cell types (such as leukocytes, endothelial cells, platelets, fibroblasts, and smooth muscle cells) [11-13].

This review briefly discusses the roles of coagulation proteases and PARs in the pathogenesis of inflammatory diseases, particularly focused on cardiometabolic diseases, and summarizes recent findings (including ours) that suggest the therapeutic potential of this system against cardiometabolic disorders. First, we introduce the effects of the pharmacological inhibition of coagulation proteases, especially thrombin and FXa, on several mouse models of cardiometabolic disease. Next, we create an overview of the

phenotype of the genetic deletion of PARs, particularly PAR-1 and PAR-2, in inflammatory disease models. Furthermore, we summarize the clinical review, including large-scale clinical trials and future perspectives of PARs as therapeutic targets for cardiometabolic diseases.

Coagulation proteases and PARs

PARs are a family of G protein-coupled, 7-transmembrane domain receptors that are activated by the proteolytic cleavage of the receptor N-terminus by several proteases, generating a novel tethered ligand that subsequently activates the receptor via intramolecular binding [14]. Four members of the PAR family have been cloned (i.e. PAR 1–4) and expressed in various cell types (**Fig. 1**). PAR-1, 3, and 4 are expressed on platelets; however, interestingly, PAR-2 is not expressed on platelets. Thrombin activates PAR-1, PAR-3, and PAR-4. FXa activates both PAR-1 and PAR-2 but has no effect on PAR-3 or PAR-4. Previous studies have reported various physiological and/or pathological participations of PARs in homeostasis, inflammation, oncogenesis, embryonic development, and so on [15]. Numerous previous studies have examined the mechanisms underlying PAR signaling. For example, PAR-1 and PAR-2 activated by thrombin or FXa promote gene expression of inflammatory molecules mainly via the

nuclear factor (NF)-κB and ERK1/2 pathways.

Many papers have reported that various cell types express tissue factor or other coagulation proteases in many organs (e.g. leukocytes, vasculature cells, cardiac fibroblasts, cardiomyocytes, lung epithelial cells, brain cells, and so on) [16]. For example, tissue factor is expressed on circulating cells, such as monocytes [17], neutrophils [18,19], or platelets [20-22]. Tissue factor is also produced by several vascular cells, including endothelial cells [23], vascular smooth muscle cells, and fibroblasts [16,24]. Of note, several studies have reported that circulating monocytes, macrophages, or vascular smooth muscle cells in atherosclerotic plaques also express FVII and FX [25-27].

Of the various proteases, thrombin and FXa will be discussed subsequently; so, here, we will discuss the relationship between other proteases and PARs. Other proteases also activate PAR-1 and PAR-2. For example, activated protein C (APC) can activate PAR-1, leading to anti-inflammatory or cellular protective responses. These effects of APC through PAR-1 are mediated via Caveolin-1 interaction with endothelial cell protein C receptor (ERCP) in lipid rafts [28]. APC administration provides cytoprotective effects in several diseases, including atherosclerosis [29], myocardial ischemia [30,31], ischemic stroke [32], and diabetic nephropathy [33]. Matrix

metalloproteases (such as MMP-1, MMP-2, and MMP-13) activate PAR-1 [34-36]. Although the direct relationship between MMP-1 and PAR-1 in the atherosclerotic plaque has not yet been demonstrated, excessive MMP expression can enhance extracellular matrix degradation and promote plaque instability. Another study revealed that either the genetic deletion of PAR-1 or the inhibition of MMP-13 could prevent β-adrenergic receptor overstimulation-dependent cardiac dysfunction [37]. Thus, the sustained activation of the MMP - PAR-1 pathway might elicit potentially harmful responses in atherosclerosis or heart failure models. Trypsin, tryptase, and matriptase are also reported to activate PAR-2, causing inflammatory responses [38-40].

Role of thrombin in cardiometabolic diseases

Thrombin is one of the major ligands of PAR-1. In the past few decades, several thrombin inhibitors were developed as anticoagulants for the prevention and treatment of cardioembolic stroke or venous thromboembolism. In addition to the antithrombic effect, many animal studies have assessed the effects of thrombin inhibitors on several inflammatory conditions (**Table 1**).

The apolipoprotein E-deficient ($ApoE^{-/-}$) mouse is a frequently used mouse model of atherosclerosis [41]. Early animal studies reported that the direct thrombin

inhibitor, melagatran, attenuated the development and destabilization of atherosclerotic lesions in the aorta of *ApoE*$^{-/-}$ mice. Melagatran also decreased inflammation in the aorta [42]. Dabigatran is a highly selective, reversible, and potent thrombin inhibitor that is orally available as the prodrug, dabigatran etexilate [43,44]. Similar to melgatran, dabigatran reduced the sizes of atherosclerotic lesions and promoted plaque stability in *ApoE*$^{-/-}$ mice [45-48]. Dabigatran also attenuated vascular inflammation in *ApoE*$^{-/-}$ mice [29]. Vorapaxar, a selective antagonist of PAR-1, also reduced the sizes of atherosclerotic lesions in *ApoE*$^{-/-}$ mice [49]. Dabigatran also ameliorated diabetes-induced endothelial dysfunction, an initiation step required for atherosclerosis in diabetic mice [50]. In contrast to the findings of these studies, another study found that another thrombin inhibitor, bivalirudin, did not reduce atherogenesis [51]. The researchers were concerned that bivalirudin had a shorter half-life than the other anticoagulants, resulting in the inadequate inhibition of thrombin activities in this study. Overall, these results suggest that pharmacological blockade of thrombin decreases atherosclerosis and vascular inflammation.

Acute myocardial infarction is one of the leading causes of death in the developed world. Atherosclerosis is a major cause of myocardial infarction. Hirudin, an effective natural thrombin inhibitor, significantly reduced infarct sizes in cardiac

ischemia-reperfusion (I/R) model mice [52]. However, it remains inconclusive whether this effect might simply be due to the anticoagulant effect of this drug or not.

Chronic low-grade inflammation and immune system activation are involved in the pathogenesis of obesity-related insulin resistance and type 2 diabetes. In high-fat diet-induced obesity mice, dabigatran significantly reduced weight gain, hepatic inflammation, and hepatic steatosis [53,54]. These results suggest that the pharmacological blockade of thrombin attenuates the chronic inflammation of the adipose tissue and hepatic tissue, ameliorating metabolic abnormalities.

The *in vitro* effects of thrombin inhibitors have been well studied, especially in endothelial cells. Thrombin increased the expression of several inflammatory molecules via the NF-κB signaling pathway in human umbilical endothelial cells (HUVEC) [50], which was suppressed by dabigatran in a dose-dependent manner [55].

Role of FXa in cardiometabolic diseases

Several FXa inhibitors are currently used for the prevention of thrombotic events associated with nonvalvular atrial fibrillation and venous thrombosis. Besides its well-known coagulant actions, FXa might contribute to chronic inflammation via the PAR-2 pathway (**Table 2**).

We investigated the effects of the FXa inhibitor, rivaroxaban, on the development and destabilization of atherosclerotic plaques in *ApoE*$^{-/-}$ mice [56]. Rivaroxaban attenuated atherosclerotic plaque progression and destabilization in 8-week-old *ApoE*$^{-/-}$ mice that were fed a western-type diet for 20 weeks. Rivaroxaban also significantly reduced the mRNA expression of inflammatory molecules in the aorta. The findings of subsequent studies are almost consistent with our results [57-59]. Another study reported that rivaroxaban administration to 26-week-old *ApoE*$^{-/-}$ mice with advanced-stage atherosclerotic lesions also reduced the expression of inflammatory molecules in the aorta and stabilized atherosclerotic plaques [60]. Rivaroxaban also attenuated atherogenesis in low-density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) mice, another standard mouse model for hyperlipidemia and atherosclerosis [61]. Fondaparinux, the selective FXa inhibitor, promoted the stability of atherosclerotic lesions and reduced the mRNA expression of inflammatory mediators in *ApoE*$^{-/-}$ mice [62]. Fondaparinux also inhibited the aortic aneurysm expansion in angiotensin II-infused hypertensive *ApoE*$^{-/-}$ mice [63]. These results suggest that the pharmacological blockade of FXa attenuates atherosclerotic plaque progression, destabilization, and vascular inflammation. We also evaluated the effects of rivaroxaban on neointima formation after wire-mediated vascular injury, another atherosclerotic

model. Rivaroxaban significantly attenuated neointima formation in the injured arteries four weeks after surgery. Rivaroxaban reduced the expression of inflammatory molecules and macrophage accumulation in injured arteries seven days after surgery [64]. Thus, the inhibition of FXa by rivaroxaban attenuates neointima formation after wire-mediated vascular injury by inhibiting vascular inflammation. Rivaroxaban also reduced vascular inflammation in a mouse model of sickle cell disease (BERK mice) characterized by hypercoagulation and vascular inflammation [65].

In cardiac I/R model mice, rivaroxaban reduced the size of myocardial infarction and fibrosis. Rivaroxaban improved cardiac systolic function and survival rate after I/R [66]. Similarly, rivaroxaban administration preserved cardiac function in mice after permanent coronary artery ligation [67,68]. We also demonstrated that rivaroxaban reduced rapid pacing-induced atrial inflammation and remodeling [69]. In spontaneously hypertensive rats that underwent atrial rapid pacing, rivaroxaban attenuated inflammation and fibrosis in atrial walls. Rivaroxaban significantly reduced the inducibility of atrial fibrillation. These results suggest that pharmacological blockade of FXa reduced myocardial inflammation or cardiac remodeling. Another study showed that edoxaban, a selective inhibitor of FXa, ameliorated diabetic nephropathy by inhibiting the expression of proinflammatory and profibrotic molecules

in the kidney [70].

Numerous *in vitro* studies have hinted at the anti-inflammatory effect of FXa inhibitors in many cell types. For instance, our *in vitro* experiments demonstrated that FXa significantly increased the expression of inflammatory molecules in endothelial cells and macrophage cell lines. Rivaroxaban reduced these proinflammatory responses [71,72]. FXa promoted both the proliferation and migration of vascular smooth muscle cells, which were blocked in the presence of rivaroxaban [64]. The beneficial effects of FXa inhibitors on cardiometabolic organs could be attributed to the anti-inflammatory effects of these inhibitors.

Role of PAR-1 in cardiometabolic diseases

The effects of genetic PAR-1 deletion in mouse models with cardiometabolic diseases are summarized in Table 3. According to one previous report, PAR-1 deficiency significantly reduced atherosclerotic lesions in $ApoE^{-/-}$ mice that were fed a high-fat diet for 16 weeks [73]. On the other hand, the genetic deletion of PAR-1 did not alter atherogenesis in $Ldlr^{-/-}$ mice that were fed a western-type diet for 12 or 24 weeks [61]. *In vitro* studies demonstrated that the thrombin-PAR-1 pathway inhibits cholesterol efflux both in macrophages and smooth muscle cells by ubiquitination and the

degradation of ABC subfamily A member 1 (ABCA1), resulting in the progression of atherosclerosis [73, 74]. Such effects of PAR-1 on cholesterol efflux might lead to the difference in the severity of atherosclerosis between the *ApoE*$^{-/-}$ model and the *Ldlr*$^{-/-}$ model.

PAR-1 deficiency ameliorated impairment of left ventricular function two weeks after I/R injury. Moreover, overexpression of PAR-1 in cardiomyocytes induced eccentric hypertrophy [52]. PAR-1 deficiency did not alter inflammation in BERK mice [65]. Several studies revealed that no genotype-dependent difference in weight gain was observed in *PAR-1*$^{-/-}$ mice compared with wild-types [53,75,76]. Additional analyses demonstrated that thrombin promotes diet-induced obesity, which depends on the function of fibrinogen and not on PAR-1 signaling [53]. Although there was no change in body weight, PAR-1 deletion reduced diet-induced hepatic inflammation and steatosis [75, 76].

Role of PAR-2 in cardiometabolic diseases

Accumulating evidence demonstrates that PAR-2, a major receptor of FXa, is expressed in both vascular cells and leukocytes, which suggests that PAR-2 may contribute to the pathogenesis of inflammatory diseases (**Table 4**).

We investigated the role of PAR-2 in vascular inflammation and atherogenesis. Systemic PAR-2 deletion in *ApoE*$^{-/-}$ mice reduced atherosclerotic lesions in the aortic arch along with features of stabilized atherosclerotic plaques (**Fig. 2**) [71]. *ApoE*$^{-/-}$*PAR-2*$^{-/-}$ mice significantly decreased the expression of inflammatory molecules in the aorta. The results of bone marrow transplantation experiments demonstrated that PAR-2 in both hematopoietic cells and nonhematopoietic cells contributed to atherogenesis in *ApoE*$^{-/-}$ mice [71]. Consistent with our data, two other groups also demonstrated that PAR-2 deficiency in *ApoE*$^{-/-}$ mice attenuated the progression and instability of atherosclerotic plaques [57,77]. Our *in vitro* experiments demonstrated that FXa or a specific peptide agonist of PAR-2 significantly increased the expression of inflammatory molecules and lipid uptake in BM-derived macrophages from wild-type mice compared with those from *PAR-2*$^{-/-}$ mice. The activation of NF-κB signaling was involved in PAR-2-associated vascular inflammation and macrophage activation [71].

PAR-2 deficiency (but not PAR-1 deficiency) also attenuated vascular inflammation, atherogenesis, and plaque destabilization in *Ldlr*$^{-/-}$ mice [61]. In this model, bone marrow transplantation experiments demonstrated that PAR-2 on nonhematopoietic cells contributed to atherosclerosis. Our *in vitro* experiments also

demonstrated that the FXa - PAR-2 pathway contributes to the proinflammatory activation of vascular smooth muscle cells or endothelial cells. *In vitro* experiments using rat vascular smooth muscle cells demonstrated that FXa promoted both proliferation and migration of this cell type, which were both blocked in the presence of rivaroxaban [64]. FXa promoted JNK phosphorylation and reduced $eNOS^{Ser1177}$ phosphorylation in human coronary artery endothelial cells (HCAEC). FXa-induced endothelial dysfunction and the reduction of $eNOS^{Ser1177}$ phosphorylation in HCAEC were partially ameliorated by a JNK inhibitor [72]. These results suggest that the genetic deletion of PAR-2 attenuates vascular inflammation, atherogenesis, and plaque destabilization. Since PAR-2 is widely expressed in vascular cells, it is conceivable that it acts to promote arteriosclerosis in both hematopoietic cells and nonhematopoietic cells. Further studies are required to elucidate the role of PAR-2 signaling in the pathogenesis of atherosclerosis.

PAR-2 deficiency reduced I/R injury-induced myocardial infarct size and cardiac remodeling [78]. Similarly, PAR-2 deficiency reduced cardiac dysfunction and myocardial remodeling in a coronary artery ligation model [67]. Based on these reports, *PAR-2*$^{-/-}$ mice exhibit less cardiac dysfunction after myocardial ischemia. Oxidative/nitrative stress, the phosphorylation of mitogen-activated protein kinase, and

the expression of proinflammatory genes were significantly attenuated in ischemic hearts of *PAR-2*$^{-/-}$ mice, which could be the underlying mechanism [78].

Badeanlou et al. reported that mice lacking PAR-2 ameliorated body weight gain and insulin resistance induced by a high-fat diet [79]. PAR-2 deletion in hematopoietic cells attenuated adipose tissue inflammation and insulin resistance. PAR-2 deficiency in nonhematopoietic cells improved diet-induced obesity. PAR-2 deletion also reduced hepatic tissue inflammation and steatosis [80]. PAR-2 deletion of nonhematopoietic cells attenuated systemic inflammation in mouse models of sickle cell disease [65]. The genetic deletion of PAR-2 in a mouse model of diabetic nephropathy (*Ins2*$^{Akita/+}$ *eNOS*$^{-/-}$ mice) attenuated the progression of renal inflammation, fibrosis, and dysfunction [70]. These results suggest that PAR-2 deficiency has protective effects against cardiometabolic disorders based on the inhibition of chronic inflammation in various cells/organs. Further studies are needed to elucidate the role of PAR-2 in inflammatory diseases.

Coagulation factors and PARs as therapeutic targets for inflammatory diseases

Now, dabigatran is an anticoagulant that is widely used for the prevention of stroke and systemic embolism in adult patients with nonvalvular atrial fibrillation. Several

subclinical studies imply that the inhibition of thrombin might attenuate atherogenesis, although it is still controversial even in animal models. However, a systematic review and meta-analysis of randomized controlled clinical trials demonstrated that dabigatran is associated with a significantly increased risk of myocardial infarction [81]. Although the precise mechanism of this increased risk is inconclusive [81], a potential contributing factor may be the paradoxical antifibrinolytic effect of dabigatran [82]. In recent years, myocardial injury after non-cardiac surgery (MINS), which is the most common major perioperative complication, is due to perioperative myocardial ischemia. Most patients with MINS are asymptomatic but have a poor prognosis [83]. Impressively, recent studies reported that dabigatran lowered the risk of MINS, post-operative cardiovascular death, and non-fatal ischemic events without significantly increasing the rate of major bleeding [84]. Further investigation is needed to clarify the underlying mechanisms, including the involvement of PAR-1 signaling.

Vorapaxar (SCH 530348) is a competitive and selective antagonist of PAR-1, the major thrombin receptor on human platelets. Vorapaxar potently inhibits thrombin-induced platelet aggregation [85,86]. In the clinical trial targeting patients with acute coronary syndromes (TRACER), the addition of vorapaxar to standard therapy did not significantly reduce the primary composite end point but significantly

increased the risk of major bleeding, including intracranial hemorrhage [87]. In contrast, the clinical trial targeting patients with chronic stable atherosclerosis (TRA 2°P-TIMI 50) demonstrated that vorapaxar add-on to standard therapy reduced the incidence of cardiovascular death or ischemic events but increased the risk of moderate or severe bleeding [88]. Several subgroup analyses of the TRA 2°P-TIMI 50 trial were performed. In patients with prior MI or peripheral artery disease (PAD) who had not had a previous stroke or TIA, vorapaxar added to standard therapy was effective for the long-term secondary prevention of thrombotic cardiovascular events; however, it increased moderate or severe bleeding [89,90]. Furthermore, in patients with prior MI or PAD without previous stroke or TIA, vorapaxar improved net clinical outcomes despite its associated bleeding risk [91-93].

Several direct FXa inhibitors are available for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. In addition to its anti-thromboembolic effect, several subclinical studies have suggested that the inhibition of FXa, especially by rivaroxaban, has protective effects against cardiometabolic disorders. Furthermore, emerging evidence suggests several beneficial effects of inhibiting FXa signaling. Previous studies have indicated that the expression of tissue factor and procoagulant activity increased in human atherosclerotic plaques,

suggesting an enhanced procoagulant state during atherosclerotic plaque progression and/or the contribution of these coagulation systems to atherogenesis [25,94,95]. In our human study, in patients who underwent coronary interventions, plasma FXa levels independently correlated with the severity of coronary atherosclerosis as determined by the Gensini score and plaque volume calculated by intravascular ultrasound [71]. Combined with the results of our animal studies, these results indicated that FXa promotes vascular inflammation through PAR-2, at least partially.

A large clinical trial targeting patients with stable atherosclerosis (COMPASS) demonstrated that low-dose rivaroxaban (2.5 mg twice daily), as an add-on to standard therapy, significantly reduced the incidence of cardiovascular death or ischemic events. Although major bleeding events occurred in more patients in the rivaroxaban add-on group than in the standard therapy group, mainly owing to gastrointestinal bleeding, there was no significant difference in the rates of intracranial or fatal bleeding between these two groups [96]. The results of subgroup analyses focused on patients with stable coronary artery disease or PAD showed that the addition of low-dose rivaroxaban reduced the incidence of major adverse cardiovascular events [97]. Although the incidence of major bleeding was increased by the rivaroxaban add-on, fatal or critical organ bleeding was not increased. In patients with stable coronary artery disease, there

was also a significant net benefit in favor of the rivaroxaban add-on, and the death rate was reduced by 23% [98]. Furthermore, in patients with PAD who underwent lower-extremity revascularization, rivaroxaban (2.5 mg twice daily), as an add-on to aspirin, significantly lowered the incidence of major adverse limb events and cardiovascular events compared to aspirin alone (VOYAGER PAD) [99]. The incidence of major bleeding did not differ significantly between the groups. Thus, the addition of vorapaxar or rivaroxaban to standard antiplatelet therapy in patients with stable atherosclerotic diseases has the potential to substantially reduce morbidity and mortality from ischemic events; however, this requires careful consideration of their bleeding risk.

Similar to antithrombogenic agents such as direct oral anticoagulants or PAR-1 agonists, a selective inhibitor of PAR-2 could be a potential therapeutic option for cardiometabolic disorders, given the abundant data from animal studies. However, because of the continuously changing PAR-2 conformation in response to several inflammatory proteases, it has taken almost 25 years for the first inhibitor to reach clinical trials; so far, no PAR-2 antagonist has been approved for human use. Recently, the selective PAR-2 antibody (MEDI0618) has been taken into first-in-human, phase I clinical trials (NCT04198558) for chronic osteoarthritis closely involving PAR-2 signaling [100]. Targeting proinflammatory responses mediated by PAR-2 is a potential

therapeutic strategy to control unwanted, disease-associated inflammation; however, further studies are needed to develop clinically-tolerated therapeutic strategies.

Conclusion

Chronic low-grade inflammation plays a central role in the pathophysiology of cardiometabolic disorders, in which various cellular and molecular mechanisms participate. This review has focused on the role of PAR pathways, such as the thrombin - PAR-1 pathway or the FXa - PAR-2 pathway, in the proinflammatory activation of immune cells and the pathogeneses of cardiovascular and metabolic diseases (**Fig. 3**). In summary, these pathways could be potential therapeutic targets and possible biomarkers for this global health threat. However, further studies are required to define the most suitable clinical application for targeting this pathway.

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Disclosures

The other authors declare no conflicts of interest.

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Figure legends

Figure 1 Overview of coagulation cascade and PARs. Thrombin activates PAR-1, PAR-3, and PAR-4. FXa activates both PAR-1 and PAR-2. PAR-1 and PAR-2 activated by thrombin or FXa promote gene expressions of inflammatory molecules, mainly via the NF-κB and ERK1/2 pathways. ERK: extracellular-signal-regulated kinase, FXa: activated coagulation factor X, NF-κB: nuclear factor-kappa B, PAR: protease-activated receptor

Figure 2. Genetic deletion of PAR-2 attenuated development of atherosclerosis. Representative figures of Sudan IV staining of the aortic arch of *ApoE*$^{-/-}$ or *PAR-2*$^{-/-}$ *ApoE*$^{-/-}$ mice. The genetic deletion of PAR-2 attenuated the development of atherosclerosis. Bar, 1 mm. ApoE: apolipoprotein E, PAR: protease-activated receptor

Figure 3. Role of PARs in development of cardiometabolic diseases. Metabolic stress enhances coagulation proteases–PARs signaling in various cell types, leading to the development of chronic inflammation. This system plays a pivotal role in the pathogenesis of cardiometabolic diseases. EC: endothelial cell, Mφ: macrophage, SMC: smooth muscle cell, PAR: protease-activated receptor

Table 1 Effects of thrombin inhibitors in mouse models of cardiometabolic disorders;

Mouse strain	Model(feeding)	Drug	Duration of Thrombin inhibitor	Major Findings	Ref
Atherosclerosis					
ApoE$^{-/-}$	normal chow	melagatran	22 weeks	inflammation↓ atherogenesis↓ plaque stability↑	42
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	dabigatran	12 weeks	atherogenesis↓ plaque stability↑	46
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	dabigatran	12 weeks	atherogenesis↓ plaque stability↑	45
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	dabigatran	4 weeks	atherogenesis↓	47
ApoE$^{-/-}$	Normal chow	dabigatran	5 or 20 weeks	atherogenesis↓ plaque stability↑	48
TM$^{Pro/Pro}$:*ApoE*$^{-/-}$	HFD, 45 kcal% fat	dabigatran	6 weeks	inflammation↓ atherogenesis↓ plaque stability↑	29
ApoE$^{-/-}$	HFD, 40 kcal% fat, 0.21% cholesterol	bivalirudin	16 weeks	atherogenesis→	51
ApoE$^{-/-}$	WTD, 21% fat, 0.2% cholesterol	vorapaxar (*PAR-1 inhibitor)	48 weeks	inflammation↓ atherogenesis↓	49
Myocardial disease					
C57BL/6J	myocardial ischemia reperfusion	hirudin	2 hours	infarct size↓	52

	injury				
Obesity/NAFLD					
C57BL/6J	HFD, 40 kcal% fat	dabigatran	12 weeks	inflammation↓ steatosis↓ body weight gain↓	[54]
C57BL/6J	HFD, 40 kcal% fat	dabigatran	12 weeks	body weight gain↓	[53]

ApoE: apolipoprotein E

HFD: high fat diet

NAFLD: nonalcoholic fatty liver disease

TM: thrombomodulin

WTD: Western-type diet

Table 2 Effects of FXa inhibitors in mouse models of cardiometabolic disorders.

Mouse strain	Model (feeding)	Drug	Duration of FXa inhibitor	Major Findings	Ref
Atherosclerosis					
ApoE$^{-/-}$	normal chow	rivaroxaban	26 weeks	inflammation↓ plaque stability↑	[60]
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	rivaroxaban	20 weeks	inflammation↓ atherogenesis↓ plaque stability↑	[56]
ApoE$^{-/-}$	WTD, 16% fat	rivaroxaban	6 or 14 weeks	inflammation↓ atherogensis↓ plaque stability↑	[59]
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	rivaroxaban	20 weeks	atherogenesis↓	[57]
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	rivaroxaban	14 weeks	inflammation↓ atherogenesis↓	[58]
ApoE$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	fondaparinux	4 weeks	inflammation↓ plaque stability↑	[62]
ApoE$^{-/-}$	angiotensin II infusion	fondaparinux	14 days	inflammation↓ atherogenesis↓	[63]
Ldlr$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	rivaroxaban	12 or 24 weeks	atherogenesis↓	[61]
C57BL/6J	wire-mediated vascular injury	rivaroxaban	5 weeks	inflammation↓ neointima formation↓	[64]
Myocardial disease					
C57BL/6J	myocardial ischemia reperfusion injury	rivaroxaban	14 days	inflammation↓ Ejection fraction↑ survival↑	[66]
C57BL/6J	myocardial infarction	rivaroxaban	28 days	Ejection Fraction↑	[67]

				remodeling↓	
SR-BI KO /*ApoeR61*$^{h/h}$	myocardial infarction	rivaroxaban	2 weeks	inflammation↓ remodeling↓ heart failure↓ survival↑	[68]
SHR (*rat)	atrial rapid pacing	rivaroxaban	2 weeks	inflammation↓ AF inducibility↓	[69]
Sickle cell disease					
sickle BERK mice	normal chow	rivaroxaban	10 days	inflammation↓	[65]
Diabetic nephropathy					
Ins2$^{Akita/+}$ *eNOS*$^{-/-}$	normal chow	edoxaban	14 weeks	inflammation↓ fibrosis↓	[70]

ApoE: apolipoprotein E

eNOS: endothelial nitric oxide synthase

Ldlr: low density lipoprotein receptor

NAFLD: nonalcoholic fatty liver disease

SHR: spontaneously hypertensive rat

WTD: Western-type diet

Table 3 Effects of genetic PAR-1 deletion in mouse models of cardiometabolic disorders.

Mouse strain	Model (feeding)	Duration of intervention	Major Findings	Reference
Atherosclerosis				
ApoE$^{-/-}$ *PAR-1*$^{-/-}$	HFD, 42% kcal% fat	16 weeks	atherogenesis↓	[73]
Ldlr$^{-/-}$ *PAR-1*$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	12 or 24 weeks	atherogenesis →	[61]
Myocardial disease				
PAR-1$^{-/-}$	myocardial ischemia reperfusion injury	2 weeks	remodeling↓	[52]
Sickle cell disease				
sickle BERK/ *PAR-1*$^{-/-}$	normal chow	10 days	inflammation →	[65]
Obesity/NAFLD				
PAR-1$^{-/-}$	methionine and choline (MCD) diet	2 weeks	body weight gain → Inflammation↓	[76]
PAR-1$^{-/-}$	HFD, 40% kcal% fat	12 weeks	body weight gain → inflammation↓ steatosis↓	[75]
PAR-1$^{-/-}$	HFD, 60% kcal% fat	20 weeks	body weight gain →	[53]

ApoE: apolipoprotein E

HFD: high fat diet

Ldlr: low density lipoprotein receptor

NAFLD: nonalcoholic fatty liver disease

PAR: protease-activated receptor

WTD: Western-type diet

Table 4 Effects of genetic PAR-2 deletion in mouse models of cardiometabolic disorders.

Mouse strain	Model (feeding)	Duration of intervention	Major Findings	Reference
Atherosclerosis				
ApoE$^{-/-}$ *PAR-2*$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	12 weeks	inflammation↓ atherogenesis↓ plaque stability↑	77
ApoE$^{-/-}$ *PAR-2*$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	20 weeks	inflammation↓ atherogenesis↓ plaque stability↑	71
ApoE$^{-/-}$ *PAR-2*$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	20 weeks	atherogenesis↓	57
Ldlr$^{-/-}$ *PAR-2*$^{-/-}$	WTD, 21% fat, 0.15% cholesterol	12 or 24 weeks	inflammation↓ atherogenesis↓ plaque stability↑	61
Myocardial disease				
PAR-2$^{-/-}$	myocardial ischemia reperfusion injury	4 weeks	inflammation↓ infarct size↓	78
PAR-2$^{-/-}$	myocardial infarction	4 weeks	Ejection Fraction↑ remodeling↓	67
Sickle cell disease				
sickle BERK mice	normal chow	10 days	inflammation↓	65
Obesity/NAFLD				
PAR-2$^{-/-}$	HFD, 60 kcal% fat	16-20 weeks	inflammation↓ body weight gain↓ insulin sensitivity↑ steatosis↓	79
PAR-2$^{-/-}$	HFD, 60 kcal% fat	16-20 weeks	inflammation↓ steatosis↓	80
Diabetic nephropathy				
Ins2$^{Akita/+}$ *eNOS*$^{-/-}$ *PAR-2*$^{-/-}$	Normal chow	28 weeks	inflammation↓ fibrosis↓ renal dysfunction↓	70

ApoE: apolipoprotein E

eNOS: endothelial nitric oxide synthase

HFD: high fat diet

Ldlr: low density lipoprotein receptor

NAFLD: nonalcoholic fatty liver disease

PAR: protease-activated receptor

WTD: Western-type diet

Graphical abstract

Highlights

- Protease-activated receptors (PARs) significantly contribute to the pathogenesis of cardiometabolic diseases.
- Pharmacological or genetic deletion of the PAR signaling pathway attenuates cardiovascular inflammation and atherogenesis.
- The PAR signaling pathway may serve as a potential therapeutic target for cardiometabolic disorders.

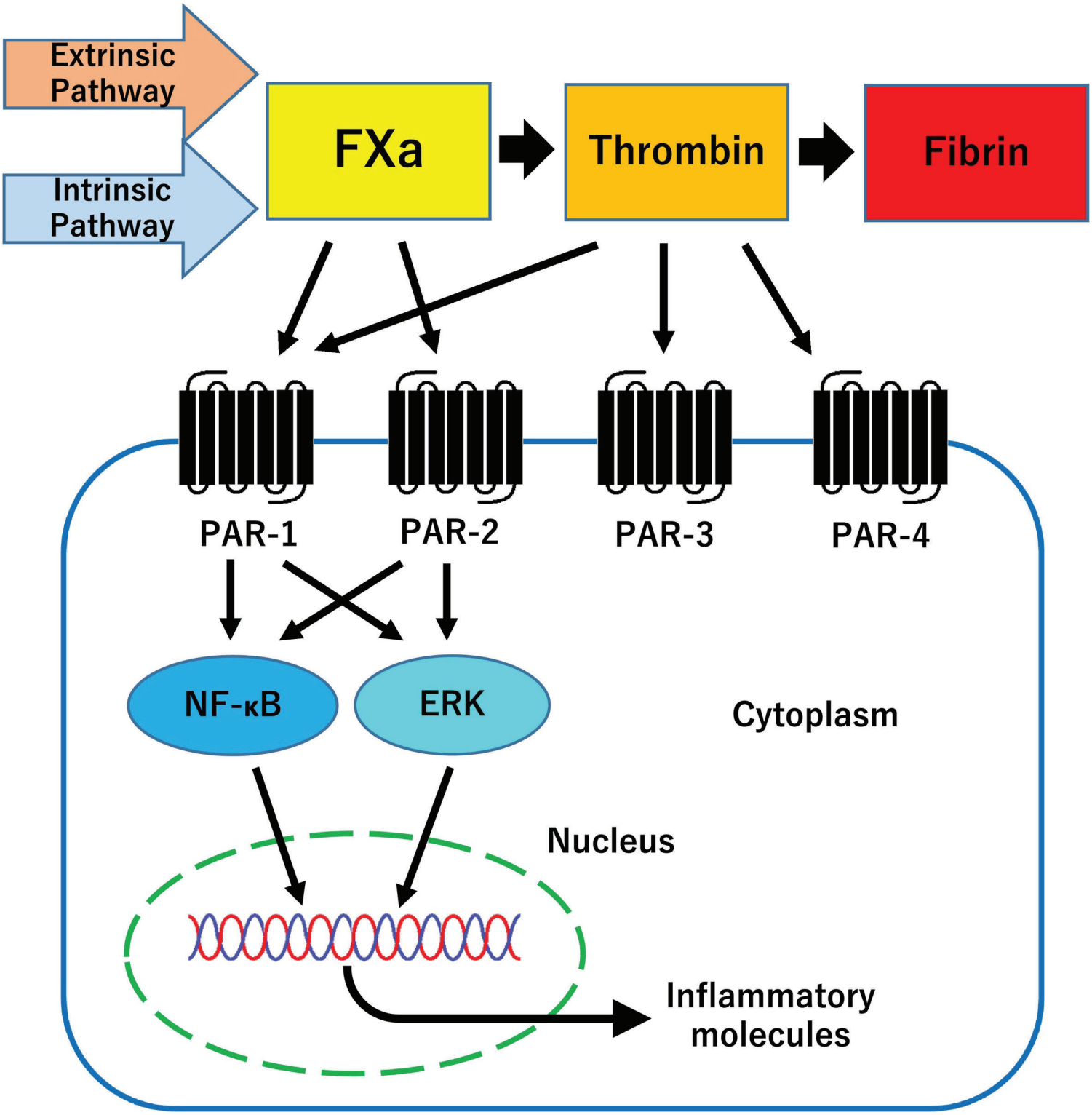
Extrinsic Pathway
Intrinsic Pathway
FXa
Thrombin
Fibrin
PAR-1
PAR-2
PAR-3
PAR-4
NF-κB
ERK
Cytoplasm
Nucleus
Inflammatory molecules

Figure 1

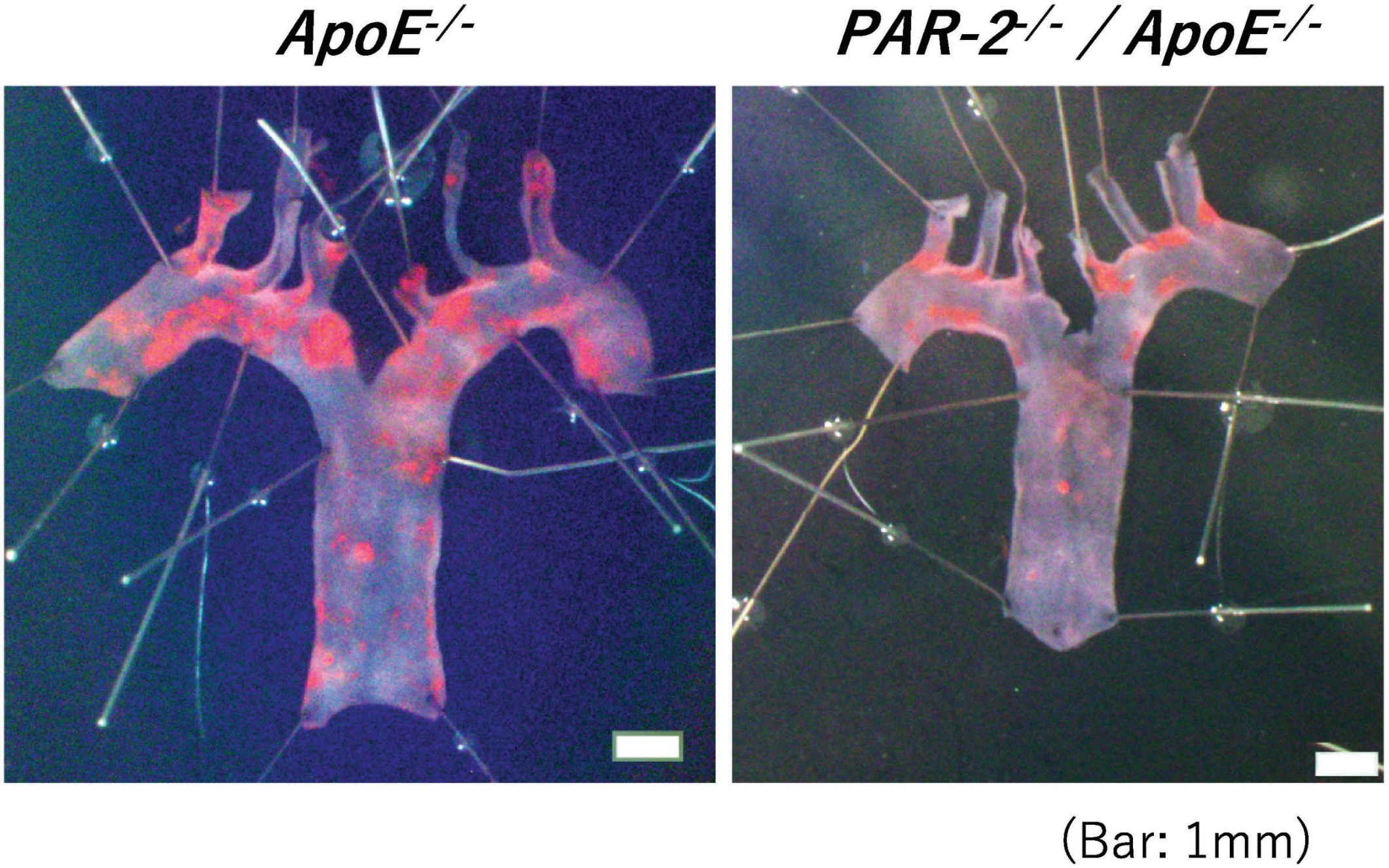

Figure 2

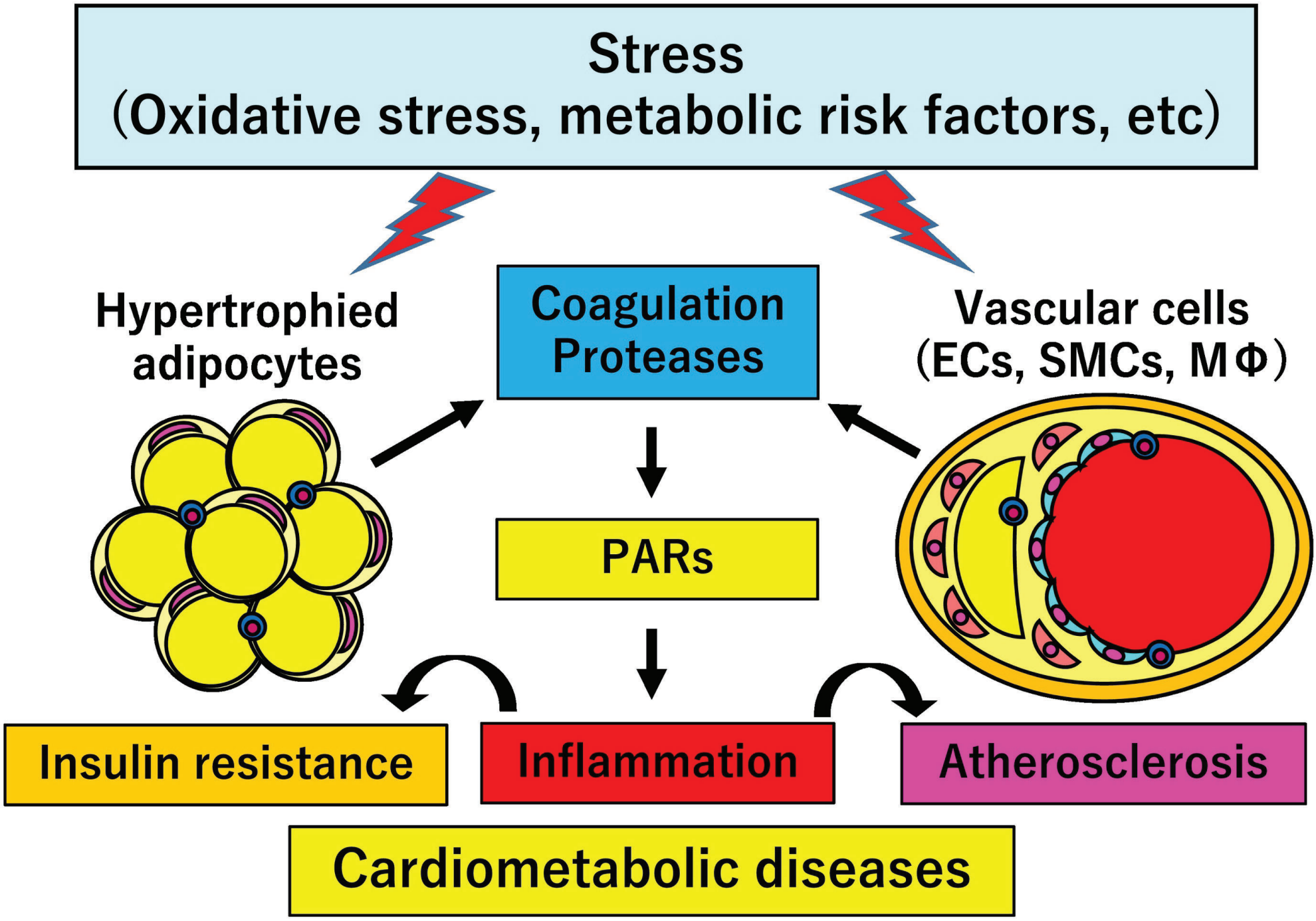
Stress
(Oxidative stress, metabolic risk factors, etc)
Hypertrophied adipocytes
Coagulation Proteases
Vascular cells (ECs, SMCs, MΦ)
PARs
Insulin resistance
Inflammation
Atherosclerosis
Cardiometabolic diseases

Figure 3