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REVIEW ARTICLE

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Pathophysiology of atherothrombosis: Mechanisms of thrombus formation on disrupted atherosclerotic plaques

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Atherothrombosis is a leading cause of cardiovascular mortality and morbidity worldwide. The underlying mechanisms of atherothrombosis comprise plaque disruption and subsequent thrombus formation. Arterial thrombi are thought to mainly comprise aggregated platelets as a result of high blood velocity. However, thrombi that develop on disrupted plaques comprise not only aggregated platelets, but also large amounts of fibrin, because plaques contain large amount of tissue factor that activate the coagulation cascade. Since not all thrombi grow large enough to occlude the vascular lumen, the propagation of thrombi is also critical in the onset of adverse vascular events. Various factors such as vascular wall thrombogenicity, local hemorheology, systemic thrombogenicity and fibrinolytic activity modulate thrombus formation and propagation. Although the activation mechanisms of platelets and the coagulation cascade have been intensively investigated, the underlying mechanisms of occlusive thrombus formation on disrupted plaques remain obscure. Pathological findings derived from humans and animal models of human atherothrombosis have uncovered pathophysiological processes during thrombus formation and propagation after plaque disruption, and novel factors have been identified that modulate the activation of platelets and the coagulation cascade. These findings have also provided insights into the development of novel drugs for atherothrombosis.

KEYWORDS

atherothrombosis, blood flow, coagulation factor, platelet, vasoconstriction

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INTRODUCTION

Atherothrombotic events such as myocardial infarction and ischemic stroke are major causes of morbidity and mortality worldwide. Atherosclerotic plaque disruption and subsequent thrombus formation are considered to be critical processes involved in the onset of atherothrombotic events. However, autopsy studies have revealed that these processes do not

always result in thrombotic occlusion followed by acute symptomatic events. These findings indicate that thrombus propagation is also critical to the onset of clinical events.[1]

Platelet activation is an initial step in hemostatic and thrombotic processes. Therefore, the development of platelet-rich thrombi has been regarded as a trigger of acute cardiovascular events.[2] Many findings of experimental animal models with 'normal' arteries damaged by chemical or physical means support the concept that activated platelets play critical roles in the establishment of arterial thrombus. However, the effects of antiplatelet therapy are clinically variable in terms of reducing the incidence of death, myocardial infarction, stroke and/or the need for further intervention.[3–5] These results suggest that factors other than platelets contribute to thrombus formation at sites of plaque disruption. According to autopsy findings, thrombi that cause myocardial infarction comprise not only platelets, but also large amounts of fibrin, suggesting increased activation of the coagulation cascade during such events.[6] Tissue factor (TF) is a trigger of the coagulation cascade. High levels of TF are expressed in human atherosclerotic lesions and that this is an important determinant of thrombogenicity contributing to thrombus formation at sites of plaque disruption.[7,8]

The mechanisms of plaque instability and the activation of platelets and coagulation pathways have been investigated in detail. Yet, how the formation of thrombus at sites of plaque disruption leads to the onset of cardiovascular events remains obscure. This article examines the underlying mechanisms of thrombus formation and propagation on disrupted atherosclerotic plaques from pathological perspectives.

PATHOLOGY OF PLAQUE DISRUPTION

Plaque disruption initiates arterial thrombus formation. Pathological studies after sudden coronary death have identified two types of plaque disruption: rupture and erosion[9,10] (Fig. 1).

Plaque rupture is the most common cause of acute coronary events. It is characterized by the disruption of a thin fibrous cap, which allows the thrombogenic necrotic core to contact circulating blood. The histological features of rupture-prone plaques include a large necrotic core, thin (<65 μm) fibrous caps, many inflammatory cells (macrophages, T lymphocytes), positive vascular remodeling, increased neovascularization, and occasional smooth muscle cells (SMC).[9,10] Accumulating evidence supports the notion that inflammation plays a key role in the pathogenesis of plaque rupture. Macrophages in plaques express proteolytic collagenases, gelatinases and elastolytic enzymes that can degrade fibrous caps. Activated T lymphocytes and macrophages can express and secrete interferon (INF)-γ that inhibits collagen synthesis and induces SMC apoptosis.[11] Therefore, inflammatory pathways significantly participate in plaque destabilization.

Plaque erosion is characterized by relatively superficial injury to plaques in 22 – 44% of patients with fatal coronary thrombi.[10,12] Such plaques are rich in SMC and proteoglycan matrix containing abundant versican and hyaluronan, whereas the necrotic core is small or absent and inflammatory cells are scant. Compared with plaque rupture, patients with plaque erosion are younger, and females are predominant.[10,12] Several factors including hemodynamic forces, endothelial apoptosis, neutrophil activation, matrix

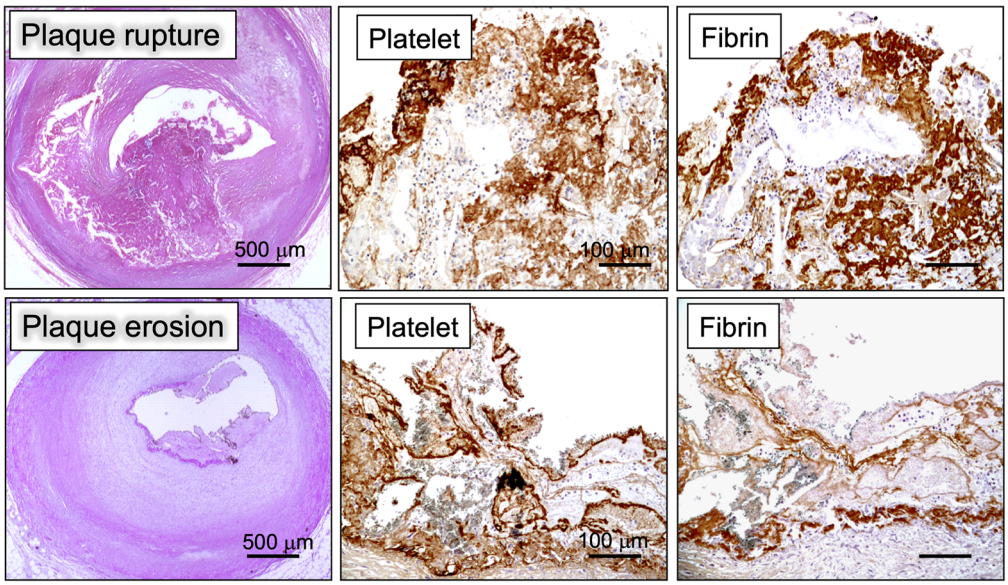

Figure 1 Microphotographs of human coronary plaque rupture and erosion with thrombi. Ruptured plaque comprises large necrotic core and disrupted thin fibrous cap accompanied by thrombus formation. Eroded plaque is fibrous and rich in smooth muscle cells, without visible atheromatous components. Both types of thrombi comprise platelets and fibrin (Ref. 13 with permission).

modification, and vasoconstriction are considered to contribute to erosion, however, details remain unclear.[12]

PATHOLOGY OF THROMBUS ON RUPTURED AND ERODED PLAQUES

Since platelets play a central role in arterial thrombus formation because of high blood velocity, arterial thrombi are generally considered to mainly comprise aggregated platelets. However, pathological results have revealed that thrombi growing on disrupted plaques consist of not only aggregated platelets, but also large amounts of fibrin (Fig. 1).[13,14] In addition, the proportions of fibrin and platelets differ in coronary thrombi on ruptured and eroded plaques, which are fibrin- and rather platelet-rich, respectively.[13] These pathological features indicate that the coagulation cascade is also activated at plaque disruption sites, and that the thrombogenetic mechanisms differ between plaque rupture and erosion. The above findings could explain why the ability of antiplatelet therapy to prevent cardiovascular events is variable and suggest that antiplatelet combined with anticoagulation therapy would be more favorable, although bleeding risk would be increased.[15]

PLATELET ACTIVATION ON DISRUPTED PLAQUES

Platelet activation on disrupted plaques is an initial step in the formation of thrombus. Under high shear conditions, von Willebrand factor (VWF) forms a bridge between exposed collagen and platelet glycoprotein (GP) Ib-IX-V receptor complexes on the platelet membrane.[16] Exposed collagen also binds directly to platelet GP Ia/IIa and GP VI receptors. The shape of activated platelets changes during this process and they release adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT, serotonin) and thromboxane A_2, all of which further promote platelet recruitment and activation. Adenosine triphosphate (ATP) released from erythrocytes and leukocytes can also activate platelets.[17] The metabolism of ADP and ATP prevents excessive platelet activation in the bloodstream. We present three new modulators of platelet activation: ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase-1), C-type lectin-like receptor 2 (CLEC-2), and a disintegrin and metalloprotease with a thrombospondin type 1 motif 13 (ADAMTS-13).

Ectonucleoside triphosphate diphosphohydrolase-1

Endothelial cells and SMC express NTPDase-1 (CD39) on the cell surface, where it rapidly hydrolyzes ADP and ATP to AMP and consequently inhibits platelet activation and SMC constriction.[18] Endothelial NTPDase-1 expression is downregulated under conditions of inflammation and disrupted blood flow.[19,20] Hatakeyama *et al.*[21] showed that NTPDase-1 expression by vascular SMCs is reduced in human atherosclerotic plaques, being more significantly decreased among patients with unstable, than stable angina. These findings suggest that NTPDase-1 expression by SMC is also reduced by inflammatory and/or oxidative stress. The local expression of NTPDase-1 due to gene transfer in the injured arterial walls of rat models of thrombotic carotid artery occlusion significantly suppresses platelet aggregation and thrombotic occlusion, and inhibits vasoconstriction induced by ADP and ATP.[22,23] Extracellular ATP and ADP also modulate the expression of plasminogen activator inhibitor-1 (PAI-1) and TF in vascular SMC and endothelial cells.[24,25] These lines of evidence suggest that reduced NTPDase-1 expression in atherosclerotic lesions augments platelet activation and thrombus formation when plaques are disrupted.

C-type lectin-like receptor 2 and endogenous ligands

C-type lectin-like receptor 2 is a type II transmembrane glycoprotein and receptor for the platelet-activating snake venom protein, rhodocytin. This receptor mediates activation signals in conjunction with Src, Syk kinases and phospholipase Cγ2, like the collagen receptor GPVI/FcRγ-chain complex.[26] Podoplanin has recently been identified as an endogenous ligand for CLEC-2.[26] Under physiological conditions, podoplanin is expressed on lymphatic, but not arterial and venous endothelial cells. However, large amounts of podoplanin are expressed in human advanced atherosclerotic lesions, and that podoplanin overexpression in rat carotid arteries caused by gene transfer enhances platelet aggregation, resulting in occlusive thrombus formation.[27,28]

Inoue *et al.*[29] identified S100A13 as another novel ligand for CLEC-2 in SMC. S100A13 belongs to the S100 family of proteins that have EF-hand Ca^{2+}-binding motifs, and it is implicated in inflammation, angiogenesis and tumor growth.[30] This protein localizes in the cytoplasm and/or nucleus but on the surface of SMC under oxidative stress.[29]

These findings indicate that increased expression of CLEC-2 ligands, podoplanin and S100A13 in advanced atherosclerotic plaques promotes thrombus formation on disrupted plaques via platelet activation.

von Willebrand factor/ADAMTS-13 axis

von Willebrand factor is essential for platelet adhesion and aggregation, and it assumes a multimeric form (MW range, 50–20 000 kDa) in circulating blood.[16] This factor is synthesized and stored in Weibel–Palade bodies of endothelial cells and upon release, is rich in ultra-large (UL) multimeric forms that hyperactively bind platelet GP Ib and can induce platelet

aggregation.[31,32] Under high shear conditions, UL-VWF multimers are rapidly cleaved by the plasma protease, ADAMTS-13, to smaller and less active multimeric forms.[32] Since ADAMTS-13 cleaves VWF multimers under high shear conditions, it is considered to protect against platelet aggregation and thrombotic occlusion in stenotic atherosclerotic arteries.[33] Clinical studies have shown decreased ADAMTS-13 activity or a higher ratio of VWF/ADAMTS-13 in patients with acute myocardial infarction (AMI).[34,35] We found that ADAMTS-13 closely localizes with VWF in coronary thrombi from patients with acute coronary syndrome (ACS), and that decreased ADAMTS-13 activity significantly augmented thrombus formation in rabbit injured arteries *in vivo* and in a flow chamber system *in vitro*.[36] ADAMTS-13 activity is reduced with aging and by systemic inflammation.[37,38]

ROLES OF COAGULATION CASCADE PATHWAY IN PLAQUES

The coagulation cascade pathway also plays a pivotal role in hemostasis and thrombus formation. The extrinsic coagulation pathway is initiated by plasma factor VII/VIIa (FVII/FVIIa) binding to TF, then TF/FVIIa complexes activate both FIX and FX. Factor Xa activates prothrombin in the presence of its activated cofactor FVa, to thrombin, which subsequently plays a central role in the coagulation protease cascade.[39] Activated platelets provide a negatively charged surface, which significantly enhances the coagulation cascade.[16]

Tissue factor is a type-I transmembrane glycoprotein that serves as a cell surface receptor and cofactor for blood coagulation factors VII and VIIa, and thus plays a central role in hemostasis and thrombogenesis.[39] This glycoprotein is widely expressed in the human brain, heart, placenta, lungs, kidneys and other organs.[40] Only adventitial fibroblasts express TF in normal arteries, whereas macrophages and SMC in atherosclerotic lesions express high levels of TF. In addition, large amounts of TF are deposited in the extracellular matrix of advanced lesions in humans[7,8] (Fig. 2). These findings indicate that TF expressed in atherosclerotic plaques largely contributes to increasing plaque thrombogenicity and promotes thrombus formation after plaque disruption. Furthermore, macrophages and SMC that express

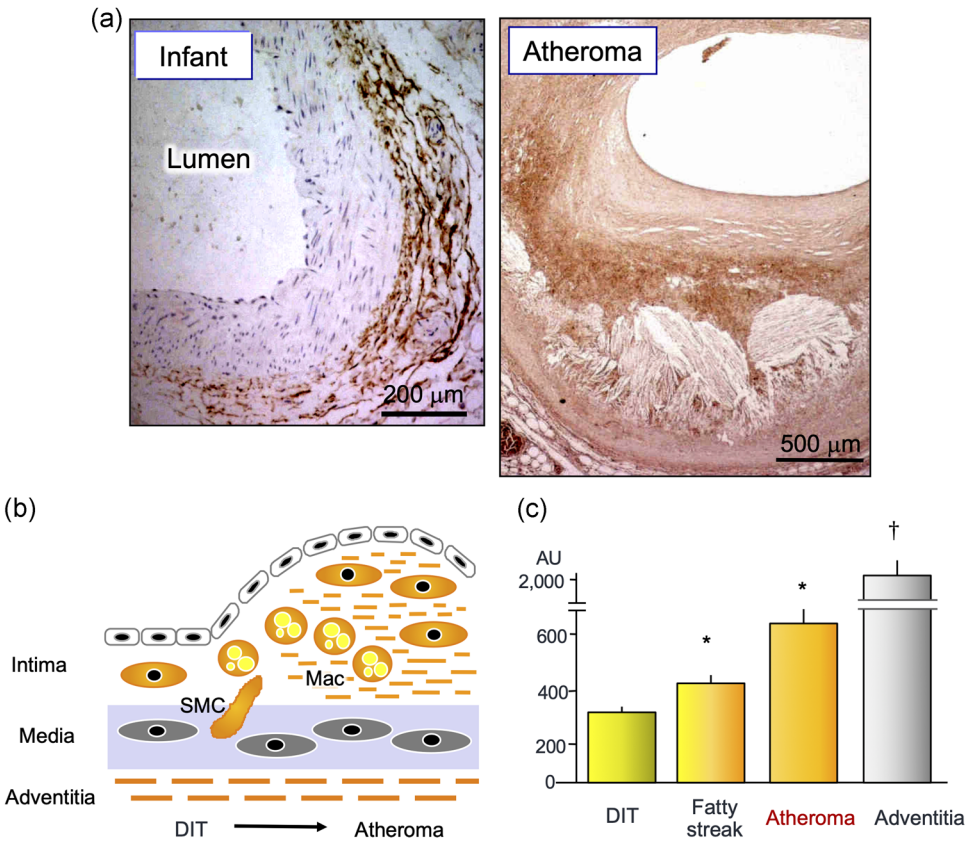

Figure 2 Localization and activity of tissue factor in human atherosclerotic lesions. (**a**) Tissue factor is localized in adventitia of nonatherosclerotic artery (infant coronary artery), but is broadly present in atheromatous lesion of coronary artery. (**b**) Schema of TF localization in atherosclerosis. Tissue factor is localized in SMC and macrophages during early to advanced stages of atherosclerosis. Large amounts of TF are localized in extracellular matrix of advanced lesions. (**c**) Tissue factor activity is found in all atherosclerotic lesions and is more prominent in fatty streaks and atheroma than in DIT. $^{*}P < 0.05$, $^{\dagger}P < 0.001$, versus DIT. (Ref. 8 with permission). DIT, diffuse intimal thickening; Mac, macrophage; SMC, smooth muscle cells.

TF can release microparticle (MP)-associated TF. Thus, atherosclerotic lesions are abundant in MP-TF, high concentrations of which circulate in the blood of patients with atherothrombotic diseases.[41,42] Since MP-TF is highly procoagulant, MP in blood could serve as a useful biomarker and predictor of cardiovascular events.[41,42] However, MP-TF contribution to arterial thrombus formation/propagation remains controversial due to the high blood velocity in arterial circulation.[42]

Tissue factor and coagulation factors also have nonhemostatic biological functions via G-protein-coupled proteinase-activated receptors (PAR) and are activated by proteolytic cleavage of the NH_2-terminal end, which exposes a cryptic tethered ligand.[43] Four forms of PAR are ubiquitously expressed in many cell types in systemic organs, where they play important roles in the physiology and pathophysiology of cardiovascular and other organ systems.[43] The TF/FVIIa complexes, FXa and thrombin activate PAR[44–47] (Fig. 3). Activated PAR in the vascular system mediates various activities including the regulation of vascular tone, the migration and proliferation of SMCs, and the production of extracellular matrix, thus contributing to the development of vascular lesions.[43,44] We found that PAR2 plays a pivotal role in SMC migration induced by TF/VIIa complexes.[46] Complexes of MP-associated TF and soluble TF with FVIIa activates PAR2, even though the soluble form does not activate coagulation. The above evidence indicates that three forms of TF and other coagulation factors contribute to the formation of plaque and thrombus.

RABBIT MODEL OF ATHEROTHROMBOSIS

Experimental animal models are important for studies of human diseases and pathophysiology, and atherosclerosis and thrombosis have been investigated, for example, in nonhuman primates, pigs, rabbits, rats and mice.[48] Ideal experimental animals should be of an appropriate size and easy to handle, and resemble humans in terms of anatomy, physiology and pathophysiology, particularly for medical and pharmaceutical research. Rabbits are valuable for studying human atherothrombosis, because they have mechanisms of lipoprotein metabolism and platelet activation that are more similar to those of humans than smaller rodents such as rats and mice.[48,49] We created plaques in rabbit arteries with intimal injury induced by a balloon catheter and fed them with a conventional or a cholesterol diet to develop SMC- or macrophage-rich plaques, respectively. Both SMC and macrophages in the plaques expressed abundant TF. The amounts of TF messenger RNA (mRNA) expression and thrombogenetic activities increased with plaque growth and were more prominent in macrophage-rich plaques,[50,51] thus mimicking the situation in human atherosclerotic plaques.

Mechanical plaque injury caused by a balloon catheter produced large thrombi comprising a mixture of platelets and fibrin, whereas small platelet-rich thrombi developed on injured normal arteries. Far more fibrin was produced on the injured macrophage-rich plaques (Fig. 4),[51] which also simulated the pathological features of human atherothrombosis. An intravenous injection of a recombinant TF pathway inhibitor (TFPI) or TFPI gene transfection into

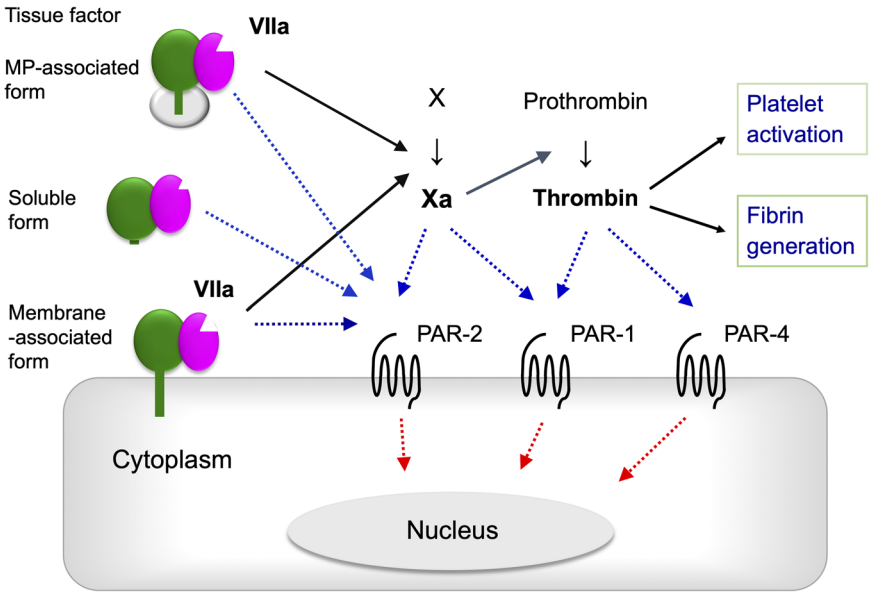

Figure 3 Tissue factor/factor VIIa complex-dependent coagulation pathway and proteinase-activated receptors (PAR). Membrane- and microparticle (MP)-associated tissue factor (TF) binding to factor VIIa triggers coagulation pathway, whereas soluble TF with factor VIIa does not. Downstream coagulation factors activate PAR that also play other noncoagulative biological roles (Ref. 47).

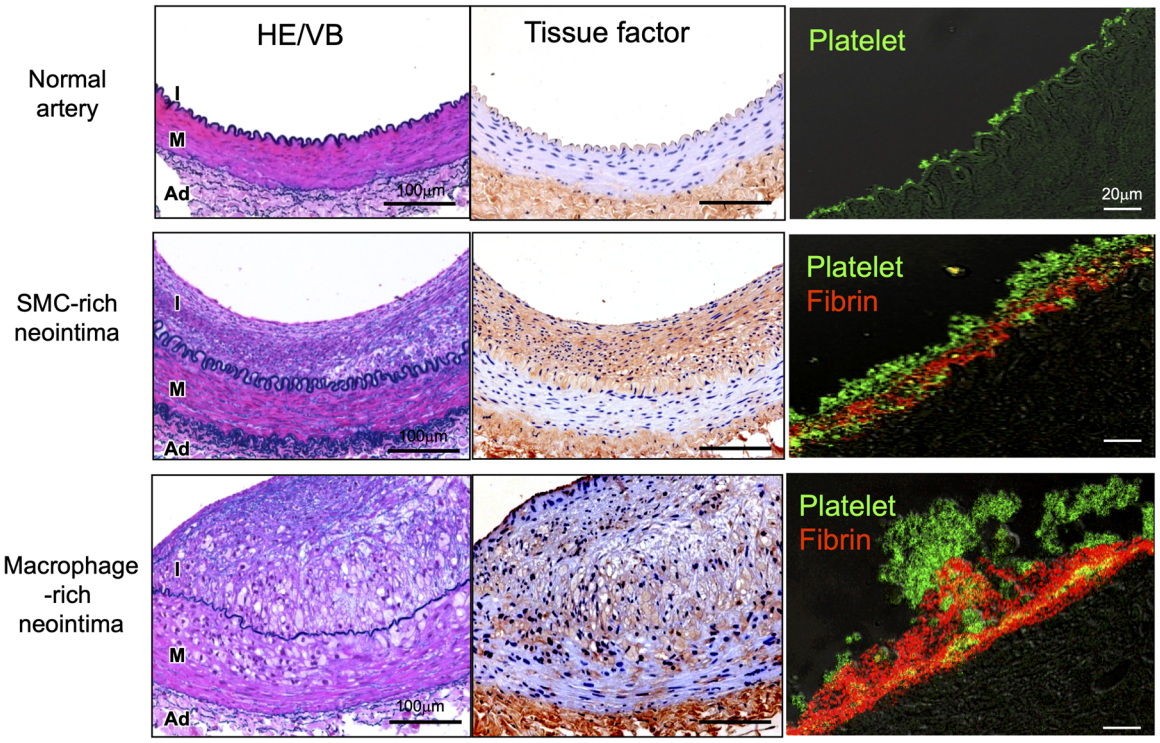

Figure 4 Immunohistochemical microphotographs of tissue factor and thrombus in rabbit normal and atherosclerotic femoral arteries. Left and middle columns: Representative immunohistochemical microphotographs of normal femoral artery and of femoral arteries at 3 weeks after balloon injury of conventional (smooth muscle cells (SMC)-rich neotima) or 0.5% cholesterol diet (Macrophage-rich neointima). Middle column: Tissue factor is expressed in SMC- and macrophage-rich neointima, and in adventitia. Right column: Thrombus at 15 min after balloon injury on normal artery comprises only small aggregated platelets, whereas that on neointima comprises platelets and fibrin. Thrombus on macrophage-rich neointima is much larger. Ad, adventitia; HE/VB, hematoxylin and eosin/Victoria blue; I, intima; M, media (Ref. 51 with permission).

injured vessel walls obviously reduced thrombus formation, indicating the crucial role of a TF-dependent coagulation pathway in thrombus formation on disrupted plaques.[52,53]

INCREASED EXPRESSION OF TISSUE FACTOR IN PLAQUES

Proinflammatory cytokines, such as interleukin-1, IFNγ, tumor necrosis factor-alpha (TNFα), lipopolysaccharide, and modified low density lipoprotein can all induce TF expression by macrophages and SMC in plaques.[54] Furthermore, the following factors also induce TF expression in plaques.

C-reactive protein

C-reactive protein (CRP) is a classical plasma protein marker that is clearly elevated during the acute phase of inflammation and tissue damage, and high levels of plasma CRP are associated with future cardiovascular events.[55] This protein is localized to macrophages, SMC and necrotic cores in atherosclerotic plaques, and it is more prevalent in plaques of patients with unstable, rather than stable angina,[56,57] implicating a critical role of CRP in the formation of plaque and thrombus. Human CRP (hCRP) overexpressed in transgenic (Tg) rabbits does not affect the development of atherosclerosis, but significantly promotes thrombus formation after plaque disruption via enhanced TF expression in SMC.[58,59]

Plaque hypoxia

Hypoxia affects the biological functions of vascular cells by regulating metabolism, inflammation, angiogenesis and several other processes.[60] Hypoxia signaling can modulate tissue remodeling or the severity of cardiovascular disorders. Sluimer *et al.*[61] found hypoxic areas in advanced human atherosclerotic lesions using pimonidazole hydrochloride, a marker of hypoxia, and correlated these areas with macrophages, as well as the expression of hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF). They also associated the HIF pathway with plaque progression and intraplaque angiogenesis. We found that the amounts of HIF-1α-positive nuclei positively correlated with TF- and PAI-1-positive areas in human coronary plaques, and were significantly more prevalent in plaques with thrombi.[62]

Plaque hypoxia has been detected in animal models of atherosclerosis. Leppänen *et al.*[63] assessed plaque hypoxia in a rabbit atherosclerotic model using (7-(4′-(2-nitroimidazol-1-yl) -butyl)-theophylline (NITP) and found that plaques >500 μm thick are hypoxic and characterized by ATP depletion, low glucose and glycogen, and high lactate concentrations. We also detected hypoxic areas in a rabbit model of atherosclerosis using pimonidazole hydrochloride. The hypoxic areas were located deep inside macrophage-rich plaques, and positively correlated with the number of HIF-1α-positive nuclei and TF- and PAI-1-positive areas, as in human plaques.[62] We also found that hypoxic conditions increase TF and PAI-1 expression in cultured atherosclerotic plaque tissues and macrophages[62] (Fig. 5). These results suggested that plaque hypoxia augments the thrombogenic potential of atherosclerotic plaques via prothrombotic factor upregulation.

Tryptophan and kynurenine pathway

Tryptophan (Trp) is an essential amino acid that is directed towards either protein synthesis or metabolized to various bioactive molecules via the serotonin or the kynurenine (Kyn) pathways, the latter of which is the major route of Trp catabolism.[64] Catabolites in the Kyn pathway play important roles in regulating immune and inflammatory reactions, and thus this pathway is associated with cardiovascular diseases.[64] Indoleamine 2,3-dioxygenase 1 (IDO1) is a rate-limiting enzyme of the Kyn pathway that is a major contributor to Trp degradation. It is overexpressed in many pathological states such as cancer, autoimmune and inflammatory bowel diseases.[65] We found that macrophages express IDO1 more predominantly within atherosclerotic plaques from patients with unstable than stable angina, and that IDO1 closely localizes with TF. We also showed that IDO1 and Kyn enhance TF expression and activity in cultured macrophages stimulated with IFNγ and TNFα.[66]

ONSET OF ACUTE CARDIOVASCULAR EVENTS

Thrombus formation on disrupted plaques is a critical process in the onset of acute cardiovascular events. However, thrombus does not always lead to complete vessel occlusion with subsequent acute symptomatic events.[1]

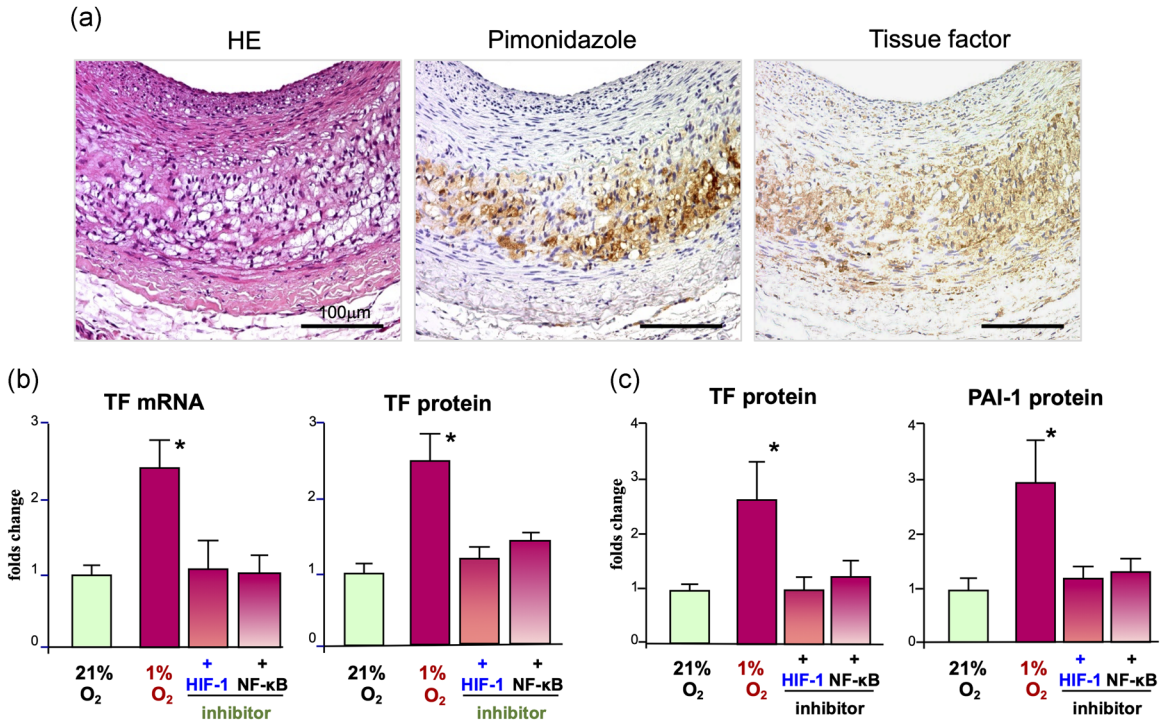

Figure 5 Plaque hypoxia and thrombogenicity in rabbit atherosclerotic lesion. (**a**) Representative histological and immunohistochemical images of rabbit atherosclerotic lesion show close proximity of pimonidazole (hypoxic marker) and tissue factor expression. (**b**) Effects of hypoxia on TF gene and protein expression in cultured macrophages. Tissue factor (TF) messenger RNA (mRNA) and protein levels in cultured THP-1 macrophages are significantly increased after 6 h under 1% O_2 (hypoxia), and suppressed by inhibitors of either HIF-1 (dimethyl-bisphenol A, 100 μmol/L) or nuclear factor-kappa B (NF-kB) (Bay 11–7085, 20 μmol/L). *$P < 0.0001$ versus normoxia (21% O_2), and HIF-1 and NF-kB inhibitors. (**c**) Effects of hypoxia on TF and PAI-1 protein expression in cultured atheromatous lesions. Protein levels of TF and PAI-1 in cultured atheromatous lesions from rabbits are significantly increased at 6 h under hypoxic, versus normoxic conditions. *$P < 0.0001$ versus atheromatous lesions cultured under normoxia (21% O_2), or with HIF-1 or NF-kB inhibitor (Ref. 62 with permission).

Autopsy studies of patients dying of non-cardiovascular diseases found a 4–10% incidence of asymptomatic disrupted plaques with nonocclusive mural thrombi in the coronary arteries.[67–69] Clinical intravascular imaging findings have revealed a far higher incidence of asymptomatic coronary plaque disruption, and the disruption of many plaques is quite prevalent in patients with ACS.[70,71] Plaque disruption at various stages of healing is also occasionally found in autopsy cases with or without ACS.[69,72] These lines of evidence indicate that asymptomatic plaque disruption is not unusual, and that occlusive thrombus formation is a critical process during the onset of clinical events.

We investigated the vascular factors that affect occlusive thrombus formation in an autopsy study of AMI and noncardiac death (asymptomatic plaque disruption), and found that the length of plaque disruption, luminal narrowing, lipid core size and the expression of TF and hexokinase-II in plaques were associated with thrombotic occlusion in human coronary arteries.[73] These results suggest that the morphological characteristics and increased TF expression in plaques are associated with thrombotic occlusion, and glucose metabolism is also associated with thrombogenicity in plaques. As noted above, advanced plaques are in a hypoxic milieu, and macrophages in hypoxic areas express HIF-1, which enhances the glycolytic pathway.[74]

Positron emission tomography (PET) using [^{18}F]-fluorodeoxyglucose (^{18}F-FDG) is useful for evaluating glucose uptake. Clinical studies have uncovered a relationship between ^{18}F-FDG uptake and future cardiovascular events.[75,76] The uptake of ^{18}F-FDG in plaques correlates significantly with hypoxic areas and macrophage content, TF expression and thrombus size after plaque disruption in rabbit models[77] (Fig. 6). These results suggest that ^{18}F-FDG uptake could be useful to assess thrombotic risk of atherosclerotic plaques in patients with cardiovascular diseases.[78]

Pathological studies of aspirated coronary thrombi obtained from patients with AMI during percutaneous coronary intervention (PCI) have shown that most of such thrombi are fresh, but cell lytic change and/or organizing reactions such as endothelialization and SMC ingrowth are evident in 33–>50% of them.[79–81] These findings indicate that thrombi are already days or weeks old; that is, plaque disruption and thrombus formation occur at least several days before symptom onset. In addition, older thrombi are independent

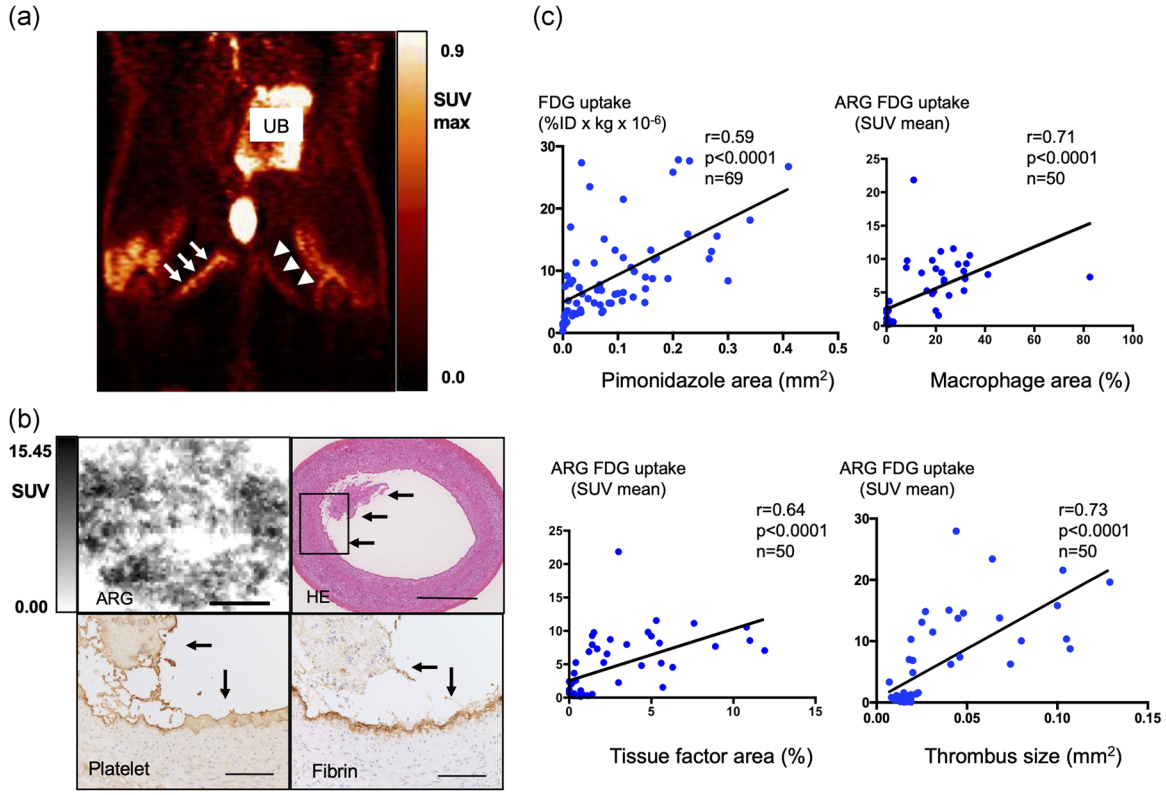

Figure 6 18F-FDG-PET imaging and radioactivity accumulation in rabbit arteries. (**a**) Coronal image shows more 18F-FDG accumulation in atherosclerotic (arrows) than normal (arrowheads) arteries. (**b**) Autoradiographic and histologic findings of atherosclerotic arteries with thrombus. Radioactivity has accumulated in sections of atherosclerotic arteries. Thrombus (arrows) consists of platelets and fibrin. (**c**) Correlations between 18F-FDG uptake and vascular (**a**) and thrombus (**b**) components in arterial sections. Uptake of 18F-FDG positively correlates with immunopositive areas for pimonidazole (hypoxia), macrophages, tissue factor and thrombus size. ARG, autoradiography; FDG, fluorodeoxyglucose; PET, positron emission tomography; SUV, standardized uptake value; UB, urinary bladder (Ref. 74,77 with permission).

predictors of mid or long-term mortality in patients with AMI.[81] These lines of evidence suggest that the underlying mechanisms of thrombotic occlusion at sites of plaque disruption are critical to the onset of clinical events. The following factors can contribute to the mechanisms.

Changes in blood flow

Altered blood flow is a major contributor to the development of thrombus. Blood flow disturbance induced by vascular narrowing or luminal surface irregularities in arteries with advanced atherosclerosis are thought to favor the activation of platelets and coagulation factors.[82] Decreased ADAMTS-13 activity under disturbed flow can augment platelet aggregation.[33] Furthermore, plaque disruption and coronary intervention can induce distal microembolisms and microvascular constriction that reduce or disrupt coronary blood flow at sites of plaque disruption.[83,84] Reduced blood flow facilitated thrombus propagation and thrombotic occlusion at plaque disruption sites in our animal models.[85]

Disturbed blood flow can also induce plaque disruption (erosion). Endothelial cells preferentially undergo apoptosis downstream of atherosclerotic plaques, where blood flow is disturbed and shear stress is lower than that upstream.[86] Experimental acute aortic stenosis of rat normal aortae (lumen reduced to 20–25%) can induce endothelial change or denudation and platelet adhesion on denuded areas.[87] We created a rabbit model with mild arterial stenosis, and showed that post-stenotic perturbed blood flow induces plaque erosion and augmented thrombus propagation resulting in thrombotic occlusion.[88,89] Furthermore, computational flow simulation has shown that increased wall shear stress, turbulence kinetic energy and the blood pressure gradient play significant roles in the onset of plaque erosion[90] (Fig. 7). These results indicate that hemodynamic forces, particularly that of disturbed blood flow induced by stenosis, could be a significant factor in the generation of

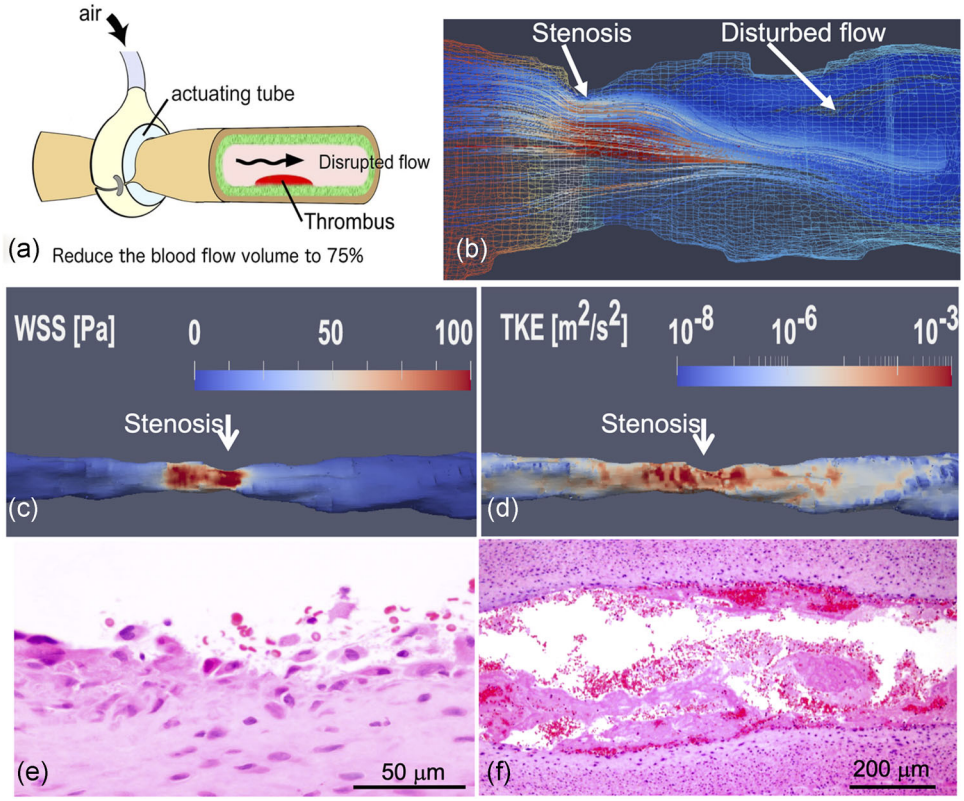

Figure 7 Computational flow simulation and microphotographs of erosive injury of rabbit stenotic femoral artery with SMC-rich plaque. (**a**) Rabbit femoral arteries at 3 weeks after balloon injury were constricted using a vascular occluder (actuating tube) to reduce blood flow volume to 75%. (**b**) Representative computational reconstructed image and flow simulation in Reynolds-Averaged Navier-Stokes model. Red and blue mesh indicates high and low wall pressure, respectively. Flow velocity in this model increases at stenosis and decreases at post-stenotic portion, resulting in disrupted flow. (**c**, **d**) Distribution of wall shear stress (WSS) and turbulence kinetic energy (TKE) of 3D-image in reconstructed artery. Magnitude of WSS is increased at stenotic portion. Magnitude of TKE is broadly and heterogeneously increased in this model and is maximal at stenotic portion. (**e**, **f**) Representative microphotographs of erosive injury and thrombus formation. Neointimal endothelial cells and SMC are broadly detached at stenotic and post-stenotic portions 15 min after vascular stenosis (**e**), and large mural thrombi formed 60 min after vascular stenosis (**f**). (Ref. 88–90 with permission).

plaque erosion and thrombosis, and suggest that altered blood flow combined with increased thrombogenicity in plaques is crucial for thrombus propagation and thrombotic occlusion.[85]

Thrombus-mediated vasoconstriction

Vascular constriction is a pivotal factor for thrombotic occlusion. If thrombus that arises after plaque disruption is not occlusive, the additional contribution of vasoconstriction can lead to vascular occlusion. Mural thrombi release many vasoactive agents including 5-HT, ADP, ATP, thromboxane A_2 and coagulation factors. Vasoconstriction induced by ADP and ATP is likely elicited in atherosclerotic vessels due to decreased NTPDase-1 activity, and FXa and thrombi increase vascular tonus via PAR.

Intima rich in SMC and extracellular matrix is a morphological feature of atherosclerotic lesions that is susceptible to vasoconstriction or vasospasm by vasoactive agents.[9,91] The coronary arteries of patients with coronary vasospasm have SMC-rich intima,[92] and platelet and blood coagulation in coronary circulation are activated after vasospastic angina.[93,94] We found that among these vasoactive agents, 5-HT induces a powerful hypercontractile response in arteries with SMC-rich neointima compared with normal arteries. Hypercontractile responses were also induced by 5-HT in SMC-rich neointima and in underlying media via 5-HT_{2A} receptors and the Rho-kinase pathway (Fig. 8).[95] These findings suggest that mural thrombus can promote vascular narrowing and occlusion via thrombus-mediated vasoconstriction.

Role of factor XI in thrombus propagation

Factor XI (FXI) is an intrinsic coagulation factor and the zymogen of a trypsin-like serine protease that is activated by FXIIa, thrombin and FXIa.[39,96] Therefore, FXI functions as an amplification factor that further generates thrombin in the coagulation cascade pathway. In addition, FXI promotes clot resistance to fibrinolysis through thrombin activatable

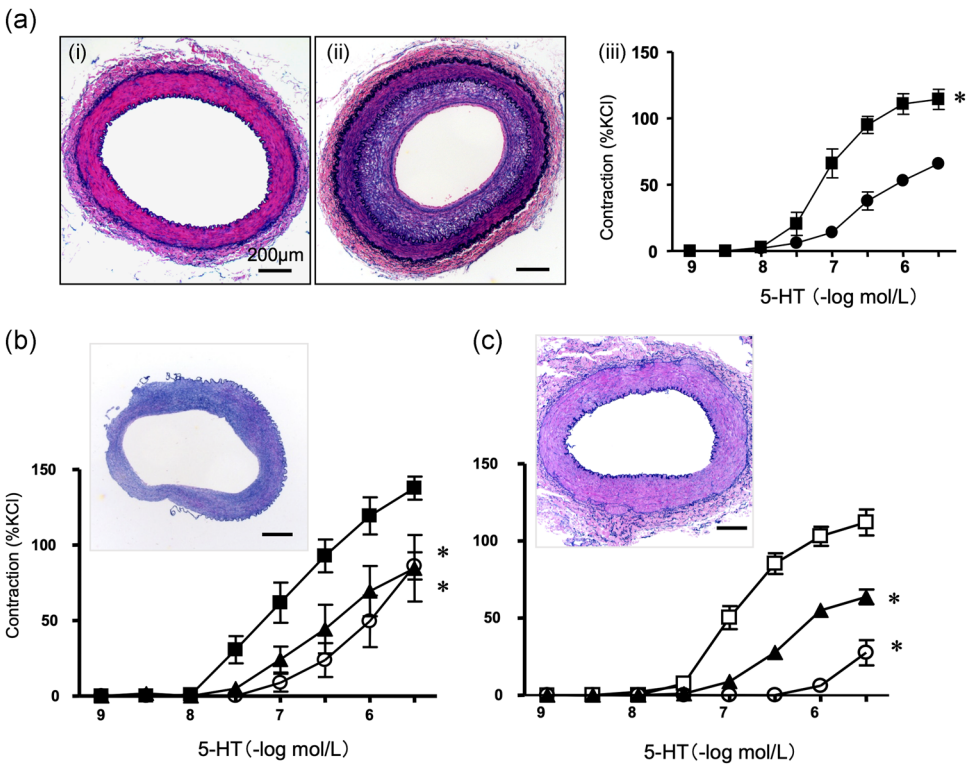

Figure 8 Vasoconstriction induced by 5-HT in rabbit femoral arteries. (**a**) Rabbit femoral artery without (**i**) and with (**ii**) smooth muscle cells (SMC)-rich neointima. Neointima was induced by balloon catheter injury 3 weeks previously. Vasocontraction induced by 5-HT is significantly more augmented in arteries with, than without (normal) SMC-rich neointima. Femoral arteries without (●) or with (■) SMC-rich neointima after endothelial denudation (**iii**). *$P < 0.01$ versus normal artery. Concentration-response curves for 5-HT of separated neointima with endothelial denudation (**b**) and separated media (**c**). Femoral artery gently separated into neointima and media. Cumulative amounts of 5-HT induces proportional contraction in neointima and media. Sarpogrelate (selective 5-HT_{2A} receptor antagonist) and fasudil (specific Rho-kinase inhibitor), significantly inhibit both contractile responses. (Neointima (■), media (□), with 1.0 μM sarpogrelate (○) or 3.0 μM fasudil (▲) *$P < 0.01$ versus neointima or media (Ref. 95 with permission).

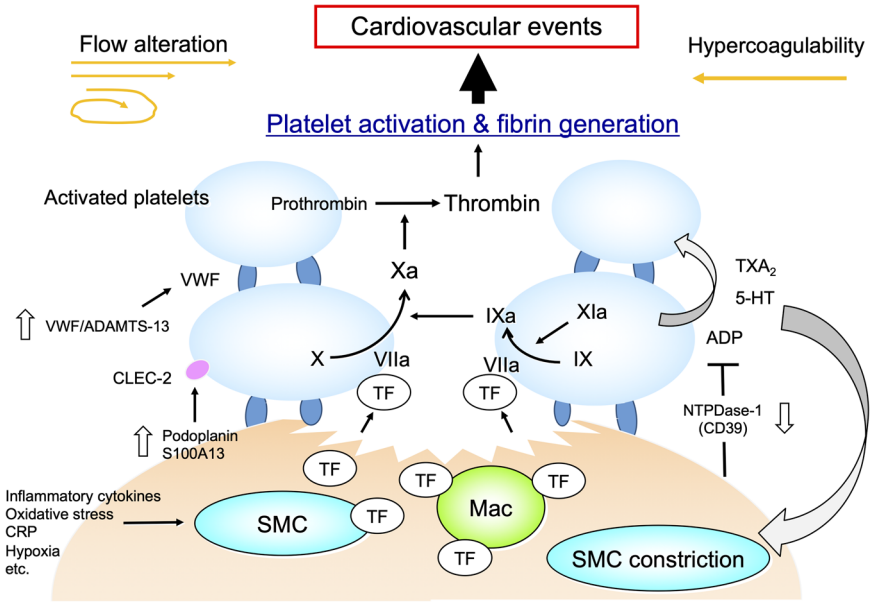

Figure 9 Activation of platelets and coagulation pathway at site of disrupted atherosclerotic plaque. 5-HT, 5-hydroxytryptamine; ADAMTS-13, a disintegrin and metalloprotease with a thrombospondin type 1 motif 13; ADP, adenosine diphosphate; CLEC-2, c-type lectin-like receptor 2; CRP, c-reactive protein; NTPDase-1, ecto-nucleoside triphosphate diphosphohydrolase-1; Mac, macrophage; SMC, smooth muscle cell; TF, tissue factor; TXA_2, thromboxane A_2; VWF, von Willebrand factor.

fibrinolysis inhibitor (TAFI).[97] Therefore, FXI plays a significant role in thrombus stabilization and propagation.

However, FXI is generally considered less critical in normal hemostasis, because an FXI deficiency usually does not lead to spontaneous bleeding.[98] Clinical studies have found that high levels of plasma FXI apparently comprise an independent risk factor for deep venous thrombosis and ischemic stroke,[99,100] whereas an FXI deficiency is associated with lower risk for these diseases.[101–103] We studied the role of FXI in thrombogenesis among rabbit models of arterial and venous thrombosis and found that inhibiting FXIa activity significantly reduces thrombus propagation without prolonging bleeding time.[104–106] Other clinical and animal studies have also shown that reducing plasma FXI activity attenuates thrombus formation without increasing bleeding risk.[107] These lines of evidence indicate that FXI contributes to thrombus propagation rather than to physiological hemostasis, and that FXI is a novel therapeutic target for antithrombotic therapy with less bleeding risk. A recent clinical study using an FXI antisense oligonucleotide found that reducing FXI levels in patients undergoing total knee arthroplasty safely prevented venous thrombosis with low risk of bleeding.[108]

CONCLUSIONS

Thrombus formation and propagation on disrupted atherosclerotic lesions are key mechanisms for the onset of acute cardiovascular events. During these processes, platelets are prone to activation by several factors including down-regulated NTPDase-1, increased CLEC-2 ligands, podoplanin and S100A13 in plaques, and disturbed blood flow associated with decreased ADAMTS-13 activity. In addition, the coagulation cascade pathway is activated by overexpressed TF in plaques under conditions of inflammation, oxidative stress and hypoxia. A combination of these factors largely promotes thrombus formation at sites of plaque disruption. Changes in blood flow and vasoconstriction can facilitate thrombus propagation and vascular narrowing, leading to the onset of cardiovascular events (Fig. 9).

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DISCLOSURE STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

YA, AY, YS and KH contributed to conception and design and drafting figures of this work. YA, AY and YS contributed to drafting the manuscript.

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