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Chapter 1

Pathophysiology of Atherothrombosis — Thrombus Growth, Vascular Thrombogenicity, and Plaque Metabolism

Atsushi Yamashita and Yujiro Asada

Additional information is available at the end of the chapter

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Abstract

Atherosclerotic plaque disruption does not always result in acute symptomatic events. Therefore, the formation of a large thrombus is a critical step in the development of atherothrombosis. However, little is known about the mechanisms involved in thrombus growth processes after plaque disruption. Studies in vivo have demonstrated that the tissue factor (TF) derived from the vascular wall contributes to the formation of thrombin-dependent platelet–fibrin thrombus on atherosclerotic arteries but not on normal arteries, and that altered blood flow in disrupted atherosclerotic arteries promotes platelet recruitment mediated by von Willebrand factor (VWF) on the thrombus surface and augmented blood coagulation resulting in thrombus growth. The thrombogenic potential of plaques is a fundamental factor in atherothrombosis. We recently found that the arterial glucose uptake reflects vascular thrombogenicity, which might be partly explained by metabolic adaptation and enhanced procoagulant activity in a hypoxic microenvironment. Hypoxic responses might link atherometabolism to vascular thrombogenicity.

Keywords: Atherometabolism, hypoxia, plaque erosion, thrombus growth, plaque thrombogenicity

1. Introduction

Thrombus formation on a ruptured or an eroded atherosclerotic plaque is a critical event that leads to atherothrombosis. However, autopsy studies have identified asymptomatic coronary thrombi on disrupted plaques and pathological differences in plaques with symptomatic and asymptomatic thrombi [1, 2]. Therefore, plaque disruption is not a final step, whereas thrombus growth processes are critical for the development of atherothrombosis. Despite intensive

investigation into the mechanisms of atherogenesis and plaque instability [3], little is known about the mechanisms involved in thrombus growth after plaque disruption. Vascular thrombogenicity and changes in blood flow and blood factors are generally thought to regulate thrombus formation. Therefore, disparities in these regulators affect the thrombus growth, and atherosclerotic plaque thrombogenicity is an essential factor for atherothrombosis. Plaque vulnerability, the upregulation of prothrombotic factors, and the downregulation of antithrombotic factors in atherosclerotic plaques have been demonstrated [3–5], and inflammatory stimuli play important roles in plaque thrombogenicity [6]. However, the determinants of vascular wall thrombogenicity are not fully understood.

Arterial inflammation has been evaluated using positron emission tomography (PET) imaging with [^{18}F]-fluorodeoxyglucose (^{18}F-FDG). The close correlation between ^{18}F-FDG uptake and plaque macrophage contents in animal models of atherosclerosis [7] suggests that the degree of ^{18}F-FDG reflects the underlying levels of vascular inflammation. Clinical studies have identified a relationship between ^{18}F-FDG uptake and numbers of cardiovascular risk factors, as well as risk for future events [8, 9]. The uptake of ^{18}F-FDG is significantly higher in aortic segments with thrombus than without thrombus in a rabbit model of advanced atherosclerosis [10]. These lines of evidence imply an association between glucose uptake and vascular thrombogenicity, although the underlying mechanism is unknown.

This article focuses on the pathophysiology of thrombus growth on disrupted plaques and discusses a conceivable relationship between atherometabolism and vascular thrombogenicity.

2. Pathology of atherothrombosis

Arterial thrombi are mainly composed of aggregated platelets as a likely result of rapid blood flow, and the development of platelet-rich thrombi is regarded as a cause of atherothrombosis. Fresh coronary thrombi in patients with acute myocardial infarction (AMI) and unstable angina have become assessable due to technological advances, distal protection, and thrombus aspiration devices. Evidence gathered from such studies indicates that such fresh atherothrombi consist of aggregated platelets, fibrin, erythrocytes, and white blood cells comprising mostly neutrophils, and that they are constitutively immunopositive for the platelet integrin αIIbβ3, fibrin, the membrane protein expressed on erythrocytes glycophorin A, and the von Willebrand factor (VWF; blood adhesion molecule). VWF and tissue factor (TF; initiator of the coagulation cascade) are closely associated with integrin αIIbβ3 and fibrin, respectively [11, 12] (Figure 1). These findings suggest that the enhanced platelet aggregation and fibrin formation result in thrombus growth and obstructive thrombus formation on disrupted atherosclerotic plaques, and that VWF and/or TF contribute to the pathological process.

Pathological findings suggest that plaque disruption and sudden coronary occlusion are often preceded by a variable period of thrombus formation. Aspirated thrombi from one-third to half of patients with AMI show cell lytic changes and/or organizing reactions, namely endothelialization with or without smooth muscle cell (SMC) ingrowth [13, 14] (Figure 2).

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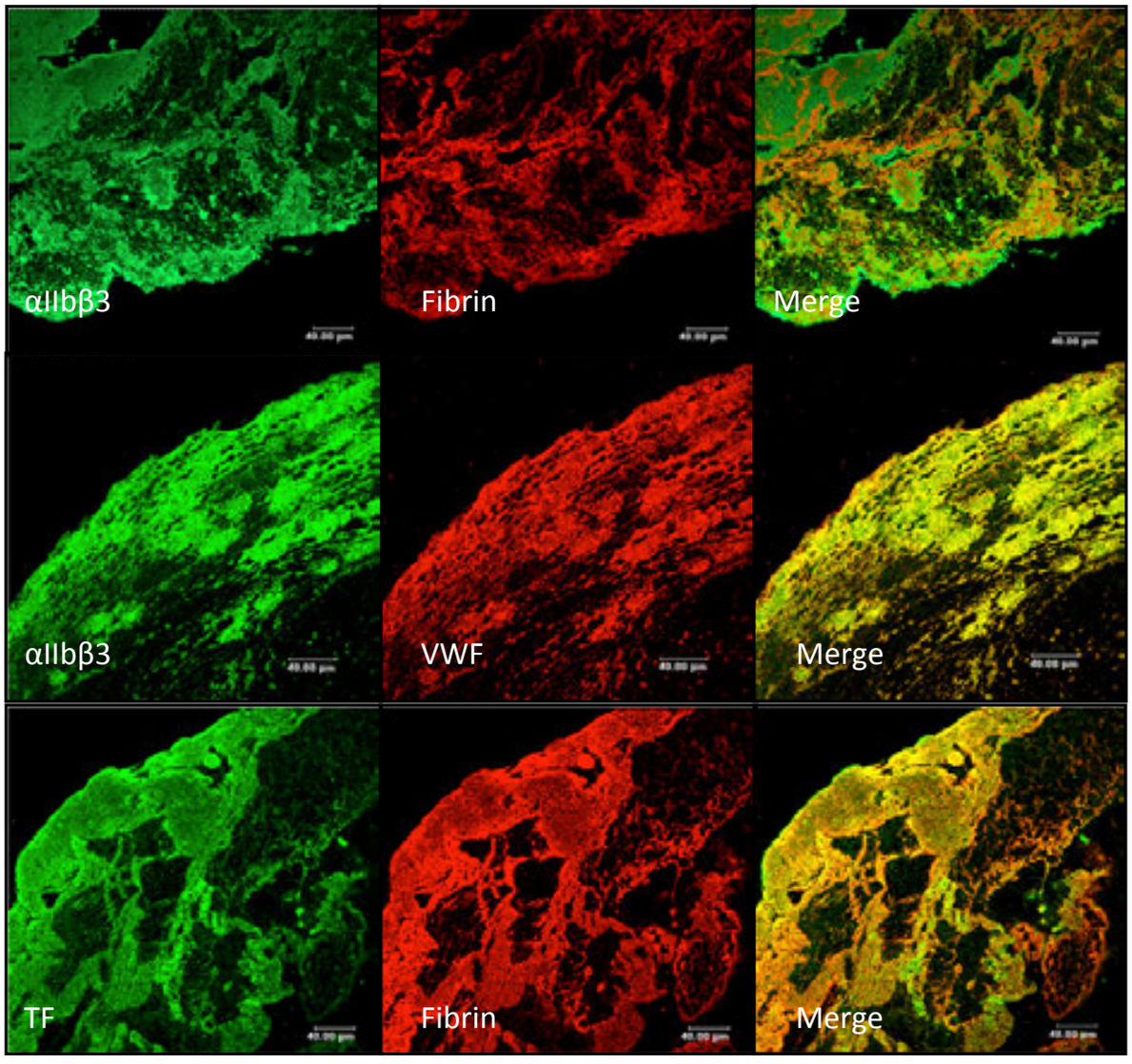

Figure 1. Immunofluorescence micrographs of fresh coronary thrombi in patients with acute myocardial infarction. Left, staining with fluorescein isothiocyanate-labeled integrin αIIbβ3, VWF, and TF (green). Center, staining with Cy3 labeled with fibrin and VWF (red). Right, merged immunofluorescence images. Areas of colocalized factors are stained yellow. Atherothrombus comprises platelets and fibrin. VWF and TF are closely associated with αIIbβ3 and fibrin, respectively. TF, tissue factor; VWF von Willebrand factor (from Ref. 11 with permission).

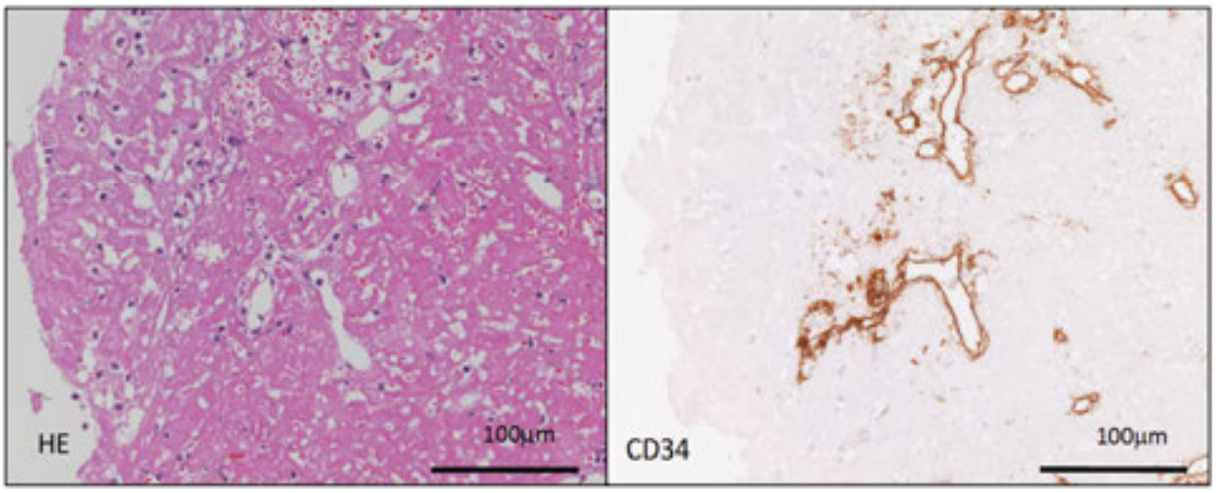

Figure 2. Light and immunohistochemical images of acute coronary thrombi in patients with acute myocardial infarction. Thrombus comprises degraded erythrocytes and leukocytes, mononuclear cell infiltrate, and small luminal structures lined with flat or spindle cells. CD34 immunostaining highlights endothelialization and microvessel formation in the thrombus, indicating organized reaction, even acute onset of AMI. HE, hematoxylin eosin stain.

American Heart Association classification of atherosclerosis. Plaques undergoing erosion are characterized by a denuded surface and thrombus formation, and lack a disrupted fibrous cap. Patients with plaque erosion are younger and are not predominantly males compared with those who have ruptured plaques. Angiography has shown that the luminal surface of eroded plaques is less narrowed and irregular. The morphological features include abundant SMCs and proteoglycan matrix, especially versican and hyaluronan, and disrupted surface endothelium. An eroded plaque contains relatively few macrophages and T cells compared with a ruptured plaque [16]. In contrast, macrophages comprise the dominant cell type at sites of both plaque rupture and erosion, and both inflammatory cells and SMCs express human leukocyte antigen (HLA-DR) antigens [18]. These findings suggest that eroded plaques are heterogeneous and that both inflammatory and noninflammatory processes contribute to the development of plaque erosion. The proportions of fibrin and platelets differ in coronary thrombi on ruptured and eroded plaques. Thrombi on ruptured and eroded plaques are rich in fibrin and platelets, respectively. The tissue factor is abundant in a ruptured plaque compared with an eroded plaque [19]. These characteristics of ruptured and eroded plaques (abundant TF, phospholipids, and less matrix protein in ruptured sites; abundant matrix protein and less TF in eroded sites) might explain the difference in the composition of thrombi in ruptured and eroded plaques.

3. Pathology of asymptomatic plaque disruption

Clinical angioscopy studies have revealed that asymptomatic multiple plaque rupture is a frequent complication among patients with coronary atherothrombosis [20]. Various stages of healed plaque disruption are also occasionally found during autopsies of individuals with and without known coronary atherothrombosis [2, 21]. Hearts at autopsy from patients who died

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of AMI have been compared with hearts form those who succumbed to noncardiac death to determine the incidence and morphological characteristics of thrombi and plaque disruption in patients with noncardiac death [1]. This study found coronary thrombi in 16% of noncardiac deaths; most of them had developed an eroded plaque and were too small to affect the coronary lumen (Figure 3). Smaller lipid areas, thicker fibrous caps, and more modest luminal narrowing were features of disrupted plaques associated with noncardiac death compared with AMI. Davies et al. [21] and Arbustini et al. [22] found coronary thrombus in 4% and 7% of noncardiac deaths, respectively, at autopsy. The coronary thrombi from noncardiac deaths were associated with eroded plaques [22]. These results suggest that plaque disruption does not always result in complete thrombotic occlusion with subsequent acute symptomatic events, and that thrombus growth is a critical step in the onset of clinical events. However, the determinants of coronary thrombus size after plaque disruption remain unknown.

The histological assessment of 157 carotid endarterectomy samples from asymptomatic patients and those with symptomatic major stroke revealed less frequent erosion in both symptomatic (3.1%) and asymptomatic plaques (6.7%) [23]. Less disrupted plaques, fewer inflammatory cells, a smaller necrotic core, and more calcification were identified in thrombotic plaques from the asymptomatic patients [23]. Because information about asymptomatic plaque disruption is limited, the features of coronary and carotid plaques are not identical.

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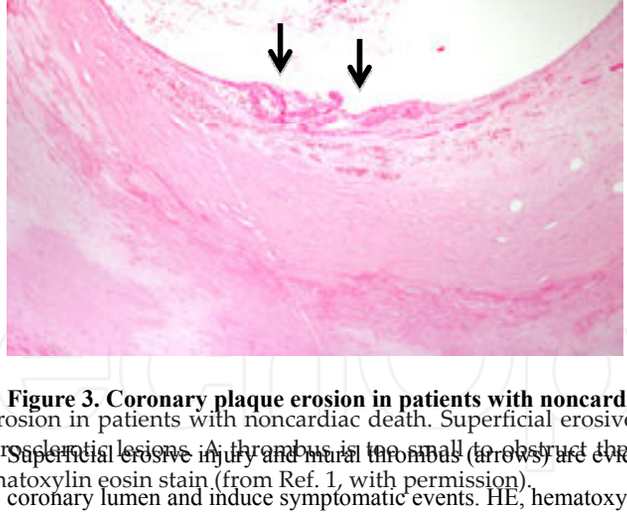

Figure 3. Coronary plaque erosion in patients with noncardiac death.

Superficial erosive injury and mural thrombus (arrows) are evident on atherosclerotic lesions. A thrombus is too small to obstruct the coronary lumen and induce symptomatic events. HE, hematoxylin eosin stain (from Ref. 1, with permission).

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4. Pathophysiology of atherothrombosis

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Atherothrombosis is initiated by plaque rupture or erosion. Accumulating evidence supports the notion that inflammation and matrix degradation contribute to plaque destabilization and that subsequent rupture of the fibrous cap triggers thrombus formation. Because the pathophysiology underlying plaque rupture is established [3], the pathophysiology of plaque erosion and thrombogenicity is the focus of this section.

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4.1. Possible mechanism of plaque erosion

Eroded plaque is characterized by a superficial plaque injury, but the mechanisms of such erosions are p

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Eroded plaque is characterized by a superficial plaque injury, but the mechanisms of such erosions are poorly understood. Approximately 80% thrombi associated with plaque erosion is nonocclusive regardless of sudden coronary death [16]. Experimental aortic stenosis can induce acute endothelial changes or damage to the normal aorta [24]. Therefore, hemodynamic forces, particularly those generated by disturbed blood flow induced by stenosis or vasoconstriction, could be a crucial factor in generating surface vascular damage and thrombosis. We investigated pathological changes after acute luminal narrowing in SMC-rich plaques in rabbits to understand the relationship between disturbed blood flow and plaque erosion. Balloon-induced injury of rabbit femoral arteries resulted in the formation of SMC-rich plaques comprising stellate- or spindle-shaped SMCs and extracellular matrix [25]. Acute vascular narrowing disrupted the blood flow in these arteries that consequently induced superficial erosive injury of the SMC-rich plaque. Figure 4 shows microscopic images of a longitudinal section of neointima at the post-stenotic region 15 min after vascular narrowing. The endothelial cells and SMCs at this region are broadly detached and associated with platelet adhesion to the subendothelium and a change in the shape of the SMCs. Endothelial cells and superficial SMCs became apoptotic within 15 min [26]. Subsequent vascular narrowing induced mural thrombi comprising platelets and fibrin, as in human plaque erosion. Thus, disrupted blood flow can induce superficial erosive damage to SMC-rich plaques and subsequent thrombus formation. We therefore designed a computational fluid simulation using the Reynolds-averaged Navier–Stokes model and calculated wall shear stress (WSS), turbulence kinetic energy (TKE), blood pressure, and blood pressure gradients (BPG) in a rabbit model to clarify the contribution of hemodynamic factors to the onset of plaque erosion in the SMC-rich plaque. The magnitude of WSS, TKE, and BPG correlated with the extent of histologically defined erosive damage. The values for WSS and TKE were significantly larger at eroded, than at noneroded sites [27]. Although direct clinical evidence has not supported the notion that coronary artery vasospasm plays a role in plaque erosion, ergonovine and norepinephrine-stimulated vasospasm of the atherosclerotic coronary artery has induced superficially damaged plaques and acute ischemic injury to the myocardium of a strain of Watanabe heritable hyperlipidemic rabbits that are prone to myocardial infarction [28]. Platelet and blood coagulation are activated in the coronary circulation after vasospastic angina [29, 30]. Although additional evidence is needed, hemodynamic factors might play an important role in the development of plaque erosion.

4.2. Thrombus growth and stability on disrupted plaques

Thrombus growth is considered critical to the onset of clinical events. Vascular wall thrombogenicity, local blood flow, and blood contents regulate thrombus formation, and thus thrombus growth seems to vary depending on the status of these three regulators. Nevertheless, a thrombogenic atherosclerotic lesion is essential for atherothrombosis.

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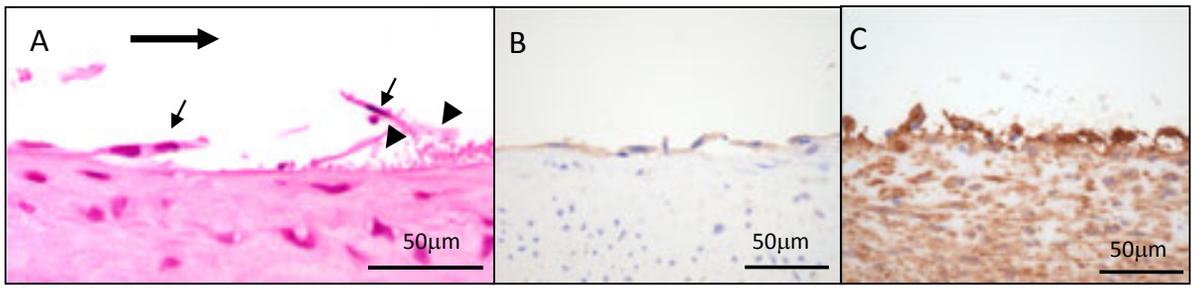

Figure 4. Representative images of the superficial erosive injury of SMC-rich plaques and thrombus formation. Smooth muscle cell-rich plaque 15 min after vascular narrowing shows endothelial detachment (small arrows) accompanied by platelet adhesion (arrow heads) at 1 mm from a region of vascular narrowing (A, hematoxylin eosin stain). Larger arrow indicates flow direction. Immunohistochemistry for VWF (marker of endothelium; B) and smooth muscle actin (marker of SMC; C) confirms detached endothelial cells and SMCs at the post-stenotic region (from Ref. 26).

atherosclerotic plaque derived TF contributes to the activation of the intravascular coagulation cascade and to thrombus size on atherosclerotic lesions.

The tissue factor pathway inhibitor and argatroban, a thrombin inhibitor, reduce both platelet and fibrin content in atherosclerotic arteries but not in normal femoral arteries [35]. Oral-activated factor X (FXa) inhibitors significantly reduce the atherothrombus growth induced by balloon injury and vascular ligation in rabbits, and plasma FXa activity correlates with the antithrombotic effects of the oral FXa inhibitors [36]. These findings indicate that excess

contributes to the activation of the intravascular coagulation cascade and to thrombus size on atherosclerotic lesions.

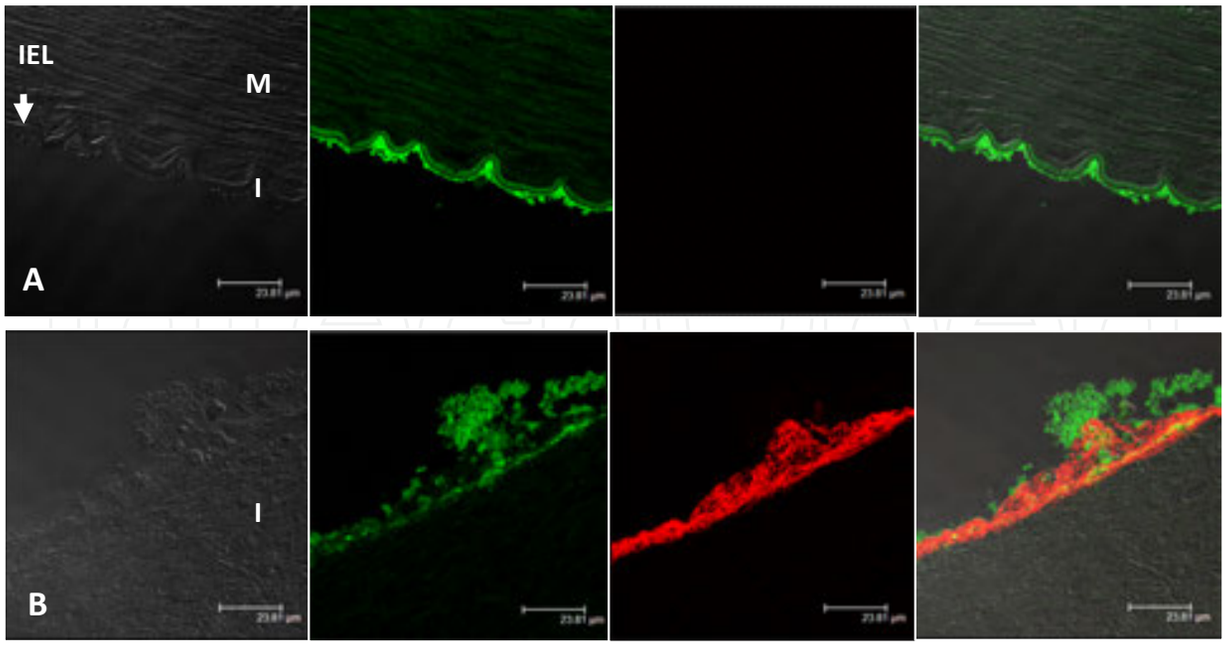

Figure 5. Immunofluorescence images of thrombus on rabbit femoral artery.

Figure 5. Immunofluorescence images of thrombus on rabbit femoral artery. Immunofluorescence microphotographs of thrombi 15 min after balloon injury of a normal femoral artery and of an atherosclerotic plaque under 0.5% cholesterol diet. Rows show differential interference contrast images, images stained with fluorescein isothiocyanate-labeled integrin αIIbβ3 (green), Cy3-labeled fibrin (red), and merged immunofluorescence images. Areas with colocalized factors are stained yellow. Thrombi on normal intima comprise small aggregated platelets (A), whereas large thrombi on atherosclerotic plaques comprise platelets and fibrin (B). I, intima; IEL, internal elastic lamina, M, media (from Ref. 35, with permission).

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thrombin generation via FXa contributes to further platelet accumulation and fibrin formation on disrupted plaques. The FXa is generated by factor VII (FVII) or activated factor IX. The feedback activation of factors VIII and XI (FXI) by thrombin amplifies further thrombin generation through FXI-, FIX-, and FX activation (Figure 6). The intrinsic coagulation pathway is initiated when the coagulation factor XII (FXII) comes in contact with collagen, nucleic acids and polyphosphate, high-molecular mass kininogen, and plasma kallikrein. Factor XI is activated by activated FXII, thrombin, and activated XI (FXIa) in vitro [37]; it is present in platelet–fibrin thrombus induced by balloon injury of atherosclerotic lesions in rabbits, and anti-FXI antibody reduces thrombus formation without prolonging bleeding [38]. Factors XI and FXII do not affect the initial phase of thrombus formation but prevent thrombus shedding and contribute to thrombus stability on carotid ruptured plaques in ApoE-deficient mice [39, 40]. In contrast, the inhibition of activated FVII participates in the initial stage of thrombus formation on ruptured plaques [40]. These findings suggest that after plaque disruption, FXII- and FXI-mediated amplification of thrombin generation plays an important role in thrombus stability on disrupted plaques. However, a clinical study discovered an inverse relationship between levels of FXII and risk of myocardial infarction [41]. A deficiency of FXI did not prevent the development of acute coronary events [42] but reduced the incidence of deep vein thrombosis and ischemic strokes [43, 44]. The role of the intrinsic coagulation pathway in atherothrombosis requires further investigation.

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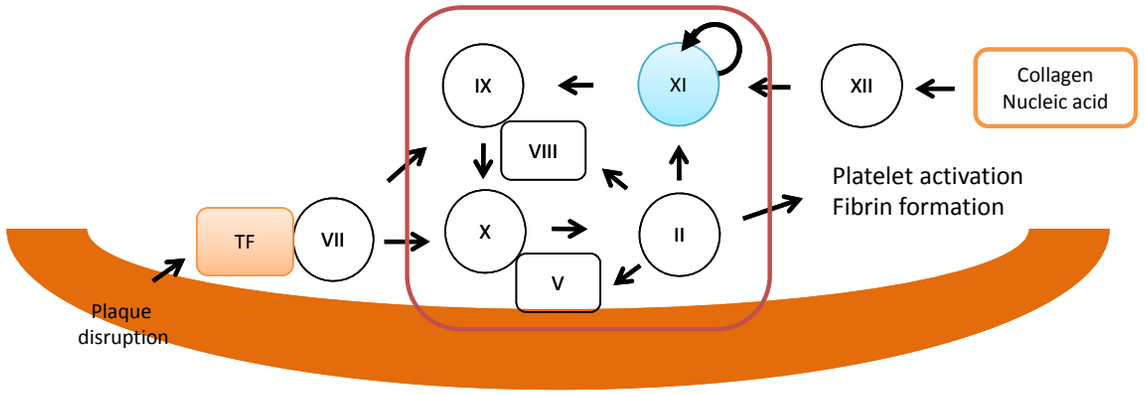

Figure 6. Exposure of prothrombotic factors and amplification of coagulation cascade on disrupted plaques. Exposure of the tissue factor, (TF) after plaque disruption, initiates blood coagulation. The activated factor X is generated by activated factors VII or IX. Feedback activation of factors VIII and XI by thrombin amplifies further thrombin generation through sequential activation of factors XI, IX, and X. Factor XI is also activated by intrinsic coagulation factor XII and activated factor XI. The excess thrombin generation via the feedback activation and intrinsic pathway contributes to further platelet accumulation and fibrin formation on disrupted plaques. Red square indicates platelet surface.

Platelets comprise a major cellular component in coronary thrombus and play an important role in not only the initial but also the growth phase of thrombus formation. Platelet recruitment to the thrombus surface and the stabilization of platelet activation are important to maintain platelet accumulation and thrombus growth. Adhesion molecules and their receptors on platelets are essential for thrombus formation, because they support platelet tethering and firm adhesion, as well as platelet aggregation and recruitment to the thrombus surface. The large, multimeric, plasma protein VWF undergoes a conformational change when bound to extracellular matrix and platelet surface, and the conformational change permits its binding to GPIbα. Studies in vitro and in vivo have shown that platelet recruitment to the thrombus surface is primarily mediated by VWF and GPIbα on flowing platelets [45, 46]. We found a large amount of VWF localized to coronary thrombi in patients with AMI [11], and that a monoclonal antibody against the VWF A1 domain that interacts with platelet GPIbα, significantly suppresses the formation of platelet–fibrin thrombi and completely inhibits the occlusive thrombus formation in rabbit atherosclerotic lesions [25, 47]. These findings indicate that VWF plays a crucial role in thrombus growth via platelet recruitment. Real-time analysis of platelet calcium flux during thrombus formation in vitro has shown that the stable adhesion between aggregated platelets and the growth of platelet thrombus depends on sustained integrin αIIbβ3 activation and cyclic calcium flux through secreted adenosine diphosphate and its $P2Y_1$ and $P2Y_{12}$ receptors [48, 49]. We administer anti-$P2Y_{12}$ antagonists to prevent secondary cardiovascular events because these drugs destabilize thrombus and limit thrombus formation.

4.3. Effects of altered blood flow on thrombus growth

Blood flow is a key modulator of thrombus growth, and coronary blood flow is altered in patients with ischemic heart diseases. Marzilli et al. [50] found that the coronary blood flow is

reduced by about 80% during ischemia in patients with unstable angina and an autopsy study frequently found intramyocardial microemboli in patients who succumbed to sudden coronary death [51]. Distal microvascular embolism and/or vasoconstriction could affect blood flow as well as thrombus formation and growth at culprit lesions. We examined the effects of the blood flow reduction on thrombus formation in an animal model and found that > 75% reduction in blood flow promoted the growth of thrombus consisting of a mixture of platelets and fibrin on atherosclerotic lesions that subsequently became occlusive, and it also induced the formation of very small thrombi composed only of platelets on normal arteries [47]. Therefore, a reduction in blood flow associated with increased vascular wall thrombogenicity is considered to contribute to thrombus growth. We also identified an important role of the 5-HT_{2A} receptor on platelets and SMCs in this process via platelet aggregation and thrombogenic vasoconstriction [52, 53].

In addition to distal vascular resistance, blood flow disturbed by acute vascular narrowing promotes thrombus growth at post-stenotic regions. As described above, vascular narrowing of the rabbit femoral artery induced superficial erosive injury to SMC-rich plaque. The progression of neointimal damage caused by disturbed blood flow was associated with thrombus growth. These findings suggest that the degree of plaque disruption affects thrombus size after the plaque erosion [26].

The rheological effect on thrombus growth might be partly explained by a shear gradient-dependent mechanism of platelet aggregation. Nesbitt et al. [54] used imaging to reveal a shear gradient-dependent, platelet-aggregation process that is preceded by the soluble agonist-dependent aggregation in stenotic microvessels in vitro and in vivo. Shear microgradients at post-stenotic regions or downstream of thrombi induce stable platelet aggregates, the sizes of which are directly influenced by shear microgradients. This process requires ligand binding to integrin αIIbβ3 and transient Ca^{2+} flux, but does not require a change in global platelet shape or soluble agonists. These findings suggest that a biomechanical mechanism is principally involved in the early phase of platelet adhesion and aggregation. Vessel and/or thrombus geometry itself might promote thrombus formation.

Platelet and/or fibrin aggregates must exceed the physical forces exerted by blood flow within the narrowing lumen for thrombus to become occlusive and VWF is a candidate in the final process. The thrombogenic activity of VWF is strictly dependent upon its multimeric structure. A critical shear stress of 35 ± 3.5 dyn/cm^2 induces VWF to change from a globular state to an extended chain conformation [55]. Such excessive shear stress can induce platelet aggregation in the absence of exogenous agonists [56], and the interaction between VWF and platelets under high shear rates might be a key determinant of the final process of thrombotic occlusion. In fact, inhibiting the plasma VWF completely prevents occlusive thrombus formation induced by neointimal injury and disrupted blood flow in rabbits [47].

5. Atherometabolism and vascular wall thrombogenicity

Atherosclerotic plaque thrombogenicity is an essential factor for the development of atherothrombosis. However, the determinants of vascular wall thrombogenicity are not yet fully

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understood. Understanding the factors that reflect vascular wall thrombogenicity will allow the detection of high-risk plaques and the development of novel therapies for atherosclerosis that target vascular thrombogenicity.

Metabolism in higher animals including humans is finely regulated by metabolic organs, a neuroendocrine system, and cellular regulatory mechanisms. Recent studies have found that distinct metabolic states maintain the functions of cancerous and inflammatory cells within specific microenvironments [57, 58]. Because enhanced glucose metabolism has been visualized by ^{18}F-FDG-PET in some atherosclerotic lesions, we speculate that the metabolically active plaque is highly thrombogenic. Therefore, this section focuses on atherometabolism and vascular wall thrombogenicity.

5.1. Glucose uptake reflects vascular wall thrombogenicity

Although recent advances in clinical imaging have revealed detailed anatomical information about atherosclerotic lesions, functional information remains limited. The uptake of ^{18}F-FDG in atherosclerosis closely correlates with macrophage contents in rabbits [7]. Clinical studies have also found a relationship between ^{18}F-FDG uptake and future cardiovascular events [9, 59]. Thus, the evidence implies an association between ^{18}F-FDG uptake and arterial thrombus formation. We therefore visualized rabbit atherosclerotic and contralateral nonatherosclerotic femoral arteries using ^{18}F-FDG-PET, and induced arterial thrombus by balloon injury. The images revealed more radioactivity along the atherosclerotic than along the contralateral nonatherosclerotic artery. Areas of arterial thrombus correlated with the amount of arterial radioactivity determined from the PET images and autoradiography. The amount of arterial radioactivity positively correlated with macrophage contents, TF expression, and the nuclear localization of NF-κB, a transcriptional factor of TF. Inhibiting NF-κB reduced TF expression in rabbit atherosclerotic plaques cultured in vitro [60]. In addition, more ^{18}F-FDG is taken up by advanced atherosclerotic segments with thrombus induced by Russell's viper venom and histamine compared with segments without thrombus in the rabbit aorta [10]. An imaging study using apoE-deficient mice showed colocalization of deoxyglucose and annexin A5, which binds phosphatidyl serine, a thrombogenic lipid, on the cell membrane in macrophage-rich atherosclerotic lesions in mice [61]. Studies of human coronary arteries using ^{18}F-FDG-PET are limited due to cardiac ^{18}F-FDG uptake. A clinical study has demonstrated higher ^{18}F-FDG uptake in culprit lesions associated with acute coronary thrombosis compared with stable coronary lesions in patients with ischemic heart disease [62]. These lines of evidence might support the notion that arterial glucose uptake reflects plaque thrombogenicity and ^{18}F-FDG-PET is a minimally invasive method of detecting highly thrombogenic plaques.

5.2. Metabolism in atherosclerotic arteries

Cells uptake ^{18}F-FDG through glucose transporters and phosphorylate it by hexokinases. Thereafter, intracellular ^{18}F-FDG accumulates without further metabolism and is thus considered a marker of glucose utilization in tissues. Although systemic metabolic disorders such as dyslipidemia and diabetes accelerate the development of atherosclerosis, little is known about metabolic changes in atherosclerotic arteries and their significance in atherogenesis and

in atherosclerotic arteries. Macrophage-rich or SMC-rich lesions were generated by balloon injury in the iliac–femoral arteries of rabbits fed with a 0.5% cholesterol diet or a conventional diet. The number of proliferative cells increased in macrophage-rich arteries compared with control and SMC-rich arteries. Metabolites of glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle, and nucleotides increased in arteries with macrophage-rich lesions compared with noninjured control arteries and those with SMC-rich lesions [65] (Figure 7).

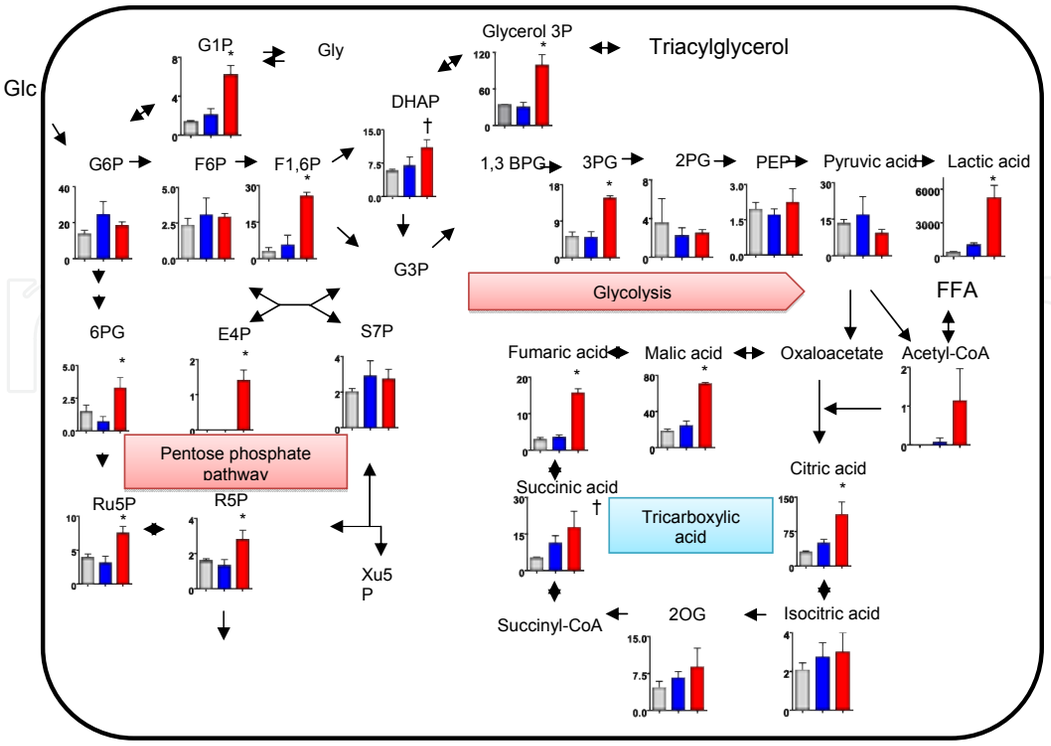

Figure 7. Levels of metabolites of glycolysis, the pentose phosphate pathway, tricarboxylic acid cycle, and glyconeogenesis/glycogenolysis in rabbit iliac–femoral arteries.

Figure 7. Levels of metabolites of glycolysis, the pentose phosphate pathway, tricarboxylic acid cycle, and glyconeogenesis/glycogenolysis in rabbit iliac–femoral arteries. Gray, blue, and red bars: iliac–femoral arteries that were not injured (control), and those with SMC-rich (conventional diet) and macrophage-rich (0.5% cholesterol diet) lesions, respectively ($n = 3$ for all). Metabolite levels are expressed as nanomole per gram; $^{*}p < 0.05$ vs. other groups, $^{\dagger}p < 0.05$ vs. noninjured femoral artery. Significant vascular metabolite changes in artery with macrophage-rich lesion. 1,3BPG, 1,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; 2OG, 2-oxoglutaric acid; 6PG, 6-phosphogluconic acid; DHAP, dihydroxyacetone phosphate; E4P, erythrose 4-phosphate; F1-6P, fructose 1,6-diphosphate; F6P, fructose 6-phosphate; FFA, free fatty acid; G1P, glucose 1-phosphate; G3P, glyceraldehyde 3-phosphate; G6P, glucose 6-phosphate; Glu, glucose; Gly, glycogen; PEP, phosphoenolpyruvic acid; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; Xu5P, xylulose 5-phosphate (from Ref. 65).

thrombosis. Glucose utilization and O_2 consumption are increased in monkey and rabbit atherosclerotic aortic segments compared with nonatherosclerotic aortic segments [63]. In contrast, adenosine triphosphate (ATP) depletion in the cores of advanced atherosclerotic plaques is associated with hypoxic areas in the rabbit aorta [64]. We analyzed levels of central carbon metabolites in rabbit atherosclerotic arteries using capillary electrophoresis–time-of-flight mass spectrometry (CE-TOFMS) to determine comprehensive metabolic changes and hypoxic effects in atherosclerotic arteries. Macrophage-rich or SMC-rich lesions were generated by balloon injury in the iliac–femoral arteries of rabbits fed with a 0.5% cholesterol diet or a conventional diet. The number of proliferative cells increased in macrophage-rich arteries compared with control and SMC-rich arteries. Metabolites of glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle, and nucleotides increased in arteries with macrophage-rich lesions compared with noninjured control arteries and those with SMC-rich lesions [65] (Figure 7).

Hypoxic areas visualized by immunostaining pimonidazole hydrochloride were detectable only in arteries with macrophage-rich lesions, and they positively correlated with vascular

associated with hypoxic areas and neovascularization.

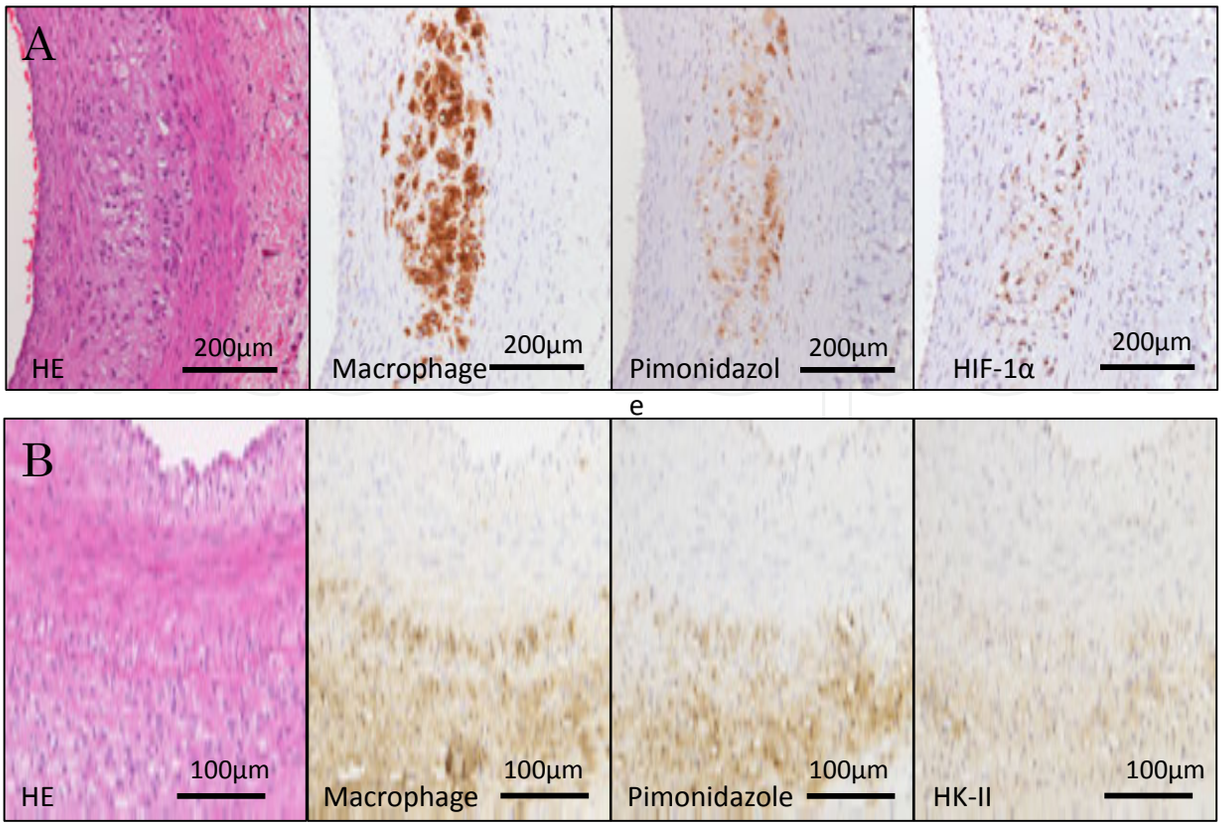

Figure 8. Immunohistochemical findings of hypoxia-inducible factor (HIF)-1α and hexokinase (HK)-II in iliac–femoral artery with macrophage-rich lesion.

Figure 8. Immunohistochemical findings of hypoxia-inducible factor (HIF)-1α and hexokinase (HK)-II in iliac–femoral artery with macrophage-rich lesion. Immunohistochemical staining for macrophages, pimonidazole and HIF-1α in paraffin sections (A) and for macrophages, pimonidazole and HK-II in frozen sections (B). Nuclear translocation of HIF-1α and expression of HK-II in macrophage-rich hypoxic area. Hematoxylin eosin (HE stain) (from Ref. 65).

pimonidazole and HK-II in frozen sections (B). Nuclear translocation of HIF-1α and expression of HK-II in macrophage-rich hypoxic area. Hematoxylin eosin (HE stain) (from Ref. 65).

^{18}F-FDG uptake. The hypoxic areas were localized deep in the wall, and distributed in macrophage-rich areas with nuclear localization of hypoxia-inducible factor (HIF)-1α and hexokinase II expression [65] (Figure 8). Hypoxic responses might partly affect the glycolytic activity in macrophage-rich arteries. The cores of more advanced plaques > 500 μm thick were necrotic and hypoxic, and characterized by ATP depletion, low glucose and glycogen concentrations, and high lactate concentrations in the rabbit aorta [64]. The depletion of ATP might contribute to macrophage death and expansion of the necrotic core in atherosclerotic lesions. A hypoxic plaque has been detected in the rabbit atherosclerotic aorta using ^{18}F-fluoromisonidazole PET imaging [66]. Higher ^{18}F-fluoromisonidazole uptake by advanced atherosclerotic lesions is associated with hypoxic areas and neovascularization.

The pentose phosphate pathway is an alternative route for glucose catabolism that functions in the formation of ribose 5-phosphate (R5P) that is required for nucleotide and deoxyribonucleic acid synthesis for cell growth and proliferation, and the formation of nicotinamide adenine dinucleotide phosphate (NADPH) for biosynthetic reactions. NADPH protects directly against oxidative stress by neutralizing reactive oxygen intermediates and indirectly via regenerating reduced glutathione. Studies have found both small and large amounts of metabolic flow through the pentose phosphate pathway in arteries compared with the glycolysis pathway [67, 68].

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Increased nucleotide levels and numbers of proliferative cells in macrophage-rich arteries [65]. The roles of the pentose phosphate pathway in atherosclerosis and

in the formation of ribose 5-phosphate (R5P) that is required for nucleotide and deoxyribo-

enzyme of pentose phosphate pathway, decreased serum cholesterol levels, vascular superoxide release, and atherosclerotic lesions

glycolysis pathway [67, 68]. Increased amounts of metabolites of the pentose phosphate pathway are compatible with increased nucleotide levels and numbers of proliferative cells in macrophage-rich arteries [65]. The roles of the pentose phosphate pathway in atherosclerosis and atherothrombosis are poorly understood. A 20% reduction in normal levels of glucose 6-phosphate dehydrogenase activity, a key enzyme of pentose phosphate pathway, decreased serum cholesterol levels, vascular superoxide release, and atherosclerotic lesions but increased blood pressure in apoE-deficient mice suggest that this pathway exerts complex effects on atherogenesis [69].

The increase in glycerol 3-phosphate is compatible with increased phospholipid and triglyceride synthesis in the rabbit atherosclerotic aorta [70]. Atherosclerotic rabbits were injected with sodium acetate-1-C^{14} to assess its incorporation into arterial phospholipids. Radioactivity levels were higher in noncholine-containing phospholipids, phosphatidylcholine, sphingomyelin, and lysolecithin as well as triglycerides in atherosclerotic lesions than in corresponding plasma phospholipids, whereas < 1% of the label found in the phospholipids was incorporated into free cholesterol taken up by atherosclerotic lesions [70]. This suggests that phospholipids and triglycerides are synthesized in atherosclerotic lesions in situ, and that cholesterol is mainly derived from the plasma. These metabolic changes in atherosclerotic arteries could partly explain the metabolic changes in activated and/or hypoxic macrophages, as described below.

5.3. Metabolism of activated and/or hypoxic macrophages

Most cells produce ATP via glycolysis and oxidative phosphorylation under normoxic conditions, and mainly via glycolysis under hypoxic conditions. However, macrophages produce ATP via glycolysis even under normoxic conditions. The contributions of glucose, glutamine, and oleate to ATP production were measured in isolated mouse peritoneal macrophages using radiolabeled probes. The production of ATP in the macrophages was mediated by anaerobic glycolysis (54%), aerobic glutamine (24%), and fatty acid oxidation (22%) [71]. At an initial glucose concentration of 5 mM, almost all utilized glucose was converted into lactate through glycolysis, whereas only ~3% of glucose was oxidized under normoxic conditions [72]. The dependence of macrophages on glycolysis might be partly explained by the impaired HIF-1 inhibition. The cytoplasmic tail of the membrane type-1 matrix metalloprotease bound to factor inhibiting HIF-1 (FIH-1) leads to the inhibition of the FIH by the adaptor protein Mint3/APBA3 under normoxic conditions [73]. Because most of the genes related to glycolysis, such as those for glucose transporter 1, hexokinase, phosphofructokinase, phosphoglycerate kinase 1, are induced by HIF-1, the constitutive activation of HIF-1 might enhance the glycolysis pathway in normoxic macrophages. However, HIF-1α was translocated to the nucleus mainly in hypoxic areas but not in all macrophages in rabbit atherosclerotic lesions. Therefore, HIF-1-dependent and -independent pathways might contribute to the enhanced glycolytic pathway in macrophages in atherosclerotic lesions.

Macrophages comprise a heterogeneous cell population in terms of protein expression and function, both of which are affected by the microenvironment, and the metabolic status is also affected by macrophage activation pathways. Classical activation by lipopolysaccharide (LPS)

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and/or interferon-γ enhances the glycolysis pathway and lactate production, whereas an alternative pathway (interleukin-4 and -13) enhances fatty acid oxidation [74–76]. Hypoxia, but not pro-inflammatory cytokines, notably stimulates glucose uptake in human macrophages and foam cells, whereas pro-inflammatory cytokines stimulate glucose uptake in SMCs and endothelial cells [77]. We analyzed levels of 110 cationic and anionic metabolites in classically activated THP-1 macrophages under normoxic and hypoxic (1% O_2) conditions to determine comprehensive metabolic changes in macrophages. Under normoxic conditions, one-third of metabolite levels differed in the macrophages stimulated by lipopolysaccharides and interferon-γ, while metabolites of the glycolysis and pentose phosphate pathways did not increase in activated macrophages compared with nonstimulated macrophages. Hypoxia increased metabolite levels of glycolysis and the pentose phosphate pathway and decreased metabolite levels of tricarboxylic acid cycle in the activated macrophages. The results of a comprehensive metabolic analysis support the notion that hypoxia augments glucose utilization through the glycolysis and pentose phosphate pathways in classically activated macrophages [65]. This study suggested that metabolic changes occur in nucleotides, amino acids, and other charged metabolites in classically activated hypoxic macrophages.

Hypoxia also affects lipid metabolism in macrophages and foam cell formation. Increases of 50% and 120% in cholesterol and triglyceride contents, respectively, under hypoxic conditions (1% O_2), were accompanied by elevated 3-hydroxy-3-methyl-glutaryl-CoA reductase activities. Cholesterol-efflux assays showed that hypoxia significantly decreased the efflux mediated by the subcellular distribution of the ATP-binding cassette subfamily A member 1 (ABCA1). The hypoxia-induced accumulation of sterol and decreased cholesterol efflux was partly mediated by HIF-1α [78]. Other pathways might also contribute to the lipid metabolism in hypoxic macrophages. Cholesterol efflux mediated by ABCA1 is regulated by HIF-1β in primary human macrophages under hypoxia [79]. Positive interaction between liver X receptor α and HIF-1α synergistically induces triglyceride accumulation and foam cell formation in human primary macrophages and RAW 264.7 cells under hypoxia [80]. The expression of the secreted proteoglycan, versican, is enhanced in hypoxic macrophages, which might increase lipoprotein retention in microenvironments as well as the lipid content of hypoxic macrophages [81].

Changes in metabolism or in metabolites can alter the inflammatory response of activated macrophages. The heterozygous disruption of inducible 6-phosphofructo-2-kinase, a rate-limiting glycolysis enzyme, enhances pro-inflammatory gene expression in adipose tissue macrophages from mice fed with a high-fat diet [82]. A kinase screening has identified downregulation of sedoheptulose kinase, an enzyme of the pentose phosphate pathway, in LPS-stimulated RAW 264.7 macrophages. The reduction of sedoheptulose kinase in activated macrophages is associated with greater flux through glycolysis and the oxidative pentose phosphate pathway, which results in an increased overall redox potential and a reduced rate of O_2 consumption [83]. The overexpression of glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, stimulates NF-κB transcriptional activity and promotes oxidative stress and inflammatory responses in RAW 264.7 macrophages [84]. These findings suggest cross talk between

macrophage activation pathways and metabolism. With respect to atherogenesis, a deficiency of fatty acid synthase or a long-chain fatty acid elongase reduces atherosclerosis in apoE- or low-density receptor-deficient mice [85, 86].

5.4. Hypoxia links atherometabolism to vascular thrombogenicity

Arterial glucose uptake reflects vascular thrombogenicity in rabbits [60]. However, the underlying mechanisms remain unclear. Hypoxia is an important microenvironmental factor that influences the progression of atherosclerosis by inducing foam cell formation and necrotic core expansion, metabolic changes in infiltrative macrophages, and plaque neovascularization. Sluimer et al. [87] used the hypoxia marker pimonidazole hydrochloride to reveal hypoxic areas in the center of an advanced human carotid plaque and found that they correlated with the presence of thrombus, angiogenesis, microvessel density and the expression of hexokinase, HIF, and vascular endothelial growth factor. Hypoxia upregulates TF in the lungs [88], plasminogen activator inhibitor (PAI)-1 in hepatocytes [89], and ecto-nucleoside triphosphate diphosphohydrolase 1 (ecto-NTPDase 1) expression in endothelial cells [90]. TF and PAI-1 promote fibrin formation by initiating the coagulation cascade and inhibiting fibrinolysis, respectively, whereas ecto-NTPDase inhibits platelet aggregation via the hydrolysis of extracellular adenosine diphosphate. Because these three molecules are expressed in human atherosclerotic plaques [4, 91, 92], hypoxic conditions in the plaques can affect vascular thrombogenicity and arterial thrombus formation. We examined the effects of hypoxia in arterial thrombus formation using rabbit model of atherosclerosis, atherothrombosis, cultured macrophages, and human coronary plaques with or without acute coronary thrombosis. Atherosclerotic lesions were induced in rabbit femoral arteries by a 0.5%-cholesterol diet and balloon injury. Hypoxic areas detected by pimonidazole immunoreactivity were localized in the lipid and macrophage-rich deep portions of the plaque and a few superficial cells, but not in uninjured arteries. The extent of the hypoxic areas correlated with the plaque size, macrophage content, the nuclear localization of HIF-1α and NF-κB p65, and TF expression. Hypoxic areas in arteries closely correlated with fibrin areas in thrombus induced by a balloon catheter and hypoxia (1% O_2) enhanced the nuclear localization of HIF-1α and NF-κB p65, as well as TF expression and procoagulant activity in cultured macrophages [93]. The enhanced TF activity was suppressed by the inhibitors of either HIF-1 or NF-κB, suggesting that HIF-1 and NF-κB exert synergic effects upon procoagulant activities in hypoxic plaques [93]. These findings suggested that hypoxia promotes arterial fibrin formation via the augmentation of vascular thrombogenicity in rabbits. Coronary plaques contain more abundant macrophages, T lymphocytes, and fibrin deposition in patients with unstable angina pectoris than in patients with stable angina pectoris. The expression of TF, PAI-1, HIF-1α, and NF-κB p65 is closely distributed in coronary thrombotic plaques, and the nuclear localization of HIF-1α correlates with the expression of NF-κB p65, TF, PAI-1, and fibrin [93]. The pathological findings support the notion that hypoxic and nonhypoxic, possibly inflammatory, stimuli enhance thrombogenicity in symptomatic plaques. Because hypoxia enhances glucose uptake and glycolysis flux in atherosclerotic lesions and macrophages as described above, hypoxia might link glucose metabolism and thrombogenicity in atherosclerotic plaques.

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6. Conclusion

The pathological findings suggest that the enhanced platelet aggregation and fibrin formation result in occlusive thrombus formation on disrupted atherosclerotic plaques. The size of the arterial thrombus would be affected by the degree of plaque disruption, the amount of exposed TF and platelet agonists, changes in blood flow, and the geographic features of disrupted plaques. Disrupted blood flow and excess thrombin generation can amplify blood coagulation and platelet recruitment to the thrombus surface and sustain platelet activation. Arterial glucose uptake reflects vascular thrombogenicity. These findings might be partly explained by metabolic adaptation and enhanced procoagulant activity under a hypoxic microenvironment, although further study is required. The relationship might provide new insight into novel therapeutic targets for atherothrombosis and the noninvasive detection of high-risk plaques.

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References

[1] Sato Y, Hatakeyama K, Marutsuka K, Asada Y. Incidence of asymptomatic coronary thrombosis and plaque disruption: comparison of non-cardiac and cardiac deaths among autopsy cases. Thromb Res. 2009;124:19–23.

[2] Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation. 2001;103:934–40.

[3] Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res. 2014;114:1852–66.

[4] Hatakeyama K, Asada Y, Marutsuka K, Sato Y, Kamikubo Y, Sumiyoshi A. Localization and activity of tissue factor in human aortic atherosclerotic lesions. Atherosclerosis. 1997;133:213–19.

[5] Oemar BS, Tschudi MR, Godoy N, Brovkovich V, Malinski T, Lüscher TF. Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. Circulation. 1998;97:2494–98.

[6] Monaco C, Andreakos E, Kiriakidis S, Mauri C, Bicknell C, Foxwell B, Cheshire N, Paleolog E, Feldmann M. Canonical pathway of nuclear factor kappa B activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. Proc Natl Acad Sci U S A. 2004;101:5634–39.

[7] Ogawa M, Ishino S, Mukai T, Asano D, Teramoto N, Watabe H, Kudomi N, Shiomi M, Magata Y, Iida H, Saji H. (18)F-FDG accumulation in atherosclerotic plaques: immunohistochemical and PET imaging study. J Nucl Med. 2004;45:1245–50.

[8] Tahara N, Kai H, Yamagishi S, Mizoguchi M, Nakaura H, Ishibashi M, Kaida H, Baba K, Hayabuchi N, Imaizumi T. Vascular inflammation evaluated by [18F]-fluorodeoxyglucose positron emission tomography is associated with the metabolic syndrome. J Am Coll Cardiol. 2007;49:1533–39.

[9] Rominger A, Saam T, Wolpers S, Cyran CC, Schmidt M, Foerster S, Nikolaou K, Reiser MF, Bartenstein P, Hacker M. 18F-FDG PET/CT identifies patients at risk for future vascular events in an otherwise asymptomatic cohort with neoplastic disease. J Nucl Med. 2009;50:1611–20.

[10] Aziz K, Berger K, Claycombe K, Huang R, Patel R, Abela GS. Noninvasive detection and localization of vulnerable plaque and arterial thrombosis with computed tomography angiography/positron emission tomography. Circulation. 2008;117:2061–70.

[11] Yamashita A, Sumi T, Goto S, Hoshiba Y, Nishihira K, Kawamoto R, Hatakeyama K, Date H, Imamura T, Ogawa H, Asada Y. Detection of von Willebrand factor and tissue factor in platelets-fibrin rich coronary thrombi in acute myocardial infarction. Am J Cardiol. 2006;97:26–28.

[12] Nishihira K, Yamashita A, Ishikawa T, Hatakeyama K, Shibata Y, Asada Y. Composition of thrombi in late drug-eluting stent thrombosis versus de novo acute myocardial infarction. Thromb Res. 2010;126:254–57.

[13] Rittersma SZ, van der Wal AC, Koch KT, Piek JJ, Henriques JP, Mulder KJ, Ploegmakers JP, Meesterman M, de Winter RJ. Plaque instability frequently occurs days or weeks before occlusive coronary thrombosis: a pathological thrombectomy study in primary percutaneous coronary intervention. Circulation. 2005;111:1160–65.

[14] Nishihira K, Hatakeyama K, Shibata Y, Kitamura K, Asada Y. Organized thrombus in aspirated coronary materials can predict in-hospital mortality of patients with acute myocardial infarction. Circ J. 2013;77:1275–80.

[15] Kramer MC, van der Wal AC, Koch KT, Ploegmakers JP, van der Schaaf RJ, Henriques JP, Baan J Jr, Rittersma SZ, Vis MM, Piek JJ, Tijssen JG, de Winter RJ. Presence of older thrombus is an independent predictor of long-term mortality in patients with ST-elevation myocardial infarction treated with thrombus aspiration during primary percutaneous coronary intervention. Circulation. 2008;118:1810–16.

[16] Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 2000;20:1262–75.

[17] Kolodgie FD, Burke AP, Farb A, Gold HK, Yuan J, Narula J, Finn AV, Virmani R. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. Curr Opin Cardiol. 2001;16:285–92.

[18] van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36–44.

[19] Sato Y, Hatakeyama K, Yamashita A, Marutsuka K, Sumiyoshi A, Asada Y. Proportion of fibrin and platelets differs in thrombi on ruptured and eroded coronary atherosclerotic plaques in humans. Heart. 2005;91:526–30.

[20] Okada K, Ueda Y, Matsuo K, Nishio M, Hirata A, Kashiwase K, Asai M, Nemoto T, Kodama K. Frequency and healing of nonculprit coronary artery plaque disruptions in patients with acute myocardial infarction. Am J Cardiol. 2011;107:1426–29.

[21] Davies MJ, Bland JM, Hangartner JR, Angelini A, Thomas AC. Factors influencing the presence or absence of acute coronary artery thrombi in sudden ischaemic death. Eur Heart J. 1989;10:203–08.

[22] Arbustini E, Grasso M, Diegoli M, Morbini P, Aguzzi A, Fasani R, Specchia G. Coronary thrombosis in non-cardiac death. Coron Artery Dis. 1993;4:751–59.

[23] Mauriello A, Servadei F, Sangiorgi G, Anemona L, Giacobbi E, Liotti D, Spagnoli LG. Asymptomatic carotid plaque rupture with unexpected thrombosis over a non-canonical vulnerable lesion. Atherosclerosis. 2011;218:356–62.

[24] Fry DL. Acute vascular endothelial changes associated with increased blood velocity gradients. Circ Res. 1968;22:165–97.

[25] Yamashita A, Asada Y, Sugimura H, Yamamoto H, Marutsuka K, Hatakeyama K, Tamura S, Ikeda Y, Sumiyoshi A. Contribution of von Willebrand factor to thrombus formation on neointima of rabbit stenotic iliac artery under high blood-flow velocity. Arterioscler Thromb Vasc Biol. 2003;23:1105–10.

[26] Sumi T, Yamashita A, Matsuda S, Goto S, Nishihira K, Furukoji E, Sugimura H, Kawahara H, Imamura T, Kitamura K, Tamura S, Asada Y. Disturbed blood flow induces erosive injury to smooth muscle cell-rich neointima and promotes thrombus formation in rabbit femoral artery. J Thromb Haemost. 2010;8:1394–402.

[27] Sameshima N, Yamashita A, Sato S, Matsuda S, Matsuura Y, Asada Y. The values of wall shear stress, turbulence kinetic energy and blood pressure gradient are associated with atherosclerotic plaque erosion in rabbits. J Atheroscler Thromb. 2014;21:831–38.

[28] Shiomi M, Ishida T, Kobayashi T, Nitta N, Sonoda A, Yamada S, Koike T, Kuniyoshi N, Murata K, Hirata K, Ito T, Libby P. Vasospasm of atherosclerotic coronary arteries precipitates acute ischemic myocardial damage in myocardial infarction-prone strain of the Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol. 2013;33:2518–23.

[29] Miyamoto S, Ogawa H, Soejima H, Takazoe K, Kajiwara I, Shimomura H, Sakamoto T, Yoshimura M, Kugiyama K, Yasue H, Ozaki Y. Enhanced platelet aggregation in the coronary circulation after coronary spasm. Thromb Res. 2001;103:377–86.

[30] Oshima S, Yasue H, Ogawa H, Okumura K, Matsuyama K. Fibrinopeptide A is released into the coronary circulation after coronary spasm. Circulation. 1990;82:2222–25.

[31] Østerud, B. Tissue factor expression in blood cells. Thromb Res. 2010;125 Suppl 1:S31–S34.

[32] Mackman, N. Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol. 2004;24:1015–22.

[33] Chou J, Mackman N, Merrill-Skoloff G, Pedersen B, Furie BC, Furie B. Hematopoietic cell-derived microparticle tissue factor contributes to fibrin formation during thrombus propagation. Blood. 2004;104:3190–97.

[34] Wang L, Miller C, Swarthout RF, Rao M, Mackman N, Taubman MB. Vascular smooth muscle-derived tissue factor is critical for arterial thrombosis after ferric chloride-induced injury. Blood. 2009;113:705–13.

[35] Yamashita A, Matsuda S, Matsumoto T, Moriguchi-Goto S, Takahashi M, Sugita C, Sumi T, Imamura T, Shima M, Kitamura K, Asada Y. Thrombin generation by intimal tissue factor contributes to thrombus formation on macrophage-rich neointima but not normal intima of hyperlipidemic rabbits. Atherosclerosis. 2009;206:418–26.

[36] Funatsu T, Yamashita A, Kaku S, Iwatsuki Y, Asada Y. Plasma factor Xa inhibition can predict antithrombotic effects of oral direct factor Xa inhibitors in rabbit atherothrombosis models. Thromb Haemost. 2012;108:896–902.

[37] Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. Science. 1991;253:909–12.

[38] Yamashita A, Nishihira K, Kitazawa T, Yoshihashi K, Soeda T, Esaki K, Imamura T, Hattori K, Asada Y. Factor XI contributes to thrombus propagation on injured neointima of the rabbit iliac artery. J Thromb Haemost. 2006;4:1496–501.

[39] van Montfoort ML, Kuijpers MJ, Knaup VL, Bhanot S, Monia BP, Roelofs JJ, Heemskerk JW, Meijers JC. Factor XI regulates pathological thrombus formation on acutely ruptured atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 2014;34:1668–73.

[40] Kuijpers MJ, van der Meijden PE, Feijge MA, Mattheij NJ, May F, Govers-Riemslag J, Meijers JC, Heemskerk JW, Renné T, Cosemans JM. Factor XII regulates the pathological process of thrombus formation on ruptured plaques. Arterioscler Thromb Vasc Biol. 2014;34:1674–80.

[41] Doggen CJ, Rosendaal FR, Meijers JC. Levels of intrinsic coagulation factors and the risk of myocardial infarction among men: opposite and synergistic effects of factors XI and XII. Blood. 2006;108:4045–51.

[42] Salomon O, Steinberg DM, Dardik R, Rosenberg N, Zivelin A, Tamarin I, Ravid B, Berliner S, Seligsohn U. Inherited factor XI deficiency confers no protection against acute myocardial infarction. J Thromb Haemost. 2003;1:658–61.

[43] Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. Blood. 2008;111:4113–17.

[44] Salomon O, Steinberg DM, Zucker M, Varon D, Zivelin A, Seligsohn U. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. Thromb Haemost. 2011;105:269-73. doi: 10.1160/TH10-05-0307.

[45] Kulkarni S, Dopheide SM, Yap CL, Ravanat C, Freund M, Mangin P, Heel KA, Street A, Harper IS, Lanza F, Jackson SP. A revised model of platelet aggregation. J Clin Invest. 2000;105:783–91.

[46] Bergmeier W, Piffath CL, Goerge T, Cifuni SM, Ruggeri ZM, Ware J, Wagner DD. The role of platelet adhesion receptor GPIbalpha far exceeds that of its main ligand, von Willebrand factor, in arterial thrombosis. Proc Natl Acad Sci U S A. 2006;103:16900–05.

[47] Yamashita A, Furukoji E, Marutsuka K, Hatakeyama K, Yamamoto H, Tamura S, Ikeda Y, Sumiyoshi A, Asada Y. Increased vascular wall thrombogenicity combined with reduced blood flow promotes occlusive thrombus formation in rabbit femoral artery. Arterioscler Thromb Vasc Biol. 2004;24:2420–24.

[48] Nesbitt WS, Giuliano S, Kulkarni S, Dopheide SM, Harper IS, Jackson SP. Intercellular calcium communication regulates platelet aggregation and thrombus growth. J Cell Biol. 2003;160:1151–61.

[49] Goto S, Tamura N, Ishida H, Ruggeri ZM. Dependence of platelet thrombus stability on sustained glycoprotein IIb/IIIa activation through adenosine 5'-diphosphate receptor stimulation and cyclic calcium signaling. J Am Coll Cardiol. 2006;47:155–62.

[50] Marzilli M, Sambuceti G, Fedele S, L'Abbate A. Coronary microcirculatory vasoconstriction during ischemia in patients with unstable angina. J Am Coll Cardiol. 2000;35:327–34.

[51] Schwartz RS, Burke A, Farb A, Kaye D, Lesser JR, Henry TD, Virmani R. Microemboli and microvascular obstruction in acute coronary thrombosis and sudden coronary death: relation to epicardial plaque histopathology. J Am Coll Cardiol. 2009;54:2167–73.

[52] Nishihira K, Yamashita A, Tanaka N, Kawamoto R, Imamura T, Yamamoto R, Eto T, Asada Y. Inhibition of 5-hydroxytryptamine receptor prevents occlusive thrombus formation on neointima of the rabbit femoral artery. J Thromb Haemost. 2006;4:247–55.

[53] Nishihira K, Yamashita A, Tanaka N, Moriguchi-Goto S, Imamura T, Ishida T, Kawashima S, Yamamoto R, Kitamura K, Asada Y. Serotonin induces vasoconstriction of smooth muscle cell-rich neointima through 5-hydroxytryptamine2A receptor in rabbit femoral arteries. J Thromb Haemost. 2008;6:1207–14.

[54] Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, Carberry J, Fouras A, Jackson SP. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. Nat Med. 2009;15:665–73.

[55] Siedlecki CA, Lestini BJ, Kottke-Marchant KK, Eppell SJ, Wilson DL, Marchant RE. Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. Blood. 1996;88:2939–50.

[56] Ikeda Y, Handa M, Kawano K, Kamata T, Murata M, Araki Y, Anbo H, Kawai Y, Watanabe K, Itagaki I. The role of von Willebrand factor and fibrinogen in platelet aggregation under varying shear stress. J Clin Invest. 1991;87:1234–40.

[57] Moreno-Sánchez R, Rodríguez-Enríquez S, Marín-Hernández A, Saavedra E. Energy metabolism in tumor cells. FEBS J. 2007;274:1393–418.

[58] Ghesquière B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. Nature. 2014;511:167–76.

[59] Grandpierre S, Desandes E, Meneroux B, Djaballah W, Mandry D, Netter F, Wahl D, Fay R, Karcher G, Marie PY. Arterial foci of F-18 fluorodeoxyglucose are associated with an enhanced risk of subsequent ischemic stroke in cancer patients: a case-control pilot study. Clin Nucl Med. 2011;36:85–90.

[60] Yamashita A, Zhao Y, Zhao S, Matsuura Y, Sugita C, Iwakiri T, Okuyama N, Ohe K, Koshimoto C, Kawai K, Tamaki N, Kuge Y, Asada Y. Arterial (18)F-fluorodeoxyglucose uptake reflects balloon catheter-induced thrombus formation and tissue factor

expression via nuclear factor-κB in rabbit atherosclerotic lesions. Circ J. 2013;77:2626–35.

[61] Zhao Y, Zhao S, Kuge Y, Strauss WH, Blankenberg FG, Tamaki N. Localization of deoxyglucose and annexin A5 in experimental atheroma correlates with macrophage infiltration but not lipid deposition in the lesion. Mol Imaging Biol. 2011;13:712–20.

[62] Rogers IS, Nasir K, Figueroa AL, Cury RC, Hoffmann U, Vermylen DA, Brady TJ, Tawakol A. Feasibility of FDG imaging of the coronary arteries: comparison between acute coronary syndrome and stable angina. JACC Cardiovasc Imaging. 2010;3:388–97.

[63] Morrison ES, Scott RF, Kroms M, Frick J. Glucose degradation in normal and atherosclerotic aortic intima-media. Atherosclerosis. 1972;16:175–84.

[64] Leppänen O, Björnheden T, Evaldsson M, Borén J, Wiklund O, Levin M. ATP depletion in macrophages in the core of advanced rabbit atherosclerotic plaques in vivo. Atherosclerosis. 2006;188:323–30.

[65] Yamashita A, Zhao Y, Matsuura Y, Yamasaki K, Moriguchi-Goto S, Sugita C, Iwakiri T, Okuyama N, Koshimoto C, Kawai K, Tamaki N, Zhao S, Kuge Y, Asada Y. Increased metabolite levels of glycolysis and pentose phosphate pathway in rabbit atherosclerotic arteries and hypoxic macrophage. PLoS One. 2014;9:e86426.

[66] Mateo J, Izquierdo-Garcia D, Badimon JJ, Fayad ZA, Fuster V. Noninvasive assessment of hypoxia in rabbit advanced atherosclerosis using ^{18}F-fluoromisonidazole positron emission tomographic imaging. Circ Cardiovasc Imaging. 2014;7:312–20.

[67] Lofland HB Jr, Moury DM, Hoffman CW, Clarkson TB. Lipid metabolism in pigeon aorta during atherogenesis. J Lipid Res. 1965;6:112–18.

[68] Whereat AF. Recent advances in experimental and molecular pathology. Atherosclerosis and metabolic disorder in the arterial wall. Exp Mol Pathol. 1967;7:233–47.

[69] Matsui R, Xu S, Maitland KA, Mastroianni R, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6-phosphate dehydrogenase deficiency decreases vascular superoxide and atherosclerotic lesions in apolipoprotein E(-/-) mice. Arterioscler Thromb Vasc Biol. 2006;26:910–16.

[70] Newman HA, McCandless EL, Zilversmit DB. The synthesis of C14-lipids in rabbit atheromatous lesions. J Biol Chem. 1961;236:1264–68.

[71] Newsholme P, Newsholme EA. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. Biochem J. 1989;261:211–18.

[72] Newsholme P, Gordon S, Newsholme EA. Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. Biochem J. 1987;242:631–36.

[73] Sakamoto T1, Seiki M. A membrane protease regulates energy production in macrophages by activating hypoxia-inducible factor-1 via a non-proteolytic mechanism. J Biol Chem. 2010;285:29951–64.

[74] Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab. 2006;4:13–24.

[75] Satomi T, Ogawa M, Mori I, Ishino S, Kubo K, Magata Y, Nishimoto T. Comparison of contrast agents for atherosclerosis imaging using cultured macrophages: FDG versus ultrasmall superparamagnetic iron oxide. J Nucl Med. 2013;54:999–1004.

[76] Tavakoli S, Zamora D, Ullevig S, Asmis R. Bioenergetic profiles diverge during macrophage polarization: implications for the interpretation of 18F-FDG PET imaging of atherosclerosis. J Nucl Med. 2013;54:1661–67.

[77] Folco EJ, Sheikine Y, Rocha VZ, Christen T, Shvartz E, Sukhova GK, Di Carli MF, Libby P. Hypoxia but not inflammation augments glucose uptake in human macrophages: implications for imaging atherosclerosis with 18fluorine-labeled 2-deoxy-D-glucose positron emission tomography. J Am Coll Cardiol. 2011;58:603–14.

[78] Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiel DM, Gassmann M, Gardner LB, Fisher EA. Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. Circ Res. 2011;109:1141–52.

[79] Ugocsai P, Hohenstatt A, Paragh G, Liebisch G, Langmann T, Wolf Z, Weiss T, Groitl P, Dobner T, Kasprzak P, Göbölös L, Falkert A, Seelbach-Goebel B, Gellhaus A, Winterhager E, Schmidt M, Semenza GL, Schmitz G. HIF-1beta determines ABCA1 expression under hypoxia in human macrophages. Int J Biochem Cell Biol. 2010;42:241–52.

[80] Na TY, Lee HJ, Oh HJ, Huh S, Lee IK, Lee MO. Positive cross-talk between hypoxia inducible factor-1α and liver X receptor α induces formation of triglyceride-loaded foam cells. Arterioscler Thromb Vasc Biol. 2011;31:2949–56.

[81] Asplund A, Stillemark-Billton P, Larsson E, Rydberg EK, Moses J, Hultén LM, Fagerberg B, Camejo G, Bondjers G. Hypoxic regulation of secreted proteoglycans in macrophages. Glycobiology. 2010;20:33–40.

[82] Huo Y, Guo X, Li H, Wang H, Zhang W, Wang Y, Zhou H, Gao Z, Telang S, Chesney J, Chen YE, Ye J, Chapkin RS, Wu C. Disruption of inducible 6-phosphofructo-2-kinase ameliorates diet-induced adiposity but exacerbates systemic insulin resistance and adipose tissue inflammatory response. J Biol Chem. 2010;285:3713–21.

[83] Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, Knapp B, Haas R, Schmid JA, Jandl C, Amir S, Lubec G, Park J, Esterbauer H, Bilban M, Brizuela L, Pospisilik JA, Otterbein LE, Wagner O. The sedoheptulose kinase CARKL directs macro-

http://dx.doi.org/10.5772/61769

phage polarization through control of glucose metabolism. Cell Metab. 2012;15:813–26.

[84] Ham M, Lee JW, Choi AH, Jang H, Choi G, Park J, Kozuka C, Sears DD, Masuzaki H, Kim JB. Macrophage glucose-6-phosphate dehydrogenase stimulates proinflammatory responses with oxidative stress. Mol Cell Biol. 2013;33:2425–35.

[85] Schneider JG, Yang Z, Chakravarthy MV, Lodhi IJ, Wei X, Turk J, Semenkovich CF. Macrophage fatty-acid synthase deficiency decreases diet-induced atherosclerosis. J Biol Chem. 2010;285:23398–409.

[86] Saito R, Matsuzaka T, Karasawa T, Sekiya M, Okada N, Igarashi M, Matsumori R, Ishii K, Nakagawa Y, Iwasaki H, Kobayashi K, Yatoh S, Takahashi A, Sone H, Suzuki H, Yahagi N, Yamada N, Shimano H. Macrophage Elovl6 deficiency ameliorates foam cell formation and reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2011;31:1973–79.

[87] Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ, Bijnens AP. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. J Am Coll Cardiol. 2008;51:1258–65.

[88] Yan SF, Zou YS, Gao Y, Zhai C, Mackman N, Lee SL, Milbrandt J, Pinsky D, Kisiel W, Stern D. Tissue factor transcription driven by Egr-1 is a critical mechanism of murine pulmonary fibrin deposition in hypoxia. Proc Natl Acad Sci U S A. 1998;95:8298–303.

[89] Kietzmann T, Roth U, Jungermann K. Induction of the plasminogen activator inhibitor-1 gene expression by mild hypoxia via a hypoxia response element binding the hypoxia-inducible factor-1 in rat hepatocytes. Blood. 1999;94:4177–85.

[90] Eltzschig HK, Köhler D, Eckle T, Kong T, Robson SC, Colgan SP. Central role of Sp1-regulated CD39 in hypoxia/ischemia protection. Blood. 2009;113:224–32.

[91] Hatakeyama K, Hao H, Imamura T, Ishikawa T, Shibata Y, Fujimura Y, Eto T, Asada Y. Relation of CD39 to plaque instability and thrombus formation in directional atherectomy specimens from patients with stable and unstable angina pectoris. Am J Cardiol. 2005;95:632–35.

[92] Raghunath PN, Tomaszewski JE, Brady ST, Caron RJ, Okada SS, Barnathan ES. Plasminogen activator system in human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 1995;15:1432–43.

[93] Matsuura Y, Yamashita A, Iwakiri T, Sugita C, Okuyama N, Kitamura K, Asada Y. Vascular wall hypoxia promotes arterial thrombus formation via augmentation of vascular thrombogenicity. Thromb Haemost. 2015; 114:158–72.

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