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Fibrinogen and fibrin in hemostasis and thrombosis

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Introduction

Despite its recognition as a key component of blood clots, the roles of fibrinogen and fibrin (collectively fibrin[ogen]) in hemostasis and thrombosis are insufficiently understood. Consequently, fibrin(ogen) remains an active focus of investigation at all levels of the research spectrum, including fundamental basic/discovery science, epidemiology, and clinical practice and applications. This article briefly reviews basic biology and biochemistry of fibrinogen and fibrin formation, structure, and stability, and highlights recent studies published in *Arteriosclerosis, Thrombosis, and Vascular Biology* and elsewhere. These have enhanced our understanding of fibrin(ogen) and revealed new potential applications for fibrin detection in thrombosis.

Fibrinogen biology and structure

The fibrinogen molecule is a 340-kDa homodimeric glycoprotein consisting of 2Aα, 2Bβ, and 2γ polypeptide chains linked by 29 disulfide bridges. Fibrinogen synthesis occurs primarily in hepatocytes (Figure 1). Assembly of the six chains takes place in a step-wise manner in which single chains assemble first into Aα-γ and Bβ-γ complexes, then into Aα/Bβ/γ half-molecules, and finally into hexameric complexes $(Aα/Bβ/γ)_2$ (reviewed in[1]). All six fibrinogen chains are assembled with their N-termini located in a central “E nodule,” and extend outward in a coiled-coil arrangement. The Bβ and γ chains terminate in globular regions known as βC and γC modules, respectively. These regions collectively comprise the so-called “D nodule.” The Aα chains are the longest; at the end of the coiled-coil region each chain extends into a highly-flexible series of repeats followed by a globular αC region. Using high-resolution atomic force microscopy, Protopopova et al obtained striking images of fibrinogen that visualize each of these structural components.[2]

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None

In healthy individuals, fibrinogen circulates in plasma at high concentrations (2–5 mg/mL). However, fibrinogen is an acute phase protein, and during acute inflammation, plasma fibrinogen levels can exceed 7 mg/mL. The fibrinogen chains are encoded in three genes that are thought to have arisen through gene duplication. Mechanisms that regulate expression of the fibrinogen genes are still largely undetermined. Genome-wide association studies have identified single nucleotide polymorphisms within the fibrinogen genes[3], as well as loci distinct from fibrinogen that implicate transcription factors (e.g., hepatocyte nuclear factors 1 and 4 [TCF1 and HNF4], signal transducer and activator of transcription 3 [STAT3])[3–5] and inflammatory signaling pathways downstream of interleukin-6[5] in fibrinogen gene expression. In addition, microRNAs (miR) in the hsa-miR-29 family and hsa-miR-409-3p down-regulate fibrinogen expression in hepatoma cells in vitro[6], revealing mechanisms that may fine-tune fibrinogen levels in response to environmental cues.

Fibrin formation, structure, and stability

During coagulation, fibrinogen is converted into insoluble fibrin (Figure 1). Fibrin formation involves thrombin-mediated proteolytic cleavage and removal of N-terminal fibrinopeptides from the Aα and Bβ chains. Insertion of these newly-exposed α- and β- “knobs” into a- and b- “holes” in the γC and βC regions of the D nodule, respectively, on another fibrin monomer permits the half-staggered association of fibrin monomers into protofibrils. Subsequent aggregation of protofibrils into fibers yields a fibrin meshwork that is essential for blood clot stability. This process has been extensively reviewed.[7–11]

Clot formation, structure, and stability are strongly influenced by the conditions present during fibrin generation. These include the concentrations of procoagulants, anticoagulants, fibrin(ogen)-binding proteins, molecules[12–20], and metal ions[21,22], as well as contributions of blood and vascular cells, and cell-derived microvesicles[23–30], and presence of blood flow[31,32] (Figure 2). Many of these mechanisms have been reviewed.[33,34]

The contribution of thrombin concentration to fibrin formation and structure has received considerable attention. High thrombin concentrations produce dense networks of highly-branched fibrin fibers, and these clots are relatively resistant to fibrinolysis. In contrast, low thrombin concentrations produce coarse networks of relatively unbranched fibrin fibers, and these clots are relatively susceptible to fibrinolysis.[12,17,35,36] Most studies have reported that compared to fibers formed from low thrombin concentrations, fibers generated by high thrombin concentrations are thinner. However, turbidimetry and microscopy studies of fully hydrated clots suggest high thrombin concentrations decrease the average protofibril content per fiber but only slightly decrease fiber size, leading to a generally less compact fiber.[37] Thus, the substantially thinner fibers observed at high thrombin concentrations in earlier studies may reflect fiber compaction or shrinkage that occurs during dehydration. Regardless, the association of fibrin clot structural parameters with clinical pathologies – dense networks of thinner/compact fibers with increased thrombotic risk[38–40] and coarse networks of thicker/less compact fibers with increased bleeding risk[36,41] – suggests fibrin structure is a critical determinant of hemostasis and thrombosis.

Endogenous mediators of thrombin generation and fibrin

Multiple mechanisms mediate thrombin generation and consequently, the thrombin concentration present during fibrin formation. First, the levels of pro- and anticoagulants present during coagulation strongly influence procoagulant activity. For example, elevated levels of prothrombin are associated with increased thrombin generation[35,42], formation of dense fibrin networks[35], and increased venous thrombus weight in mice[43]. These studies, designed to model the clinical situation in humans with the G20210A prothrombin mutation associated with increased circulating prothrombin levels[44], suggest increased thrombin generation enhances venous thrombosis risk in part by promoting abnormal fibrin deposition and structure. Second, the location of thrombin generation impacts fibrin network formation. Effective assembly and activity of the prothrombinase complex (factors Xa, Va, and prothrombin) requires a lipid surface.[45] Localization of prothrombinase on a cell surface establishes a thrombin concentration gradient that influences both fibrin formation and network structure. In vitro experiments employing in situ thrombin generation on fibroblasts and endothelial cells reveal a significantly denser fiber network proximal versus distal to the cell surface.[29] These structural differences give rise to substantially different fibrinolytic susceptibilities in different regions of the clot; fibrin located near the cell surface is significantly more resistant to lysis than fibrin located distal to the cell surface.[29] Third, blood flow (shear) present during fibrin formation influences local thrombin concentrations by (re)supplying procoagulant proteins and removing activated enzymes (reviewed in[46]). Flow also aligns fibrin fibers[31,32], which may have profound effects on fibrin formation and mechanical and fibrinolytic stability[47]. Furthermore, the shear rate affects clot formation triggered on tissue factor- plus collagen-coated plates, resulting in different fibrin deposition in different regions of a thrombus.[48] Nanoindentation analysis to evaluate clot biophysical properties shows this fibrin distribution pattern determines clot microelasticity, which may impact thrombus stability and risk of embolization.[48] Fourth, thrombin movement through the thrombus is substantially influenced by solute transport mechanisms mediated by cell packing density; this may also influence the amount of fibrin deposition in different regions of the clot.[49]

Effects of antithrombotic and hemostatic agents on fibrin

Given the prominent role of thrombin concentration in determining fibrin network formation and structure, it is not surprising that antithrombotic agents that reduce thrombin activity reduce fibrin deposition and consequently, thrombus formation. Since factor XI (FXI[a]) augments thrombin generation, in part by synergizing tissue factor-mediated procoagulant activity[50], FXI inhibition strategies to reduce thrombosis have received considerable attention (reviewed in[51]). These approaches include conventional anti-FXI inhibitory antibodies, as well as technology in which antisense oligonucleotides (ASOs) result in the specific degradation of a target mRNA and corresponding reduction in target protein level. These studies reveal surprisingly specific effects of FXI inhibition in models of thrombosis and bleeding. Pharmacologic FXI inhibition does not reduce local platelet adhesion in tissue factor and collagen-coated capillary tubes, but reduces platelet activation and aggregation downstream of the growing thrombus.[52] Similarly, in an arteriovenous shunt model of thrombosis in non-human primates, neither anti-FXI antibodies nor ASOs alter platelet

deposition on a collagen-rich segment of graft, but both decrease thrombus propagation (platelet accumulation and fibrin deposition) downstream of the collagen-rich region.[53,54] A promising phase I clinical trial demonstrated success of anti-FXI ASO treatment in humans undergoing elective total knee arthroplasty; ASO-mediated reduction of plasma FXI levels decreased symptomatic or asymptomatic venous thrombosis/thromboembolism (VTE) incidence, and the higher ASO dose tested was superior to enoxaparin.[55] In addition to its role in VTE, FXI also appears to contribute to atherogenesis in mice. Mice with deficiency in apolipoprotein E ($Apoe^{-/-}$) spontaneously develop atherosclerotic lesions, but $Apoe^{-/-}$ mice with genetic FXI deficiency show reduced atherosclerosis progression.[56] Moreover, anti-FXI ASO treatment reduces thrombus formation and fibrin deposition in a model of plaque rupture in $Apoe^{-/-}$ mice.[57] Thus, FXI inhibition may also be effective for reducing arterial thrombosis in humans. Notably, FXI reduction has not been associated with increased bleeding in any of these studies, suggesting FXI antagonism may be safer than current antithrombotics. However, given that bleeding occurs in a subset of patients with FXI deficiency[58,59] and the finding of altered structure and stability of plasma clots from these patients[60], the safety of FXI inhibition should be carefully monitored in future trials. Regardless, these findings collectively support continued efforts to investigate and advance FXI inhibition strategies into the clinic.

Heparin and heparin-like compounds are used to prevent thrombosis, presumably due to their ability to reduce thrombin activity. However, heparin binds to the central E nodule of fibrin[61], and both unfractioned heparin and low molecular weight heparin can also directly alter fibrin structure in an antithrombin-independent manner.[62] Observed changes include effects on fibrin fiber thickness, as well as porosity. These changes are not observed with the pentasaccharide, fondaparinux. Demonstration of these direct effects of unfractioned heparin and low molecular weight heparin on fibrin structure suggest tests that assess efficacy based solely on thrombin inhibition may not fully capture therapeutic effects of these drugs. Global assays that assess both thrombin generation and fibrin formation[63] may more closely reflect therapeutic effects of these drugs.

The common heparin reversal agent, protamine, also modulates fibrin network structure and stability. Protamine interacts directly with fibrinogen and is incorporated into clots, resulting in the production of thicker fibrin fibers in clots that are more susceptible to fibrinolysis.[64] Recently, Kalathottukaren et al characterized a synthetic polycation they termed universal heparin reversal agent (UHRA), as an alternative to protamine. UHRA can neutralize both heparin anticoagulant activity and polyphosphate procoagulant activity without the off-target effects on fibrin quality observed with protamine.[64] Further studies to evaluate the therapeutic potential of UHRA are anticipated.

Effect of cells and cell components on fibrin formation, structure, and stability

Clot quality is heavily influenced by cells and cell-derived components present at the injury site.[23–30] Recent studies have revealed previously unrecognized effects of red blood cells

(RBCs) and neutrophil extracellular traps (NETs) on fibrin formation, structure and stability. These may have substantial implications for understanding coagulation disorders.

RBCs are present in hemostatic and thrombotic clots, but their ability to influence clot formation or function have been unclear. RBCs can support thrombin generation[65–67] and thereby alter procoagulant activity at the site of clot formation. The presence of RBCs during clot formation also increases fibrin network heterogeneity, but whether RBCs increase[28] or decrease[27] fibrin fiber thickness is unclear. Once in the clot, RBCs alter clot viscoelastic properties[28] and by reducing plasminogen activation, increase resistance of clots to fibrinolysis[27]. Effects of RBCs on fibrin structure are reduced in the presence of eptifibatide, suggesting these effects are modulated in part by an interaction between fibrin(ogen) and a cell surface integrin.[27] This observation is consistent with observations suggesting fibrin(ogen) binds to RBCs via a β_3-like molecule on the RBC surface[68], although neither the fibrin(ogen) binding site, nor the putative RBC receptor, have been identified. Studies of contracted whole blood clots show profound compression of resident RBCs into structures termed “polyhedrocytes.”[25,69] Adoption of this tight packing arrangement reduces clot permeability and may explain the resistance of older, compact clots to thrombolysis.[25]

NETs are composed of DNA, histones, and antimicrobial proteins and have surfaced as an intriguing link between inflammation and coagulation. NETs can be detected within venous thrombi[70], and levels of cell-free DNA (CFDNA) in plasma are increased in patients with deep vein thrombosis, suggesting NETs contribute to thrombosis pathogenesis.[71] However, specific effects of NETs and NET components on coagulation are complex. Briefly, NETs can interact with cells and coagulation factors and influence their activation and activity. NET components promote thrombin generation by activating the intrinsic pathway of coagulation and by inducing platelet-dependent mechanisms in toll-like receptors-2 and -4-dependent mechanisms.[72–74] Histones also enhance activated protein C generation by thrombin/thrombomodulin in vitro and in mice.[75] By altering these procoagulant and anticoagulant pathways, NETs may alter local thrombin levels and indirectly alter fibrin formation and quality. In vitro studies suggest CFDNA promotes formation of densely-packed networks of thick fibrin fibers.[74] This observation is interesting, given that dense fibrin networks are more typically associated with decreased fiber thickness/compaction. Thus, this finding suggests CFDNA, like its highly-charged cousin polyphosphate, modulates fibrin structure at least partly through a direct interaction with fibrin(ogen). In addition, clots formed in the presence of CFDNA exhibit delayed fibrinolysis via a mechanism that involves a CFDNA-dependent reduction in plasmin fibrinolytic activity.[74] These effects may have substantial clinical implications. For example, CFDNA levels are elevated in patients with sepsis, and the effects of CFDNA on fibrin structure and fibrinolysis are also observed in plasmas from sepsis patients.[74,76] The role of NETs and potential utility of DNA-dissolving treatment (e.g., DNase) remains an active area of investigation.

Alternatively-spliced fibrinogen

Multiple alternatively-spliced forms of fibrinogen can be detected in plasma. Of these, an alternatively-spliced form of the γ chain (γ′) is the most prevalent and has received the most attention. The γ′ chain has the final 4 amino acids of the native γ chain replaced with 20 amino acids that add substantial negative charge.[77–79] Molecules containing the γ′ chain circulate as a heterodimer with the γA chain (2Aα, 2Bβ, and γA/γ′) and comprise 8–15% of total fibrinogen in healthy individuals.[79,80]

Epidemiologic studies have associated altered arterial and venous thrombosis risk with the level of circulating γA/γ′ fibrinogen. For example, elevated levels of γA/γ′ fibrinogen have been associated with increased incidence of coronary artery disease[81], myocardial infarction[82], and ischemic stroke[83], leading to the hypothesis that γA/γ′ fibrinogen promotes arterial thrombosis. However, a recent prospective study showed that while γA/γ′ fibrinogen is associated with increased incidence of cardiovascular disease (CVD), peripheral arterial disease, and heart failure, this association is lost when the analysis is adjusted for total fibrinogen and C-reactive protein.[84] Thus, the association of elevated fibrinogen with these pathologies may be mediated, at least in part, by a co-existing inflammatory reaction. In contrast, reduced levels of γA/γ′ fibrinogen and decreased γ′-to-total fibrinogen ratio have been fairly consistently associated with increased risk of VTE[85] and thrombotic microangiopathy[86]. These findings suggest γA/γ′ fibrinogen is protective against venous thrombosis, and raise interesting questions about the operant mechanism.

In vitro studies investigating the relative role(s) of the fibrinogen isoforms during clot formation indicate both γA/γA and γA/γ′ isoforms are incorporated into the fibrin network; however, the γA/γ′ isoform has unique properties that modify its role during clotting and subsequently, the function of the fibrin clot. Studies have generally reported thinner fibrin fibers in clots containing the γA/γ′ isoform and associated this effect with increased resistance to fibrinolysis.[87,88] However, Domingues et al detected reduced packing of γA/γ′ molecules in fully-hydrated protofibrils, suggesting reduced packing results in the appearance of decreased fiber diameter in dehydrated clots.[37] Moreover, in contrast to clots formed by γA/γA fibrinogen, characteristics of fibers formed by γA/γ′ fibrinogen are relatively unaffected by the thrombin concentration.[37]

The γA/γ′ isoform supports high-affinity binding to thrombin exosite II[89,90] that led to its recognition as “antithrombin I.” Thrombin binding to the γ′ chain competitively inhibits thrombin-mediated platelet activation[91], reduces thrombin-mediated fibrinopeptide B cleavage[92], and decreases factor VIII[93] and V[94] activation. In in vitro microfluidic models, γA/γ′ fibrinogen reduces clot growth primarily at venous, but not arterial, wall shear rates, suggesting the impact of its antithrombin I activity depends in part on the location of the thrombotic event (vein or artery).[94] Studies with mice have documented antithrombotic effects of γA/γ′ in models of both venous and arterial thrombosis. Expression of the human γA/γ′ fibrinogen isoform in mice that are heterozygous for the factor V Leiden mutation reduces thrombus volume following electrolytic injury to the femoral vein.[95] In a model of $FeCl_3$-induced carotid artery injury, healthy mice infused with unfractionated human fibrinogen have a shortened time to vessel occlusion, and this effect is recapitulated by

infusion of γA/γA, but not γA/γ′, fibrinogen. Although γA/γ′-infused mice are not protected against thrombus formation in this model, they do have lower levels of circulating plasma thrombin-antithrombin complexes compared to γA/γA-infused mice, consistent with increased thrombin-binding capacity of γA/γ′ fibrin(ogen).[96] Collectively, these studies suggest γA/γ′ has generally antithrombotic roles during coagulation and its expression may serve to downregulate inflammation-induced prothrombotic activity.

Fibrin crosslinking

Covalent crosslinking of fibrin chains is a critical determinant of fibrin stability. Crosslinking is mediated predominantly by transglutaminase factor XIII (FXIII) found in plasma and platelets. Plasma FXIII is a 320-kDa heterotetrameric zymogen (FXIII-A_2B_2) composed of two catalytic subunits (FXIII-A_2) tightly-associated (K_d~10^{-10} M)[97] with two non-catalytic subunits (FXIII-B_2). FXIII-A_2B_2 circulates at ~70 nM (14–28 μg/mL)[98] in complex with fibrinogen. Although early data suggested FXIII-A_2B_2 preferentially binds the alternatively-spliced fibrinogen γ′ chain, more recent studies have localized binding to γ-chain residues 390–396 with additional contributions from the Aα-chain.[99–101]

Catalytically-active FXIII (FXIIIa) induces ε-N-(γ-glutamyl)-lysyl crosslinks between glutamine and lysine residues on fibrin γ- and α-chains, yielding γ-γ dimers and high molecular weight (HMW) species (γ-multimers, α-polymers, and αγ-hybrids). FXIII can also crosslink other plasma proteins (e.g., $α_2$-antiplasmin, fibronectin) to fibrin. Covalent crosslinking of $α_2$-antiplasmin to fibrin prevents expulsion of $α_2$-antiplasmin from the clot during clot compression or contraction[102] and is essential for clot stability. For example, in a mouse model of middle cerebral artery occlusion in which plasma clots formed ex vivo are placed into $α_2$-antiplasmin-deficient mice, clots made from $α_2$-antiplasmin sufficient plasma are more resistant to dissolution than clots made from $α_2$-antiplasmin-deficient plasma.[103] The importance of FXIIIa-mediated crosslinking for clot stability has been reviewed.[104–106]

Recently, we discovered that FXIIIa-mediated fibrin crosslinking also promotes RBC retention in clots, exposing a newly-recognized role for this activity during VTE.[99,107] Briefly, compared to FXIII-sufficient mice (*F13a*$^{+/+}$), FXIII-deficient (*F13a*$^{-/-}$) mice produce thrombi that have reduced RBC retention and consequently, are smaller.[99] This effect of FXIII on RBC retention in clots is mediated specifically by fibrin α-chain crosslinking.[107]

The timing of fibrin crosslinking also appears to be integral to RBC retention in clots. Mice that have reduced binding of FXIII to fibrinogen and *delayed* FXIII activation and fibrin crosslinking (*Fibγ*$^{390–396A}$) show significantly decreased RBC retention and thrombus size, similar to that seen in *F13a*$^{-/-}$ mice.[99] Interestingly, the FXIII Val34Leu polymorphism that exhibits *accelerated* FXIII activation, paradoxically conveys moderate protection against VTE by modulating clot structure in a fibrinogen concentration-dependent manner.[108] These interesting and apparent paradoxical findings raise important and clinically-relevant questions regarding the role of FXIII activation kinetics and fibrin crosslinking in thrombosis. Duval et al tested the contribution of the Val34Leu polymorphism to thrombus formation in mice.[109] Despite observing increased FXIII activation and crosslinking in vitro

and in vivo, *F13a*$^{-/-}$ mice infused with recombinant FXIII-Leu34 showed no difference in thrombus size compared to FXIII-Val34-infused mice in the $FeCl_3$ model of femoral vein thrombosis.[109] These data suggest the Leu34 variant does not alter thrombus size; however, since $FeCl_3$ injury induces rapid formation of platelet-rich thrombi, effects of the Val34Leu polymorphism on the slow process of RBC- and fibrin-rich venous thrombus formation remain unknown. Further investigations on the contribution of the FXIII Leu34 polymorphism to fibrin formation and thrombosis are warranted.

Clot contraction

An essential function during coagulation is the platelet-mediated consolidation of clots in a process known as clot contraction (or retraction). This process involves fibrin(ogen) binding to platelet integrin receptor $\alpha_{IIb}B_3$ and is influenced by both platelet and fibrin(ogen) concentrations.[110] Although recognized as a fundamental process during coagulation, clot contraction has received little attention, particularly in a clinical setting. This gap is noteworthy given findings that associate platelet aggregation and clot contraction with decreased clot permeability and increased resistance to fibrinolysis, two parameters thought to impact thrombosis risk.[25,111] Tutwiler et al evaluated the kinetics of clot contraction in blood samples collected from patients with recent acute ischemic stroke, and correlated parameters with hemostatic and hematological laboratory characteristics.[69] Surprisingly, compared to clots from healthy individuals, whole blood clots from patients with recent ischemic stroke exhibit reduced clot contraction.[69] However, since samples were collected after symptom onset, these changes may reflect a consequence, rather than cause, of the thrombotic event. Ischemic stroke patients had quantitative and qualitative defects in circulating platelets (decreased platelet count, shape change and P-selectin exposure in unstimulated platelets, and decreased fibrinogen-binding capacity of activated platelets)[69], suggesting the ischemic event may consume platelets and/or induce a refractory phenotype in circulating platelets that are not incorporated into the thrombus. Prospective analysis of blood samples prior to stroke onset is necessary to determine if altered contraction promotes occlusive thrombus formation.

Abnormal fibrinogen and fibrin structure in thrombosis

Production of clots with abnormal structure and stability has been demonstrated in plasma samples from patients with increased CVD risk.[38–40] Following percutaneous coronary intervention, patients who develop in-stent thrombosis demonstrate abnormal plasma clot characteristics (e.g., permeability, turbidity, lysis time) compared to patients who did not develop in-stent thrombosis.[112] Similarly, compared to healthy controls, plasma clots from patients with abdominal aortic aneurysm have more densely-packed fibrin networks with smaller pores, and were more resistant to lysis.[113] Moreover, effects are aneurysm-size-dependent; patients with larger aneurysms have more densely-packed fibers compared to patients with smaller aneurysms.[113] In both in-stent thrombosis and abdominal aortic aneurysm patients, these effects on clot properties are independent of total fibrinogen levels, but may be related to effects of other plasma proteins on fibrin formation.[112,113] It remains unclear whether these fibrin clot abnormalities are only a biomarker for an operant

pathophysiologic mechanism, or whether abnormal fibrin clot structure is causative in the disease etiology.

Post-translational modifications of fibrinogen

Although genetic mutations in fibrinogen (congenital dysfibrinogenemia) have been associated with abnormal fibrin clot formation and bleeding and/or thrombosis (reviewed in[114]), acquired fibrinogen abnormalities are likely far more prevalent. The high concentration of fibrinogen in circulation makes it a frequent target of enzymes and activities that modify its structure and function. These post-translational modifications include nitration, homocysteinylation, and glycation, and are reviewed elsewhere.[115]

Importantly, fibrinogen modifications have been observed in plasma clots from individuals with increased cardiovascular risk. For example, cigarette smoking is associated with abnormal fibrinogen levels and is a major risk factor for CVD.[116] A multi-ethnic cohort of current, former, and non-smokers, observed that current chronic smokers with a longer number of pack-years have fibrinogen levels higher than either former smokers or non-smokers[117], suggesting smoke exposure causes an acquired, but reversible, hyperfibrinogenemia that increases cardiovascular risk. Interestingly, even acute exposure to cigarette smoke is associated with the production of plasma clots with thinner fibrin fibers and increased platelet aggregation[118] suggesting cigarette smoke exposure also induces immediate (post-translational) functional changes in fibrin formation and structure that promote thrombogenicity.[118] Although the specific mechanisms were not determined, the authors speculated that free radicals (reactive oxygen species) present in cigarette smoke modify fibrinogen in circulation. Consistent with that premise, other studies have specifically associated oxidative modification of fibrinogen with CVD and increased thrombotic risk.[119,120] Compared to age-, sex- and risk factor-matched controls, patients with post-acute myocardial infarction have elevated plasma markers of oxidative stress, including increased fibrinogen carbonylation. Moreover, fibrinogen isolated from these plasma samples demonstrates abnormal clotting characteristics, including the production of clots with thinner fibrin fibers.[120] Fibrinogen carbonyl content correlates negatively with fibrin clot turbidity (a proxy for fibrin network structure) and positively with extent of fibrin β-chain remaining during fibrinolysis (a proxy for resistance to fibrinolysis).

Cirrhosis is also associated with elevated thrombosis risk, and fibrinogen isolated from patients with cirrhosis demonstrates carbohydrate modifications and increased carbonyl content.[119,121] Compared to controls, both plasma clots and clots made from purified fibrinogen from cirrhotic patients demonstrate abnormal clotting characteristics, including decreased clot permeability and shorter clot lysis times.[119] Somewhat paradoxically, patients included in this study[119] reported bleeding, mostly variceal, rather than thrombosis. However, the more frequent association of the observed clot characteristics with thrombotic risk suggests abnormal clot structure ultimately contributes to thrombosis in these patients.

Patients with chronic kidney disease also have increased risk of thrombotic events. Fibrinogen purified from patients with chronic kidney disease on hemodialysis shows evidence of glycosylation and guanidinylation.[122] Compared to fibrin clots from healthy controls, clots made with guanidinylated fibrinogen have significantly thinner, or perhaps

more compact, fibrin fibers. Notably, formation of denser fibrin networks was independently associated with mortality risk in the hemodialysis patients.[122]

Collectively, these findings implicate fibrin(ogen) modification in thrombosis associated with multiple pathologies. Additional studies are needed to demonstrate direct pathologic contributions of each of these modifications to fibrin(ogen) function in vivo.

Fibrin(ogen) detection as a diagnostic tool

VTE diagnosis includes imaging technologies such as Doppler ultrasound or computed tomography to detect deep vein thrombosis or pulmonary embolism, respectively.[123,124] These technologies show vascular abnormalities and flow disturbances around the thrombus, but do not reveal information about thrombus composition. Development of technologies that can detect thrombus composition may have clinical utility. Notably, whereas early thrombi have substantial crosslinked fibrin content, this fibrin is replaced with collagen during thrombus resolution.[125] Since fibrin-rich thrombi are more susceptible to fibrinolysis than collagen-rich thrombi[126], distinguishing early, fibrin-rich thrombi from older, collagen-rich thrombi may aid in identifying thrombi that are susceptible to fibrin-degrading thrombolytic therapy. Currently, assessment of thrombus age is highly-subjective and only poorly able to identify patients who may respond to thrombolytic treatment.[126] However, two recent studies of thrombosis detection in rodents have advanced methods to detect intravascular thrombi and reveal information about thrombus fibrin content. Blasi et al demonstrated the ability of a fibrin-binding probe, ^{64}Cu-FBP8, to detect both venous and arterial thrombi in a single whole-body positron emission tomography (PET) scan.[127] Probe uptake correlated positively with fibrin content in both arterial and venous clots, distinguishing young (high probe uptake) from old (low probe uptake) thrombi.[127] This PET-based imaging method enables imaging of multiple thrombi in one examination and may be a noninvasive and sensitive approach to assess changes in thrombus composition over time. Similarly, the spatial and temporal uptake of a gadolinium-based fibrin-specific MRI contrast agent, EP-2104R, also correlates positively with time-dependent changes in thrombus fibrin content.[128] Furthermore, thrombi that exhibit high EP-2104R uptake are more susceptible to tissue plasminogen activator-mediated dissolution, suggesting EP-2104 can be used to identify thrombi that are susceptible to thrombolytic therapy.[128] Additional studies are warranted to determine if these methods can be used to identify human patients with greatest potential benefit of thrombolytic therapy.

Summary

This review has highlighted both established and newer findings on fibrin(ogen) expression and function that demonstrate its central role in clot formation during hemostasis and thrombosis. Additional studies beyond the scope of this review have exposed intriguing roles for fibrin(ogen) in inflammation, infection, neurologic disease, cancer, and other pathologies. Collectively, these discoveries have uncovered critical links between disease pathways and rationalize the significant association of many diseases with increased bleeding and/or thrombosis risk. Identification of these pathways may yield new therapeutic targets with enhanced specificity and safety. Consequently, efforts to advance both basic

research in fibrin(ogen) genetics, biology, biochemistry, and biophysics, as well as translational applications for fibrin(ogen) detection and altering fibrin(ogen) function are likely to have broad impact on health and disease.

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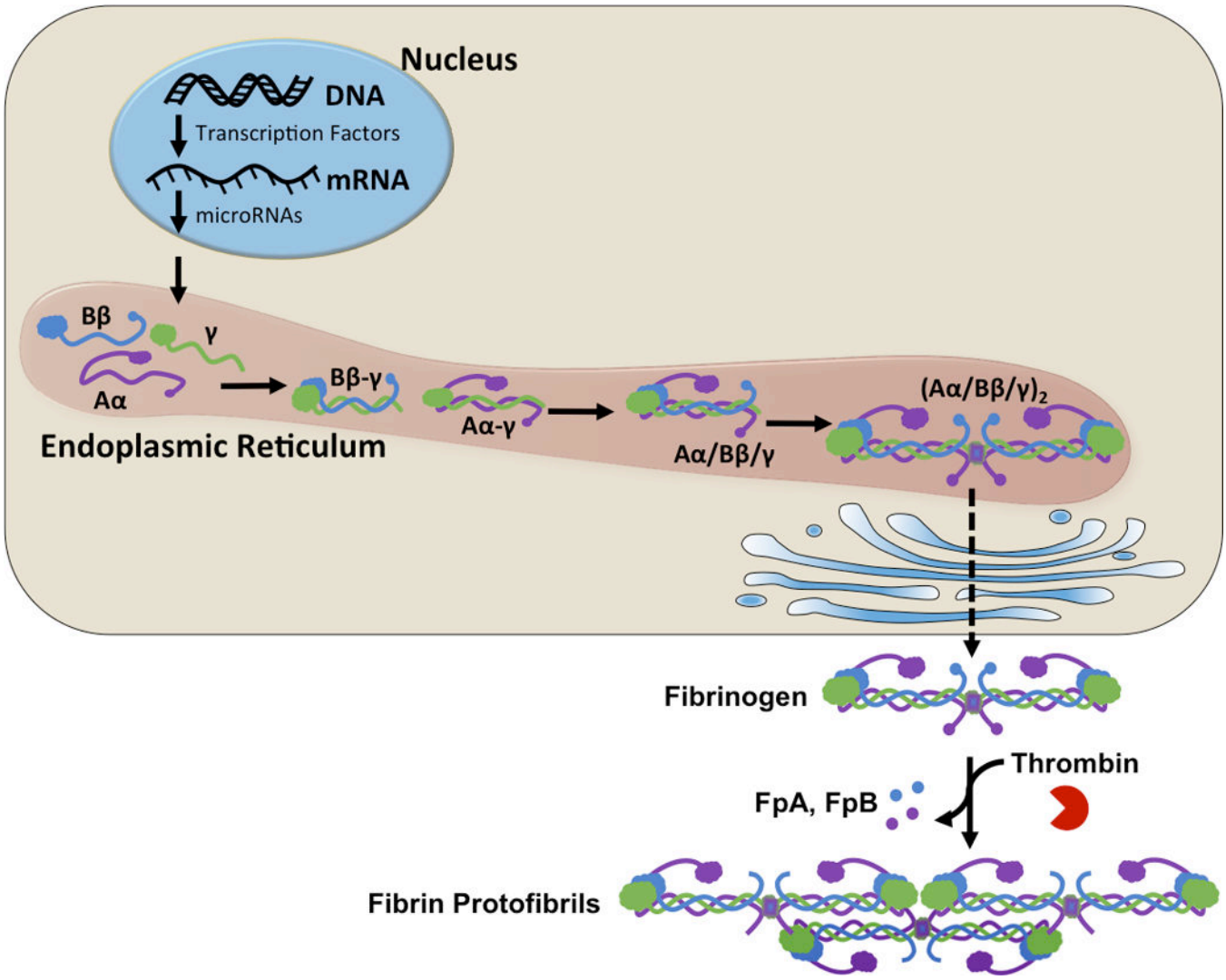

Figure 1. Fibrinogen synthesis and expression

Fibrinogen synthesis is regulated by both transcriptional and translational mechanisms. After individual fibrinogen chains are translated, fibrinogen assembly occurs step-wise. Single chains assemble first into Aα-γ and Bβ-γ precursors, then into Aα/Bβ/γ half-molecules, and finally into hexameric complexes $(Aα/Bβ/γ)_2$. Once fibrinogen is released into blood, it circulates until thrombin cleaves fibrinopeptides from the Aα and Bβ chains (FpA and FpB, respectively) to form fibrin monomers. These monomers then polymerize in a half-staggered arrangement to form fibrin protofibrils and ultimately, the fibrin network at a site of tissue injury.

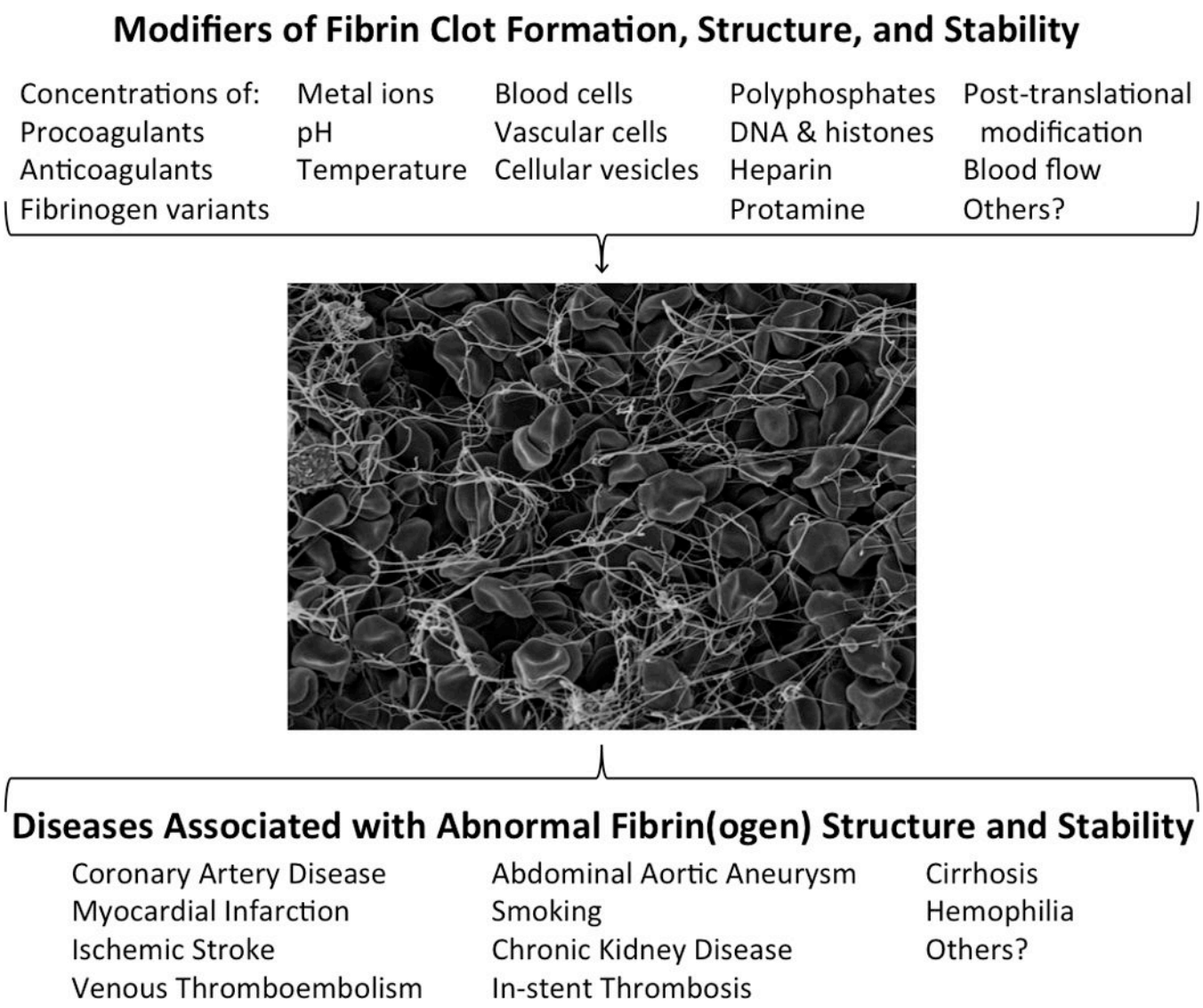

Figure 2. Modifiers of fibrin(ogen) and association with disease

Clot formation, structure, and stability are influenced by conditions present during fibrin generation. Abnormal clot formation is observed in a number of diseases.