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Fibrinolysis and the control of blood coagulation

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Abstract

Fibrin plays an essential role in hemostasis as both the primary product of the coagulation cascade and the ultimate substrate for fibrinolysis. Fibrinolysis efficiency is greatly influenced by clot structure, fibrinogen isoforms and polymorphisms, the rate of thrombin generation, the reactivity of thrombus-associated cells such as platelets, and the overall biochemical environment.

Regulation of the fibrinolytic system, like that of the coagulation cascade, is accomplished by a wide array of cofactors, receptors, and inhibitors. Fibrinolytic activity can be generated either on the surface of a fibrin-containing thrombus, or on cells that express profibrinolytic receptors. In a widening spectrum of clinical disorders, acquired and congenital defects in fibrinolysis contribute to disease morbidity, and new assays of global fibrinolysis now have potential predictive value in multiple clinical settings. Here, we summarize the basic elements of the fibrinolytic system, points of interaction with the coagulation pathway, and some recent clinical advances.

Keywords

Fibrinolysis; Thrombosis; Coagulation; Fibrin(ogen); Hemostasis

1. Introduction

Platelets are activated upon contact with subendothelial matrix proteins, including collagen, von Willebrand factor, and fibronectin, in response to vascular injury [1]. Platelet activation leads to exposure of cell surface anionic phospholipids, which serve as a nidus for the assembly of procoagulant proteins. In the ensuing activation of the coagulation cascade, a sequential series of serine protease-mediated cleavage events, thrombin is activated from its zymogen prothrombin [2]. Active thrombin can then catalyze the polymerization of fibrin by

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cleaving small peptides from two of its three subunits. Polymerization converts soluble fibrinogen into insoluble fibrin, which stems the flow of blood, thus achieving “hemostasis,” the prevention of major blood loss [3]. As the clot or “thrombus” forms, circulating red blood cells, white blood cells, and platelets become incorporated into its structure. In addition, fibrin becomes cross-linked through the action of factor XIIIa, which is also activated by thrombin, and provides further structural stability [4]. Upon healing of the injured blood vessel, the effete thrombus is lysed through the action of plasmin. Plasmin is generated from the zymogen plasminogen on the surface of the fibrin clot, or on cell surfaces, by either tissue plasminogen activator (tPA) or urokinase (uPA) [5]. Proteolysis of fibrin gives rise to soluble fibrin degradation products (FDPs), some of which have immunomodulatory and chemotactic functions. The coagulation and fibrinolytic systems are highly regulated and inter-related through mechanisms that insure balanced hemostasis.

2. Fibrin formation and clot structure

Fibrinogen, a soluble 340-kDa protein, circulates in whole blood at concentrations of 2–4 mg/mL [6]. It consists of two sets of three distinct disulfide-linked polypeptide chains ($\text{A}\alpha$, $\text{B}\beta$, and γ), whose synthetic programs are directed by three separate genes on chromosome 4. Thrombin’s major molecular target is fibrinogen, which is converted to fibrin monomers as thrombin removes N-terminal fibrinopeptides A and B. The resulting monomer is a disulfide-linked trinodular protein whose N- and C-termini converge at the E- and D-nodes, respectively.

Assembly of fibrin fibers then proceeds in a stepwise fashion. After an initial lag phase, release of fibrinopeptide A encourages protofibril formation by the lateral aggregation of fibrin fibers, wherein the E domain of one homodimer interacts with the D domain of a second to generate a half-staggered, overlapping fibrillar pattern within the developing thrombus [6]. Fibrin is cross-linked at lysine residues by factor XIIIa and forms fibrillar aggregates, which, together with platelets and red blood cells, provide structural integrity to the growing thrombus [7]. Turbidity and circulatory flow assist in fibrin polymerization and protofibril assembly by orienting the fibers as the growing thrombus forms [8–12].

Many factors, including local calcium concentration, pH, and platelet numbers, affect clot stability [6]. Stability is also based partly on fibrin fiber diameter, and the geometry of the fibrin network. Local thrombin concentration also impacts clot structure, as higher thrombin concentrations generate more stable clots [6,11,13]. Fragile clots are more susceptible to fibrinolysis and bleeding, whereas firm clots are more resistant, but may promote thrombosis [14–16]. For example, hemophilia patients have both spontaneous bleeding and poor clot formation resulting from impaired peak thrombin generation; the resulting thrombi are porous and more susceptible to fibrinolysis [17–19]. The variables that affect fiber architecture are ultimately important for fibrinolysis, since both fiber size and arrangement impact tissue plasminogen activator (tPA) binding and rates of fibrinolysis [20–23].

γ' -Fibrinogens are γ -chain splice variants that compromise approximately 8–15% of γ -fibrinogen ($\gamma\text{A}/\gamma'$), compared to the more common fibrinogen $\gamma\text{A}/\gamma\text{A}$. γ' -Fibrinogens result in the formation of thinner fibers with increased branching [24]. Epidemiologic data initially

indicated that γ' -fibrinogen isoforms were elevated in patients with arterial thrombosis, but more recently both human and murine models suggest a relative antithrombotic role for $\gamma A/\gamma'$, possibly as a result of thrombin sequestration [25,26]. Thrombus formation depends upon not only the total fibrinogen concentration, but also the isoform composition of the fibrinogen pool.

Clot structure, therefore, reflects the complex interplay of many factors ranging from polymorphisms in fibrinogen itself, to the efficiency of thrombin generation, the reactivity of associated cells, such as platelets, and the biochemical milieu. These components define fibrin clot architecture, which is a key determinant of the efficiency of clot lysis [27].

3. Regulation of fibrinolysis

Like the coagulation cascade, fibrinolysis is tightly controlled by a series of cofactors, inhibitors, and receptors [5]. Plasmin is the primary fibrinolysin, and is activated from plasminogen by either of two primary serine proteases, tPA and uPA. Whereas tPA is synthesized and released by endothelial cells, uPA is produced by monocytes, macrophages, and urinary epithelium. Both activators have exceedingly short half-lives in circulation (4–8 minutes) due to the presence of high concentrations of specific inhibitors, such as plasminogen activator inhibitor-1 (PAI-1). Compared to tPA, uPA has lower affinity for plasminogen, does not require fibrin as a cofactor, and, under normal conditions, appears to act mainly in extravascular locations. Both tPA and uPA are cleared by the liver after forming complexes with a low density lipoprotein (LDL)-receptor-like protein [28]. Because plasmin increases activator activity by converting single-chain tPA and uPA to their two-chain counterparts, plasminogen exerts positive feedback on its own activation [29–31].

Inhibitors are also important to prevent excess unregulated plasmin or plasminogen activator activity. Circulating plasmin and plasminogen activators are neutralized by serine protease inhibitors, or serpins, which are present in excess concentrations [32]. Serpins form covalent complexes with their unique target enzymes that are subsequently cleared from the circulation. The three serpins most important in fibrinolysis are plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and $\alpha 2$ -antiplasmin (A2AP). Plasmin and A2AP bind with 1:1 stoichiometry, whereupon both become inactive. When plasmin is bound to fibrin, however, it is protected from A2AP inhibition, allowing for fibrinolysis to proceed [33]. Similarly, the plasminogen activators tPA and uPA are rapidly inhibited by PAI-1, which is released into the circulation from endothelial cells, platelets, and other cells [34]. PAI-1 is upregulated by a large number of proinflammatory cytokines as well [5]. In pregnancy, PAI-2 is also a major tPA and uPA inhibitor, and its concentrations increase as the pregnancy progresses. Deficiencies in PAI-2 have been associated with adverse pregnancy outcomes [35,36]. Other non-serpin plasmin inhibitors include $\alpha 2$ -macroglobulin, C1-esterase inhibitor, and members of the contact pathway of the coagulation cascade, which also play minor roles in plasmin inhibition.

Thrombin activated fibrinolysis inhibitor (TAFI) is a non-serpin fibrinolysis inhibitor that is activated by thrombomodulin-associated thrombin. TAFI is a carboxypeptidase that removes C-terminal lysine and arginine residues on fibrin, thereby decreasing the number of available

plasminogen binding sites, slowing plasmin generation, and stabilizing clots. TAFI is found at reduced levels in hemophilia patients as a result of impaired thrombin burst, and leads to increased fibrinolysis [37,38].

3.1. Thrombus-based fibrinolysis

Fibrinolysis is a highly regulated enzymatic process that prevents unnecessary accumulation of intravascular fibrin and enables the removal of thrombi. Fibrin surfaces are key activation sites for fibrinolysis that modulate the binding of plasminogen and plasmin [29]. Fibrin-bound tPA, for example, shows an approximately 500-fold increase in catalytic efficiency of plasminogen activation compared to tPA in the fluid phase [30]. Similarly, plasmin is protected from inhibition by A2AP upon binding to fibrin, while initially fibrin-bound A2AP protects the clot from fibrinolysis [39–41]. Because both fibrin and fibrinogen increase conversion of plasminogen to plasmin, they facilitate their own destruction [42,43]. Fibrin clearance is also accelerated by providing new binding sites for plasminogen, as C-terminal lysine residues become exposed at an increasing rate during fibrinolysis.

3.2. Cell surface fibrinolysis

While plasmin, tPA, and uPA are all neutralized by soluble circulating inhibitors, the surfaces of endothelial cells and the fibrin thrombus offer a safe haven for preserving their fibrinolytic activity. Several cell surface molecules, including a variety of plasminogen receptors, the uPA receptor (uPAR), and the annexin A2 complex bind plasminogen and/or its activators on endothelial cells, monocytes, and many other cell types [29]. Some receptors, such as uPAR and the transmembrane plasminogen receptor (PlgR-KT) may modulate additional non-fibrinolytic functions, some as diverse as cell-matrix adhesion and catecholamine release [44–46].

Annexin A2, an important component of cell-based fibrinolysis, is a member of the annexin family of calcium-binding proteins that fulfill diverse physiologic functions [47–50]. On the surface of endothelial cells and monocytes, annexin A2 forms a heterotetrameric complex with another protein, S100A10 (also known as p11); the complex serves as a profibrinolytic receptor that binds plasminogen and tPA, but not uPA [51]. The (annexin A2₂-S100A10₂) complex strongly promotes the tPA-dependent activation of plasmin independently of fibrin [47,52,53].

Inhibition of the annexin A2 complex's function may increase thrombosis risk by impairing fibrinolysis. High-titer antibodies directed against annexin A2 have been observed with increased frequency in patients with antiphospholipid syndrome and a history of thrombosis, and also in a cohort of patients with cerebral venous thrombosis [54,55]. Polymorphisms in annexin A2 have also been associated with vascular occlusion in patients with sickle cell disease [56,57]. Conversely, abnormally high levels of annexin A2 are expressed by blast cells in acute promyelocytic leukemia (APL) and appear to contribute to increased fibrinolysis and bleeding (Fig. 1) [58,59]. On APL cells, annexin A2 probably increases fibrinolysis in concert with protein p11, which is also upregulated in an autonomous APL cell line [60].

4. Fibrin degradation products

Fibrin degradation products (FDPs) begin to form as plasminogen is activated and plasmin begins to degrade the thrombus. Multiple FDPs, including fibrinopeptide B and other fibrin degradation monomers and dimers are released [61–63]. When fibrin polymers are cleaved by plasmin at the D fragment site, the resulting D-dimer fragment reflects the degree of thrombosis and plasmin activity. D-dimer assays have found predictive and prognostic value in a number of disease states, including disseminated intravascular coagulation (DIC), pulmonary embolism, deep vein thrombosis, and cancer-associated thrombosis [64–67].

Individual FDPs may have immunomodulatory effects [68]. Fibrinopeptide B can serve as a chemoattractant for neutrophils, monocytes, and macrophages [69,70]. The B β 15-42 fragment of the N-terminal B chain has both cytokine-inducing and immunosuppressive attributes that preserve endothelial barriers and may protect organs from shock-related ischemia [71–73]. The anti-inflammatory properties of FDPs are an interesting prospect for future exploration and potentially therapeutic utility.

Some FDPs appear to have thromboregulatory properties in animal models. For example, synthetic peptides based on the degradation product fibrin B knob have been shown to impair fibrinolysis [74]. Addition of FDPs to arterial canine blood or plasma *ex vivo* prolongs clot formation as assayed by multiple coagulation tests [75,76]. The mechanism of the anticoagulant effect of FDPs remains unclear, but based on prior studies, it is unlikely that FDPs exert any target-specific feedback inhibition or amplification of thrombosis [77].

5. Points of intersection: Where coagulation meets fibrinolysis

5.1. Fibrinolysis and coagulation cofactor activity

In vitro evidence suggests that plasmin may inactivate factor Va by cleaving both its heavy and light chains. Similarly, it appears that plasmin can inactivate factor VIIIa, another procoagulant cofactor that is structurally related to factor Va [78,79]. These cleavage events occur at sites distinct from those targeted by activated protein C [80].

5.2. Fibrinolysis and platelet function

Platelet glycoproteins IIb/IIIa and Ib, the cell surface receptors for fibrinogen and von Willebrand factor, respectively, are also plasmin substrates [81,82], raising the question of whether plasmin serves to modulate the formation of the primary hemostatic plug. Indeed, in patients receiving tPA infusion for thrombolysis, bleeding times were found to be prolonged within 90 minutes [83]. On the other hand, platelets may initiate thrombotic reocclusion of blood vessels following successful thrombolytic therapy [84]. The role of platelet function as it relates to fibrinolysis is an area for future study.

5.3. Fibrinolysis and the thrombin-protein C-thrombomodulin system

Thrombomodulin (TM) is a transmembrane endothelial cell protein that has been extensively studied in relation to its role in conversion of protein C into its activated anticoagulant form. Unlike free thrombin, TM-bound thrombin is unable to cleave fibrinogen, activate platelets, modify factors V and VIII, or interact with protease-activated

receptors [85–87]. Instead, TM-bound thrombin acquires an anticoagulant role by two mechanisms; — first, by producing activated protein C, which can inactivate procoagulant cofactors Va and VIIIa, and, second, by activating TAFI which limits fibrin degradation as described above [88]. TM-bound thrombin can also catalyze the inactivation of pro-urokinase, thereby dampening fibrinolysis and tissue remodeling [89–91]. Thus, the anticoagulant, antifibrinolytic, and anti-inflammatory actions of TM-bound thrombin are complex and must be considered in the context of free thrombin's more widely recognized prothrombotic effects [92].

6. Lessons from hemophilia and inherited disorders of fibrinogen

There are numerous disease states that illustrate the importance of balanced fibrin formation and fibrin degradation. Although inherited bleeding disorders, such as hemophilia, reflect defects in the coagulation cascade upstream of fibrin formation, delayed bleeding develops as a result of abnormal fibrin structures yielding clots that are poorly adherent and easily dissolved. Impaired clot formation and structure can be restored by hemostatic treatments like recombinant factor VIIa [17,18].

Dysfibrinogenemias result from rare autosomal dominant mutations in any of the three fibrinogen chains. The majority are missense mutations or small deletions, and many do not have clinical manifestations. However, both bleeding disorders and thrombosis have been described in dysfibrinogenemias and are related to the structural change of the mutation [93,94]. For example, a congenital γ -chain molecular defect, or γ -dysfibrinogenemia like fibrinogen Dusard (Arg554Cys), results in the impaired binding of tPA to fibrin [95]. The result of this dysfibrinogenemia is reduced plasminogen activation, impaired fibrinolysis, and an increased tendency for thrombosis [96]. γ -Chain dysfibrinogenemias are also associated with abnormal fibrin assembly and fibrinolysis [95]. While these disorders are rare, they offer a unique insight into fibrinogen's role in hemostasis.

Afibrinogenemia is a rare bleeding disorder that results from a congenital absence of fibrinogen. Although umbilical bleeding and menorrhagia are often reported, thrombosis has also been described [97]. This paradox may be explained by a sequestration effect, whereby, under normal conditions, thrombin is sequestered by binding to fibrinogen. In patients with afibrinogenemia, there may be greater availability of active thrombin from a lack of fibrinogen binding, resulting in a prothrombotic tendency upon exposure to exogenous fibrin [26,98]. This theory may explain reports of afibrinogenemia patients who develop thrombosis when receiving fibrinogen concentrates [99–101].

7. Acquired fibrinolytic disorders

Acquired disorders of fibrinolysis and fibrinolytic components have been described in many disease states. These disorders can be subdivided into hyper- and hypofibrinolytic groups as described below.

7.1. Hyperfibrinolysis

Hyperfibrinolysis can result in bleeding, and may be based on dysregulation at either the cell surface or in the fluid phase. One of the most common hyperfibrinolytic states is disseminated intravascular coagulation (DIC), where systemic inflammation results in increased consumption of fibrin in microthrombi. This consumptive coagulopathy leads to a deficiency of circulating fibrinogen and increased bleeding tendencies [102]. Another hyperfibrinolytic state results from the loss of fibrinolytic inhibitors due to deficient synthesis. The decreased synthesis of A2AP, which can occur in chronic liver disease, results in hyperfibrinolytic bleeding [103]. The nephrotic syndrome is also associated with several fibrinolytic perturbations, including urinary loss of A2AP, which leads to bleeding, and increased thrombosis from loss of TAFI [104,105]. In metastatic prostate cancer, a hyperfibrinolytic state may reflect excessive local production of urokinase [106]. Heat stroke has a coagulopathic and fibrinolytic component, although the exact mechanism is unclear [107,108]. Coagulopathies of trauma are also associated with hyperfibrinolysis, which may reflect the combined effects of endothelial tPA release following extensive tissue injury and inhibition of PAI-1 release in association with shock [109]. Medical interventions, such as cardiopulmonary bypass, have been observed to cause hyperfibrinolysis; in this situation, blood contact with non-endothelial cell surfaces appears to initiate thrombin generation, which subsequently stimulates endothelial cell release of tPA, and activation of plasminogen [110].

7.2. Hypofibrinolysis

Hypofibrinolysis resulting in impaired clot dissolution is a recognized acquired cause of thrombosis in multiple disease states. The production of auto-antibodies directed against a plasminogen activator, such as tPA, or against a fibrinolytic receptor component, such as annexin A2, may result in major thrombosis, and has been observed in the antiphospholipid syndrome [55,111]. In addition, alcoholic liver disease has been associated with elevated levels of PAI-1 that may increase risk of thrombosis and promote liver inflammation in response to injury [112]. Hypothyroidism has been associated with hypofibrinolysis, where alterations in levels of A2AP, TAFI, tPA, PAI-1, and fibrinogen levels have been observed. Correction of the hypothyroid state resolves this coagulopathy [113]. Finally, in multiple myeloma hypofibrinolysis has been demonstrated in patients upon exposure to induction chemotherapy, and reverses after stem cell transplantation [114]. The mechanism for this observation remains unclear.

Acquired defects in fibrinolysis are pervasive in multiple chronic and acute medical conditions, and are poorly understood. Understanding alterations of the fibrinolytic system, especially in malignancy, may improve risk-based stratification and allow more effective tailoring thromboprophylaxis and transfusion therapies.

8. Congenital defects and variation in fibrinolysis

Numerous congenital defects in the fibrinolytic system have been described (Table 1A). Among congenital hypofibrinolytic disorders, livedoid vasculopathy is an occlusive vascular disorder affecting small blood vessels of the lower extremities that has been associated with

elevated levels of PAI-1 due to a promoter polymorphism (4G/4G) that increases its production [115]. Ulcerations that occur in this disorder lead to development of white atrophic scars (atrophie blanche) as a result of a marked defect in post-venous occlusion release of tPA [116]. Curiously, congenital deficiency of plasminogen is not associated with thrombosis, but causes liginous mucositis of the conjunctiva, oral surface, and other mucous membranes [117–119]. In patients affected by a congenital plasminogen deficiency, plasminogen replacement results in improvement in mucositis.

In congenital hyperfibrinolysis, bleeding is usually due to an inhibitor deficiency. Congenital A2AP deficiency can present as medullary bone hemorrhage or delayed umbilical bleeding in the neonate [120,121]. Similarly, PAI-1 deficiency typically results in mild to moderate bleeding, including epistaxis, menorrhagia, and delayed bleeding after surgery or trauma [117,122–124].

There are no reports of human congenital deficiencies in uPA, tPA, or TAFI, suggesting that null mutations in these genes may be lethal *in utero*. Murine knockout models have demonstrated increased intravascular deposition of fibrin in adult mice that are deficient in tPA or uPA [125]. Deficiencies of uPA may also promote cancer progression in experimental models of inflammatory colon cancer [126]. In murine models, deficiency of TAFI has been linked with increased fibrin deposition and inflammation leading to liver damage [127]. Other polymorphisms in TAFI and PAI-1 have been described that may contribute to thrombotic risk, but the strength of their association is unclear (Table 1B).

9. Measuring global fibrinolysis in clinical practice

Fibrinolysis is difficult to measure directly, and assays remain poorly predictive of thrombosis or bleeding. In addition, developing a reliable test of fibrinolysis for clinical use has been difficult, and this may have precluded the identification of some fibrinolytic disorders. The complex interplay of hemostatic and fibrinolytic proteins makes it difficult to predict the development of thrombosis or bleeding. Moreover, interference from other variables such as inflammatory mediators and hyperlipidemia has made creating a predictive fibrinolytic assay especially challenging. Part of this difficulty lies in the fact that plasma levels of individual fibrinolytic components, including tPA, PAI-1, and plasmin–antiplasmin complexes, are poor predictors of venous thrombosis [128–130]. Only TAFI levels have marginal predictive value, but not in all studies [131].

9.1. Clot lysis time

In an attempt to solve these issues, the clot lysis time (CLT) assay has been developed as a global test of plasma fibrinolysis. This assay is performed on plasma samples with the addition of calcium and phospholipid vesicles, and the resulting fibrin clot is then lysed by the addition of exogenous tPA. In patients with recurrent venous thrombosis, hypofibrinolysis measured by this test accurately predicted a two-fold increased risk of thrombosis, independent of age or sex [132]. The assay has also been used in larger numbers of patients, and has retained its predictive value for venous thrombosis in the MEGA study of over 1000 patients [133]. Hypofibrinolysis, as defined by CLT, has also demonstrated predictive value in Budd–Chiari syndrome and portal vein thrombosis [134]. In arterial

thrombosis, the CLT assay was useful in predicting myocardial infarction (MI) in men under the age of 50 [135]. Individual components of the fibrinolytic system, conversely, had inconsistent predictive value [135]. A2AP and TAFI levels were independently associated with MI, but other fibrinolytic components were not significant when adjusting for other cardiovascular risk factors [136,137].

Unfortunately, excessive fibrinolytic activity, as defined by the CLT assay, has not shown robust predictive power in bleeding disorders. For example, hyperfibrinolysis failed to correlate with bleeding scores in von Willebrand disease in a recent study [138]. Further studies using the CLT assay in a larger population with bleeding disorders may prove useful, especially around the time of surgery, or after treatment with antifibrinolytic agents.

A theoretical problem with CLT is the reliance on high doses of exogenous tPA and the absence of other whole blood components in the assay. A whole blood global fibrinolysis assay (Global Fibrinolysis Capacity; GFC) avoids this problem and is conducted in the presence of cellular elements like red blood cells and platelets. The GFC assay has been evaluated in patients with liver disease and predicts hyperfibrinolysis as accurately as CLT [139]. However, the GFC assay has not been used to measure clinical bleeding and thrombotic outcomes in large populations. This assay may be useful in future studies, but needs more extensive clinical validation.

9.2. Thromboelastometry

Another assay of global fibrinolysis is rotational thromboelastometry (ROTEM). Clot strength, amplitude, and maximal firmness are assessed by a modified ROTEM procedure referred to as fibrinolysis thromboelastometry (FibTEM), which has had some success in identifying patients with disseminated intravascular coagulation [140]. FibTEM can also quickly determine whether hypofibrinogenemia is present the peri-operative period after cardiac surgery, and results correlate with plasma fibrinogen concentrations [141]. Thromboelastometry has shown the most promise in directing blood product use in the peri-operative period, predicting massive transfusion requirements due to trauma-associated bleeding, and determining hypercoagulability in later trimesters of pregnancy [142–144]. Finally, hyperfibrinolysis, as assayed by FibTEM, was associated with increased mortality after major surgery [145]. At present, FibTEM is limited by the fact that it can be performed only at specialty centers by trained personnel, by its variable reproducibility, and by the requirement for fresh samples of blood.

10. Summary and future directions

The fibrinolytic system is as complicated and multifaceted as the coagulation cascade, and is equally relevant thrombotic disease and bleeding. Dysregulation of the fibrinolytic system is associated with diverse and unpredictable clinical phenotypes ranging from the coagulopathies of liver disease and DIC to rare congenital bleeding disorders. To date, global assays of fibrinolysis have shown promise in predicting risk of thrombosis but not bleeding, and more research in this area is needed. In addition, the potential role of the fibrinolytic system in cell signaling pathways, inflammation, and malignancy remains to be fully explored. The fibrinolytic system represents a true frontier in understanding the

complex interactions of biological systems, and, as we gain a better understanding of its role in biology, we will be able to improve the care of patients with diverse medical conditions.

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Practice points

- Disorders of fibrinolysis can be congenital or acquired in association with numerous medical conditions including malignancy, liver disease, and renal failure.
- Hypofibrinolysis is more often associated with thrombosis, while hyperfibrinolysis may result in a bleeding tendency.
- Defects in fibrinolytic components have been associated with non-hematologic manifestations such as ligneous mucositis.
- Global assays of fibrinolysis developed to predict thrombosis should be validated in larger populations, and further studied for use in bleeding disorders.

Research agenda

- Improved understanding of hematologic and non-hematologic pathways of the fibrinolytic system in human disease.
- Validation of fibrinolysis testing to predict bleeding and thrombosis.

Fig. 1.

Human acute promyelocytic leukemia (APL) cells overexpress annexin A2. Formalin-fixed APL cells cultured from a human bone marrow aspirate were stained with either rabbit polyclonal IgG directed against annexin A2 (A) or pre-immune IgG (B). Primary antibody binding was detected with fluorescein isothiocyanate-labeled goat anti-rabbit IgG (green). Nuclei were counterstained with propidium iodide (red). Original magnification 100 $\times$.

Table 1A

Some congenital disorders of fibrinolysis.

Congenital deficiency	Hemostatic phenotype in humans	Hemostatic phenotype in mice	References
Plasminogen	Homozygous: ligneous mucositis, pseudomembranes of gingiva, eye, ear, respirator tract infections, impaired wound healing	Runting, fibrin deposition, and ligneous mucositis	[119,156,157]
	Heterozygous: no symptoms	None	[119]
tPA	Not reported	Increased fibrin deposition, reduced clot lysis	[125]
uPA	Not reported	Mild fibrin deposition, increased susceptibility to colon cancer and decreased cartilage remodeling	[125,126,146]
A2AP	Homozygous: severe bleeding, especially umbilical; delayed bleeding after surgery/trauma; intramedullary hemorrhage into diaphysis of long bones	Enhances clot lysis, resistance to endotoxin-induced thrombosis	[120,121,158]
	Heterozygous: asymptomatic or minor bleeding	None	[120,121]
PAI-1	Homozygous: mild-moderate bleeding, menorrhagia, spontaneous miscarriage, impaired wound healing	Enhances clot lysis, resistance to endotoxin-induced thrombosis	[159]
	Heterozygous: asymptomatic	None	[159]
PAI-2	Not reported	None	[147]
TAFI	Not reported	Increased clot lysis	[160,161]
Annexin A2	Not reported	Increased fibrin deposition, reduced clot lysis	[148]

Table 1B

Some examples of human polymorphisms associated with thrombosis.

Polymorphism	Hemostatic phenotype	References
PAI-1 4G promoter	Increased venous thrombosis risk, slightly increased arterial thrombosis risk, no apparent increase in stroke risk	[149–151]
tPA enhancer 7531 C/T	Increased arterial thrombosis in select populations	[152]
TAFI Thr325Ile	Increased circulating TAFI, but no increase in thrombosis	[153–155]
TAFI Ala147Thr	Increased risk for coronary artery disease	[153]