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REVIEW ARTICLE

Regulation of coagulation by tissue factor pathway inhibitor: Implications for hemophilia therapy

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Abstract

Tissue factor pathway inhibitor (TFPI) is an alternatively spliced anticoagulant protein that primarily dampens the initiation phase of coagulation before thrombin is generated. As such, TFPI's actions are localized to cells expressing TF and to sites of injury, where it is an important regulator of bleeding in hemophilia. The major splice isoforms TFPIα and TFPIβ localize to different sites within and surrounding the vasculature. Both forms directly inhibit factor Xa (FXa) via their Kunitz 2 domain and inhibit TF-FVIIa via their Kunitz 1 domain in a tight complex primarily localized to cells. By forming complexes localized to distinct cellular microenvironments and engaging additional cell surface receptors, TFPI alters cellular trafficking and signaling pathways driven by coagulation proteases of the TF pathway. TFPIα, which circulates in complex with FV and protein S, also serves an inhibitor of FXa independent of the TF initiation complex and prevents the formation of an active prothrombinase. This regulation of thrombin generation in the context of vessel injury is effectively blocked by antibodies to Kunitz 2 domain of TFPI and exploited as a therapy to restore efficient hemostasis in hemophilia.

KEYWORDS
blood coagulation, hemophilia, hemostasis, signal transduction, tissue factor pathway inhibitor

1 | INTRODUCTION

Tissue factor pathway inhibitor (TFPI) is an alternatively spliced anticoagulant protein present in plasma,[1] platelets,[2,3] and extracellular matrix,[4] as well as on the surface of endothelial cells,[5,6] monocytes, and macrophages.[7] It is a multifunctional Kunitz-type serine protease inhibitor that acts at several steps of the blood coagulation cascade.[8] TFPI primarily inhibits initiation of coagulation, dampening procoagulant stimuli before thrombin is generated.[9–11] As such, it is an important regulator of bleeding in hemophilia,[12–14] and antibodies that block TFPI activity are under development for hemophilia treatment.[15–18] In phase 2 clinical studies, an anti-TFPI antibody prevented bleeding episodes in people with hemophilia A and B, with or without inhibitors.[17] As an inhibitor of blood coagulation, TFPI also alters cellular trafficking and signaling pathways driven by coagulation proteases of the TF pathway.[19,20] Here, we review the biochemistry and physiology underpinning the effects of TFPI in blood coagulation and cellular signaling pathways, and how these may be altered by anti-TFPI therapeutic strategies.

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2 | TFPI BIOCHEMISTRY

2.1 | Overview of TFPI isoforms and structures

mRNA encoding three isoforms of TFPI are produced in humans. The isoforms, TFPIα, TFPIβ, and TFPIδ, result from three alternative splicing events that produce distinctive C-terminal ends of the TFPIα and TFPIβ proteins. However, *in vivo* production of TFPIδ has not been demonstrated and its physiological relevance is uncertain.[21] This variation in the C-termini targets the individual isoforms to different locations within the vasculature and alters their anticoagulant activities.[8] The two major isoforms have identical N-termini consisting of an acidic stretch of amino acids followed by two Kunitz-type serine protease inhibitory domains (Figure 1). These two Kunitz domains (K1 and K2) inhibit the tissue factor-factor VIIa (TF-FVIIa) catalytic complex in a factor Xa (FXa)-dependent manner. The isoform structures diverge after the K2 domain (Figure 1). TFPIα has a third Kunitz domain (K3) that binds protein S (PS),[22] followed by a stretch of basic amino acids that bind glycosaminoglycans and other negatively charged polymers.[23,24] The basic C-terminus of TFPIα also binds to an acidic region of the FV B-domain allowing it to inhibit early forms of the FVa-FXa catalytic complex (prothrombinase) that assemble before thrombin is generated.[10] TFPIβ has a stretch of amino acids that encode a glycosylphosphatidylinositol (GPI) cell-surface attachment sequence.[25] TFPIβ rapidly inhibits TF-FVIIa procoagulant activity when both proteins are expressed on the same cell.[19]

2.2 | Localization of TFPI isoforms

The distinctive C-termini of TFPIα and TFPIβ localize them to different portions of the vasculature. TFPIα circulates in plasma as a soluble protein at ~0.4 nM (~12.5 ng/ml).[1,26] TFPI circulates in association with PS and FV.[27,28] Consistently, the plasma concentration of TFPIα correlates with the plasma concentrations of PS and FV.[26] TFPIα is also a heparin-releasable protein, and its plasma concentration rapidly

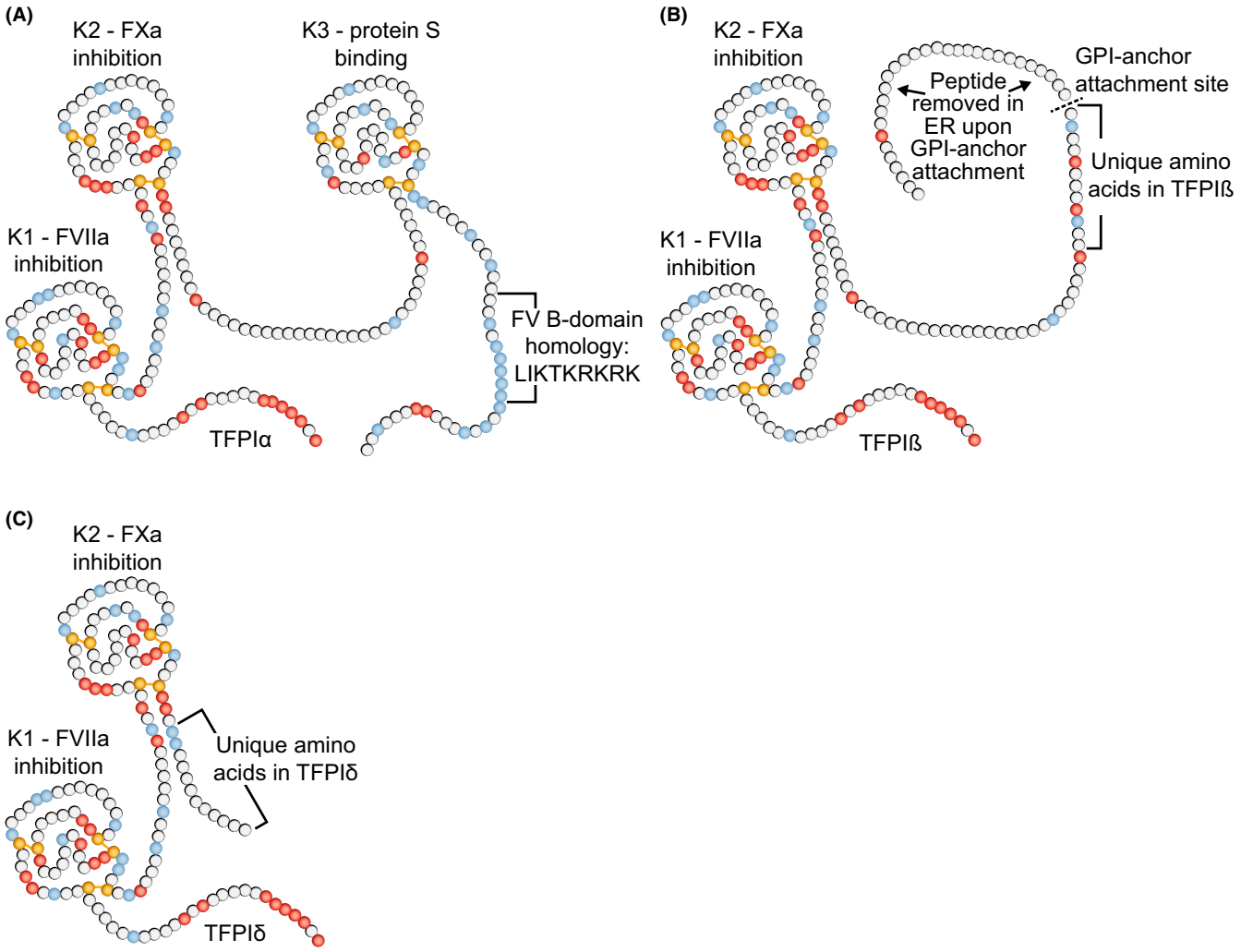

FIGURE 1 TFPI isoforms in humans. Yellow residues represent cysteine residues and disulfide bridges. Blue residues are basic. Red residues are acidic. a, active; F, factor; GPI, glycophosphatidylinositol; K, Kunitz domain; TFPI, tissue factor pathway inhibitor. Adapted from Maroney SA and Mast AE. *J Thromb Haemost*. 2015;13(S1):S200–S207[113]

increases two- to four-fold following heparin infusion.[19,29] It has been suggested that the heparin-releasable pool of TFPIα is bound to the endothelial surface via interactions between its basic C-terminal region and glycosaminoglycans within the endothelial glycocalyx. TFPIα also interacts with cell surface glycosaminoglycan receptors,[20] which can be either GPI-anchored (glypican 3)[30] or transmembrane domain-anchored (syndecan-3 and syndecan-4)[31,32] through its third Kunitz and carboxyl terminal domains.[33] However, TFPIα is not detectable on the surface of cultured endothelial cells or human kidney endothelium, and recent data indicate the extracellular matrix is the major source of heparin releasable TFPIα.[4] This localizes TFPIα within the extravascular space, where it is available to regulate the activity of endogenously expressed TF on cells exposed to blood following endothelial injury.

Although TFPIα is not present on the endothelial surface, it is present within granule-like structures distinct from Weibel-Palade bodies in cultured Ea.hy926 cells.[4,34] TFPIα is also present within platelets.[3] It is released from activated platelets and accumulates at the site of vascular injury, with a portion localizing to the platelet surface.[2,3] Interestingly, TFPIα is not present in platelet α-granules,[3] and, accordingly, the platelet TFPIα concentration does not correlate with platelet PS or FV, both of which are within α-granules.[26] TFPIα anticoagulant function is also regulated by polyphosphates that are released from activated platelets.[10,35]

TFPIβ is localized to the surface of endothelial cells and monocytes via a GPI anchor.[5,7,25] Expression of TFPIβ on the surface of cultured endothelial cells is not altered by heparin,[4,36] and TFPIβ does not contribute to the heparin-releasable pool of TFPI observed in vivo.[37] TFPIβ is not present in platelets[3] or within the extracellular matrix.[4]

3 | TFPI INHIBITS COAGULATION THROUGH DISTINCT BIOCHEMICAL MECHANISMS

TFPI inhibits coagulation proteases at several steps of the blood coagulation cascade (Figure 2).[8,9] The direct binding of K2 to the FXa active site provides the foundation for its anticoagulant activities,[38] the inhibition of TF-FVIIa performed by TFPIα and β,[11] and the inhibition of early forms of prothrombinase performed by only TFPIα.[10] These catalytic complexes assemble early in a procoagulant response, and their inhibition is unique to TFPI because they are relatively resistant to inhibition by other anticoagulant proteins, such as antithrombin[39–41] and activated protein C.[42,43] The inability to overcome TFPI inhibitory activity through generation of additional FXa via the FVIIIa-FIXa catalytic complex reduces thrombin generation and results in the bleeding experienced by hemophilia patients.[44] This provides the mechanistic basis for inhibition of TFPI as a treatment for hemophilia bleeding.

3.1 | TFPI inhibition of TF-FVIIa

TF-FVIIa initiates the blood coagulation cascade by activating FX of the common blood coagulation pathway[11] and FIX of the intrinsic blood coagulation pathway[45] (Figure 2). The inhibition of TF-FVIIa is mediated by direct binding of K1 to the FVIIa active site.[38] However, K1 is a poor inhibitor of TF-FVIIa and efficient inhibition requires FXa.[11] Thus, TFPI has been described as a two-stage inhibitor where K2 binds to FXa, and in a subsequent inhibitory step the TFPI-FXa complex inhibits TF-FVIIa. However, the rate-limiting step for inhibition of TF-FVIIa is the inhibition of FXa.[46] This indicates that instead of occurring in a two-step process, TFPI simultaneously inhibits the ternary TF-FVIIa-FXa complex in a single reaction occurring immediately after FX is activated by TF-FVIIa but before FXa dissociates from the ternary TF-FVIIa-FXa complex.[46] In this manner, TFPI blocks TF-FVIIa mediated generation of prothrombinase, FVa-FXa, in the common blood coagulation pathway. However, because TFPI does not inhibit activation of FIX by TF-FVIIa, TF-FVIIa-mediated initiation of blood coagulation can also proceed through the intrinsic pathway. Additionally, the TF-FVIIa-FXa ternary complex can directly activate FVIII in a process that is poorly inhibited by TFPI and occurs before the dissociation of the nascent product FXa.[47] This provides a mechanism for TF-FVIIa–mediated activation of both antihemophilic factors and production of the FVIIIa-FIXa intrinsic tenase complex independent of thrombin providing feedback activation of FVIII.[47]

Because TFPIα and TFPIβ have K1 and K2, they both inhibit TF-FVIIa through this FXa-dependent mechanism. The localization of TFPIβ to cell surfaces via its GPI anchor greatly enhances its TF-FVIIa inhibitory activity; soluble forms of TFPI that mimic TFPIβ and contain only K1 and K2 are much weaker inhibitors.[19] Similarly, the TFPIα K3 domain binds to PS,[22,48] which contains a Gla domain that localizes TFPIα to cell surfaces and enhances its ability to inhibit FXa.[49] Additionally, about two-thirds of plasma TFPIα is C-terminally degraded and associated with lipoproteins.[37] This pool of plasma-truncated TFPIα has reduced anticoagulant activity in TF-FVIIa-initiated plasma coagulation assays. However, it is a potent inhibitor of the propagation phase in thrombin generation assays.[50] At this point, the physiological importance of lipoprotein-associated plasma TFPIα remains uncertain.

3.2 | TFPIα inhibition of early forms of prothrombinase

Prothrombinase is the FVa-FXa catalytic complex that converts prothrombin to thrombin at the convergence of the intrinsic and extrinsic blood coagulation pathways. The discovery that TFPIα is the only TFPI isoform present in platelets[3] and recognition of amino acid homology between the TFPIα C-terminus and the FV B-domain[9] led to description of the inhibitory mechanism of early forms of prothrombinase (i.e., those that assemble before thrombin is generated[10] and that involves FXa dependent activation of FV).[51]

In this mechanism, the TFPIα basic region binds tightly to partially activated forms of FV that lack this basic region but still contain the acidic region of the B-domain, allowing interaction with TFPIα as well as protein S.[52] This allows prothrombinase

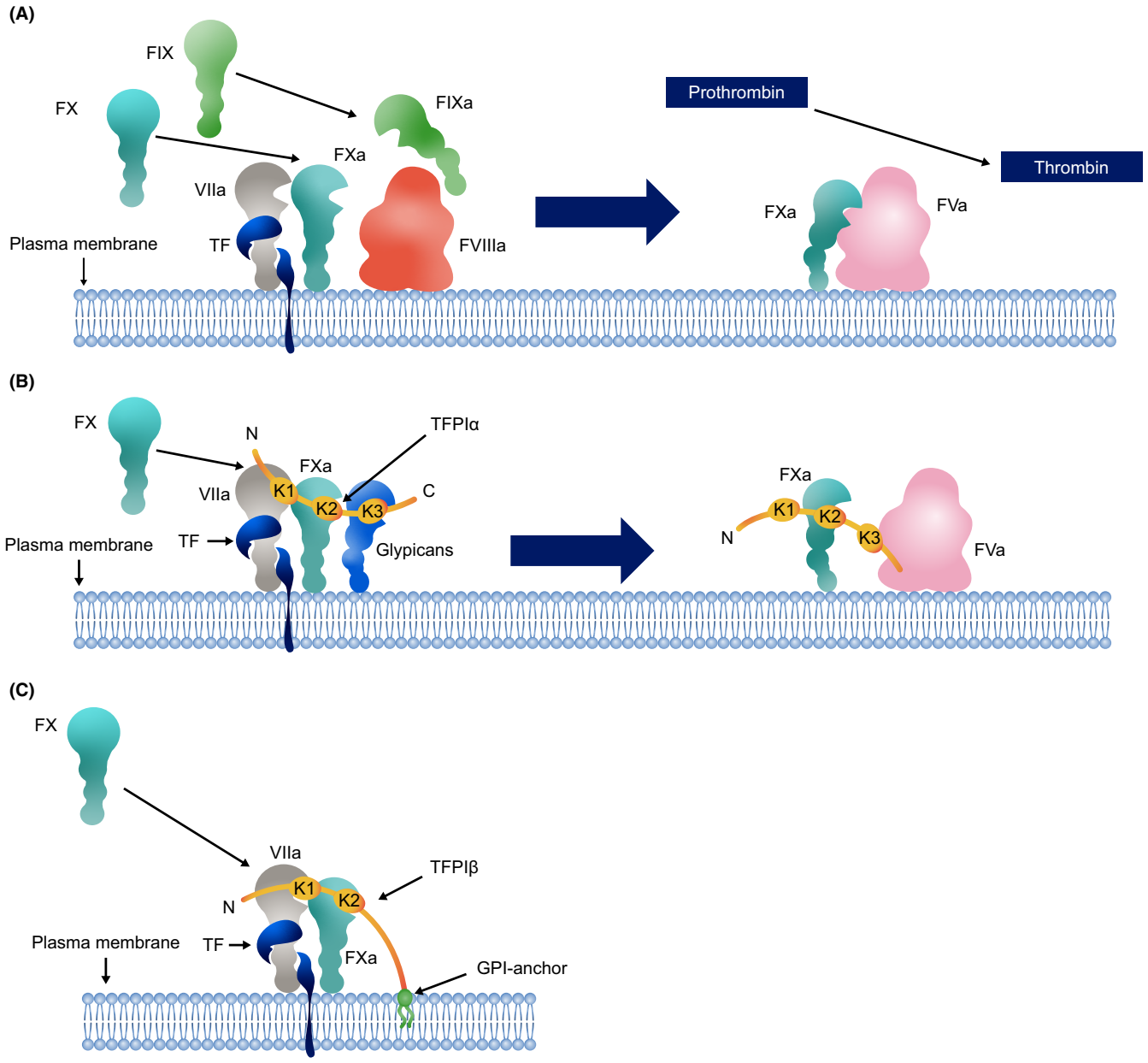

FIGURE 2 Presence of TFPI isoforms result in the inhibition of the TF-FVIIa-FXa ternary complex. (A) The TF-FVIIa complex activates FIX (part of the intrinsic coagulation pathway) and FX (part of the common coagulation pathway). This results in plasma membrane association of FXa and FVa, forming the prothrombinase complex, and subsequent cleavage of prothrombin to thrombin. (B) The K2 domain of TFPIα binds to FXa, which supports inhibition of the TF-FVIIa complex. The binding of the K3 domain to PS supports membrane association of TFPIα and thus further promotes inhibition of the TF-FVIIa complex. The basic C-terminus of TFPIα can also interact with FVa, resulting in inhibition of the early prothrombinase complex. (C) TFPIβ localizes to the cell surface via its GPI anchor, increasing its ability to inhibit FXa. a, active; C, carboxy; F, factor; GPI, glycophosphatidylinositol; K, Kunitz; N, amino; TF, tissue factor; TFPI, tissue factor pathway inhibitor

inhibition mediated through K2 binding of the FXa active site and a lower affinity interaction between conserved uncharged amino acids in the TFPIα C-terminus and the FV heavy chain in the region of FV arginine 506.[53] This later interaction is weakened in patients with the FV Leiden mutation, which decreases the threshold for initiation of coagulation and thereby increases thrombotic risk in these patients.[54,55] Thrombin rapidly cleaves and removes the entire FV B-domain[56] after which TFPIα can no longer inhibit prothrombinase. However, the binding of TFPIα to FV slows this thrombin cleavage,[57] further contributing to TFPIα anticoagulant activity. Three FV variants that lack the basic region of the B-domain have been identified: FV east Texas,[58,59] FV Amsterdam,[60] and FV Atlanta.[61] TFPIα binds tightly to these forms of FV, and consequently, patients have greatly increased plasma TFPIα concentrations and an associated bleeding disorder. Thus, it appears that TFPIα inhibition of prothrombinase is a physiologically important anticoagulant activity occurring at this early point in the coagulation cascade when nascent forms

of prothrombinase have assembled, but before thrombin is generated. Interestingly, the saliva of the black fly, *Simulium vittatum*, contains a single Kunitz-type inhibitory domain that binds the active site of FXa followed by a basic C-terminal region with homology to TFPIα and the FV B-domain.[62] This is a fascinating convergent evolutionary event whereby the fly prevents coagulation through the same mechanism used by TFPIα to inhibit early forms of prothrombinase.

3.3 | TFPI inhibition of thrombin generation in the cell-based model of coagulation

The cell-based model proposed by Hoffman and Monroe emphasizes that blood coagulation is regulated by the properties of the cell surfaces on which coagulation reactions occur.[63] In line with this model, the presence of TFPIα and TFPIβ on different cell surfaces allows inhibition at different stages of the procoagulant response[19] (Figure 2). TFPIβ inhibits the initiation of coagulation on endothelial cells that express intravascular TF following inflammatory stimuli or reactive oxidant stress,[64] while TFPIα in the extracellular matrix is positioned to dampen the initiation of coagulation by the TF-bearing cell that is exposed to circulating blood following vascular injury.[4] However, TFPI is a poor inhibitor of TF-mediated activation of FIX and FVIII,[46,47] which can diffuse to the surface of activated platelets accumulating at the injury site. The FVIIIa-FIXa catalytic complex on the platelet then activates FX.[65] The FXa then interacts with FVa released from platelets to form prothrombinase.[63] Because some forms of FVa released from platelets lack the basic region of B-domain while retaining the acidic region,[13,66] platelet or plasma TFPIα can block the procoagulant response by inhibition of early prothrombinase, thereby preventing the propagation of clotting by the subsequent burst of thrombin generation.[10] There is evidence that this process is enhanced by interactions between TFPIα and PS that enable localization of TFPIα to the platelet surface.[67] Thus, TFPI prevents inappropriate coagulation by acting early in a procoagulant response on both the TF-bearing cell and the platelet.

By exerting anticoagulant activity at these early stages of the procoagulant response, TFPI is the primary physiological regulator of bleeding in hemophilia,[14] and blocking TFPI activity is an effective approach for hemostatic prophylaxis in these patients.[16–18] Several therapeutics that block TFPI have been evaluated in clinical trials. An aptamer, BAX 499, which bound to K1, K3, and the TFPIα C-terminus, was withdrawn from development in 2012 because it paradoxically increased bleeding frequency in people with hemophilia.[68] This aptamer increased plasma TFPI levels up to 25-fold, possibly by causing release of endothelial stores of TFPI and/or by altering its clearance. Because the K2 domain was not blocked, the circulating aptamer-bound TFPI could potentially exert anticoagulant activity by inhibiting FXa. A monoclonal antibody, BAY1093448, that bound to K1 and K2 was terminated in clinical development in 2020 because of thrombotic events in three people with hemophilia. There are two monoclonal antibodies, concizumab and marstacimab, that bind to K2 and are currently in phase 3 clinical trials. Marstacimab was given to 58 people with hemophilia in phase 2 studies with no episodes of thrombosis.[69] Concizumab was given to 54 people with hemophilia in phase 2 studies with no episodes of thrombosis.[17] Phase 3 studies of concizumab were paused in March 2020 after three enrolled subjects with hemophilia had nonfatal thrombotic events. These patients also had preexisting risk factors and used concomitant hemostatic medication with FVIII or FVIIa.[70] Phase 3 studies resumed in August 2020 after safety issues were addressed and guidelines for the management of bleeding episodes with concomitant hemostatic agents and updates to the concizumab prophylactic dosing regimen were implemented.

Importantly, targeting TFPI does not alter the anticoagulant activity of activated protein C or antithrombin, which inhibits later steps of the coagulation cascade, and are still in place to limit thrombosis. Although inhibition of TFPI activity on both the TF-bearing cell and the platelet likely contributes to its efficacy in hemophilia prophylaxis, the cell-based model of coagulation suggests that diminished activation of FX on the platelet surface is a major contributor to bleeding in patients with hemophilia.[63] In support of this model, inhibition of platelet TFPIα alone is sufficient for restoring hemostasis in a mouse model of hemophilia,[71] suggesting that specifically targeting platelet TFPIα rather than endothelial TFPIβ may help to restore hemostasis while limiting the potential for thrombosis.

4 | ROLE OF TF IN CELLULAR SIGNALING

The TF coagulation pathway is directly connected to cell signaling by protease-activated receptors (PARs) located on the surface of endothelial cells and platelets within the vasculature, as well as on the surface of monocytes/macrophages, other immune and epithelial cells in the extravascular space. PARs are activated by TF-associated proteases FVIIa and FXa, as well as by thrombin. The TF-FVIIa catalytic complex can directly activate PAR2 in a reaction independent of FX, and prolonged endosomal signaling through this pathway is dependent on the association with integrin β1 through a binding site in the FVIIa protease domain (Figure 3).[72,73] Direct TF-FVIIa signaling through PAR2 is particularly relevant for extravascular processes, such as epithelial cell migration, wound healing responses, and tumor progression.[72,74–76] In addition, TF-FVIIa directly influences growth factor signaling and angiogenesis[77,78] and alters cell-cell interactions independently of PAR2 by cleaving ephrin receptors.[79]

In a distinct signaling pathway, TF-FVIIa generated nascent product FXa (TF-FVIIa-FXa) cleaves PAR2 in the presence of the endothelial protein C receptor (EPCR).[80,81] This signaling pathway plays a pivotal role in the innate immune system and is essential for the induction of interferon responses downstream of Toll-like receptor 4 signaling.[81,82] In this context, TF signaling is regulated at the level

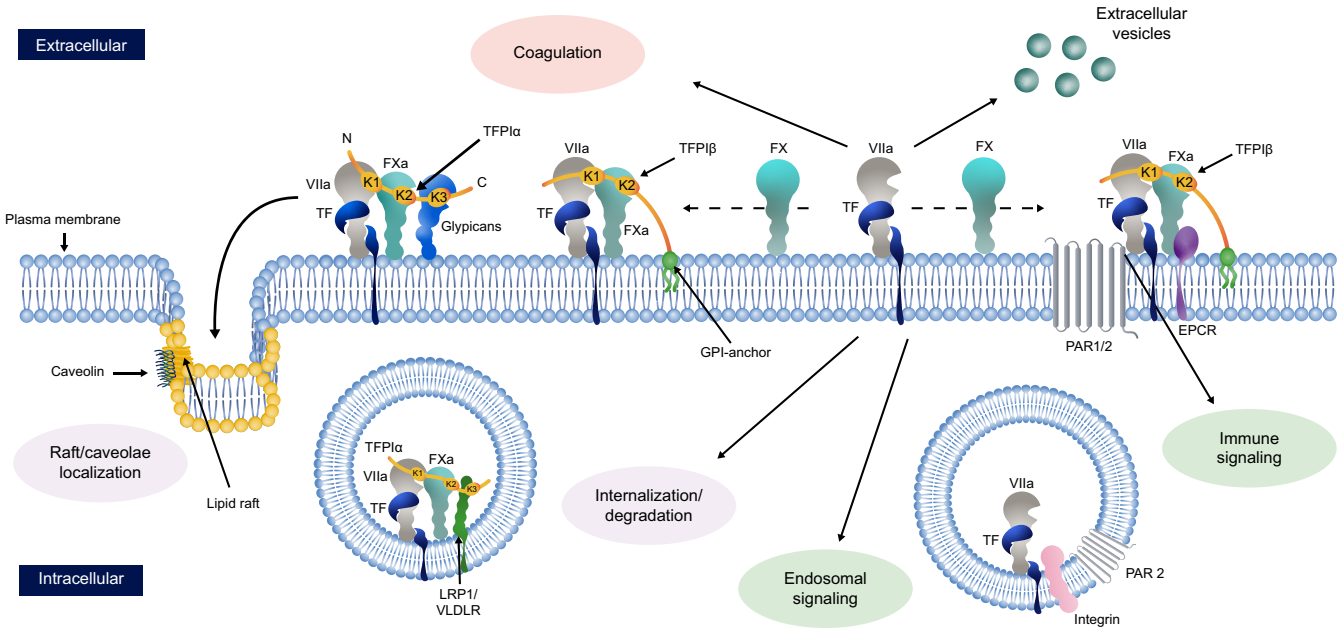

FIGURE 3 Signaling and interactions of TF and TFPI. TFPIα binds coreceptors that support its cell-surface functions. Binding to GPI-anchored glycosaminoglycan receptors (e.g., glypicans) via the K3 domain mediates membrane association. GPI-anchored TFPIα or TFPIβ can translocate to sphingolipid-rich raft domains and caveolae. Binding to receptors involved in internalization and degradation of FXa (e.g., lipoprotein receptor related protein 1 and VLDLR) can modulate cellular responses to various stimuli. TFPIβ is a GPI anchored via its C-terminal domain and appears to regulate cell-intrinsic functions of TF, such as immune signaling of FXa in complex with TF-FVIIa via protease activated receptor (PAR) 2 in the presence of the endothelial protein C receptor (EPCR) or signaling of the TF-FVIIa associated with integrin receptors via PAR2 after endosomal uptake. a, active; C, carboxy; F, factor; GPI, glycophosphatidylinositol; K, Kunitz; LRP1, low density lipoprotein receptor-related protein-1; TF, tissue factor; TFPI, tissue factor pathway inhibitor; VLDLR, very low-density lipoprotein receptor

of EPCR by the anticoagulant protein C pathway with FV and PS as cofactors.[83] This PAR2 signaling pathway requires only very low concentrations of FVIIa[80] and may therefore occur not only in intravascular but also extravascular milieus when TF-expressing cells are exposed to exudated plasma components. Moreover, monocytes and macrophages autonomously synthesize FVII and FX, and macrophage polarization in the tumor microenvironment is critically dependent on macrophage FX synthesis and activation of PAR2.[84] The synthesis of coagulation factors by immune cells is differentially regulated. Whereas FVII is constitutively expressed in tissue resident macrophages of the peritoneal cavity and lungs,[85,86] FX is transcriptionally induced in response to inflammatory stimuli.[84] The innate immune cell signaling roles of the TF-VIIa complex can therefore involve either cell autonomous, extravascular FX synthesis or FX from plasma sources.

TF-initiated coagulation also induces procoagulant signaling by thrombin activating PARs on endothelial and immune cells. Intravascular thrombin signaling is counterbalanced by multiple mechanisms, including the endothelial cell-localized anticoagulant protein C pathway as well as plasma anticoagulants, platelet receptors and fibrin(ogen).[87] There is also expanding evidence that thrombin generation occurs in extravascular locations and can control stem cell activity through PAR1 signaling at steady state and in response to injury.[88] Similarly, tissue macrophages synthesize FV and assemble a functional prothrombinase complex.[89] No clear roles have emerged for TFPI in controlling these thrombin-dependent vascular and extravascular signaling events.

5 | ROLE OF TFPI IN MODULATING TF-DEPENDENT CELLULAR SIGNALING

TFPI is a relatively weak inhibitor of TF-FVIIa.[11,90] However, its affinity for TF-FVIIa is greatly increased by binding to PS in reactions that lack FX but include FIX.[91] Similarly, TFPI associated with the matrix can support cell adhesion through interaction with TF-FVIIa expressed by tumor cells. Remarkably, this process studied in cell culture is entirely independent of FXa.[76] Thus, there are no compelling data that regulation of extravascular signaling functions of the TF-FVIIa complex are influenced by antibody blockade of TFPI binding to FXa.

TFPI is a potent inhibitor of the TF-FVIIa-FXa ternary complex and thereby modulates cellular signaling at vascular interfaces.[20] TFPI also decreases TF-initiated thrombin generation and may thereby dampen intravascular coagulation signaling mediated by thrombin in the context of sepsis. Indeed, leukocyte proteases released in this context are known to degrade TFPI and thus contribute to disseminated intravascular coagulation in severe infections.[92,93]

Pathological mechanisms that release TF from the tight inhibition by TFPI are not restricted to TFPI degradation in infections. TF-dependent cell signaling is also stimulated in the context of autoimmune disease, where antiphospholipid antibodies destabilize the TF-FVIIa-FXa complex inhibited by TFPI. Remarkably, blocking TFPI with polyclonal antibodies did not trigger but rather prevented

the same autoimmune signaling,[7] emphasizing that TF control on immune cells can be achieved by alternative mechanisms in the absence of functional TFPI (e.g., by TF cellular degradation or release on extracellular vesicles).[94]

The two major TFPI isoforms distinctly modulate cell signaling through their different mechanisms for cell-surface association (Figure 3). TFPIα binds GPI-anchored co-receptors on the cell surface, whereas TFPIβ is directly GPI-anchored to cellular microdomains.[3,4] The mode for cell-surface association determines the subcellular and membrane microdomain localization of TFPI isoforms, which, in turn, affects intracellular trafficking relevant for signaling and protein degradation. TFPIα also binds to transmembrane receptors mediating the cellular uptake and degradation of FXa (e.g., lipoprotein receptor related protein)[95] or regulating angiogenesis (i.e., very low density lipoprotein receptor [VLDLR]).[96] In this manner, soluble TFPIα from various sources may bind to target cells and modulate cellular responses. In contrast, cell-intrinsic functions of TF appear to be predominantly regulated by GPI-anchored TFPIβ and/or endogenously synthesized TFPIα tightly bound to the cell surface through GPI-anchored receptors.[20,25,36,97]

Monocytes express low levels of TF in the absence of inflammatory stimuli that can assemble into a TF-FVIIa-FXa complexes inhibited by membrane-anchored TFPI.[7] Genetic evidence indicates that EPCR is required for the formation of the quaternary TF-FVIIa-FXa-TFPI complex on the monocyte cell surface.[81] Antiphospholipid antibodies interact with EPCR and release the TF-FVIIa-FXa complex from TFPI inhibition, triggering TF and PAR1/2-dependent procoagulant responses and pathogenic signaling.[81] Thus, TFPI has a paradoxical prothrombotic role by priming responses in the autoimmune antiphospholipid syndrome.[7] However, genetic deletion of TFPI from monocytes has no apparent prothrombotic effect in other models of experimental thrombosis, indicating that TFPI has specific functions in disease pathologies that are unrelated to its physiological regulatory roles.

Endogenously synthesized, membrane-anchored TFPI efficiently regulates signaling of the TF-FVIIa-FXa complex in cytokine stimulated endothelial cells,[20] in line with data demonstrating highly efficient inactivation of cellular TF by TFPIβ.[25] In contrast, exogenously added TFPIα appears to be relatively ineffective in blocking cell signaling in comparison to suppressing TF-initiated coagulation.[20] Thus, TFPI isoforms have different potencies in regulating TF-dependent cell signaling. However, loss of TFPI in endothelial cells, similar to the observations in monocytes, does not necessarily result in increased thrombosis because deletion of TFPI in endothelial cells downregulates TF mRNA expression in the vessel wall.[98]

6 | IMPLICATIONS OF TFPI LOSS ON SIGNALING PATHWAYS, AND PATHOLOGICAL AND PHYSIOLOGICAL PROCESSES

Deletion of K1 in mice causes embryonic lethality,[99] implicating TFPI as a crucial regulator of TF activity during developmental processes. In addition, more detailed studies on mid-gestational lethality of TFPI-deficient mouse embryos demonstrate roles of TFPI in vascular development and angiogenesis. TFPI deletion causes vascular abnormalities specifically in the central nervous system and defects in the cerebrovascular development are prevented by genetic deletion of FV,[100] suggesting that TFPI is an important regulator of thrombin-dependent signaling events that modulate cerebrovascular development. These vascular development defects cannot be rescued by genetic overexpression of platelet TFPIα,[101] which is efficient in preventing death during early embryonic development. In addition, genetic reduction of TFPI expression leads to thrombotic perinatal lethality in mice carrying the prothrombotic FV_{Leiden} mutation,[102] further emphasizing the crucial role of TFPI as a regulator of the common coagulation pathway during pre- and postnatal development.

The extent to which TFPI regulates vascular processes by modulating TF pathway signaling is not fully understood. TF supports postnatal vascular development in part through PAR2 signaling, and TFPI-like inhibitors specifically suppress hypoxia-induced angiogenic sprouting[78] and tumor angiogenesis.[103] Locally applied high concentrations of TFPI also suppress tumor growth, whereas FXa inhibitors were not effective in this study.[103] It is conceivable that under pathological conditions with elevated TF levels, such as in cancer and pathological angiogenesis, extravascular TFPI is not produced at sufficiently high levels to control TF signaling and that these pharmacological inhibitory strategies restore functional control of increased TF expression and pathological signaling. In addition, TFPI interactions with endothelial cells modulate proangiogenic growth factor signaling[104] and interaction of TFPI with VLDLR induces endothelial cell apoptosis.[96] These regulatory roles of TFPI have been mapped to carboxyl terminal domains of TFPIα, which are not directly targeted by anti-hemophilic strategies to neutralize TFPI's inhibitory functions towards FXa.

TFPI degradation is observed in pathological processes. The connecting region between K1 and K2 is protease sensitive,[93] and therapeutic intervention with fibrinolytics leads to TFPI degradation with a shift toward a procoagulant state on monocytes.[105] Proteolytic inactivation of TFPI serves important functions in host defense against intravascular pathogens,[106] but may ultimately lead to disseminated intravascular coagulation in severe sepsis.[107] Whereas TFPI degradation plays a role in the host defense to infection and inflammatory processes, loss of TFPI function leads to pathologies and disease particularly in the context of concomitant activation or upregulation of TF.

TFPI is expressed by cancer cell lines *in vitro* and bound to transmembrane syndecan-3[32] or GPI-anchored glypican-3[108] receptors. Cancer cell-associated TFPI appears to play a minor role in regulating cancer cell TF clotting activity and rather supports leukemia dissemination by promoting chemokine-dependent cell motility in patient-derived cell lines.[108] In contrast, downregulation of TFPIβ enhances breast cancer cell line migration,[109] supporting the notion that TFPI isoforms regulate distinct aspects of cancer biology. Consistently, deletion of presumably membrane

anchored TFPI in endothelial cells enhances experimental metastasis in mice.[98] Thus, the role of TFPI in these processes is diverse and does not point to uniform functions in disease pathologies. TFPI also plays critical regulatory roles within the vessel wall and TFPI deletion exacerbates atherosclerosis,[110] whereas overexpression in smooth muscle cells prevents atherosclerosis and injury-induced hyperplasia in mouse models.[111,112] There is no evidence that the intravascular neutralization of TFPI for improved hemostasis in hemophilia influences these regulatory functions of vessel wall expressed TFPI in vascular inflammation.

7 | CONCLUSIONS

Structural differences in the C-terminal regions of TFPIα and TFPIβ splice isoforms affect how they localize to cells or circulate in blood and regulate the coagulation cascade. The C-terminal region of TFPIα binds tightly to nascent forms of FVa, allowing for inhibition of prothrombinase, in a reaction that also involves binding of K2 to the FXa active site. The C-terminal region of TFPIβ encodes a GPI-anchor attachment sequence localizing it to the cell surface where it is a highly effective inhibitor of TF-FVIIa complexes that assemble on the same cells. Because TF-FVIIa can be inhibited by K1 of TFPIα when bound to PS in the absence of FXa, therapeutic strategies directed to K2 may avoid, in part, the crucial function of TFPI in inhibiting TF-dependent coagulation reactions and cell signaling.

Directly or indirectly membrane anchored forms of TFPI regulate activity of TF-FVIIa and the TF-FVIIa-FXa coagulation initiation complex and their functions in cell signaling. TF-FVIIa regulates cellular functions occurring outside the vasculature, such as angiogenesis, tumor biology, and inflammation. In these extravascular processes, TFPI is particularly important for control of the TF-FVIIa-FXa coagulation initiation complex. The TFPI-TF-FVIIa-FXa complex has remarkable stability and forms rapidly to control excessive signaling of TF; however, other mechanisms may regulate TF on immune or vascular cells, and anti-TFPI antibodies do not act within the extravascular space.

Within the intravascular space, TFPI exerts anticoagulant activity early in the coagulation cascade, before thrombin is generated, and at steps that do not require FVIII or FIX. Inhibition of TFPI allows amplification of coagulation through pathways that bypass FVIII and FIX. Therefore, TFPI is an attractive target for management of hemophilia-related bleeding. Currently, several anti-TFPI antibodies that target the intravascular activity of TFPI are in clinical trials for hemophilia prophylaxis. They can be dosed subcutaneously and effectively prevent bleeding episodes in patients with hemophilia A and B, with or without inhibitors.

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CONFLICTS OF INTEREST

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Alan E. Mast and Wolfram Ruf wrote the manuscript and provided the outline of the manuscript figures.

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