Published in final edited form as:

Semin Hematol. 2008 April ; 45(2 Suppl 1): S21–S24.

Factor VIIa Interaction with Tissue Factor and Endothelial Cell Protein C Receptor on Cell Surfaces

Usha R. Pendurthi, PhD¹ and L. Vijaya Mohan Rao, PhD²

¹Associate Professor of Molecular Biology, The University of Texas Health Center at Tyler, Tyler, TX

²Professor of Biochemistry, Biomedical Research, The University of Texas Health Center at Tyler, Tyler, TX

Abstract

Factor VIIa (FVIIa) is the enzyme that triggers activation of the clotting cascade that eventually leads to fibrin deposition and platelet activation. Association of FVIIa with its cellular receptor, tissue factor (TF), which greatly increases FVIIa enzymatic activity, is essential for the effective initiation of the coagulation pathway. FVIIa also complexes with endothelial cell protein C receptor (EPCR), but this association does not increase the enzymatic activity of FVIIa. This article reviews current knowledge of FVIIa interaction with TF and EPCR on cell surfaces with a specific focus on how these interactions may contribute to FVIIa and TF clearance, thereby regulating TF–FVIIa activity.

Introduction

Although much progress has been made in understanding the molecular structure and functions of factor VIIa (FVIIa) and tissue factor (TF), our understanding of the cellular interactions of FVIIa with TF and other receptors, and their role in clearance mechanisms, is limited. Recent observation of FVIIa interaction with endothelial cell protein C receptor (EPCR), a known receptor for protein C/activated protein C (APC), raises an important question concerning the functional significance and consequences of this interaction. Greater understanding of the cellular interactions between FVIIa, TF, and EPCR is likely to provide considerable clinical benefit, particularly regarding the use of recombinant FVIIa (rFVIIa; NovoSeven®, Novo Nordisk, Bagsværd, Denmark) in treating bleeding episodes not only in patients with congenital and acquired hemophilia with antibodies to FVIII or FIX, but also in other patient groups.

This review focuses on FVIIa interaction with TF and EPCR on cell surfaces, with a specific focus on how these interactions may contribute to FVIIa clearance and regulation of TF expression at the cell surface. It is important to point out here that our current understanding of the consequences of cellular interactions (in relation to FVIIa and TF clearance) associated with FVIIa, TF, and EPCR are limited to cell model systems. The review focuses primarily on studies performed in the authors' laboratories. Further studies are needed to determine whether FVIIa interaction with TF or EPCR on cell surfaces plays an *in vivo* role in regulating their levels.

Address correspondence to: Usha R. Pendurthi, PhD, Biomedical Research, The University of Texas Health Center at Tyler, 11937 US Highway 271, Tyler, TX 75708, USA, Tel.: 903-877-7342, Fax: 903-877-7426, E-mail: usha.pendurthi@uthct.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FVIIa Association with TF-Expressing Cells

TF is the essential co-factor in FVIIa-mediated initiation of blood coagulation (1). It is constitutively expressed in fibroblasts of the adventitia and variably present in the outer layer of medial smooth muscle cells, but absent from blood cells and the endothelial cells that line blood vessel walls (2,3). However, TF expression is induced in vivo in monocytes and endothelial cells under various pathological conditions (4–8). Thus, vessel wall injury that disrupts the endothelial cell barrier, or pathological expression of TF in monocytes and endothelial cells, will result in the circulating blood coming into contact with TF on cell surfaces, allowing TF–FVIIa complex formation and subsequent activation of the coagulation pathway.

Studies using cell lines that constitutively express TF and radiolabeled FVIIa revealed that FVIIa binds to these cells in a time- and temperature-dependent manner, with binding reaching saturation at about 1 h (9–11). However, TF–FVIIa activity on cell surfaces reached the maximum level long before the saturation of TF-specific binding of FVIIa (11). While binding of FVIIa at lower concentrations correlated well with the generation of factor Xa (FXa), the relationship between FVIIa binding and FXa generation at higher FVIIa concentrations is nonstoichiometric (9). This suggests that TF exists in two forms: cryptic and functional. FVIIa can bind to both forms of TF, but at different rates. A recent review discusses in detail TF encryption and the processes that convert cryptic TF to functional TF (12).

Binding of FVIIa to TF on cell surfaces not only triggers the coagulation cascade, but also induces intracellular signaling; however, the latter process depends on the availability of specific protease-activated receptors (PAR) on the cell surface, and on other cell-associated proteins near TF–FVIIa complexes on the cell membrane (13). Thus, not all cells that express TF are capable of transmitting cell signaling in response to FVIIa binding.

FVIIa interaction with TF on cell surfaces may also lead to regulation of FVIIa and TF levels through receptor-mediated endocytosis. FVIIa bound to TF is internalized in many cell types (e.g. fibroblasts, monocytes, and baby hamster kidney [BHK] cells) (14–16). The majority of internalized FVIIa enters the endocytotic pathway and gets degraded but a small portion of it is recycled back to the cell surface as intact protein (14). Although both FVIIa and active site-inhibited FVIIa (ASIS) bind to TF, there seems to be subtle differences between them in their internalization and recycling patterns (17). Interestingly, despite the continuous internalization of FVIIa via TF, TF levels on the cell surface remain relatively constant (14–16). It may be important to note that the rate of TF-mediated FVIIa endocytosis is not as rapid as one would expect for classical receptor-mediated endocytosis. However, formation of the ternary complex with tissue factor pathway inhibitor (TFPI)-FXa significantly increases the rate of FVIIa internalization in fibroblasts (14) and down-regulates TF levels at the cell surface in both fibroblasts (14) and activated monocytes (15). Antibodies against low density lipoprotein receptor-related protein (LRP) or the receptor-associated protein, which block LRP-dependent internalization, reduce TFPI-mediated FVIIa internalization but not the FVIIa internalization observed in the absence of TFPI (14). Thus, it appears that FVIIa bound to TF may undergo endocytosis via two distinct pathways. First, FVIIa complexed with TF in the absence of its inhibitor, TFPI, internalizes via an LRP-independent and probably a non-coated pit pathway; second, FVIIa in a quaternary complex internalizes via the LRP-dependent, coated pit pathway (18). However, these processes may operate in a cell-specific fashion, since TFPI-FXa was shown not to influence FVIIa internalization in BHK cells transfected with TF (16).

Although the early conception was that TF antigen and its activity were entirely localized at the cell surface (19–21), recent studies found that a substantial amount of TF is localized intracellularly in several cell types (16,22–25). In fibroblasts, approximately 75% of the total

TF antigen is stored intracellularly, primarily in the Golgi compartment (26). In the absence of ligand, there was no appreciable recycling of TF between the cell surface and the intracellular pools (26). Interestingly, FVIIa binding to TF at the cell surface mobilizes the TF from the Golgi compartment (Figure 1). At least, some of the TF mobilized from the Golgi compartment in response to FVIIa binding is transported to the outer cell surface plasma membrane (26). This could explain why TF levels are not depleted from the cell surface despite the endocytosis of cell surface TF upon binding to FVIIa. It is important to note that not only the binding of FVIIa to the cell surface TF, but also the catalytic activity of FVIIa, is essential for the mobilization of TF from the Golgi compartment, as evidenced by the failure of ASIS to deplete TF from the Golgi compartment (26). Since FVIIa bound to cell surface TF can proteolytically activate PAR-mediated cell signaling, and since FVIIa protease activity is required for TF mobilization from the Golgi compartment, it is likely that FVIIa-induced PAR-mediated cell signaling is responsible for TF cellular transport. Consistent with this hypothesis, blocking PAR2 with PAR2-specific antibodies or PAR2-specific siRNA completely attenuates FVIIa-induced TF mobilization (27). In the absence of FVIIa, activation of PAR1 or PAR2 by specific proteases such as thrombin and trypsin, respectively, can also mobilize TF from the Golgi compartment.

Exocytosis of TF in response to pathophysiological stimuli might play an important role in hemostasis and thrombosis. Based on the studies described above, we hypothesize that FVIIa binding to TF, or traces of thrombin formed at an injury site, increases TF levels at the cell surface where and when it is needed. The ability of thrombin and other proteases to regulate externalization of TF via PAR-mediated cell signaling, independent of FVIIa, may play an important role in cells that express little or no TF at the cell surface but which might have TF in intracellular storage compartments. In this context, it may be pertinent to note that recent studies suggest that unstimulated monocytes (24) and endothelial cells (28) contain low levels of TF antigen in intracellular compartments. In addition to PAR-mediated cell signaling, activation of other signaling pathways may also modulate TF exocytosis. At present, available data on TF exocytosis is limited to fibroblasts and it is unknown whether such a mechanism operates in other cell types.

FVIIa Association with EPCR on Endothelium

Until recently, TF was the only known receptor for FVIIa. Its expression under normal conditions is strictly limited to extra-vascular cells (2,3) and thus it is unlikely that FVIIa would come into contact with TF-expressing cells. The endothelium, which constitutes the interface between the circulating blood and the vascular tissue, is optimally positioned to interact with circulating clotting factors. In their quiescent state, endothelial cells provide a non-thrombogenic surface and express receptors, such as EPCR, which participate in the anticoagulant mechanism; they are, however, devoid of procoagulant receptors, such as TF. Early studies on FVIIa binding to various cell types showed that only nonvascular cells that express TF possess receptors for FVIIa, while cells derived from vascular tissue, such as aortic endothelial cells, do not (29). However, Reuning et al (30) found that FVIIa binds to non-stimulated human umbilical vein endothelial cells (HUVEC) in a time- and dose-dependent manner. These studies suggested that the FVIIa binding site on HUVEC might be a common binding site for vitamin K-dependent proteins, since prothrombin and other vitamin K-dependent proteins reduce FVIIa binding to endothelial cells.

Our recent studies revealed that FVIIa binds specifically to non-stimulated HUVEC, and that EPCR—a known receptor for protein C/APC—provides the binding site for FVII/FVIIa (31). FVIIa interacts with EPCR on endothelial cell surfaces with a similar affinity to that of protein C/APC (32). This suggests that EPCR acts as a true receptor for FVIIa. However, in comparison with FVIIa affinity for cell-associated TF (K_d of approximately 100 pM–3 nM)

(10,11,33), the affinity of FVIIa for EPCR on endothelium is relatively weak (K_d of approximately 40–50 nM). Nonetheless, as protein C also binds to EPCR with a similar affinity, even at plasma concentrations of 70 nM (34), FVIIa will still be able to bind to EPCR. The amount of each ligand associated with the EPCR would be approximately proportional to their respective concentrations. Thus, at normal plasma concentrations, about 15% of EPCR will be occupied by FVII and the rest by protein C. However, this would ultimately depend on many other factors, such as the on- and off- rates of the two ligands and how their respective plasma inhibitors modulate their interaction with the EPCR.

Analysis of the interaction between protein C/APC recombinant variants and soluble EPCR reveal that the Leu-8 residue in the ω -loop of the Gla domain of protein C/APC plays a critical role in EPCR recognition (35). The complete conservation of this ω -loop in FVII/FVIIa is thought to be responsible for the binding of FVII/FVIIa to EPCR. This could also explain a comparable affinity of FVII/FVIIa and protein C/APC for EPCR (35).

Unlike FVIIa association with TF, FVIIa association with EPCR may not result in allosteric conformational changes in FVIIa that would otherwise markedly enhance the catalytic activity of FVIIa. EPCR–FVIIa complexes neither activate FX (31) nor induce cell signaling through activation of PAR1 or PAR2 (31,36). FVIIa binding to EPCR has no influence on the formation of TF–FVIIa complexes on activated endothelial cells (31). However, Lopez-Sagasetta et al (37) recently reported that soluble EPCR dose-dependently inhibited TF–FVIIa activation of FX. They also showed that blocking the EPCR binding site on the endothelial cell surface increased the generation of FXa. Further investigation is needed on this subject, as these results conflict with our own published (31) and unpublished data. At present, it is unknown whether FVIIa binding to EPCR either on non-stimulated or stimulated endothelium facilitates TFPI inhibition of FVIIa.

The physiological importance of FVII/FVIIa binding to EPCR is currently unclear. Since FVII circulates in plasma at a 7-fold lower concentration than protein C, it is unlikely that FVII acts as the major ligand for EPCR. However, in therapeutic conditions, FVIIa concentration in the circulation would be elevated to a level close to that of protein C, and thus may compete with protein C for EPCR binding. Even under these conditions, one would not expect a severe reduction in protein C binding to EPCR, since FVIIa would first bind to unoccupied EPCR sites before it could compete with protein C. Thus, it is conceivable that therapeutic concentrations of FVIIa may partially, but not completely, down-regulate the protein C pathway. This could provide an additional beneficial effect of rFVIIa treatment in achieving the cessation of hemorrhage. The hypothesis described above warrants further testing.

Even if FVII/FVIIa binding to EPCR has no consequences on the generation or modulation of FVIIa or protein C activities, FVIIa association with EPCR on the endothelium could still be relevant. More FVIIa is endocytosed in cells expressing EPCR than in cells that lack EPCR. Furthermore, blocking EPCR on endothelial cells significantly reduces FVIIa internalization in HUVEC (31), suggesting that FVIIa association with EPCR facilitates FVIIa endocytosis. If this is confirmed in in vivo experiments using animal model systems, then novel therapeutic options to prolong circulatory levels of FVIIa might arise.

Summary

Recent data from cell model systems suggest that interaction of FVIIa with TF and EPCR on cell surfaces plays a role in regulating the activities and levels of cell surface FVIIa, TF, and EPCR via receptor-mediated endocytosis. Further studies using appropriate animal model systems are necessary to evaluate the importance of receptor-mediated endocytosis in FVIIa clearance and in modulating the cellular expression of TF and EPCR. Greater understanding of the cellular activities and mechanisms associated with FVIIa, TF, and EPCR would be

helpful in refining the use of existing therapeutic agents—such as rFVIIa—in bleeding disorders, and would also expedite the development of novel and improved therapeutic agents for a variety of clinical indications.

References

1. Rapaport SI, Rao LV. The tissue factor pathway: how it has become a "prima ballerina". *Thromb Haemost* 1995;74:7–17. [PubMed: 8578528]
2. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 1989;134:1087–1097. [PubMed: 2719077]
3. Fleck RA, Rao LV, Rapaport SI, Varki N. Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thromb Res* 1990;59:421–437. [PubMed: 2237820]
4. Contrino J, Hair G, Kreutzer DL, Rickles FR. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nat Med* 1996;2:209–215. [PubMed: 8574967]
5. Drake TA, Cheng J, Chang A, Taylor FB Jr. Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal *Escherichia coli* sepsis. *Am J Pathol* 1993;142:1458–1470. [PubMed: 7684196]
6. Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. *Thromb Haemost* 1983;49:5–7. [PubMed: 6845273]
7. Leatham EW, Bath PM, Tooze JA, Camm AJ. Increased monocyte tissue factor expression in coronary disease. *Br Heart J* 1995;73:10–13. [PubMed: 7888247]
8. Osterud B. Tissue factor expression by monocytes: regulation and pathophysiological roles. *Blood Coagul Fibrinolysis* 1998;9:S9–S14. [PubMed: 9819023]
9. Ploplis VA, Edgington TS, Fair DS. Initiation of the extrinsic pathway of coagulation. Association of factor VIIa with a cell line expressing tissue factor. *J Biol Chem* 1987;262:9503–9508. [PubMed: 3496335]
10. Sakai T, Lund-Hansen T, Paborsky L, Pedersen AH, Kisiel W. Binding of human factors VII and VIIa to a human bladder carcinoma cell line (J82). Implications for the initiation of the extrinsic pathway of blood coagulation. *J Biol Chem* 1989;264:9980–9988. [PubMed: 2785997]
11. Le DT, Rapaport SI, Rao LV. Relations between factor VIIa binding and expression of factor VIIa/tissue factor catalytic activity on cell surfaces. *J Biol Chem* 1992;267:15447–15454. [PubMed: 1639786]
12. Bach RR. Tissue factor encryption. *Arterioscler Thromb Vasc Biol* 2006;26:456–461. [PubMed: 16397140]
13. Rao LV, Pendurthi UR. Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol* 2005;25:47–56. [PubMed: 15569823]
14. Iakhiaev A, Pendurthi UR, Voigt J, Ezban M, Vijaya Mohan Rao L. Catabolism of factor VIIa bound to tissue factor in fibroblasts in the presence and absence of tissue factor pathway inhibitor. *J Biol Chem* 1999;274:36995–37003. [PubMed: 10601255]
15. Hamik K, Setiadi H, Bu G, McEver RP, Morrissey JH. Down-regulation of monocyte tissue factor mediated by tissue factor pathway inhibitor and the low density lipoprotein receptor-related protein. *J Biol Chem* 1999;274:4962–4969. [PubMed: 9988740]
16. Hansen CB, Pyke C, Petersen LC, Rao LV. Tissue factor-mediated endocytosis, recycling, and degradation of factor VIIa by a clathrin-independent mechanism not requiring the cytoplasmic domain of tissue factor. *Blood* 2001;97:1712–1720. [PubMed: 11238112]
17. Iakhiaev A, Pendurthi UR, Rao LV. Active site blockade of factor VIIa alters its intracellular distribution. *J Biol Chem* 2001;276:45895–45901. [PubMed: 11590156]
18. Rao LV, Pendurthi UR. Regulation of tissue factor-factor VIIa expression on cell surfaces: a role for tissue factor-factor VIIa endocytosis. *Mol Cell Biochem* 2003;253:131–140. [PubMed: 14619963]

19. Drake TA, Ruf W, Morrissey JH, Edgington TS. Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. *J Cell Biol* 1989;109:389–395. [PubMed: 2663880]
20. Rao LV, Robinson T, Hoang AD. Factor VIIa/tissue factor-catalyzed activation of factors IX and X on a cell surface and in suspension: a kinetic study. *Thromb Haemost* 1992;67:654–659. [PubMed: 1509405]
21. Carson SD. Manifestation of cryptic fibroblast tissue factor occurs at detergent concentrations which dissolve the plasma membrane. *Blood Coagul Fibrinolysis* 1996;7:303–313. [PubMed: 8735137]
22. Schecter AD, Giesen PL, Taby O, Rosenfield CL, Rossikhina M, Fyfe BS, et al. Tissue factor expression in human arterial smooth muscle cells. TF is present in three cellular pools after growth factor stimulation. *J Clin Invest* 1997;100:2276–2285. [PubMed: 9410905]
23. Camera M, Giesen PLA, Fallon J, Aufiero BM, Taubman M, Tremoli E, et al. Cooperation between VEGF and TNF-alpha is necessary for exposure of active tissue factor on the surface of human endothelial cells. *Arterioscler Thromb Vasc Biol* 1999;19:531–537. [PubMed: 10073954]
24. Egorina EM, Sovershaev MA, Bjørkøy G, Gruber FX, Olsen JO, Parhami-Seren B, et al. Intracellular and surface distribution of monocyte tissue factor: application to intersubject variability. *Arterioscler Thromb Vasc Biol* 2005;25:1493–1498. [PubMed: 15860742]
25. Fortin JP, Rivard GE, Adam A, Marceau F. Studies on rabbit natural and recombinant tissue factors: intracellular retention and regulation of surface expression in cultured cells. *Am J Physiol Heart Circ Physiol* 2005;288:H2192–H2202. [PubMed: 15653755]
26. Mandal SK, Pendurthi UR, Rao LV. Cellular localization and trafficking of tissue factor. *Blood* 2006;107:4746–4753. [PubMed: 16493004]
27. Mandal SK, Pendurthi UR, Rao LV. Tissue factor trafficking in fibroblasts: involvement of protease-activated receptor-mediated cell signaling. *Blood* 2007;110:161–170. [PubMed: 17384202]
28. Atkinson BT, Kos CA, Furie BC, Furie B. Rapid activation of unstimulated endothelial cells containing tissue factor following laser-induced injury as monitored via calcium mobilization. *Blood* 2006;108:507a.(abstr)
29. Rodgers GM, Broze GJ Jr, Shuman MA. The number of receptors for factor VII correlates with the ability of cultured cells to initiate coagulation. *Blood* 1984;63:434–438. [PubMed: 6692043]
30. Reuning U, Preissner KT, Müller-Berghaus G. Two independent binding sites on monolayers of human endothelial cells are responsible for interaction with coagulation factor VII and factor VIIa. *Thromb Haemost* 1993;69:197–204. [PubMed: 8456434]
31. Ghosh S, Pendurthi UR, Steinoe A, Esmon CT, Rao LV. Endothelial cell protein C receptor acts as a cellular receptor for factor VIIa on endothelium. *J Biol Chem* 2007;282:11849–11857. [PubMed: 17327234]
32. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem* 1994;269:26486–26491. [PubMed: 7929370]
33. Broze GJ Jr. Binding of human factor VII and VIIa to monocytes. *J Clin Invest* 1982;70:526–535. [PubMed: 6980899]
34. Stenflo J. Structure and function of protein C. *Semin Thromb Hemost* 1984;10:109–121. [PubMed: 6429858]
35. Preston RJ, Ajzner E, Razzari C, Karageorgi S, Dua S, Dahlbäck B, et al. Multifunctional specificity of the protein C/activated protein C Gla domain. *J Biol Chem* 2006;281:28850–28857. [PubMed: 16867987]
36. Bae JS, Yang L, Manithody C, Rezaie AR. The ligand occupancy of endothelial protein C receptor switches the protease-activated receptor 1-dependent signaling specificity of thrombin from a permeability-enhancing to a barrier-protective response in endothelial cells. *Blood* 2007;110:3909–3916. [PubMed: 17823308]
37. López-Sagaseta J, Montes R, Puy C, Díez N, Fukudome K, Hermida J. Binding of factor VIIa to the endothelial cell protein C receptor reduces its coagulant activity. *J Thromb Haemost* 2007;5:1817–1824. [PubMed: 17723119]

Figure 1.

Mobilization of tissue factor (TF) from intracellular pools in response to FVIIa. Fibroblasts were exposed to a control vehicle (top panel) or factor VIIa (FVIIa; 10 nM) for 60 min. The cells were fixed, permeabilized and immunostained with rabbit polyclonal anti-human TF and monoclonal anti-human golgin-97 antibodies, followed by Rhodamine Red- and Oregon Green-conjugated secondary antibodies. Insets show a magnified view of TF localization in the Golgi compartment.