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# Splice variants of Tissue Factor and integrin-mediated signaling

Ramprasad Srinivasan, Vladimir Y. Bogdanov *

*Division of Hematology/Oncology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

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ABSTRACT

Full-length Tissue Factor (flTF) – the obligatory co-factor for the serine protease (factor) VII/VIIa – serves as the initiator of blood coagulation. The flTF/VIIa complex triggers a sequence of proteolytic events that lead to the formation of a hemostatic plug. Aside from hemostatic maintenance, flTF can contribute to thrombogenesis in some settings. The proteolytic properties of the flTF/VIIa complex (as well as the flTF/VIIa/Xa complex) account for non-hemostatic functions of flTF, largely exerted through activation of intracellular signaling via Protease Activated Receptors (PARs). The flTF-PAR nexus impacts several kinases highly significant in the pathobiology of cancer and cardiovascular disease. Over the past decade, many advances have been made in the understanding of PAR-mediated functions of flTF, an important highlight of which was the finding that a sub-set of integrins – a diverse family of integral membrane proteins – cross-regulate flTF-elicited signaling. Concomitantly, an alternatively spliced TF form (asTF) was discovered in human and mouse. Initial studies characterizing asTF revealed that it is differentially expressed during development, continuously present in circulating blood and solid tissues, and possesses very low pro-coagulant activity. Hypomorphic nature of asTF's cofactor activity is the source of an ongoing controversy over whether asTF is pro-coagulant, and how it may contribute to hemostatic maintenance and/or its aberrations. Very recently, a novel concept emerged in asTF biology: asTF can evidently trigger intracellular signaling that promotes the formation of new vessels from the existing ones (angiogenesis) and monocyte-endothelial interactions, via interaction with integrins. We provide a brief overview of the fl/asTF-integrin nexus with an emphasis on asTF's non-proteolytic, integrin-mediated biological activity.



## Tissue Factor gene expression, alternative splicing, and integrins: an evolving paradigm

The human and the murine Tissue Factor (TF) gene (*F3* and *Cf3*, respectively) comprise six protein-coding exons; two protein-coding mRNA forms are generated by *F3* and *Cf3* as a result of alternative pre-mRNA splicing. Full-length TF (flTF) mRNA features all six exons, whereas alternatively spliced TF (asTF) mRNA lacks the fifth exon. In asTF mRNA, an open reading frame shift caused by the exclusion of the fifth exon yields a unique C-terminus that replaces the transmembrane and the intracellular domains of flTF (Fig. 1A,B). The structure of asTF is analogous in human and mouse, yet murine asTF (masTF) has a C-terminus significantly longer than that of human asTF (hasTF) [1,2]. hasTF and masTF rapidly accumulate in thrombi formed by whole blood *in vitro* and *in vivo*, indicating that asTF is readily available in circulation; recombinant hasTF and masTF exhibit a low pro-coagulant activity that requires phospholipids [1,2]. While earlier studies demonstrated that hasTF protein is present in the circulation of healthy donors [1], a more recent study documented that circulating hasTF levels vary significantly in patients on chronic hemodialysis (HD) and correlate with the rate of access thrombosis; interestingly, the patient with the highest number of thrombotic incidents had the highest plasma hasTF concentration in the entire HD cohort, in excess of 1 ng/mL [3]. Last year, we developed a monoclonal hasTF-specific ELISA and determined that in some patients with sickle cell disease, hasTF plasma concentrations vastly exceed 1 ng/mL [4] (Ozhegov et al., manuscript in preparation). Although the discernible pro-coagulant activity of naturally occurring hasTF has been demonstrated in sensitive and specific assays [5,6], no data is available regarding the cofactor activity of native masTF. hasTF and masTF proteins are detectable in various tissues including heart, lung, brain, kidney, liver, spleen, as well as in arterial thrombi, atherosclerotic plaques, and cancerous lesions [1,2,7-10] (Fig. 1C). In systemic circulation, monocytes appear to be the major source of hasTF [1,11]. To date, no tissue and/or naturally occurring biological setting has been described in which hasTF or masTF is present without its full-length counterpart – hlfTF and mflTF, respectively [12].

## flTF-β1 integrin crosstalk and intracellular signaling

The importance of the flTF-β1 integrin crosstalk became increasingly evident in the late 90's: flTF was shown to impact β1 integrin-mediated signaling via proteolytic as well as non-proteolytic mechanisms comprising activation of PAR-2 and inhibition of integrin functions via flTF's intracellular C-terminus, respectively [13-15]. Dorfleutner and colleagues dissected the mechanistic determinants

* Corresponding author at: University of Cincinnati College of Medicine, Department of Internal Medicine, Division of Hematology/Oncology, The Vontz Center for Molecular Studies, 3125 Eden Ave., Cincinnati, OH 45267, USA. Tel.: +1 513 558 6276; fax: +1 513 558 6703.
*E-mail address:* vladimir.bogdanov@uc.edu (V.Y. Bogdanov).

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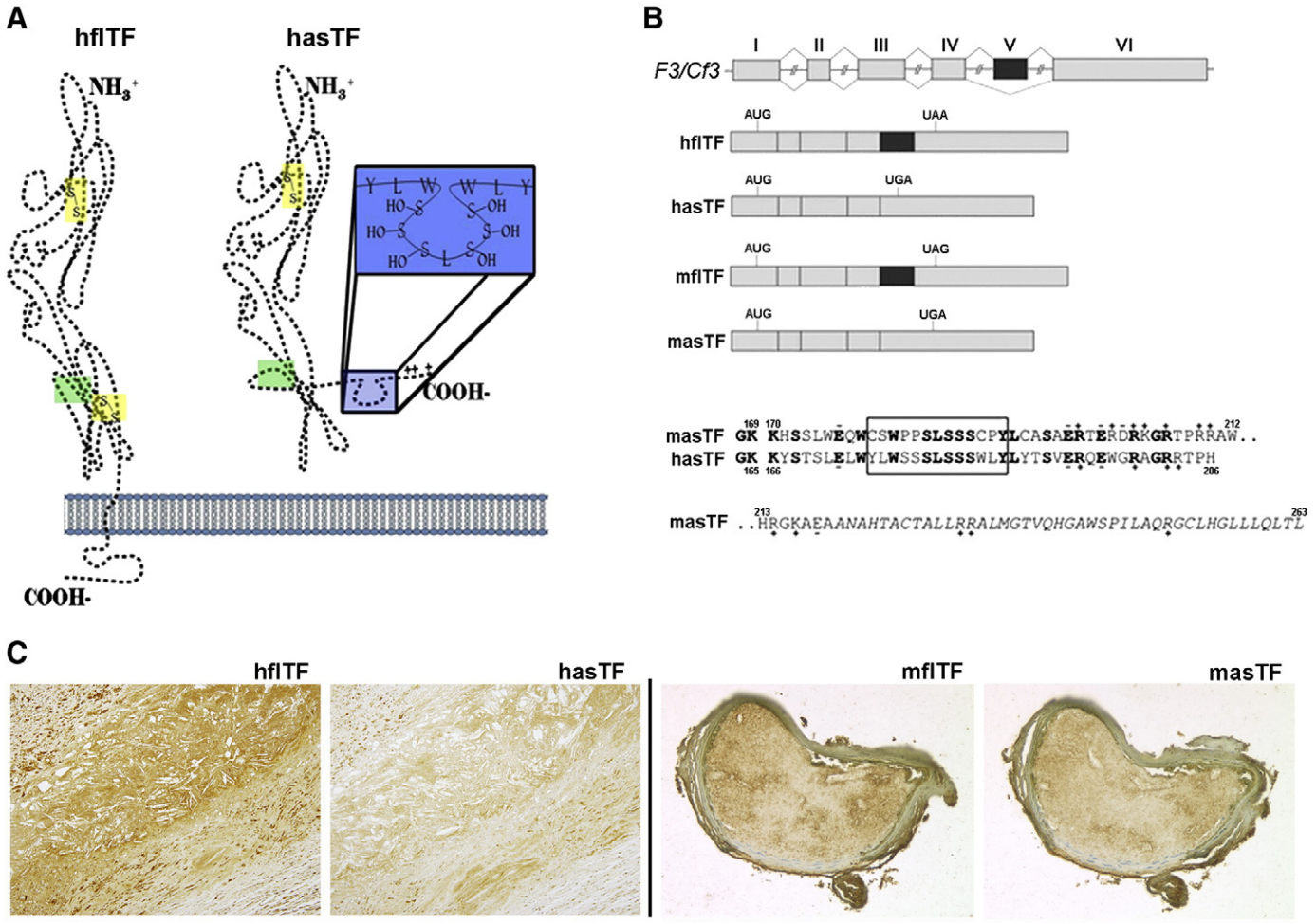


**Fig. 1.** A, Schematic structures of hflTF and hasTF proteins; yellow boxes – evolutionarily conserved disulphide bonds; green box – the lysine doublet critical for macromolecular substrate binding preserved in hasTF; highlighted in blue box – the serine-rich palindrome core (see ref. [31] for details). B, Schematic structure of *F3/Cf3* and the resultant protein-encoding mRNA variants (exons are identified by Roman numerals); lower panel: amino acid structures of the hasTF and masTF unique C-termini; the gap in the amino acid sequence demarcates the start of the regions unique to masTF and hasTF; in box – the 13 amino acid serine-rich palindrome partially conserved in masTF (adapted from ref. [2]). C, hasTF/hflTF and masTF/mflTF co-localize in lipid-rich carotid plaques (left panel), and occlusive arterial thrombi ($FeCl_3$ vessel injury model, right panel).

of flTF's induction of cell migration: they employed *in vitro* assays carried out in the presence of human TF-specific mouse monoclonal antibodies termed 6B4 and 5G9 (6B4 blocks the flTF epitope involved in VIIa binding whereas 5G9 blocks macromolecular substrate binding by flTF/VIIa, 6B4 disrupts flTF-integrin interactions whereas 5G9 enhances flTF-integrin complex formation) [16]. In the case of HaCaT cells, whose migration on fibronectin is relatively specific for $\alpha5\beta1$ and on laminin – for $\alpha3\beta1$, 5G9 promoted HaCaT cell migration in a $\alpha3\beta1$-dependent fashion [16]. Phosphorylation of flTF's C-terminus, as well as the physical association of flTF's extracellular domain with $\alpha3\beta1$ were found to be involved in the flTF-$\alpha3\beta1$ crosstalk. Remarkably, the integrin axis ties in proteolytic and non-proteolytic properties of flTF inasmuch as activation of PAR-2 by flTF/VIIa leads to phosphorylation of flTF'c C-terminus, which removes the inhibitory influence of flTF on $\beta1$ integrins [15,16]. Recently, the flTF-$\beta1$ nexus was rigorously investigated by Versteeg and colleagues in a cancer setting: flTF was found to be constitutively associated with $\beta1$ integrins in breast cancer cells whereas in non-cancerous cells, flTF-integrin interactions were dependent on the binding of the flTF's extracellular ligand – VIIa [17]. In basic cancer research, the emphasis is currently being placed on flTF-triggered PAR-2 signaling in promoting the angiogenic switch and cancer cell-favoring profile of host immune cell microenvironment [18]; the complexity of this functional interplay is underscored by the fact that flTF-$\beta1$ complex formation potentiates PAR-2 mediated signaling while, concomitantly, flTF positively regulates integrin function, thereby influencing cell-matrix interactions [17-19].

## asTF and angiogenesis

Two structural features of asTF are important with regard to integrin biology. First, asTF comprises most of flTF's extracellular domain that interacts with integrins on cell surfaces [15,16]. Second, asTF lacks the transmembrane and the intracellular domains present in flTF; thus, asTF i) can be secreted as a soluble protein and deposited/accumulated in the extracellular matrix, and ii) is not likely to exert an inhibitory action on integrins as such an action appears to require the intracellular C-terminus of flTF [15]. In fact, there is evolutionary evidence that the ancestral form of TF – present in prokaryotes – lacked a membrane-spanning domain, and served as an extracellular chaperone protein in the bacterial secretion system [20]. Thus, a concept could be postulated whereby asTF might *serve* as a *ligand* for integrins, rather than *interacting* with integrins in a *ligand-dependent manner*, as happens to be the case with flTF [15]. In 2007, hasTF-overexpressing pancreatic ductal adenocarcinoma cells injected subcutaneously in nude mice developed solid tumors that were significantly larger than those overexpressing hflTF, or no human TF whatsoever [21]. Staining for CD34 in "hasTF-enriched" tumors revealed increased angiogenesis [21], yet the mechanisms underlying this phenomenon were not elucidated. In 2009, a report by