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Full Length Article

Interplay between alternatively spliced Tissue Factor and full length Tissue Factor in modulating coagulant activity of endothelial cells

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ABSTRACT

Background: Full length Tissue factor (flTF) is a key player in hemostasis and also likely contributes to venous thromboembolism (VTE), the third most common cardiovascular disease. flTF and its minimally coagulant isoform, alternatively spliced TF (asTF), have been detected in thrombi, suggesting participation of both isoforms in thrombogenesis, but data on participation of asTF in hemostasis is lacking. Therefore, we assessed the role of asTF in flTF cofactor activity modulation, using a co-expression system.
Objective: To investigate the interplay between flTF and asTF in hemostasis on endothelial cell surface.
Methods: Immortalized endothelial (ECRF) cells were adenovirally transduced to express asTF and flTF, after which flTF cofactor activity was measured on cells and microvesicles (MVs). To study co-localization of flTF/asTF proteins, confocal microscopy was performed. Finally, intracellular distribution of flTF was studied in the presence or absence of heightened asTF levels.
Results: Levels of flTF antigen and cofactor activity were not affected by asTF co-expression. asTF and flTF were found to localize in distinct subcellular compartments. Only upon heightened overexpression of asTF, lower flTF protein levels and cofactor activity were observed. Heightened asTF levels also induced a shift of flTF from non-raft to lipid raft plasma membrane fractions, and triggered the expression of ER stress marker BiP. Proteasome inhibition resulted in increased asTF – but not flTF – protein expression.
Conclusion: At moderate levels, asTF appears to have negligible impact on flTF cofactor activity on endothelial cells and MVs; however, at supra-physiological levels, asTF is able to reduce the levels of flTF protein and cofactor activity.

1. Introduction

Venous thromboembolism (VTE) belongs to the top three most common cardiovascular diseases in industrialized nations [1]. VTE mainly comprises deep vein thrombosis and pulmonary embolism, which occurs with an incidence rate of approximately 1–2 events per 1000 individuals per year. Thrombosis is initiated after changes in blood flow, composition of blood, and/or damage to the vessel wall, also known as the triad of Virchow [2]. Hypercoagulability may be caused by increased microvesicle (MV) levels in blood and is associated with VTE in cancer patients, as reviewed elsewhere [3,4].

MVs can be shed via e.g. blebbing of the cell membrane and fall within a size range of 10–1000 nm [5]. In numerous disease settings these MVs can be shed from platelets, monocytes, and/or endothelial cells, and are believed to contribute to pathological processes such as cancer and VTE [6]. To date, it is unclear whether MVs are a cause or a consequence of VTE. Depending on the origin of the cell, these MVs may contain negatively charged phosphatidylserine (PS) and/or Tissue Factor full length form (TF/flTF) in the outside leaflet of their lipid bilayer, which increases the procoagulant potential of these vesicles [7].

flTF, a glycosylated transmembrane protein, is the only initiator of the extrinsic coagulation cascade in vivo [8], and a key player in thrombosis [3,9]. flTF is also required for vessel formation and maturation, as flTF deficiency results in the abrogation of mouse embryonic development between days 8.5 and 10.5 due to defects in the yolk sac vasculature [10,11]. In 2003, the structure of an alternative isoform of TF termed alternatively spliced Tissue Factor (asTF), was reported [12]. During TF pre-mRNA processing, exon 5 is spliced out, resulting in a frameshift that yields a soluble TF isoform with a unique C-terminal domain. We have previously shown that recombinant asTF induces angiogenesis in an integrin-dependent manner. asTF binding to αvβ3-integrins promotes endothelial cell migration, while capillary formation is induced by asTF via α6β1-integrin activation [13]. In cancer, asTF

Abbreviations: asTF, alternatively spliced Tissue Factor; ECRF, immortalized human umbilical vein endothelial cells; ER, endoplasmic reticulum; FVIIa, activated coagulation factor VII; FXa, activated coagulation factor X; flTF, full length Tissue Factor; HUVEC, human umbilical vein endothelial cells; IL-1α, interleukin-α; MVs, microvesicles; PS, phosphatidylserine; VTE, venous thromboembolism.

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induces tumor growth and metastasis in a β1-integrin dependent manner, and recruits monocytes to the tumor stroma [14–16]. asTF also increases the coagulant potential of pancreatic ductal adenocarcinoma cells and MVs via an indirect mechanism [17].

Even though asTF promotes cancer progression non-proteolytically, coagulant properties of asTF and thus its involvement in thrombosis and/or hemostasis are controversial [18–20]. Both flTF and asTF accumulate in occlusive thrombi [21], suggesting a role for asTF in thrombus formation. asTF may in principle influence coagulation as it retains the first 166 residues of flTF critical to forming a complex with FVII(a), including the 165–166 lysine doublet involved in the binding of FVII(a) and FX [22,23], but asTF lacks a complete binding site for the macromolecular substrates FIX and FX [12,24]. Initial functional studies showed that high concentrations of recombinant asTF shorten clotting times in the presence of PS-containing phospholipid vesicles [12], but these studies did not uncover how and whether asTF influences flTF-dependent clotting. In arterial lipid-rich plaques, the functional activity of asTF likely contributes to thrombus formation in a slow and long-term manner; however, in these settings asTF more likely serves to recruit monocytes that destabilize the plaque (reviewed in [25]). Another study showed that coagulant activity in supernatants from cytokine-stimulated endothelial cells decreased upon asTF depletion [20], but again this study did not explore whether asTF and flTF may synergize, or alternatively, have opposing functions in coagulation initiation. Finally, a study by Böing and colleagues found that asTF expressed in flTF-null HEK293 cells did not influence coagulation initiation, but again, this study did not evaluate the possible effects of asTF on flTF function [18].

While the above studies investigated TF isoforms in isolation, in vivo, *F3* expression results in simultaneous biosynthesis of flTF and asTF. Although the relative abundance of asTF can vary widely, asTF is never exclusively expressed [25]. The lack of functional data on TF isoform-

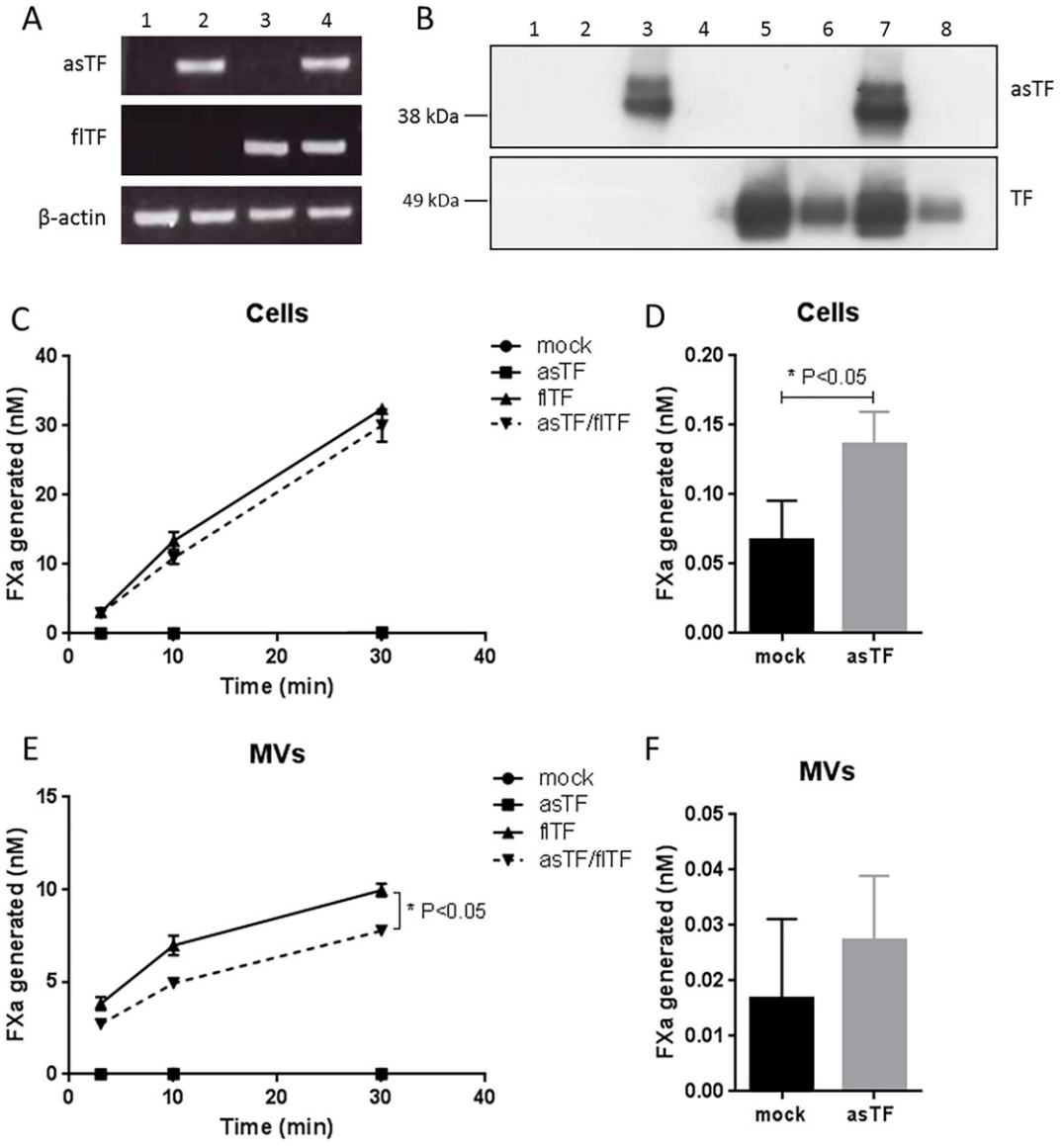

Fig. 1. Characterization of asTF and flTF in ECRF cells after adenoviral transduction. A) mRNA levels of asTF and flTF were determined in mock-transduced (1), asTF-transduced (2), flTF-transduced (3) and asTF/flTF transduced cells (4) via RT-PCR. B) asTF and flTF protein expression in cell lysates and MVs assessed using western blot. Odd numbers represent total lysates, and even numbers – MVs. C) and E) FXa generation on cells and MVs in the presence of 1 nM FVIIa and 50 nM FX. D) and F) Bar graphs represent FXa generation on mock (black) versus asTF (grey) ECRF cells and MVs. (*$p < 0.05$).