Tissue factor isoforms and cancer

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Chapter 1 - General introduction and outline of the thesis

Full-length tissue factor (flTF) is a 47 kDa transmembrane glycoprotein that is primarily responsible for activation of the coagulation cascade along the extrinsic pathway. Upon vessel damage, subendothelial expressed flTF forms a complex with circulating factor VII/VIIa (FVII/FVIIa) that can subsequently activate factor X (FX) and thrombin, resulting in localized formation of fibrin required for hemostasis. flTF expression is classically thought to be limited to subendothelial tissues in order to serve as a hemostatic envelope surrounding the vasculature[1;2]. However, in specific conditions, circulating monocytes, endothelium and circulating microparticles can bear membrane-bound flTF as well[3;4].

Besides its important role in initiating the coagulation cascade, flTF is involved in numerous physiological and pathological processes like angiogenesis and inflammation. flTF expression on endothelium and circulating microparticles underlies thrombotic complications in cancer and sepsis due to flTF's coagulant activity, but non-hemostatic properties of flTF also influence tumor biology and inflammatory processes.

The study of flTF-/- mice showed that flTF is indispensable for embryonic pericyte function and therefore to be critical for embryonic angiogenesis[5]. In adults, flTF's role in angiogenesis comprises wound healing, neovascularization and tumor angiogenesis. The last years, extensive research provided insight in how flTF triggers several cellular signaling pathways involved in cell growth, cell migration and cell survival[6-12]. Examination of mice that express TF lacking the cytoplasmic domain indicated that this domain is not required for and may even inhibit angiogenesis. These results support the notion that flTF may only affect angiogenesis indirectly via downstream coagulation factors[13;14]. In subsequent studies, however, direct activation of the membrane bound G-protein coupled protease activated receptor 2 (PAR2) by either the flTF/FVIIa/fXa-complex or the flTF/FVIIa-complex was reported[15;16]. These direct activation routes obviate the need for downstream coagulation factors and the formation or breakage of the disulfide bond between Cys186 and Cys209 has been claimed to switch between either signaling or pro-coagulant activity of flTF. It should be noted though that there remains controversy regarding the role of disulfide switching and this remains an area of ongoing research[12;17-19].

Binding and activation of integrins is a second FVII/FVIIa-independent and non-hemostatic property of flTF. Integrins are heterodimeric complexes consisting of an α and β subunit that play a critical role in cellular mechanisms involved in angiogenesis, wound healing and cancer biology[20]. flTF can directly bind to β1 integrins and this binding results in activation of integrins, but also in an active state of the flTF:FVIIa:PAR2 complex, thus allowing for flTF signaling[21].

In 2003 Bogdanov and colleagues reported a soluble, circulating splicing variant of flTF[22]. Through alternative splicing, exon 5 is excluded, leading to a frameshift that alters the amino acid sequence. This alternatively spliced tissue factor (asTF) lacks a transmembrane domain and has a unique C-terminus of which the consequences remain unknown. Since its discovery, the role of asTF in coagulation has been a matter of debate, although a role in tumor growth, potentially through angiogenesis by integrin ligation, has been suggested[22-24].

Outline

In this thesis, the properties of asTF regarding angiogenesis, inflammatory processes and cancer are investigated. In addition, the relative contributions of each TF isoform to tumor angiogenesis are assessed in a large cohort of breast cancer patients.

Chapter 2 examines how recombinant human asTF promotes angiogenesis in a non-hemostatic, but integrin-dependant fashion. **Chapter 3** is a review that describes the current debate on the role of asTF in coagulation and its suggested non-hemostatic properties, with a focus on cancer, but also a potential role in atherosclerosis will be discussed. **Chapter 4** outlines how asTF promotes the interaction between inflammatory cells and the endothelium. In addition, data are provided that support the notion that asTF mediates adherence of inflammatory cells to the endothelium, which is relevant to both atherosclerosis and cancer. Whether murine asTF has similar properties as human asTF is investigated in **chapter 5**. Furthermore, this chapter describes the distribution of asTF in experimental murine models for atherosclerosis and cancer. **Chapter 6** proceeds with an assessment of the role of asTF in human breast cancer. A large cohort of human breast cancer is studied in order to provide insight in the clinical and pathological relevance of both asTF and flTF. **Chapter 7** continues with a study on ectopically produced FVII in breast cancer. The results suggest that auto-activation of the flTF:FVIIa:PAR2 axis influences the prognosis of young women with breast cancer. In **chapter 8** the evolutionary conservation of the cysteines involved in flTF disulfide bonding is assessed and a comparison is made with other proteins of which the activity is also regulated by isomerization of an allosteric disulfide. **Chapter 9** reviews the current knowledge on the non-hemostatic effects of TF isoforms in cancer. The chapter describes the regulation of TF expression in cancer, its effects *in vitro* and in mice, and how studies on human tumor material support the findings from experimental studies. **Chapter 10** provides a general summary and discussion.

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