Interplay between cancer and thrombosis; identification of key factors
Ünlü, B.

Citation
Ünlü, B. (2019, January 29). *Interplay between cancer and thrombosis; identification of key factors*. Retrieved from https://hdl.handle.net/1887/68470

Cover Page

Universiteit Leiden

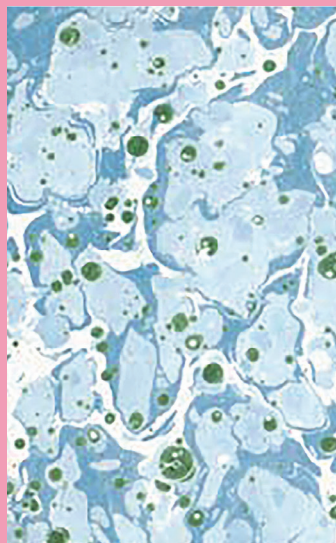The handle http://hdl.handle.net/1887/68470 holds various files of this Leiden University dissertation.
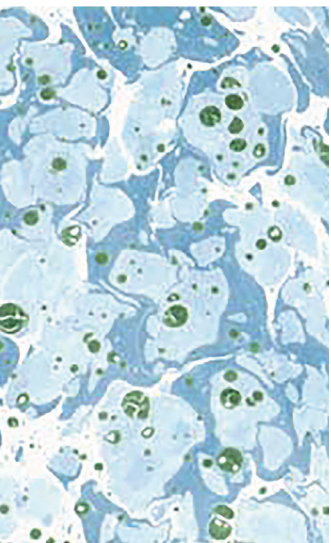
Author: Ünlü, B.
Title: Interplay between cancer and thrombosis; identification of key factors
Issue Date: 2019-01-29

Chapter 1

General introduction

In 1823 the link between cancer and thrombosis was first reported by Jean Baptiste Bouil-laud [1]. Although being the first, Armand Trousseau was the one who got recognized for the association between gastric cancer and thrombosis in 1865 [2]. Ironically, this French internist diagnosed himself with venous thromboembolism (VTE) and predicted his own death, which took place in 1867. For these reasons cancer-associated thrombosis (CAT) is now referred to as Trousseau's Syndrome.

Blood clots in the deep veins (formation of which is termed deep vein thrombosis; DVT) can detach and ultimately travel through the bloodstream and occlude an artery in the lung, which is called a pulmonary embolism (PE). Among all patients with VTE (DVT + PE) one in five is diagnosed with cancer [3, 4]. Furthermore, cancer patients have on average a 7-fold increased risk for developing VTE when compared to healthy subjects [5, 6]. CAT contrib-utes to high morbidity and mortality [7]. After cancer itself, CAT is the second cause of death in cancer patients [8]. Unfortunately, the mechanism underlying CAT is poorly understood. Until now only a few clinical and patient-related factors have been uncovered that may con-tribute to VTE in cancer patients, such as high age, obesity, and prior history of VTE [7, 9]. Risk factors directly associated with the presence of a tumor further contribute to an increased risk of VTE, like the location of the primary tumor, increase in tumor stage and cancer treatment [10, 11]. Assessment tools to estimate the risk of CAT have been developed, however they do not perform well. Furthermore, thromboprophylaxis of cancer patients increases the risk of spontaneous bleedings. Therefore, it is key to unravel the mechanism behind cancer-associated thrombosis in order to select those patients that are at high risk for CAT and those who could benefit from prophylactic anticoagulant treatment.

Nowadays, it is well accepted that the transmembrane protein Tissue Factor (TF), the phys-iological activator of the coagulation cascade, is the linking pin between coagulation and cancer. Under normal conditions, TF is expressed on subendothelial cells. Upon external stimuli (e.g. injury), TF is exposed to the bloodstream and forms a binary complex with its ligand Factor VII (FVII) that eventually results in thrombin activation, fibrin deposition and platelet activation in order to close the site of the wound [12]. Apart from its function in blood coagulation, TF is expressed by tumors and has been shown to play a pivotal role in tumor progression. Overexpression has been associated with reduced survival, increased angiogenesis, migration and invasive capacity of tumor cells [13].

TF influences cancer progression through two distinct pathways. First, it activates cellular signaling events to promote tumor growth via activation of Protease activated Receptor-2

(PAR2), facilitating the angiogenic switch and affecting migration of cancer cells. Upon entering the blood vessel, coagulant properties of TF become important, leading to the formation of a platelet and fibrin shield around the tumor cell to prevent shear stress and immune cell mediated apoptosis, thereby promoting survival of metastatic cells [14].

A soluble alternatively spliced isoform of TF has been discovered in 2003, the so-called alternatively spliced Tissue Factor (asTF). This isoform lacks the transmembrane region of TF − as exon 5 is spliced out − and due to a frameshift it contains a unique C-terminal tail that differs from that of full-length TF protein (flTF) [15]. Studies have shown involvement of asTF in angiogenesis and tumor growth in an integrin-dependent manner [16, 17]. Although the first 166 amino acids are identical between flTF and asTF, the procoagulant properties of asTF have remained controversial as it lacks the binding site for macromolecular coagulation factors IX and X [18-20].

Outline

In this thesis the focus is on the interplay between coagulation factors and cancer. First, the role of asTF in flTF activity modulation is addressed. Second, the contribution of TF signaling in breast cancer metastasis and its underlying mechanism has been elucidated. Finally, tumor-expressed contributors to VTE were studied and an *in vivo* model was developed with spontaneous thrombosis in combination with cancer.

Chapter 2 shows that co-expression of asTF has a negligible impact on flTF coagulant activity of endothelial cells and extracellular vesicles. Analysis shows that asTF and flTF do not localize to the same cellular compartments, explaining why asTF does not influence flTF function. **Chapter 3** summarizes the current literature on tumor-expressed coagulation factors in cancer progression and the elevated risk of VTE. Whether TF signaling promotes metastasis in breast cancer is described in **Chapter 4.** By using *in vivo, ex vivo* and *in vitro* models it is shown that inhibition of TF signaling reduces i) epithelial-to-mesenchymal transition and cancer stem cell transcriptional programs, ii) primary tumor-resident cancer stem cells and iii) subsequent metastasis *in vivo.* In addition, metastasis is significantly associated with TF expression in estrogen receptor negative tumors in a large breast cancer cohort. Furthermore, the underlying mechanism, i.e. TF-dependent modulation of integrin function, is elucidated. In **Chapter 5** a spontaneous *in vivo* model was developed in order to provide insight in the evolution of cancer-associated thrombosis. Due to elevated platelet counts in mice with breast cancer, a severe phenotype with consumption of coagulation factors is reduced. **Chapter 6** reveals new genes that were associated with VTE in colorectal

1

cancer patients. Through the use of laser capture microdissection method to isolate RNA from tumor cells only, a comparison in tumor cell-expression profiles could be made between individually matched colorectal cancer patients with and without VTE. In **Chapter 7** current knowledge on links between genetic alterations and risk of cancer-associated thrombosis is reviewed. In addition, future directions are discussed in order to increase the accuracy of cancer-associated thrombosis prediction models. Finally, **Chapter 8** provides a general summary and discussion of this thesis.

REFERENCES

1. Bouillaud, J.B., *De l'Obliteration des veines et de son influence sur la formation des hydropisies partielles: consideration sur la hydropisies passive et general.* Arch Gen Med, 1823(1): p. 188-204.
2. Trousseau, A., *Phlegmasia alba dolens.* Clin Med Hotel-dieu Paris., 1865. **3**: p. 654-712.
3. Streiff, M.B., *Thrombosis in the setting of cancer.* Hematology Am Soc Hematol Educ Program, 2016. **2016**(1): p. 196-205.
4. Khorana, A.A. and G.C. Connolly, *Assessing risk of venous thromboembolism in the patient with cancer.* J Clin Oncol, 2009. **27**(29): p. 4839-47.
5. Heit, J.A., et al., *Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study.* Arch Intern Med, 2002. **162**(11): p. 1245-8.
6. Falanga, A. and L. Russo, *Epidemiology, risk and outcomes of venous thromboembolism in cancer.* Hamostaseologie, 2012. **32**(2): p. 115-25.
7. Timp, J.F., et al., *Epidemiology of cancer-associated venous thrombosis.* Blood, 2013. **122**(10): p. 1712-23.
8. Khorana, A.A., et al., *Thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy.* J Thromb Haemost, 2007. **5**(3): p. 632-4.
9. Ikushima, S., et al., *Trousseau's syndrome: cancer-associated thrombosis.* Jpn J Clin Oncol, 2016. **46**(3): p. 204-8.
10. Khorana, A.A., et al., *Development and validation of a predictive model for chemotherapy-associated thrombosis.* Blood, 2008. **111**(10): p. 4902-7.
11. Khalil, J., et al., *Venous thromboembolism in cancer patients: an underestimated major health problem.* World J Surg Oncol, 2015. **13**: p. 204.
12. Versteeg, H.H., et al., *New fundamentals in hemostasis.* Physiol Rev, 2013. **93**(1): p. 327-58.
13. van den Berg, Y.W., et al., *The relationship between tissue factor and cancer progression: insights from bench and bedside.* Blood, 2012. **119**(4): p. 924-32.
14. Han, X., et al., *Tissue factor in tumor microenvironment: a systematic review.* J Hematol Oncol, 2014. **7**: p. 54.
15. Bogdanov, V.Y., et al., *Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein.* Nat Med, 2003. **9**(4): p. 458-62.
16. van den Berg, Y.W., et al., *Alternatively spliced tissue factor induces angiogenesis through integrin ligation.* Proc Natl Acad Sci U S A, 2009. **106**(46): p. 19497-502.
17. Kocaturk, B., et al., *Alternatively spliced tissue factor promotes breast cancer growth in a beta1 integrin-dependent manner.* Proc Natl Acad Sci U S A, 2013. **110**(28): p. 11517-22.
18. Boing, A.N., et al., *Human alternatively spliced tissue factor is not secreted and does not trigger coagulation.* J Thromb Haemost, 2009. **7**(8): p. 1423-6.
19. Censarek, P., et al., *Alternatively spliced human tissue factor (asHTF) is not pro-coagulant.* Thromb Haemost, 2007. **97**(1): p. 11-4.
20. Szotowski, B., et al., *Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines.* Circ Res, 2005. **96**(12): p. 1233-9.

1