The role of blood coagulation in cancer, inflammation and embryonic development
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High FVIIa levels do not promote tumor metastasis

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A critical role for coagulation factors in cancer biology has been suspected for years, and recently it became evident that anticoagulant treatment of end-stage cancer patients with LMWH may significantly improve survival [1-2]. In agreement with this observation, anticoagulant treatment inhibits metastasis in experimental animal models [3-4] whereas the intravenous injection of minute concentrations of thrombin enhances murine experimental pulmonary metastasis [5]. Although the mechanism by which the coagulation system affects metastasis is not completely understood, current evidence suggests that the coagulation cascade interacts with metastasis by two alternative mechanisms. First, fibrin clots produced by blood coagulation may provide protection for cancer cells to help these escape from the immune system [6-10]. Second, individual activated coagulation factors may modify the survival of circulating cancer cells by inducing intracellular signal transduction [11-13].

Common to both mechanisms is that coagulation factors need to be activated, probably through initiation of the extrinsic pathway of coagulation. The key component involved is tissue factor (TF), which is a membrane bound glycoprotein that on exposure to blood (and via formation of an enzymatic complex with factor VIIa (FVIIa)) activates coagulation, leading to thrombin activation and fibrin deposition [14-16]. In addition, however, TF induces intracellular signal transduction pathways involved in cancer progression either by its cytoplasmic domain or by TF/FVIIa-dependent proteolytic cleavage of protease activated receptor (PAR)-2 [12].

FVII levels are under normal circumstances not rate limiting in haemostasis. However, as cancer cells express large amounts of TF one could speculate that FVII might become rate limiting in malignancy and that endogenous FVIIa would contribute to a coagulant phenotype thereby promoting metastasis. In agreement with this notion, ectopic synthesis of FVII by cancer cells promotes cell migration and invasion by complex formation with TF [17]. Based on these intriguing findings, we hypothesized that high exogenous FVIIa levels promote cancer progression. To test this hypothesis, mice were treated with recombinant FVIIa (rFVIIa; 10 mg/kg; NovoSeven, Novo Nordisk, Denmark), a dose known to normalize bleeding time and blood loss in FVIII-deficient mice [18], prior to subjecting them to an experimental murine pulmonary metastasis model [19]. As shown in figure 1A, the injection of $1 \times 10^7$ murine TF expressing B16F0 melanoma cells (American Type Culture Collection, Manassas, Virginia U.S.A.) [20] into the tail vein of C57Bl/6J mice resulted in a substantial number of tumor foci in the lungs after 14 days. However, pretreatment with rFVIIa did not affect the number of metastases, suggesting that FVIIa levels are not rate-limiting in TF-dependent metastasis.

To exclude experimental and/or technical problems as an explanation for lack of tumor promoting activity of rFVIIa, we verified our pulmonary metastasis model. To this end, we first confirmed that our model is indeed TF dependent by treating mice with active site-inhibited FVIIa (2.5 mg/kg; FVIIai; Novo Nordisk, Denmark). As shown in figure 1B, the competitive inhibition of FVIIa by FVIIai reduced the number of tumor foci significantly ($p=0.03$) indicating that TF/FVIIa complex formation is an important determinant of the number of pulmonary metastases in this model. In addition, inhibiting downstream thrombin formation by hirudin (10 mg/kg) (almost) completely abrogated the formation of pulmonary metastasis (fig 1B).
High FVIIa levels do not promote tumor metastasis as shown before [9] thereby further confirming the validity of our experimental model. A limitation to this study is that the pulmonary metastasis model, although well accepted and extensively used in the past [9,19,20], is a surrogate for hematogeneous metastasis and future experiments in alternative models should validate our findings.

Overall our data show that a FVIIa bolus injection has no effect on pulmonary metastasis of melanoma cells in mice and suggests that increased FVIIa levels do not play an important role in tumor progression, indicating that FVIIa is not rate-limiting. As rFVIIa is nowadays a serious treatment option of patients suffering from serious bleeding complications, such as hemophilia [21-23], intracerebral hemorrhage [24], obstetrical and gynaecological haemorrhage [25], and thrombocytopenia [26], long-term follow-up studies of patients treated with rFVIIa might give more definitive answers.

**Figure 1:** Effect of rFVIIa (A), FVIIai and hirudin (B) on pulmonary metastasis in C57Bl/6 mice. B16F0 cells (1-3×10^5) were injected intravenously into the lateral tail vein. Thirty minutes before administration of the melanoma cells, mice were injected with a single intravenous bolus of 10 mg/kg rFVIIa (Novoseven, Novo Nordisk, Norway), 2.5 mg/kg FVIIai or 10 mg/kg PEG-hirudin (subcutaneously). Shown is the number of pulmonary foci after 14 days (n=8, mean+/−SEM). p=0.9 for rFVIIa versus control, p=0.03 for FVIIai versus control and p=0.001 for hirudin versus control.
Chapter 3

References
