Coagulation, angiogenesis and cancer

Niers, T.M.H.

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General introduction
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The relationship between cancer and thrombosis is twofold. First, cancer patients have an increased risk to develop venous thromboembolism (VTE). Second, the coagulation system affects cancer progression and metastasis. Furthermore, various coagulation factors, like tissue factor (TF) and thrombin are involved in angiogenesis, new vessel formation, which facilitates tumor growth and metastasis\(^1\). This thesis is focused on three major cancer progression-stimulating factors: platelets, TF/thrombin and angiogenesis -and the two-way interactions between cancer and coagulation.

Increased risk of thrombosis in cancer patients

Cancer patients have an increased risk to develop VTE. This was first described by Bouillaud (1823)\(^3\) and forty years later (1865) interpreted and published by Trousseau\(^5\). An unprovoked VTE can be the first sign of occult cancer, which was described for the first time in 1935\(^6\). According to Virchow, the hallmarks of VTE are pathological changes in blood flow, coagulability and the condition of the vessel wall. All three phenomena may occur in cancer patients. The proposed mechanisms to explain hypercoagulation associated with cancer include a reaction of the patients body to the tumor such as abnormal protein synthesis, angiogenesis and necrosis and more specific processes related to tumor-mediated haemostatic activities (cancer cells interacting with platelets, endothelial cells, monocytes and with the coagulation and fibrinolytic systems)\(^7\). Furthermore, cancer treatment -irradiation, chemotherapy and surgery- may further upset the balance between procoagulant and anticoagulant factors\(^8\).

The incidence of VTE is also associated with the use of central venous catheters (CVC). Cancer patients frequently have to use CVCs for chemotherapy, stem cell infusion, blood supply, medication, parenteral hyperalimentation and blood sampling. Risk factors for CVC-related thrombosis include the type of malignancy, chemotherapy and CVC and insertion sites of the catheter tip\(^9\). Many studies have addressed the incidence and associated risk factors of CVC-related infections and VTE in patients with solid tumors but only few data are available on haemato-oncological patients. These patients may differ from patients with solid tumors, because of the more severe and prolonged thrombocytopenia and leukopenia. Therefore, an important matter of debate is whether haematological cancer patients should receive thrombosis prophylaxis or not. This issue is dealt with in chapter 9.

The coagulation system affects cancer progression and metastasis

Until the mid nineties of the last century, the initial treatment of VTE consisted of a brief course of unfractionated heparin (UFH) followed by a course of vitamin K antagonists for several months. In 1992, Prandoni et al compared the relative safety and efficacy of
low molecular weight heparin (LMWH) and UFH for the treatment of VTE and concluded that fixed-dose subcutaneous LMWH is at least as effective and safe as UFH for the initial treatment of VTE\textsuperscript{11}. This has been confirmed by others\textsuperscript{12-14}. Unexpectedly, LMWH showed a favourable effect on the survival of cancer patients. At 3 months, 44\% (8 of 18) of the cancer patients died in the UFH group vs. 7\% (1 of 15) in the LMWH group (p=0.021)\textsuperscript{11}. These findings were confirmed in meta-analyses of 9 studies that compared LMWH with UFH in the treatment of VTE\textsuperscript{15,16}. These studies initiated clinical trials evaluating the effect of anticoagulants on survival of cancer patients without thrombosis\textsuperscript{17-20}. Thus, cancer favours thrombosis and the coagulation system promotes cancer as suggested by the marked survival advantage of patients using anticoagulants.

Various experimental studies showed the inhibitory effects of anticoagulants on cancer progression and metastasis. The results are reviewed in chapter 2. However, the mechanisms by which anticoagulants may interfere with tumor growth and metastasis are diverse, remain poorly defined and seem to be dependent on the type of cancer and individual anticoagulant\textsuperscript{21-27}. In this thesis, experimental studies are described that focus in particular on the phase of haematogenous dissemination when cancer cells are present in the circulation. These studies are described in chapters 4, 5, 6 and 7. Chapter 3 reviews validation of noninvasive bioluminescence imaging (BLI) for quantitative assessment of tumor load in time in small animals, a technique we used in two of our studies.

**Platelets**

Platelet aggregation on cancer cells takes place rapidly when cancer cells have entered the circulation. Cancer cells are thus masked for the immune system, protected against shear stress in the vasculature and adhesion to vessel walls is facilitated. The process starts with the interaction of activated platelets with cancer cells that express P-selectin ligands, such as glycoprotein ligand-1, CD24, heparan sulphate proteoglycan (HSPG) and sialyl-Lewis a/x\textsuperscript{28,29}. The interactions of platelets and cancer cells may also involve ß3-containing integrins binding von Willibrand factor (vWF), thrombomodulin and fibrinogen to form molecular bridges\textsuperscript{30,31}. Then, activation of procoagulant proteins such as TF can occur as is described below\textsuperscript{32,33}. These interactions enable rolling of cancer cells or cancer cell-platelet complexes along vessel walls where endothelial cells constitutively express low amounts of P-selectin\textsuperscript{34} that facilitates adhesion of cancer cells to the wall and transmigration into the subendothelium\textsuperscript{35} or protect cancer cells within vessels against mechanical stress and the immune system\textsuperscript{36-40}.

P-selectin also occurs in a soluble form in blood plasma. Ferroni et al demonstrated that soluble P-selectin (sP-selectin) plays a pivotal role in the pathogenesis of metastasis by formation of cancer cell-platelet complexes. sP-selectin is considered to be a marker for platelet activation and sP-selectin correlated inversely with prognosis in patients with cancer\textsuperscript{41}. It has been suggested that anticoagulants inhibit metastasis by blocking P-selectin\textsuperscript{42}. A major issue is whether the life-prolonging effects of LMWH in cancer patients
can be explained by its interference in P-selectin-mediated interactions between platelets and cancer cells. This is described in chapter 8.

**Thrombin**

When TF is expressed on the plasma membrane of cancer cells, it activates circulating liver-derived coagulation factors VII, V and X that leads to the generation of thrombin from prothrombin (Figure 1). Thrombin has distinct effects on cells. Intracellular effects of thrombin are mediated by protease activated receptors (PARs), members of the family of G-coupled receptors. Four PARs have been described: PAR-1, -2, -3 and -4. Thrombin seems to be the major physiological activator of PAR-1 and PAR-4, but it can also activate PAR-3, that functions as a cofactor of PAR-4. PAR-2 is not directly activated by thrombin but via trypsin, coagulation factor VIIa and factor X. TF and PARs play an important role in cancer progression.

PAR signalling upregulates adhesion molecules on endothelial surfaces and triggers production of chemokines by activating neutrophils and monocytes. This leads to binding, rolling and attachment of platelets and leukocytes on the surface of endothelium. High
concentrations of locally-produced thrombin may lead to direct release of P-selectin stored in Weibel bodies in endothelial cells through PAR-4-dependent signalling, resulting in increased platelet aggregation and cancer cell-platelet binding. Local aggregates of cells expressing TF\textsuperscript{53} along with procoagulant activity of platelets\textsuperscript{54} may trigger further thrombin formation\textsuperscript{1} and increased permeability of the endothelium\textsuperscript{2}. Thrombin also activates platelets to release growth factors that may sustain tumor development and may aid angiogenesis by production of platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)\textsuperscript{9,57-59}. Furthermore, thrombin converts fibrinogen into fibrin, the end product of the coagulation cascade. Fibrin depositions have been found in and around many types of tumors, providing a scaffold for angiogenesis and possibly also protecting the cancer cells against the host defence system\textsuperscript{60,61}. Thus, possible mechanisms by which anticoagulants prolong survival of cancer patients may also be reduction of thrombin and fibrin formation. The relationship between thrombin and cancer is described in the chapters 5 and 7.

**Angiogenesis**

Several coagulation factors, such as TF and thrombin play a role in angiogenesis, that is required for tumor growth and metastasis\textsuperscript{1,2}. First, thrombin can activate angiogenesis by reduction of endothelial cell attachment to lamina basalis proteins and activation of matrix metalloproteinases\textsuperscript{62}. Second, thrombin has chemotactic and apoptotic effects on endothelial cells and upregulates expression of VEGF receptors (VEGFR). Third, thrombin upregulates expression of αvβ3 integrin, the marker of the angiogenic phenotype of endothelial cells\textsuperscript{63}. Platelets may contribute to this process because they also release angiogenic factors like VEGF upon activation by thrombin via PAR-1\textsuperscript{64}.

VEGF is one of the most important angiogenic factors. It binds to specific tyrosine kinase receptors on the surface of endothelial cells including VEGFR-1 (Flt-1) and VEGFR-2 (KDR/flk-1) on vascular endothelium and VEGFR-3 (Flt-4) expressed on lymphatic endothelium, resulting in cell migration, proliferation and survival\textsuperscript{65,66}. Clinical research on angiogenesis has two major directions in cancer patients. First, quantification of angiogenesis for diagnosis, prognosis as well as for the monitoring of responses. Second, the inhibition of angiogenesis to halt tumor growth\textsuperscript{2}. However, serum, plasma and whole blood have been indiscriminately used to determine VEGF levels in the body. Because coagulation results in the release of VEGF from platelets, serum VEGF levels include plasma-derived VEGF and platelet-derived VEGF\textsuperscript{67}. Therefore, VEGF levels in serum do not reflect the true circulating levels of VEGF. In citrate or EDTA plasma, where less platelet activation and subsequent VEGF release occurs than in serum, VEGF levels were found to be higher in cancer patients than in controls and this was interpreted as a reflection of the higher levels of VEGF in the circulation of cancer patients\textsuperscript{69}. However, the release of VEGF from platelets may contribute to increased VEGF levels in plasma as well under these conditions. Therefore, the effects of the different blood collection protocols on the measurement of circulating VEGF and
their impact on VEGF release from platelets by in vitro platelet activation is described in chapter 10.

VEGF expression is regulated by a number of factors. In renal cell carcinoma (RCC), VEGF expression is a consequence of inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene, resulting in a remarkable overexpression of VEGF. Because of the high levels of VEGF occurring in RCC, VEGF may be identified as a critical component of angiogenesis in RCC and as a potential therapeutic target to treat RCC. The strategy to inhibit the activity of VEGF includes binding of the VEGF protein and blockade of VEGFR. Besides the classical prognostic markers for advanced RCC, novel validated biomarkers are needed to predict the outcome of targeted therapy and the development of drug resistance. Circulating levels of VEGF, placenta growth factor (PlGF), sVEGFR-1 and -2 and bFGF are potential candidates to predict outcome of the various therapies. We correlated base line levels and changes in the levels during treatment of these potential markers with disease outcome and the development of resistance during therapy in chapter 11.
Chapter 1

References


Chapter 1


Chapter 1


