Cell. Mol. Life Sci. 63 (2006) 1024–1027
1420-682X/06/0901024-4
DOI 10.1007/s00018-005-5570-9

Cellular and Molecular Life Sciences

Visions & Reflections

Cancer and blood coagulation

C. Boccaccio[a, *] and E. Medico[b]

[a] Division of Molecular Oncology, Institute for Cancer Research and Treatment (IRCC), University of Turin Medical School, Str. Prov. 142, 10060 Candiolo (Italy), Fax: +39 011 9933 225, e-mail: carla.boccaccio@ircc.it
[b] Oncogenomics Center, Institute for Cancer Research and Treatment (IRCC), University of Turin Medical School, Str. Prov. 142, 10060 Candiolo (Italy)

Received 30 November 2005; received after revision 7 February 2005; accepted 8 February 2006
Online First 14 April 2006

Abstract. In human patients, blood coagulation disorders often associate with cancer, even in its early stages. Recently, *in vitro* and *in vivo* experimental models have shown that oncogene expression, or inactivation of tumour suppressor genes, upregulate genes that control blood coagulation. These studies suggest that activation of blood clotting, leading to peritumoral fibrin deposition, is instrumental in cancer development. Fibrin can indeed build up a provisional matrix, supporting the invasive growth of neoplastic tissues and blood vessels. Interference with blood coagulation can thus be considered as part of a multifaceted therapeutic approach to cancer.

Keywords. MET, oncogene, cancer, invasive growth, blood coagulation, haemostasis, PAI-1, COX-2, fibrin.

In human patients, a blood disorder involving hyperactivation of the coagulation system and formation of intravenous fibrin clots (thrombosis) can be the first manifestation of a tumour [1]. The association of venous thrombosis with cancer was named Trousseau's syndrome after Armand Trousseau, the French clinician who first described it in 1865 [2]. Since then, a long list of medical publications testifies to the occurrence of blood coagulation disorders at every stage of tumour progression. It is almost intuitive that the carefully balanced haemostatic system could collapse in advanced cancer, as a result of systemic spread of neoplastic cells, extensive tissue damage and severe overall decay of the organism. In contrast, it is harder to connect an otherwise unexplained thrombosis with cancer onset. Clinicians can miss this connection, as the early stages of cancer can escape the sensitivity of sophisticated diagnostic tools. Biologists can miss this connection as well because it is difficult to envision a transformed cell affecting the haemostasis system while remaining confined within its tissue of origin, usually an epithelium. However, authoritative clinical studies of patients with Trousseau's syndrome led to the striking conclusion that 'either premalignant changes promote thrombosis, or cancer and thrombosis share common risk factors' [3]. Thus, two questions merit the attention of tumour biologists: First, what is the mechanistic link between cancer and haemostasis activation? Second, is the procoagulant activity of tumours a mere coincidence, or is it instrumental to tumour growth? An answer to the latter question was already proposed in the late 1800s by the pathologist Theodor Billroth, who found cancer cells embedded in circulating microthrombi, and thus suggested that these clots could safely ship cancer cells through the bloodstream, favouring metastasis [4]. If tumours exploit blood coagulation to their advantage, the search for cancer-associated molecules responsible for thrombosis could unveil targets to fight both the side effect (thrombosis, which can be lethal in itself) as well as the primary disease (cancer).

But how do cancer cells trigger blood coagulation? Explanations provided so far include inappropriate expression of factors directly involved in blood coagulation, or molecules inducing platelet aggregation, or cytokines, which modulate endothelial and inflammatory processes

* Corresponding author.

(for review see [5, 6]). Among the best-documented events is expression of tissue factor (TF) on the surface of transformed epithelial cells ([6] and references therein). TF is usually expressed by endothelial cells in response to severe injury (e.g. endotoxin exposure etc.), and initiates blood clotting by triggering activation of the blood coagulation factor cascade, leading to thrombin formation. Thrombin catalyses conversion of circulating fibrinogen into insoluble fibrin. Fibrin is further modified by other enzymes to form a gel-like provisional matrix that seals vessel and tissue ruptures, providing the first support for tissue regeneration (reviewed in [7]).

TF is membrane-bound, and thus its increased expression by cancer cells may explain the peritumoral activation of blood coagulation favoured by newly formed vessels, which are often leaky and permeable to coagulation factors and fibrinogen [8]. However, in cancer patients with Trousseau's syndrome, thrombosis usually occurs in regions (such as deep veins of the legs) that are distant from the epithelial organs that usually host the primary tumour. This suggests that the tumour also exerts a systemic procoagulant effect. Interestingly, TF can be released into the blood circulation, mainly in association with membrane microparticles shed from the surface of neoplastic cells, or cells of the tumour microenvironment [9]. Recently it was shown in cell lines that TF expression is increased by genetic lesions responsible for human cancers. This is the case with Ras activation or p53 inactivation, in colorectal cancer [10], and with Pten loss in glioblastomas [11].

Besides TF, oncogenes regulate the expression of other molecules that can be responsible for Trousseau's syndrome, as shown by a new mouse model which incorporates stepwise cancer progression in association with a progressive haemostatic disturbance [12]. This mouse model was generated through somatic transduction of the *MET* oncogene, which encodes the tyrosine kinase receptor for hepatocyte growth factor. *MET* is an unconventional oncogene, able not only to transform cells, but also to induce 'invasive growth', a process supporting cell motility and survival through foreign tissues, and metastasis to distant sites [13]. *MET* was transduced in the liver of adult mice by lentiviral vectors, which can integrate into non-dividing cells and drive expression through a tissue-specific promoter (in this case, albumin). With respect to conventional germ-line transgenesis, lentiviral transduction induced transformation of a small fraction of hepatocytes which remained interspersed among normal cells. *MET*-transduced mice developed a stepwise tumorigenic process, arising from single transduced hepatocytes. This process was associated with haemostatic disturbances, which started with venous thrombosis, noticeably, before the appearance of the first preneoplastic lesions. This observation suggested that the genetic program activated by the *MET* oncogene in hepatocytes was responsible for both cell transformation and the concomitant procoagulant activity. The transcriptome of cells expressing the activated *MET* was then examined, and it was discovered that, among the 12,000 genes analysed by Affymetrix microarray, the two most upregulated genes were plasminogen activator inhibitor-type 1 (PAI-1) and cyclooxygenase 2 (COX-2). Nearly the entire subset of genes involved in haemostasis regulation was analysed in the array (71 genes), and found to be significantly modulated as a whole. However, each single gene (including tissue factor) was only weakly affected.

PAI-1 and COX-2, which are expressed *in vivo* by *MET*-transduced mice, are both suitable candidates to support the systemic haemostasis disturbance associated with cancer. PAI-1 is secreted into the blood where it prevents generation of plasmin, the enzyme that dissolves fibrin clots [14]. Therefore, the net effect of an increase in PAI-1 is to promote the persistence and expansion of thrombi. This is confirmed by studies that correlate high levels of plasmatic PAI-1 with an increased risk of venous and artery thrombosis (reviewed in [15]). COX-2 encodes an inducible form of prostaglandin synthase that catalyses an intermediate step in the synthesis of prostacylins and thromboxane. These molecules are also systemically released and modulate functions of platelets (for a review see [16]). Interestingly, the prothrombotic state of cancer patients also depends on increased platelet activation, which is a consequence of several factors. These include circulating molecules such as glycosylated proteins and lipids, and cytokines, released by cancer cells or by their microenvironment [5]. The prominence of PAI-1 and COX-2 in the *MET* transcriptome led to speculation that the same proteins could be effectors not only for haemostasis disturbances but also for tumour progression itself. In fact, many clues implicate COX-2 and PAI-1 in cancer onset and progression. The case of COX-2 is typical, as its inhibition by specific drugs (e.g. Rofecoxib) can prevent colorectal cancer, both in mouse models and in human patients (for a review see [17]). Circumstantial evidence also implicates PAI-1 in tumorigenesis, as documented by the association of high levels of PAI-1 with cancer metastasis and poor prognosis [18]. In *MET*-transduced mice, it was shown that inhibition of COX-2 or PAI-1 with specific drugs reduced the haemostasic disturbance, and, in the case of COX-2 inhibition, also the cancer phenotype. The effect of PAI-1 inhibition on cancer growth is still inconclusive, due to the limited availability of inhibitors suitable for long-term treatment.

Taken together, the above studies support the conclusion that, on the one hand, the genetic lesions responsible for cancer onset and progression control genes involved in blood coagulation; on the other hand, blood coagulation promotes cancer onset and progression. But what are the mechanisms involved? It is likely that the fibrin matrix that is quickly deposited around the growing cells provides two advantages (Fig. 1). First, it offers an adhesive

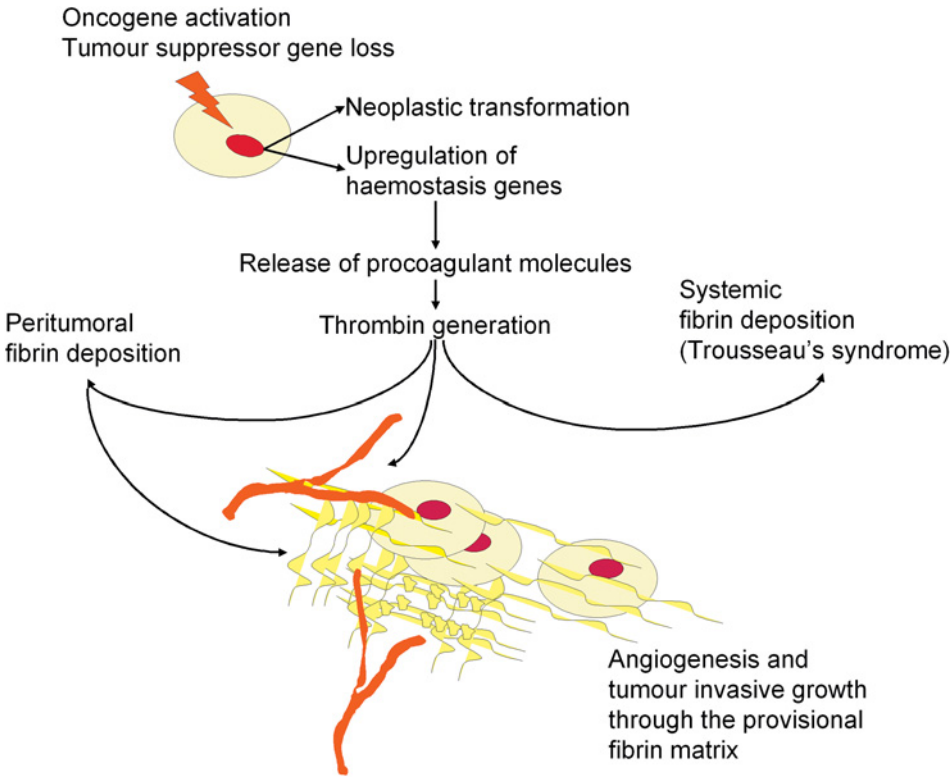

Figure 1. Oncogene activation or loss of tumour suppressor genes activate haemostasis genes. This is followed by the release of procoagulant molecules that induce local and systemic activation of the blood coagulation process, ending in thrombin formation and fibrin deposition. Peritumoral fibrin forms a quick-setting extracellular matrix that promotes angiogenesis and tumour invasive growth.

support for cell anchorage during local expansion and emigration, and a possible trail towards blood vessels. Second, the fibrin clot and the associated coagulation factors provide a highly pro-angiogenic environment [19]. In particular TF, which is critical for embryo vessel development [20], includes a cytoplasmic tail providing signals that upregulate vascular endothelial growth factor (VEGF) expression [21]. In addition to cleaving fibrinogen, thrombin cleaves and activates specific receptors (proteinase-activated receptors, or PARs), expressed by endothelium, platelets and other cells. These receptors induce vessel sprouting and morphogenesis, via mechanisms involving VEGF upregulation (for a review see [22, 23]). Thus, increasing evidence indicates that mechanisms of blood clotting can be targeted within the frame of a multifaceted therapeutical approach to cancer. The finding of a genetic link between oncogenic events and specific genes that modulate haemostasis (TF, COX-2 and PAI-1) highlights defined molecular targets. However, involvement of specific molecules in human cancer patients can be hard to assess, and the identified molecules can be difficult to target. At present, realistic approaches include direct inhibition of thrombin, or general interference with blood coagulation. Whatever the *primum movens* of haemostatic imbalance, thrombin is the end of the 'clotting factor cascade' and, as noted above, catalyses fibrin formation and stimulates cellular responses (angiogenesis) relevant for cancer growth. The use of specific thrombin inhibitors (such as hirudin) in experimental systems has shown that they can prevent cancer growth, metastasis and angiogenesis [24–26]. This should foster their employment in human clinical trials. Broad-spectrum inhibitors of blood coagulation such as low molecular weight heparins have already been tested in large clinical trials, displaying the ability not only to prevent haemostasis disturbances associated with cancer, but also cancer itself (reviewed in [27, 28]). The dialogue between research bench and bedside is thus (hopefully) destined to grow. What if the mechanism of action of heparins entails inhibition of molecules other than coagulation factors?

Acknowledgements. We thank P. M. Comoglio for constant advice and support, R. Srinivasan for editing the manuscript and A. Cignetto for excellent assistance. The work from the authors' laboratory is supported by AIRC, FIRB-MIUR, Ministero della Sanità and Regione Piemonte.

1 Rickles F. R. and Levine M. N. (2001) Epidemiology of thrombosis in cancer. Acta haematol. **106:** 6–12
2 Trousseau A. (1865) Phlegmasia alba dolens. In: Clinique Médicale de l'Hotel-Dieu de Paris, vol. 3, pp. 654–712, JB Ballière et Fils, Paris
3 Baron J. A., Gridley G., Weiderpass E., Nyrén O. and Linet M. (1998) Venous thromboembolism and cancer. Lancet **351:** 1077–1080

4 Billroth T. (1878) Lectures on Surgical Pathology and Therapeutics: A Handbook for Students and Practictioners, 8 edn., New Sydenham Society, London
5 De Cicco M. (2004) The prothrombotic state in cancer: pathogenic mechanisms. Crit. Rev. Oncol. Hematol. **50:** 187–196
6 Levine M. N., Lee A. Y. and Kakkar, A. K. (2003) From Trousseau to targeted therapy: new insights and innovations in thrombosis and cancer. J. Thromb. Haemost. **1:** 1456–1463
7 Mann K. G. (1999) Biochemistry and physiology of blood coagulation. Thromb. Haemost. **82:** 165–174
8 Dvorak H. F. (1986) Tumors: wounds that do not heal. NEJM **315:** 1650–1659
9 Yu J. L. and Rak J. W. (2004) Shedding of tissue factor (TF)-containing microparticles rather than alternatively spliced TF is the main source of TF activity released from human cancer cells. J. Thromb. Haemost. **2:** 2065–2067
10 Yu J. L., May L., Lhotak V., Shahrzad S., Shirasawa S., Weitz J. I. et al. (2005) Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood **105:** 1734–1741
11 Rong Y., Post D. E., Pieper R. O., Durden D. L., Van Meir E. G. and Brat D. J. (2005) PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. Cancer Res. **65:** 1406–1413
12 Boccaccio C., Sabatino G., Medico E., Girolami F., Follenzi A., Reato G. et al. (2005) The MET oncogene drives a genetic programme linking cancer to haemostasis. Nature **434:** 396–400
13 Trusolino L. and Comoglio P. M. (2002) Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat. Rev. Cancer **4:** 289–300
14 Durand M. K., Bodker J. S., Christensen A., Dupont D. M., Hansen M., Jensen J. K. et al. (2004) Plasminogen activator inhibitor-I and tumour growth, invasion and metastasis. Thromb. Haemost. **91:** 438–449
15 Kohler H. P. and Grant P. J. (2000) Plasminogen-activator inhibitor type 1 and coronary artery disease. NEJM **342:** 1792–1801
16 Smith W. L., De Witt D. L. and Garavito R. M. (2000) Cyclooxygenases: structural, cellular and Molecular Biology. Annu. Rev. Biochem. **69:** 145–182
17 FitzGerald G. A. (2003) COX-2 and beyond: approaches to prostaglandin inhibition in human disease. Nat. Rev. Drug Discov. **2:** 879–890
18 Look M. P., van Putten W. L., Duffy M. J., Harbeck N., Christensen I. J., Thomssen C. et al. (2002) Pooled analysis of prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in 8377 breast cancer patients. J. Natl. Cancer Inst. **94:** 116–128
19 Dvorak H. F. (2003) Rous-Whipple Award Lecture. How tumors make bad blood vessels and stroma. Am. J. Pathol. **162:** 1747–1757
20 Carmeliet P., Mackman N., Moons L., Luther T., Gressens P., Van Vlaenderen I. et al. (1996) Nature **383:** 73–75
21 Rickles F. R., Shoji M. and Abe K. (2001) The role of the hemostatic system in tumor growth, metastasis and angiogenesis: tissue factor is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. Int. J. Hematol. **73:** 145–150
22 Carmeliet P. (2001) Biomedicine. Clotting factors build blood vessels. Science **293:** 1602–1604
23 Nash G. F., Walsh D. C. and Kakkar A. K. (2001) The role of the coagulation system in tumour angiogenesis. Lancet Oncol. **2:** 608–613, 2001
24 Hu L., Lee M., Campbell W., Perez-Soler R. and Karpatkin S. (2004) Role of endogenous thrombin in tumor implantation, seeding and spontaneous metastasis. Blood **104:** 2746–2751
25 Im J. H., Fu W., Wang H., Bhatia S. K., Hammer D. A., Kowalska M. A. et al. (2004) Coagulation facilitates tumor cell spreading in the pulmonary vasculature during early metastatic colony formation. Cancer Res. **64:** 8613–8619
26 Caunt M., Huang Y. Q., Brooks P. C. and Karpatkin S. Thrombin induces neoangiogenesis in the chick chorioallantoic membrane. J. Thromb. Haemost. **1:** 2097–2102
27 Kakkar A. K. and Levine M. N. (2004) Thrombosis and cancer: implications beyond Trousseau. J. Thromb. Haemost. **2:** 1261–1262
28 Petralia G. A., Lemoine N. R. and Kakkar A. K. (2005) Mechanisms of disease: the impact of antithrombotic therapy in cancer patients. Nat. Clin. Pract. Oncol. **2:** 356–363