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REVIEW

Tissue factor as an effector of angiogenesis and tumor progression in hematological malignancies

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In the last few years, it has become clear that the processes of tumor angiogenesis, metastasis and invasiveness are highly dependent on components of the blood coagulation cascade. One of the key proteins in coagulation is tissue factor (TF). In addition, TF is also known as a mediator of intracellular signaling events that can alter gene expression patterns and cell behavior. TF significantly participates in tumor-associated angiogenesis and its expression levels have been correlated with the metastatic potential of many types of hematological malignancies. Signaling pathways initiated by both, tissue factor-activated factor VII (TF-FVII(a)) protease activation of protein-activated receptors (PARs), and phosphorylation of the TF-cytoplasmic domain, appear to regulate these tumoral functions. Advances in antiangiogenic therapies and preclinical studies with TF-targeted therapeutics are hopeful in the control of tumor growth and metastasis, but continued studies on the regulation of TF are still needed. In the last few years, the use of approaches of functional genomics and proteomics has allowed the discovery of new proteins involved in the origin of the neoplasia and their participation in the development of the disease. This review attempts to establish a cellular and molecular causal link between cancer coagulopathy, angiogenesis and tumor progression in hematological malignancies.
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General overview of TF

Tissue factor (TF) is a 47 kDa transmembrane glycoprotein, found on the surface of various cells and is the principal initiator of the coagulation cascade. The TF protein consists of 295 amino acids, from which 32 amino acids serve as a signal peptide for intracellular transport. The mature peptide consists of 263 amino acids with an extracellular domain (residues 1–219) that contribute to the binding of its natural ligand, the FVII(a): a membrane spanning domain (residues 220–242) necessary for stabilization of the molecule, and a cytoplasmic domain (residues 243–263), playing a key role in intracellular signaling.[1,2] Structurally, TF belongs to the class II cytokine receptor superfamily, sharing a significant degree of homology with the interferon class of receptors. Moreover, the fact that the intracellular part of TF contains two putative phosphorylation sites suggests a role for this protein in intracellular processes.[3] TF is a key player in blood coagulation; one of the consequences of the disruption of the vessel wall is the exposure of TF-expressing cells located in the underlying cell layers to the bloodstream, thus enabling binding of activated factor VII to TF. This protein complex then initiates the extrinsic coagulation pathway, thereby initiating the formation of a blood clot. In the last few years, it has become clear also that TF-mediated conversion of FIX to FIXa is pivotal for the activation of the intrinsic coagulation pathway. Thus, TF ensures that the extrinsic and intrinsic pathways operate simultaneously.[1,3] In the adult organism, TF is constitutively expressed in a variety of extravascular tissues. In addition, TF expression can be transiently upregulated by growth factors and cytokines in intravascular cells as endothelial cells and monocytes.[4] TF expression itself is under cell-type-specific control through different transcriptional pathways, which is also reflected by different activation patterns in response to different stimuli. In addition to its regulation of the clotting cascade, TF has recently been shown to participate in a variety of physiological processes distinct from hemostasis, including embryogenesis, inflammation, cellular signaling, cell migration, tumor growth, metastasis and angiogenesis.[4,5] Furthermore, recent investigations have revealed that intracellular activities induced by FVII(a) bound to cell surface TF may well be related to these events.[5–7] In addition, TF can often exerts its role independently from binding to its natural ligand, FVII(a)[8] (Figure 1).

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TF/FVII(a)-induced intracellular signaling

Binding of FVII(a) to cell surface-associated TF results in the production of intracellular signals through cytosolic calcium alteration, mitogen activated protein kinase (MAPK) phosphorylation, p38 MAPK phosphorylation, protein kinase B phosphorylation and upregulation of multiple genes, such as early growth response gene-1, Cyr61 and connective tissue growth factor gene (Table 1 and Figure 2). Signaling activities of TF are mainly mediated by the protein-activated receptors (PARs) family of G-protein-coupled receptors, in particular protease-activated receptor 1 (PAR1) and protease-activated receptor 2 (PAR2). To date, four subtypes of PAR have been identified in humans (named PAR1 to PAR4), whose expression has been detected in most tissues and in numerous cells.[9] In hematological malignancies, PAR3 has been shown to be upregulated by the induction of megakaryocyte phenotype in human erythroleukemia cells,[10] and PAR1 expression has been verified by flow cytometry analysis in the promyelocytic cell line HL-60,[11] and after induced differentiation in the monocytic cell line U937.[12] Recent studies have shown that the TF-VII(a) complex activates PAR2, and the product of initiation of coagulation, Xa, while still assembled in the transient ternary TF-VII(a)-Xa complex, signals through PAR1 or PAR2.[13] Moreover, PAR1 and PAR2 are

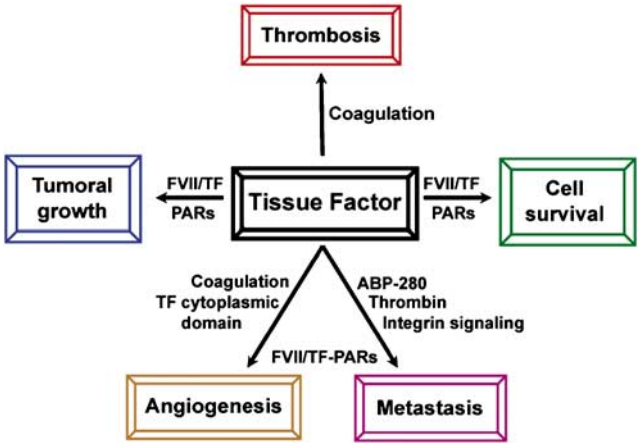

Figure 1 TF participates in numerous biological processes. Intracellular activities induced by Factor VII(a) bound to cell surface TF contributes to thrombosis, angiogenesis, tumor growth and metastasis.

coexpressed both in tumor cells and in cells of the tumor microenvironment, and their activation also contributes to a variety of biological processes, including inflammation, angiogenesis, metastasis and cell migration.[14] Finally, in some tumor cells it has been demonstrated that TF can also exerts its role independently of ligand activation, via its cytoplasmic tail, which can be phosphorylated *in vitro*, having the potential to transduce intracellular signals.[5,8]

Aberrant TF expression in hematological malignancies

It is now recognized that patients with malignant lymphoma, acute myeloid leukemia (AML) and multiple myeloma have a hypercoagulable state and frequently have associated thrombotic disorders during the clinical course of the disease.[15,16] Hypercoagulability in patients with hematological malignancies could be owing to the activation of TF, which may be tumor cell-derived or owing to the tumor-associated environment. In the early 1990s it was established that monocytes from lymphoma patients are endowed with functional abnormalities leading to the simultaneous expression of TF and antifibrinolytic activity.[17] Recent studies have further demonstrated that the incidence of severe complications, such as disseminated intravascular coagulation (DIC) in malignant lymphoma, differs between clinical stages and histological types of the disease, but they occur frequently in stage IV or natural killer (NK) cell lymphoma. Patients with stage IV or NK cell lymphoma exhibit abnormal thrombotic and hemostatic states. One of the mechanisms in DIC might involve elevated cytokine expression by lymphoma cells stimulating the expression of TF in blood cells or surrounding tissue.[16,18] It has been also demonstrated an increased expression of TF in AML cells, particularly in leukemic blast from subtypes M3 and M5. This overexpression accounted for an increased risk of thrombohemorragic alterations in these patients.[19,20] Some other hematological malignancies (such as multiple myeloma and myeloproliferative disorders) have also been described to be associated to the development of thrombotic events.[15] The prothrombotic status in these malignancies has been mostly attributed to procoagulant autoantibody production, acquired activated protein C resistance or altered platelet production and function.[21] However, to date, no relationship with TF expression has been reported.

Role of TF in tumor angiogenesis

The switch to an angiogenic phenotype requires a shift in balance between endogenous proangiogenic and antiangiogenic factors that regulate vessel growth and development. Aberrant expression of TF in tumors contributes to the angiogenic phenotype, in part, by upregulating the expression of the proangiogenic protein vascular endothelial growth factor (VEGF) and downregulating the expression of the antiangiogenic protein thrombospondin.[8] Moreover, some transcription factors, including specificity protein-1 (SP-1), activator protein-1 (AP-1) and nuclear factor-kappa B (NFκB), are involved in the regulation of both TF and VEGF, offering a potential mechanism for their colocalization and co-regulation in many tumors.[4] The accumulating data indicate that TF expressed by tumor cells as well as host cells initiates direct or indirect signaling events that support tumor angiogenesis[22] Coagulation activation produces proteolytic fragments of proteases, inhibitors and extracellular matrix components with potent regulatory effects on angiogenesis.[23] *In vivo*, TF may drive local thrombin generation and thus indirectly induce VEGF signaling either by paracrine PAR1 signaling in stromal cells or by PAR1 activation of tumor cell in an autocrine manner.[24]

The role of TF in tumor angiogenesis, however, is still controversial. TF plays a role in tumor angiogenesis at least in some tumors, but is definitely not the only player that contributes to vessel formation. Consistently, in those tumor cells that are incapable of expressing TF, VEGF transcription was not completely abolished. Thus, additional factors are operative in angiogenesis and tumor vascularization, which is consistent with the clinical observation that some but not all tumors show colocalization of TF and VEGF. The diversity of pathways identified to date illustrates the complexity of TF signaling, which, in addition, is dependent on the cell types.

Differential mechanisms of TF-induced tumor angiogenesis

TF contributes to tumor angiogenesis via both clotting-dependent and -independent mechanisms.[8] Clotting-dependent induction of tumor angiogenesis is primarily mediated by TF-induced generation of thrombin and subsequent deposition of crosslinked fibrin. A crosslinked fibrin network provides a provisional proangiogenic matrix that facilitates blood vessel infiltration. Clotting-independent pathways are primarily mediated by the constitutive and aberrant expression of TF observed in many tumor cells and associated vascular endothelial cells, and can be divided into three independent, although interconnected pathways (Figure 3) that appear to involve: (i) phosphorylation of the cytoplasmic domain of the TF receptor and subsequent downstream signaling events that occur independently of thrombin production or clot formation, and possibly even independently of ligand activation;[25] (ii) activation of intracellular signaling as a consequence of TF-FVII(a) interaction and subsequent actin reorganization and increased endothelial cell adhesion and migration;[26] and (iii) via thrombin generation, which can induce angiogenesis by cleaving the cell membrane-bound PARs, thus leading to the transcriptional activation of a number of genes that are involved in angiogenesis.[9] *In vivo*, PAR signaling has generally been attributed to activation by thrombin.[9] However, recent studies have provided clear evidence for relevant thrombin-independent, TF-mediated PAR signaling in angiogenesis *in vivo*.[27] In this context, the TF-dependent signaling occurs either by the TF-FVIIa protease complex specifically through PAR2[28,29] or by

Table 1 TF-FVII(a)-induced intracellular signaling and their contribution to pathophysiological processes

Intracellular signaling induced	*Cell type*	*Pathophysiological effects*	*References*
PAR1 and PAR2 activation			Bromberg[35]
	VSMC cells	Inflammation	Marutsuka, Thromb Res 107:271; 2002
	Macrophages	Angiogenesis	Shi[13]
	Various tumor cell types	Migration	Hjortoe[28]
		Tumor metastasis	Morris, Cancer Res 66:307; 2006
P42/44 MAPK phosphorylation– p21 ras/MAPK pathway activation	BHK cells	Cell survival	Sorensen, J Biol Chem 274:21349; 1999
	Fibroblasts	Tumor metastasis	Camerer[29]
	HaCaT keratinocytes		Versteeg, J Thromb Haemost 1:1012; 2003
Intracellular Ca^{2+} oscillations	Endothelial cells	Angiogenesis	Rottingen, J Biol Chem 270:4650; 1995
	J82 bladder carcinoma		
	MDCK cells		Camerer, J Biol Chem 271:29034; 1996
P38 ^{MAPK}/JNK phosphorylation	HaCaT keratinocytes	Inflammation	Camerer, J Biol Chem 274:32225; 1999
Pl3K/Akt/PKB pathway activation	BHK cells	Cell survival	Versteeg, J Biol Chem 275:28750; 2000
	Fibroblasts (A14 cells)		
	HaCaT keratinocytes		
STAT phosphorylation via Jak2 activation	BHK(TF) cells	Cell survival	Versteeg, Circ Res 94:1032; 2004
P70/p85(S6K) and p90(RSK) phosphorylation	BHK cells	Protein synthesis	Versteeg, J Biol Chem 277:27065; 2002
	HaCaT keratinocytes		
Gene transcripts: VEGF, uPAR, Egr-1, IL-8, etc	Fibroblasts	Angiogenesis	Ollivier, Blood 91:2698; 1998
	Keratinocytes	Tumor growth	Taniguchi, Cancer Res 58:4461; 1998
	Various tumor cell types	Tumor metastasis	Wang, J Biol Chem 277:23620; 2002
Altered expression of selected genes: CCN1 and CCN2	Fibroblasts	Cell adhesion, proliferation	Pendurthi, J Biol Chem 275:14632; 2000
	Keratinocytes	Angiogenesis, tumor metastasis	
		Wound healing	
Phosphorylation of the TF cytoplasmic domain	Endothelial cells	Angiogenesis	Ahamed[27]
	CHO/TF cells		

Abbreviations: HaCaT, human keratinocyte cell line; Jak2, Janus tyrosine kinase 2; JNK, Jun-N-terminal kinase; MAPK, mitogen activated protein kinase; PAR1, protease-activated receptor 1; PAR2, protease-activated receptor 2; TF, tissue factor; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells

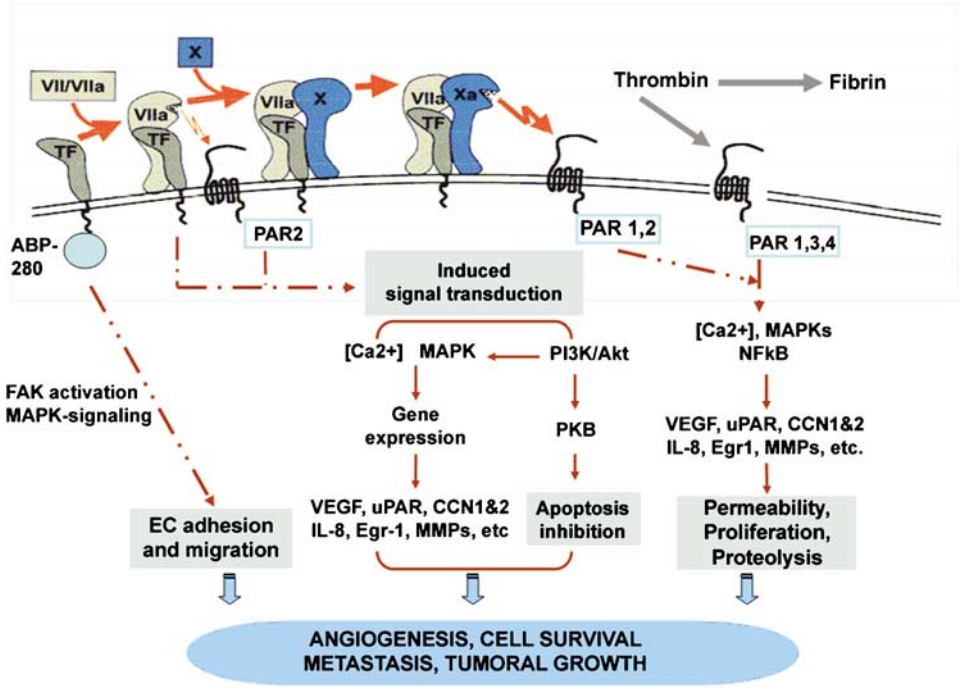

Figure 2 TF/FVIIa-induced intracellular signaling. Binding of FVII(a) to TF results in the activation of several MAPK pathways and subsequent gene transcription. PI3 kinase activation constitutes also an important branching point for further signal transduction routes, mainly related to anti-apoptotic processes. On the other hand, ABP-280 may play a role in TF-signaling as well since this protein has a high affinity for the TF cytoplasmic domain. In addition, TF-dependent generation of coagulation proteases activates multiple pathways involved in coagulation and signaling. The overall activation of this multiple cell signaling is responsible for the induction of angiogenesis, tumor growth and metastasis.

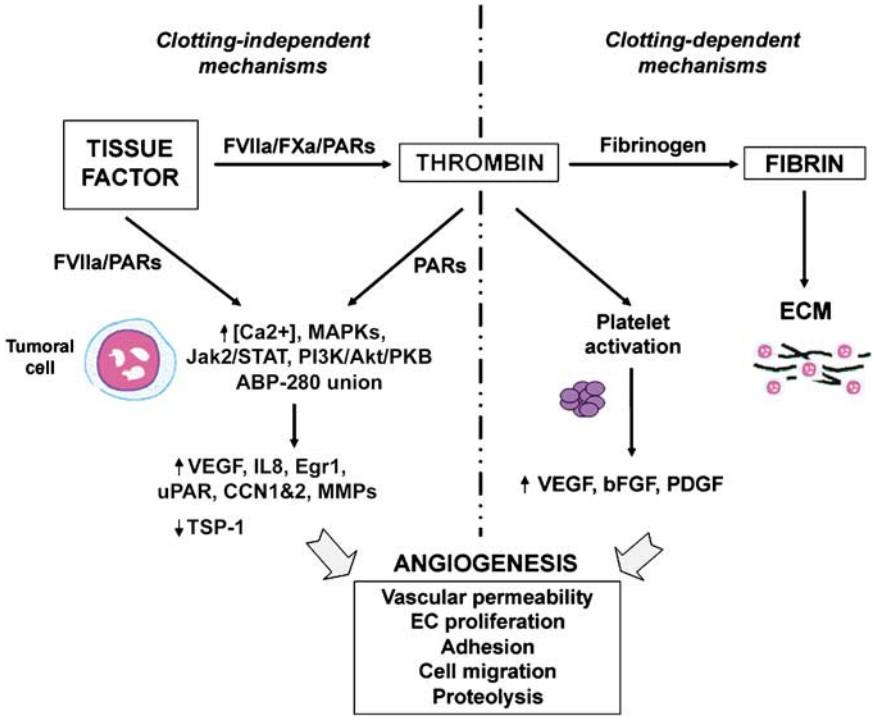

Figure 3 Differential mechanisms of TF-induced tumor angiogenesis. Both clotting-dependent and independent mechanisms induce angiogenesis. TF induces nonclotting-dependent mechanisms via phosphorylation of its cytoplasmic tail and subsequent signal transduction cascades. TF induces angiogenesis also via clotting-independent mechanisms by downstream generation of proteases such as thrombin, proteolytic cleavage of the PARs and subsequent activation of G-protein-coupled signal transduction cascades that induce angiogenesis-related genes. Clotting-dependent mechanisms induced by TF are mediated via fibrin deposition and platelet activation.

the nascent product Xa in the ternary TF-VIIa-Xa complex that signals through PAR2[30,31] as well as PAR1,[29–32] the first recognized thrombin receptor. The studies performed by Belting *et al* have further demonstrated that TF cytoplasmic domain plays a crucial role in these effects, so that its genetic deletion results in accelerated physiological and pathological angiogenesis. TF cytoplasmic domain exerts a potent negative regulatory control on PAR2 to prevent PAR2-dependent proangiogenic signaling. Because PAR2 signaling leads to TF phosphorylation, it is assumed that hyperphosphorylation of TF may shut off the angiogenesis-suppressive functions of the TF cytoplasmic domain in pathological angiogenesis.

Tumor progression: TF as an effector of metastasis

In the last few years, it has become clear that the processes of cancer metastasis and invasion are highly dependent on components of the blood coagulation cascade. As indicated previously, one of the key proteins in coagulation is TF. TF upregulation has frequently been associated with tumorigenesis, and tumor cells show a high procoagulant activity.[33] Moreover, the accumulating data indicate that TF expressed by tumor cells as well as hosts cells initiates direct or indirect signaling events that support tumor development by distinct mechanisms. A molecular pathway by which TF might support cell migration and cellular trafficking is the binding of the protein ABP-280 to the cytoplasmic domain of TF. Recruitment of ABP-280 results in the reorganization of actin filaments, cell spreading and migration.[25] These effects are mediated by interactions of the TF cytoplasmic tail with cytoskeletal adaptor proteins, and thus might explain the functional significance of the TF cytoplasmic domain in metastasis and vasculogenesis. On the other hand, the TF pathway inhibitor (TFPI) and its homologous Kunitz-type inhibitor, TFPI-2, which are associated with the extracellular matrix, interact with the TF-FVII(a) complex and thereby influence migratory and adhesive properties of tumor cells.[7] The TF pathway can also upregulate the expression of the urokinase-type plasminogen activator receptor (uPAR), a key factor in the plasminogen activation system that promotes the production of matrix-lysing enzymes, and which therefore may have a role in promoting tumor cell, migration and metastasis.[34] On the other hand, regulation of cell motility is a possible pathway by which TF directly influences tumor cell metastasis. Experimental hematogeneous metastasis is dependent on both TF-VIIa-driven thrombin generation as well as TF cytoplasmic domain signaling.[35,36] It has been recently demonstrated that the TF cytoplasmic domain, in its unphosphorylated state, inhibits the migration on laminin-5 that is dependent on the activation of integrin alpha-3β1. TF phosphorylation is specifically induced by PAR2 signaling, and TF-FVIIa-mediated activation of PAR2 is sufficient to release integrin inhibition in a pathway that required phosphorylation of the TF cytoplasmic domain. Thus, TF-VIIa signaling may simultaneously counteract integrin suppression by phosphorylating the TF cytoplasmic domain and locally triggering proinvasive PAR2 activation.[37] Finally, thrombin generation downstream of TF also plays a central role in the metastatic process, and thrombin's effects on tumor cells are generally assumed to be mediated by PAR1. Expression of PAR1 in tumor cells has been associated with increased tumor invasiveness and metastasis. However, a very recent study has shown that thrombin markedly enhances migration of metastatic tumor cells and that PAR1 activation is necessary but not sufficient. Rather, the simultaneous activation of PAR1 and PAR2 by thrombin is required to cause the chemokinetic effect.[13]

Angiogenesis in hematologic malignancies

Current studies indicate that angiogenesis is critical in the pathogenesis of numerous different hematologic malignancies, including acute leukemia, chronic leukemia, non-Hodgkin's lymphoma, myelodysplastic syndrome and multiple myeloma.[38–44] Moreover, some markers of angiogenesis such as increased expression of VEGF and basic fibroblast growth factor (bFGF) have been correlated with clinical characteristics in leukemia, serving as predictors of poor prognosis.[45] Angiogenesis is tightly regulated by proangiogenic and antiangiogenic molecules, which arise from cancer cells, stromal cells, endothelial cells, the extracellular matrix (ECM) and blood. Importantly, the relative contribution of these molecules is dependent on the tumor type and site, and their expression changes with tumor growth, regression and relapse.[46] The imbalance of angiogenic regulators (i.e., VEGF and angiopoietin) accounts for abnormal structure of tumor vessels, which in turn results in chaotic, variable blood flow and vessel leakiness, thereby lowering drug delivery and selecting more malignant tumor cells.[47]

In addition to stimulating the production of new blood vessels, it has been suggested that VEGF may be involved in complex autocrine and paracrine interactions in the bone marrow microenvironment. One possibility is that there may be paracrine interactions in which VEGF produced by neoplasic cells promotes endothelial cell migration and proliferation and that the endothelial cells, in turn, produce factors that promote tumor cell growth (Figure 4). This paracrine interaction would result in a positive feedback loop that may enhance both angiogenesis and tumor cell proliferation.[48,49] Co-expression of both VEGF and VEGF receptors in leukemia, lymphoma and multiple myeloma coupled with its direct effects on tumor cell survival migration, and proliferation, confirms the pivotal role for autocrine VEGF loops in the pathogenesis of these malignancies.[50] On the other hand, it should be mentioned that in addition to its role as an essential regulator of physiologic and pathologic angiogenesis, as demonstrated for TF, VEGF holds a number of recently proved additional roles. For example, it (i) triggers growth, survival and migration of leukemia and multiple myeloma cells;[51,52] (ii) plays a pivotal role in hematopoiesis and differentiation of multiple hematopoietic lineage;[53] (iii) inhibits maturation of dendritic cells;[54] and (iv) increases osteoclastic bone–resorbin activity as well as osteoclast chemotaxis.[55,56]

TF and angiogenesis in acute leukemia: molecular mechanisms involved

It has been demonstrated that the 'angiogenic switch' is triggered by oncogene-mediated tumor expression of angiogenic proteins including TF, VEGF and angiopoietin, as well as by metabolic stress, mechanical stress, genetic mutations, altered intracellular signaling and the immune response.[45,57] Actually, a number of different signaling routes seem to be triggered simultaneously in leukemia cells, with distinct and overlapping activities.[58,59] As stated above, TF significantly participates in tumor-associated angiogenesis and its expression levels have been correlated with the invasive and metastatic potential or many types of hematological malignancies.[6] Indeed,

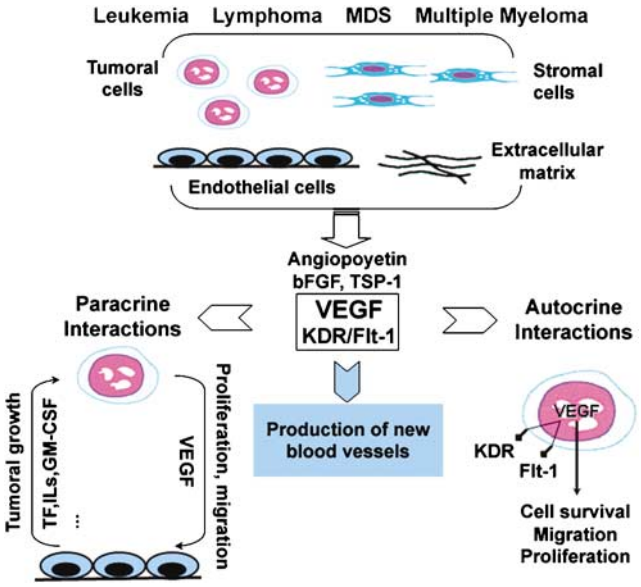

Figure 4 Angiogenesis in hematological malignancies. Angiogenesis is critical in the pathogenesis of several hematological malignancies, including leukemia, lymphoma, SMD and multiple myeloma. Pro- and antiangiogenic molecules secreted by tumoral, stromal and endothelial cells contribute to tumor growth and progression. Tumor-derived angiogenic factors like VEGF promote tumor angiogenesis. In addition, VEGF may be involved in complex autocrine and paracrine interactions in the bone marrow microenvironment. Paracrine processes involve secretion of multiple growth factors, cytokines and chemokines, including IL-6, VEGF and TNFα, as well as direct tumor-EC contact. Alternatively, VEGF may act in an autocrine fashion. Binding of VEGF to its receptors results in their dimerization and activation. Such activation of the receptors initiates a cascade of cellular events, leading to cell survival, proliferation and migration of neoplasic cells.

upregulation of VEGF and angiogenesis has been recently demonstrated in acute leukemia patients.[37,38] Both TF and VEGF can be induced by constitutive activation of oncogenic proteins such as Raf, MEK or PI3K, acting at various levels of the Ras signaling pathway.[60]

In that way, a recent study has investigated in a subset of acute leukemia samples the expression and activation status of TF, VEGF and two intracellular proteins involved in their regulation: the extracellular-regulated kinase (ERK1/2) and the NFκB (Barbarroja *et al*. *Pathophysiol Haemost Thromb* 2004: **33**; abstract). Significantly higher VEGF and TF-mRNA levels were found in the majority of the samples analyzed compared to controls, with a positive correlation between them. The extent of VEGF and TF expression varied among the different samples tested, but was higher in all subtypes of the AMLs FAB classification. That data correlated with previous studies showing that both TF and VEGF are co-expressed under similar conditions and in some solid tumors.[5] Concomitantly, fresh leukemia samples also expressed the VEGF receptor KDR, thus indicating, as above suggested, the possibility of an autocrine pathway in which the tumor cells may stimulate their own growth after VEGF exposure.[37,38] In addition, almost all the blast samples showing increased TF and VEGF levels also exhibited enhanced ERK phosphorylation in agreement with previous studies, which demonstrated that some transcription factors regulating TF can also induce VEGF transcription.[5] Accordingly, MAPK seems to be a common pathway that regulates, independently, VEGF and TF expression and, at the same time, is involved in the control of TF or VEGF expression induced by each other. In this study, it was also demonstrated that, of the investigated leukemia cases, NFκB was frequently constitutively activated, and that this activation correlated significantly with the increased expression of mRNA-TF. Many studies have shown that increased TF expression can be explained by induction of different transactivating transcription factors, such as members of the NFκB.[61,62] Moreover, a recent study has described the involvement of the Ras-induced Raf/MEK/ERK pathway in activating NFκB DNA binding and transactivation.[63] Thus, the simultaneous activation of the ERK pathway found in the leukemia samples could help to explain this concomitant activation. Furthermore, recent data suggest that in a variety of tumors, several NFκB target genes encode secreted growth factors that induce NFκB activation.[64] Thus, autocrine loops might be also important in the constitutive activation of NFκB in cancer. In this work, clinical studies demonstrated a higher incidence of recurrence and fatal outcome among patients in which a coordinated increase in the expression and activation of the above-mentioned proteins was observed. Both TF and VEGF, remarkably elevated and simultaneously expressed in blast AML cells, might determine an increase in tumor angiogenesis, thus linking both proteins to pathophysiological processes. Furthermore, the constitutive activation status of intracellular pathways such as MEK/ERK MAP kinase or NFκB, which are also linked to the activation of many other oncogenic proteins, may contribute to the adverse development of the disease. Activation of these pathways might be triggered by autocrine production of growth factors or by a positive synergistic relationship between leukemic and endothelial cells, through the paracrine production by each of mitogenic growth factors. Therefore, deregulation of TF and VEGF expression and their inducing signaling pathways seems to play an important role in the pathogenesis of acute leukemia.

Proteomic approaches to the altered expression of TF and VEGF in leukemia

From the facts above stated, it may be concluded that many elements of the coagulation/fibrinolytic system in general, and TF in particular, seem to be implicated in the regulation of angiogenesis, as well as tumor growth and metastasis in various tumoral models. TF expressed by cancer cells appears to act as both a regulatory target and an important mediator of oncogene-driven tumor growth and neovascularization. However, two main questions remain unanswered. First, what caused the upregulation of TF in human cancer cells, with the subsequent increase in their malignancy? Second, what are the consequences of TF expression related to the phenotypic changes induced by underlying 'cancer-causing' genetic alterations?

It has been demonstrated that TF expression by colorectal carcinoma cells is directly linked to their genetic status.[21] In recent years, a considerable research effort has been directed towards the identification of markers to guide an effective consideration of AML treatment. DNA microarray analysis has been applied to identify molecular markers of human hematological malignancies.[65] The use of approaches of functional genomics and proteomics has allowed the discovery of new proteins involved in the origin of the neoplasia and their participation in the development of the disease. Emerging studies have identified the changes in the proteasome associated with the action of Bcr-Abl tyrosine kinases in chronic myeloid leukemia,[66] protein targets of transcription factors involved in myeloid stem cell development and leukemia,[67] or new protein

expressed and/or activated in response to various anticancer reagents.[68–70]

In that way, in a recent study,[71] the protein profile of the AML subtypes has been analyzed, in an attempt to delineate the aberrant gene expression underlying the pathogenesis of this malignancy. The proteomic analysis, performed in blast from newly diagnosed acute myeloid leukemia patients, allowed to the identification of several proteins differentially expressed in the leukemia subtypes. Proteins identified as more significantly altered between the different AML subtypes belonged to the groups of suppressor genes, metabolic enzymes, antioxidants, structural proteins and signal transduction mediators. Among them, catalase, an antiapoptotic enzyme,[72] and annexin I, a calcium- and phospholipid-binding protein that specifically regulates signaling components of the Ras/MEK/ERK signal-transduction pathway,[73] were found to be expressed in M1, M3 and M5 AML samples at higher levels than in normal monocytes and than in other AML subtypes. On the contrary, tropomyosin, a family of cytoskeletal proteins whose inhibition contribute to the invasive and metastatic properties of cancer cells,[74] was found concomitantly reduced in these AML subtypes. Furthermore, complementary genetic and protein studies demonstrated that blast samples showing increased levels of annexin I and reduced levels of tropomyosin also exhibited enhanced ERK phosphorylation as well as increased expression of both TF and VEGF (Lopez-Pedrera *et al*. *Seminars in Proteomics* 2005; **2**: 215; abstract). Pathological changes in acute leukemia may thus be reflected in proteomic patterns in cells, and identification of these altered proteins represents a way of discovering new tumoral markers. The identification of novel proteins related to the hematological malignancies by proteomic approaches may enable to establish a comprehensive diagnostic approach that will yield a key to the precise pathobiologic nature of these neoplasias, and the spot of new therapeutic targets.

TF- and VEGF-targeted cancer therapy in hematological malignancies

Selective expression of TF on vascular endothelial cells and tumor cells of malignant but not benign tumors makes TF an ideal target for directed cancer therapeutics.

The direct targeting of this receptor with, for example, monoclonal antibodies, or inactivating peptides, has been already demonstrated to be efficacious in several models *in vivo*.[75,76] Although TF-targeted therapeutics have only been evaluated in animal models to date, phase I clinical studies are in progress; these trials represent a novel strategy that holds significant promise for treating patients with cancer. In addition, it is becoming increasingly evident that agents that interfere with blood vessel formation also block tumor progression, and accordingly, antiangiogenic therapy has gained much interest as a potential adjunct to conventional therapy of many hematological malignancies. Major principles of the antiangiogenic treatment include interference with the following elements of the angiogenic cascade: angiogenic stimulators (e.g. VEGF and bGFG), angiogenic factor receptors (e.g. VEGF-receptor signaling), extracellular matrix interactions (e.g. blocking of endothelial integrins), control of angiogenesis (e.g. inhibiting oncogenes controlling the angiogenic response) and proteolysis (e.g. inhibitors of MMP activity).[77]

At the moment, there are only a few clinical studies in hematologic malignancies underway, mainly based on the use of competitive inhibitors of VEGF receptors (SU11248) and antiangiogenic molecules (Thalidomide). SU11248 is an orally active multitarget inhibitor of Flt3, kit, VEGF receptors and PDGF receptors.[78,79] This kinase inhibitor has been recently evaluated in a phase 1 study in the treatment of patients with refractory or resistant AML or not amenable to conventional therapy for disease.[80] Monotherapy with SU11248 showed molecular and clinical activity in AML, although responses were of short duration. Thus, SU11248 shows promise in the treatment of AML, although future studies are still required to evaluate if the addition of this compound to conventional AML therapy can improve treatment outcome. Thalidomide, which has antiangiogenic effects and direct cytotoxic effects, has been found to be effective in multiple myeloma and is considered as an established treatment modality for patients with refractory or relapsed multiple myeloma. Thalidomide has also a significant therapeutic effect in myelodysplastic syndrome (MDS) by improving cytopenia and achieving independence of transfusion therapy in a subset of patients.[45] Moreover, partial responses to thalidomide treatment have been recorded in patients with lymphoma.[81]

In contrast to standard chemotherapeutic agents, antiangiogenic agents generally elicit few toxic side effects. However, an unexpected high incidence of both arterial and venous thrombosis has recently been reported in a number of clinical trials in which patients were treated with both antiangiogenic agents and standard chemotherapy.[82,83] Although any pathophysiologic study explains this high rate of thrombosis, it is possible that synergistic vascular toxicity might occur between antiangiogenic agents and chemotherapy drugs.[8] Thus, adding anticoagulants to combination drug regimens including agents that interact with the endothelium may help to prevent some of these thrombotic complications by blocking thrombin generation. In addition, antiangiogenic agents can be combined with other novel treatment strategies, such as immunologic approaches, and agents that interfere with signal transduction. Preclinical and clinical studies have demonstrated that a variety of clinically applicable cell cycle inhibitors (interferon, vitamin D, retinoids and bryostatin-1) preferentially augment growth factor-mediated induction of myeloid leukemia terminal differentiation, as well as blocks growth factors' effects on leukemia proliferation.[84] In that way, it has been recently shown that in acute promyelocytic leukemia (APL) cells, ATRA therapy inhibits VEGF production and suppresses angiogenesis.[49] It is well known that VEGF also stimulates TF production, which is significantly increased in this AML subtype as well. ATRA also reduces the procoagulant potential of malignant cells and modulates a number of hemostatic properties of normal endothelium and monocytes, including the increase in thrombomodulin (TM) expression and enhanced profibrinolytic functions. Moreover, it has been previously demonstrated that there is a parallel modulation of differentiation, TF and TM expression after retinoid treatment in promyelocytic cells.[85,86] Furthermore, retinoids are able to enhance the antithrombotic potential of vascular endothelial cells by inducing the synthesis of TM,[87] thus providing further evidence of their likely antithrombotic effects in tumors. Thus, the use of retinoids for their differentiating and angiogenic activities in conjunction with specific antiangiogenic agents such as anti-VEGF antibodies would be useful in ameliorating both the angiogenesis and the coagulopathy seen in APL and other types of myeloid leukemias.

Recent *in vitro* studies have also shown that treatment of leukemic cells with arsenic trioxide interrupts a reciprocal stimulatory loop between leukemic cells and endothelial cells by causing apoptosis in both cell types and by inhibiting leukemic cell VEGF production.[88] Furthermore, in APL cells

arsenic trioxide has been demonstrated to be effective in suppressing TF expression by blast cells, thus contributing to the improvement of the coagulopathy found in these patients.[89] Multiple events involved in the pathogenesis of acute leukemia and multiple myeloma coincide with pathways targeted by arsenic trioxide, and early results have suggested that clinical responses and safety in patients with advanced disease are promising.[90,91] Although these are just emerging *in vitro* studies, they open the possibility of using a combination of direct toxicity against leukemic cells with antiangiogenic activity, which may be the optimum characteristic of a successful antileukemic agent. Targeting one or more of these proteins may thus provide added benefit for control of tumor growth, while reducing the risks for serious thrombotic complications.

Summary

TF significantly participates in thrombosis and tumor-associated angiogenesis and its expression levels have been correlated with the invasive and metastatic potential of many types of hematological malignancies. Indeed, upregulation of VEGF and angiogenesis has been demonstrated in hematological neoplasias. However, the origin of constitutive TF and VEGF expression, as well as their association with other molecular markers relevant to the development of these neoplasias remains unknown. TF is also capable of transducing intracellular signals and regulating gene expression. Although TF does not seem to possess any overt cell autonomous transforming or growth regulatory properties, it clearly possesses the ability to modify tumor cell behavior *in vivo*, likely through interaction with host-derived entities. The two-way association between tumor-induced coagulation activation and tumor growth, angiogenesis and metastasis leaves open the possibility that these latter processes may be positively affected by some antiangiogenic and/or antithrombotic strategies. Emerging studies have identified the changes in the proteasome associated with the origin and/or the evolution of these neoplasias and have allowed the identification of altered proteins that represents new tumoral markers and might thus represent new therapeutic targets. Understanding these complex changes taking place at the molecular level – the molecular anatomy of the disease – should lead to the identification of precise prognostic markers and novel drug intervention sites and, ultimately, to a specific tailored therapy, individually designed for a patient based on his or her particular molecular anatomy.

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References

1 Versteeg HH, Peppelenbosch MP, Spek CA. The pleiotropic effects of tissue factor: a possible role for factor VIIa-induced intracellular signalling? *Thromb Haemost* 2001; **86**: 1353–1359.
2 Ruf W, Edgington TS. Structural biology of TF, the initiator of thrombogenesis *in vivo*. *FASEB J* 1994; **8**: 385–390.
3 Peppelenbosch MP, Versteeg HH. Cell biology of tissue factor, and unusual member of the cytokine receptor family. *Trends Cardiovasc Med* 2001; **11**: 335–339.
4 Drake TA, Morrisey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 1989; **134**: 1087–1097.
5 Chen J, Bierhaus A, Schiekofer S, Andrassy M, Chen B, Stern DM *et al.* Tissue factor – a receptor involved in the control of cellular properties, including angiogenesis. *Thromb Haemost* 2001; **86**: 334–345.
6 Versteeg HH, Spek CA, Peppelenbosch MP, Richel DJ. Tissue factor and cancer metastasis: the role of intracellular and extracellular signalling pathways. *Mol Med* 2004; **10**: 6–11.
7 Sampson MT, Kakkar AK. Coagulation proteases and human cancer. *Biochem Soc Trans* 2002; **30**: 201–207.
8 Rickles FR, Shoji M, Abe K. The role of the hemostatic system in tumor growth, metastasis, and angiogenesis: TF is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. *Int J Hematol* 2001; **73**: 145–150.
9 Coughlin SR. Thrombin signalling and protease-activated-receptors. *Nature* 2000; **407**: 258–264.
10 Cupit LD, Schmidt VA, Gnatenko DV, Bahou WF. Expression of protease activated receptor 3 (PAR3) is upregulated by induction of megakaryocyte phenotype in human erythroleukemia (HEL) cells. *Exp Hematol* 2004; **32**: 991–999.
11 Schiller H, Bartscht T, Arlt A, Zahn MO, Seifert A, Bruhn T *et al.* Thrombin as a survival factor for cancer cells: thrombin activation in malignant effusions *in vivo* and inhibition of idarubidin-induced cell death *in vitro*. *Int J Clin Pharmacol Ther* 2002; **40**: 329–335.
12 Naldini A, Sower L, Bocci V, Meyers B, Carney DH. Thrombin receptor expression and responsiveness of human monocytic cells to thrombin is linked to interferon-induced cellular differentiation. *J Cell Physiol* 1998; **177**: 76–84.
13 Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM. Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. *Mol Cancer Res* 2004; **2**: 395–402.
14 Yin Y-J, Salah Z, Maoz M, Cohen Even Ram S, Ochayon S, Neufeld G *et al.* Oncogenic transformation induces tumor angiogenesis: a role for PAR1 activation. *FASEB J* 2003; **17**: 163–174.
15 Tallman MS, Kwaan HC. Intravascular clotting activation and bleeding in patients with hematologic malignancies. *Rev Clin Exp Hematol* 2004; **8**: E1.
16 Sase T, Wada H, Yamaguchi M, Ogawa S, Kamidura Y, Nishikawa M *et al.* Haemostatic abnormalities and thrombotic disorders in malignant lymphoma. *Thomb Haemost* 2005; **93**: 153–159.
17 Semeraro N, Montemurro P, Giordano P, Santoro N, De Mattia D, Colucci M. Increased mononuclear cell tissue factor and type-2 plasminogen activator inhibitor and reduced plasma fibrinolytic capacity in children with lymphoma. *Thromb Haemost* 1994; **72**: 54–57.
18 Wada H, Sase T, Yamaguchi M. Hypercoagulant states in malignant lymphoma. *Exp Oncol* 2005; **27**: 179–185.
19 López-Pedrera CH, Jardí M, Malagón MM, Inglés-Esteve J, Dorado G, Torres A *et al.* Involvement of tissue factor (TF) and urokinase receptor (uPAR) in bleeding complications of leukemic patients. *Thromb Haemost* 1997; **771**: 62–70.
20 Nadir Y, Katz T, Sarig G, Hoffman R, Oliven A, Rowe JM *et al.* Hemostatic balance on the surface of leukemic cells: the role of tissue factor and urokinase plasminogen activator receptor. *Haematologica* 2005; **90**: 1549–1556.
21 Zangari M, Saghafifar F, Mehta P, Barlogie B, Fink L, Tricot G. The blood coagulation mechanism in multiple myeloma. *Semin Thromb Hemost* 2003; **29**: 275–282.
22 Yu JL, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz JI *et al.* Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood* 2005; **105**: 1734–1741.
23 Browder T, Folkman J, Pirie-Shepherd S. The hemostatic system as a regulator of angiogenesis. *J Biol Chem* 2000; **275**: 1521–1524.
24 Tsopanoglou NE, Maragoudakis ME. On the mechanism of thrombin-induced angiogenesis. Potentiation of vascular endothelial growth factor activity on endothelial cells by up-regulation of its receptors. *J Biol Chem* 1999; **274**: 23969–23976.
25 Abe K, Shoji M, Chen J, Bierhaus A, Danave I, Micko C *et al.* Regulation of vascular endothelial growth factor production and angiogenesis by the cytoplasmic tail of tissue factor. *Proc Natl Acad Sci USA* 1999; **96**: 8663–8668.

26 Ott I, Fischer E, Miyagi Y, Mueller BM, Ruf W. A role for TF in cell adhesion and migration mediated by interaction with actin-binding protein 280. *J Cell Biol* 1998; **140**: 1241–1253.
27 Belting M, Dorrel MI, Sandgren S, Aguilar E, Ahamed J, Dorfleutner A *et al.* Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat Med* 2004; **10**: 502–509.
28 Hjortoe GM, Petersen LC, Albrektsen T, Sorensen BB, Norby PL, Mandal SK *et al.* Tissue factor-factor VIIa-specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated by PAR-2 and results in increased cell migration. *Blood* 2004; **103**: 3029–3037.
29 Camerer E, Huang W, Coughlin SR. Tissue factor and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc Natl Acad Sci USA* 2000; **97**: 5255–5260.
30 Riewald M, Ruf W. Mechanistic couple of protease signaling and initiation of coagulation by tissue factor. *Proc Natl Acad Sci USA* 2001; **98**: 7742–7747.
31 Ahamed J, Ruf W. Protease-activated receptor 2-dependent phosphorylation of the tissue factor cytoplasmic domain. *J Biol Chem* 2004; **279**: 23038–23044.
32 Riewald M, Krawchenko VV, Petrovan RJ, O'Brien PJ, Brass LF, Ulevitch RJ *et al.* Gene induction by coagulation factor Xa is mediated by activation of protease-activated receptor 1. *Blood* 2001; **97**: 3109–3116.
33 Rickes FR, Hair GA, Zeff RA, Lee E, Bona RD. Tissue Factor expression in human leukocytes and tumor cells. *Thromb Haemost* 1995; **74**: 391–395.
34 Cantero D, Friess H, Deflorin J, Zimmermann A, Brundler MA, Riesle E *et al.* Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic carcinoma. *Br J Cancer* 1997; **75**: 388–395.
35 Bromberg ME, Sundaram R, Homer RJ, Garen A, Konisgsberg WH. Role of TF in metastasis functions of the cytoplasmic and extracellular domains of the molecule. *Thromb Haemost* 1999; **82**: 88–92.
36 Wang H, Fu W, Im JH, Zhou Z, Santoro SA, Iyer V *et al.* Tumor cell alpha3beta1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. *J Cell Biol* 2004; **164**: 935–941.
37 Dorfleutner A, Hintermann E, Tarui T, Takada Y, Ruf W. Crosstalk of integrin alpha-3,1 and tissue factor in cell migration. *Mol Biol Cell* 2004; **15**: 4416–4425.
38 Pérez-Atayde AR, Sallan SE, Tedrow U, Connors S, Allred E, Folkman J. Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. *Am J Pathol* 1997; **150**: 815–821.
39 Hussong JW, Rodgers GM, Shami PJ. Evidence of increased angiogenesis in patients with acute myeloid leukemia. *Blood* 2000; **95**: 309–313.
40 Aguayo A, Kantarjian H, Manshouri T, Gidel G, Estey E, Thomas D *et al.* Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. *Blood* 2000; **96**: 2240–2245.
41 Lundberg LG, Lerner R, Sundelin P, Rogers R, Folkman J, Palmblad J. Bone marrow polycythemia vera, chronic myelocytic leukemia, and myelofibrosis has an increased vascularity. *Am J Pathol* 2000; **157**: 15–19.
42 Vacca A, Ribatti D, Roncali L, Dammacco F. Angiogenesis in B cell lymphoproliferative diseases: biological and clinical studies. *Leukemia Lymphoma* 1995; **20**: 27–38.
43 Pruneri G, Bertolini F, Soligo D, Carboni N, Cortelezzi A, Ferrucci PF *et al.* Angiogenesis in myelodysplastic syndromes. *Br J Cancer* 1999; **81**: 1398–1401.
44 Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R *et al.* Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. *Blood* 1999; **93**: 3064–3073.
45 Moehler TM, Hillengass J, Goldschmidt H, Ho AD. Antiangiogenic therapy in hematologic malignancies. *Curr Pharm Des* 2004; **10**: 1221–1234.
46 Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**: 401–410.
47 Podar K, Anderson KC. The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. *Blood* 2005; **105**: 1383–1395.
48 Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T *et al.* Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. *Blood* 2001; **98**: 428–435.
49 Kini AR, Peterson LC, Tallman MS, Lingen MW. Angiogenesis in acute promyelocytic leukemia: induction by VEGF and inhibition by all-*trans* retinoic acid. *Blood* 2001; **97**: 3919–3924.
50 Dias S, Shmelkov SV, Lam G, Rafii S. VEGF(165) promotes survival of leukemic cells by Hsp-90-mediated induction of Bcl-2 expression and apoptosis inhibition. *Blood* 2002; **99**: 2532–2540.
51 Ria R, Roccaro AM, Merchionne F, Vacca A, Dammacco F, Ribatti D. VEGF and its receptors in multiple myeloma. *Leukemia* 2003; **17**: 1961–1966.
52 List AF. Vascular endothelial growth factor signaling pathway as an emerging target in hematologic malignancies. *Oncologist* 2001; **6**: 24–31.
53 Gerber HP, Malik AK, Solar GP, Sherman D, Liang XH, Meng G *et al.* VEGF regulates hematopoietic stem cell survival by an internal autocrine loop mechanism. *Nature* 2002; **417**: 954–958.
54 Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S *et al.* Production of VEGF by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 1996; **2**: 1096–1103.
55 Nakagawa M, Kaneda T, Arakawa T, Morita S, Sato T, Yomada T *et al.* Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. *FEBBS lett* 2000; **473**: 161–164.
56 Henriksen K, Karsdal M, Delaisse JM, Engsig MT. RANKL and vascular endothelial growth factor (VEGF) induce osteoclast chemotaxis through and ERK1/2 dependent mechanism. *J Biol Chem* 2003; **278**: 48745–48753.
57 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.
58 Lee JT, McCubrey JA. The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. *Leukemia* 2002; **16**: 486–507.
59 Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BRC-ABL in cell cycle progression and leukemogenesis. *Leukemia* 2004; **18**: 189–218.
60 Rak J, Yu JL, Klement G, Kerbel RS. Oncogenes and angiogenesis: signaling three-dimensional tumor growth? *J Invest Dermatol Symp Proc* 2000; **5**: 24–33.
61 Hall AJ, Vos HL, Bertina RM. Lipopolysaccharide induction of tissue factor in THP-1 cells involves Jun protein phosphorylation and nuclear factor kappaB nuclear translocation. *J Biol Chem* 1999; **274**: 376–383.
62 Oeth P, Parry GC, Mackman N. Regulation of the tissue factor gene in human monocytic cells. Role of AP-1, NFkappaB/Rel, and SP1 proteins in uninduced and lipopolysaccharide-induced expression. *Arterioscler Thromb Vasc Biol* 1997; **17**: 365–374.
63 Birkenkamp KU, Geugien M, Schepers H, Westra J, Lemmink HH, Vellenga E. Constitutive NFkappaB DNA-binding activity in AMLs is frequently mediated by Ras/PI3K/PKB-dependent pathway. *Leukemia* 2004; **18**: 103–112.
64 Lu T, Sathe SS, Swiatkowski SM, Hampole CV, Stark GR. Secretion of cytokines and growth factors as a general cause of constitutive NFkappaB activation in cancer. *Oncogene* 2004; **23**: 2138–2145.
65 Mano H. Stratification of acute myeloid leukemia based on gene expression profiles. *Int J Hematol* 2004; **80**: 389–394.
66 Smith DL, Evans CA, Pierce A, Gaskell SJ, Whetton AD. Changes in the proteasome associated with the action of Brc-Abl tyrosine kinase are not related to transcriptional regulation. *Mol Cell Proteomics* 2002; **11**: 876–884.
67 Behre G, Reddy VA, Tenen DG, Hiddemann W, Zada AA, Singh SM. Proteomic analysis of transcription factor interactions in myeloid stem cell development and leukemia. *Expert Opin Ther Targets* 2002; **6**: 491–495.
68 Seong JK, Kim do K, Choi KH, Oh SH, Kim KS, Lee SS *et al.* Proteomic analysis of the cellular proteins induced by adaptive concentrations of hydrogen peroxide in human U937 cells. *Exp Mol Med* 2002; **34**: 374–378.
69 Harris HN, Ozpolat B, Abdi F, Gu S, Legler A, Mawuenyega KG *et al.* Comparative proteomic analysis of all-*trans* retinoic acid treatment reveals systematic posttranscriptional control mechanisms in acute promyelocytic leukemia. *Blood* 2004; **104**: 1314–1323.

70 García-Echevarría C, Pearson MA, Hofmann F, Anderson KC, Kung AL. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell* 2004; **5**: 221–230.
71 López-Pedrera CH, Villalba JM, Siendones E, Barbarroja N, Gómez-Díaz C, Rodríguez-Ariza A *et al*. Proteomic analysis of acute myeloid leukemia: identification of potential early biomarkers and therapeutic targets. *Proteomics* 2006; **Suppl 1**: S293–S299.
72 Hachiya M, Akashi M. Catalase regulates cell growth in HL60 human promyelocytic cells: evidence for growth regulation by H_2O_2. *Radiat Res* 2005; **163**: 271–282.
73 Aldridge LC, Harris HJ, Plevin R, Hannon R, Bryant CE. The annexin protein lipocortin I regulates the MAPK/ERK pathway. *J Biol Chem* 1999; **274**: 37620–37628.
74 Shah V, Braverman R, Prasad GL. Suppression of neoplastic transformation and regulation of cytoskeleton by tropomyosins. *Somat Cell Mol Genet* 1998; **24**: 273–280.
75 Hu Z, Garen A. Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostatic cancers. *Proc Natl Acad Sci USA* 2001; **98**: 12180–12185.
76 Nilsson F, Kosmehl H, Zardi L, Neri D. Targeted delivery of tissue factor to the ED-B domain of fibronectin, a marker of angiogenesis, mediates the infarction of solid tumors in mice. *Cancer Res* 2001; **61**: 711–716.
77 Moehler TM, Neben K, Ho AD, Goldschmidt H. Angiogenesis in hematologic malignancies. *Ann Hematol* 2001; **80**: 695–705.
78 O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW *et al*. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* 2003; **101**: 3597–3605.
79 Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G *et al*. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targetins vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003; **9**: 327–337.
80 Fiedler W, Serve H, Döhner H, Shwittay M, Ottmann OG, O'Farrel AM *et al*. A phase I study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for disease. *Blood* 2005; **105**: 986–993.
81 Prentice HG, Sacchi S, Russel N. Future directions in hematology: beyond multiple myeloma. *Acta Hematol* 2005; **114**: 27–32.
82 Zangari M, Siegel E, Barlogie B, Anaissie E, Saghafifar F, Fassas A *et al*. Thrombogenic activity of doxorubicin in myeloma patients receiving thalidomide: implications for therapy. *Blood* 2002; **100**: 1168–1171.
83 Kuenen BC, Rosen L, Smith EF, Parson MR, Levi M, Ruijter R *et al*. Dose-finding and pharmacokinetic study of cisplatin, gemcitabine, and SU5416 in patients with solid tumors. *J Clin Oncol* 2002; **20**: 1657–1667.
84 Gorin NC, Estey E, Jones RJ, Levitsky HI, Borrello I, Slavin S. New developments in the therapy of acute myelocytic leukemia. *Hematology (Am Soc Hematol Educ Program)* 2000, 69–89.
85 López-Pedrera CH, Dobado-Berrios PM, Ros R, Torres A, García-Navarro S, Jardí M *et al*. Signal transduction pathways underlying the expression of tissue factor and thrombomodulin in differentiating promyelocytic cells induced to differentiate by retinoid acid and dibutyryl cAMP. *Thromb Haemost* 2001; **85**: 1031–1036.
86 López-Pedrera CH, Barbarroja N, Buendia Bello P, Torres A, Dorado G, Velasco F. Promyelocytic leukemia retinoid signaling targets regulate apoptosis, tissue factor and thrombomodulin expression. *Haematologica* 2004; **89**: 286–295.
87 Marchetti M, Vignoli A, Bani MA, Balducci D, Barbui T, Falanga A. All-*trans* retinoic acid modulates microvascular endothelial cell hemostatic properties. *Haematologica* 2003; **88**: 895–905.
88 Roboz GJ, Dias S, Lam G, Lane WJ, Soignet SL, Varrell RP *et al*. Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert and antileukemic effect via inhibition of angiogenesis. *Blood* 2000; **96**: 1525–1530.
89 Chen Z, Chen G-Q, Shen Z-X, Chen S-J, Wang Z-Y. Treatment of acute promyelocytic leukemia with arsenic compounds: *in vitro* and *in vivo* studies. *Semin Hematol* 2001; **38**: 26–36.
90 Shen Z-X, Shi Z-Z, Fang J, Gu B-W, Li J-M, Zhu Y-M *et al*. All-*trans* retinoic acid/As_2O_3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci* 2004; **101**: 5328–5335.
91 Anderson KC, Boise LH, Louie R, Waxman S. Arsenic trioxide in multiple myeloma: rationale and future directions. *Cancer J* 2002; **8**: 12–25.