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Review

Matrix metalloproteinases in bone marrow: roles of gelatinases in physiological hematopoiesis and hematopoietic malignancies

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Summary. Turnover balance of extracellular matrix (ECM) is a prerequisite for the structural and functional homeostasis of bone marrow (BM) microenvironment. The role of ECM in physiologic hematopoiesis and its pathologic change in hematopoietic malignancies are very important and under extensive investigation. Accumulating evidence suggests that matrix metalloproteinases (MMPs), a family of zinc-dependent proteinases, take an active part in the physiological and pathological hematopoiesis through remodeling the ECM in BM hematopoietic microenvironment. In this review, we will focus on the roles of MMPs in physiological hematopoiesis, hematopoietic stem cells mobilization/transplantation, and hematological malignancies. Furthermore, the preclinical studies on the role of synthetic MMP inhibitors in the treatment of hematological malignancies will be discussed.

Key words: Matrix metalloproteinases, Metalloproteinase inhibitors, Gelatinase, Hematopoiesis, Hematological malignancies

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral endopeptidases which can degrade various components of the extracellular matrix (ECM) and basement membrane. MMPs play important roles in multiple physiological and pathological processes including embryo development, wound healing, angiogenesis, immunity, inflammation, tumor invasion and metastasis (Klein, 1995; Moses, 1997; John and Tuszynski et al., 2001; Beaudeux et al., 2004). Bone marrow (BM), hosting heterogenous cell populations, cytokines, growth factors and ECM, is the major reservoir for hematopoietic stem cells (HSCs). In BM hematopoietic microenvironment, ECM is mainly made up of collagens (type I, III, IV, V), fibronectin, vitronectin, laminin, thrombospondin, glycosaminoglycans and proteoglycans (Janowska-Wieczorek et al., 2000a). MMPs participate in the turnover of ECM in the hematopoietic microenvironment regulating the release of HSCs and mature leukocytes from BM into peripheral blood (PB).

Hematological malignancies are clonal disorders resulting from the neoplastic transformation of blood progenitor cells. There are a few reviews discussing the roles of MMPs in the pathogenesis and progression of hematological malignancies, including acute and chronic lymphoblastic/myeloid leukemia, Hodgkin's and non-Hodgkin's lymphomas, myeloma, and myelodysplastic syndromes (Guedez et al., 1996; Ries et al., 1999; Beaudeux et al., 2004; Klein et al., 2004). Here, for the first time, we will systematically discuss the role of MMPs, especially gelatinases, in the regulation of normal and pathological hematopoiesis, HSCs mobilization/transplantation, and explore the potential of MMP inhibitors in the treatment of related diseases.

Classification and structural characteristics of MMPs

Based on the substrate specificity and primary structure, human MMPs family (over 20 identified at present) is divided into five classes: collagenases, gelatinases, stromelysins, membrane type MMPs (MT-MMPs) and novel MMPs (Table 1).

Most MMPs share several conserved domains, including hydrophobic signal peptide for secretion, propeptide domain rendering enzyme latency, catalytic domain with a highly conserved zinc-binding site, and hemopexin-like domain (HLD) linked to the catalytic domain with a short hinge region (Fig. 1). Additionally,

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gelatinases (MMP-2, also called gelatinase A, and MMP-9, also called gelatinase B) contain repeats of fibronectin type II-like domain which interact with collagen and gelatin. MMP-23 has no signal peptide and the hemopexin-like domain is substituted by a cysteine-rich/praline-rich and interleukin-1 receptor-like motif (Ohnishi et al., 2001). MMP-7 (matrilysin) and MMP-26 (matrilysin-2), the smallest MMPs, lack the hinge region and hemopexin-like domain. MT-MMPs contain a transmembrane domain at the carboxy-terminal end of the hemopexin-like domain, anchoring them to the cell surface.

Regulation of MMPs

The expression of most MMPs is not present at high levels in normal tissues and their activity is highly regulated on three levels: transcriptional regulation, latent precursor activation and proteolysis activity inhibition by natural inhibitors (Sternlicht and Werb, 2001). Biochemical stimuli, including cytokines, growth factors, hormones, cell matrix components, and cell-cell contacts can modulate the expression of MMPs at transcriptional level (Ries and Petrides, 1995). Several *cis*-acting elements (AP-1, PEA3, κB) upstream of the promoter participate in the transcriptional regulation of MMPs (Borden and Heller, 1997; Westermarck et al., 1997; Bond et al., 1998). Moreover, the expression of gelatinases (MMP-2, MMP-9) is also regulated by post-transcriptional modification (Overall et al., 1991; von Luttichau et al., 2002).

MMPs are generally expressed as latent precursors in soluble or membrane bound form (Nagase and Woessner, 1999). Activation is achieved via proteolysis, induced by extracellular proteinases including plasmin and urokinase, to remove the amino-terminal propeptide domain and displace the cysteine-zinc interaction with a water-zinc interaction, termed "cysteine-switch" mechanism (Van Wart and Birkedal-Hansen, 1990). Some activated MMPs can further activate other pro-MMPs, constituting a positive feedback.

With transmembrane domain, MT-MMPs can act as

Table 1. Human matrix metalloproteinase family.

GROUP	TRIVIAL NAME	SUBSTRATES
collagenases		
MMP-1	Interstitial collagenase	type I, II, III, VII and X collagen
MMP-8	Neutrophil collagenase	type I, II, III, VII and X collagen
MMP-13	Collagenase-3	type I, II, III, VII, X and XIV collagen. Tenascin, fibronectin, aggrecan core protein
Gelatinases		
MMP-2	Gelatinase A	type I, IV, V, X collagen, gelatin, laminin V
MMP-9	Gelatinase B	type I, II, V collagen, gelatin, laminin V
Stromelysin		
MMP-3	Stromelysin-1	type III, IV, VII, IX and X collagen, gelatin, elastin, laminin, proteoglycan core proteins, pro-MMP-1, fibronectin
MMP-10	Stromelysin-2	type III, IV, VII, IX and X collagen, gelatin, elastin, laminin, proteoglycans core proteins, pro-MMP-1, fibronectin
MMP-11	Stromelysin-3	serine-proteinase inhibitors, α2-antitrypsin, α1-proteinase inhibitor
MMP12	metalloelastase	elastin, type IV collagen, fibronectin, vitronectin, laminin
MMP-7	matrilysin	elastin, type IV collagen, fibronectin, laminin, nidogen, proteoglycan core proteins
MMP-23		
MMP-26	matrilysin-2	type IV collagen, fibronectin, fibrinogen, gelatin, pro-MMP-9
MT-MMP		
MMP-14	MT1-MMP	pro-MMP-2, pro-MMP-13, gelatin, fibronectin, Laminin, type I collagen
MMP-15	MT2-MMP	pro-MMP-2, pro-MMP-13, gelatin, fibronectin, tenascin, laminin
MMP-16	MT3-MMP	gelatin, type III collagen, pro-MMP-2, fibronectin
MMP-17	MT4-MMP	pro-MMP-2, gelatin
MMP-24	MT5-MMP	pro-MMP-2, gelatin
MMP-25	MT6-MMP	type IV collagen, fibronectin, gelatin, fibrin
Novel MMP		
MMP-19	RAS-1	not known
MMP-27		not known
MMP-28	epilysin	gelatin

receptors and activators for other MMPs and serve to localize extracellular matrix proteolysis at the pericellular region (Morgunova et al., 1999; Murphy et al., 1999). MT-MMPs can activate pro-MMP-2, and in the case of MT1-MMP, this process requires the participation of small amounts of tissue inhibitors of the metalloproteinase 2 (TIMP-2) as shown in Figure 2 (Butler et al., 1998; Kayano et al., 2004). Like other human MMPs, MT1-MMP is synthesized as a zymogen which can be activated intracellularly by furin or extracellularly by plasmin (Nagase and Woessner, 1999; Yana and Weiss, 2000). A model for the activation of pro-MMP-2 on the cell surface presumes that the catalytic domain of MT1-MMP binds to the N-terminal portion of TIMP-2, leaving the C-terminal section of TIMP-2 available for binding with the HLD of pro-MMP-2. The formation of MT1-MMP/TIMP-2/pro-MMP-2 ternary complex facilitates the partial cleavage of the pro-MMP-2 propeptide domain by a vicinal TIMP-2-free MT1-MMP. The intermediate MMP-2 is fully activated by further autoproteolysis or plasmin (Sato et al., 1996; Bulter et al., 1998; Jo et al., 2000). However, superfluous TIMP-2 may inhibit the activation by blocking all free MT1-MMP molecules. In addition, MT2-MMP can activate pro-MMP-2 in a TIMP-2-independent mode (Morrison et al., 2001).

A number of studies have revealed complex molecular interactions between MMPs and PA/plasminogen system. MMP-3, 7, 9, 12 cleave plasminogen to generate angiostatin-like fragments with receptor-binding domains (Dong et al., 1997; Lijnen et al., 1998). MMP-3 and MMP-7 can also process u-PA, PAI-1, α_2-antiplasmin, fibrinogen and cross-linked fibrin (Bini et al., 1996, 1999; Ugwu et al., 1998; Lijnen et al., 2000). Therefore, MMPs may be implicated in the regulation of cellular fibrinolysis by the proteolysis of PA/plasminogen components. On the other hand, plasmin directly activates several pro-MMPs, including pro-MMP-1, -3, -9, -10, -12, -13 and pro-MT-MMP1 (Baramova et al., 1997; Devy et al., 1997; Nagase, 1997; Okumura et al., 1997). Plasmin can active pro-MMP-9 and pro-MMP-2 without the action of other MMPs, and this process is accelerated by the binding of u-PA or plasmin to the cell surface. In addition, PA/plasmin system further activates pro-MMP-2 by converting the intermediate form of MMP-2 into a mature, active form (Baramova et al., 1997). Furthermore, plasmin can down-regulate gelatinase activity by degrading both MMP-9 and MMP-2 in the soluble phase (Mazzieri et al., 1997). Therefore, PA/plasmin system is a physiological modulator of gelatinase activity in the extracellular microenvironment.

The proteolytic activity of MMPs can be inhibited by natural inhibitors, including specific tissue inhibitors of the metalloproteinases (TIMPs), TIMP-like molecules (such as tissue factor pathway inhibitor 2, TFPI-2), thrombospondin-2 (TSP-2), α1-antiprotease, α2-macroglobulin and endostatin (Baker et al., 2002; Kim et al., 2000). The balance of MMPs/TIMPs is critical for the maintenance of ECM homeostasis. The four known TIMPs (TIMP-1, -2, -3 and -4) form non-covalent high affinity complexes with all active MMPs as well as some pro-MMPs, thereby inhibiting the activity of MMPs and the activation of pro-MMPs (Baker et al., 2002).

MMPs in physiological hematopoiesis

During normal hematopoiesis, the proliferation, differentiation and migration of HSCs are strictly regulated by their complex interactions with the

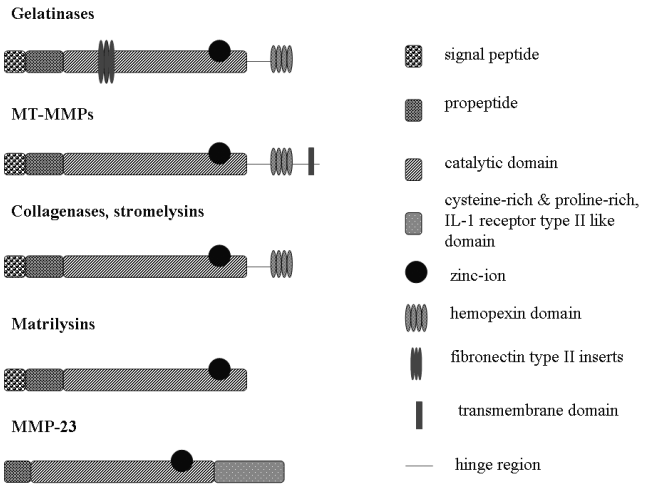

Fig. 1. General structure of the human matrix metalloproteinases (MMPs).

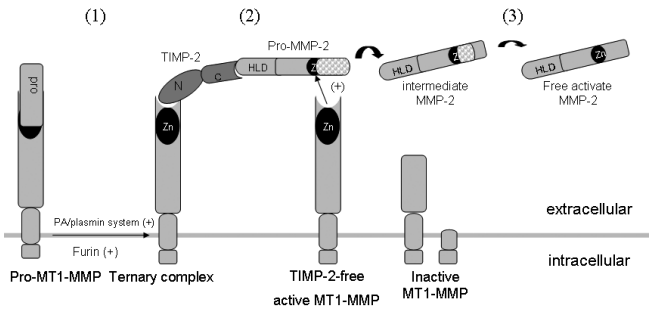

Fig. 2. Model of pro-MMP-2 activation by MT1-MMP. (1) pro-MT1-MMP can be activated extracellularly by plasmin or intracellularly by furin. (2) The complex of TIMP-2 and active MT1-MMP serves as a "receptor" for pro-MMP-2 on the cell surface. The formation of ternary complex (MT1-MMP/TIMP-2/pro-MMP-2) facilitates the partial cleavage of the pro-MMP-2 propeptide domain by a neighboring TIMP-2-free active MT1-MMP. MT1-MMP can be autoproteolysis to an inactive form without the catalytic domain or the entire extracellular fraction. (3) Full activation of the intermediate form MMP-2 is achieved by an autocatalytic process or the action of plasmin.

surrounding BM microenvironment, such as marrow stromal cells, cytokines and extracellular matrix proteins. Under steady-state conditions, most stem cells are maintained in G_0 phase of cell cycle by contact with BM stromal cells (Cheng et al., 2000). Release of cytokines and growth factors from ECM/cell membrane by MMPs can alter the stem cells-stromal cells interaction and promote HSCs migration and differentiation (Bergers et al., 2000).

In normal BM tissue, both gelatinases (MMP-2 and MMP-9) are detected in myeloid cells and megakaryocytes, and MMP-2 is also detected in erythroblasts (Ogawa et al., 2000). Marquez-Curtis et al. (2001) investigated the production of MMPs in long-term marrow cultures (LTMCs) established from normal donors and found that pro-MMP-9 and pro-MMP-2 were constitutively expressed in LTMC and the expression of pro-MMP-9 decreased while pro-MMP-2 increased with culture time. In addition, MT1-MMP, MT2-MMP, MT3-MMP and MT4-MMP could be detected at different levels in erythroid, megakaryocytic and myeloid precursors expanded from normal BM $CD34^+$ cells. These MMPs may play an important role in intercellular cross-talk in hematopoiesis.

Although the precise role of MMPs in the normal hematopoiesis remains to be elucidated, it appears that they may participate in the hematopoietic reconstitution after BM suppression. Both pro-MMP-9 and MMP-9 are up-regulated in BM cells after 5-FU-induced BM ablation. Active MMP-9 cleaves stromal cells membrane-bound KitL (mKitL) to a soluble form (sKitL), enhancing the proliferation and motility of HSCs and thereby promoting the hematopoietic recovery (Heissig et al., 2002; Rafii et al., 2003).

MMPs are also concerned with thrombopoiesis. Stromal cell derived factor 1 (SDF-1) can promote the transendothelial migration of megakaryocytes (MKs) and enhance platelet production through inducing the expression of MMP-9 in mature MKs (Lane et al., 2000; Majka et al., 2000). Incubation of mature MKs with synthetic MMP inhibitor, 5-phenyl-1, 10-phenanthrolene, resulted in the inhibition of platelet production, suggesting that the expression of MMPs is not only critical for MKs migration but also important in subsequent platelet release.

Briefly, MMPs modulate the proliferation, differentiation, and migration of hematopoietic stem/progenitor cells of different lineages through ECM proteolysis and growth factors/cytokines release. Therefore, inhibition of MMPs provides a novel approach for hematopoiesis regulation in myelo-proferative or thrombotic disorders.

MMPs in the mobilization and transplantation of HSCs

HSCs transplantation is one of the most effective treatments for hematological malignancies and hereditary metabolic disorders. The process of homing plays key roles during HSCs transplantation and hematopoietic reconstitution. Following transplantation, HSCs home to and lodge in the specific "niches" of the BM environment and start hematopoietic reconstitution (Quesenberry and Becker, 1998). Mobilization of HSCs, mediating their release from BM to PB, is widely used to get enough HSCs. MMPs have been proven to take active part in the important process of HSCs mobilization and homing.

MMPs are indispensable in HSCs mobilization induced by a number of agents, including growth factors, interleukins, chemokines and chemotherapeutic drugs (Lapidot and Petit, 2002). Up-regulation of gelatinases (MMP-2 and MMP-9) by GM-CSF, G-CSF, SCF, TNF-α, MIP-1α, IL-6 and IL-8 stimuli increases the transmigration activity of $CD34^+$ cells in Matrigel model, suggesting that these growth factors and cytokines may mobilize HSCs from BM into PB through gelatinase pathways (Janowska-Wieczorek et al., 1999b, 2000b; Carstanjen et al., 2002). IL-8, a chemoattractant and activator of neutrophils, can rapidly induce HSCs mobilization within a few hours, which relies on the action of MMP-9, since anti-MMP-9 antibody significantly reduced HSCs mobilization in rhesus monkey models (Pruijt et al., 1999). Similarly, the synergistic mobilization effect of G-CSF and chemokines GROß/$GRO\beta_T$ is mediated through polymorphonuclear neutrophil (PMN)–derived MMP-9 (van Os et al., 2002; Pelus et al., 2004). Pertussis toxin (Ptx) mobilizes HSCs via G-protein signaling pathway, which can be dramatically enhanced with the combination of G-CSF in a MMP-9-dependent manner (Papayannopoulou et al., 2003). However, IL-8 or G-CSF-induced HSCs mobilization is normal in $MMP\text{-}9^{-/-}$ mice, suggesting the presence of other MMP-independent HSCs trafficking pathway (Pruijt et al., 2002; Levesque et al., 2004).

MMPs are also vital for the homing of transplanted HSCs to BM. SDF-1, a CXC chemokine, is a key regulator of HSCs migration and homing. SDF-1 regulates the production of MMPs, specifically gelatinases (MMP-2, MMP-9) to enhance the migration of HSCs. On the other hand, gelatinases could inactive SDF-1 by the cleavage at its NH_2-terminus (McQuibban et al., 2001). Clear correlation has been found between the number of circulation $CD34^+$ cells and MMP-9 plasma levels both in steady condition and mobilization (Carion et al., 2003). Cord blood (CB)/PB-derived $CD34^+$ cells, regardless of mobilization, express high level of gelatinases. CB $CD34^+$ cells with higher MMP-9 expression show higher migration capacity than BM $CD34^+$ cells. And the migration of $CD34^+$ cells over ECM can be inhibited by MMP inhibitor or anti-MMP-9 monoclonal antibody (Voermans et al., 1999; Rao et al., 2004). Moreover, CB $CD34^+$ cells having homed to BM after transplantation strongly express both gelatinases (Zheng et al., 2003). And pretreatment of CB $CD34^+$ cells with specific MMP inhibitor KB8301 can completely block human cell engraftment in

xenotransplanted NOD/SCID recipients (Zheng et al., 2005).

In conclusion, MMPs function as the mediator of HSCs mobilization induced by various stimuli and homing process through releasing HSCs from matrix attachment, facilitating HSCs migration, and unclear mechanisms. Therefore, MMPs may be a future target for the modulation of stem cell mobilization and transplantation.

Gelatinases and hematological malignancies

The balance of ECM turnover is crucial for maintaining the structural and functional homeostasis of bone marrow. The alteration in the expression or activity of MMPs will destroy the hematopoietic micro-environment resulting in hematopoiesis aberration (Freireich, 1984). Leukemia can be characterized as a prototype of disseminated cancer with uncontrolled proliferation of immature hematopoietic cells in BM. Malignant transformation of the HSCs results in a blockade in their ability to terminally differentiate, causing a rapid accumulation of immature proliferative cells (Sawyers et al., 1991). The abnormal blood cell aggregation may induce a collapse of cell-stroma interaction, leading to the subsequent egress of excessive immature cells from BM into PB and the infiltration to extramedullary organs.

Recent studies imply a crucial role of MMPs in the progression of hematological malignancies. Most studies performed so far have focused on the roles of gelatinases (MMP-9 and MMP-2) in leukemia, and the role of other MMPs is not extensively investigated (Fig. 3). Like in other cancer, MMPs participate in the main processes of leukemia progression through ECM proteolysis and growth factors/cytokines release (Klein et al., 2004). However, MMPs have dual effects. Numerous fragments of ECM (e.g. tumstatin and endostatin), fragments of plasminogen (e.g. angiostatin) and fragments of MMPs (e.g. PEX) inhibit angiogenesis and ensued metastasis (Klein et al., 2004), which may partially account for the discrepancy of MMPs expression levels in different leukemic cell lines and patients. Human leukemic cell lines are important for the in vitro study of MMPs in hematopoietic malignances, on the base of which, more research works are focused on the role of MMPs in the leukemia patients.

MMPs in leukemia cell lines

Human promyelocytic HL-60, which constitutively secret considerable pro-MMP-9 and moderate pro-MMP-2, is the most commonly used cell line for acute leukemia differentiation-induction research (Devy et al., 1997). Butyric acid (BA), all-trans-retinoic acid (ATRA) and aclarubicin (ACLA) can induce the granulocytic/monocytic differentiation of HL-60, accompanied by increased in vitro migration/invasion as well as enhanced MMP-9 expression/activation (Devy et al., 2002; Richard et al., 2002a,b). Reactive oxygen species (ROS) has been implicated in ACLA and BA-induced differentiation and MMP-9 overexpression in HL-60. Enhanced MMP-9 expression is also associated with phorbol 12-myristate 13-acetate (PMA)-induced macrophage differentiation of HL-60, suggesting that the proteolytic activity of MMP-9 may be necessary for macrophage functions, such as extravasation, migration and tissue remodeling (Xie et al., 1998a,b).

As compared with HL-60, K562 (erythroleukemia cell line) exhibits four times higher invasive capacity. Both cell lines produce similar amounts of MMP-2 and MMP-9, but only K562 cells express MMP-7 (Matrilysin), suggesting a potential role of MMP-7 in leukemia invasion (Lynch and McDonnell, 2000). Although an enhanced expression of latent/active form of gelatinases is detected in stimulated NB4 (promyelocytic), PL-21 (myeloid) and KG-1 (myeloid) cell lines, the invasion activity of these cells is mainly related to the level of active MMP-2 (Sawicki et al., 1998; Shibakura et al., 2002).

In addition to the myeloid leukemic cell lines, seven T-leukemia cell lines representing different stages of differentiation have been analyzed for their migration and invasion properties (Ivanoff et al., 1999). These cell lines produce diverse amounts and types of MMPs. However, there is no correlation between MMPs level and migration activity in these distinct stage cell lines, suggesting that the function of MMPs in lymphocyte infiltration is more complex than we thought.

Therefore, MMPs expression may be a phenotypic marker for differentiation and invasion of leukemia cells. Except these in vitro cell models, the precise roles of MMPs, especially gelatinases in the pathogenesis of hematological diseases have been extensively studied in patients.

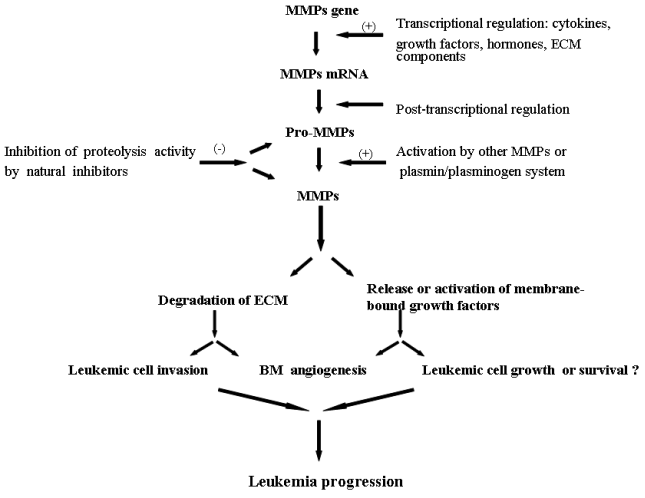

Fig. 3. A schematic overview of the regulation of MMPs and their roles in leukemia progression. (+): positive regulation; (-): negative regulation.

Acute myelogenous leukemia (AML)

MMP-9 expression has been detected in the conditioned media of acute myelogenous leukemia blasts (Janowska-Wieczorek et al., 1999a; Ries et al., 1999; Lin et al., 2002). The expression level of MMP-9 in the BM plasma is significantly lower in AML patients at preliminary diagnosis as compared to normal controls, and recovers to normal level at complete remission while declining again at relapse (Lin et al., 2002; Aref et al., 2003). Therefore, the plasma MMP-9 level is a surrogate marker of leukemia status in AML patients. On the other hand, the BM plasma MMP-9 level is significantly higher in patients with extramedullary infiltration than that in those without, showing that the premature production of MMP-9 in leukemic cells may contribute to their dissemination from BM (Aref et al., 2003).

MMP-2 expression has been detected in the isolated BM/PB AML blast cells, but not in the normal BM-MNCs. Primary AML blast cells can invade Matrigel in a MMP-2 dependent manner, suggesting that MMP-2 is an invasion marker for AML as well as for solid tumors (Janowska-Wieczorek et al., 1999a; Sawicki et al., 1998). On the other hand, there is also a study showing the correlation between MMP-2 level and favorable AML prognosis (Kuittinen et al., 1999). However, usually MMP mRNA and protein levels instead of active MMP-2 levels have been measured in studies performed so far, which may explain the discrepancy between MMP-2 protein expression and prognosis in AML. Advanced studies are required to evaluate the prognostic implications of active MMP-2 in AML patients.

Acute lymphoblastic leukemia (ALL)

Like in AML patients, plasma MMP-9 level in ALL patients is significantly lower than normal controls. Whereas, MMP-2 level in ALL patients is much higher than normal level (Lin et al., 2002). Kuittinen et al. (2001) have analyzed the expression of MMP-2 and MMP-9 in 22 adult and 55 pediatric ALL patients. Correlation between MMP-2 expression and multi-organs extramedullary infiltration has been found in adult ALL patients, suggesting an important role of MMPs in the extravasation of the leukemia cells in adult ALL. In pediatric ALL patients, however, the expression of both gelatinases is correlated with high-risk disease and T-cell immunophenotype but not with survival rate or extramedullary infiltrates. These findings support the hypothesis that adult ALL and pediatric ALL may be biologically distinct diseases.

Adult T-cell leukemia (ATL)

Adult T-cell leukemia (ATL) is a progressive and lethal malignancy caused by mature peripheral CD4+ cells infected with human T-cell leukemia virus type I (HTLV-I) (Yoshida et al., 1982). Rapid infiltration of infected cells into various tissues and secondary lymphoid organs is a common manifestation of ATL. Malignant human T cells produce both MMP-9 and a low level of MMP-2, while normal T cells only express the former. Transmembrane glycoprotein Emmprin overexpressed on T-lymphoma cells can enhance the infiltration of lymphoma cells by up-regulating self MMP-2 production (Nabeshima et al., 2004). HLTV-I-encoded transactivator oncoprotein Tax activates the MMP-9 promoter through NF-κB and SP-1, which may contribute to the clinical aggressiveness of ATL (Hayashibara et al., 2002; Mori et al., 2002). In ATL patients, the elevated plasma MMP-9 and VEGE are closely associated, and synergistically promote ATL extra-medullar infiltration (Hayashibara et al., 2002). HTLV-I infection stimulates T-cells to secrete VEGF and b-FGF, inducing the growth of T-cells and contiguous endothelial cells (Bazarbachi et al., 2004). Moreover, viral oncoprotein Tax transactivates the promoter of gag-junction protein connexin-43 and enhances gag-junction-mediated heterocellular communication with endothelial cells. Taken together, the HTLV-I transformed T-cells up-regulate gelatinases' activities on the endothelial cell through both paracrine stimulation and direct gag-junction-mediated communication to facilitate their extravasation.

Chronic myeloid leukemia (CML) and myelodysplastic syndromes (MDS)

Primary human Bcr/Abl (CML) cells and Bcr/Abl transfected murine FL5.12 cells express high levels of VEGF and gelatinases, resulting in a high angiogenic potential *in vivo* (Janowska-Wieczorek et al., 2002). The only study about the role of gelatinases in CML and MDS patients showed that the BM MNCs from these patients constitutively secrete MMP-9 (Ries et al., 1999). On the contrary, MMP-2 could only be detected in the CML patients in blast crisis or MDS patients undergoing disease progression. Thus, MMP-2 may represent a potential marker for dissemination in myeloproliferative malignancies.

Chronic lymphocytic leukemia (CLL)

CLL cells secrete variable amounts of pro-MMP-9 in monomeric, dimeric or complex forms with lipocalin. High levels of intracellular MMP-9 are associated with advanced (stage C) clinical state involving extensive BM infiltration, hematopoietic suppression and poor survival (Kamiguti et al., 2004). One specific MMP-9 inhibitor, Ro31-9790 can inhibit the transmigration of CLL cells across type IV collagen-coated membrane and endothelial monolayer, suggesting that the enzyme may participate in CLL cells egress and infiltration.

B-CLL is characterized by accumulation of proliferating monoclonal B cells in the peripheral blood and their progressive infiltration into lymphoreticular tissue, which is associated with an increased angiogenesis and unfavorable prognosis. There is a

much higher pro-MMP-9 level in B-CLL cells than in normal B cells. And the constitutive expression of MMP-9 is dependent on p38 MAPK, also an important mediator of B-CLL cells survival (Ringshausen et al., 2004). High levels of VEGF, b-FGF, TNF-α have also been detected in B-CLL culture supernatant, which may account for the up-regulation of MMP-9 (Bauvois et al., 2002). These studies suggest that a network of angiogenic cytokines may play complex roles in the leukemogenesis and progression of B-CLL through the MMP-9 activity control. Exogenous IFNs type I and II can inhibit the expression of MMP-9 at a transcriptional level, but have no effect on the production of other angiogenic factors, which may explain the inefficacy of IFN treatment in B-CLL patients. Elevated MMP-9 in the serum of untreated early B-CLL patients (grade A according to Binet's classification) plays an important role in the invasion and angiogenesis of this disease. Molica et al. (2003) have found a positive correlation of MMP-9 levels in early B-CLL patients with hemoglobin and platelet levels (P=0.03) but not with other clinical hematological features, such as BM microvessel density, genotypic aberrations. They proposed that serum MMP-9 level should be used for the prognostic assessment of individual patients with early B-CLL but not as a standard practice.

Hodgkin disease (HD) and Non-Hodgkin's lymphoma (NHL)

In patients with HD or NHL, serum MMP-9 levels are significantly elevated. However, in Hodgkin and Reed-Sternberg (HRS) cells, only different levels of MMP-7, -10, -11 but not gelatinases can be detected. In contrast, MMP-2 and MMP-9 are secreted from surrounding non-neoplastic cells including fibroblasts and macrophages (Hazar et al., 2004). Emmprin, a cell surface glycoprotein on HRS cells may induce MMP-2 expression in the surrounding non-neoplastic cells (Thorns et al., 2003). The strong MMP-2 expression correlates with favorable prognosis in HD, contrary to that in the solid tumors (Kuittinen et al., 2002).

More recently, Epstein-Barr virus (EBV) infection has been linked to the development of HD, and EBV encoded latent membrane protein 1 (LMP-1) can increase MMP-9 expression in lymphoma cell lines (Yoshizaki et al., 1998). However, the high expression of MMP-9 by HRS cells in HD patients is not associated with either EBV infection status or patient outcome (Flavell et al., 2000).

NHL represents a heterogeneous group of neoplasmas. ECM composition differs in different types of NHL and different NHL types produce different amounts of ECM-degrading enzymes (Kossakowska et al., 2000). MMP-9 is important for i*n vitr*o degradation of ECM by NHL cells (Kossakowska et al., 1998). Overexpression of MMP-9 in a subset of aggressive NHL prefigures poor clinical outcome (Kuittinen et al., 2003; Sakata et al., 2004). The elevated IL-6 in high-grade NHL can induce the transcription of MMP-2 and MMP-9 in lymphoid cells and enhance the in vitro invasion capacity of these cells. These data demonstrate that IL-6 may play a role in the pathogenesis of NHL not only through its growth factor activity but also by stimulating production of gelatinases to facilitate the invasive behaviors (Kossakowska et al., 1999).

Multiple myeloma (MM)

Multiple myeloma is a devastating B-cell cancer characterized by the proliferation of malignant plasma cells in the BM and the destruction of bone matrix. Several osteoclast-activating factors and angiogenic factors produced by myeloma cells may account for the development of osteolytic lesions and pathological angiogenesis. MMPs function in different processes of MM, including myeloma cell homing, tumor growth, invasion, osteolytic bone disease and the formation of new blood vessels. Both myeloma cell induced-BM angiogenesis and MMP-2 overexpression may account for intra/extramedullary spreading of myeloma cells during active MM (Vacca et al., 1999). High expressions of MMP-2, -8, -9 and -13 accompany enhanced microvessel density (MVD) have been detected in the BM of murine 5T2MM models (Van Valckenborgh et al., 2004). Treatment with specific competitive MMPs inhibitor SC-964 not only reduces the tumor growth by a significant inhibition of angiogenesis but also restrains the development of osteolytic bone disease. Co-culture of murine myeloma 5T33MM-vt cells with STR10 bone marrow endothelial cells (BMECs) can induce MMP-9 expression in MM cells, suggesting that the up-regulation of MMP-9 *in vivo* may be dependent on the BM microenvironment (Van Valckenborgh et al., 2002). BMECs-derived hepatocyte growth factor (HGF) increases the transmigration and invasion capacity of myeloma cells through MMP-9 up-regulation, suggesting a role of MMPs in the homing of MM cells to the BM (Vande Broek et al., 2004). Moreover, the myeloma cells-derived MMP-9 can increase the production of MMP-1 and the activation of pro-MMP-2 in the BM stromal cells from MM patients (Barille et al., 1997). In addition, MMP-8 and MMP-13 also participate in the malignant bone-destructive lesions in MM patients (Wahlgren et al., 2001). And MMP-7 produced by myeloma cells may take part in bone destruction and tumor diffusion in MM patients, not only through proteolysis but also through pro-MMP-2 activation (Barille et al., 1999). These studies raise the possibility that pharmacological modulation of particular MMPs production and function may provide new strategies for the treatment of MM.

Taken together, studies performed so far have been dedicated to elucidate the roles of MMPs, especially gelatinases, in the progression of hematological malignancies, including angiogenesis, extravasation/dissemination and their correlation with disease

outcome, which found the rationale of novel therapeutics for malignant hematopoietic diseases with MMPs expression/activity regulation.

Preclinical studies on the role of synthetic MMP inhibitors in leukemia treatment

Because MMPs play a pivotal role in cancer progression, drugs targeting these proteinases are expected to specifically restrain the growth of tumors but have no effect on normal tissues (John and Tuszynski, 2001). Synthetic MMP inhibitors (MMPIs) designed for cancer treatment, such as AG3340 and Batimastat, are undergoing clinical evaluation (Hidalgo and Eckhardt, 2001).

Deregulation of MMPs as well as MMPs/TIMPs imbalance may promote pathological angiogenesis in the BM and facilitate egress of premature leukemia blasts from BM and dissemination into peripheral tissue. Therefore, MMPIs are candidates for the treatment of hematological malignancies. SI-27, a new MMPI, can dose-dependently suppress the growth of several human leukemia cell lines such as U937, NB-4 and HL-60. SI-27 induces a variety of changes including activation of caspase-8, -9 and -3, the decrease of mitochondrial transmembrane potential ($\Delta\psi m$), the appearance of hypodiploid DNA, and cleavage of PARP and IκBα. Furthermore, albeit inducing no direct apoptosis at a lower concentration, SI-27 sensitizes these leukemia cells to TNF-α-mediated apoptosis by blocking NF-κB activation through IκBα cleavage (Nakamura et al., 2001). Another synthetic MMPI METVAN [bis (4, 7-dimethyl-1, 10 phenanthroline) sulfatooxovanadium (IV); VO (SO4) (Me2-Phen) 2], can induce apoptosis in leukemic cell lines as well as in primary leukemic cells from ALL, AML and CML patients (Narla et al., 2001b). *In vivo*, METVAN exhibits a significant anti-tumor activity on human glioblastoma and breast cancer xenograft models on SCID mice. METVAN induces apoptosis through ROS generation, glutathione depletion and $\Delta\psi m$ depolarization (Narla et al., 2001a). In addition to apoptosis inducing potency, METVAN inhibits leukemia cells from integrin-mediated adhesion to the ECM and invasion through Matrigel. Inhibition of invasion by METVAN may be associated with the reduction of proteolytic activities of MMP-9 and MMP-2.

Induction of leukemia differentiation is used as an adjunctive therapeutics to the conventional cytotoxic chemotherapy. ATRA induces differentiation of human acute promyelocytic leukemia (APL) cells and eventual complete remission in patients. Nevertheless, ATRA treatment may enhance the migration and invasion properties of leukemic cells by increasing their MMPs expression and activities (Devy et al., 2002). This may be a trigger for ATRA syndrome, characterized by a hyper-inflammatory reaction and mobilization of leukemia cells into extramedullary organs (De Botton et al., 1998). Therefore, the combination of MMPIs with ATRA may be an efficient prophylaxis for the ATRA syndrome in APL patients.

Recently, the important role of angiogenesis has been confirmed in the leukemogenesis and progression of certain hematological malignancies, just like in solid tumors (Bertolini et al., 2000; Yang and Han, 2000). Despite intensive chemotherapy, the vast majority of patients continue to relapse or become refractory to traditional chemotherapeutics. Both synthetic MMPIs and neutralizing monoclonal antibodies against human VEGF can inhibit tumor invasion (Rosen, 2000; Hidalgo and Eckhardt, 2001; Thomas et al., 2001). These findings strengthen the idea that anti-angiogenic/anti-MMPs therapy has a clinical application potential alone or in combination with chemotherapy.

In addition, MMPIs have also been proved to be effectual in animal models of lethal acute graft-versus-host disease (GVHD). TNF-α and Fas ligand (FasL) digested from cytomembrane by some MMPs, have been implicated in the pathogenesis of GVHD. Administration of KB-R7785, a hydroxamic acid-based MMP inhibitor for collagenases, stromelysins and gelatinases, along with allogeneic BMT, can inhibit tumor infiltration into liver and spleen and prevent acute GVHD and reduce the mortality of mice recipients by blocking the release of FasL and TNF-α while preserving the graft-versus-leukemia effect (GVL) in a murine model of lethal acute GVHD (Hattori et al, 1997, 1999).

Future directions

Numerous studies have shown that MMPs contribute to several processes of tumor progression including tumor angiogenesis, tumor growth and metastasis. The balance between MMPs and TIMPs is critical for the appropriate maintenance of structural and functional homeostasis in hematopoietic tissue. A number of MMPs play important roles in physiological hematopoiesis, HSCs mobilization and homing. However, most studies on the roles of MMPs in hematological malignancies have been focused on their effects on the invasion and transmigration behavior of the leukemia cells (Fig. 4). MMPs may also take part in pathological BM angiogenesis and leukemic cell survival/growth through

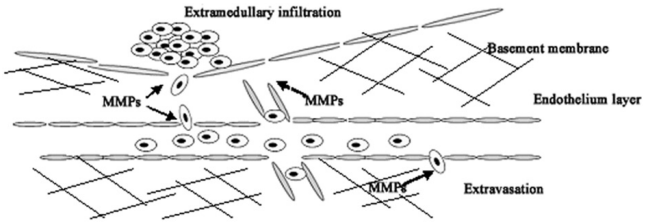

Fig. 4. The role of MMPs in leukemic cells extravasation, invasion and angiogenesis.

growth factors release, such as VEGF and SCF, promoting leukemia progression. Therefore, it is essential to elucidate the complex effects of MMPs/TIMPs and the biological roles of MMPs in pathological BM angiogenesis and leukemic cell survival/growth.

At present, bio-available oral MMPIs have been designed and tested clinically for the treatment of cancer and arthritis (Hidalgo and Eckhardt, 2001). Unfortunately, none has met the high expectations for the untoward side effects and moderate efficacy, which may result from non-specific inhibition of MMPs and ineffective local drug concentration in the tumor (Brown, 2000). Several MMPIs have shown anti-leukemia activity *in vitro* and anti-GVHD activity *in vivo*. So far, none of the MMPIs has been clinically tried in hematological malignancies due to the limited understanding about the roles of MMPs in these diseases. In addition, the application of MMPIs in hematological malignancies may face more problems in comparison with that in solid tumors. Since MMPs are key regulators in physiological hematopoiesis, any overcorrection may lead to a negative effect. Therefore, future studies should first elucidate the dysregulated MMPs production by different BM subsets in hematological malignancies pathogenesis, and then identify specific MMP targets to improve anti-leukemia efficacy and reduce side effects. Moreover, it's likely that MMPIs alone are insufficient in treatment of late-stage hematopoietic diseases. The application of one or more selective-targeted MMP inhibitors in combination with conventional anti-leukemia treatment may represent a promising strategy in the combat against hematopoietic malignancies.

References

Aref S., El-Sherbiny M., Mabed M., Menessy A. and El-Refaei M. (2003). Urokinase plasminogen activator receptor and soluble matrix metalloproteinase-9 in acute myeloid leukemia patients: a possible relation to disease invasion. Hematology 8, 385-391.

Baker AH., Edwards D.R. and Murphy G.. (2002). Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J. Cell Sci. 115, 3719-3727.

Baramova E.N., Bajou K., Remacle A., L'Hoir C., Krell H.W., Weidle U.H., Noel A. and Foidart J.M. (1997). Involvement of PA/plasmin system in the processing of pro-MMP-9 and in the second step of pro-MMP-2 activation. FEBS Lett. 405, 157-162.

Barille S., Akhoundi C., Collette M., Mellerin M.P., Rapp M.J., Harousseau J.L., Bataille R. and Amiot M. (1997). Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. Blood 90, 1649-1655.

Barille S., Bataille R., Rapp M.J., Harousseau J.L. and Amiot M. (1999). Production of metalloproteinase-7 (matrilysin) by human myeloma cells and its potential involvement in metalloproteinase-2 activation. J. Immunol. 163, 5723-5728.

Bauvois B., Dumont J., Mathiot C. and Kolb J.P. (2002). Production of matrix metalloproteinase-9 in early stage B-CLL: suppression by interferons. Leukemia 16, 791-798.

Bazarbachi A., Abou Merhi R., Gessain A., Talhouk R., El-Khoury H., Nasr R., Gout O., Sulahian R., Homaidan F., de The H., Hermine O. and El-Sabban M.E. (2004). Human T-cell lymphotropic virus type I-infected cells extravasate through the endothelial barrier by a local angiogenesis-like mechanism. Cancer Res. 64, 2039-2046.

Beaudeux J.L., Giral P., Bruckert E., Foglietti M.J. and Chapman M.J. (2004). Matrix metalloproteinases, inflammation and atherosclerosis: therapeutic perspectives. Clin. Chem. Lab. Med. 42, 121-131.

Bergers G., Brekken R., McMahon G., Vu T.H., Itoh T., Tamaki K., Tanzawa K., Thorpe P., Itohara S., Werb Z. and Hanahan D. (2000). Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat. Cell Biol. 2, 737-744.

Bertolini F., Mancuso P., Gobbi A. and Pruneri G. (2000). The thin red line: angiogenesis in normal and malignant hematopoiesis. Exp. Hematol. 28, 993-1000.

Bini A., Itoh Y., Kudryk B.J. and Nagase H. (1996). Degradation of cross-linked fibrin by matrix metalloproteinase 3 (stromelysin 1): hydrolysis of the gamma Gly 404-Ala 405 peptide bond. Biochemistry 35, 13056-13063.

Bini A., Wu D., Schnuer J. and Kudryk B.J. (1999). Characterization of stromelysin 1 (MMP-3), matrilysin (MMP-7), and membrane type 1 matrix metalloproteinase (MT1-MMP) derived fibrin(ogen) fragments D-dimer and D-like monomer: NH2-terminal sequences of late-stage digest fragments. Biochemistry 38, 13928-139236.

Bond M., Fabunmi R.P., Baker A.H. and Newby A.C. (1998). Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: an absolute requirement for transcription factor NF-kappa B. FEBS Lett. 435, 29-34.

Borden P. and Heller R.A. (1997). Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit. Rev. Eukaryot. Gene Expr. 7, 159-178.

Brown P.D. (2000). Ongoing trials with matrix metalloproteinase inhibitors. Expert. Opin. Investig. Drugs 9, 2167-2177.

Butler G.S., Butler M.J., Atkinson S.J., Will H., Tamura T., van Westrum S.S., Crabbe T., Clements J., d'Ortho M.P. and Murphy G. (1998). The TIMP2 membrane type 1 metalloproteinase "receptor" regulates the concentration and efficient activation of progelatinase A. A kinetic study. J. Biol. Chem. 273, 871-880.

Carion A., Benboubker L., Herault O., Roingeard F., Degenne M., Senecal D., Desbois I., Colombat P., Charbord P., Binet C. and Domenech J. (2003). Stromal-derived factor 1 and matrix metalloproteinase 9 levels in bone marrow and peripheral blood of patients mobilized by granulocyte colony-stimulating factor and chemotherapy. Relationship with mobilizing capacity of haematopoietic progenitor cells. Br. J. Haematol. 122, 918-926.

Carstanjen D., Ulbricht N., Iacone A., Regenfus M. and Salama A. (2002). Matrix metalloproteinase-9 (gelatinase B) is elevated during mobilization of peripheral blood progenitor cells by G-CSF. Transfusion 42, 588-596.

Cheng T., Rodrigues N., Shen H., Yang Y., Dombkowski D., Sykes M. and Scadden D.T. (2000). Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science 287, 1804-1808.

de Botton S., Dombret H., Sanz M., Miguel J.S., Caillot D., Zittoun R., Gardembas M., Stamatoulas A., Conde E., Guerci A., Gardin C., Geiser K., Makhoul D.C., Reman O., de la Serna J., Lefrere F., Chomienne C., Chastang C., Degos L. and Fenaux P. (1998). Incidence, clinical features, and outcome of all trans-retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic

leukemia. The European APL Group. Blood 92, 2712-2718.

Devy L., Hollender P., Munaut C., Colige A., Garnotel R., Foidart J.M., Noel A. and Jeannesson P. (2002). Matrix and serine protease expression during leukemic cell differentiation induced by aclacinomycin and all-trans-retinoic acid. Biochem. Pharmacol. 63, 179-189.

Devy L., Noel A., Baramova E., Bajou K., Trentesaux C., Jardillier J.C., Foidart J.M. and Jeannesson P. (1997). Production and activation of matrix metalloprotease-9 (MMP-9) by HL-60 promyelocytic leukemia cells. Biochem. Biophys. Res. Commun. 238, 842-846.

Dong Z., Kumar R., Yang X. and Fidler I.J. (1997). Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell 88, 801-810.

Flavell J.R., Baumforth K.R., Williams D.M., Lukesova M., Madarova J., Noskova V., Prochazkova J., Lowe D., Kolar Z., Murray P.G. and Nelson P.N. (2000). Expression of the matrix metalloproteinase 9 in Hodgkin's disease is independent of EBV status. Mol. Pathol. 53, 145-149.

Freireich E.J. (1984) Acute leukemia. A prototype of disseminated cancer. Cancer 53, 2026-2033.

Guedez L., Lim M.S. and Stetler-Stevenson W.G. (1996). The role of metalloproteinases and their inhibitors in hematological disorders. Crit. Rev. Oncog. 7, 205-225.

Hattori K., Hirano T., Ushiyama C., Miyajima H., Yamakawa N., Ebata T., Wada Y., Ikeda S., Yoshino K., Tateno M., Oshimi K., Kayagaki N., Yagita H. and Okumura K. (1997). A metalloproteinase inhibitor prevents lethal acute graft-versus-host disease in mice. Blood 90, 542-548.

Hattori K., Hirano T., Miyajima H., Yamakawa N., Ikeda S., Yoshino K., Tateno M., Oshimi K., Kayagaki N., Yagita H. and Okumura K. (1999). A metalloproteinase inhibitor prevents acute graft-versus-host disease while preserving the graft-versus-leukaemia effect of allogeneic bone marrow transplantation. Br. J. Haematol. 105, 303-312.

Hayashibara T., Yamada Y., Onimaru Y., Tsutsumi C., Nakayama S., Mori N., Miyanishi T., Kamihira S., Tomonaga M. and Maita T. (2002). Matrix metalloproteinase-9 and vascular endothelial growth factor: a possible link in adult T-cell leukaemia cell invasion. Br. J. Haematol. 116, 94-102.

Hazar B., Polat G., Seyrek E., Bagdatoglglu O., Kanik A. and Tiftik N. (2004). Prognostic value of matrix metalloproteinases (MMP-2 and MMP-9) in Hodgkin's and non-Hodgkin's lymphoma. Int. J. Clin. Pract. 58, 139-143.

Heissig B., Hattori K., Dias S., Friedrich M., Ferris B., Hackett N.R., Crystal R.G., Besmer P., Lyden D., Moore M.A., Werb Z. and Rafii S. (2002). Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 109, 625-637.

Hidalgo M. and Eckhardt S.G. (2001). Development of matrix metalloproteinase inhibitors in cancer therapy. J. Natl. Cancer Inst. 93, 178-193.

Ivanoff A., Ivanoff J., Hultenby K. and Sundqvist K.G. (1999). Infiltrative capacity of T leukemia cell lines: a distinct functional property coupled to expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1). Clin. Exp. Metastasis 17, 695-711.

Janowska-Wieczorek A., Marquez L.A., Matsuzaki A., Hashmi H.R., Larratt L.M., Boshkov L.M., Turner A.R., Zhang M.C., Edwards D.R. and Kossakowska A.E. (1999a). Expression of matrix metalloproteinases (MMP-2 and -9) and tissue inhibitors of metalloproteinases (TIMP-1 and -2) in acute myelogenous leukaemia blasts: comparison with normal bone marrow cells. Br. J. Haematol. 105, 402-411.

Janowska-Wieczorek A., Marquez L.A., Nabholtz J.M., Cabuhat M.L., Montano J., Chang H., Rozmus J., Russell J.A., Edwards D.R. and Turner A.R. (1999b). Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. Blood 93, 3379-3390.

Janowska-Wieczorek A., Matsuzaki A. and Marquez L. A. (2000a). The hematopoietic microenvironment: Matrix metalloproteinases in the hematopoietic microenvironment. Hematology 4, 515-527.

Janowska-Wieczorek A., Marquez L.A., Dobrowsky A., Ratajczak M.Z. and Cabuhat M.L. (2000b). Differential MMP and TIMP production by human marrow and peripheral blood CD34(+) cells in response to chemokines. Exp. Hematol. 28, 1274-1285.

Janowska-Wieczorek A., Majka M., Marquez-Curtis L., Wertheim J.A., Turner A.R. and Ratajczak M.Z. (2002). Bcr-abl-positive cells secrete angiogenic factors including matrix metalloproteinases and stimulate angiogenesis in vivo in Matrigel implants. Leukemia 16,1160-1166.

Jo Y., Yeon J., Kim H.J. and Lee S.T. (2000). Analysis of tissue inhibitor of metalloproteinases-2 effect on pro-matrix metalloproteinase-2 activation by membrane-type 1 matrix metalloproteinase using baculovirus/insect-cell expression system. Biochem. J. 345 Pt 3, 511-519.

John A. and Tuszynski G. (2001). The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol. Oncol. Res. 7, 14-23.

Kamiguti A.S., Lee E.S., Till K.J., Harris R.J., Glenn M.A., Lin K., Chen H.J., Zuzel M. and Cawley J.C. (2004). The role of matrix metalloproteinase 9 in the pathogenesis of chronic lymphocytic leukaemia. Br. J. Haematol. 125, 128-140.

Kayano K., Shimada T., Shinomiya T., Nakai S., Hisa Y., Aoki T., Seiki M. and Okada Y. (2004). Activation of pro-MMP-2 mediated by MT1-MMP in human salivary gland carcinomas: possible regulation of pro-MMP-2 activation by TIMP-2. J. Pathol. 202, 403-411.

Kim Y.M., Jang J.W., Lee O.H., Yeon J., Choi E.Y., Kim K.W., Lee S.T. and Kwon Y.G.. (2000). Endostatin inhibits endothelial and tumor cellular invasion by blocking the activation and catalytic activity of matrix metalloproteinase. Cancer Res. 60, 5410-5413.

Klein G. (1995). The extracellular matrix of the hematopoietic microenvironment. Experientia 51, 914-926.

Klein G., Vellenga E., Fraaije M.W., Kamps W.A. and de Bont E.S. (2004). The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. Crit. Rev. Oncol. Hematol. 50, 87-100.

Kossakowska A.E., Hinek A., Edwards D.R., Lim M.S., Zhang C.L., Breitman D.R., Prusinkiewicz C., Stabbler A.L., Urbanski L.S. and Urbanski S.J. (1998). Proteolytic activity of human non-Hodgkin's lymphomas. Am. J. Pathol. 152, 565-576.

Kossakowska A.E., Edwards D.R., Prusinkiewicz C., Zhang M.C., Guo D., Urbanski S.J., Grogan T., Marquez L.A., and Janowska-Wieczorek A. (1999). Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. Blood 94, 2080-2089.

Kossakowska A.E., Urbanski S.J. and Janowska-Wieczorek A. (2000).

Matrix metalloproteinases and their tissue inhibitors - expression, role and regulation in human malignant non-Hodgkin's lymphomas. Leuk. Lymphoma 239, 485-493.

Kuittinen O., Savolainen E.R., Koistinen P., Mottonen M. and Turpeenniemi-Hujanen T. (2001). MMP-2 and MMP-9 expression in adult and childhood acute lymphatic leukemia (ALL). Leuk. Res. 25, 125-131.

Kuittinen O., Savolainen E.R., Koistinen P. and Turpeenniemi-Hujanen T. (1999). Gelatinase A and B (MMP-2, MMP-9) in leukaemia MMP-2 may indicate a good prognosis in AML. Anticancer Res. 19, 4395-4400.

Kuittinen O., Soini Y. and Turpeenniemi-Hujanen T. (2002). Diverse role of MMP-2 and MMP-9 in the clinicopathological behavior of Hodgkin's lymphoma. Eur. J. Haematol. 69, 205-212.

Kuittinen O., Apaja-Sarkkinen M. and Turpeenniemi-Hujanen T. (2003). Gelatinases (MMP-2 and MMP-9), TIMP-1 expression and the extent of neovascularization in aggressive non-Hodgkin's lymphomas. Eur. J. Haematol. 71, 91-99.

Lane W.J., Dias S., Hattori K., Heissig B., Choy M., Rabbany S.Y., Wood J., Moore M.A. and Rafii S. (2000). Stromal-derived factor 1-induced megakaryocyte migration and platelet production is dependent on matrix metalloproteinases. Blood 96, 4152-4159.

Lapidot T. and Petit I. (2002). Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp. Hematol. 30, 973-981.

Levesque J.P., Liu F., Simmons P.J., Betsuyaku T., Senior R.M., Pham C. and Link D.C. (2004). Characterization of hematopoietic progenitor mobilization in protease-deficient mice. Blood 104, 65-72.

Lijnen H.R., Ugwu F., Bini A. and Collen D. (1998). Generation of an angiostatin-like fragment from plasminogen by stromelysin-1 (MMP-3). Biochemistry 37, 4699-4702.

Lijnen H.R., Arza B., Van Hoef B., Collen D. and Declerck P.J. (2000). Inactivation of plasminogen activator inhibitor-1 by specific proteolysis with stromelysin-1 (MMP-3). J. Biol. Chem. 275, 37645-37650.

Lin L.I., Lin D.T., Chang C.J., Lee C.Y., Tang J.L. and Tien H.F. (2002). Marrow matrix metalloproteinases (MMPs) and tissue inhibitors of MMP in acute leukaemia: potential role of MMP-9 as a surrogate marker to monitor leukaemic status in patients with acute myelogenous leukaemia. Br. J. Haematol. 117, 835-841.

Lynch C.C. and McDonnell S. (2000). The role of matrilysin (MMP-7) in leukaemia cell invasion. Clin. Exp. Metastasis 18, 401-406.

Majka M., Janowska-Wieczorek A., Ratajczak J., Kowalska M.A., Vilaire G., Pan Z.K., Honczarenko M., Marquez L.A., Poncz M. and Ratajczak M.Z. (2000). Stromal-derived factor 1 and thrombopoietin regulate distinct aspects of human megakaryopoiesis. Blood 96, 4142-4151.

Marquez-Curtis L.A., Dobrowsky A., Montano J., Turner A.R., Ratajczak J., Ratajczak M.Z. and Janowska-Wieczorek A. (2001). Matrix metalloproteinase and tissue inhibitors of metalloproteinase secretion by hematopoietic and stromal precursors and their production in normal and leukemic long-term marrow cultures. Br. J. Haematol. 115, 595-604.

Mazzieri R., Masiero L., Zanetta L., Monea S., Onisto M., Garbisa S. and Mignatti P. (1997). Control of type IV collagenase activity by components of the urokinase-plasmin system: a regulatory mechanism with cell-bound reactants. EMBO J. 16, 2319-2332.

McQuibban G.A., Butler G.S., Gong J.H., Bendall L., Power C., Clark-Lewis I. and Overall C.M. (2001). Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. J. Biol. Chem. 276, 43503-43508.

Molica S., Vitelli G., Levato D., Giannarelli D., Vacca A., Cuneo A., Cavazzini F., Squillace R., Mirabelli R. and Digiesi G.. (2003). Increased serum levels of matrix metalloproteinase-9 predict clinical outcome of patients with early B-cell chronic lymphocytic leukaemia. Eur. J. Haematol. 70, 373-378.

Mori N., Sato H., Hayashibara T., Senba M., Hayashi T., Yamada Y., Kamihira S., Ikeda S., Yamasaki Y., Morikawa S., Tomonaga M., Geleziunas R. and Yamamoto N. (2002). Human T-cell leukemia virus type I Tax transactivates the matrix metalloproteinase-9 gene: potential role in mediating adult T-cell leukemia invasiveness. Blood 99, 1341-1349.

Morgunova E., Tuuttila A., Bergmann U., Isupov M., Lindqvist Y., Schneider G. and Tryggvason K. (1999). Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. Science 284, 1667-1670.

Morrison C.J., Butler G.S., Bigg H.F., Roberts C.R., Soloway P.D. and Overall C.M. (2001). Cellular activation of MMP-2 (gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. J. Biol. Chem. 276, 47402-10.

Moses M.A. (1997). The regulation of neovascularization of matrix metalloproteinases and their inhibitors. Stem Cells 15, 180-189.

Murphy G., Stanton H., Cowell S., Butler G., Knauper V., Atkinson S. and Gavrilovic J. (1999). Mechanisms for pro matrix metalloproteinase activation. APMIS 107, 38-44.

Nabeshima K., Suzumiya J., Nagano M., Ohshima K., Toole B.P., Tamura K., Iwasaki H. and Kikuchi M. (2004). Emmprin, a cell surface inducer of matrix metalloproteinases (MMPs), is expressed in T-cell lymphomas. J. Pathol. 202, 341-351.

Nagase H. (1997). Activation mechanisms of matrix metalloproteinases. Biol. Chem. 378, 151-60.

Nagase H. and Woessner J.F. Jr. (1999). Matrix metalloproteinases. J. Biol. Chem. 274, 21491-21494.

Nakamura Y., Sato K., Wakimoto N., Kimura F., Okuyama A. and Motoyoshi K. (2001). A new matrix metalloproteinase inhibitor SI-27 induces apoptosis in several human myeloid leukemia cell lines and enhances sensitivity to TNF alpha-induced apoptosis. Leukemia 15, 1217-1224.

Narla R.K., Chen C.L., Dong Y. and Uckun F.M. (2001a). In vivo antitumor activity of bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) (METVAN [VO(SO4)(Me2-Phen)2]). Clin. Cancer Res. 7, 2124-2133.

Narla R.K., Dong Y., Klis D. and Uckun F.M. (2001b). Bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(I.V.) as a novel antileukemic agent with matrix metalloproteinase inhibitory activity. Clin. Cancer Res. 7, 1094-1101.

Ogawa M., Kawamoto M. and Yamanaka N. (2000). Matrix metalloproteinase and tissue inhibitor of metalloproteinase in human bone marrow tissues-an immunohistochemical study. J. Nippon Med. Sch. 67, 235-41.

Ohnishi J., Ohnishi E., Jin M., Hirano W., Nakane D., Matsui H., Kimura A., Sawa H., Nakayama K., Shibuya H., Nagashima K. and Takahashi T. (2001). Cloning and characterization of a rat ortholog of MMP-23 (matrix metalloproteinase-23), a unique type of membrane-anchored matrix metalloproteinase and conditioned switching of its expression during the ovarian follicular development. Mol. Endocrinol. 15,747-764.

Okumura Y., Sato H., Seiki M. and Kido H. (1997). Proteolytic activation of the precursor of membrane type 1 matrix metalloproteinase by human plasmin. A possible cell surface activator. FEBS Lett. 402, 181-194.

Overall C.M., Wrana J.L. and Sodek J. (1991). Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. J. Biol. Chem. 266, 14064-14071.

Papayannopoulou T., Priestley G.V., Bonig H. and Nakamoto B. (2003). The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization. Blood 101, 4739-4747.

Pelus L.M., Bian H., King A.G. and Fukuda S. (2004). Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. Blood 103, 110-119.

Pruijt J.F., Fibbe W.E., Laterveer L., Pieters R.A., Lindley I.J., Paemen L., Masure S., Willemze R. and Opdenakker G. (1999). Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). Proc. Natl. Acad. Sci. USA 96, 10863-10868.

Pruijt J.F., Verzaal P., van Os R., de Kruijf E.J., van Schie M.L., Mantovani A., Vecchi A., Lindley I.J., Willemze R., Starckx S., Opdenakker G. and Fibbe W.E. (2002). Neutrophils are indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice. Proc. Natl. Acad. Sci. USA 99, 6228-33.

Quesenberry P.J. and Becker P.S. (1998). Stem cell homing: rolling, crawling, and nesting. Proc. Natl. Acad. Sci. USA 95, 15155-15157.

Rafii S., Avecilla S., Shmelkov S., Shido K., Tejada R., Moore M.A., Heissig B. and Hattori K. (2003). Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. Ann. NY Acad. Sci. 996, 49-60.

Rao Q., Zheng G.G., Lin Y.M. and Wu K.F. (2004). Production of matrix metalloproteinase-9 by cord blood CD34+ cells and its role in migration. Ann. Hematol. 83, 409-413.

Richard D., Hollender P. and Chenais B. (2002a). Involvement of reactive oxygen species in aclarubicin-induced differentiation and invasiveness of HL-60 leukemia cells. Int. J. Oncol. 21, 393-399.

Richard D., Hollender P. and Chenais B. (2002b). Butyric acid increases invasiveness of HL-60 leukemia cells: role of reactive oxygen species. FEBS Lett. 518, 159-163.

Ries C. and Petrides P.E. (1995). Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction in disease. Biol. Chem. Hoppe Seyler 376, 345-355.

Ries C., Loher F., Zang C., Ismair M.G. and Petrides P.E. (1999). Matrix metalloproteinase production by bone marrow mononuclear cells from normal individuals and patients with acute and chronic myeloid leukemia or myelodysplastic syndromes. Clin. Cancer Res. 5, 1115-1124.

Ringshausen I., Dechow T., Schneller F., Weick K., Oelsner M., Peschel C. and Decker T. (2004). Constitutive activation of the MAPkinase p38 is critical for MMP-9 production and survival of B-CLL cells on bone marrow stromal cells. Leukemia 18, 1964-1970.

Rosen L. (2000). Antiangiogenic strategies and agents in clinical trials. Oncologist. 5 Suppl 1, 20-27.

Sakata K., Satoh M., Someya M., Asanuma H., Nagakura H., Oouchi A., Nakata K., Kogawa K., Koito K., Hareyama M. and Himi T. (2004). Expression of matrix metalloproteinase 9 is a prognostic factor in patients with non-Hodgkin lymphoma. Cancer 100, 356-365.

Sato H., Takino T., Kinoshita T., Imai K., Okada Y., Stetler Stevenson W.G. and Seiki M. (1996). Cell surface binding and activation of gelatinase A induced by expression of membrane-type-1-matrix metalloproteinase (MT1-MMP). FEBS Lett. 385, 238-240.

Sawicki G., Matsuzaki A. and Janowska-Wieczorek A. (1998). Expression of the active form of MMP-2 on the surface of leukemic cells accounts for their in vitro invasion. J. Cancer. Res. Clin. Oncol. 124, 245-252.

Sawyers C.L., Denny C.T. and Witte O.N. (1991). Leukemia and the disruption of normal hematopoiesis. Cell 64, 337-350.

Shibakura M., Niiya K., Kiguchi T., Shinagawa K., Ishimaru F., Ikeda K., Namba M., Nakata Y., Harada M. and Tanimoto M. (2002). Simultaneous induction of matrix metalloproteinase-9 and interleukin 8 by all-trans retinoic acid in human PL-21 and NB4 myeloid leukaemia cells. Br. J. Haematol. 118, 419-425.

Sternlicht M.D. and Werb Z. (2001). How matrix metalloproteinases regulate cell behavior. Annu. Rev. Cell. Dev. Biol. 17, 463-516.

Thomas D.A., Giles F.J., Cortes J., Albitar M. and Kantarjian H.M. (2001). Antiangiogenic therapy in leukemia. Acta Haematol. 106, 190-207.

Thorns C., Bernd H.W., Hatton D., Merz H., Feller A.C. and Lange K. (2003). Matrix-metalloproteinases in Hodgkin lymphoma. Anticancer Res. 23, 1555-1558.

Ugwu F., Van Hoef B., Bini A., Collen D. and Lijnen H.R. (1998). Proteolytic cleavage of urokinase-type plasminogen activator by stromelysin-1 (MMP-3). Biochemistry 37, 7231-7236.

Vacca A., Ribatti D., Presta M., Minischetti M., Iurlaro M., Ria R., Albini A., Bussolino F. and Dammacco F. (1999). Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood 93, 3064-3073.

Vande Broek I., Asosingh K., Allegaert V., Leleu X., Facon T., Vanderkerken K., Van Camp B. and Van Riet I. (2004). Bone marrow endothelial cells increase the invasiveness of human multiple myeloma cells through upregulation of MMP-9: evidence for a role of hepatocyte growth factor. Leukemia 18, 976-982.

van Os R., van Schie M.L., Willemze R. and Fibbe W.E. (2002). Proteolytic enzyme levels are increased during granulocyte colony-stimulating factor-induced hematopoietic stem cell mobilization in human donors but do not predict the number of mobilized stem cells. J. Hematother. Stem. Cell Res. 11, 513-521.

Van Valckenborgh E., Bakkus M., Munaut C., Noel A., St Pierre Y., Asosingh K., Van Riet I., Van Camp B. and Vanderkerken K. (2002). Upregulation of matrix metalloproteinase-9 in murine 5T33 multiple myeloma cells by interaction with bone marrow endothelial cells. Int. J. Cancer 101, 512-518.

Van Valckenborgh E., Croucher P.I., De Raeve H., Carron C., De Leenheer E., Blacher S., Devy L., Noel A., De Bruyne E., Asosingh K., Van Riet I., Van Camp B. and Vanderkerken K. (2004). Multifunctional role of matrix metalloproteinases in multiple myeloma: a study in the 5T2MM mouse model. Am. J. Pathol. 165, 869-878.

Van Wart H.E. and Birkedal-Hansen H. (1990). The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc.

Natl. Acad. Sci. USA 87, 5578-5582.

Voermans C., Gerritsen W.R., von dem Borne A.E. and van der Schoot C.E. (1999). Increased migration of cord blood-derived CD34$^+$ cells, as compared to bone marrow and mobilized peripheral blood CD34$^+$ cells across uncoated or fibronectin-coated filters. Exp. Hematol. 27, 1806-1814.

von Luttichau I., Djafarzadeh R., Henger A., Cohen C.D., Mojaat A., Jochum M., Ries C., Nelson P.J. and Kretzler M. (2002). Identification of a signal transduction pathway that regulates MMP-9 mRNA expression in glomerular injury. Biol. Chem. 383, 1271-1275.

Wahlgren J., Maisi P., Sorsa T., Sutinen M., Tervahartiala T., Pirila E., Teronen O., Hietanen J., Tjaderhane L. and Salo T. (2001). Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. J. Pathol. 194, 217-224.

Westermarck J., Seth A. and Kahari V.M. (1997). Differential regulation of interstitial collagenase (MMP-1) gene expression by ETS transcription factors. Oncogene 14, 2651-2660.

Xie B., Laouar A. and Huberman E. (1998a). Fibronectin-mediated cell adhesion is required for induction of 92-kDa type IV collagenase/gelatinase (MMP-9) gene expression during macrophage differentiation. The signaling role of protein kinase C-beta. J. Biol. Chem. 273, 11576-11582.

Xie B., Laouar A. and Huberman E. (1998b). Autocrine regulation of macrophage differentiation and 92-kDa gelatinase production by tumor necrosis factor-alpha via alpha5 beta1 integrin in HL-60 cells. J. Biol. Chem. 273, 11583-11588.

Yana I. and Weiss S.J. (2000). Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. Mol. Biol. Cell 11, 2387-2401.

Yang R. and Han Z.C. (2000). Angiogenesis in hematologic malignancies and its clinical implications. Int. J. Hematol. 75, 246-256.

Yoshida M., Miyoshi I. and Hinuma Y. (1982). Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc. Natl. Acad. Sci. USA 79, 2031-2035.

Yoshizaki T., Sato H., Furukawa M. and Pagano J.S. (1998). The expression of matrix metalloproteinase 9 is enhanced by Epstein-Barr virus latent membrane protein 1. Proc. Natl. Acad. Sci. USA 95, 3621-3626.

Zheng Y., Sun A. and Han Z.C. (2005). Stem cell factor improves SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem/progenitor cells in xenotransplanted NOD/SCID mouse model. Bone Marrow Transplant. 35, 137-142.

Zheng Y., Watanabe N., Nagamura-Inoue T., Igura K., Nagayama H., Tojo A., Tanosaki R., Takaue Y., Okamoto S. and Takahashi T.A. (2003). Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their transmigratory potential. Exp. Hematol. 31, 1237-1246.

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