

# Cell-surface association between matrix metalloproteinases and integrins: role of the complexes in leukocyte migration and cancer progression

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Leukocyte motility is known to be dependent on both  $\beta_2$ -integrins and matrix metalloproteinases MMP-2/-9 or gelatinases, which mediate leukocyte adhesion and the proteolysis needed for invasion, respectively. Gelatinases not only play an important role in cell migration, tissue remodeling, and angiogenesis during de-

velopment, but are also involved in the progression and invasiveness of many cancers, including leukemias. The concept that MMPs associate with integrins, as well as their importance in some physiologic and pathologic conditions, has been advanced previously but has not been examined on leukocytes. This re-

view will examine mainly the function of the MMP-integrin complexes in normal leukocyte migration and the effect of integrin and broad-spectrum MMP inhibitors in tumor progression. (Blood. 2006;108:1441-1450)

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## Leukocyte adhesion and migration

Neutrophils, also known as polymorphonuclear leukocytes (PMNs) originate from stem cells in the bone marrow. They represent 60% to 70% of the total circulating leukocytes and are the first cells to be recruited to the sites of infection or injury within minutes to hours after maturation, forming a primary defense against infectious agents or “foreign” substances that invade our body’s physical barriers. The initiation of an inflammatory response involves 3 major steps: (1) increased blood flow by dilation of capillaries; (2) escape of plasma proteins from the bloodstream; and (3) extravasation of neutrophils through the endothelium and accumulation at the site of injury. Elimination of invading microorganisms is accomplished by phagocytosis, generation of reactive oxygen metabolites, as well as through release of proteolytic enzymes and microbicidal substances, all stored in intracellular granules of mature PMNs.<sup>1</sup>

The main functions of neutrophils involve adhesion, extravasation, chemotaxis, phagocytosis, and production of oxidative agents. Like all leukocytes, these functions can be triggered by appropriate stimuli and the synergistic action of different adhesion molecules that are present on the surface of both neutrophils and activated endothelial cells.<sup>2</sup> Interactions of neutrophils with the activated endothelium have been extensively studied either under static conditions or under physiologic conditions (flow shear forces). Neutrophil tethering and capture have been shown to be mediated by P-selectin binding to its ligand PSGL-1; neutrophil activation by chemokines, such as IL-8; and firm adhesion by ICAM-1 binding to  $\alpha_L\beta_2$ - and  $\alpha_M\beta_2$ -integrins.<sup>3</sup> Chemokines capable of triggering rapid arrest of T cells, B cells, and monocytes on endothelial cells under physiologic conditions include SLC/CCL21, RANTES, and SDF-1/CXCL12, respectively. Unlike other leukocytes, arrest chemokines for neutrophils have been much more difficult to define, even though the neutrophil adhesion cascade has been studied longer and by more groups.

## Role of integrins and MMPs in leukocyte migration

### Structure and function of leukocyte $\beta_2$ -integrins

The structural characteristics and functional roles of leukocyte  $\beta_2$ -integrins have been extensively reviewed.<sup>4,5</sup> The  $\beta_2$ -integrins ( $\alpha_L\beta_2$ ,  $\alpha_M\beta_2$ ,  $\alpha_X\beta_2$ , and  $\alpha_D\beta_2$ ) consist of  $\alpha$ - (1063, 1137, 1144, and 1084 residues, respectively) and  $\beta$ - (747 residues) subunits (Figure 1A). Divalent cations are essential for integrin functions by regulating the integrin structure in a state in which they increase or suppress binding to physiologic ligands. A recent crystal structure of  $\alpha_V\beta_3$ -integrin showed that the bent form is capable of binding a physiologic ligand in a  $Mn^{2+}$ -dependent manner.<sup>6</sup> To date, the primary structures of all 4  $\beta_2$ -integrin  $\alpha$ - and  $\beta$ -subunits have been described by molecular cloning.<sup>7,8</sup> Each integrin  $\alpha$ -subunit contains 7, 60-amino-acid long, homologous segments in the amino-terminal region, and with resemblance to a domain present in the trimeric G protein  $\beta$ -subunit, that are predicted to fold into a 7-bladed  $\beta$ -propeller domain.<sup>9</sup> Along with the I-like domain ( $\beta$ A) from the  $\beta$ -subunit, they both interact to form the “head” of the integrin (Figure 1B). Half of all integrin  $\alpha$ -subunits contain an additional, 200-amino-acid long, I domain that is inserted between the propeller  $\beta$  sheets 2 and 3<sup>7,9</sup> and is homologous to (domains within) a plasma glycoprotein von Willebrand factor.<sup>10</sup> The 3-dimensional architecture of the extracellular domains of the integrin  $\alpha$ - and  $\beta$ -subunits has been revealed by crystallization, electron microscopy, and nuclear magnetic resonance (NMR).<sup>11</sup> Based on the crystal structure of the extracellular domains of  $\alpha_V\beta_3$ , it has been predicted that the I domain lies on top of the  $\beta$ -propeller domain (Figure 1C).<sup>9</sup>

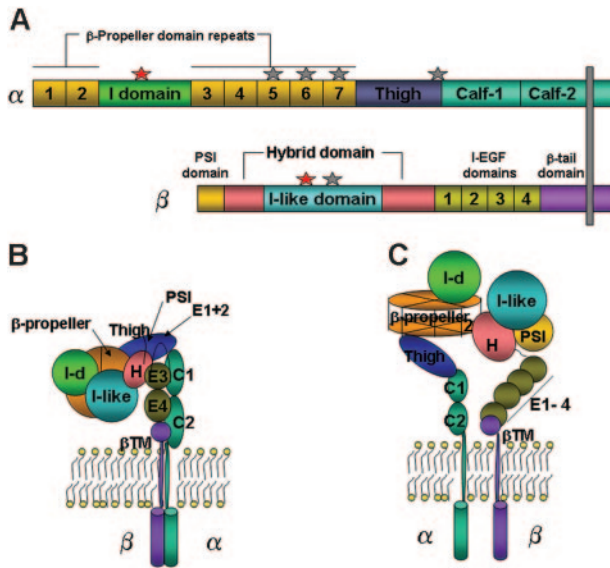
Loss of heteromerization of the integrin during biosynthesis caused by mutations in the gene encoding the  $\beta$ -subunit resulted in reduced  $\beta_2$ -integrin cell-surface expression and function on leukocytes, leading to a rare human inherited disease called leukocyte

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**Figure 1. Schematic structure of the leukocyte integrin.** (A) The integrin's primary structure, including divalent cation-binding sites ( $Mg^{2+}$  as red stars, and  $Ca^{2+}$  as gray stars). (B,C) Schematic representations of the bent (inactive) and straightened (active) conformations of the integrin, respectively. The arrangement of domains is based on the 3-dimensional crystal structure of  $\alpha_v\beta_3$ -integrin, with an I domain added between the second and third  $\beta$ -propeller repeats. Each domain is colored as in panel A. I-d indicates I domain; I-EGF, integrin-epidermal growth factor domain; PSI, plexin/semaphorin/integrin; and  $\beta$ TM,  $\beta$ -tail domain.

adhesion deficiency-I (LAD-I).<sup>12</sup> Expression of nonfunctional  $\beta_2$ -integrins was also observed in LAD-I patients carrying mutations in the MIDAS motif of the I-like domain in the  $\beta$ -subunit.<sup>13</sup> PMNs and monocytes from LAD-I patients fail to migrate through the vascular endothelium or become fully activated because of lack of adherence, actin cytoskeleton rearrangement, and spreading on ICAM-1- or ECM-coated surfaces. This explains why LAD-I patients are susceptible to life-threatening bacterial infections. The same phenotype was observed in  $\beta_2$ -integrin knock-out mice.<sup>14</sup>

### Regulation of cell adhesion and migration

Leukocyte migration is a complex process, controlled by a wide spectrum of leukocyte and endothelial cell adhesion molecules and by the presence of chemotactic molecules. These molecules, as well as growth factors, are responsible for the establishment of a polarized cell migration and there is enough evidence to prove that signaling from both phospholipids and proteins from the Rho family of small GTPases are also involved in directed cell motility.<sup>15</sup> Migration of leukocytes is essential for immune responses, tissue repair, and embryonic development.

A polarized morphology of leukocytes was first described to be similar to that of a migrating amoeba, with a leading edge at the front and a uropod at the rear of a migrating cell.<sup>16</sup> T cells recognize and bind to antigen presenting cells (APCs) through their leading edge. A number of receptors are concentrated at the leading edge, including  $\alpha_v\beta_3$ , uPAR, and fMLP-R in neutrophils; CCR2, CCR5, and FAK in T cells; and CXCR4 in B cells, which are able to sense chemotactic gradients, thus guiding leukocytes to migrate in a polarized manner. At the uropod, several reports show localization of ICAMs, L-selectin,  $\alpha_M\beta_2$ , PSGL-1, Fc $\gamma$ R-IIIb, CD2, CD43, and CD44,<sup>17,18</sup> which play a pivotal role in cell adhesion, thus facilitating cell migration. Release of the uropod triggers cell migration. Some of these receptors, when bound to the substratum, become linked to the actin cytoskeleton during cell migration. Interactions between the cytoskeleton and the cell-surface receptors are required for the formation of membrane protrusions, such

as lamellipodia (broad, sheetlike structures) and filopodia (thin cylindrical needlelike projections), both structures located at the leading edge.<sup>16</sup> Several MMPs, including MT1-MMP and MMP-2, were found to colocalize at membrane protrusions. Interaction of MMPs with their natural inhibitors, TIMPs, at these sites might be the key mechanism for the regulation of cell-surface MMP activation and, eventually, the control of the invasive phenotype of cells.<sup>19</sup>

### Cell-surface association of MMPs and other proteases

Matrix metalloproteinases (MMPs) are a family of structurally related and highly conserved zinc-dependent endopeptidases collectively capable of degrading most components of the basement membrane and ECM.<sup>20</sup> MMP substrates also include a wide variety of proteins, such as chemotactic molecules, adhesion molecules, proteinase inhibitors, cell-surface receptors, blood clotting factors, latent growth factors, and growth factor-binding proteins. Most human MMPs can be divided according to their sequence homology, substrate specificity, and cellular location into several subclasses: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and others. The basic multidomain structure of MMPs comprises the following: (1) an amino-terminal domain; (2) a catalytic domain; and (3) a carboxy-terminal domain. To date, there are at least 25 secreted or membrane-bound known human MMPs.<sup>21</sup> The expression, secretion, and activity of MMPs in normal tissues are subject to tight control. Data generated from intensive studies on MMP activities in different cells and tissues, as well as studies from knock-out animals, witness the importance of these enzymes in many normal physiologic processes (eg, embryonic development, bone resorption, angiogenesis, and wound healing) and pathologic processes (rheumatoid arthritis, multiple sclerosis, periodontal disease, and tumor growth and metastasis).<sup>20,22,23</sup>

MMPs are secreted as zymogens from inside the cell to the cell surface and into the extracellular environment where they are able to degrade both ECM and non-ECM proteins. It remains unclear how these enzymes make it to the correct location at the cell surface and how the proteolytic activity is controlled at the pericellular space. However, it has been suggested that MMP binding to cell-surface proteins can have an effect on intracellular signaling, facilitate proenzyme localization and activation, mediate cell motility by disruption of cell contacts with the ECM, and promote internalization of the enzyme. For example, integrins are shown to act as receptors for several proteases, including MMPs. Such interactions have been detected in caveolae, in invadopodia, and at the leading edge of migrating cells, where directed proteolytic activity is needed. The first interaction between an integrin ( $\alpha_v\beta_3$ ) and an MMP (MMP-2) was identified on the surface of melanoma cells and angiogenic blood vessels (Table 1). This complex was shown to be involved in tumor growth and angiogenesis *in vivo*.<sup>82</sup> Caveolae are membrane invaginations known to serve as sites for clustering of various integrins and proteases.<sup>83</sup> MT1-MMP was shown to activate  $\alpha_v\beta_3$  through proteolytic cleavage, suggesting that coordinated expression and localization of these molecules may be important for cancer cell invasion and metastasis. Furthermore, there is evidence that the  $\alpha_v\beta_3$ -integrin has modulatory properties on MMP-2 activity by binding to its C-terminal domain.<sup>28,82</sup> Inhibition of the  $\alpha_v\beta_3$ /MMP-2 complex formation by either the MMP-2 C-terminal domain<sup>84</sup> or a small molecule inhibitor, TSRI265,<sup>85</sup> dramatically suppressed angiogenesis *in vivo*, demonstrating that this interaction is essential for endothelial cell proliferation and migration. Since then, several other important protease associations with integrins have been reported (Table 1),

Table 1. Proteinase association with integrins

Soluble proteases/associated proteins	Cell-surface expression	Source
<b>MMPs</b>		
<b>MMP-1</b>		
$\alpha_1\beta_1$	Myocytes	Stricker et al <sup>24</sup>
$\alpha_2\beta_1$	Keratinocytes	Dumin et al <sup>25</sup>
EMMPRIN	Lung carcinoma	Guo et al <sup>26</sup>
PAR1	Breast carcinoma	Boire et al <sup>27</sup>
<b>MMP-2</b>		
$\alpha_v\beta_3$	Melanoma, endothelial	Brooks et al <sup>28</sup>
LRP	Fibroblasts	Yang et al <sup>29</sup>
Collagen chains	Fibroblasts	Steffensen et al <sup>30</sup>
TSP-2	Fibroblasts	Yang et al <sup>29</sup>
TIMP-2	Malignant cells	Olson et al <sup>31</sup>
Caveolin-1	Endothelial	Puyraimond et al <sup>32</sup>
Hsp90 $\alpha$	Fibrosarcoma	Eustace et al <sup>33</sup>
MT1-MMP	Fibrosarcoma	Strongin et al <sup>34</sup>
BS	—	Fedarko et al <sup>35</sup>
<b>MMP-3</b>		
Osteopontin	—	Fedarko et al <sup>35</sup>
<b>MMP-7</b>		
CD44HSPG	Epithelial	Yu et al <sup>36</sup>
TM4SF	—	Maecker et al <sup>37</sup>
CD151	Rectal carcinoma	Shiomi et al <sup>38</sup>
<b>MMP-9</b>		
Collagen chains	Epithelial/fibrosarcoma	Okada et al <sup>39</sup>
RECK	Fibrosarcoma	Takahashi et al <sup>40</sup>
CD44	Melanoma	Yu et al <sup>41</sup>
ICAM-1	Leukemias	Fiore et al <sup>42</sup>
LRP	Fibroblasts	Hahn-Dantona et al <sup>43</sup>
Ku protein	Macrophages/leukemia	Monferran et al <sup>44</sup>
TIMP-1	Fibroblasts	O'Connell et al <sup>45</sup>
TSP-1	Malignant cells	Rodriguez-Mazaneque et al <sup>46</sup>
$\alpha_{M\beta_2}$	Neutrophils/leukemias	Stefanidakis et al <sup>47</sup>
$\alpha_5\beta_1$	Epithelial	Wang et al <sup>48</sup>
$\alpha_3\beta_1$	Mammary carcinoma	Morini et al <sup>49</sup>
$\alpha_v\beta_5$	Fibrosarcoma	Bjorklund et al <sup>50</sup>
DMP-1	—	Fedarko et al <sup>35</sup>
<b>MT1-MMP</b>		
$\alpha_v\beta_3$	Endothelial	Galvez et al <sup>51</sup>
$\beta_1$ -subunit	Endothelial	Galvez et al <sup>51</sup>
CD44	Fibrosarcoma	Mori et al <sup>52</sup>
TIMP-2	Breast carcinoma	Imai et al <sup>53</sup>
Collagen type I	Gingival fibroblasts	Tam et al <sup>54</sup>
RECK	Fibrosarcoma	Oh et al <sup>55</sup>
<b>Serine proteases</b>		
<b>uPA</b>		
uPAR*	Malignant	Ellis et al <sup>56</sup>
$\alpha_{M\beta_2}$	Neutrophils	Xue et al <sup>57</sup>
$\alpha_v\beta_3$	Fibrosarcoma	Xue et al <sup>58</sup>
$\alpha_v\beta_5$	Mammary carcinoma	Carriero et al <sup>59</sup>
$\alpha_3\beta_1$	Mammary carcinoma	Wei et al <sup>60</sup>
<b>Elastase</b>		
$\alpha_M\beta_2$	Neutrophils	Cai and Wright <sup>61</sup>
<b>Seprase</b>		
uPAR	Melanoma	Artym et al <sup>62</sup>
$\alpha_3\beta_1$	Melanoma	Monsky et al <sup>63</sup>
<b>Dipeptidyl peptidase IV</b>		
$\alpha_3\beta_1$	Fibroblasts	Gherzi et al <sup>64</sup>
<b>Cathepsin G</b>		
FPR	Leukemias	Sun et al <sup>65</sup>
HIV-1 gp120	Leukemias	Avril et al <sup>66</sup>
Membrane Gp	Platelets/neutrophils	Molino et al <sup>67</sup>
<b>Proteinase 3</b>		
$\alpha_M\beta_2$	Neutrophils	David et al <sup>68</sup>
<b>Plasmin</b>		
Annexin II	Kidney cells	MacLeod et al <sup>69</sup>

Table 1. Proteinase association with integrins (continued)

Soluble proteases/associated proteins	Cell-surface expression	Source
<b>Cysteine proteases</b>		
<b>Cathepsin B</b>		
Annexin II	Tumors	Mai et al <sup>70</sup>
$\alpha_2$ -M	Bone metastases	Arkona et al <sup>71</sup>
<b>Caspase-8</b>		
$\alpha_3\beta_1$	Neuroblastoma	Stupack et al <sup>72</sup>
<b>ADAMs</b>		
<b>ADAM-2</b>		
$\alpha_6\beta_1$	Oocytes	Chen et al <sup>73</sup>
<b>ADAM-7</b>		
$\alpha_4\beta_1$	T-cell leukemia	Bridges et al <sup>74</sup>
$\alpha_9\beta_1$	T-cell leukemia	Bridges et al <sup>74</sup>
$\alpha_4\beta_7$	T-cell leukemia	Bridges et al <sup>74</sup>
<b>ADAM-9</b>		
$\alpha_6\beta_1$	Fibroblasts	Nath et al <sup>75</sup>
$\alpha_9\beta_1$	Oocytes	Eto et al <sup>76</sup>
$\alpha_v\beta_5$	Myeloma	Zhou et al <sup>77</sup>
<b>ADAM-12</b>		
$\alpha_9\beta_1$	Hematopoietic	Zhang et al <sup>78</sup>
<b>ADAM-15</b>		
$\alpha_v\beta_3$	Hematopoietic	Nath et al <sup>79</sup>
$\alpha_5\beta_1$	Hematopoietic	Nath et al <sup>79</sup>
$\alpha_9\beta_1$	Oocytes	Eto et al <sup>76</sup>
<b>ADAM-17</b>		
$\alpha_5\beta_1$	Epithelial	Bax et al <sup>80</sup>
<b>ADAM-23</b>		
$\alpha_v\beta_3$	Neuroblastoma	Cal et al <sup>81</sup>
<b>ADAM-28</b>		
$\alpha_4\beta_1$	Lymphocytes	Bridges et al <sup>74</sup>
$\alpha_9\beta_1$	T-cell leukemia	Bridges et al <sup>74</sup>
$\alpha_4\beta_7$	T-cell leukemia	Bridges et al <sup>74</sup>
<b>ADAM-33</b>		
$\alpha_9\beta_1$	T-cell leukemia	Bridges et al <sup>74</sup>

Many of the functions and binding mechanisms of these complexes have not yet been elucidated.

PAR1 indicates protease-activated receptor 1; Hsp, heat shock protein; BS, bone sialoprotein; HSPG, heparan sulfate proteoglycans; RECK, reversal-inducing cysteine-rich protein with kazal motifs; DMP-1, dentin matrix protein-1; FPR, formyl peptide receptor;  $\alpha_2$ -M,  $\alpha_2$ -macroglobulin; ADAM, a disintegrin and metalloproteinase; Gp, membrane glycoproteins; and —, not studied.

\*uPAR, in turn, interacts with  $\alpha_{M\beta_2}$ ,  $\alpha_v\beta_3$ ,  $\alpha_v\beta_5$ , and  $\alpha_3\beta_1$ .

suggesting that pericellular proteolysis may be activated and targeted by integrins and other cell-surface receptors.

In leukocytes, uPA could bind to its receptor, uPAR, and to  $\alpha_M\beta_2$  simultaneously, forming a trimolecular complex where  $\alpha_M\beta_2$  could serve as a signaling receptor.<sup>86</sup> This interaction is likely to be mediated by both the kringle and proteolytic domains for uPA and the I-domain for  $\alpha_M\beta_2$ . This complex plays an essential role in the migration of inflammatory cells and vascular homeostasis. The uPA/uPAR complex was also found to be associated with the  $\alpha_5\beta_1$ -integrin and capable of promoting adhesion and migration of Chinese hamster ovary cells as well as intracellular signal transduction through the integrin. In addition, a cyclic peptide DDGW discovered by phage display and an MMP-9–derived peptide motif HFDDDE both inhibited proMMP-9/ $\alpha_M\beta_2$  complex formation and leukocyte migration in vitro and in vivo.<sup>47,87</sup> However, this motif did not block leukocyte adhesion to ICAM-1 and fibrinogen, suggesting the integrin-bound MMP is essential for degradation of integrin-directed bonds to matrix proteins. Recently, proMMP-9 was found to be associated with ICAM-1<sup>42</sup> and DNA repair protein Ku<sup>44</sup> on the surface of leukemic cells. ICAM-1 cleavage by MMP-9 resulted in tumor cell resistance to natural killer cell–

mediated cytotoxicity. Also, a chaperone heat shock protein 90 (Hsp90) was found to interact with MMP-2 on the cell surface of fibrosarcoma cells, thus promoting MMP-2 activation, which is critical for tumor invasiveness.<sup>33</sup> The binding mechanism of most of these interactions has not yet been elucidated.

Several cell-surface hyaluronan receptor CD44 isoforms, RECK, TSP-1, LRP, and cell-surface collagen IV chains also serve as MMP-9-docking molecules. The CD44/MMP-9 complex was found to be associated with invasiveness of mouse mammary carcinoma and human melanoma cells *in vivo*,<sup>41</sup> suggesting that CD44 helps to localize MMP-9 activity to the cell surface. The GPI-linked proteins RECK and TSP-1 were not only identified as cell-surface receptors for MMP-9 but also were found to block their enzymatic activity.<sup>46,55</sup> Interaction of MMPs with the cell surface not only may be needed for proenzyme activation and targeting at specific sites for degradation of cell-surface substrates, but also could promote intracellular degradation via receptor-mediated endocytosis (RME). Regulation of the cell-surface activity of proteolytic enzymes that are involved in cancer progression, including MMP-2, -9, -13, tPA, and uPA by endocytosis, has led to suppression of tumor cell invasion.<sup>88</sup>

A disintegrin and a metalloproteinase (ADAMs) and ADAM with a thrombospondin motif (ADAMTS) comprise a large family of proteins capable of interacting with integrins and involved in processes such as angiogenesis, fertilization, myogenesis, neurogenesis, and inflammation. Unlike the transmembrane proteins ADAMs, ADAMTS proteins are soluble ECM proteases consisting of a prodomain, metalloprotease, and disintegrin domains, but devoid of ADAMs' cysteine-rich, EGF-like transmembrane and cytoplasmic domains.<sup>89</sup> ADAM2 or fertilin  $\beta$  was one of the first disintegrins identified and found to interact with  $\alpha_6\beta_1$ -integrin.<sup>73</sup> To date, several other ADAM-integrin interactions have been identified: ADAM9 with  $\alpha_5\beta_5$  and  $\alpha_6\beta_1$ , ADAM12 and ADAM15 with  $\alpha_9\beta_1$ , ADAM15 and ADAM23 with  $\alpha_v\beta_3$ , and ADAM15 with  $\alpha_5\beta_1$  (Table 1).<sup>90</sup>

## Role of integrins and gelatinases in cancer progression

Early events in tumor progression are characterized by increases in cell proliferation, insensitivity to growth-inhibitory signals, reduced ability for differentiation, as well as the ability to escape from apoptosis and immune surveillance.<sup>91</sup> Proteinases that degrade components of the ECM and are capable of processing nonmatrix substrates (eg, growth factors and their receptors, chemokines, adhesion molecules, and apoptotic mediators) have long been considered to be important at all stages of tumorigenesis.<sup>92</sup> The combined participation of integrins and MMPs is required for invasion of tumor cells into surrounding connective tissues, intravasation and extravasation from blood vessels, and metastasis to distant organs.<sup>93</sup> Indeed, studies on TIMPs have shown that overexpression or administration of these inhibitors as recombinant proteins inhibited experimental invasion and metastasis.<sup>94</sup> In most cases, the stage of tumor progression correlates with the expression levels of gelatinases, as the invasive and metastatic potential of tumor cells is strongly affected by changes in gelatinase expression in animal models. Expression of MMP-2 and MMP-9 was found to be strongly up-regulated in cancers of lung, colon, breast, skin, and prostate, which correlated with increased tumor invasiveness and metastasis.<sup>22</sup> Inhibition of MMP-9 expression in a model of experimental metastasis reduced the number of colonies formed in the lungs of mice.<sup>95</sup> Further evidence supporting

this hypothesis came from studies on MMP-2 and -9 null mice. These mice developed fewer tumors than the wild type.<sup>21</sup>

## Integrins and gelatinases in invasion and metastasis

The initial step of tumor cell invasion is characterized by the breakdown of the basement membrane, a process known to be dependent on type IV collagen-degrading enzymes, mainly MMP-2 and MMP-9. Liotta et al obtained results where type IV gelatinase activity correlated with cancer metastasis.<sup>96</sup> Endothelial cell proliferation and migration into the tumor tissue are mediated by angiogenic (eg, MMP-9, VEGF, and basic fibroblast growth factor [bFGF]) and lymphangiogenic factors that are released by tumor cells. Using DNA microarrays, primary tumor-gene expression profiles could be arranged in classes of "good" and "poor" prognosis. DNA-microarray analysis on human breast carcinoma cell lines that have metastasized to bone revealed some of the genes (eg, MMP-1, MMP-2, CXCR4, IL-11, and CTGF) responsible for the increased metastatic potential of breast cancer cells.<sup>97,98</sup> Videomicroscopy studies showed that MMPs play a significant role in tumor metastasis, as TIMP-1 and MMP inhibitor batimastat (BB-94) blocked the formation of tumors in secondary sites.<sup>99</sup> The role of MMPs in tumor invasion and metastasis has also been studied using small-interfering RNAs and antisense technology.<sup>100,101</sup> Gelatinases and MT-MMPs revealed a new mechanism to control metastasis by cleavage of the metastasis suppressor gene, KiSS-1.<sup>102</sup> Finally, recent studies supporting the *in vitro* data from double MMP-2/MMP-9-deficient mice demonstrated that these enzymes cooperate in promoting the invasive phenotype of malignant keratinocytes in an experimental model *in vivo*.<sup>103</sup>

Changes in integrin expression and localization can also influence invasion and metastasis of tumor cells.<sup>104</sup> Integrins were shown to be involved in the migration and liver metastasis of large cell lymphoma cells and angiogenesis, as  $\alpha_v\beta_3$  antagonists induced apoptosis and blocked cancer cell invasion.<sup>105</sup>  $\alpha_4\beta_1$ -integrin has a dual role in cancer progression as it inhibited the initial invasive growth while promoting metastatic spread of melanoma cells. A different study showed that increased expression of this integrin could inhibit the invasive stage of metastasis formation.<sup>106</sup> Blocking integrins with synthetic peptides containing an RGD sequence, antibodies, or disintegrins (integrin-binding proteins isolated from snake venom) has been demonstrated to interfere with tumor cell invasion and metastasis *in vitro* and *in vivo*.<sup>107</sup> Of importance, cooperation between  $\alpha_5\beta_3$  and MMP-9 increased migration of metastatic breast cancer cells.<sup>108</sup> Also, several reports show that uPA binding to its receptor uPAR is a requirement for tumor cell invasion and metastasis, as this process is efficiently inhibited either by an amino-terminal fragment of urokinase or a mutant plasminogen activator inhibitor-2 (PAI-2).<sup>109</sup> Finally, a recent study highlights the importance of chemokine receptors in breast cancer metastasis *in vitro* and *in vivo*.<sup>110</sup>

## Integrins and gelatinases in cancer-associated inflammation

Chronic inflammation is also associated with a variety of cancers, including breast, liver, prostate, and skin.<sup>92</sup> In human cancer, tumor cells are not the only source of MMPs. MMPs, mainly gelatinases, are predominantly produced by stromal cells, ranging from immune (lymphocytes and dendritic cells), inflammatory (granulocytes and monocytes), and vascular cells (vascular- and lymphoendothelial cells and pericytes). MMPs have been involved in the escape of cancer cells from immune surveillance. The escape mechanism occurs through MMP-9-induced cleavage of the interleukin-2 receptor (IL-2R $\alpha$ ),<sup>111</sup> TGF- $\beta$  activation,<sup>112</sup> and ICAM-1

and ICAM-2 shedding,<sup>42,113</sup> thus suppressing T-cell proliferation and immune response against tumors.

Chemokines play an essential role in regulating directional migration of leukocytes. Proteolytic cleavage of chemokines by MMPs can lead to enhanced or reduced leukocyte recruitment into tumors. For example, a cleaved form of MCP-3 produced by MMP-2 can bind to CC-chemokine receptors, and unlike intact MCP-3, it abrogates chemotaxis and suppresses inflammation.<sup>114</sup> ET-1 processing by MMP-9 generates endothelin-1 (ET-1) that induces secretion of MMP-9 from neutrophils,<sup>115</sup> suggesting that MMPs are both effectors of leukocyte migration and regulators of the inflammatory response. The importance of chemokine receptors in metastasis was demonstrated by inhibition of SDF-1 binding to its receptor. Dissociation of SDF-1/CXCR-4 complex by blocking antibodies strongly reduced breast cancer metastasis to lungs and lymph nodes *in vivo*.<sup>110</sup> MMP-9 and VEGF are produced by mammary tumor-infiltrating immune cells.<sup>116</sup> Expression of MMP-9 by tumor-infiltrating macrophages promotes angiogenesis as well as growth and invasion of xenografted ovarian cancer cells *in vivo*.<sup>117</sup> Several studies show that cancer cells can promote the secretion of MMPs by stromal cells in a paracrine manner via secretion of growth factors, interleukins, and EMMPRIN.<sup>21</sup> Recruitment of hematopoietic precursor cells is also required for tumor angiogenesis.<sup>118</sup>

## Role of integrins and gelatinases in acute leukemias

Leukemia can be described as the uncontrolled proliferation of hematopoietic cells that lack the ability to differentiate into mature blood cells. The precise role of gelatinase expression in acute leukemias is not clear. So far, it is known that invasiveness of many hematologic malignancies, including myelo-monocytic leukemias, involves overexpression of proteolytic enzymes, such as MMP-2 and MMP-9.<sup>119</sup> MMP-9 is induced and secreted in conditioned media of leukemic cell lines in response to extracellular stimuli, after pretreatment of cells with chemokines, and after cell adhesion to the ECM.<sup>119</sup> Higher gelatinase expression levels were detected in the bone marrow plasma of patients with leukemia compared with healthy controls. After chemotherapy, the levels of TIMP-1 and TIMP-2 were significantly increased, whereas MMP-9 levels were lower in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. Accordingly, AML patients who achieved a complete remission showed significantly lower MMP-9 levels, suggesting that MMP-9 could be a surrogate marker of leukemic status in these patients. Also, the low MMP-9 expression levels in patients with leukemia correlated with increased survival.<sup>120</sup>

Several reports have demonstrated the involvement of both MMP-2/9 gelatinases and  $\beta_2$ -integrins in the growth and progression of myeloid and lymphoid neoplasms.<sup>121,122</sup> Selective MMP-9 expression is induced as a result of  $\alpha_M\beta_2$ -integrin ligation in PMNs<sup>123</sup> and  $\alpha_L\beta_2$ -integrin ligation in T-lymphoma cells.<sup>124</sup> Also, studies from  $\alpha_M$ - and  $\alpha_L$ -integrin knock-out mice confirm the importance of  $\beta_2$ -integrins in mediating leukocyte adhesion and migration.<sup>125</sup> In accordance, high infiltration of leukemic blasts in patients with AML strongly correlated with increased expression of both  $\alpha_L\beta_2$ - and  $\alpha_M\beta_2$ -integrins.<sup>121</sup> AML cell adhesion to bone marrow fibroblast monolayers seems to require both  $\beta_1$ - and  $\beta_2$ -integrins, as antibodies against them inhibited the binding.<sup>126</sup> Interaction between leukemic cells and bone marrow stroma cells has been shown to increase leukemic cell survival and chemotherapy-induced leukemia cell resistance.<sup>127</sup>

Increased vessel density was detected in the bone marrow of acute and chronic leukemia patients compared with normal bone marrow, and is known to be mediated by angiogenic factors such as VEGF and bFGF.<sup>128,129</sup> Both increased plasma MMP-9 and VEGF correlated with high leukemia cell infiltration, suggesting that MMP-9 and VEGF act cooperatively in the process of leukemia cell invasion.<sup>122</sup> Another study showed that increased vessel density was mediated by MMP-2 and MMP-9 overexpression in primary AML blasts by promoting endothelial cell migration.<sup>130</sup> After achieving complete remission, the vessel number in AML patients was restored to normal levels. Furthermore, a gene therapy approach using a retroviral vector encoding for gelatinase inhibitors, endostatin and angiostatin, strongly inhibited bone marrow angiogenesis and leukemia tumor growth *in vivo*.<sup>131</sup> These data suggest that gelatinases could be involved in leukemia progression. As a result, inhibitors of MMPs may be useful in treating hematologic malignancies.

## Therapeutic intervention with MMP and integrin inhibitors

Due to the fact that integrins and MMPs are involved in tumor cell invasion and metastasis, over the past 20 years a lot of effort has been put into designing integrin and MMP inhibitors (MMPIs). Although endogenous inhibitors, such as TIMPs, inhibited tumor growth in transgenic mouse models, their use in cancer was limited due to poor pharmacokinetics, difficulties in protein administration, and broad spectrum of inhibition. To date, several synthetic MMPIs have been developed, tested widely in clinical trials, and classified into the following pharmacologic groups: collagen peptidomimetics, nonpeptidomimetics, tetracycline derivatives, and biphosphonates.<sup>132</sup> The efficacy of these inhibitors in clinical trials is summarized in Table 2.

The design of collagen peptidomimetic MMPIs is based on the collagen-peptide backbone with zinc-binding hydroxamate moiety that coordinates the  $Zn^{2+}$  ion, thus inhibiting the MMP catalytic activity. An oral MMPI, marimastat, significantly increased survival of patients with gastric carcinoma. Treatment with marimastat was well tolerated by the patients, except for some minor side effects such as musculoskeletal pain, probably because of the need of MMPs in normal remodeling of the connective tissue of tendons and joints. In patients with advanced pancreatic cancer (a phase 2 study), marimastat showed comparable therapeutic effects as conventional therapy with gemcitabine that was used.<sup>133</sup> The survival of patients suffering from glioblastoma multiforme was also improved by using marimastat in combination with temozolomide, a cytotoxic drug.<sup>134</sup> Several nonpeptidomimetic MMP inhibitors, including BMS-275291, AG3340, and MMI270, have also been tested in clinical trials (Table 2).

Tetracyclines and biphosphonates have also been shown to block MMP activity.<sup>135</sup> For example, a broad spectrum MMP inhibitor, metastat (or Col-3), showed increased tumor cell toxicity, reduced tumor-induced angiogenesis, as well as antimetastatic activity,<sup>136</sup> and is currently being tested in patients with Kaposi sarcoma and brain cancer in a phase 2 clinical trial. Periostat, a tetracycline used for the treatment of periodontal diseases, is the only MMPI on the market. Of interest, compounds (TSRI265) capable of inhibiting interactions between MMPs and integrins showed promising results in animal experiments.<sup>85</sup> Also, a cyclic peptide, CTTHWGFTLC, discovered by phage display technology as a selective gelatinase inhibitor, could block cell migration and tumor growth in a gelatinase-dependent manner.<sup>137</sup>

**Table 2. MMP and integrin antagonists in clinical trials**

Inhibitors	Structure	Specificity	Status/indication
<b>MMPs</b>			
Batimastat (BB-94)	Peptidomimetic	MMP-1, -2, -3, -7, -9	Development halted
Marimastat (BB-2516)	Peptidomimetic	MMP-1, -2, -7, -9	Phase 3/gastric cancer; phase 2/pancreatic cancer
BAY12-9566	Nonpeptidomimetic	MMP-2, -3, -9	Development halted
AG3340	Nonpeptidomimetic	MMP-2, -3	Phase 2/3/no benefit
BMS-275291	Nonpeptidomimetic	Broad spectrum	Phase 1/2/NSCL
MMI270	Nonpeptidomimetic	Broad spectrum	Phase 1/advanced cancer
Metastat (Col-3)	Tetracycline derivative	MMP-2, -9	Phase 2/Kaposi sarcoma
Periostat	Tetracycline derivative	Broad spectrum	Phase M/periodontal disease
Neovastat (AE-941)	Shark cartilage extract	Broad spectrum	Phase 2/multiple myeloma; phase 3/NSCL
Not known	Green tea extract	MMP-2, -9	Phase 3/cancer
<b>Integrins</b>			
Efalizumab/Hu1124	Humanized MAb	CD11 $\alpha$ subunit	Phase 3/psoriasis
Anti-CD18	Humanized MAb	CD18	Phase 2/myocardial infarction
Anti-LFA1	Murine	CD18	Phase 3/allograft rejection
Hu23F2G	Humanized MAb	CD11/CD18 integrin	Phase 2/multiple sclerosis; phase 2/myocardial infarction; phase 3/stroke
LDP-01	Humanized MAb	CD18 integrin subunit	Phase 2/allograft rejection/stroke
LDP-02	Humanized MAb	$\alpha_4\beta_7$ -integrin	Phase 2/ulcerative colitis
Volociximab & erlotinib	MAb	$\alpha_5\beta_1$ -integrin	Phase 2/metastatic NSCL
ATN-161	PHSRN motif from FN	$\alpha_5\beta_1$ -integrin	Phase 1/NSCL
M200	MAb	$\alpha_5\beta_1$ -integrin	Phase 2/kidney cancer
Vitaxin/LM609	Humanized MAb	$\alpha_v\beta_3$ -integrin	Phase 2/sarcoma
Antegren	Humanized MAb	$\alpha_4\beta_1$ -integrin	Phase 3/multiple sclerosis; phase 2/colitis, Crohn disease
Tysabri/natalizumab	MAb	$\alpha_4\beta_1$ -integrin	Phase M/multiple sclerosis
Abciximab	Chimeric Ab	$\alpha_{IIb}\beta_3$ , $\alpha_v\beta_3$ , $\alpha_M\beta_2$	FDA approved
Eptifibatide	Cyclic heptapeptide	$\alpha_{IIb}\beta_3$ -integrin	FDA approved
Tirofiban	Peptidomimetic	$\alpha_{IIb}\beta_3$ -integrin	FDA approved/myocardial infarction
Cilengitide	Cyclic RGD peptide	$\alpha_v\beta_3/\alpha_v\beta_5$ -integrin	Phase 2/GBM
Altacor/lovastatin	Chemical	$\alpha_L\beta_2$ -integrin	FDA approval/atherosclerosis

Phase M indicates on the market; NSCL, non-small-cell lung cancer; Mab, monoclonal antibody; FN, fibronectin; GBM, glioblastoma multiforme; and FDA, Food and Drug Administration.

The involvement of integrins in tumor cell invasion and metastasis became clear after using  $\alpha_v$ -<sup>138</sup> or  $\beta_1$ -<sup>139</sup> subunit-blocking antibodies or small synthetic antagonists generated from the ligand's recognition sequence. Humanized mAbs, vitaxin and efalizumab against  $\alpha_v\beta_3$ -<sup>140</sup> and  $\alpha_L$ -subunit of  $\alpha_L\beta_2$ ,<sup>141</sup> respectively, and the synthetic, cyclic Arg-Gly-Asp (RGD) peptide motif<sup>142</sup> present in many integrin ligands, were the 3 among many other integrin-binding agents that have entered cancer clinical trials (Table 2). Efalizumab and a recombinant mAb against  $\alpha_4\beta_1$ , natalizumab, have shown a great promise in the treatment of psoriasis,<sup>141</sup> as well as in multiple sclerosis and Crohn disease, respectively.<sup>143,144</sup> However,  $\beta_3$ - and  $\beta_5$ -integrin knock-out mice showed increased expression of VEGFR-2 receptor, leading to enhanced tumor angiogenesis.<sup>145</sup> Taken together, MMP and integrin knock-out models and inhibitors can increase our understanding of the multiple functions of these molecules in several diseases, including cancer. Such studies may be used to develop therapeutic agents that can interfere with the integrin and MMP function on invasive tumor cells and blood vessels.

## Concluding remarks

The failure of MMPi in several cancer clinical trials is not surprising.<sup>146</sup> Most MMPi were used to treat patients with late-stage tumors, whereas most results obtained from animal experiments show the need for targeting MMPs in early stages of cancer progression. Also, these inhibitors target all MMPs, many of which are needed for the processing of antiangiogenic factors, including angiostatin and endostatin. For that, increasing the selectivity of these compounds (for example, for gelatinases

involved in metastasis) could solve the problem of side effects reported so far. MMPi are known to target also ADAMTS, enzymes capable of reducing tumor growth by blocking tumor angiogenesis.<sup>147</sup> It should be taken into consideration that other proteases are up-regulated during tumor progression that could compensate for the loss of MMPs. These proteases should be identified and targeted along with MMPs.

Extensive effort has been made in developing small molecules, peptides, and peptidomimetics capable of inhibiting interactions that occur on the cell surface. Several linear and cyclic peptides derived from sequences of  $\beta_2$ -integrins, ICAMs, and ECM proteins have been shown to have inhibitory effects in vitro and in vivo. Indeed, ICAM-1-derived peptides can control immune responses in autoimmune diseases and allograft rejection by simply blocking ICAM-1 binding to  $\alpha_L\beta_2$ -integrin. Peptides derived from the sequence of  $\beta_1$ - and  $\beta_2$ -integrins have also been shown to be potent anti-inflammatory agents by blocking integrin-mediated adhesion of leukocytes.<sup>148</sup> Furthermore, inhibition of an integrin-MMP cell-surface complex,  $\alpha_v\beta_3$ /MMP-2, dramatically suppressed tumor angiogenesis in vivo, suggesting that this interaction is essential for endothelial cell proliferation and migration.<sup>85</sup> Such reagents were reported not only to interfere with ligand binding, but also could stabilize integrin conformations. Also, integrin-directed small molecules have entered phase 1 and 2 clinical cancer trials, as they showed strong inhibition of tumor angiogenesis.<sup>149</sup> Peptides containing the RGD sequence have been demonstrated to inhibit experimental tumor metastasis in animal models.<sup>107</sup>

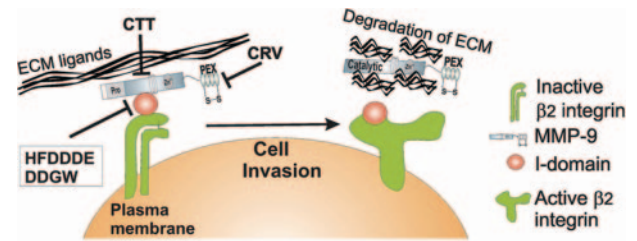
Studies on the role of MMP-9 in leukocyte migration have been controversial. For example, some reports have supported MMP-9 function in leukocyte migration,<sup>150,151</sup> whereas others have not.<sup>152,153</sup> These findings are not surprising as MMPs are known to have

overlapping functions and other MMPs within the family could compensate for the loss of MMP-9.

The physiologic role of MMP-2 and -9 is not fully understood, but to our current knowledge they are involved in the processing of the extracellular matrix during growth and tissue differentiation, probably as critical factors for cell motility. Proteases and integrins for such a function have been expected to be colocalized at the surface of migrating leukocytes and other cells. Most MMPs, however, are secreted enzymes and the search for cell-surface receptors for MMPs has been going on for years. At the moment there are some hundred publications describing receptors, such as integrins for various MMPs, among them MMP-2 and -9. Likewise, gelatinase activity has been found in the membrane of leukocytes, but the identification of the leukocyte integrins as gelatinase receptors is new to our knowledge<sup>47,87</sup> and likely to extend our understanding of further mechanisms involved in leukocyte migration. Studies from knock-out models for integrins, including leukocyte  $\beta_2$ -integrins, confirm their involvement in various steps of cancer development. Eventually, tumor growth and metastasis could be blocked by interfering with integrin function on tumor cells and blood vessels.

MMP-9 has been reported to cooperate with  $\alpha_v\beta_3$ - and  $\alpha_3\beta_1$ -integrins. MMP-9 was able to stimulate  $\alpha_v\beta_3$ -integrin-dependent migration of metastatic breast cancer cells and  $\alpha_3\beta_1$ -integrin-induced tumor invasion.<sup>108</sup> MMP-9 and uPA have been also identified as critical players in the invasion of tumor cells to the blood circulation (a process called intravasation). These proteases may act in concert with integrins, such as  $\alpha_v\beta_5$ , which, in turn, is also required for tumor cell dissemination in a chicken choriocallan-toic membrane assay.<sup>82</sup>

We have recently shown that proMMP-9/ $\alpha_M\beta_2$  complex is stored within the intracellular granules in resting PMNs and translocated to the cell surface upon cell stimulation (Figure 2). This is a more plausible mechanism for the MMP/integrin complex formation than binding of a secreted MMP to an unoccupied integrin on the cell surface. Also, leukocyte integrins could play a role in targeting of proMMPs to a site where proteolytic activity is



**Figure 2. Schematic summary of the integrin/MMP complex in PMNs.** The  $\alpha_M\beta_2$ /MMP-9 complex is formed in PMN intracellular granules and can be rapidly mobilized to the cell surface after exposure to degranulation stimuli, such as TNF- $\beta$ , LPS, and fMLP. PMN degranulation can also be achieved when cells are in contact with ECM proteins. Upon PMN activation, cell-surface receptors are routinely shed from PMNs. Loss of these receptors is mainly due to PMN-derived MMP activity, a process that facilitates PMN rolling and migration via degradation of the vascular basement membrane during PMN extravasation. Disruption of the  $\alpha_M\beta_2$ -integrin/MMP-9 complex by integrin- (HFDDDE and DDGW) and MMP-9- (CTT and CRV) binding peptides strongly reduced PMN migration *in vitro* and *in vivo*.

needed. However, it remains to be determined by which mechanism (pro)MMP-9 is located at the surface of cells lacking  $\beta_2$ -integrins. Based on our findings, new and more effective cancer therapeutics could be achieved by blocking, not only MMPs alone but their association with integrins or other cell-surface receptors. A peptide motif derived from the MMP-9 catalytic domain, HFDDDE, and a cyclic peptide, DDGW, discovered by phage display both inhibited proMMP-9/ $\alpha_M\beta_2$  complex formation and leukocyte migration *in vitro* and in a thioglycolate-induced peritonitis model *in vivo*.<sup>47,87</sup> Also, a C-terminal domain MMP-9-binding peptide, CRVYGPYLLC (or CRV), inhibited the interaction between MMP-9 and  $\alpha_v\beta_5$ -integrin, resulting in reduced angiogenesis and tumor invasion.<sup>50</sup> Finally, a cyclic peptide, CTTHWGFTLC, discovered by phage display technology as a selective gelatinase inhibitor, could block leukemia cell migration and tumor growth in a gelatinase-dependent manner.<sup>87,137</sup> Selective antagonists of the MMP-9/ $\alpha_M\beta_2$ -integrin interaction may be therapeutic not only in leukemias but also in other types of malignancies where tumor-infiltrating leukocytes enhance tumor growth.

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