The role of adhesion molecules in acute myeloid leukemia and (hemato)oncology: A systematic review

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**Background.** The treatment of malignancies like acute myeloid leukemia (AML) is often complicated by the heterogeneity of the disease and the mechanisms of the disease progression. This heterogeneity is often not reflected in standard treatment approaches which provide predictable outcomes in the majority of patients but fail in individual cases even with high-dose multi-agent chemotherapy regimens and allogeneic stem cell transplantation. Further, the unselective effect of chemotherapy causes high treatment-related toxicity and accelerates the risk of infection during prolonged pancytopenia, preventing further dose escalation. Despite rapid progress in therapeutic strategies, the fatality of high-grade malignancies remains enormous.

**Objectives.** Adhesive interactions trigger signal transduction pathway activation and this prevents the apoptosis of both normal and malignant cells. A correlation between expression of defined adhesion molecules and patient outcome has been found for several malignant diseases including AML. We aim to describe how disruption of these signalling pathways can overcome the high resistance to treatment and increase the selectivity of targeting malignant cells. This could effectively reduce the overall treatment-related toxicity and improve the general outcome.

**Conclusions.** Adhesion molecules facilitate growth of malignant diseases. This review provides a deeper insight into these processes. Modulation of adhesion molecules-mediated interactions is an innovative and feasible approach in treatment of AML and many other malignancies. Due to expected low toxicity it is an acceptable addition to standard chemotherapeutical regimens for all age groups of patients. This approach could improve the overall treatment outcome in the future.

**Key words:** AML, adhesion molecules, prognosis, treatment approaches

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**INTRODUCTION**

Acute myeloid leukemia (AML) is an aggressive disorder characterized by accumulation of immature malignant cells in the bone marrow. Standard chemotherapy regimens provide predictable outcomes in the majority of patients but the relapse rate is enormous. Even high-dose multiagent chemotherapy and allogeneic stem cell transplantation often fail to prevent relapses and most adult patients die from the disease due to high resistance to treatment.

Adhesion molecules (AM) are crucial components of cell-cell, the same as cell-matrix interactions. In this review, we highlight AM mediated interactions important for AML cell survival and disease progression. On binding to their adhesive counterparts, AM moderate attachment of immune cells to vessel walls under blood flow and enable tissue infiltration in a nonspecific inflammatory response. Specific modulations of adhesive molecules enable more selective cellular trafficking into sophisticated structures of skin, lymphnodes and bone marrow. AM are classified into four major families: cadherins, selectins, integrins, and imunoglobulin family AM (ref.\textsuperscript{1}). To cover the whole spectrum of adhesive interactions relevant in hematooncology, we also provide the most important data on the roles of chemokine receptor CXCR4 and CD44 molecules in cellular adhesion.

As we accumulated interesting data, we found that adhesive interactions are also critical for disease progression in solid tumors. Further, we found that, like cytokines, adhesive interactions trigger activation of several signal transduction pathways. The influence of inflammation and altered cytokine signalling on oncogenesis, leading to tumor progression, have been documented\textsuperscript{2,3}. In fact, adhesive interactions with platelets and endothelial cells help malignant cells to acquire protection from both apoptosis and destruction by the immune system. We identified malignant disease-mediated positive feedback loops causing adhesion molecule inflammatory overexpression on endothelial cells. Here we describe these mechanisms and clarify the ability of malignant cells to sustain toxic concentrations of several broadly used chemotherapeutic agents. We also focus on adhesion molecule-dependent mechanisms that modulate selective trafficking of defined cells into various compartments in macroorganisms on the understanding that these processes may be the pivot of innovative treatment approaches. Above all, what we consider to be the most important, is the fact that a limited
count of adhesion molecules and their clinically relevant modifications, act with the same patterns in several and very probably the vast majority of malignant diseases. We anticipate that therapeutic approaches combining specific modulation of defined adhesive interactions with standard treatment could be successful in a broad spectrum of solid tumors and hematologic malignancies. This reasoning led us to include relevant data on adhesive interactions in solid tumors in this review which was originally aimed at covering their role in AML and hematopoiecity.

We have previously reviewed the role of cytokines in AML. Cytokines are soluble molecules carrying specific information for target cells with the capacity to modulate expression of adhesion molecules on endothelial cells in an inflammatory response. Cytokines may be produced by AML blasts. There is evidence that AML activity influences serum levels of cytokines, growth factors and soluble adhesion molecules. Experimentally, leukemic cytokines enhanced endothelial cell growth in a co-culture of AML and endothelial cells. Together with AML induced endothelial overexpression of AM, these mechanisms may underlie leukemogenesis. We demonstrate that cytokines and adhesion molecules form a unique interacting functional network that, when deregulated, supports the growth and survival of malignant cells. We shed light on how disruption of these mechanisms may restore tumor sensitivity to therapy.

**CADHERINS**

Cadherins are glycoprotein macromolecules that participate in very compact intercellular junctions (tight junctions) providing sophisticated isolation of different compartments in macroorganisms. Cadherine AM are bound to the cytoskeleton. Vascular endothelial cadherin (VE-cadherin) is a subtype expressed in endothelia. VE-cadherin is essential for the maintenance of the endothelial barrier and prevents leukocyte infiltration of the tissue. Antibodies against VE-cadherin dissociate the contacts of endothelial cells in culture. Administration of monoclonal antibodies (mAb) against VE-cadherin accelerates neutrophil migration into the inflamed peritoneum in rodents. The anti-VE-cadherin mAb mediated increase in vascular permeability is concentration and time dependent. Further, upon Vascular Endothelial Growth Factor (VEGF) binding to endothelial receptors, the intracellular domain of VE-cadherin is phosphorylated and the interaction with cytoskeleton is modulated. These steps further increase endothelial permeability. This mechanism seems to be important in AML biology. VEGF secretion by AML cells was confirmed in adults as for pediatric AML patients. In a pediatric model, significant increase in VEGF secretion for M4/M5 AML according to the FAB classification was found. Both VEGF levels and age at diagnosis had independent significant effect on relapse free survival. VEGF activates downstream kinase pathways. VEGF receptors 1 and 2 are expressed on subsets of acute myeloid and acute lymphoid leukemia. Activation of VEGF receptor-1 (VEGFR-1) on acute myeloid leukemia cells was shown to involve p38 and Erk1/2 activation. In vitro it has been demonstrated that VEGFR-1 expression in AML is linked to cholesterol accumulation in membrane domains and that cholesterol-rich domains regulate VEGFR-1 signaling on subsets of AML in vitro. Primary leukemia cells were also found to accumulate significantly more cholesterol than do normal cells and that this accumulation correlates with disease aggressiveness. Coinciding expression of VEGFR-2 on AML cells may multiply the effects of this autocrine loop. The neutralizing mAb, specific to human VEGFR-2 inhibited leukemic cell survival and VEGF-induced proliferation and migration in vitro. From the foregoing, we conclude that AML is capable of influencing vascular permeability by modulating AM functions and the same mechanism also provides AML clone expansion. These findings may clarify the effect of anti-VEGF therapy on leukemia regression in experimental AML models.

**SELECTINS AND SIALYL LEWIS X**

Selectins are glycoprotein macromolecules with short C-terminal intracellular domain. At the N-terminus of selectin molecules, a domain similar to C-type lectin is the site of adhesive interactions. Underneath this domain, the EGF-like chain (Epidermal Growth Factor-like) and a variable number of SCR (Short Consensus Repeat) domains are located. According to the SCR-domain count, P-, E- and L- selectin are distinguished. Soluble forms of selectin AM probably arise from proteolytic cleavage of surface-expressed molecules. In lymphoma-bearing mice, the lymphoma cells are the major source of soluble L-selectins. Selectins bind to a surface tetrasaccharide structure called “Sialyl Lewis x” epitope (sLeX) and sequentially cooperate to support leukocyte tethering and rolling along inflamed vascular walls by mediating interactions with counter-receptors expressed by endothelium, adherent platelets, or leukocytes. SLLeX epitope may be integrated as a part of molecules CD15, CD24, CD43 and CD162. The full details of the synthesis of (sLeX/CD15x) from core molecules (sialylation) were described by Gege et al. These structures are broadly expressed in human tissues and sLeX is constitutively expressed on granulocytes and monocytes and mediates inflammatory extravasation of these cells. Resting T and B lymphocytes lack its expression but are strongly induced to express sLeX upon activation. Among T lymphocytes, the sLeX determinant is known to be preferentially expressed on activated Th1 cells but not on Th2 cells.

Defective synthesis of the sLeX is caused by the loss of fucosyltransferase (FucT) activity. Impaired glycosylation of core proteins results in leukocyte adhesion deficiency type 2 (ref.,23). Furthermore, specific peracetylated disaccharide precursor reduced sLeX expression on tumor cells and diminished binding to selectin-coated surface.

Sialylation has profound implications for immunoreactivity and biologic functions. Although bearing a common trisaccharide core, antibodies to sLeX do not recognize
LeX and vice versa. Identification of sLeX with monoclonal antibodies such as HECA-452 has been useful in defining subsets of cells that bind both E- and P-selectin (not only P-selectin as do unsialylated molecules) and display specialized tissue migration patterns, such as dermatotrophic lymphocytes\(^{30-32}\) and osteotropic stem cells\(^{33,34}\). The sLeX determinant is associated with the most primitive subset of the resident bone marrow cells in humans. Myeloid cell maturation is accompanied by relative loss of sLeX/CD15s and gain of LeX/CD15 expression. During myeloid differentiation, both sLeX/CD15s synthesis and sialidase activity are increased; however, the increase in sialidase activity dominates so that the overall expression pattern is an increase in LeX and a decrease in sLeX expression\(^{35}\). Changes in the sialylation pattern also occur on the CD43 molecule, a sialomucin expressed on dermatotropic T-lymphocytes. Bearing of the CLA epitope (CLA, cutaneous lymphocyte-associated antigen) is associated with the acquisition of both P-selectin and E-selectin ligand functions\(^{36}\).

New insights on adhesive interactions and the prognostic relevance of tumor CD24 expression have been recently documented for various nonhematological malignancies\(^{37-43}\). Knowledge of sLeX mediated adhesive interactions with endothelial cells and platelets is important for understanding the pathophysiology of tumor metastasis\(^{44-46}\). Both CD24 and CD44 molecules that serve as selectin ligands are strongly expressed in solid tumors. In postresection patients, an inverse correlation between survival and sLeX expression in tumor cells was found\(^{46-51}\). P-selectin deficient mice showed slower growth of subcutaneously implanted human colon carcinoma cells and generated fewer lung metastases after intravenous administration. In vitro experiments demonstrated that normal mouse platelets but not P-selectin-deficient platelets, bound to control tumor cells and protected them from leukocyte-mediated cytolysis\(^{46}\).

### E-selectin

E-selectin (CD62E) is expressed by endothelia. Binding to sLeX plays an important role in AML growth and tumor progression. The E-selectin ligand CD65 was found critical for AML blast transmigration and extravasation\(^{52}\). The E-selectin expression is regulated on the transcription level and is induced by TNF\(\alpha\), IL-1 and Oncostatin M (ref.\(^{1}\)). In response to G-CSF, endothelia increase expression of E-selectin, VCAM-1 and ICAM-1 and promote augmented leukocyte adhesion in a p38 MAPK dependent manner\(^{53}\). Both E- and P-selectin are permanently expressed in bone marrow (BM) endothelia and together with VCAM-1 mediate rolling of hematopoietic stem and progenitor cells (HPCs) on BM endothelium.

Among human hematopoietic progenitors, two well-described glycoprotein E-selectin ligands are known to be expressed: HECA-452 reactive PSGL-1 (CLA) and HECA-452 reactive glycoform of CD44 termed HCELL. In contrast to PSGL-1 that displays CLA on O-glycans, the CLA determinants and the E/L-selectin binding sites of HCELL are on N-glycans. The CLA epitope located on PSGL-1 is expressed in the majority of skin homing lymphocytes. Only CLA - PSGL-1 functions as an E-selectin ligand whereas both CLA + and CLA - glycoforms of PSGL-1 can bind P-selectin\(^{30,32,33}\). Both E- and P-selectin are constitutively expressed on dermal microvasculature. Hence it is CLA modification of PSGL-1 that promotes homing of memory T-lymphocytes to skin. CLA is upregulated in malignant T cells in patients with cutaneous T cell lymphoma and this directly correlates with the unique pattern of skin involvement, providing the opportunity to target these sLeX moieties and attenuate the dermal dissemination of malignant T cells. Subsequent experiments modulated P- and E-selectin ligand activities by fluorosugar analogues and the influence on E- and P-selectin recognition by malignant human T cells was studied\(^{34,35}\).

E-selectin also mediates critical steps in cellular homing and engraftment into the BM. Both CLA and HCELL are involved in these processes. HCELL is a glycoform of an integral membrane glycoprotein, CD44, that also expresses the CLA epitope (recognized by mAb HECA452). HCELL expression is characteristic of the most primitive hematopoietic cells, especially the earliest subset of HSC lacking CD38 expression\(^{46,57}\). All human cells that express HCELL, including hematopoietic progenitor cells (HPCs), de novo AML cells, the AML-derived cell line KG1a and G-CSF-mobilized peripheral blood leukocytes, display higher binding to E-selectin than cells lacking HCELL. Selectin-independent rolling in BM sinusoid is mediated by alpha-beta1 integrin (see below). All three mechanism involving VLA-4, PSGL-1 and HCELL were necessary to gain full homing activity in lethally irradiated mice\(^{34-46}\).

In case the bone marrow trafficking is not included into the model, the P- and E-selectin mediated adhesion resulted in growth inhibition of HPCs and a subpopulation of more differentiated cells underwent apoptosis following adhesion to E-selectin. These processes were found to be PSGL-1 independent\(^{51}\), probably employing HCELL molecule (author’s comment). Recent data show these mechanisms may support AML expansion. AML cells were found to trigger positive feedback loops and activate endothelial cells. Activated endothelia overexpress E-selectin so that AML cells may adhere to activated endothelia. The adherent AML cells are in a quiescent state, escape chemotherapy and later detach and become proliferative again. Anti E-selectin treatment completely abrogated this protection. This mechanism suggests how E-selectin protects AML cells from chemotherapy\(^{52}\). The selectin-dependent protection of malignant cells is not restricted to hematologic malignancies. Like sLeX mediated adhesion of neutrophils, pancreatic cancer cells also adhere to endothelial E-selectin, which is increased after TNF\(\alpha\) stimulation\(^{53}\). In conclusion, surgical trauma may promote the hematogenic spreading of pancreatic cancer cells via TNF\(\alpha\) induced E-selectin expression. On the other hand, the upregulation of E-selectin expression is dependent on the p38 MAPK signaling pathway and may be inhibited pharmacologically\(^{54}\). At the experimental level, FucT-I transduction into malignant cells decreased sLeX expression and dramatically modified interactions with
E-selectin and this may form the basis for antimetastatic gene therapy\(^{64}\). Overall, it is clear that some populations of cells may be rescued from apoptosis following adhesion to E-selectin. Further studies of TNFα overproduction and selectin AM expression in patients with malignancies may provide substantial progress in understanding the complex mechanisms of tumor biology. As a possible novel treatment approach of broad therapeutic application, the mechanisms involved in these processes should be further investigated.

**L-selectin**

L-selectin (CD 62L) is “leukocyte” selectin, a glycoprotein constitutively expressed on granulocytes, monocytes and lymphocytes. L-selectin is involved in targeted migration (homing) of lymphocytes to lymph nodes and sites of chronic inflammation. L-selectin is a PSGL-1 ligand but it also binds αβ, integrin Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1). L-selectin mediates leukocyte homing to lymphnodes and HPC homing to bone marrow\(^{1,66-68}\). The leukocyte homing is based on the selective attachment of lymphocytes to high endothelial venules (HEV) of peripheral lymph nodes. A sulfotransferase that is highly restricted to HEV is essential for L-selectin ligand activity\(^{69}\). L-selectin also binds the sLe\(^x\) motif attached to the endothelial CD34 molecule\(^{70}\). Alternatively, in sites of chronic inflammation β\(^{-}\) integrin mediated pathway also participates in leukocyte recruitment\(^{71}\).

L-selectin deficit results in the complete loss of lymphocyte ability to bind HEV and populate peripheral lymph nodes (PLNs). Memory T cells that lack L-selectin or CC-chemokine receptor 7 cannot enter PLNs (ref.\(^{27}\)). In a murine model of T-cell lymphoma, the lack of L-selectin expression delayed the dissemination to peripheral tissues. This resistance of selectin-deficient mice to lymphoma progression was restricted to variants with lower malignancy. Highly tumorigenic variants were insensitive to the absence of L-selectin. In general, the role of L-selectin in the spread of T-cell lymphomas is probably less important compared to ICAM-1/LFA-1 interaction\(^{72}\). Interestingly, L-selectin mediated signalling enhanced CXCR4 surface expression in lymphocytes. L-selectin-induced CXCR4 emanates from intracellular stores because most of the CXCR4 in freshly prepared lymphocytes is inside the cell and it is induced to mobilize to the surface within minutes\(^{28}\).

The prognostic significance of L-selectin and soluble (s)L-selectin in AML has also been studied. AML blasts were found to express L-selectin but the expression is variable. In a study of 36 AML patients at diagnosis, a correlation of low L-selectin expression and adverse cytogenetics was found\(^{73}\). The low L-selectin expressers had a lower probability to achieve complete remission and had shorter relapse-free survival. In a different study on 50 AML patients, those with higher sE- and sL-selectin levels at diagnosis had higher relapse rate and shorter event free survival\(^{74}\). Despite convincing data in both studies, the cohorts were rather small and multivariate analysis including other adhesion molecules was not performed. We conclude that at the moment, we lack sufficient data to judge the prognostic role of L- and sL-selectin in AML.

**P-selectin**

P-selectin (CD62P) is a 140kD glycoprotein expressed by activated platelets and endothelia. P-selectin is stored in intracellular vesicles (Weibel-Palade bodies) and is rapidly expressed on the surface after activation. P-selectin containing storage granules were also found in endothelial cells\(^{75}\). Upon thrombin triggered platelet activation, the p38 MAPK is phosphorylated and thus activated but the mechanism that mobilizes P-selectin from intracellular stores is p38 MAPK independent\(^{76}\). P-selectin was found to support leukocyte rolling along postcapillary venules at the earliest phase of inflammation\(^{77}\).

The activators of P-selectin transcription are interleukins IL-4 and IL-13 and a Oncostatin M, a pleiotropic cytokine belonging to the IL-6 group of cytokines\(^{1,80,81}\). The best characterized selectin ligand is P-selectin glycoprotein ligand-1 (PSGL-1, CD162) that is expressed in the majority of leukocytes. PSGL-1 can bind all three selectins with specific requirements for E-selectin binding capacity\(^{10,82}\). The role of FucT-IV and -VII in the synthesis of carbohydrate PSGL-1 binding sites has been documented\(^{83}\).

**INTEGRINS**

The Integrin family of adhesion molecules represents noncovalently linked surface heterodimers of α and β subunits allowing interactions with components of the intercellular (IC) matrix (various types of collagen, laminine and fibronectine). Further classification is based on the β-subunit type\(^{1}\). Each integrin appears to have a specific, nonredundant function\(^{84}\). Integrin mediated adhesions participate in signal transduction. For example, upon αβ interaction with cognate ligands, the FAK (Focal Adhesion Kinase), phosphatidylinositol-3 kinase (PI3K) and Ca\(^{2+}\)/calmodulin-dependent protein kinase pathways are activated and boost expression of the Bcl-2 oncogene\(^{85}\). Integrin mediated adhesion facilitates PDGF, EGF and VEGF receptor stimulation. Cellular responses to soluble growth factors are dependent on integrin-mediated cellular adherence. In fact, many integrin-stimulated pathways are very similar to those coupled with growth factor receptors\(^{84}\). Understanding these mechanisms explains how apoptosis is blocked in normal cells by integrin-mediated signalling. In malignant cells the antiapoptotic signals are provided by disregulation in oncogene/tumor-supression gene function but one may speculate that integrin- mediated signalling further enhances protection from apoptosis.

Beta-2 (CD18) integrins are preferably expressed on leukocytes. The Leukocyte Function associated Antigen-1 (LFA-1) is αβ integrin providing interactions dominantly with the Inter Cellular Adhesion Molecule-1 (ICAM-1). Recent studies clarified that LFA-1 is not an E-selectin ligand in hematopoietic stem and progenitor cells\(^{86,87}\).
Leukocyte adhesion deficiency type I arises from mutations in the β<sub>1</sub> subunit<sup>84</sup>. Activated endothelial cells express AM involved in leukocyte rolling (P- and E-selectin) leukocyte adhesion (VCAM-1, ICAM-1), same as chemoattractants MCP-1 and IL-8. The molecular mechanism of leukocyte transmigration from the vasculature into tissues has been described<sup>89</sup>. Recently, the macrophage migration inhibitory factor (MIF) and its’ p38 MAPK dependent contribution to endothelial expression of E-selectin, ICAM-1, VCAM-1 and production of IL-8 and MCP-1 was documented<sup>90</sup>.

Very Late Antigens (VLA) are β<sub>1</sub> (CD29) integrins with inducible expression upon leukocyte activation. The α<sub>4</sub>β<sub>1</sub> integrin (VLA-4) is the major counterpart of Vascular Cell Adhesion Molecule-1 (VCAM-1). Further it binds fibronectine. VLA-4 facilitates the stem/progenitor cell retention in bone-marrow niches. This is critical for attachment of leukemic blasts to the vessel wall and together with CXCR-4/SDF-1 interaction it mediates migration of CD34+ cells (also malignant myeloid cells) beneath marrow stromal cells<sup>91</sup>. Integrin-mediated adhesion in the bone marrow microenvironment affects signal transduction, protects AML blasts from chemotherapy-induced apoptosis and provides resistance to several chemotherapeutic agents such as cytarabine, etoposide, daunorubicine or mitoxantrone. Adhesion of U937 AML cells to human osteoblasts upregulated the Wnt pathway antagonist and supported resistance to daunorubicin. Several potential mechanisms of resistance, including VLA-4 triggered activation of the phosphatidylinositol-3-kinase (PI3K)/Akt/bcl-2 pathway, were studied. Blocking antibody to VLA-4 restored chemotherapy sensitivity to cytarabine in a murine AML model<sup>92,93</sup>. In a B-cell lymphoma model, targeting of VLA-4 overcame stromal cell mediated protection against rituximab and other cytotoxic drugs<sup>84</sup>. High levels of VLA-4 expression were documented on AML blasts but the expression was not significantly associated with response to chemotherapy or patient outcome. Subsequent analyses including soluble (s)VCAM-1 revealed that increased binding of sVCAM-1 to VLA-4 was significantly associated with longer overall survival<sup>95</sup>. In a study on 216 pediatric AML patients, high VLA-4 expression was associated with lower FLT3 internal tandem duplication prevalence and higher likelihood of extramedullary disease. Multivariate analysis showed that high VLA-4 expressors had a lower relapse rate and better disease-free survival (DFS). Low VLA-4 expression was an independent adverse prognostic factor for DFS and relapse rate. The role of VLA-4 expression was most prominent in patients with standard-risk AML. A similar trend was seen in low-risk but not high-risk patients<sup>89</sup>. In contrast, another study on relapsed pediatric patients with B-cell precursor acute lymphoblastic leukemia (ALL) showed that high VLA-4 expression was associated with poor molecular response to therapy. The event-free and overall survival were significantly worse in high expressors. In vitro blockade of VLA-4 with specific antibodies abolished the protective effect of stromal cells in coculture and restored sensitivity to cytarabine also in relapsed B-ALL (ref.97). Evaluation of sVCAM-1 levels was not included in these studies. We conclude that integrin-mediated adhesion in context with sVCAM-1 binding to VLA-4 or at least sVCAM-1 levels is worth further investigation. VLA-4 targeting in hematologic malignancies seems to be a promising therapeutic approach.

## IMMUNOGLOBULIN ADHESION MOLECULE FAMILY

Immunoglobulin AM are subclassified according to their ligand and number of immunoglobulin domains in the structure. The presence of heavy glycosylation and specific structural motifs of ICAM-1 (CD54) supports its interactions with numerous ligands. In general, binding to ICAM-1 facilitates extravasation of leukocytes across vascular endothelia during the inflammatory response. All types of ICAM molecules (ICAM 1, 2, 3, 4) interact with LFA-1. Blocking of LFA-1 attenuates T-cell lymphoma migration through ICAM-1 coated barrier<sup>99</sup>. ICAM-1-deficient mice are resistant to the development of lymphoma infiltration of kidneys, spleen and liver after intravenous inoculation of LFA-1 expressing T-lymphoma cells<sup>95</sup>. ICAM-1 expression is induced by IL-1, TNF-α, IFN-γ. ICAM-2 (CD 102) is non-inducibly expressed on leukocytes and endothelia<sup>1</sup>. With ICAM-1 described as an adhesion and viral entry molecule<sup>100</sup>, a role in signal transduction was hypothesized and further studied. In lymphocytes, ICAM-1 stimulation leads to B-cell receptor signalling with subsequent tyrosine phosphorylation and activation of STP with possible cytokine release<sup>101</sup>. In astrocytes, ICAM-1 signalling promotes recruitment of inflammatory immune cells through TNF-α secretion<sup>102</sup>. TNF-α triggers phosphorylation of the p38 MAPK and further up-regulates expression of ICAM-1 and other adhesion molecules via the same mechanisms as MIF (ref.90,103). In addition, the expression of RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) mRNA and protein was also found to be upregulated by ICAM-1 ligation in a p38 MAPK independent manner<sup>104</sup>. These data suggest that ICAM-1 dependent binding induces STP activation and de novo synthesis of ICAM-1 itself, providing signal amplification.

VCAM-1 (CD 106) is primarily the VLA-4 ligand but it also has affinity to α<sub>4</sub>β<sub>1</sub> integrin and has been shown to interact with ezrin and moesin<sup>105</sup>. The endothelial VCAM-1 expression is upregulated by increased gene transcription after IL-1β, TNF-α or Oncostatin M stimulation or through mRNA stabilization by IL-4 and IL-13 (ref.106,107). The VLA-4/VCAM-1 interaction enables tight leukocyte adhesion to endothelia. Myeloblasts can activate endothelial cells and promote their own adhesion to endothelia through cytokine secretion which is remarkable in the pathophysiology of life-threatening leukostasis and tissue infiltration by myeloblasts<sup>108</sup>.
CXCR4

CXCR4 is a chemokine receptor for stromal derived factor-1α (SDF-1α) also known as CXCL-12. Homing of cells into BM is a coordinated, multistep process which involves SDF-1α signaling, activation of LFA-1, VLA-4 and VLA-5 and cytoskeleton rearrangement. Both normal and cancer cells share this mechanism. CXCR4 expression is a documented prognostic marker in AML (ref.117). Mechanisms including CXCR4/SDF-1α axis and VLA-4/VCAM-1 pathway mediate interactions with bone marrow stromal cells protect malignant cells from chemo- and radiotherapy. The role of CXCR4 in AML was illustrated by experiments showing reduction in engraftment of primary human AML cells into NOD/SCID mice recipients treated with antibody to CXCR4 (ref.117). Further, the prognostic significance of FAK expression was described. FAK is a nonreceptor tyrosine kinase with an important role in cell motility and survival. Tumor cells overexpressing FAK present with increased proliferation, motility and invasiveness. The adhesion phenotype of AML determining CXCR4, FAK and VLA-4 expression was studied by flow-cytometry in a group of 36 patients. Overall survival (OS) was negatively influenced by overexpression of all of these markers in univariate analysis. Combination of these markers revealed two prognostic subgroups. Patients overexpressing 2 or 3 factors had shorter OS (ref.118).

A peptide inhibitor of the CXCR4 exhibited direct cytotoxicity against AML and multiple myeloma cells in vitro and in xenografts. Another CXCR4 inhibitor, AMD3100, worked synergistically with histone deacetylase inhibitor panobinostat to induce apoptosis of AML cells in vitro. The AMD 3100 is a SDF-1α analogue known as plerixafor that is used in mobilizing normal progenitor cells into peripheral circulation. Various SDF-1α antagonists have been investigated. The polypeptide RCP168 had strong antagonistic effect on the stromal cell-induced chemotaxis of leukemic cells. Furthermore, RCP168 inhibited SDF-1α-induced AKT and ERK phosphorylation. Equivalent results were obtained with the small-molecule CXCR4 inhibitor AMD3465, a second generation CXCR4 inhibitor.

FLT3-ITD is a marker of poor prognosis in AML. We draw attention to the fact that the association between FLT3-ITD and higher CXCR4 expression has been documented. Various tyrosinkinase inhibitors have been tested in FLT3-ITD positive AML. Despite some benefit, these drugs have not provided significant improvement in patient prognosis. The resistance to treatment is high and concerns about toxicity prevent further dose escalation. AMD 3465 antagonized SDF-1α and stroma-induced chemotaxis and suppressed stroma activated PI3K/AKT and MEK/ERK pathways which effectively mobilized leukemia cells and stem cells into circulation and enhanced the sensitivity to chemotherapy or FLT3-inhibitor-induced cell death. Based on these data, the inhibition of CXCR4/SDF-1α axis is logically a novel therapeutic target in AML. The therapeutic efficacy of CXCR4 inhibition was tested in a phase 1/2 study with 52 with relapsed/refractory AML patients. Combined chemotherapy with CXCR4 inhibitor plerixafor provided convincing rates of complete remissions, demonstrating in vivo the profound impact of the CXCR4/SDF-1α axis disruption.

It was hypothesized that cytotoxic chemotherapeutic agents induce dynamic changes in surface CXCR4 expression. Chemotherapy-induced upregulation of CXCR4 may represent a mechanism of acquired therapeutic resistance. This hypothesis was explored in the AML cell line MOLM-14 and clinical specimens of pediatric AML. Chemotherapy-induced upregulation of surface CXCR4 was confirmed and it was shown that cell lines variably upregulate CXCR4 with chemotherapy treatment. Those that upregulated CXCR4 were protected from chemotherapy-induced apoptosis when cocultured with bone marrow stromal cells. Treatment with AMD3100 decreased stromal protection of myeloblasts.

There are several other mechanisms for overcoming the CXCR4/SDF-1α axis. CXCR4 mediated signaling activates PI3K that can be selectively inhibited by isoform-selective inhibitors. Another option in targeting CXCR4 is modulation of posttranslational phosphorylation of the intracellular domain at serine339. Phosphorylation of CXCR4 Serine339 in bone marrow biopsies correlated with poor prognosis. Experimental Kasumi-1 AML cells with mutations in the 339 position had increased CXCR4 expression but significantly reduced bone marrow homing. Engraftment of mutant cells into immunodeficient recipient was also impaired. CXCR4 phosphorylation at serine339 is regulated by serine/threonine kinase PIM1. PIM1 serine/threonine kinase activity is essential for CXCR4 surface expression and migration towards SDF-1α gradient. The FLT3-ITD cells with the inhibited PIM1 failed to reconstitute lethally irradiated recipients. Experimentally, PIM1 may be inhibited by small molecule inhibitors which is promising for future therapeutic applications. Thus, influencing posttranslational modulation might be an independent mechanism in CXCR4 inhibition.

CD44

CD44 is a receptor for extracellular matrix components such as hyaluronic acid, but can also interact with osteopontin, collagens, and matrix metalloproteinases. The standard isoform, designated CD44s, comprising exons 1–5 and 16–20, is expressed in most mammalian cell types. Alternative splicing is the basis for the structural and functional diversity of this protein. CD44 molecule undergoes numerous posttranslational modifications. CD44 glycosylation directly controls its binding capacity to fibrin and immobilized fibrinogen. One critical modification involves discrete sulfationcyses rendering the selectin-binding glycoform of CD44 called HCELL (for Hematopoietic cell E-selectin/L-selectin ligand). The HCELL was originally discovered in human hematopoietic stem cells and leukemic blasts and was found to direct migration of these cells into bone marrow. This migration occurred despite absence of CXCR4 expression.
on these cells. Engagement of HCELL with E-selectin triggers VLA-4 adhesiveness, resulting in shear-resistant adhesion to ligand VCAM-1. This VLA-4 activation and subsequent GTPase signaling pathway activation represent molecular molecular effectors in this process. HCELL thus functions as a "bone homing receptor".

Administration of antibody to CD44 blocked engraftment of AML cells in NOD-SCID mice and directly eliminated the engrafted leukemic stem cells (LSC). CD44 has been identified as a key regulator of AML LSC with no documented effect on engrafted normal hematopoietic cells derived from cord blood or human bone marrow. Very recently, a humanized monoclonal antibody specific for CD44 that targets and directly kills chronic lymphocytic leukemia cells, was identified. The cytotoxic effect of anti-CD44 treatment was not mitigated by interaction with mesenchymal stromal cells or hyaluronic acid. HCELL was also identified in colon carcinoma cells. Cancer cells characteristically express CD44, and there is increasing evidence that HCELL serves as their major selectin ligand. This finding clarifies the ability of solid tumors to infiltrate bone marrow that is both HCELL and CXCR4 dependent. Variations in CD44 are reported as cell surface markers for breast cancer stem cells. In breast cancer CD44+/CD24− expression is a marker for cancer stem cells (CSCs)-like characteristics. CSC are likely to have a central role in both tumorigenesis and metastasis. The CD44+/CD24− cells showed increased migration and invasivity. Variant 6 isoform of CD44 is a cancer stem cell-selective marker in prostate cancer, associated with proliferation, invasivity, metastasis and chemoresistance. These effects are mediated through PI3K activation and the Wnt signaling pathway. Variant isoforms of CD44 are also relevant to the progression of head and neck squamous cell carcinoma. In contrast, in epithelial ovarian cancer CD44 upregulation seems to be associated with well-differentiated tumor and favorable outcome.

Glycosyltransferase-programmed stereosubstitution (GPS) is a procedure allowing us to modify the surface of live cells expressing CD44 and enco HCELL expression. The utility and applicability of GPS for glycoengineering of HCELL expression has been reviewed recently. Understanding the mechanisms of HCELL-mediated organ-specific dissemination of tumor cells may help to develop effective treatment of hematogenous spread of solid tumors. Ex vivo glycan engineering of HCELL expression may then open the 'avenues' for the efficient vascular delivery of cells into bone marrow. These procedures may form the basis of sophisticated cellular therapies in hematology and oncology.

CONCLUSIONS

We attempted to elucidate how adhesion molecule dependent factors support malignant cell growth and survival. We described the origin of adhesion molecule overexpression in malignancies and demonstrated how malignant cells hijack these mechanisms and support their own growth and survival. Understanding these processes provides deeper insight into the contributing factors triggering malignant cell proliferation, migration, tissue infiltration and resistance to treatment as these are the basic questions in clinical and experimental oncology with treatment consequences.

We believe that further investigation of adhesion molecules will help us define novel therapeutic approaches allowing more accurate targeting of the origin of tumor progression and leukemogenesis and will provide better outcomes and better quality of life for oncological patients.

ABBREVIATIONS

AM, Adhesion molecules; AML, Acute myeloid leukemia; EGF, Epidermal growth factor; ERK, Extracellular signal regulated kinase; FAK, Focal adhesion kinase; FLT3-ITD, FMS-like tyrosine kinase 3 internal tandem duplications; G-CSF, Granulocyte colony stimulating factor; HCELL, Hematopoietic cell E-selectin/L-selectin ligand; ICAM, Intercellular adhesion molecule; IL, Interleukin; LIC, Leukemia initiating cell; HSC, Hematopoietic stem cell; MAPK, Mitogen activated protein kinase; MIF, Matrix migration inhibitory factor; NOD-SCID, Non-obese diabetic-severe combined immunodeficiency; PAK, Protein kinase A; PDGF, Platelet derived growth factor; P13K, Phosphatidylinositol 3-kinase; Raf; RAS activated; SDF-1, Stromal derived factor-1; STP, Signal transduction pathway; VACM, Vascular cell adhesion molecule; VEGF, Vascular endothelial growth factor; VLA, Very late antigen.

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