

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 immunoglobulin family AM (ref.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^{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 hematocarcinology.

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^{4,6}. 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^{7,9}. 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 patients¹⁰⁻¹². 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 leu-

mia cells was shown to involve p38 and Erk1/2 activation¹³. 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 signalling 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 aggressiveness¹⁴. 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 vitro¹⁵. 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^{16,17}.

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 distinguished¹. Soluble forms of selectin AM probably arise from proteolytic cleavage of surface-expressed molecules¹⁸. In lymphoma-bearing mice, the lymphoma cells are the major source of soluble L-selectins¹⁹. Selectins bind to a surface tetrasaccharide structure called "Sialyl Lewis^x" epitope (sLe^x) 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^{20,21}. sLe^x epitope may be integrated as a part of molecules CD15, CD24, CD43 and CD162. The full details of the synthesis of (sLe^x/CD15s) from core molecules (sialylation) were described by Gege et al.²². These structures are broadly expressed in human tissues and sLe^x is constitutively expressed on granulocytes and monocytes and mediates inflammatory extravasation of these cells^{23,24}. Resting T and B lymphocytes lack its expression but are strongly induced to express sLe^x upon activation²⁵. Among T lymphocytes, the sLe^x determinant is known to be preferentially expressed on activated Th1 cells but not on Th2 cells²⁶.

Defective synthesis of the sLe^x is caused by the loss of fucosyltransferase (FucT) activity²⁷. Impaired glycosylation of core proteins results in leukocyte adhesion deficiency type 2 (ref.^{23,28}). Furthermore, specific peracetylated disaccharide precursor reduced sLe^x expression on tumor cells and diminished binding to selectin-coated surface²⁹.

Sialylation has profound implications for immunoreactivity and biologic functions. Although bearing a common trisaccharide core, antibodies to sLe^x do not recognize

Le^x and vice versa. Identification of sLe^x 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 dermatotropic lymphocytes^{30,32} and osteotropic stem cells^{33,34}. The sLe^x 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 sLe^x/CD15s and gain of Le^x/CD15 expression. During myeloid differentiation, both sLe^x/CD15s synthesis and sialidase activity are increased; however, the increase in sialidase activity dominates so that the overall expression pattern is an increase in Le^x and a decrease in sLe^x expression³⁵. 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 functions³⁶.

New insights on adhesive interactions and the prognostic relevance of tumor CD24 expression have been recently documented for various nonhematological malignancies³⁷⁻⁴³. Knowledge of sLe^x mediated adhesive interactions with endothelial cells and platelets is important for understanding the pathophysiology of tumor metastasis⁴⁴⁻⁴⁸. 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 sLe^x expression in tumor cells was found⁴⁸⁻⁵¹. 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 cytotoxicity⁴⁶.

E-selectin

E-selectin (CD62E) is expressed by endothelia. Binding to sLe^x plays an important role in AML growth and tumor progression. The E-selectin ligand CD65 was found critical for AML blast transmigration and extravascular spreading⁵². The E-selectin expression is regulated on the transcription level and is induced by TNF α , 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 manner⁵³. 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 sLe^x 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^{54,55}.

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^{56,57}. 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 α 4 β 1 integrin (see below). All three mechanism involving VLA-4, PSGL-1 and HCELL were necessary to gain full homing activity in lethally irradiated mice⁵⁸⁻⁶⁰.

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 independent⁶¹, 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 chemotherapy⁶². The selectin-dependent protection of malignant cells is not restricted to hematologic malignancies. Like sLe^x mediated adhesion of neutrophils, pancreatic cancer cells also adhere to endothelial E-selectin, which is increased after TNF α stimulation⁶³. In conclusion, surgical trauma may promote the hematogenic spreading of pancreatic cancer cells via TNF α 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 pharmacologically⁶⁴. At the experimental level, FucT-I transduction into malignant cells decreased sLe^x expression and dramatically modified interactions with

E-selectin and this may form the basis for antimetastatic gene therapy⁶⁵. Overall, it is clear that some populations of cells may be rescued from apoptosis following adhesion to E-selectin. Further studies of TNF α 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 $\alpha_4\beta_7$ 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 activity⁶⁹. L-selectin also binds the sLe^x motif attached to the endothelial CD34 molecule⁷⁰. Alternatively, in sites of chronic inflammation β_1 integrin mediated pathway also participates in leukocyte recruitment⁷¹.

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.⁷²). 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 interaction⁷³. 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 minutes⁷⁴.

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 found⁷⁵. The low L-selectin expressors 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 survival⁷⁶. 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 cells⁷⁷. 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 independent⁷⁸. P-selectin was found to support leukocyte rolling along postcapillary venules at the earliest phase of inflammation⁷⁹.

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^{30,82}. The role of FucT-IV and -VII in the synthesis of carbohydrate PSGL-1 binding sites has been documented⁸³.

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 type¹. Each integrin appears to have a specific, nonredundant function⁸⁴. Integrin mediated adhesions participate in signal transduction. For example, upon $\alpha_5\beta_1$ interaction with cognate ligands, the FAK (Focal Adhesion Kinase), phosphatidylinositol-3 kinase (PI3K) and Ca²⁺/calmodulin-dependent protein kinase pathways are activated and boost expression of the Bcl-2 oncogene⁸⁵. 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 receptors⁸⁴. Understanding these mechanisms explains how apoptosis is blocked in normal cells by integrin-mediated signalling. In malignant cells the antiapoptotic signals are provided by dysregulation in oncogene/tumor-suppression 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 $\alpha_1\beta_2$ 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_2 subunit⁸⁸. 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 described⁸⁹. 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 documented⁹⁰.

Very Late Antigens (VLA) are β_1 (CD29) integrins with inducible expression upon leukocyte activation. The $\alpha_4\beta_1$ 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 cells⁹¹. 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^{92,93}. In a B-cell lymphoma model, targeting of VLA-4 overcame stromal cell mediated protection against rituximab and other cytotoxic drugs⁹⁴. 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 survival⁹⁵. 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 patients⁹⁶. 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 co-culture 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 barrier⁹⁸. 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 cells⁹⁹. ICAM-1 expression is induced by IL-1, TNF- α , IFN- γ . ICAM-2 (CD 102) is non-inducibly expressed on leukocytes and endothelia¹. With ICAM-1 described as an adhesion and viral entry molecule¹⁰⁰, 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 release¹⁰¹. In astrocytes, ICAM-1 signalling promotes recruitment of inflammatory immune cells through TNF- α secretion¹⁰². 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 manner¹⁰⁴. 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 $\alpha_4\beta_7$ integrin and has been shown to interact with ezrin and moesin¹⁰⁵. The endothelial VCAM-1 expression is upregulated by increased gene transcription after IL-1 β , 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 myeloblasts¹⁰⁸.

CXCR4

CXCR4 is a chemokine receptor for stromal derived factor-1 α (SDF-1 α) also known as CXCL-12. Homing of cells into BM is a coordinated, multistep process which involves SDF-1 α signaling, activation of LFA-1, VLA-4 and VLA-5 and cytoskeleton rearrangement. Both normal and cancer cells share this mechanism^{91,109-111}. CXCR4 expression is a documented prognostic marker in AML (ref.¹¹²⁻¹¹⁴). Mechanisms including CXCR4/SDF-1 α axis and VLA-4/VCAM-1 pathway mediate interactions with bone marrow stromal cells protect malignant cells from chemo- and radiotherapy^{92,115,116}. 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 invasiveness¹¹⁸. 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.¹¹⁹).

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^{120,121}. The AMD 3100 is a SDF-1 α analogue known as plerixafor that is used in mobilizing normal progenitor cells into peripheral circulation¹²². Various SDF-1 α antagonists have been investigated. The polypeptide RCP168 had strong antagonistic effect on the stromal cell-induced chemotaxis of leukemic cells. Furthermore, RCP168 inhibited SDF-1 α -induced AKT and ERK phosphorylation¹²³. 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 documented¹²⁴. 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-1 α 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 death¹²⁵. Based on these data, the inhibition of CXCR4/SDF-1 α 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-1 α axis disruption¹²⁶.

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 myeloblasts¹²⁷.

There are several other mechanisms for overcoming the CXCR4/SDF-1 α axis. CXCR4 mediated signaling activates PI3K that can be selectively inhibited by isoform-selective inhibitors¹²⁸. Another option in targeting CXCR4 is modulation of posttranslational phosphorylation of the intracellular domain at serine339. Phosphorylation of CXCR4 Serine 339 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 impaired¹²⁹. 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-1 α 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^{130,131}. 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 fibrinogen¹³². One critical modification involves discrete sialofucosylations 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^{34,133}. 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 effectors in this process¹³⁴. 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 marrow¹³⁵. Very recently, a humanized monoclonal antibody specific for CD44 that targets and directly kills chronic lymphocytic leukemia cells, was identified¹³⁶. 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 dependent¹³⁷⁻¹³⁹. 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^{140,141}. 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-like marker in prostate cancer, associated with proliferation, invasiveness, metastasis and chemo-/radioresistance. These effects are mediated through PI3K activation and the Wnt signalling pathway¹⁴². Variant isoforms of CD44 are also relevant to the progression of head and neck squamous cell carcinoma¹⁴³. In contrast, in epithelial ovarian cancer CD44 upregulation seems to be associated with well-differentiated tumor and favorable outcome¹⁴⁴.

Glycosyltransferase-programmed stereosubstitution (GPS) is a procedure allowing us to modify the surface of live cells expressing CD44 and enforce HCELL expression¹⁴⁵. The utility and applicability of GPS for glycoengineering of HCELL expression has been reviewed recently¹⁴⁶. Understanding the mechanisms of HCELL-mediated organ-specific dissemination of tumor cells may help to develop effective prevention of hematogenous spread of solid tumors¹⁴⁷. 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; PI3K, Phosphatidylinositol 3-kinase; Raf, RAS activated factor; SDF-1, stromal derived factor-1; STP, Signal transduction pathway; VCAM, Vascular cell adhesion molecule; VEGF, Vascular endothelial growth factor; VLA, Very late antigen.

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REFERENCES

1. Krejsek J. Imunitní systém jako informační soustava. In: Krejsek J, Kopecky O. Klinická imunologie. Nukleus HK, 2004, p. 41-52.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357(9255):539-45.
3. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420(6917):860-7.
4. Kupsa T, Horacek JM, Jebavy L. The role of cytokines in acute myeloid leukemia: a systematic review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2012;156(4):291-301.
5. Sanchez-Correa B, Bergua JM, Campos C, Gayoso I, Arcos MJ, Bañas H, Morgado S, Casado JG, Solana R, Tarazona R. Cytokine profiles in

- acute myeloid leukemia patients at diagnosis: survival is inversely correlated with IL-6 and directly correlated with IL-10 levels. *Cytokine* 2013;61(3):885-91.
6. Hatfield K, Øyan AM, Ersvaer E, Kalland KH, Lassalle P, Gjertsen BT, Bruserud Ø. Primary human acute myeloid leukaemia cells increase the proliferation of microvascular endothelial cells through the release of soluble mediators. *Br J Hematol* 2009;144(1):53-68.
 7. Lampugnani MG, Resnati M, Raikeri M, Pigott R, Pisacane A, Houen G, Ruco LP, Dejana E. A novel-endothelial specific membrane protein is a marker of cell-cell contacts. *J Cell Biol* 1992;118(6):1511-22.
 8. Gotsch U, Borges E, Bosse R, Böggemeyer E, Simon M, Mossmann H, Vestweber D. VE-cadherin antibody accelerates neutrophil recruitment in vivo. *J Cell Sci* 1997;110:583-8.
 9. Corada M, Mariotti M, Thurston G, Smith K, Kunkel R, Brockhaus M, Lampugnani MG, Martin-Padura I, Stoppacciaro A, Ruco L, McDonald DM, Ward PA, Dejana E. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. *Proc Natl Acad Sci USA* 1999;96(17):9815-20.
 10. Esser S, Lampugnani MG, Corada M, Dejana E, Risau W. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *J Cell Sci* 1998;111:1853-65.
 11. Girgis EH, Mahoney JP, Rafaat KH, Soliman MR. Effect of thalidomide and arsenic trioxide on the release of tumor necrosis factor- α and vascular endothelial growth factor from the KG-1a human acute myelogenous leukemia cell line. *Oncology Letters* 2010;1(4):663-7.
 12. de Bont ES, Fidler V, Meeuwse T, Scherpen F, Hählen K, Kamps WA. Vascular Endothelial Growth Factor Secretion Is an Independent Prognostic Factor for Relapse-free Survival in Pediatric Acute Myeloid Leukemia Patients. *Clin Cancer Res* 2002;8(9):2856-61.
 13. Casalou C, Fragoso R, Nunes JFM, Dias S. VEGF/PLGF induces leukemia cell migration via P38/ERK1/2 kinase pathway, resulting in Rho GTPases activation and caveolae formation. *Leukemia* 2007;21(7):1590-4.
 14. Casalou C, Costa A, Carvalho T, Gomes AL, Zhu Z, Wu Y, Dias S. Cholesterol Regulates VEGFR-1 (FLT-1) Expression and Signaling in Acute Leukemia Cells. *Mol Cancer Res* 2011;9(2):215-24.
 15. Dias S, Hattori K, Zhu Z, Heissig B, Choy M, Lane W, Wu Y, Chadburn A, Hyjek E, Gill M, Hicklin DJ, Witte L, Moore MA, Rafii S. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. *J Clin Invest* 2000;106(4):511-21.
 16. Golay J, Cuppini L, Leoni F, Micò C, Barbui V, Domenghini M, Lombardi L, Neri A, Barbui AM, Salvi A, Pozzi P, Porro G, Pagani P, Fossati G, Mascagni P, Introna M, Rambaldi A. The histone deacetylase inhibitor ITF2357 has anti-leukemic activity in vitro and in vivo and inhibits IL-6 and VEGF production by stromal cells. *Leukemia* 2007;21(9):1892-900.
 17. Madlambayan GJ, Meacham AM, Hosaka K, Mir S, Jorgensen M, Scott EW, Siemann DW, Cogle CR. Leukemia regression by vascular disruption and antiangiogenic therapy. *Blood* 2010;116(9):1539-47.
 18. Morandini R, Ghanem G, Portier-Lemarié A, Robaye B, Renaud A, Boeynaems JM. Action of cAMP on expression and release of adhesion molecules in human endothelial cells. *Am J Physiol* 1996;270(3):807-16.
 19. Aubé C, Bélanger SD, St-Pierre Y. Lymphoma cells contribute to the augmentation of plasma sL-selectins in the serum of lymphoma-bearing mice. *Leuk Lymphoma* 2010;51(1):125-31.
 20. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med* 2003;9(6):263-8.
 21. Lowe JB. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr Opin Cell Biol* 2003;15(5):531-8.
 22. Gege C, Geyer A, Schmidt RR. Synthesis and molecular tumbling properties of sialyl Lewis X and derived neoglycolipids. *Chemistry* 2002;8(11):2454-63.
 23. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC, Gershoni-Baruch R. Recurrent Severe Infections Caused by a Novel Leukocyte Adhesion Deficiency. *N Engl J Med* 1992;327(25):1789-92.
 24. Sarangapani KK, Qian J, Chen W, Zarnitsyna VI, Mehta P, Yago T, McEver RP, Zhu C. Regulation of catch bonds by rate of force application. *J Biol Chem* 2011;286(37): 32749-61.
 25. Ohmori K, Takada A, Ohwaki I, Takahashi N, Furukawa Y, Maeda M, Kiso M, Hasegawa A, Kannagi M, Kannagi R. A distinct type of sialyl Lewis X antigen defined by a novel monoclonal antibody is selectively expressed on helper memory T cells. *Blood* 1993;82(9):2797-805.
 26. Austrup F, Vestweber D, Borges E, Löhning M, Bräuer R, Herz U, Renz H, Hallmann R, Scheffold A, Radbruch A, Hamann A. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature* 1997;385(6611):81-3.
 27. Nakayama F, Nishihara S, Iwasaki H, Kudo T, Okubo R, Kaneko M, Nakamura M, Karube M, Sasaki K, Narimatsu H. CD15 expression in mature granulocytes is determined by alpha 1,3-fucosyltransferase IX, but in promyelocytes and monocytes by alpha 1,3-fucosyltransferase IV. *J Biol Chem* 2001;276(19):16100-6.
 28. Yakubenia S, Wild MK. Leukocyte adhesion deficiency II. Advances and open questions. *FEBS J* 2006;273(19):4390-8.
 29. Fuster MM, Brown JR, Wang L, Esko JD. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. *Cancer Res* 2003;63(11):2775-81.
 30. Fuhlbrigge RC, Kieffer JD, Armerding D, Kupper TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature* 1997;389(6654):978-81.
 31. Dimitroff CJ, Bernacki RJ, Sackstein R. Glycosylation-dependent inhibition of cutaneous lymphocyte-associated antigen expression: implications in modulating lymphocyte migration to skin. *Blood* 2003;101(2):602-10.
 32. Fuhlbrigge RC, King SL, Dimitroff CJ, Kupper TS, Sackstein R. Direct real-time observation of E- and P-selectin-mediated rolling on cutaneous lymphocyte-associated antigen immobilized on Western blots. *J Immunol* 2002;168(11):5645-51.
 33. Sackstein R. The bone marrow is akin to skin: HCELL and the biology of hematopoietic stem cell homing. *J Invest Dermatol* 2004;122(5):1061-9.
 34. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, Wohlgemuth R. Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med* 2008;14(2):181-7.
 35. Gadhoum SZ, Sackstein R. CD15 expression in human myeloid cell differentiation is regulated by sialidase activity. *Nat Chem Biol* 2008;4(12):751-7.
 36. Fuhlbrigge RC, King SL, Sackstein R, Kupper TS. CD43 is a ligand for E-selectin on CLA+ human T cells. *Blood* 2006;107(4):1421-6.
 37. Nagy B, Szendroi A, Romics I. Overexpression of CD24, c-myc and phospholipase 2A in prostate cancer tissue samples obtained by needle biopsy. *Pathol Oncol Res* 2009;15(2):279-83.
 38. Kim KH, Choi JS, Kim JM, Choi YL, Shin YK, Lee HC, Seong IO, Kim BK, Chae SW, Kim SH. Enhanced CD24 expression in endometrial carcinoma and its expression pattern in normal and hyperplastic endometrium. *Histol Histopathol* 2009;24(3):309-16.
 39. Baumhoer D, Riener MO, Zlobec I, Tornillo L, Vogetseder A, Kristiansen G, Dietmar W, Hartmann A, Wuensch PH, Sessa F, Ruemmele P, Terracciano LM. Expression of CD24, P-cadherin and S100A4 in tumors of the ampulla of Vater. *Mod Pathol* 2009;22(2):306-13.
 40. Sano A, Kato H, Sakurai S, Sakai M, Tanaka N, Inose T, Saito K, Sohda M, Nakajima M, Nakajima T, Kuwano H. CD24 expression is a novel prognostic factor in esophageal squamous cell carcinoma. *Ann Surg Oncol* 2009;16(2):506-14.
 41. Yang XR, Xu Y, Yu B, Zhou J, Li JC, Qiu SJ, Shi YM, Wang XY, Dai Z, Shi GM, Wu B, Wu LM, Yang GH, Zhang BH, Qin WX, Fan J. CD24 is a novel predictor for poor prognosis of hepatocellular carcinoma after surgery. *Clin Cancer Res* 2009;15(17):5518-27.
 42. Athanassiadou P, Grapsa D, Gonidi M, Athanassiadou AM, Tsipis A, Patsouris E. CD24 expression has a prognostic impact in breast carcinoma. *Pathol Res Pract* 2009;205(8):524-33.
 43. Wang W, Wang X, Peng L, Deng Q, Liang Y, Qing H, Jiang B. CD24-dependent MAPK pathway activation is required for colorectal cancer cell proliferation. *Cancer Sci* 2010;101(1):112-9.
 44. Rice GE, Bevilacqua MP. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science* 1989;246(4935):1303-6.
 45. Stone JP, Wagner DD. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. *J Clin Invest* 1993;92(2):804-13.
 46. Kim YJ, Borsig L, Varki NM, Varki A. P-selectin deficiency attenuates tumor growth and metastasis. *Proc Natl Acad Sci USA* 1998;95(16):9325-30.
 47. Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. *Proc Natl Acad Sci USA* 2001;99(4):3352-57.

48. Takahashi S, Oda T, Hasebe T, Sasaki S, Kinoshita T, Konishi M, Ueda T, Nakahashi C, Ochiai T, Ochiai A. Overexpression of sialyl Lewis x antigen is associated with formation of extratumoral venous invasion and predicts postoperative development of massive hepatic metastasis in cases with pancreatic ductal adenocarcinoma. *Pathobiology* 2001;69(3):127-35.
49. Ogawa J, Sano A, Koide S, Shohtsu A. Relation between recurrence and expression of proliferating cell nuclear antigen, sialyl Lewis(X), and sialyl Lewis(a) in lung cancer. *J Thorac Cardiovasc Surg* 1994;108(2):329-36.
50. Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T. Involvement of carbohydrate antigen sialyl Lewis(x) in colorectal cancer metastasis. *Dis Colon Rectum* 1997;40(4):420-31.
51. Baldus SE, Mönig SP, Hanisch FG, Zirbes TK, Flucke U, Oelert S, Zilkens G, Madejczik B, Thiele J, Schneider PM, Hölscher AH, Dienes HP. Comparative evaluation of the prognostic value of MUC1, MUC2, sialyl-Lewis(a) and sialyl-Lewis(x) antigens in colorectal adenocarcinoma. *Histopathology* 2002;40(5):440-9.
52. Noguchi M, Sato N, Sugimori H, Mori K, Oshimi K. A minor E-selectin ligand, CD65, is critical for extravascular infiltration of acute myeloid leukemia cells. *Leuk Res* 2001;25(10):847-53.
53. Fusté B, Mazzara R, Escolar G, Merino A, Ordinas A, Díaz-Ricart M. Granulocyte colony-stimulating factor increases expression of adhesion receptors on endothelial cells through activation of p38 MAPK. *Haematologica* 2004;89(5):578-85.
54. Yamaguchi T, Ohshima K, Tsuchiya T, Suehiji H, Karube K, Nakayama J, Suzumiya J, Yoshino T, Kikuchi M. The comparison of expression of cutaneous lymphocyte-associated antigen (CLA), and Th1- and Th2-associated antigens in mycosis fungoides and cutaneous lesions of adult T cell leukemia/lymphoma. *Eur J Dermatol* 2003;13(6):553-9.
55. Descheny L, Gainers ME, Walcheck B, Dimitroff CJ. Ameliorating skin-homing receptors on malignant T Cells with a fluorosugar analog of N-acetylglucosamine: P-selectin ligand is a more sensitive target than E-selectin ligand. *J Invest Dermatol* 2006;126(9):2065-73.
56. Dimitroff CJ, Lee JY, Rafii S, Fuhlbrigge RC, Sackstein R. Cd44 Is a Major E-Selectin Ligand on Human Hematopoietic Progenitor Cells. *J Cell Biol* 2001;153(6):1277-86.
57. Greenberg AW, Kerr W, Hammer DA. Relationship between selectin-mediated rolling of hematopoietic stem and progenitor cells and progression in hematopoietic development. *Blood* 2000;95(2):478-86.
58. Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, Wagner DD, von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule 1. *J Exp Med* 1998;188(3):465-74.
59. Katayama Y, Hidalgo A, Furie BC, Vestweber D, Furie B, Frenette PS. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha4 integrin. *Blood* 2003;102(6):2060-7.
60. Dagia NM, Gadhoun SZ, Knoblauch CA, Spencer JA, Zamiri P, Lin CP, Sackstein R. G-CSF induces E-selectin ligand expression on human myeloid cells. *Nat Med* 2006;12(10):1185-90.
61. Winkler IG, Snapp KR, Simmons PJ, Lévesque JP. Adhesion to E-selectin promotes growth inhibition and apoptosis of human and murine hematopoietic progenitor cells independent of PSGL-1. *Blood* 2004 ;103(5):1685-92.
62. Pezeshkian B, Donnelly C, Tamburo K, Geddes T, Madlambayan GJ. Leukemia Mediated Endothelial Cell Activation Modulates Leukemia Cell Susceptibility to Chemotherapy through a positive Feedback Loop Mechanism. *Plos One* 2013; 8(4):e60823. doi:10.1371/journal.pone.0060823
63. Nozawa F, Hirota M, Okabe A, Shibata M, Iwamura T, Haga Y, Ogawa M. Tumor necrosis factor alpha acts on cultured human vascular endothelial cells to increase the adhesion of pancreatic cancer cells. *Pancreas* 2000;21(4):392-8.
64. Tanigawa N, Hagiwara M, Tada H, Komatsu T, Sugiura S, Kobayashi K, Kato Y, Ishida N, Nishida K, Ninomiya M, Koketsu M, Matsushita K. Acacetin inhibits expression of E-selectin on endothelial cells through regulation of the MAP kinase signaling pathway and activation of NF- κ B. *Immunopharmacol Immunotoxicol* 2013;35(4):471-7.
65. Mathieu S, Prorok M, Benoliel AM, Uch R, Langlet C, Bongrand P, Gerolami R, El-Battari A. Transgene expression of $\alpha(1,2)$ -fucosyltransferase-I (FUT1) in tumor cells selectively inhibits sialyl-Lewis x expression and binding to E-selectin without affecting synthesis of sialyl-Lewis a or binding to P-selectin. *Am J Pathol* 2004;164(2):371-83.
66. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, Butcher EC. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MadCAM-1. *Cell* 1993;74(1):185-95.
67. Arbonés ML, Ord DC, Ley K, Ratech H, Maynard-Curry C, Otten G, Capon DJ, Tedder TF. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* 1994;1(4):247-60.
68. Katayama Y, Hidalgo A, Peired A, Frenette PS. Integrin alpha4beta7 and its counterreceptor MadCAM-1 contribute to hematopoietic progenitor recruitment into bone marrow following transplantation. *Blood* 2004;104(7):2020-6.
69. Hemmerich S, Bistrup A, Singer MS, van Zante A, Lee JK, Tsay D, Peters M, Carminati JL, Brennan TJ, Carver-Moore K, Leviten M, Fuentes ME, Ruddle NH, Rosen SD. Sulfation of L-selectin ligands by an HEV-restricted sulfotransferase regulates lymphocyte homing to lymph nodes. *Immunity* 2001;15(2):237-47.
70. Hernandez Mir G, Helin J, Skarp KP, Cummings RD, Mäkitie A, Renkonen R, Leppänen A. Glycoforms of human endothelial CD34 that bind L-selectin carry sulfated sialyl Lewis x capped O- and N-glycans. *Blood* 2009;114(3):733-41.
71. Rivera-Nieves J, Olson T, Bamias G, Bruce A, Solga M, Knight RF, Hoang S, Cominelli F, Ley K. L-Selectin, alpha 4 beta1, and alpha 4 beta 7 integrins participate in CD4+ T cell recruitment to chronically inflamed small intestine. *J Immunol* 2005;174(4):2343-52.
72. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. *Nat Rev Immunol* 2003;3(11):867-78.
73. Bélanger SD, St-Pierre Y. Role of selectins in the triggering, growth, and dissemination of T-lymphoma cells: implication of L-selectin in the growth of thymic lymphoma. *Blood* 2005;105(12):4800-6.
74. Ding Z, Issekutz TB, Downey GP, Waddell TK. L-selectin stimulation enhances functional expression of surface CXCR4 in lymphocytes: implications for cellular activation during adhesion and migration. *Blood* 2003;101(11):4245-52.
75. Graf M, Reif S, Hecht K, Pelka-Fleischer R, Pfister K, Nuessler V, Schmetzer H. Low L-selectin (CD62L) expression in acute myeloid leukemia correlates with a bad cytogenetic risk. *Eur J Hematol* 2003;71(5):366-76.
76. Aref S, Salama O, Al-Tonbary Y, Fouda M, Menessy A, El-Sherbiny M. L and E selectins in acute myeloid leukemia: expression, clinical relevance and relation to patient outcome. *Hematology* 2002;7(2):83-7.
77. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest* 1989;84(1):92-99.
78. Kaur J, Woodman RC, Kubes P. P38 MAPK: critical molecule in thrombin-induced NF-kappaB-dependent leukocyte recruitment. *Am J Physiol Heart Circ Physiol* 2003;284(4):H1095-103.
79. Ley K, Bullard DC, Arbonés ML, Bosse R, Vestweber D, Tedder TF, Beaudet AL. Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. *J Exp Med* 1995;181(2):669-75.
80. Yao L, Pan J, Setiadi H, Patel KD, McEver RP. Interleukin 4 or oncostatin M induces a prolonged increase in P-selectin mRNA and protein in human endothelial cells. *J Exp Med* 1996;184(1):81-92.
81. Tanaka M, Miyajima A. Oncostatin M, a multifunctional cytokine. *Rev Physiol Biochem Pharmacol* 2003;149: 39-52.
82. Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. *Cell* 2000;103(3):467-79.
83. Martinez M, Joffraud M, Giraud S, Baïsse B, Bernimoulin MP, Schapira M, Spertini O. Regulation of PSGL-1 interactions with L-selectin, P-selectin, and E-selectin: role of human fucosyltransferase-IV and -VII. *J Biol Chem* 2005;280(7):5378-90.
84. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;110(6):673-87.
85. Lee BH, Ruoslahti E. Alpha5beta1 integrin stimulates Bcl-2 expression and cell survival through Akt, focal adhesion kinase, and Ca2+/calmodulin-dependent protein kinase IV. *J Cell Biochem* 2005;95(6):1214-23.
86. Rothlein R, Dustin ML, Marlin SD, Springer TA. A human intercel-

- lular adhesion molecule (ICAM-1) distinct from LFA-1. *J Immunol* 1986;137(4):1270-4.
87. Merzaban JS, Burdick MM, Gadhoum SZ, Dagia NM, Chu JT, Fuhlbrigge RC, Sackstein R. Analysis of glycoprotein E-selectin ligands on human and mouse marrow cells enriched for hematopoietic stem/progenitor cells. *Blood* 2011;118(7):1774-83.
 88. Etzioni A, Doershuk CM, Harlan JM. Of man and mouse: leukocyte and endothelial adhesion molecule deficiencies. *Blood* 1999;94(10):3281-8.
 89. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. *Microcirculation* 2009;16(1):31-42.
 90. Cheng Q, McKeown SJ, Santos L, Santiago FS, Khachigian LM, Morand EF, Hickey MJ. Macrophage migration inhibitory factor increases leukocyte-endothelial interactions in human endothelial cells via promotion of expression of adhesion molecules. *J Immunol* 2010;185(2):1238-47.
 91. Burger JA, Spoo A, Dwenger A, Burger M, Behringer D. CXCR4 chemokine receptors (CD184) and alpha4beta1 integrins mediate spontaneous migration of human CD34+ progenitors and acute myeloid leukaemia cells beneath marrow stromal cells (pseudoemperipolesis). *Br J Haematol* 2003;122(4):579-89.
 92. Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A, Akiyama T, Kuroda H, Kawano Y, Kobune M, Kato J, Hirayama Y, Sakamaki S, Kohda K, Miyake K, Niitsu Y. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. *Nat Med* 2003;9(9):1158-65.
 93. De Toni F, Racaud-Sultan C, Chicanne G, Mas VM, Cariven C, Mesange F, Salles JP, Demur C, Allouche M, Payrastra B, Manenti S, Ysebaert L. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. *Oncogene* 2006;25(22):3113-22.
 94. Mraz M, Zent CS, Church AK, Jelinek DF, Wu X, Pospisilova S, Ansell SM, Novak AJ, Kay NE, Witzig TE, Nowakowski GS. Bone marrow stromal cells protect lymphoma B-cells from rituximab-induced apoptosis and targeting integrin α 4- β 1 (VLA-4) with natalizumab can overcome this resistance. *Br J Hematol* 2011;155(1):53-64.
 95. Becker PS, Kopecky KJ, Wilks AN, Chien S, Harlan JM, Willman CL, Petersdorf SH, Stirewalt DL, Papayannopoulou T, Appelbaum FR. Very late antigen-4 function of myeloblasts correlates with improved overall survival for patients with acute myeloid leukemia. *Blood* 2009;113(4):866-74.
 96. Walter RB, Alonzo TA, Gerbing RB, Ho PA, Smith FO, Raimondi SC, Hirsch BA, Gamis AS, Franklin JL, Hurwitz CA, Loken MR, Meshinchi S. High expression of the very late antigen-4 integrin independently predicts reduced risk of relapse and improved outcome in pediatric acute myeloid leukemia: a report from the children's oncology group. *J Clin Oncol* 2010;28(17):2831-8.
 97. Shalapour S, Hof J, Kirschner-Schwabe R, Bastian L, Eckert C, Prada J, Henze G, von Stackelberg A, Seeger K. High VLA-4 expression is associated with adverse outcome and distinct gene expression changes in childhood B-cell precursor acute lymphoblastic leukemia at relapse. *Haematologica* 2011;96(11):1627-35.
 98. Soede RD, Wijnands YM, Van Kouteren-Cobzaru I, Roos E. ZAP-70 tyrosine kinase is required for LFA-1-dependent T cell migration. *J Cell Biol* 1998;142(5):1371-9.
 99. Aoudjit F, Potworowski EF, Springer TA, St-Pierre Y. Protection from lymphoma cell metastasis in ICAM-1 mutant mice: a posthoming event. *J Immunol* 1998;161(5):2333-8.
 100. Bella J, Kolatkar PR, Marlor CW, Greve JM, Rossmann MG. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc Natl Acad Sci USA* 1998;95(8):4140-5.
 101. Holland J, Owens T. Signaling through intercellular adhesion molecule 1 (ICAM-1) in a B cell lymphoma line. The activation of Lyn tyrosine kinase and the mitogen-activated protein kinase pathway. *J Biol Chem* 1997;272(14):9108-12.
 102. Etienne-Manneville S, Chaverot N, Strosberg AD, Couraud PO. ICAM-1-coupled signaling pathways in astrocytes converge to cyclic AMP response element-binding protein phosphorylation and TNF-alpha secretion. *J Immunol* 1999;163(2):668-74.
 103. Scaldaferrri F, Sans M, Vetrano S, Correale C, Arena V, Pagano N, Rando G, Romeo F, Potenza AE, Repici A, Malesci A, Danese S. The role of MAPK in governing lymphocyte adhesion to and migration across the microvasculature in inflammatory bowel disease. *Eur J Immunol* 2009;39(1):290-300.
 104. Blaber R, Stylianou E, Clayton A, Steadman R. Selective regulation of ICAM-1 and RANTES gene expression after ICAM-1 ligation on human renal fibroblasts. *J Am Soc Nephrol* 2003;14(1):116-27.
 105. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, Furthmayr H, Sanchez-Madrid F. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. *J Cell Biol* 2002;157(7):1233-45.
 106. Fritz DK, Kerr C, Tong L, Smyth D, Richards CD. Oncostatin-M up-regulates VCAM-1 and synergizes with IL-4 in eotaxin expression: involvement of STAT6. *J Immunol* 2006;176(7):4352-60.
 107. Fukuda K, Nishida T, Fukushima A. Synergistic induction of eotaxin and VCAM-1 expression in human corneal fibroblasts by staphylococcal peptidoglycan and either IL-4 or IL-13. *Allergol Int* 2011;60(3):355-63.
 108. Stucki A, Rivier AS, Gikic M, Monai N, Schapira M, Spertini O. Endothelial cell activation by myeloblasts: molecular mechanisms of leukostasis and leukemic cell dissemination. *Blood* 2001;97(7):2121-9.
 109. Pardo-Cabañas M, Bartolome RA, Wright N, Hidalgo A, Dräger AM, Teixidó J. Integrin α 4beta1 involvement in stromal cell-derived factor-1 α -promoted myeloma cell transendothelial migration and adhesion: role of cAMP and the actin cytoskeleton in adhesion. *Exp Cell Res* 2004;294(2):571-80.
 110. Kucia M, Reza R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, Ratajczak J, Ratajczak MZ. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. *Stem Cells* 2005;23(7):879-94.
 111. Ngo HT, Leleu X, Lee J, Jia X, Melhem M, Runnels J, Moreau AS, Burwick N, Azab AK, Roccaro A, Azab F, Sacco A, Farag M, Sackstein R, Ghobrial IM. SDF-1/CXCR4 and VLA-4 interaction regulates homing in Waldenstrom macroglobulinemia. *Blood* 2008;112(1):150-8.
 112. Konoplev S, Rassidakis GZ, Estey E, Kantarjian H, Liakou CI, Huang X, Xiao L, Andreoff M, Konopleva M, Medeiros LJ. Overexpression of CXCR4 predicts adverse overall and event-free survival in patients with unmutated FLT3 acute myeloid leukemia with normal karyotype. *Cancer* 2007;109(6):1152-6.
 113. Spoo AC, Lübbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in acute myelogenous leukemia. *Blood* 2007;109(2):786-91.
 114. Mannelli F, Cutini I, Gianfaldoni G, Bencini S, Scappini B, Pancani F, Ponziani V, Bonetti MI, Biagiotti C, Longo G, Bosi A. CXCR4 expression accounts for clinical phenotype and outcome in acute myeloid leukemia. *Cytometry B Clin Cytom* 2014;86(5):340-9.
 115. Hazlehurst LA, Valkov N, Wisner L, Storey JA, Boulware D, Sullivan DM, Dalton WS. Reduction in drug-induced DNA double-strand breaks associated with beta1 integrin-mediated adhesion correlates with drug resistance in U937 cells. *Blood* 2001;98(6):1897-903.
 116. Macanas-Pirard P, Leisewitz A, Broekhuizen R, Cautivo K, Barriga FM, Leisewitz F, Gidi V, Riquelme E, Montecinos VP, Swett P, Besa P, Ramirez P, Ocqueteau M, Kalergis AM, Holt M, Rettig M, DiPersio JF, Nervi B. Bone marrow stromal cells modulate mouse ENT1 activity and protect leukemia cells from cytarabine induced apoptosis. *PLoS One* 2012;7(5):e37203. doi: 10.1371/journal.pone.0037203.
 117. Tavor S, Petit I, Porozov S, Avigdor A, Dar A, Leider-Trejo L, Shemtov N, Deutsch V, Naparstek E, Nagler A, Lapidot T. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res* 2004;64(8):2817-24.
 118. Owens LV, Xu L, Craven RJ, Dent GA, Weiner TM, Kornberg L, Liu ET, Cance WG. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer Res* 1995;55(13):2752-5.
 119. Tavernier-Tardy E, Cornillon J, Campos L, Flandrin P, Duval A, Nadal N, Guyolat D. Prognostic value of CXCR4 and FAK expression in acute myelogenous leukemia. *Leukemia Research* 2009;33(6):764-8.
 120. Mandawat A, Fiskus W, Buckley KM, Robbins K, Rao R, Balusu R, Navenot JM, Wang ZX, Ustun C, Chong DG, Atadja P, Fujii N, Peiper SC, Bhalla K. Pan-histone deacetylase inhibitor panobinostat depletes CXCR4 levels and signaling and exerts synergistic anti-myeloid activity in combination with CXCR4 antagonists. *Blood* 2010;116(24):5306-15.

121. Beider K, Begin M, Abraham M, Wald H, Weiss ID, Wald O, Pikarsky E, Zeira E, Eizenberg O, Galun E, Hardan I, Engelhard D, Nagler A, Peled A. CXCR4 antagonist 4F-benzoyl-TN14003 inhibits leukemia and multiple myeloma tumor growth. *Exp Hematology* 2011;39(3):282-92.
122. Flomenberg N, Devine SM, DiPersio JF, Liesveld J, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005;106(5):1867-74.
123. Zeng Z, Samudio IJ, Munsell M, An J, Huang Z, Estey E, Andreeff M, Konopleva M. Inhibition of CXCR4 with the novel RCP 168 peptide overcomes stroma-mediated chemoresistance in chronic and acute leukemias. *Mol Cancer Ther* 2006;5(12):3113-21.
124. Rombouts EJ, Pavic B, Löwenberg B, Ploemacher RE. Relation between CXCR-4 expression, Flt3 mutations, and unfavorable prognosis of adult acute myeloid leukemia. *Blood* 2004;104(2):550-7.
125. Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, Levis M, Rubin JB, Negrin RR, Estey EH, Konoplev S, Andreeff M, Konopleva M. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. *Blood* 2009;113(24):6215-24.
126. Uy GL, Rettig MP, Motabi IH, McFarland K, Trinkaus KM, Hladnik LM, Kulkarni S, Abboud CN, Cashen AF, Stockerl-Goldstein KE, Vij R, Westervelt P, DiPersio JF. A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood* 2012;119(17):3917-24.
127. Sison EA, McIntyre E, Magoon D, Brown P. Dynamic chemotherapy-induced upregulation of CXCR4 expression: a mechanism of therapeutic resistance in pediatric AML. *Mol Cancer Res* 2013;11(9):1004-16.
128. Niedermeier M, Hennessy BT, Knight ZA, Henneberg M, Hu J, Kurtova AV, Wierda WG, Keating MJ, Shokat KM, Burger JA. Isoform-selective phosphoinositide 3'-kinase inhibitors inhibit CXCR4 signaling and overcome stromal cell-mediated drug resistance in chronic lymphocytic leukemia: a novel therapeutic approach. *Blood* 2009;113(22):5549-57.
129. Brault L, Rovó A, Decker S, Dierks C, Tzankov A, Schwaller J. CXCR4-SERINE339 regulates cellular adhesion, retention and mobilization, and is a marker for poor prognosis in acute myeloid leukemia. *Leukemia* 2014;28(3):566-76.
130. Grundler R, Brault L, Gasser C, Bullock AN, Dechow T, Woetzel S, Pogacic V, Villa A, Ehret S, Berridge G, Spoo A, Dierks C, Biondi A, Knapp S, Duyster J, Schwaller J. Dissection of PIM serine/threonine kinases in FLT3-ITD-induced leukemogenesis reveals PIM1 as regulator of CXCL12-CXCR4-mediated homing and migration. *J Exp Med* 2009;206(9):1957-70.
131. Decker S, Finter J, Forde A, Kissel S, Schwaller J, Mack TS, Kuhn A, Gray NS, Follo M, Jumaa H, Burger M, Zirlik K, Pfeifer D, Miduturu C, Eibel H, Veelken H, Dierks C. PIM kinases are essential for chronic lymphocytic leukemia cell survival (PIM2/3) and CXCR4 mediated microenvironmental interactions (PIM1). *Mol Cancer Ther* 2014;13(5):1231-45.
132. Alves CS, Yakovlev S, Medved L, Konstantopoulos K. Biomolecular characterization of CD44-fibrin(ogen) binding: distinct molecular requirements mediate binding of standard and variant isoforms of CD44 to immobilized fibrin(ogen). *J Biol Chem* 2009;284(2):1177-89.
133. Sackstein R, Dimitroff CJ. A hematopoietic cell L-selectin ligand that is distinct from PSGL-1 and displays N-glycan-dependent binding activity. *Blood* 2000;96(8):2765-74.
134. Thankamony SP, Sackstein R. Enforced hematopoietic cell E- and L-selectin ligand (HCELL) expression primes transendothelial migration of human mesenchymal stem cells. *Proc Natl Acad Sci USA* 2011;108(6):2258-63.
135. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* 2006;12(10):1167-74.
136. Zhang S, Wu CC, Fecteau JF, Cui B, Chen L, Zhang L, Wu R, Rassenti L, Lao F, Weigand S, Kipps TJ. Targeting chronic lymphocytic leukemia cells with a humanized monoclonal antibody specific for CD44. *Proc Natl Acad Sci USA* 2013;110(15):6127-32.
137. Burdick MM, Chu JT, Godar S, Sackstein R. HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells. *J Biol Chem* 2006;281(20):13899-905.
138. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. *J Biol Chem* 2008;283(23):15647-55.
139. Al-Souhibani N, Al-Ghamdi M, Al-Ahmadi W, Khabar KS. Post-transcriptional control of the chemokine receptor CXCR4 expression in cancer cells. *Carcinogenesis* 2014;35(9):1983-92.
140. Li F, Tiede B, Massagué J, Kang Y. Beyond tumorigenesis: cancer stem cells in metastasis. *Cell Res* 2007;17(1):3-14.
141. Yan W, Chen Y, Yao Y, Zhang H, Wang T. Increased invasion and tumorigenicity capacity of CD44+/CD24- breast cancer MCF7 cells in vitro and in nude mice. *Cancer Cell Int* 2013;13(1):62.
142. Ni J, Cozzi PJ, Hao JL, Beretov J, Chang L, Duan W, Shigdar S, Delprado WJ, Graham PH, Bucci J, Kearsley JH, Li Y. CD44 variant 6 is associated with prostate cancer metastasis and chemo-/radioreistance. *Prostate* 2014;74(6):602-17.
143. Wang SJ, Wong G, de Heer AM, Xia W, Bourguignon LY. CD44 variant isoforms in head and neck squamous cell carcinoma progression. *Laryngoscope* 2009;119(8):1518-30.
144. Sillanpää S, Anttila MA, Voutilainen K, Tammi RH, Tammi MI, Saarikoski SV, Kosma VM. CD44 expression indicates favorable prognosis in epithelial ovarian cancer. *Clin Cancer Res* 2003;9(14):5318-24.
145. Sackstein R. Glycosyltransferase-programmed stereosubstitution (GPS) to create HCELL: engineering a roadmap for cell migration. *Immunol Rev* 2009;230(1):51-74.
146. Sackstein R. Engineering cellular trafficking via glycosyltransferase-programmed stereosubstitution. *Ann NY Acad Sci* 2012;1253(1):193-200.
147. Jacobs PP, Sackstein R. CD44 and HCELL: Preventing Hematogenous metastasis at step 1. *FEBS Lett* 2011;585(20):3148-58.