[Frontiers in Bioscience 16, 3233-3251, June 1, 2011]

Interactions between endothelial selectins and cancer cells regulate metastasis

Catherine A. St. Hill[1]

[1]*Department of Veterinary Clinical Sciences, University of Minnesota, Room C339, Veterinary Medical Center, 1352 Boyd Avenue, St. Paul, MN 55108, USA*

TABLE OF CONTENTS

1. ABSTRACT

The selectins: E-selectin, P-selectin, and L-selectin are adhesion molecules that are crucial for binding of circulating leukocytes to vascular endothelium during the inflammatory response to injury or infection. Accumulated evidence indicates that selectins regulate adhesion of circulating cancer cells to the walls of blood vessels. Selectin ligands are transmembrane glycoproteins expressed on leukocytes and cancer cells that promote bond formations with selectins to mediate inflammatory processes. Selectins and selectin ligands also participate in signal transduction to regulate diverse cellular functions. Sialyl Lewis X (sLe^x) and sialyl Lewis A (sLe^a) tetrasaccharides are carbohydrate motifs displayed on protein or lipid scaffolds that are critical components of functional selectin ligands. Selectin binding to sLe^x and sLe^a present on colon, gastric, bladder, pancreatic, breast, and prostate carcinomas enhances distant organ metastasis. High expression of sialyl Lewis ligands on these cancers is significantly correlated with a poor post-operative prognosis. This review will focus on the roles of E-selectin and P-selectin in cancer progression. Understanding the role of selectins in cancer supports the development of novel selectin-based therapies to control metastasis.

2. INTRODUCTION

The majority of cancer deaths are attributed to the metastatic spread of malignant cells to vital organs rather than to primary tumor growth. Control of metastasis is a significant challenge of current anti-cancer strategies. Therefore, understanding the mechanisms that regulate metastasis is crucial to the discovery of successful therapies that could prolong the lives of cancer patients. The mechanisms involved in the movement of leukocytes from the circulation into tissue sites of injury or infection are similar to those used by circulating tumor cells to enter target organs in the early events of metastasis (1, 2). Several cell adhesion molecules and soluble mediators such as chemokines facilitate contact of leukocytes in flowing blood with the vascular wall and arrest on endothelial cells. The cell adhesion molecules are grouped into three families: the selectins, the integrins, and the immunoglobulin supergene family. The selectin family: E-selectin, P-selectin, and L-selectin mediate the initial cell contact between leukocytes and endothelium which results in transient tethering and rolling of leukocytes on cytokine activated endothelial cells. These binding events require the presence of free calcium ions and occur under dynamic shear forces of blood flow that exist in the vasculature.

Interruption of these initial events prevents subsequent leukocyte adhesion and emigration. The rolling cells sense signals produced by chemokines and other factors that activate leukocyte integrins. Integrins bind to immunoglobulin superfamily members on endothelial cells resulting in firm adhesion of leukocytes to the endothelium. Leukocytes subsequently migrate out of blood vessels and into tissues through the layer of endothelial cells and underlying basal membrane (3).

For epithelial cancers known as carcinomas, the establishment of metastases in secondary organs is a complex multistep process that involves tumor transformation; separation of tumor cells from the primary tumor and local invasion; intravasation and dissemination of circulating cancer cells in blood; stasis, adhesion to blood vessels, extravasation; proliferation and growth; and vascularization of the metastatic tumor (4). All steps must be completed sequentially in order for metastasis to be successful and the cancer must also overcome numerous host defenses. In addition, tumor cells can remain dormant for many years after primary cancer treatment before metastases recur.

Overall, metastasis is an inefficient process and very few cancer cells actually establish tumors at secondary sites. The early steps in metastasis are completed more efficiently than the later steps involving growth of malignant cells in the secondary organ, vascularization, and formation of visible metastases. The attachment of circulating tumor cells to vascular endothelium in target organs is a key step in metastasis and is necessary for subsequent extravasation and invasion into target tissues. The sialyl Lewis x antigen and similar carbohydrate structures are selectin ligands on several types of carcinomas that have been identified as markers of cancer progression, particularly for gastrointestinal carcinomas (5). E-selectin is the best studied selectin and mediates the adhesion of tumor cells to activated endothelial cells (6). However, P-selectin and L-selectin also participate in tumor dissemination as studies have shown that metastasis is impaired in P-selectin and L-selectin deficient mice (7). P-selectin and L-selectin mediate early interactions of platelets and leukocytes with circulating tumor cells (8). Although L-selectin is an important contributor to cancer progression, it will not be thoroughly discussed here. Instead, the focus of this review will be to discuss recent evidence that supports the importance of E-selectin and P-selectin interactions with selectin ligands in promotion of metastasis in a permissive microenvironment. The impact of this information on the development of novel selectin-based therapies to control cancer dissemination will also be reviewed.

3. CHARACTERISTICS OF SELECTINS AND THEIR LIGANDS

3.1. Structure and localization of selectins

Selectins are type-I transmembrane glycoproteins that bind to carbohydrate ligands in a calcium-dependent manner. E-selectin, P-selectin, and L-selectin comprise this family of cell adhesion molecules and share similar structures. Each selectin has a C-type lectin domain at the amino terminus, an epidermal growth factor (EGF)-like domain, variable numbers of short consensus repeat domains (P-selectin has nine, E-selectin has six, and L-selectin has two), a single-pass transmembrane domain, and a short cytoplasmic tail at the carboxyl terminus (9). The lectin domains of the selectins are 60% homologous and this feature is responsible for differences in carbohydrate binding and selectin specificity (10). E-selectin (CD62E, ELAM-1, LECAM-2) is expressed exclusively on the surface of endothelial cells but is not constitutively expressed. Instead, E-selectin is rapidly induced by inflammatory stimuli such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta), or disturbed blood flow and regulates leukocyte adhesion at sites of inflammation or injury (11, 12). After expression on the cell surface, E-selectin is slowly internalized and is targeted for degradation by lysosomes (13). P-selectin (CD62P, LECAM-3, GMP-140, PADGEM) is constitutively expressed on endothelial cells and platelets and is stored in Weibel-Palade bodies and alpha-granules respectively in these cell types. The activation of endothelial cells or platelets (e.g. by stimulation with histamine or thrombin) causes exocytosis and fusion of these secretory granules with the cell membrane, leading to rapid cell surface expression of P-selectin (14, 15). Subsequently, P-selectin molecules are rapidly internalized by endocytosis (16) and can be recycled (17). Soluble forms of E- and P-selectin are found in serum and plasma. Soluble E-selectin is released by activated or damaged human endothelial cells (18, 19), and is chemotactic for neutrophils, assists in their migration, and activates beta 2-integrins. High levels of soluble P-selectin in plasma results in a pro-coagulant state, is associated with hypercholesterolemia, and may be a useful marker of endothelial dysfunction in these patients (20, 21). Both E-selectin and P-selectin are constitutively expressed in certain tissues in the absence of inflammatory stimuli. For instance, the endothelium of human hematopoietic organs constitutively expresses E-selectin (22). Venules in non-inflamed skin support significant rolling of leukocytes that are partly mediated by P-selectin (23). Thus, inflammatory stimuli are not strictly required for some blood vessels to express P-selectin. L-selectin (CD62L, LAM-1, LECAM-1) is constitutively expressed on peripheral blood leukoctytes as well as many lymphocytes and is rapidly shed by proteolytic cleavage upon their activation (24). In addition to its function in recruitment of leukocytes and lymphocytes to sites of inflammation, L-selectin is required for lymphocyte recirculation and homing to allow binding to high endothelial venules (HEV) of peripheral lymph nodes. Soluble L-selectin is produced by leukocytes located in tissues and high concentrations inhibit leukocyte attachment to endothelium (25). L-selectin mediates the rolling of leukocytes on leukocytes adherent to the blood vessel wall in a process known as “secondary tethering” that will be discussed in section 3.3.

3.2. Expression and function of selectin ligands

The C-type lectin domain of selectins binds with high affinity to ligands modified with particular carbohydrate motifs. Selectin ligands are transmembrane glycoproteins that require fucosylation to be properly functional and this is achieved by the fucose-generating FX

enzyme, which supplies 90% of cellular fucose. Fucose decorates the termini of O-, N-, or lipid-linked glycans of selectin ligands and fucosylation determines their selectin binding ability which in turn, controls fundamental steps of selectin-dependent leukocyte adhesion and trafficking (26). Mice null for the FX enzyme are immunodeficient and have impaired leukocyte recruitment similar to patients with leukocyte adhesion deficiency type II (LAD-II) (27). Patients with this uncommon disease have a congenital defect in fucose processing and cannot produce functional fucosylated selectin ligands. Their neutrophils do not roll effectively and the syndrome is associated with severe recurrent bacterial infections (28). Sialyl Lewis X (sLe^x or CD15s) and its isomer sialyl Lewis A (sLe^a) tetrasaccharides are two of the minimum carbohydrate motifs displayed on protein or lipid scaffolds that are critical components of functional selectin ligands. These motifs enable the presentation of several selectin ligands in clusters. Mucin-type O-glycans, N-glycans, or neolactosphingolipids expressed on the surface of leukocytes or cancer cells present sLe^x and sLe^a carbohydrates at their termini. In inflammatory diseases and in certain cancers, sLe^x expressed on leukocytes and sLe^x and sLe^a on circulating tumor cells promote better recognition and high-affinity binding to selectins (29, 30). Carbohydrate enzymes known as alpha1,3-fucosyltransferases (FucT), alpha2,3-sialyltransferases, beta1,4-galactosyltransferases, and N-acetylglucosaminyltransferases synthesize the carbohydrate motifs that comprise selectin ligands (11). At least nine human FucT enzymes exist and five of them FucT-III – FucT-VII are involved in the biosynthesis of sLe^x. In myeloid cells the most important carbohydrate modifying enzymes are FucT-IV and FucT-VII. E-selectin, P-selectin, and L-selectin dependent rolling is abolished in mice deficient in FucT-IV and FucT-VII. This absence of rolling demonstrates the importance of alpha1,3 fucosylation for the generation of selectin ligands (26). Interestingly, other selectin ligands may exist in mice deficient in FucT-IV and FucT-VII as platelets of these doubly deficient mice are still able to bind to and roll on P-selectin expressed on endothelium *in vivo* (31). FucT-III and FucT-VI appear to be the predominant fucosyltransferases that synthesize sLe^x in multiple cancer cell lines and in colorectal cancer tissues (32, 33). Thus, these fucosyltransferases may be useful targets to develop therapies for reduction of the metastatic potential of cancer cells.

All three selectins bind to the well characterized ligand P-selectin glycoprotein ligand-1 (PSGL-1) which is expressed on myeloid, lymphoid, and dendritic cells (34) (Figure 1). PSGL-1 is the main physiological ligand for P-selectin although high strength binding occurs to both P-selectin and L-selectin due to sulfation of tyrosine residues and O-glycosylation in the N-terminus of PSGL-1 (35). Glycosylation regulates PSGL-1 binding and glycosylated PSGL-1 binds to E-selectin. Binding is insensitive to sulfation of PSGL-1 indicating that the epitope recognized by E-selectin is different from that recognized by P-selectin and L-selectin (36). CD24, expressed on neutrophils, is a small O-linked oligosaccharide-modified glycoprotein that functions as another P-selectin ligand and mediates rolling on P-selectin when PSGL-1 is absent. Using $CD24^+$ cell lines, Aigner *et al.* determined that this rolling interaction requires expression of the sLe^x antigen (37). In addition to PSGL-1, E-selectin binds many ligands including E-selectin ligand-1 (ESL-1), CD44, CD43, beta 2-integrins, and L-selectin (38-43) however the physiological relevance of these interactions requires further investigation. L-selectin binds to sulfated sLe^x epitopes (6-sulfo- sLe^x) expressed on O-glycans of glycoprotein ligands and sulfation is essential for binding. These interactions allow lymphocyte rolling along HEV of peripheral lymph nodes and Peyer's patches. In the HEV of peripheral lymph nodes, L-selectin binds to sialomucins known as peripheral node addressins (PNAds). PNAds include glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1 or Sgp50), CD34 (Sgp90), podocalyxin, endomucin and nepmucin (44). L-selectin also interacts with the endothelial mucin mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1) in Peyer's patches. Sgp200 is another HEV-expressed L-selectin ligand that participates in lymphocyte homing and exists in both cell associated and secreted forms. CD34 is expressed on the surface of endothelial cells. Sgp200 and CD34 may control the initial loose tethering of lymphocytes to HEV (45). GlyCAM-1 is secreted and may primarily participate in signal transduction mechanisms of leukocytes. MAdCAM-1 interacts with both L-selectin and alpha4, beta7 integrin to support lymphocyte tethering and rolling. The selectin-selectin ligand interactions that are well characterized are shown in Figure 1.

Recently, Mantovani and colleagues reported that the pentraxin protein PTX3 which augments innate immunity, also diminishes neutrophil recruitment *in vivo* (46, 47). PTX3 appears to be a selectin ligand that limits inflammation by binding to P-selectin and inhibiting the rolling of neutrophils on P-selectin. P-selectin may bind with slower on- and off-rates to PTX3 than to PSGL-1 but this phenomenon remains to be confirmed. PTX3 may be as effective in inhibition of P-selectin function as P-selectin function blocking antibodies or genetic deficiency in P-selectin. Endothelial cells, macrophages, and dendritic cells stimulated with cytokines or endotoxins can synthesize PTX3 and neutrophils are able to store PTX3 in specific granules. PTX3 exerts anti-inflammatory effects as demonstrated by the increased susceptibility of PTX3-deficient mice to ischemia-reperfusion injury (48) however the mechanisms are not well understood. It is unknown whether the binding interactions of PTX3 with P-selectin are Ca^{2+} ion dependent. Interestingly, recombinant PTX3 has been reported to bind to P-selectin but not E-selectin and L-selectin but the reasons for this discrepancy are unclear (46). Although much still needs to be elucidated about the function of PTX3 as a selectin ligand, this molecule may have potential therapeutic usefulness in the regulation of inflammatory reactions *in vivo*.

3.3. Interactions of selectins with endothelial cells

The selectins are key adhesion molecules that mediate hemostasis and initiate tethering of circulating blood cells to each other or to endothelium during migration of leukocytes from the blood vessel and recruitment into inflamed tissues. Binding of leukocytes to the endothelium of blood vessels is tightly regulated by a complex cascade of

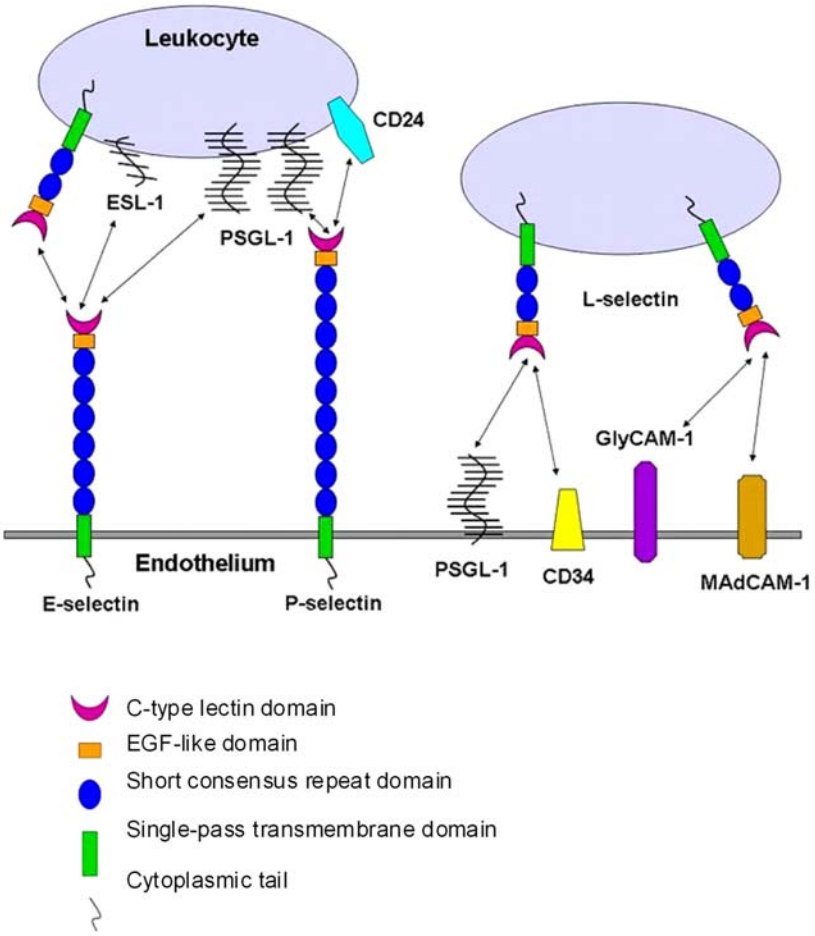

Figure 1. Structure of selectins and interactions with selectin ligands. Selectin binding is calcium dependent and occurs through the C-type lectin domain. E-selectin and P-selectin are expressed on activated vascular endothelium. Ligands for E-selectin include E-selectin ligand-1 (ESL-1), P-selectin glycoprotein-1 (PSGL-1), and L-selectin. P-selectin binds to PSGL-1 with high affinity and also binds to CD24. L-selectin is present on leukocytes and binds to PSGL-1 strongly. L-selectin also binds to CD34 and the homing receptors glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), and mucosal addressin cell adhesion molecule 1 (MAdCAM-1) on endothelial cells. These CAMs play key roles in leukocyte trafficking into sites of inflammation and the mucosal immune compartment. The arrows indicate the binding interactions of selectins with their ligands.

dynamic interactions between leukocytes and endothelial cells that are mediated by selectins, integrins, and the immunoglobulin supergene family of cell adhesion molecules (IgCAMs). The selectins mediate low affinity transient and reversible rolling adhesions of leukocytes on activated endothelium. The integrins regulate firm adhesion and migration of leukocytes through the endothelium. Integrins are heterodimers composed of an alpha and a beta chain that recognize multiple ligands including extracellular matrix proteins, cell surface glycoproteins, complement factors, and other soluble hematogenous factors. Integrins are present on many cell types including leukocytes which express beta 2-integrins (CD11/CD18) and some leukocyte subpopulations express beta 1, beta 7 and alpha 4 integrins on their surface. The endothelial IgCAMS directly bind to leukocyte cell-surface integrins and also play key roles in adhesion and transmigration of leukocytes (49, 50). At sites of inflammation, the rolling contacts of leukocytes with the vascular endothelium are facilitated by chemokines that are secreted from the inflamed environment. The chemokines transcytose through the endothelium and bind to the endothelial cell surface associated with proteoglycans. These chemokines interact with the G-protein coupled chemokine receptors on leukocytes and induce intracellular signals leading to inside-out integrin activation and firm leukocyte adhesion. The leukocytes undergo shape changes associated with the conversion of G-actin to F-actin and pseudopod formation which enable leukocytes to adhere to endothelium and complete transmigration (51).

In addition to the direct capture (primary tethering) of circulating leukocytes on the vascular endothelium as described above, leukocytes can be arrested from free-flowing blood indirectly by previously attached leukocytes and platelets (secondary tethering) (52, 53). Thus, a free-flowing leukocyte can be captured by an attached leukocyte and after collision, advances over the attached leukocyte before accumulation downstream of the

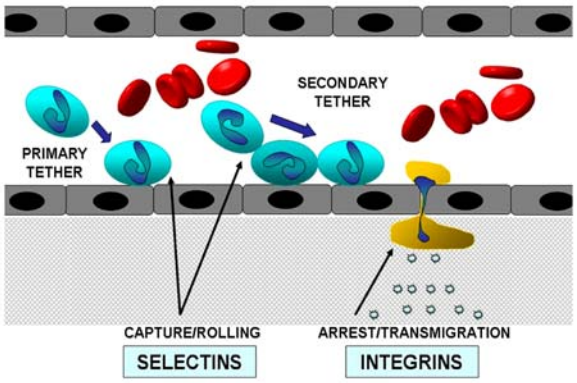

Figure 2. Mechanisms of leukocyte accumulation on activated vascular endothelium. Selectins initiate the tethering of leukocytes to endothelial cells. Leukocytes that are captured from free-flowing blood roll along the blood vessel wall and sense chemokines which are deposited on the endothelial cell surface. In primary tethers, leukocytes bind directly to E-selectin or P-selectin expressed by endothelial cells. In secondary tethers, a free-flowing leukocyte binds to and rolls on an already adherent leukocyte through L-selectin and PSGL-1 interactions before depositing on the endothelium downstream of the collision. These rolling adhesions result in activation of leukocyte integrins that bind to members of the immunoglobulin gene superfamily. The integrins mediate arrest of the rolling leukocyte and firm adhesion to endothelial cells. Leukocytes then migrate through the spaces between endothelial cells to enter tissues by using integrins for traction and by following directed cues from chemoattractants.

interaction. Secondary tethering occurs through binding of L-selectin to PSGL-1 and accounts for up to 70% of leukocyte accumulation on activated endothelium (54). We have recently demonstrated that under hydrodynamic shear flow conditions similar to the forces that are present in blood vessels, secondary tethering significantly augments leukocyte accumulation on P-selectin in flowing whole blood (55). This mechanism may enhance leukocyte accumulation on activated endothelium and increase the density of leukocytes within these discrete areas at sites of inflammation (Figure 2).

3.4. Functions of E- and P-selectin in disease

The study of selectin and selectin ligand deficient mice has revealed the important functions of selectins in many physiological processes including inflammation, immunity, hemostasis, and wound repair (56-58). Selectins also play important roles in many disease states including cancer which will be discussed in the next section. E-selectin may play a role in cardiovascular disease as elevated levels have been found in hypertension, diabetes and hyperlipidemia (59). Raised levels of soluble E-selectin are detected in children with severe *Plasmodium falciparum* malaria compared to children with mild malaria (60). High E-selectin or soluble E-selectin levels are also associated with acute ischemic stroke (61), bronchial asthma (62), psoriasis (63), eczema (64), atopic and allergic dermatitis (63), Kawasaki disease (65), Guillain-Barre syndrome (66), and Graves' disease (67) compared with healthy subjects. The means by which soluble E-selectin may enhance symptoms of inflammatory disease may be through activation of beta 2-integrins, control of leukocyte movement, or stimulation of the respiratory burst and release of reactive oxygen species (68).

P-selectin or soluble P-selectin may also be important in clinical disease as elevated levels are found in many inflammatory states including systemic sclerosis (69), malaria (70), acute lung injury (71), adult respiratory distress syndrome, ischemia-reperfusion injury, gram-negative septic shock, rheumatoid arthritis (72), thrombotic diseases (73), and connective tissue diseases (74). E- or P-selectin deficient mice are protected from acute inflammation and tissue damage in models of ischemia/reperfusion-induced acute renal failure (75) and endotoxemia (76) thus demonstrating the key roles that these selectins play in inflammation. It is thought that E- and P-selectin work together to allow neutrophils and monocytes to bind to endothelial cells at sites of acute inflammation (56, 77). In an experimental septic shock model, significantly decreased disseminated intravascular coagulation and mortality was observed in after inhibition of E- or P-selectin by antibodies or heparin (78). Both E- and P-selectin have been implicated in the pathogenesis of atherosclerosis whereas the absence of these selectins reduces atherosclerotic lesions (79, 80). This is in part because P-selectin expressed by activated platelets mediates their rolling on activated endothelial cells and monocyte recruitment during atherogenesis (81, 82). The role of L-selectin in disease has been extensively reviewed elsewhere and will not be addressed in detail in this article (72, 83).

3.5. Factors that regulate E- and P-selectin expression

Several transcription factors regulate the transcription of E-selectin such as TNF-alpha, IL-1, nuclear factor kappa B (NF-kappa B), and activator protein 1 (AP-1) (84, 85). Peak levels of E-selectin on the endothelial cell surface are expressed by 2 – 6 hours after stimulation by *de novo* synthesis and return to basal levels within 24 hours (86). Mouse E-selectin appears to be regulated by a similar mechanism and kinetics when assessed on mouse endothelioma cells (87). There are four regulatory elements in the human E-selectin promoter: three elements are NF-kappa B binding sites and one is an activating transcription factor (ATF)-binding element. NF-kappa B is necessary but not sufficient for cytokine-stimulated induction of E-selectin transcription and ATF is also involved in this process (88). E-selectin expression is induced by other factors such as shear stress (89), vascular endothelial growth factor (VEGF) (90), high mobility group 1 B-box (HMGB1) (91), and monocytes (92). Furthermore, activation of Rho family GTPases by various stimuli up-regulates E-selectin expression (93). E-selectin expression is blocked by inhibitors of transcription and translation including actinomycin D and cyclohexamide, cytokines such as transforming growth factor-beta (TGF-beta), glucocorticoids (94), elevations of cyclic adenosine monophosphate (95), and the histamine H2-receptor antagonist cimetidine (96-98). TNF-alpha stimulation of E-selectin expression is

inhibited by IL-4 through the actions of STAT6, which antagonizes the binding of NF-kappa B (99).

The transcription of P-selectin and protein expression is regulated by IL-4, IL-13, and oncostatin M in human endothelial cells which lasts 72 hours (100, 101). TNF-alpha was found to stimulate transcription and protein expression of P-selectin in murine and bovine endothelial cells with similar kinetics to that of E-selectin (87). These data were confirmed *in vivo* for the mouse (102) and the rat (103). Unlike the murine P-selectin promoter, the human promoter does not contain a NF-kappa B binding site and thus P-selectin mRNA synthesis is regulated by TNF-alpha and LPS in murine cells but not in human cells (104, 105). In mice, IL-4 or oncostatin M stimulates P-selectin expression more slowly than TNF-alpha. Oncostatin M is also reported to stimulate the transport of P-selectin from storage granules to the cell surface (106).

3.6. Selectins and selectin ligands mediate signal transduction

In addition to their roles in leukocyte adhesion, selectins and their ligands may function as signal transduction receptors. All three selectins contain a cytoplasmic tail and thus may receive outside-in signals from selectin ligands (107, 108). L-selectin and PSGL-1 are the best characterized signal transduction receptors that when ligated with physiological ligands or antibodies, result in diverse responses including up-regulation of beta 2-integrin-mediated adhesion to the IgCAM intercellular adhesion molecule-1 (ICAM-1) (109), oxidative burst, and secretion of cytokines such as TNF-alpha and IL-8 (110). L-selectin and PSGL-1 also activate the cytoplasmic tyrosine kinase c-Abl leading to F-actin redistribution and assembly (111, 112) which underscores the importance of L-selectin- and PSGL-1-dependent signaling in leukocyte rolling.

Phosphorylation of serine or tyrosine residues in the cytoplasmic tail of E-selectin is regulated by engagement of E-selectin by leukocyte counter-receptors, cross-linking by anti-E-selectin antibodies, and with P-selectin glycoprotein ligand-1 (PSGL-1) coated beads (107, 113, 114). E-selectin directly transduces signals into endothelial cells by p38 and p42/p44 mitogen-activated protein kinase (MAPK) pathways (115). Neutrophil slow rolling is initiated by E-selectin binding to PSGL-1 which activates beta 2-integrins through the SYK and Src kinase pathways (116). E-selectin clustering leads to its association with the endothelial actin cytoskeleton and several proteins such as alpha-actinin, filamin, vinculin, paxillin, and focal adhesion kinase (FAK) (117). Thus, E-selectin is a functional signaling receptor on activated endothelial cells. In addition, TNF-alpha stimulation of endothelial cells activates NF-kappa B, Jun NH2-terminal kinase (JNK1), and p38 kinase signaling pathways that are critical for cytokine-induced maximal E-selectin gene expression. (118). The selectins may also have co-stimulatory functions on cells. The binding of P-selectin to PSGL-1 on mouse neutrophils results in the PSGL-1 mediated transmission of signals that cause the activation of beta 2-integrins (119). The interaction of P-selectin with monocytes is sufficient to initiate signal transduction and up-regulation of tissue factor expression because inhibitory antibodies to P-selectin block cell-cell interactions and the tissue factor response (120). Binding of P-selectin on platelets to PSGL-1 on monocytes initiates signaling that leads to nuclear translocation of the NF-kB/Rel family of transcription factors and monocyte chemotactic protein -1 (MCP-1) secretion (121). In contrast, little information is available on direct P-selectin mediated signaling. P-selectin binding to PSGL-1 activates leukocyte integrins through the Src kinase family, Nef-associated factor 1 and phosphoinositide-3-OH-kinase signal transduction pathways (122, 123). Ligation of P-selectin transmits signals to endothelial cells and platelets by an unclear mechanism (124). Thus, P-selectin is able to activate signals in human and in mouse neutrophils however, only mouse neutrophils have a signaling mechanism that directly connects stimulation of PSGL-1 with the activation of beta 2 integrins.

Selectin ligands also actively participate in signal transduction. Purified or recombinant E-selectin or P-selectin delivers signals to cells expressing their selectin ligands. Adhesion of the HT-29 human colorectal carcinoma cell line to an E-selectin-Ig fusion protein stimulates tyrosine phosphorylation of several proteins in cells including c-src (125). The binding of human colorectal cancer cells to human umbilical vein endothelial cells expressing E-selectin or to a recombinant E-selectin/Fc chimera leads to the activation of SAPK2/p38 in the cancer cells (126). Blocking the activation of SAPK2/p38 of these cells inhibits their trans-endothelial migration.

4. ROLES OF SELECTIN: SELECTIN LIGAND INTERACTIONS IN CANCER METASTASIS

4.1. Selectins regulate tumor cell extravasation

Metastatic disease is an urgent clinical concern because metastasis rather than primary tumor growth is the predominant cause of many cancer deaths. The metastatic cascade is a series of well-characterized steps that include local invasiveness, cell detachment, hematogenous or lymphogenic vascular invasion, circulation, cell arrest, extravasation, survival, proliferation, and angiogenesis. All steps must be completed in order for a malignant cell to successfully metastasize (127). In 1889, Stephen Paget proposed in his “seed-and-soil theory” from a study of breast cancers whereby the microenvironment of the target tissue provides the “soil” necessary to allow tumor cells to establish metastases or “seed” at specific secondary sites (128). This theory was later challenged by James Ewing in 1928 who proposed that cancer cells become established at particular metastatic sites due to the direction of blood flow and lymphatics which cause circulating tumor cells to become lodged in capillaries (mechanical tumor arrest) (129). Although tumor cells are almost twice the diameter of leukocytes and may not easily travel through the microvasculature, there is much evidence to support the occurrence of specific adhesions with endothelial cells that facilitate the exit of tumor cells from circulating blood into distant tissues (organ-specific metastasis) (2, 6, 8, 130). Both mechanisms may participate in metastasis depending on tumor type and the metastatic environment (4).

Although still controversial, accumulated data supports the concept that cancer cells utilize similar processes in metastasis that are engaged during leukocyte extravasation. Tumor cell transendothelial migration is dependent on the heterotypic interactions of tumor cells expressing selectin ligands, endothelial cells expressing E- and P-selectin, and other blood components (2, 8, 130-132). The modulation of these interactions using selectin-ligand mimetics (133), alterations in selectin ligand biosynthesis (134), inhibition of carbohydrates that participate in selectin interactions with their ligands (135), or inhibition of E-selectin expression (136) altered tumor cell adhesion and metastasis formation. A study demonstrated that metastases were redirected from the lung to the liver in mice over-expressing E-selectin in the liver, which provides evidence that E-selectin present on activated endothelium can facilitate tumor cell seeding (137). Early evidence using human colon carcinoma cells demonstrated that the development of experimental liver metastases was dependent on E-selectin (6). P-selectin may also promote the metastatic process by helping colorectal cancer cells to evade the inflammatory response but its function is not entirely clear. In colorectal cancer specimens, P-selectin expression and leukocyte infiltration were almost undetectable in liver metastases when compared to primary tumors that expressed high levels of P-selectin and high numbers of leukocytes (138). P-selectin also appears to mediate metastasis through platelet and tumor cell interactions and a synergistic effect of P- and L-selectin has been reported in the metastasis of colon carcinoma (7). P-selectin expression is increased in gastric and breast cancers but decreased expression in melanoma and colorectal cancer is associated with tumor progression (138-142). In melanomas, P-selectin is not expressed in the microvasculature of advanced primary tumors and metastases compared to benign melanocytic lesions. The observed decrease in P-selectin expression may be due to its enhanced shedding into the circulation (142).

The tumor-promoting role of selectins in cancer progression is not clear cut as some evidence indicates that selectins do not participate in tumor progression. For instance, clinical studies conclude that levels of soluble E-selectin in circulation are not different from controls in samples obtained from colorectal cancer, breast cancer, and hepatocellular cancer patients (143-145). In selectin-deficient mice, the primary growth of human colon carcinoma or melanoma is significantly enhanced which is thought to be due to a lack of monocyte/macrophage infiltration into tumors (146). Glinskii *et al.* found that selectins did not significantly participate in the arrest of breast and prostate carcinoma cells in murine lung microvasculature because function-blocking antibodies directed against E-selectin, L-selectin, and P-selectin did not inhibit the formation of sub-pleural metastatic deposits (147). Lastly, another study reported that multiple prostate carcinoma cell lines did not adhere to selectins although they expressed sLe^x antigens (148) however static adhesion assays were used which may not accurately reflect the dynamics of attachment of cells to activated vascular endothelium. Thus, tumor type, location, physiological shear, factors in the tumor microenvironment, and other conditions all appear to be important in determining the role of selectins in tumor adhesion.

4.2. The involvement of selectin ligands in cancer metastasis

Tumor cells are abnormally glycosylated and display alterations in sialylated and fucosylated selectin ligands. This pattern was first described in 1969 by Meezan *et al.* with the observation that healthy fibroblasts have smaller membrane glycoproteins than transformed fibroblasts (149). Selectin ligands such as PSGL-1, ESL-1, CD24, sLe^x, sLe^a, CD34, MAdCAM-1, lysosomal membrane glycoproteins LAMP-1 and LAMP-2, sulfatides, CD44, and death receptor-3 (DR-3) that are expressed on leukocytes are also up-regulated on the surface of cancer cells (150). However, in many cases their biological significance remains to be elucidated. The carbohydrate determinants sLe^x and sLe^a are ligands for endothelial E-selectin and both are expressed on carcinomas of the large bowel. These determinants are synthesized by glycosyltransferases: GlcNac transferase, Gal transferase, sialyl transferase and fucosyltransferase and altered functioning of these enzymes may result in changes in selectin-mediated metastasis (151). Selectin binding to sLe^x and sLe^a carbohydrates present on several carcinomas including colon, gastric, bladder, pancreatic, breast, and prostate carcinomas has been shown to regulate distant organ metastasis and furthermore, high expression of sialyl Lewis ligands on these cancers is significantly correlated with a poor post-operative prognosis (140, 152-156).

On leukocytes, the enzyme core 2 β1,6-N-acetylglucosaminyltransferase (C2GnT1) facilitates core 2 carbohydrate branching and is required for PSGL-1 to recognize L-and P-selectin but not E-selectin (157). Expression of C2GnT1 results in synthesis of sLe^x decorated core 2 branched O-linked carbohydrates (C2-O-sLe^x) on leukocytes that bind with much greater affinity to selectins than sLe^x alone. Our group has recently reported that C2-O-sLe^x carbohydrates are tumor-associated antigens on colon and hepatic carcinoma cells that regulate their invasive properties (158), strongly bind to E-selectin (159), are predominantly expressed at the advancing edge of invasive colorectal adenocarcinomas, and that high mRNA levels of C2GnT1 are present in these carcinomas compared to normal colonic tissues (160). Our findings support other studies where high C2GnT1 expression in carcinomas has been correlated with vessel invasion, depth of tumor invasion, and metastasis (161-163). However, for other tumor types, the link between selectin-ligand interactions and tumor cell extravasation and metastasis is less clear. Highly metastatic ras-transformed NIH/3T3 fibroblasts and control cells have similar kinetics of extravasation that are independent of the metastatic capacity or the transformation status of the cells (164).

CD44 transmembrane glycoproteins are a family of molecules consisting of several isoforms expressed on epithelial, endothelial, and tumor cells. The extracellular domains of these isoforms contain combinations of at least 10 alternatively spliced exons. CD44 functions as a receptor for hyaluron, collagen, laminin and fibronectin, and interacts

with other molecules such as osteopontin, matrix metalloproteinases and selectins to regulate cell adhesion. CD44 appears to play important roles in cell survival, invasiveness, migration, and angiogenesis (165). The CD44 variant isoform CD44v is over-expressed in gastric, pancreatic, lung, and renal cancer and modulates tumor growth and metastasis (166). *In vitro* studies with colon carcinoma cells demonstrated that CD44v isoforms facilitate tumor cell adhesion to platelets, leukocytes, and endothelial cells by binding with E-selectin, P-selectin, L-selectin, and fibrin (167, 168).

DR-3 is a member of the TNFR family and was recently recognized as a sialylated and signaling counter receptor for E-selectin expressed by metastatic colon cancer cells (169). It is expressed mainly by peripheral blood lymphocytes as alternatively spliced isoforms and aberrant expression induces signals in mammalian cells that result in caspase-induced apoptosis or activation of NF kappa B (170, 171). DR-3 is expressed on colorectal cancer cells but not on normal colon tissues (169). The binding of DR-3 to E-selectin activates both DR-3 and E-selectin, activates the MAP kinases p38 and ERK, induces signaling in endothelial cells, and ultimately increases endothelial permeability which allows transendothelial migration of cancer cells (169, 172). Overall, these studies underscore the importance of selectins and selectin ligands in cancer cell adhesion and metastasis.

5. THE ROLE OF SELECTINS AND THEIR LIGANDS IN CANCER INFLAMMATION

The tumor microenvironment of primary tumors and distant metastases often contain a diverse inflammatory cell population consisting of infiltrates of platelets, neutrophils, macrophages, dendritic cells, and lymphocytes. These cells can all produce pro-inflammatory cytokines that can up-regulate selectin expression in the microvasculature, along with other mediators including chemokines, cytotoxic factors such as reactive oxygen species, serine and cysteine proteases, matrix metalloproteinases, membrane perforating agents, mediators of cell killing, and coagulation factors (173). Thus, there appears to be a link between inflammation and cancer progression.

In models of experimental liver and lung metastasis, within a few hours after the arrest of cancer cells in the microvasculature, markers of endothelial cell activation and inflammation are up-regulated (174, 175). Activated endothelial cells mediate the recruitment of leukocytes, promote inflammation and enhance metastatic colonization (41, 176). In an *in vivo* mouse model of liver metastasis, highly metastatic murine lung carcinoma and human colorectal carcinoma cell lines rapidly induced TNF-α production by Kupffer cells located in sinusoidal vessels around the invading tumor cells. Furthermore, high TNF-α levels resulted in increased tumor-specific expression of E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) on sinusoidal endothelial cells (176). Increased levels of soluble E-selectin, (ICAM-1), and (VCAM-1) found in the serum of colon cancer patients are markers indicating the activated state of endothelial cells (177). In contrast, inhibition of endothelial activation attenuates metastasis (97). Activated endothelial cells up-regulate cell adhesion molecules and the production of chemokines, which recruit leukocytes, neutrophils, and monocytes from the microcirculation (178).

Recently, it was shown that selectin-mediated interactions of tumor cells with platelets and leukocytes caused activation of endothelial cells, induced production of C-C chemokine ligand 5 (CCL5) and promoted metastasis (179). Soluble inflammatory mediators can also increase the expression of selectin ligands on cancer cells (2, 180, 181). McDonald *et al.* reported that two interrelated processes: direct endothelial selectin-selectin ligand interactions and activated and adherent neutrophils within the sinusoids both significantly facilitated the sinusoidal arrest and adhesion of lung carcinoma cells in the inflamed liver vasculature (182). Our group observed that neutrophils provided a source of TNF-alpha that significantly up-regulated the selectin ligand sLe^x on transfected non-small cell lung cancer cell lines expressing sLe^x. TNF-alpha stimulation also increased binding to E-selectin. The TNF-alpha stimulated tumor cells were more invasive and displayed morphological changes consistent with increased motility (manuscript in press). Overall, these changes indicated that neutrophil-derived TNF-alpha up-regulated selectin-mediated metastatic behavior of the non-small cell lung cancer cells. The studies reported here suggest that the inflammatory response augments the metastatic potential of cancer cells. A potential model for the inflammatory mechanisms that facilitate extravasation, invasion, and metastasis of circulating cancer cells is shown in Figure 3.

6. CLINICAL APPLICATIONS OF SELECTIN-LIGAND INTERACTIONS IN CANCER

Targeting selectins and their ligands to control cancer progression is an attractive prospect because much evidence suggests that these interactions drive tumor progression. Therefore, the development of drugs to inhibit selectin binding could potentially interrupt the metastatic cascade. A limitation of this strategy is that selectins play a central physiological role in inflammation and thrombosis and therefore potential risk exists of causing undesired and unexpected effects on host immune function with long-term drug administration. Furthermore, selectins act in concert with other adhesion molecules and their ligands to mediate metastasis of circulating tumor cells which needs to be taken into account when considering therapeutic intervention.

Varied anti-selectin therapies targeting interactions between leukocytes, platelets, and endothelial cells have been attempted for the treatment of inflammatory diseases. Promising results have been observed for treatment of conditions such as psoriasis, graft-versus-host-disease, arthritis, asthma, atherosclerosis, or ischemia-reperfusion injury using experimental animal models but clinical trials have overall been disappointing. These studies are described in detail in excellent recent reviews (45, 72, 183) and only highlights will be briefly discussed here. Several selectin antagonists have been tested but are not cell

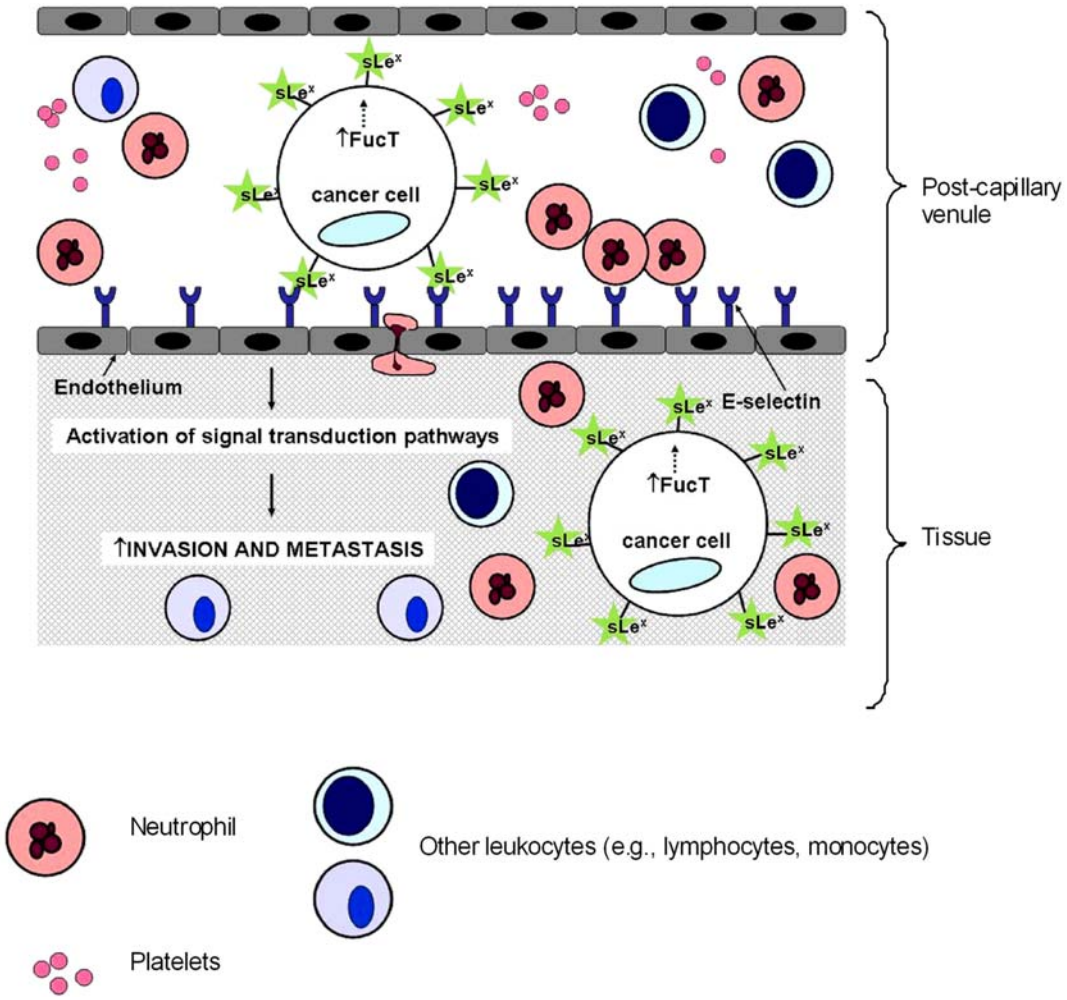

Figure 3. A potential model of selectin-mediated extravasation of cancer cells into an inflammatory tissue microenvironment during metastasis. Epithelial cancer cells intravasate into a blood vessel from a primary tumor, circulate, and interact with blood components such as leukocytes and platelets before arrest on endothelium in the peripheral vasculature including post-capillary venules. Cytokines and other factors produced by leukocytes in the inflammatory microenvironment may up-regulate fucosyltransferases and other glycosyltransferases in cancer cells resulting in increased expression of the sLe^x antigen on the cancer cell surface and high-affinity selectin binding. The binding of sLe^x on cancer cells to E-selectin may activate signal transduction pathways that result in enhanced invasion and metastasis. Neutrophils and other leukocytes that have extravasated into tissues may also facilitate invasion and metastasis through the secretion of cytokines that promote sLe^x presentation on cancer cells.

type specific. In mouse models of ischemia-reperfusion injury, anti-E-selectin and anti-P-selectin monoclonal antibodies were protective against ischemia-reperfusion-induced severe acute renal failure by reducing neutrophil infiltration into the kidney after ischemia (75, 184). SPLAT-1 (CDP850) is an engineered human antibody to E-selectin that was effective in inhibiting leukocyte recruitment to inflamed human skin grafted on SCID mice but failed to decrease leukocyte numbers in psoriasis patients or to improve symptoms and therefore further studies were not conducted (185, 186). Another anti-E-selectin antibody decreased inflammation in subarachnoid hemorrhage in mice and could have been potentially used to treat cerebral ischemia in patients but was not further characterized (187). In an atherosclerosis model using apolipoprotein E-deficient mice, blockade of P-selectin or PSGL-1 using monoclonal antibodies RB 40.34 and 4RA10 respectively significantly reduced neointima formation, plaque macrophage content, and vessel wall thickening after arterial injury which has applications as a treatment against restenosis (188).

A recombinant soluble form of human PSGL-1 covalently linked to immunoglobulin G (rPSGL-1-Ig) was developed that blocks leukocyte rolling *in vivo* and prevents inflammation but this product was discontinued due to high production costs (45, 72, 183). Biamosiamose is a pan-selectin antagonist that targets all three selectins and is effective in animal models of asthma and reperfusion injury after myocardial infarction (189, 190). Clinical improvement

was documented after biamosiamose was administered to psoriasis patients and was thought to be due to inhibition of leukocyte extravasation or mediated by E-selectin. Other glycomimetics directed against selectins have been tested including the sLe^x mimetics cylexine in phase II and III clinical trials and efomycine M in preclinical trials. Cylexine reduced reperfusion lung injury after pulmonary thromboendartectomy but was not effective in improving early mortality or post-operative recovery in infant heart surgery for congenital heart defects and thus further testing is needed. Efomycine M was non-toxic, demonstrated the most selective inhibition of selectin-mediated adhesion of leukocytes to endothelium *in vitro*, and diminished inflammatory responses in animal models of psoriasis, myocardial infarction and reperfusion injury, and T cell mediated-allergic reactions (183).

The induction of E-selectin tolerance has also been attempted to achieve an anti-inflammatory effect at selectin-positive target sites. In this system, the host's own immune system produces regulatory T cells against E-selectin. A low dose of E-selectin was repetitively administered by nasal instillation to spontaneously hypertensive stroke-prone rats which reduced the incidence of ischemic and hemorrhagic strokes (191). In humans, the safety and efficacy of an intranasal E-selectin spray in healthy patients with previous stroke was evaluated in a phase II study that was subsequently terminated.

Limited studies have been performed on the use of strategies targeting selectins as anti-cancer agents. Therapies aimed at blocking the effects of all three selectins and their ligand PSGL-1 simultaneously rather than as individual molecules may have the greatest effect in modulating metastasis. Drug design is difficult because the same cancer cell type may have multiple selectin ligands (32, 192). One study found that anti-P-selectin monoclonal antibodies suppressed metastasis of gastric cancer in mice orthotopically implanted with human gastric cancer tissues and did not adversely affect immune function (193). Another approach used glycometabolic inhibitors to inhibit O-glycosylation of mucins and fucosyltransferases and thereby indirectly reduce the production of selectin ligands (194). Metastasis was attenuated after treatment of cancer cells with a disaccharide-based inhibitor that was a decoy for glycan synthesis (195, 196). Low molecular weight heparins and unfractionated heparin have been developed that inhibit lung metastasis in experimental mouse models most likely by inhibiting the binding of cancer cells to L- and P-selectin (197). Heparins are also associated with a better survival of cancer patients in clinical trials (198).

7. CONCLUSIONS AND FUTURE PERSPECTIVES

The selectin-mediated adhesion of circulating cancer cells to endothelial cells is crucial for the homing of cancer cells to specific target organs during metastasis. However, much remains to be learned about the metastatic process. Unraveling the molecular mechanisms involved in metastasis will allow effective and highly specific selectin-based anti-cancer treatments to be developed in the future. As outlined above, a diverse array of inhibitors that target selectins and selectin ligands has been identified to potentially treat tumor metastasis using animal models. However, progress into clinical trials is slow in part because of technical issues created by the fact that these molecules participate in numerous physiological functions and their inhibition could potentially cause many unexpected or unwanted complications *in vivo*. The expression of selectins and their ligands is regulated by many factors in the microenvironment that are involved in inflammatory reactions and therefore the identification of specific targets to successfully inhibit selectins is challenging. In spite of these limitations, pursuit of selectin-based therapies to control cancer metastasis and inflammatory diseases is an exciting and novel scientific direction that deserves further study.

8. ACKNOWLEDGEMENTS

The author apologizes to colleagues whose work could not be cited due to space limitations and thanks are extended to Dr. Mariya Farooqui for review of the manuscript. This work was supported in part by funds from the National Institutes of Health, National Cancer Institute grant 5KO8CA111829-04.

9. REFERENCES

1. R. M. Lafrenie, M. R. Buchanan and F. W. Orr: Adhesion molecules and their role in cancer metastasis. *Cell Biophys*, 23, 3-89 (1993)

2. G. Mannori, P. Crottet, O. Cecconi, K. Hanasaki, A. Aruffo, R. M. Nelson, A. Varki and M. P. Bevilacqua: Differential colon cancer cell adhesion to E-, P-, and L-selectin: role of mucin-type glycoproteins. *Cancer Res*, 55, 4425-4431 (1995)

3. E. C. Butcher: Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell*, 67, 1033-6 (1991)

4. I. J. Fidler: The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer*, 3, 453-8 (2003)

5. F. G. Hanisch, C. Hanski and A. Hasegawa: Sialyl Lewis(x) antigen as defined by monoclonal antibody AM-3 is a marker of dysplasia in the colonic adenoma-carcinoma sequence. *Cancer Res* 52, 3138-44 (1992)

6. P. Brodt, L. Fallavollita, R. S. Bresalier, S. Meterissian, C. R. Norton and B. A. Wolitzky: Liver endothelial E-selectin mediates carcinoma cell adhesion and promotes liver metastasis. *Int J Cancer*, 71, 612-619 (1997)

7. L. Borsig, R. Wong, R. O. Hynes, N. M. Varki and A. Varki: 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*, 99, 2193-2198 (2002)

8. Y. J. Kim, L. Borsig, H.-L. Han, N. M. Varki and A. Varki: Distinct selectin ligands on colon carcinoma mucins

can mediate pathological interactions among platelets, leukocytes, and endothelium. *Am J Pathol*, 155, 461-472 (1999)

9. T. A. Springer: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell*, 76, 301-14 (1994)

10. K. Ley and G. S. Kansas: Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. *Nat Rev Immunol*, 4, 325-35 (2004)

11. G. S. Kansas: Selectins and their ligands: current concepts and controversies. *Blood*, 88, 3259-3287 (1996)

12. M. Morigi, C. Zoja, M. Figliuzzi, M. Foppolo, G. Micheletti, M. Bontempelli, M. Saronni, G. Remuzzi and A. Remuzzi: Fluid shear stress modulates surface expression of adhesion molecules by endothelial cells. *Blood*, 85, 1696-703 (1995)

13. M. Subramaniam, J. A. Koedam and D. D. Wagner: Divergent fates of P- and E-selectins after their expression on the plasma membrane. *Mol Biol Cell*, 4, 791-801 (1993)

14. D. B. Cines, E. S. Pollak, C. A. Buck, J. Loscalzo, G. A. Zimmerman, R. P. McEver, J. S. Pober, T. M. Wick, B. A. Konkle, B. S. Schwartz, E. S. Barnathan, K. R. McCrae, B. A. Hug, A. M. Schmidt and D. M. Stern: Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood*, 91, 3527-61 (1998)

15. P. von Hundelshausen and C. Weber: Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res*, 100, 27-40 (2007)

16. R. Hattori, K. K. Hamilton, R. D. Fugate, R. P. McEver and P. J. Sims: Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. *J Biol Chem*, 264, 7768-71 (1989)

17. D. Vestweber and J. E. Blanks: Mechanisms that regulate the function of the selectins and their ligands. *Physiol Rev*, 79, 181-213 (1999)

18. J. F. Leeuwenberg, E. F. Smeets, J. J. Neefjes, M. A. Shaffer, T. Cinek, T. M. Jeunhomme, T. J. Ahern and W. A. Buurman: E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology*, 77, 543-9 (1992)

19. R. Pigott, L. P. Dillon, I. H. Hemingway and A. J. Gearing: Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun*, 187, 584-9 (1992)

20. G. Davi, M. Romano, A. Mezzetti, A. Procopio, S. Iacobelli, T. Antidormi, T. Bucciarelli, P. Alessandrini, F. Cuccurullo and G. Bittolo Bon: Increased levels of soluble P-selectin in hypercholesterolemic patients. *Circulation*, 97, 953-957 (1998)

21. P. André, D. Hartwell, I. Hrachovinová, S. Saffaripour and D. D. Wagner: Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A*, 97, 13835-40 (2000)

22. K. M. Schweitzer, A. M. Drager, P. van der Valk, T. SF, A. Zevenbergen, A. P. Theijsmeijer, C. E. van der Schoot and M. M. Langenhuijsen: Constitutive expression of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues. *Am J Pathol*, 148, 165-175 (1996)

23. D. Nolte, P. Schmid, U. Jäger, A. Botzlar, F. Roesken, R. Hecht, E. Uhl, K. Messmer and D. Vestweber: Leukocyte rolling in venules of striated muscle and skin is mediated by P-selectin, not by L-selectin. *Am J Physiol*, 267, H1637-42 (1994)

24. K. Ley: The role of selectins in inflammation and disease. *Trends Mol Med*, 9, 263-8 (2003)

25. B. Schleiffenbaum, O. Spertini and T. F. Tedder: Soluble L-selectin is present in human plasma at high levels and retains functional activity. *J Cell Biol*, 119, 229-38 (1992)

26. J. W. Homeister, A. D. Thall, B. Petryniak, P. Malý, C. E. Rogers, P. L. Smith, R. J. Kelly, K. M. Gersten, S. W. Askari, G. Cheng, G. Smithson, R. M. Marks, A. K. Misra, O. Hindsgaul, U. H. von Andrian and J. B. Lowe: The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity*, 15, 115-26 (2001)

27. P. L. Smith, J. T. Myers, C. E. Rogers, L. Zhou, B. Petryniak, D. J. Becker, J. W. Homeister and J. B. Lowe: Conditional control of selectin ligand expression and global fucosylation events in mice with a targeted mutation at the FX locus. *J Cell Biol*, 158, 801-15 (2002)

28. A. Etzioni, M. Frydman, S. Pollack, I. Avidor, M. L. Phillips, J. C. Paulson and R. Gershoni-Baruch: Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med*, 327, 1789-92 (1992)

29. A. Takada, K. Ohmori, T. Yoneda, K. Tsuyuoka, A. Hasegawa, M. Kiso and R. Kannagi: Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. *Cancer Res*, 53, 354-361 (1993)

30. R. P. McEver and C. Zhu: Rolling cell adhesion. *Annu Rev Cell Dev Biol*, 26, 363-96 (2010)

31. P. S. Frenette, C. Moyna, D. W. Hartwell, J. B. Lowe, R. O. Hynes and D. D. Wagner: Platelet-endothelial

interactions in inflamed mesenteric venules. *Blood*, 91, 1318-24 (1998)

32. X. Yin, K. Rana, V. Ponmudi and M. R. King: Knockdown of fucosyltransferase III disrupts the adhesion of circulating cancer cells to E-selectin without affecting hematopoietic cell adhesion. *Carbohydr Res*, 345, 2334-42 (2010)

33. M. Trinchera, N. Malagolini, M. Chiricolo, D. Santini, F. Minni, A. Caretti and F. Dall'olio: The biosynthesis of the selectin-ligand sialyl Lewis x in colorectal cancer tissues is regulated by fucosyltransferase VI and can be inhibited by an RNA interference-based approach. *Int J Biochem Cell Biol*, 43, 130-9 (2011)

34. R. P. McEver and R. D. Cummings: Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest*, 100, 485-491 (1997)

35. A. Leppänen, S. P. White, J. Helin, R. P. McEver and R. D. Cummings: Binding of glycosulfopeptides to P-selectin requires stereospecific contributions of individual tyrosine sulfate and sugar residues. *J Biol Chem*, 275, 39569-39578 (2000)

36. K. R. Snapp, H. Ding, K. Atkins, R. Warnke, F. W. Luscinskas and G. S. Kansas: A novel P-selectin glycoprotein ligand-1 monoclonal antibody recognizes an epitope within the tyrosine sulfate motif of human PSGL-1 and blocks recognition of both P- and L-selectin. *Blood* 91, 154-64 (1998)

37. S. Aigner, C. L. Ramos, A. Hafezi-Moghadam, M. B. Lawrence, J. Friederichs, P. Altevogt and K. Ley: CD24 mediates rolling of breast carcinoma cells on P-selectin. *FASEB J*, 12, 1241-51 (1998)

38. P. Kotovuori, E. Tontti, R. Pigott, M. Shepherd, M. Kiso, A. Hasegawa, R. Renkonen, P. Nortamo, D. C. Altieri and C. G. Gahmberg: The vascular E-selectin binds to the leukocyte integrins CD11/CD18. *Glycobiology*, 3, 131-6 (1993)

39. C. J. Dimitroff, J. Y. Lee, S. Rafii, R. C. Fuhlbrigge and R. Sackstein: CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol*, 153, 1277-86 (2001)

40. L. J. Picker, R. A. Warnock, A. R. Burns, C. M. Doerschuk, E. L. Berg and E. C. Butcher: The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell*, 66, 921-33. Erratum in: Cell 1991 Dec 20;67(6):1267. (1991)

41. H. Läubli and L. Borsig: Selectins promote tumor metastasis. *Semin Cancer Biol*, 20, 169-77 (2010)

42. R. C. Fuhlbrigge, S. L. King, R. Sackstein and T. S. Kupper: CD43 is a ligand for E-selectin on CLA+ human T cells. *Blood*, 107, 1421-6 (2006)

43. M. Steegmaier, A. Levinovitz, S. Isenmann, E. Borges, M. Lenter, H. P. Kocher, B. Kleuser and D. Vestweber: The E-selectin-ligand ESL-1 is a variant of a receptor for fibroblast growth factor. *Nature*, 373, 615-20 (1995)

44. S. D. Rosen, D. Tsay, M. S. Singer, S. Hemmerich and W. M. Abraham: Therapeutic targeting of endothelial ligands for L-selectin (PNAd) in a sheep model of asthma. *Am J Pathol*, 166, 935-44 (2005)

45. C. Kneuer, C. Ehrhardt, M. W. Radomski and U. Bakowsky: Selectins-potential pharmacological targets? *Drug Discov Today*, 11, 1034-40 (2006)

46. L. Deban, R. C. Russo, M. Sironi, F. Moalli, M. Scanziani, V. Zambelli, I. Cuccovillo, A. Bastone, M. Gobbi, S. Valentino, A. Doni, C. Garlanda, S. Danese, G. Salvatori, M. Sassano, V. Evangelista, B. Rossi, E. Zenaro, G. Constantin, C. Laudanna, B. Bottazzi and A. Mantovani: Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol*, 1, 328-34 (2010)

47. R. P. McEver: Rolling back neutrophil adhesion. *Nat Immunol*, 11, 282-4 (2010)

48. M. Salio, S. Chimenti, N. De Angelis, F. Molla, V. Maina, M. Nebuloni, F. Pasqualini, R. Latini, C. Garlanda and A. Mantovani: Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*, 117, 1055-64 (2008)

49. K. Ebnet and D. Vestweber: Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. *Histochem Cell Biol*, 112, 1-23 (1999)

50. A. Zarbock and K. Ley: Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol*, 172, 1-7 (2008)

51. I. G. Colditz, M. A. Schneider, M. Pruenster and A. Rot: Chemokines at large: in-vivo mechanisms of their transport, presentation and clearance. *Thromb Haemost*, 97, 688-93 (2007)

52. R. F. Bargatze, S. Kurk, E. C. Butcher and M. A. Jutila: Neutrophils roll on adherent neutrophils bound to cytokine-induced endothelial cells via L-selectin on the rolling cells. *J Exp Med*, 180, 1785-1792 (1994)

53. S. M. Buttrum, R. Hatton and G. B. Nash: Selectin-mediated rolling of neutrophils on immobilized platelets. *Blood*, 82, 1165-74 (1993)

54. R. Alon, R. C. Fuhlbrigge, E. B. Finger and T. A. Springer: Interactions through L-selectin between leukocytes and adherent leukocytes nucleate rolling adhesions on selectins and VCAM-1 in shear flow. *J Cell Biol*, 135, 849-65 (1996)

55. C. A. St. Hill, S. R. Alexander and B. Walcheck: Indirect capture augments leukocyte accumulation on P-

selectin in flowing whole blood. *J Leukoc Biol*, 73, 464-471 (2003)

56. T. N. Mayadas, R. C. Johnson, H. Rayburn, R. O. Hynes and D. D. Wagner: Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell*, 74, 541-54 (1993)

57. M. L. Arbonés, D. C. Ord, K. Ley, H. Ratech, C. Maynard-Curry, G. Otten, D. J. Capon and T. F. Tedder: Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity*, 1, 247-60 (1994)

58. M. A. Labow, C. R. Norton, J. M. Rumberger, K. M. Lombard-Gillooly, D. J. Shuster, J. Hubbard, R. Bertko, P. A. Knaack, R. W. Terry, M. L. Harbison, F. Kontgen, C. L. Stewart, K. W. McIntyre, P. C. Will, D. K. Burns and B. A. Wolitzky: Characterization of E-selectin-deficient mice: demonstration of overlapping function of the endothelial selectins. *Immunity*, 1, 709-20 (1994)

59. V. Roldán, F. Marín, G. Y. Lip and A. D. Blann: Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. *Thromb Haemost* 90, 1007-20 (2003)

60. P. H. Jakobsen, S. Morris-Jones, A. Rønn, Hviid, L,, T. G. Theander, I. M. Elhassan, I. C. Bygbjerg and B. M. Greenwood: Increased plasma concentrations of sICAM-1, sVCAM-1 and sELAM-1 in patients with Plasmodium falciparum or P. vivax malaria and association with disease severity. *Immunology*, 83, 665-9 (1994)

61. K. Fassbender, R. Mössner, L. Motsch, U. Kischka, A. Grau and M. Hennerici: Circulating selectin- and immunoglobulin-type adhesion molecules in acute ischemic stroke. *Stroke*, 26, 1361-4 (1995)

62. T. Kobayashi, S. Hashimoto, K. Imai, E. Amemiya, M. Yamaguchi, A. Yachi and T. Horie: Elevation of serum soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin levels in bronchial asthma. *Clin Exp Immunol*, 96, 110-5 (1994)

63. W. Czech, E. Schöpf and A. Kapp: Soluble E-selectin in sera of patients with atopic dermatitis and psoriasis--correlation with disease activity. *Br J Dermatol*, 134, 17-21 (1996)

64. L. Kowalzick, A. Kleinheinz, K. Neuber, M. Weichenthal, I. Köhler and J. Ring: Elevated serum levels of soluble adhesion molecules ICAM-1 and ELAM-1 in patients with severe atopic eczema and influence of UVA1 treatment. *Dermatology*, 190, 14-8 (1995)

65. D. S. Kim and K. Y. Lee: Serum soluble E-selectin levels in Kawasaki disease. *Scand J Rheumatol*, 23, 283-6 (1994)

66. N. Oka, I. Akiguchi, T. Kawasaki, K. Ohnishi and J. Kimura: Elevated serum levels of endothelial leukocyte adhesion molecules in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *Ann Neurol*, 35, 621-4 (1994)

67. C. Wenisch, D. Myskiw, B. Parschalk, T. Hartmann, K. Dam and W. Graninger: Soluble endothelium-associated adhesion molecules in patients with Graves' disease. *Clin Exp Immunol*, 98, 240-4 (1994)

68. M. H. Ruchaud-Sparagano, E. M. Drost, S. C. Donnelly, M. I. Bird, C. Haslett and I. Dransfield: Potential pro-inflammatory effects of soluble E-selectin upon neutrophil function. *Eur J Immunol*, 28, 80-9 (1998)

69. M. S. Gruschwitz, O. P. Hornstein and P. von Den Driesch: Correlation of soluble adhesion molecules in the peripheral blood of scleroderma patients with their in situ expression and with disease activity. *Arthritis Rheum*, 38, 184-9 (1995)

70. C. A. Facer and A. Theodoridou: Elevated plasma levels of P-selectin (GMP-140/CD62P) in patients with Plasmodium falciparum malaria. *Microbiol Immunol*, 38, 727-31 (1994)

71. F. Sakamaki, A. Ishizaka, M. Handa, S. Fujishima, T. Urano, K. Sayama, H. Nakamura, M. Kanazawa, T. Kawashiro, M. Katayama and Y. Ikeda: Soluble form of P-selectin in plasma is elevated in acute lung injury. *Am J Respir Crit Care Med*, 151, 1821-6 (1995)

72. C. Ehrhardt, C. Kneuer and U. Bakowsky: Selectins-an emerging target for drug delivery. *Adv Drug Deliv Rev*, 56, 527-49 (2004)

73. G. Wu, F. Li, P. Li and C. Ruan: Detection of plasma alpha-granule membrane protein GMP-140 using radiolabeled monoclonal antibodies in thrombotic diseases. *Haemostasis*, 23, 121-8 (1993)

74. I. Takeda, S. Kaise, T. Nishimaki and R. Kasukawa: Soluble P-selectin in the plasma of patients with connective tissue diseases. *Int Arch Allergy Immunol*, 105, 128-3 (1994)

75. K. Singbartl, S. A. Green and K. Ley: Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. *FASEB J*, 14, 48-54 (2000)

76. P. Mangell, A. Mihaescu, Y. Wang, R. Schramm, B. Jeppsson and H. Thorlacius: Critical role of P-selectin-dependent leukocyte recruitment in endotoxin-induced intestinal barrier dysfunction in mice. *Inflamm Res*, 56, 189-94 (2007)

77. K. Ley, D. C. Bullard, M. L. Arbonés, R. Bosse, D. Vestweber, T. F. Tedder and A. L. Beaudet: Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. *J Exp Med* 181, 669-75 (1995)

78. K. E. Norman, M. J. Cotter, J. B. Stewart, K. B. Abbitt, M. Ali, B. E. Wagner, W. A. Wallace, S. B. Forlow and P.

G. Hellewell: Combined anticoagulant and antiselectin treatments prevent lethal intravascular coagulation. *Blood*, 101, 921-8 (2003)

79. R. G. Collins, R. Velji, N. V. Guevara, M. J. Hicks, L. Chan and A. L. Beaudet: P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med*, 191, 189-94 (2000)

80. Z. M. Dong, A. A. Brown and D. D. Wagner: Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. *Circulation*, 101, 2290-5 (2000)

81. Y. Huo, A. Schober, S. B. Forlow, D. F. Smith, M. C. Hyman, S. Jung, D. R. Littman, C. Weber and K. Ley: Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med*, 9, 61-7 (2003)

82. J. Rivera-Nieves, T. L. Burcin, T. S. Olson, M. A. Morris, M. McDuffie, F. Cominelli and K. Ley: Critical role of endothelial P-selectin glycoprotein ligand 1 in chronic murine ileitis. *J Exp Med*, 203, 907-17 (2006)

83. T. H. Rainer: L-selectin in health and disease. *Resuscitation*, 52, 127-41 (2002)

84. T. Collins, M. A. Read, A. S. Neish, M. Z. Whitley, D. Thanos and T. Maniatis: Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *FASEB J*, 9, 899-909 (1995)

85. K. Higai, A. Shimamura and K. Matsumoto: Amadori-modified glycated albumin predominantly induces E-selectin expression on human umbilical vein endothelial cells through NADPH oxidase activation. *Clin Chim Acta*, 367, 137-43 (2006)

86. M. P. Bevilacqua, J. S. Pober, D. L. Mendrick, R. S. Cotran and M. A. J. Gimbrone: Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA*, 84, 9238-42 (1987)

87. M. Hahne, U. Jäger, S. Isenmann, R. Hallmann and D. Vestweber: Five tumor necrosis factor-inducible cell adhesion mechanisms on the surface of mouse endothelioma cells mediate the binding of leukocytes. *J Cell Biol* 121, 655-64 (1993)

88. W. Kaszubska, R. Hooft van Huijsduijnen, P. Ghersa, A. M. DeRaemy-Schenk, B. P. Chen, T. Hai, J. F. DeLamarter and J. Whelan: Cyclic AMP-independent ATF family members interact with NF-kappa B and function in the activation of the E-selectin promoter in response to cytokines. *Mol Cell Biol*, 13, 7180-90 (1993)

89. C. Gaucher, C. Devaux, C. Boura, P. Lacolley, J. F. Stoltz and P. Menu: In vitro impact of physiological shear stress on endothelial cells gene expression profile. *Clin Hemorheol Microcirc*, 37, 99-107 (2007)

90. A. K. Stannard, R. Khurana, I. M. Evans, V. Sofra, D. I. Holmes and I. Zachary: Vascular endothelial growth factor synergistically enhances induction of E-selectin by tumor necrosis factor-alpha. *Arterioscler Thromb Vasc Biol*, 27, 494-502 (2007)

91. C. J. Treutiger, G. E. Mullins, A. S. Johansson, A. Rouhiainen, H. M. Rauvala, H. Erlandsson-Harris, U. Andersson, H. Yang, K. J. Tracey, J. Andersson and J. E. Palmblad: High mobility group 1 B-box mediates activation of human endothelium. *J Intern Med*, 254, 375-85 (2003)

92. G. E. Rainger, M. P. Wautier, G. B. Nash and J. L. Wautier: Prolonged E-selectin induction by monocytes potentiates the adhesion of flowing neutrophils to cultured endothelial cells. *Br J Haematol*, 92, 192-9 (1996)

93. E. Cernuda-Morollón and A. J. Ridley: Rho GTPases and leukocyte adhesion receptor expression and function in endothelial cells. *Circ Res*, 98, 757-67 (2006)

94. B. N. Cronstein, S. C. Kimmel, R. I. Levin, F. Martiniuk and G. Weissmann: A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A*, 89, 9991-5 (1992)

95. P. Ghersa, R. Hooft van Huijsduijnen, J. Whelan, Y. Cambet, R. Pescini and J. F. DeLamarter: Inhibition of E-selectin gene transcription through a cAMP-dependent protein kinase pathway. *J Biol Chem*, 269, 29129-37 (1994)

96. M. P. Bevilacqua, S. Stengelin, M. A. J. Gimbrone and B. Seed: Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science*, 243, 1160-5 (1989)

97. K. Kobayashi, S. Matsumoto, T. Morishima, T. Kawabe and T. Okamoto: Cimetidine inhibits cancer cell adhesion to endothelial cells and prevents metastasis by blocking E-selectin expression. *Cancer Res*, 60, 3978-84 (2000)

98. J. R. Gamble, Y. Khew-Goodall and M. A. Vadas: Transforming growth factor-beta inhibits E-selectin expression on human endothelial cells. *J Immunol*, 150, 4494-503 (1993)

99. B. L. Bennett, R. Cruz, R. G. Lacson and A. M. Manning: Interleukin-4 suppression of tumor necrosis factor alpha-stimulated E-selectin gene transcription is mediated by STAT6 antagonism of NF-kappaB. *J Biol Chem*, 272, 10212-9 (1997)

100. L. Yao, J. Pan, H. Setiadi, K. D. Patel and R. P. McEver: Interleukin 4 or oncostatin M induces a prolonged increase in P-selectin mRNA and protein in human endothelial cells. *J Exp Med*, 184, 81-92 (1996)

101. G. Woltmann, C. A. McNulty, G. Dewson, F. A. Symon and A. J. Wardlaw: Interleukin-13 induces PSGL-1/P-selectin-dependent adhesion of eosinophils, but not neutrophils, to human umbilical vein endothelial cells under flow. *Blood*, 95, 3146-52 (2000)

102. U. Gotsch, U. Jäger, M. Dominis and D. Vestweber: Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo. *Cell Adhes Commun*, 2, 7-14 (1994)

103. J. A. Auchampach, M. G. Oliver, D. C. Anderson and A. M. Manning: Cloning, sequence comparison and in vivo expression of the gene encoding rat P-selectin. *Gene*, 145, 251-5 (1994)

104. J. Pan and R. P. McEver: Regulation of the human P-selectin promoter by Bcl-3 and specific homodimeric members of the NF-kappa B/Rel family. *J Biol Chem*, 270, 23077-83 (1995)

105. J. Pan, L. Xia and R. P. McEver: Comparison of promoters for the murine and human P-selectin genes suggests species-specific and conserved mechanisms for transcriptional regulation in endothelial cells. *J Biol Chem*, 273, 10058-67 (1998)

106. V. Modur, M. J. Feldhaus, A. S. Weyrich, D. L. Jicha, S. M. Prescott, G. A. Zimmerman and T. M. McIntyre: Oncostatin M is a proinflammatory mediator. In vivo effects correlate with endothelial cell expression of inflammatory cytokines and adhesion molecules. *J Clin Invest*, 100, 158-68 (1997)

107. M. Yoshida, B. E. Szente, J. M. Kiely, A. Rosenzweig and M. A. J. Gimbrone: Phosphorylation of the cytoplasmic domain of E-selectin is regulated during leukocyte-endothelial adhesion. *J Immunol*, 161, 933-41 (1998)

108. S. I. Simon, V. Cherapanov, I. Nadra, T. K. Waddell, S. M. Seo, Q. Wang, C. M. Doerschuk and G. P. Downey: Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and colocalization with CD18. *J Immunol*, 163, 2891-901 (1999)

109. A. Levinovitz, J. Mühlhoff, S. Isenmann and D. Vestweber: Identification of a glycoprotein ligand for E-selectin on mouse myeloid cells. . *J Cell Biol*, 121, 449-459 (1993)

110. C. Laudanna, G. Constantin, P. Baron, E. Scarpini, G. Scarlato, G. Cabrini, C. Dechecchi, F. Rossi, M. A. Cassatella and G. Berton: Sulfatides trigger increase of cytosolic free calcium and enhanced expression of tumor necrosis factor-alpha and interleukin-8 mRNA in human neutrophils. Evidence for a role of L-selectin as a signaling molecule. *J Biol Chem*, 269, 4021-6 (1994)

111. X. Ba, C. Chen, Y. Gao and X. Zeng: Signaling function of PSGL-1 in neutrophil: tyrosine-phosphorylation-dependent and c-Abl-involved alteration in the F-actin-based cytoskeleton. *J Cell Biochem*, 94, 365-73 (2005)

112. C. Chen, X. Shang, T. Xu, L. Cui, J. Luo, X. Ba, S. Hao and X. Zeng: c-Abl is required for the signaling transduction induced by L-selectin ligation. *Eur J Immunol*, 37, 3246-58 (2007)

113. Y. Hu, J. M. Kiely, B. E. Szente, A. Rosenzweig and M. A. J. Gimbrone: E-selectin-dependent signaling via the mitogen-activated protein kinase pathway in vascular endothelial cells. *J Immunol*, 165, 2142-8 (2000)

114. Y. Hu, B. E. Szente, J. M. Kiely and M. A. J. Gimbrone: Molecular events in transmembrane signaling via E-selectin. SHP2 association, adaptor protein complex formation and ERK1/2 activation. *J Biol Chem*, 276, 48549-53 (2001)

115. S. I. Simon, Y. Hu, D. Vestweber and C. W. Smith: Neutrophil tethering on E-selectin activates beta 2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. *J Immunol*, 164, 4348-58 (2000)

116. A. Zarbock, C. L. Abram, M. Hundt, A. Altman, C. A. Lowell and K. Ley: PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. *J Exp Med*, 205, 2339-47 (2008)

117. M. Yoshida, W. F. Westlin, N. Wang, D. E. Ingber, A. Rosenzweig, N. Resnick and M. A. J. Gimbrone: Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J Cell Biol*, 133, 445-55 (1996)

118. M. A. Read, M. Z. Whitley, S. Gupta, J. W. Pierce, J. Best, R. J. Davis and T. Collins: Tumor necrosis factor alpha-induced E-selectin expression is activated by the nuclear factor-kappaB and c-JUN N-terminal kinase/p38 mitogen-activated protein kinase pathways. *J Biol Chem*, 272, 2753-61 (1997)

119. J. E. Blanks, T. Moll, R. Eytner and D. Vestweber: Stimulation of P-selectin glycoprotein ligand-1 on mouse neutrophils activates beta 2-integrin mediated cell attachment to ICAM-1. *Eur J Immunol*, 28, 433-43 (1998)

120. A. Celi, G. Pellegrini, R. Lorenzet, A. De Blasi, N. Ready, B. C. Furie and B. Furie: P-selectin induces the expression of tissue factor on monocytes. *Proc Natl Acad Sci U S A*, 91, 8767-71 (1994)

121. A. S. Weyrich, M. R. Elstad, R. P. McEver, T. M. McIntyre, K. L. Moore, J. H. Morrissey, S. M. Prescott and G. A. Zimmerman: Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest*, 97, 1525-34 (1996)

122. H. B. Wang, J. T. Wang, L. Zhang, Z. H. Geng, W. L. Xu, T. Xu, Y. Huo, X. Zhu, E. F. Plow, M. Chen and J. G.

Geng: P-selectin primes leukocyte integrin activation during inflammation. *Nat Immunol*, 8, 882-92 (2007)

123. V. Evangelista, Z. Pamuklar, A. Piccoli, S. Manarini, G. Dell'elba, R. Pecce, N. Martelli, L. Federico, M. Rojas, G. Berton, C. A. Lowell, L. Totani and S. S. Smyth: Src family kinases mediate neutrophil adhesion to adherent platelets. *Blood*, 109, 2461-9 (2007)

124. B. Furie and B. C. Furie: P-selectin and signal transduction. In: *The Selectins*. Ed M. A. Vadas&J. Harlan. Harwood, New York (1997)

125. S. A. Soltesz, E. A. Powers, J. G. Geng and C. Fisher: Adhesion of HT-29 colon carcinoma cells to E-selectin results in increased tyrosine phosphorylation and decreased activity of c-src. *Int J Cancer*, 71, 645-53 (1997)

126. J. Laferriere, F. Houle, M. M. Taher, K. Valerie and J. Huot: Transendothelial migration of colon carcinoma cells requires expression of E-selectin by endothelial cells and activation of stress-activated protein kinase-2 (SAPK2/p38) in the tumor cells. *J Biol Chem*, 276, 33762-72 (2001)

127. G. Poste and I. J. Fidler: The pathogenesis of cancer metastasis. *Nature*, 283, 139-46 (1980)

128. S. Paget: The distribution of secondary growth in cancer of the breast. *Lancet*, 1, 571-73 (1889)

129. J. Ewing: A treatise on tumors, neoplastic diseases. W.B. Saunders, Philadelphia (1928)

130. M. M. Burdick, J. M. McCaffery, Y. S. Kim, B. S. Bochner and K. Konstantopoulos: Colon carcinoma cell glycolipids, integrins, and other glycoproteins mediate adhesion to HUVECs under flow. *Am J Phys - Cell Phys*, 284, C977-87 (2003)

131. M. L. Majuri, P. Mattila and R. Renkonen: Recombinant E-selectin-protein mediates tumor cell adhesion via sialyl-Le(a) and sialyl-Le(x). *Biochem Biophys Res Commun*, 182, 1376-82 (1992)

132. C. J. Dimitroff, M. Lechpammer, D. Long-Woodward and J. L. Kutok: Rolling of human bone-metastatic prostate tumor cells on human bone marrow endothelium under shear flow is mediated by E-selectin. *Cancer Res*, 64, 5261-9 (2004)

133. O. Insug, L. Otvos, T. Kieber-Emmons and M. Blaszczyk-Thurin: Role of SA-Le(a) and E-selectin in metastasis assessed with peptide antagonist. *Peptides*, 23, 999-1010 (2002)

134. S. Mathieu, M. Prorok, A. M. Benoliel, R. Uch, C. Langlet, P. Bongrand, R. Gerolami and A. El-Battari: 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*, 164, 371-83 (2004)

135. F. Haroun-Bouhedja, F. Lindenmeyer, H. Lu, C. Soria, J. Jozefonvicz and C. Boisson-Vidal: In vitro effects of fucans on MDA-MB231 tumor cell adhesion and invasion. *Anticancer Res*, 22, 2285-92 (2002)

136. T. Nübel, W. Dippold, H. Kleinert, B. Kaina and G. Fritz: Lovastatin inhibits Rho-regulated expression of E-selectin by TNFalpha and attenuates tumor cell adhesion. *FASEB J*, 18, 140-2 (2004)

137. L. Biancone, M. Araki, K. Araki, P. Vassalli and I. Stamenkovic: Redirection of tumor metastasis by expression of E-selectin in vivo. *J Exp Med*, 183, 581-587 (1996)

138. C. F. Peeters, T. J. Ruers, J. R. Westphal and R. M. de Waal: Progressive loss of endothelial P-selectin expression with increasing malignancy in colorectal cancer. *Lab Invest*, 85, 248-56 (2005)

139. S. B. Fox, G. D. Turner, K. C. Gatter and A. L. Harris: The increased expression of adhesion molecules ICAM-3, E- and P-selectins on breast cancer endothelium. *J Pathol*, 177, 369-76 (1995)

140. J. Renkonen, T. Paavonen and R. Renkonen: Endothelial and epithelial expression of sialyl Lewis(x) and sialyl Lewis(a) in lesions of breast carcinoma. *Int J Cancer*, 74, 296-300 (1997)

141. B. Mayer, H. Spatz, I. Funke, J. P. Johnson and F. W. Schildberg: De novo expression of the cell adhesion molecule E-selectin on gastric cancer endothelium. *Langenbecks Arch Surg*, 383, 81-6 (1998)

142. P. T. Nooijen, J. R. Westphal, A. M. Eggermont, C. Schalkwijk, R. Max, l. R. M. de Waa and D. J. Ruiter: Endothelial P-selectin expression is reduced in advanced primary melanoma and melanoma metastasis. *Am J Pathol*, 152, 679-82 (1998)

143. G. Velikova, R. E. Banks, A. Gearing, I. Hemingway, M. A. Forbes, S. R. Preston, N. R. Hall, M. Jones, J. Wyatt, K. Miller, U. Ward, J. Al-Maskatti, S. M. Singh, P. J. Finan, N. S. Ambrose, J. N. Primrose and P. J. Selby: Serum concentrations of soluble adhesion molecules in patients with colorectal cancer. *Br J Cancer*, 77, 1857-63 (1998)

144. M. Hebbar, F. Révillion, M. M. Louchez, M. O. Vilain, C. Fournier, J. Bonneterre and J. P. Peyrat: The relationship between concentrations of circulating soluble E-selectin and clinical, pathological, and biological features in patients with breast cancer. *Clin Cancer Res*, 4, 373-80 (1998)

145. M. Cervello, L. Virruso, G. Lipani, L. Giannitrapani, M. Soresi, A. Carroccio, R. Gambino, R. Sanfililippo, L. Marasà and G. Montalto: Serum concentration of E-selectin in patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *J Cancer Res Clin Oncol*, 126, 345-51 (2000)

146. D. Taverna, H. Moher, D. Crowley, L. Borsig, A. Varki and R. O. Hynes: Increased primary tumor growth in mice null for beta3- or beta3/beta5-integrins or selectins. *Proc Natl Acad Sci U S A*, 101, 763-8 (2004)

147. O. V. Glinskii, V. H. Huxley, G. V. Glinsky, K. J. Pienta, A. Raz and V. V. Glinsky: Mechanical entrapment is insufficient and intercellular adhesion is essential for metastatic cell arrest in distant organs. *Neoplasia*, 7, 522-7 (2005)

148. M. Satoh, K. Numahata, S. Kawamura, S. Saito and S. Orikasa: Lack of selectin-dependent adhesion in prostate cancer cells expressing sialyl Le(x). *Int J Urol*, 5, 86-91 (1998)

149. E. Meezan, H. C. Wu, P. H. Black and P. W. Robbins: Comparative studies on the carbohydrate-containing membrane components of normal and virus-transformed mouse fibroblasts. II. Separation of glycoproteins and glycopeptides by sephadex chromatography. *Biochemistry*, 8, 2518-24 (1969)

150. I. P. Witz: The selectin-selectin ligand axis in tumor progression. *Cancer Metastasis Rev*, 27, 19-30 (2008)

151. S. Hakomori: Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res*, 56, 5309-5318 (1996)

152. S. Nakamori, M. Kameyama, S. Imaoka, H. Furukawa, O. Ishikawa, Y. Sasaki, T. Kabuto, T. Iwanaga, Y. Matsushita and T. Irimura: Increased expression of sialyl Lewis^x antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. *Cancer Res*, 53, 3632-3637 (1993)

153. T. Jørgensen, A. Berner, O. Kaalhus, K. J. Tveter, H. E. Danielsen and M. Bryne: Up-regulation of the oligosaccharide sialyl LewisX: a new prognostic parameter in metastatic prostate cancer. *Cancer Res*, 55, 1817-9 (1995)

154. K. Numahata, M. Satoh, K. Handa, S. Saito, C. Ohyama, A. Ito, T. Takahashi, S. Hoshi, S. Orikasa and S. I. Hakomori: Sialosyl-Le(x) expression defines invasive and metastatic properties of bladder carcinoma. *Cancer*, 94, 673-85 (2002)

155. T. Kishimoto, H. Ishikura, C. Kimura, T. Takahashi, H. Kato and T. Yoshiki: Phenotypes correlating to metastatic properties of pancreas adenocarcinoma in vivo: the importance of surface sialyl Lewis(a) antigen. *Int J Cancer*, 69, 290-4 (1996)

156. S. Nakamori, H. Furukawa, M. Hiratsuka, T. Iwanaga, S. Imaoka, O. Ishikawa, T. Kabuto, Y. Sasaki, M. Kameyama, S. Ishiguro and T. Irimura: Expression of carbohydrate antigen sialyl Le(a): a new functional prognostic factor in gastric cancer. *J Clin Oncol*, 15, 816-25 (1997)

157. R. K. Jain, C. F. Piskorz, B. G. Huang, R. D. Locke, H. L. Han, A. Koenig, A. Varki and K. L. Matta: Inhibition of L- and P-selectin by a rationally synthesized novel core 2-like branched structure containing GalNAc-Lewisx and Neu5Acalpha2-3Galbeta1-3GalNAc sequences. *Glycobiology*, 8, 707-17 (1998)

158. C. A. St. Hill, D. Baharo-Hassan and M. Farooqui: C2-O-sLeX glycoproteins Are E-selectin ligands that regulate invasion of human colon and hepatic carcinoma cells. *PloS ONE*, 6, e16281 (2011) doi:10.1371/journal.pone.0016281

159. C. A. St. Hill, K. M. Bullard and B. Walcheck: Expression of the high-affinity selectin glycan ligand C2-O-sLeX by colon carcinoma cells. *Cancer Lett*, 217, 105-113 (2005)

160. C. A. St. Hill, M. Farooqui, G. Mitcheltree, H. E. Gulbahce, J. Jessurun, Q. Cao and B. Walcheck: The high affinity selectin glycan ligand C2-O-sLex and mRNA transcripts of the core 2 beta-1,6-N-acetylglucosaminyltransferase (C2GnT1) gene are highly expressed in human colorectal adenocarcinomas. *BMC Cancer*, 9, 79 (2009)

161. K. Shimodaira, J. Nakayama, N. Nakamura, O. Hasebe, K. Tsutomu and M. Fukuda: Carcinoma-associated expression of core 2 β-1,6-*N*-acetylglucosaminyltransferase gene in human colorectal cancer: role of *O*-glycans in tumor progression. *Cancer Res*, 57, 5201-5206 (1997)

162. E. Machida, J. Nakayama, J. Amano and M. Fukuda: Clinicopathological significance of core 2 β1,6-*N*-acetylglucosaminyltransferase messenger RNA expressed in the pulmonary adenocarcinoma determined by *in situ* hybridization. *Cancer Res*, 61, 2226-2231 (2001)

163. J. Renkonen, J. Räbinä, P. Mattila, R. Grenman and R. Renkonen: Core 2 β1,6-N-acetylglucosaminyltransferases and α1,3-fucosyltransferases regulate the synthesis of O-glycans on selectin ligands on oral cavity carcinoma cells. *APMIS*, 109, 500-506 (2001)

164. S. Koop, E. E. Schmidt, I. C. MacDonald, V. L. Morris, R. Khokha, M. Grattan, J. Leone, A. F. Chambers and A. C. Groom: Independence of metastatic ability and extravasation: metastatic ras-transformed and control fibroblasts extravasate equally well. *Proc Natl Acad Sci U S A*, 93, 11080-4 (1996)

165. B. F. Haynes, H. X. Liao and K. L. Patton: The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. *Cancer Cells*, 3, 347-50 (1991)

166. S. Gout, P. L. Tremblay and J. Huot: Selectins and selectin ligands in extravasation of cancer cells and organ selectivity of metastasis. *Clin Exp Metastasis*, 25, 335-44 (2008)

167. S. L. Napier, Z. R. Healy, R. L. Schnaar and K. Konstantopoulos: Selectin ligand expression regulates the

initial vascular interactions of colon carcinoma cells: the roles of CD44v and alternative sialofucosylated selectin ligands. *J Biol Chem*, 282, 3433-41 (2007)

168. C. S. Alves, M. M. Burdick, S. N. Thomas, P. Pawar and K. Konstantopoulos: The dual role of CD44 as a functional P-selectin ligand and fibrin receptor in colon carcinoma cell adhesion. *Am J Physiol Cell Physiol*, 294, C907-16 (2008)

169. S. Gout, C. Morin, F. Houle and J. Huot: Death receptor-3, a new E-selectin counter-receptor that confers migration and survival advantages to colon carcinoma cells by triggering p38 and ERK MAPK activation. *Cancer Res*, 66, 9117-9124 (2006)

170. A. M. Chinnaiyan, K. O'Rourke, G. L. Yu, R. H. Lyons, M. Garg, D. R. Duan, L. Xing, R. Gentz, J. Ni and V. M. Dixit: Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science*, 274, 990-2 (1996)

171. G. R. Screaton, X. N. Xu, A. L. Olsen, A. E. Cowper, R. Tan, A. J. McMichael and J. I. Bell: LARD: a new lymphoid-specific death domain containing receptor regulated by alternative pre-mRNA splicing. *Proc Natl Acad Sci U S A*, 94, 4615-9 (1997)

172. P. L. Tremblay, F. A. Auger and J. Huot: Regulation of transendothelial migration of colon cancer cells by E-selectin-mediated activation of p38 and ERK MAP kinases. *Oncogene*, 25, 6563-6573 (2006)

173. L. M. Coussens and Z. Werb: Inflammation and cancer. *Nature*, 420, 860-7 (2002)

174. A. M. Khatib, M. Kontogiannea, L. Fallavollita, B. Jamison, S. Meterissian and P. Brodt: Rapid induction of cytokine and E-selectin expression in the liver in response to metastatic tumor cells. *Cancer Res*, 59, 1356-1361 (1999)

175. H. Läubli and L. Borsig: Selectins as mediators of lung metastasis. *Cancer Microenviron*, 3, 97-105 (2010)

176. A. M. Khatib, P. Auguste, L. Fallavollita, N. Wang, A. Samani, M. Kontogiannea, S. Meterissian and P. Brodt: Characterization of the host proinflammatory response to tumor cells during the initial stages of liver metastasis. *Am J Pathol*, 167, 749-59 (2005)

177. D. Alexiou, A. J. Karayiannakis, K. N. Syrigos, A. Zbar, A. Kremmyda, I. Bramis and C. Tsigris: Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer*, 37, 2392-7 (2001)

178. J. S. Pober and W. C. Sessa: Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol*, 7, 803-15 (2007)

179. H. Läubli, K. S. Spanaus and L. Borsig: Selectin-mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes. *Blood*, 114, 4583-91 (2009)

180. R. Giavazzi, M. Foppolo, R. Dossi and A. Remuzzi: Rolling and adhesion of human tumor cells on vascular endothelium under physiological flow conditions. *J Clin Invest*, 92, 3038-44 (1993)

181. A. Tözeren, H. K. Kleinman, D. S. Grant, D. Morales, A. M. Mercurio and S. W. Byers: E-selectin-mediated dynamic interactions of breast- and colon-cancer cells with endothelial-cell monolayers. *Int J Cancer*, 60, 426-31 (1995)

182. B. McDonald, J. Spicer, B. Giannais, L. Fallavollita, P. Brodt and L. E. Ferri: Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. *Int J Cancer*, 125, 1298-305 (2009)

183. B. Rossi and G. Constantin: Anti-selectin therapy for the treatment of inflammatory diseases. *Inflamm Allergy Drug Targets*, 7, 85-93 (2008)

184. K. Singbartl and K. Ley: Protection from ischemia-reperfusion induced severe acute renal failure by blocking E-selectin. *Crit Care Med*, 28, 2507-14 (2000)

185. R. Owens, E. Ball, R. Ganesh, A. Nesbitt, D. Brown, C. Gofton, S. Stephens, L. Chaplin, M. Christofidou-Solomidou, S. Blake, D. Howat, W. A. Buurman, S. Albelda and M. K. Robinson: The in vivo and in vitro characterisation of an engineered human antibody to E-selectin. *Immunotechnology*, 3, 107-16 (1997)

186. M. Bhushan, T. O. Bleiker, A. E. Ballsdon, M. H. Allen, M. Sopwith, M. K. Robinson, C. Clarke, R. P. Weller, R. A. Graham-Brown, M. Keefe, J. N. Barker and C. E. Griffiths: Anti-E-selectin is ineffective in the treatment of psoriasis: a randomized trial. *Br J Dermatol*, 146, 824-31 (2002)

187. C. L. Lin, A. S. Dumont, T. Calisaneller, A. L. Kwan, S. L. Hwong and K. S. Lee: Monoclonal antibody against E selectin attenuates subarachnoid hemorrhage-induced cerebral vasospasm. *Surg Neurol*, 64, 201-6 (2005)

188. J. W. Phillips, K. G. Barringhaus, J. M. Sanders, S. E. Hesselbacher, A. C. Czarnik, D. Manka, D. Vestweber, K. Ley and I. J. Sarembock: Single injection of P-selectin or P-selectin glycoprotein ligand-1 monoclonal antibody blocks neointima formation after arterial injury in apolipoprotein E-deficient mice. *Circulation*, 107, 2244-9 (2003)

189. E. Aydt and G. Wolff: Development of synthetic pan-selectin antagonists: a new treatment strategy for chronic inflammation in asthma. *Pathobiology*, 70, 297-301 (2002-2003)

190. Y. Onai, J. Suzuki, Y. Nishiwaki, R. Gotoh, K. Berens, R. Dixon, M. Yoshida, H. Ito and M. Isobe: Blockade of cell adhesion by a small molecule selectin antagonist attenuates myocardial ischemia/reperfusion injury. *Eur J Pharmacol*, 481, 217-25 (2003)

191. H. Takeda, M. Spatz, C. Ruetzler, R. McCarron, K. Becker and J. Hallenbeck: Induction of mucosal tolerance to E-selectin prevents ischemic and hemorrhagic stroke in spontaneously hypertensive genetically stroke-prone rats. *Stroke*, 33, 2156-63 (2002)

192. S. N. Thomas, R. L. Schnaar and K. Konstantopoulos: Podocalyxin-like protein is an E-/L-selectin ligand on colon carcinoma cells: comparative biochemical properties of selectin ligands in host and tumor cells. *Am J Physiol Cell Physiol*, 296, C505-13 (2009)

193. J. L. Chen, W. X. Chen, J. S. Zhu, N. W. Chen, T. Zhou, M. Yao, D. Q. Zhang and Y. L. Wu: Effect of P-selectin monoclonal antibody on metastasis of gastric cancer and immune function. *World J Gastroenterol*, 9, 1607-10 (2003)

194. S. R. Barthel, J. D. Gavino, L. Descheny and C. J. Dimitroff: Targeting selectins and selectin ligands in inflammation and cancer. *Expert Opin Ther Targets*, 11, 1473-91 (2007)

195. J. R. Brown, M. M. Fuster, R. Li, N. Varki, C. A. Glass and J. D. Esko: A disaccharide-based inhibitor of glycosylation attenuates metastatic tumor cell dissemination. *Clin Cancer Res*, 12, 2894-901 (2006)

196. M. M. Fuster, J. R. Brown, L. Wang and J. D. Esko: A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. *Cancer Res*, 63, 2775-81 (2003)

197. J. L. Stevenson, S. H. Choi and A. Varki: Differential metastasis inhibition by clinically relevant levels of heparins--correlation with selectin inhibition, not antithrombotic activity. *Clin Cancer Res*, 11, 7003-11 (2005)

198. N. M. Kuderer, T. L. Ortel and C. W. Francis: Impact of venous thromboembolism and anticoagulation on cancer and cancer survival. *J Clin Oncol*, 27, 4902-11 (2009)

Key Words: Cytokines, Inflammation, Leukocytes, Metastasis, Neoplasms, Neutrophils, Oligosaccharides, Selectins, Signal Transduction, Review

Send correspondence to: Catherine A. St. Hill, Department of Veterinary Clinical Sciences, University of Minnesota, Room C339, Veterinary Medical Center, 1352 Boyd Avenue, St. Paul, MN 55108, USA, Tel: 612-626-5787, Fax: 612-624-0751, E-mail: sthil001@umn.edu