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The selectin–selectin ligand axis in tumor progression

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Abstract This review will document that the selectin–selectin ligand axis is actively involved in tumor progression and drives this process. The involvement of selectins and their ligands in tumor progression takes place at three levels which will be reviewed: Interaction of tumor cells with platelets and leukocytes resulting in the formation of circulating emboli; interaction of tumor cells with endothelial cells leading to extravasation of the tumor cells; and utilization of reciprocal pro malignancy signals delivered by the selectins or by their ligands to interacting cells that express the corresponding co-receptor. We propose that the selectin–selectin ligand mediated interactions between cells in the tumor microenvironment constitute an axis of evil, that it be included in the list of pro malignancy factors, and that molecules associated with this axis serve as targets for cancer therapy.

Keywords Selectin–selectin ligand · Tumor progression · Cancer cells · Receptor–ligand interactions · Cancer therapy · Metastasis · Extravasation · Transendothelial migration

1 Introduction

Interactions of cancer cells with components of their microenvironment are crucial determinants in the decision making process that determines if cancer cells will progress towards metastasis or whether they will stay dormant or disappear altogether [1].

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There are scores of receptor–ligand interactions that play anti or pro malignancy roles. These include interactions that lead to or drive cell proliferation or death, angiogenesis, motility, chemotaxis, invasion, protective immunity, inflammation and metastasis to name a few. Most of these interactions were extensively reviewed in leading journals.

The metastatic cascade [2–4] is initiated when cells from the primary tumor detach and penetrate the extracellular matrix (ECM). Much of this invasive behavior depends upon the secretion of a variety of degradative enzymes, on alterations in the expression of adhesion molecules, on responses to cytokines and chemokines and on gene products regulating motility. A fraction of the invading cells penetrates into lymphatics or into the systemic circulation (intravasation). The tumor cells in the blood form emboli with platelets and leukocytes, adhere to the endothelium of certain organs, extravasate and migrate into secondary sites to form site specific metastasis [5–8].

The selectin–selectin ligand axis seems to play important roles in tumor progression and metastasis [9–43]. However whereas the role in tumor progression and metastasis of other factors such as integrins, proteases, angiogenic factors, cytokines or chemokines is highlighted in most review articles, the selectin–selectin ligand axis is still not situated in the forefront of the stage.

In this review we propose that the selectin–selectin ligand axis plays indeed prominent roles in tumor progression and that at least some of the molecules involved in this axis may serve as candidates for targeted tumor therapy and metastasis prevention.

Selectins, a family of mammalian lectins engaged in adhesion reactions [30, 44–52] are expressed by leukocytes, endothelial cells, and platelets. They are type I membrane proteins that contain a N-terminal C-type lectin domain, followed by an epidermal growth factor (EGF)-like motif, a

series of short consensus repeats, a transmembrane domain, and a cytoplasmatic tail. Selectins interact with cell-surface glycoconjugates and mediate tethering, rolling and adhesion of several types of cells. L-selectin is constitutively expressed by leukocytes, E-selectin by activated endothelial cells and P-selectin by platelets and activated endothelial cells [53].

Soluble E-selectin can be detected in blood where its presence is considered as evidence of endothelial activation [54]. Patients with a variety of diseases have elevated plasma levels of soluble selectins [55]. Levels of soluble E-selectin were significantly higher in patients with ovarian, breast and gastrointestinal cancer compared to controls [56]. In view of the fact that the level of soluble selectins often correlates with disease severity as in the case of liver metastasis in breast cancer [57] one may hypothesize that the soluble selectins contribute to the pathogenesis of cancer. However, to date no satisfactory mechanism for a causal relationship between high levels of circulating selectins and tumor progression has been proposed.

Selectin ligands such as PSGL-1, ESL-1, CD24, sialyl Lewis-a, sialyl Lewis-x, CD34, MAdCAM-1, lysosomal membrane glycoproteins LAMP-1 and LAMP-2, sulfatides, HCELL and other CD44 variant isoforms, to name a few [41, 44, 45, 58–71] are expressed mainly by leukocytes. These ligands are composed of a protein or a lipid carrier molecule, modified by certain oligosaccharides. The oligosaccharides have to be correctly assembled on the carrier in order to be recognized by the selectins. Specific glycoproteins, modified with certain oligosaccharides (and in some cases with other posttranslational modifications), constitute the physiological and functional ligands for the selectins [50]. Selectin binding requires sulfation on tyrosine residues, or on lactosamine units [72, 73]. The proper functioning of many selectin ligands requires their fucosylation [63, 74, 75] mainly by the fucose-generating FX enzyme [17, 74–76]. Fucose is a component of many surface-localized and secreted molecules. It decorates the terminal portions of N-, O-, or lipid-linked glycans and modifies the core of some N-linked glycans [74, 75]. Terminal fucosylated glycans in humans constitute several blood group antigens and function as selectin ligands [74, 75]. Fucosylation of these ligands determines their ability to bind to the selectin family of cell adhesion molecules and therefore controls pivotal steps of selectin-dependent leukocyte adhesion and trafficking [74, 75].

It is known for a relatively long time that tumors express aberrant glycosylation patterns [24] and that high levels of sialylated fucosylated selectin ligands are expressed on cancer cells, notably epithelial cancer. It is also known that the interaction of selectin ligands with the corresponding selectins is involved in conferring a high malignancy phenotype upon tumors [20, 22, 31, 34–37, 40, 77]. Selectin ligands such as sLe-a serve as tumor markers [38, 78]. The CA19-9 antibody recognizing a sLe-a epitope, is probably one of the better known ones used in the diagnostics of gastrointestinal and pancreatic cancer [78].

Based on the correlation between a high expression of selectin ligands and a high malignancy phenotype of cancer cells it was logical to hypothesize that the selectin–selectin ligand axis is mechanistically involved in tumor progression.

This involvement could conceivably occur at three levels:

1. Interaction of tumor cells with platelets and leukocytes resulting in the formation of circulating emboli.
2. Interaction of tumor cells with endothelial cells leading to transendothelial migration (extravasation).
3. Utilization of pro-malignancy signals delivered by the selectin–selectin ligand axis.

This review will focus on the interactions between selectins and their carbohydrate ligands expressed by tumor cells and on the down stream effects of such interactions.

2 Interactions between tumor cells, platelets and leukocytes

Platelets play multiple roles in tumor progression and metastasis [21, 79, 80]. Together with leukocytes, they form aggregates with circulating tumor cells thereby protecting the tumor cells from immune insults and shear stress. The aggregates also facilitate the binding of tumor cells to endothelial cells thereby promoting extravasation [21, 79, 80]. P-selectin (CD62P) is expressed on the membrane of endothelial cells and platelets following cellular activation. P-selectin binds to several types of human cancer cells such as colon, lung and breast cancer, as well as to melanoma and neuroblastoma [15]. Focusing specifically on the role of platelet P-selectin in the metastatic process it was found that the *in vitro* interaction between activated mouse platelets and human CRC cells expressing P-selectin ligands is markedly diminished when the platelets originated from P-selectin-deficient mice [12, 13]. Similar differences were seen in *in-vivo* experiments. Labeled CRC cells were injected into wild-type and P-selectin-deficient mice. Immunohistochemical analysis showed that the tumor cells were surrounded by mouse platelets. Fewer tumor cells were arrested in the lungs of P-selectin-deficient mice, and the tumor cells were surrounded by a lower number of platelets [12, 25]. The decreased metastatic load in P-selectin-deficient mice is probably due to impaired platelet function in these animals rather than to impairment of cancer cell adhesion to endothelium [13].

In view of the potential role of P-selectin in metastasis (and in the patho-physiology of other diseases) it was

suggested that targeting P-selectin may offer novel therapeutic strategies to treat metastatic cancer [27, 81].

L-selectin (CD62L), is structurally and functionally similar to other selectins [82]. L-selectin is essential for homing of lymphocytes to secondary lymphoid organs. This homing is mediated by the adherence of the L-selectin expressing lymphocytes to the L-selectin ligand expressed by high endothelial venules of the lymphoid organs [82]. L-selectin is constitutively expressed on most leukocytes. In addition to its role in lymphocyte homing, L-selectin is involved in several instances of inflammatory leukocyte trafficking [82].

Tumor cells express sialomucins that can serve as L, P or E-selectin ligands [33]. Human colorectal carcinoma cells, for example, express HCELL and other CD44 glycoforms that function as a high affinity E- and L-selectin ligands [67, 68]. It has been suggested that at least some of the pro malignancy functions of these CD44 molecules are due to the fact that they serve as selectin ligands [67, 71].

The vivo significance of these (and possibly other) selectin ligands was explored in mouse models of metastasis. It was found that L-selectin deficiency attenuated metastasis [13]. Although the mechanism for this attenuation was not elucidated, it was suggested that L-selectin enhances metastasis by recruiting leukocytes to platelet-tumor emboli in the circulation (see above). The increased size of the emboli could facilitate their mechanical trapping in the microvasculature. Alternatively, L-selectin expressing leukocytes could bridge the emboli to the vascular endothelial cells expressing L-selectin ligands [82]. Subsequent studies indicated that L-selectin facilitates metastasis by induction of as yet unidentified L-selectin ligands on endothelial cells via fucosyltransferase-7. These ligands, in turn, promote pro-malignancy leukocyte–endothelial interactions at the docking sites of the tumor cell emboli [29].

3 Tumor cell extravasation

The interactions of tumor and endothelial cells are one of the major driving forces of tumor progression and metastasis.

Angiogenesis is the best known and most extensively studied tumor–endothelium interaction. It is indeed a pivotal determinant of tumor progression. Other critical tumor–endothelial interactions, possibly not less important, are invasion into the blood or lymph node vessels (intravasation) and exit of the cancer cells from the circulation otherwise known as transendothelial migration or extravasation. Among these interactions, extravasation is studied more thoroughly than intravasation.

The most important physiological extravasation process is that of leukocytes interacting with and exiting the vascular endothelium in the inflammatory process and in autoimmunity. Extravasating leukocytes migrate through the endothelial barrier by first adhering to the endothelial cell surface and then transmigrating through the endothelial cell layer. Adhesion to the endothelium usually requires a cascade of steps mediated by the selectins, leukocyte activating chemotactic factors, and endothelial activating proinflammatory cytokines as well as by integrins [50, 83–86]. These molecules act in concert and regulate the sequence of distinct steps. Subsequently, leukocytes penetrate the underlying tissue in an active process, involving other molecules [87, 88].

Transendothelial migration of leukocytes requires their binding to endothelial cells. P-selectin and its glycoprotein ligand-1 (PSGL-1) are involved in this interaction. PSGL-1, is a mucin glycoprotein expressed on the leukocyte membrane. The interaction between endothelial P-selectin and leukocyte PSGL-1 mediates the tethering and rolling of the leukocytes on the endothelial cells under flow conditions [27].

Signals delivered to the endothelial cells by this binding promote the opening of endothelial cell junctions thus facilitating their transendothelial passage (diapedesis) [82, 89, 90]. However there is no agreement as to the mechanism by which leukocytes migrate across the endothelium. According to the para-cellular mechanism, leukocyte transmigration takes place at the endothelial junctions, with the traversing leukocyte transiently replacing the interactions of membrane proteins localized within the endothelial junctions. This would permit the exit of the leukocyte between adjacent endothelial cells. The trans-cellular mechanism, on the other hand, postulates that leukocytes migrate through individual intact endothelial cells [91].

There are two major schools of thought regarding tumor extravasation. The prevailing assumption is that the extravasation strategy of pre-metastatic tumor cells from blood vessels follows that of leukocytes. Namely, pre metastatic tumor cells expressing selectin ligands bind to a selectin-expressing endothelium. This interaction mediates the tethering and rolling of the tumor cells on the endothelial cells. This is then followed by additional molecular interactions with endothelial cells that culminate in extravasation [19, 26, 41, 92, 93].

The other school of thought can be designated as anatomical–mechanical. The arguments in this case are that depending on the blood-flow pattern from a primary tumor site, cancer cells that leave this tumor are taken passively to a capillary bed. Since such cells usually have diameters larger than the capillaries, they will be arrested at the narrow capillary due to size restriction. Tumor cells may be exported to the extra vascular compartment either by vessel rupture or by mechanical factors causing the membrane of the tumor cells to stretch thereby deforming the it enough to enable its passage through the vessel wall [94–96].

Additional mechanisms for tumor cell extravasation were suggested. It was demonstrated that tumor cells attached to

pulmonary endothelium proliferated in the intravascular space and gave rise to metastatic foci without the requirement for extravasation. At early stages, micrometastasis are contained entirely within the vasculature. With time, cells from metastatic colonies would "extravasate" by outgrowing the vasculature thereby destroying the vascular walls [97]. The second study showed that cells derived from solid tumors may alter the endothelium integrity by inducing endothelial cell apoptosis. This may enhance tumor cell extravasation [98].

Is the mode by which tumor cells extravasate from the circulation similar to that employed by leukocytes?

Although this controversial question is still open, the bottom line answer to this question seems to be a yes. This answer is supported by quite a large body of experimental and clinical data demonstrating that the interaction of sLe-a and sLe-x-expressing tumor cells with E- or P-selectin-expressing endothelial cells is an initial and crucial event in the interaction of tumor cells with endothelium resulting in tumor cell transendothelial migration [16, 23, 24, 28, 41, 99, 100].

An useful approach to answer the question if tumor cells utilize the leukocyte extravasation strategy was to modify selectin–selectin ligand interactions by various means and then examine the effects of such modifications on tumor cell adhesion and on metastasis. Among the approaches used were selectin–ligand mimetics [39, 101], diversion of the biosynthesis of selectin ligands toward non–adhesive structures [102], modification or inhibition of the carbohydrate moieties that participate in selectin–selectin–ligand interactions [103] or inhibition of E-selectin expression [104]. Most of these modifications blocked tumor cell adhesion and reduced metastasis. In this connection it is of interest to note that cimetidine, a drug exhibiting beneficial effects on the survival of colorectal cancer patients, down-regulated the expression of E-selectin on endothelial cells, blocked the *in vitro* adhesion of colorectal tumor cells to an endothelial cell monolayer and suppressed metastasis by these tumor cells in nude mice [105].

Do tumor cells expressing high levels of selectin ligands extravasate better than tumor cells expressing low levels of such ligands? Are the former cells more metastatic than the latter ones?

Although some of the studies linking expression levels of selectin ligands on tumor cells with their ability to extravasate and form metastasis, are based on correlative data and lack direct *in vivo* evidence, the answer to the above questions is a general yes for breast and colorectal carcinoma [27, 32, 42, 106, 107]. In a recent study performed in our laboratory (unpublished) we used metastatic CRC variants that expressed high levels of the sLe-a selectin ligand and non-metastatic CRC variants that expressed low levels of this selectin ligand. Also used were CRC cells that were transfected with siRNA of the fucose generating FX enzyme and as a result, expressed low levels of sLe-a (42). The various high and low sLe-a expressing tumor cells were inoculated i.v into mice. The arrest on lung endothelium and the extravasation of these cells was analyzed by confocal microscopy. These *in vivo* experiments indicated that the arrest of the tumor cells on the endothelium and their extravasation depended on the expression levels of the sLe-a selectin ligand. High sLe-a expressers extravasated better than low sLe-a expressing CRC cells.

For other tumor types the evidence linking selectin ligand expression, extravasation and metastasis is less convincing and even contrary to the above. Highly metastatic, ras-transformed NIH 3T3 cells, non-transformed and non-tumorigenic but immortalized NIH 3T3 cells and non-immortalized embryonic fibroblasts, showed the same kinetics of extravasation [108]. These results show that the extravasation from the microcirculation did not correlate with the metastatic capacity or even the transformation status of NIH 3T3 cells. However we are not convinced that such cells faithfully represent the *in vivo* reality of metastasis.

4 Regulation of E- and P-selectin expression

Both E- as well as P-selectins are inducible membrane proteins not expressed on quiescent endothelial cells or platelets. In both cells it is stored in intracellular granules.

Membrane expression of these molecules is induced in endothelial cells upon exposure to various proinflammatory stimuli. This ensures that these selectins are only expressed by endothelium in inflamed tissues. Expression is induced at the transcriptional level and maximal levels of E-selectin protein are expressed at the cell surface within 3–4 h after stimulation. Expression decreases and reaches basal levels about 20 h later [50, 109].

Among the additional factors that induce or co-induce, directly or indirectly, E-selectin expression by endothelial cells are shear stress [110]; VEGF [111]; HMGB1 [112]; monocytes [113] and various cytokines [50]. A down regulation of E-selectin expression can be achieved by cimetidine [105].

P-selectin, similarly to E-selectin is also regulated by cytokines. However, whereas proinflammatory cytokines such as TNFα and IL-1β induce the expression of E-selectin on human endothelial cells, these cytokines do not induce P-selectin expression on these cells. The expression of P-selectin on endothelial cells is induced by IL-3; IL-4 and oncostatin M [114].

Upon *in vitro* stimulation with histamine; thrombin; phorbol esters or Ca^{2+} ionophores, P-selectin rapidly translocates to the membrane of endothelial cells [50]. Shortly thereafter (about 60 min) the protein is cleared from the membrane by endocytosis. Internalized P-selectin molecules can be recycled [50].

The above mentioned external stimuli induce activation of Rho family GTPases including RhoA. The GTPases, in turn up regulate E-selectin expression [115].

Following the stimulation of platelets, P-selectin becomes expressed on the platelet surface from where it is rapidly shed [116].

Inflammation is a cancer promoting process [29, 117–131]. Many of the selectin-inducing components mentioned above are functional components of cancer-associated inflammation. In colorectal cancer E-selection expression is up regulated on endothelial cells that are located in close physical contact to invading tumor cells [132]. This is probably due to the secretion of pro inflammatory cytokines by the invading tumor cells. Moreover, tumor cells, similarly to normal cells [133], can indirectly induce the up regulation of selectin expression on endothelial cells by engaging third party cells. Liver-metastasizing CRC cells trigger the release of proinflammatory TNFα and IL-1β from Kupffer cells thereby up regulating the expression of E-selectin and other adhesion molecules on endothelial cells of hepatic sinusoidal vessels [134]. This up regulation enhances metastasis [135].

All these data point to a previously unconsidered pathway linking selectin ligands, inflammation and enhanced tumor progression.

5 Glycosylation and outside-in signals control the expression, structure and function of selectin ligands

Post translational modifications of proteins or lipids are necessary in order for them to function as selectin ligands [50, 136]. Whereas most of these modifications involve glycosylations, some involve sulfation of carbohydrates. These posttranslational events, in particular glycosylations constitute the major regulatory mechanism of their expression and function. Fucosyltransferases (FucT), the enzymes responsible for the addition of fucose to carbohydrates on protein or lipid are the main effectors of this regulation [50]. FucT VII plays an essential role in L-, E-, and P-selectin ligand biosynthesis. Mice deficient in FucT VII exhibit a leukocyte adhesion deficiency characterized by absent leukocyte E- and P-selectin ligand activity and deficient L-selectin ligand activity of high endothelial venules [137].

The fucose generating FX enzyme [74, 75] also takes part in the regulation of selectin ligand expression on certain tumor cells and in lymphocytes. Studies performed in our laboratory demonstrated that the FX enzyme controls the expression of sLe-x in activated T or B cells [76]. In head and neck squamous cell carcinomas, the FX enzyme is a limiting factor in the biosynthesis of sLe-a and plays a key role in the interaction of these cancer cells with endothelial cells [17, 43]. In lymphocytes, as well as in head and neck squamous cell carcinomas, the FX enzyme is regulated by outside-in activating signals [17, 76]. We also reported that a functional relationship between the expression level of the FX enzyme and that of the selectin ligand sLe-a exists in colorectal cancer (CRC) cells [42]. This conclusion was based on a direct and positive correlation between expression levels of the FX enzyme and those of sLe-a by several CRC cell lines. Furthermore, down-regulating FX expression by FX siRNA transfection decreased sLe-a expression. We also documented a functional axis linking FX and sLe-a expression to the capacity of CRC cells to adhere *in vitro* to E-selectin and to activated endothelial cells [42]. Additional results showed that highly metastatic CRC variants, expressing high levels of the FX enzyme and of sLe-a, adhered better to endothelial cells than low metastatic variants originating from the same tumor and expressing low levels of the FX enzyme and of sLe-a [42]. Similar results were obtained with hepatocellular carcinoma and hepatoma [138].

Based on the studies summarized above, one may hypothesize that the fucose-generating FX enzyme controls, by regulating selectin ligand expression, the interaction of at least certain tumor cells with endothelium and thus the capacity to extravasate and metastasize.

The FX enzyme supplies about 90% of the cellular fucose while the rest is supplied by the salvage pathway [74, 139]. In this pathway, extra cellular fucose is taken up by the cell, phosphorylated and subsequently converted to GDP L-fucose. The GDP L-fucose generated by the salvage pathway undergoes an identical biosynthetic route as the GDP L-fucose generated by the FX enzyme [74, 75]. It is still unknown if the salvage pathway is involved in the regulation of selectin ligand expression.

Cytokines regulate selectin ligand expression on T cells. Macrophage-derived IL-12 which causes the *in vitro* differentiation of mouse CD4-positive T cells into Th1 cells also induces the expression of P-selectin ligands. STAT4 is strongly involved in these processes [140]. The induction of P-selectin ligands on Th1 cells by IL-12 was apparently mediated through the induction of FucT VII by this cytokine [141]. The involvement of IL-12 in the induction of selectin ligands is still controversial. Induction of P-selectin ligands on mouse CD8-positive T cells *in vivo*, is IL-12 independent [142]. TGFβ also induces the expression of ligands for endothelial selectins on human CD4-positive T cells through the induction of FucT VII [143]. HCELL and anther E-selectin ligand were up regulated on human leukocytes by G-CSF treatment *in vitro*. The activity of FucT IV and FucT VII was also enhanced in these cells [144].

6 Signaling through selectins and selectin ligands

Selectins Although selectins are usually described as mediating low-affinity interactions, there is ample evidence

for the signaling capacity of these molecules. Selectins, being equipped with a cytoplasmic tail, may transduce outside-in signals delivered by selectin ligands [145–147]. Antibody-mediated cross linking of E- and P-selectin on endothelial cells affected the shape of the cells [148] and caused certain cytoskeletal proteins to physically interact with E-selectin [149]. E-selectin-dependent leukocyte adhesion and antibody-mediated cross-linking of cell surface E-selectin activated the MAPK signaling cascade in cultured HUVEC [150]. E-selectin was also reported to transduce signals to neutrophils thereby activating integrins [151, 152]. Other studies showed that that ligation of E-selectin on endothelial cells under flow conditions leads to signaling which can regulate subsequent leukocyte trafficking on the endothelial cells [153]. Sulfatides function as ligands for L-selectin. The binding of these ligands to L-selectin expressing neutrophils, triggered the increase of cytosolic free calcium in these cells. The involvement of L-selectin in this signaling event was supported by results showing that the cross-linking of L-selectin on neutrophils by anti-L-selectin antibodies also triggered an increase of cytosolic free calcium [69].

Put together these results demonstrate that signal transduction through selectin can activate cells by various mechanisms.

Selectin ligands Selectin ligands also function as signal-transducing molecules. Several reports indicate that purified or recombinant E- or P-selectin have the capacity to deliver signals to cells expressing the corresponding ligands. Antibody-mediated cross linking of selectin ligands generates similar results.

The following examples illustrate the fact that signals can be delivered to leukocytes through selectin ligands.

Binding of recombinant E-selectin to polymorphonuclear cells elicited co-clustering of L-selectin and PSGL-1 on these cells and activated β_2-integrin in high-avidity clusters. This activation brought about an efficient neutrophil arrest in shear flow [154].

Antibody-mediated cross-linking of PSGL-1 induced tyrosine phosphorylation of Syk and SRE-dependent transcriptional activity in human T cell lymphoblasts and in U937 myeloid cells. Treatment of cells with a Syk inhibitor and over expression of either a Syk dead kinase mutant or an ITAM-mutated moesin abrogated PSGL-1-induced transcriptional activation [155]. Triggering the P-selectin ligand PSGL-1 on mouse neutrophils by P-selectin, induced the activation of integrins in these cells [156].

Antibody-mediated cross-linking of the PSGL-1 on mouse Th1 cells increased the avidity of LFA-1 to ICAM-1 thereby enhancing the reciprocal binding of these adhesion molecules. Moreover, PSGL-1-mediated rolling on P-selectin enhanced the Th1 cell accumulation on ICAM-1 under flow conditions. PSGL-1 cross-linking induced activation of PKC isoforms. The increased Th1 cell adhesion was strongly inhibited by PKC inhibitors suggesting that this kinase participates in the signaling through PSGL-1 [157].

In addition to the direct signaling effects of selectins on selectin ligand expressing cells, the selectins may exert costimulatory functions on cells in conjunction with other costimulatory molecules such as certain cytokines [133, 156, 158].

Do selectin ligands expressed by tumor cells transduce signals? Increasing evidence shows that this is indeed the case.

Binding of CRC cells to an E-selectin-Ig fusion protein stimulated tyrosine phosphorylation of several proteins in these cells [159]. The adhesion of human CRC cells to E-selectin-expressing HUVEC or to a recombinant E-selectin/Fc chimera led to the activation of SAPK2/p38 in the tumor cells. Blocking the activation of SAPK2/p38 of the CRC cells inhibited their transendothelial migration. These results demonstrate that the interaction of CRC cells with E-selectin confers an increased motogenic potential upon the tumor cells through the transduction of signals that activate the SAPK2/p38 pathway [160].

Similar results were recently obtained using P-selectin rather than E-selectin. A soluble P-selectin-IgG chimeric protein stimulated activation of $\alpha_5\beta_1$ integrin resulting in a specific increase of adhesion and spreading of cultured human CRC cells on fibronectin substrates. P-selectin binding also induced activation of p38 MAPK and PI3-K. PI3-K inhibitors blocked P-selectin-mediated integrin activation, cell attachment, and cell spreading. Inhibition of p38 MAPK activation blocked cell spreading, but not cell attachment [161]. These results suggest that P-selectin ligands may serve as tumor cell signaling molecules that modulate integrin-mediated cell adhesion in the metastatic process.

Death receptor-3 (DR3), a member of the TNF receptor (TNFR) superfamily [162] was recently identified as a novel E-selectin ligand on CRC cells [163]. Activation of DR3 by E-selectin triggered the activation of p38 and ERK MAPK and conferred migration and survival advantages upon the CRC cells. The E-selectin-DR3 interaction was reciprocal as it delivered signals also to the interacting endothelial cells [164]. The neutralization of DR3 with anti DR3 antibodies or its knockdown by siRNA decreased the adhesion of CRC to E-selectin and to E-selectin-expressing HUVEC. This inhibition also impaired transendothelial migration of CRC cells and blocked the activation of p38 and ERK by E-selectin. Most importantly, DR3 was found to be expressed in primary human CRC but not in normal colon tissue [163].

Identifying the target genes of the selectin-derived signals delivered to tumor cells via selectin ligands is obviously an important task.

Recent unpublished experiments from our laboratory using cDNA microarrays indicated that several genes were down-regulated in metastatic CRC cells incubated on immobilized rE-selectin. The main finding of this study was that the number of genes altered by signals delivered by E-selectin was 10 times higher in metastatic CRC variants than in non-metastatic variants from the same tumors. This suggests that metastatic CRC cells may be more amenable to signals delivered from the microenvironment than non metastatic cells. Ongoing work focuses on the functional genomics of the genes that were exclusively altered in the metastatic CRC variants.

Put together, the studies on signaling via selectins and their ligands clearly demonstrate that the selectin–selectin ligand axis may activate multiple signaling pathways and downstream effector functions in cells expressing either of these co receptors. This field is widely open as there is much more to learn about the specific pathways that link the selectin–selectin ligand axis to downstream functional events, to the cross talk between this axis and other signaling cascades and the identification of additional (to DR3) selectin ligands involved in these signaling events.

7 Conclusions and future directions

By controlling various adhesion functions and initiating the extravasation process of leukocytes, the selectin–selectin ligand axis plays pivotal roles in the physiological and pathological functions of innate and adaptive immunity. The realization that pre metastatic cells hijacked at least some of the functional properties of this axis and use it to progress towards metastasis is a recent development in cancer biology.

Three facts are already firmly established: The selectins and their ligands are instrumental in the formation of circulating emboli composed of tumor cells, platelets and leukocytes. These emboli protect tumor cells from shear stress and their leukocyte component facilitates the binding of tumor cells to endothelium. The selectin–selectin ligand axis initiates the contact between extravasating pre-metastatic cells and endothelial cells. The cellular interactions mediated by the selectin–selectin ligand axis are involved in transducing reciprocal signals to the cellular interaction partners and in regulating gene expression in them.

The expression of selectins and their ligands is regulated by a variety of microenvironmental factors, many of which are involved in inflammatory reactions. Furthermore, several of the gene products whose expression is regulated by the selectin–selectin ligand axis are involved in inflammatory reactions. This creates a previously unconsidered link between this axis and cancer associated inflammation.

In view of the fact that the interaction between selectins and their ligands can be characterized as yet another factor driving tumor progression, it is not surprising that the selectin–selectin ligand axis and its down stream molecules have been marked as potential targets for cancer therapy, specifically for halting and attenuating the metastatic cascade [32, 39, 47, 55, 78, 80, 101, 104, 105, 165, 166].

In order to identify additional effective therapy targets much more has to be learnt about the activity spectrum of the selectin–selectin ligand axis in cancer progression.

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