Selectins Facilitate Carcinoma Metastasis and Heparin Can Prevent Them

Lubor Borsig
Institute of Physiology, University of Zürich, CH-8057 Zürich, Switzerland

Selectins are cell adhesion molecules mediating attachment of leukocytes to activated endothelium as well as the adhesion reaction of tumors during malignancy. Heparin, which is known to attenuate metastasis, is a potent blocker of selectins. Here, the role of selectins in metastasis and the potential of heparin to modulate malignancy are discussed.

Several lines of evidence indicate that the process of tumorigenesis consists of several steps, which reflect genetic alterations driving the transformation of normal cells into malignant derivatives. Physiological alterations associated with carcinoma malignancy encompassed several characteristics of invasion and metastasis such as enhanced migration due to loss of cell-cell interactions (loss of E-cadherin), enhanced degradation of extracellular matrix by matrix metalloproteinases, remodeling and alterations of integrins and cell adhesion molecules, and finally, altered cell-surface glycosylation, adhesion of tumor cells to the vasculature of distant organs, and their extravasation. Metastatic spread of tumor cells is the predominant cause of cancer patient death. For metastasis to occur, interactions of tumor cells with surrounding platelets, leukocytes, and endothelia are of crucial importance. Although the mechanistic understanding of platelet-leukocyte-tumor cell emboli creation remains less clear, the vascular cell adhesion molecules called selectins are one possible facilitator of these interactions. The recently identified potential of heparin to block selectins at therapeutic concentrations together with the experimental proof of the antimetastatic effect of heparin in mouse models encourages researchers to revisit heparin therapy for prevention of metastasis in certain human cancers.

Platelets and leukocytes participate in tumor cell emboli formation

Circulating platelets are known to be involved in many physiological processes, such as homeostasis, thrombosis, wound healing, etc. Early studies in the 1980s suggested that platelets could facilitate metastasis (e.g., Ref. 8). Formation of tumor cell emboli consisting of platelets and leukocytes was proposed to be a way for a tumor cells to evade host defense, to promote retention in the capillaries, and eventually to colonize distant organs. Initial support for an active role of platelets in metastasis was obtained from a mouse model in which experimental thrombocytopenia led to attenuation of metastasis (8). The significance of the tumor cell-microemboli formation for metastasis was shown in mice where the intravenous injection of tumor cells led to a rapid association of platelets, whereas in the absence of this interaction tumor cells were cleared by natural killer (NK) cells (14). The intravenous tumor cell injection also coincided with a temporary reduction of peripheral platelet counts. Interfering with platelet-tumor cell interactions also resulted in the attenuation of metastasis, further supporting the protective role of microemboli formation for tumor cell survival (3). Participation of leukocytes in the tumor cell emboli is already a part of a current textbook description of blood-borne metastasis, but their role in this process requires further analysis. Interestingly, patients with enhanced mucin-producing carcinomas experience a frequent thrombotic incidence.

Mucin-producing human carcinomas correlate with poor prognosis

Typically, epithelial cells are covered with mucins, which are high-molecular-weight molecules containing the protein core substituted with a large number of O-linked carbohydrates (glycans). Soluble forms of mucins are also secreted in large amounts to apical surfaces, which line the lumen of the respective organ. During tumorigenesis, loss of cell polarity leads to exposure and secretion of aberrantly glycosylated tumor mucins to the bloodstream. Enhanced production of altered mucins is a leading feature of many carcinomas (e.g., colon, pancreas, stomach, biliary tree, bladder, prostate, breast, lung, esophagus, or ovary). Serological detection of carcinoma mucins (e.g., CEA, CA19-9) is a relevant diagnostic factor and is especially recommended for cancer patients with negative endoscopy results or unexplained abdominal pain (15).

In particular, mucin-rich carcinomas often suffer from accompanying thromboembolism, suggesting that mucin might be actively involved in this process. Clinical manifestations by malignancies vary from venous thromboembolism to a disseminated intravascular coagulation (DIC) known as thrombophlebitis migrans or Trouseau syndrome. In this condition, platelet-rich microthrombi formation is observed. The excessive coagulation associated with cancer is still thought to be caused by activated procoagulants and/or the enhanced activation of the fluid-phase coagulation via tissue factor (5). Idiopathic venous thromboembolism correlates...
with a significant incidence of malignancy, particularly in mucin-secreting adenocarcinomas. Recently, carcinoma mucins were shown to trigger selectin-dependent platelet aggregations in mice (18). Thus carcinoma mucins could serve as templates for platelet-rich thrombi formation and could potentially explain the causal relationship between mucin-rich carcinomas and Trousseau syndrome often observed in cancer patients.

Altered cell-surface glycosylation is a prominent feature of carcinoma progression (for a review, see Ref. 10). Although the altered glycosylation per se seems not to be crucial for the early stages of tumorigenesis, changes in the glycosylation are more likely to have a functional consequence in the later stages of invasion and metastasis. Indeed, many studies have shown that the enhanced expression of sialylated and fucosylated glycans on carcinomas correlate with a poor prognosis due to a high rate of metastasis. Despite a wide range of possibilities in glycosylation pathways, there are relatively few types of glycan alterations associated with epithelial cancer. Most cancer-associated glycan alterations are either truncated versions of glycans with a dominant presence of Tn antigen, sialyl-Tn antigen, or altered terminal oligosaccharides with prevailing sialyl Lewisα and sialyl Lewisβ (sLeαβ) epitopes. The presence of sLeαβ structures on carcinoma mucins is frequently associated with an advanced cancer and further metastatic progression (10).

Selectins as receptors for carcinoma mucins

The selectins are a family of vascular adhesion receptors comprising L-, E-, and P-selectin, that recognize carbohydrate structures (11). Their physiological function in mediation of cell adhesion was shown during inflammation, immune responses, hemostasis, and wound repair. Since the initial part of cell adhesion consists of leukocyte tethering and rolling on activated endothelial cells, platelets, or other leukocytes, selectins need to support rapid and reversible interaction with their carbohydrate ligands under the hydrodynamic flow. Studies of selectin-deficient mice have shown that each individual selectin is required for mediation of the very first interactions of leukocytes with endothelium. In the absence of selectins, all subsequent steps mediated by integrins and other adhesion molecules are either substantially delayed or not occurring. In addition, L-selectin is also required for an effective recirculation of leukocytes into the lymph nodes. Although L-selectin is constitutively expressed essentially on all leukocytes, P-selectin is rapidly exteriorized on activated platelets and endothelial cells. E-selectin is only expressed on the activated endothelium. The lectin domain of selectins recognizes sialylated, fucosylated structures displayed mostly on mucin-type glycoproteins (11). The core carbohydrate structure recognized by selectins contains a terminal tetrasaccharide sLeα and sLeβ. In addition, natural ligands of L- and P-
Selectins require additional sulfate groups either on the sLe\(^x\) alone or on the protein backbone in close proximity to a sLe\(^x\) structure.

Many studies have demonstrated that all three members of the selectin family also recognize carcinoma cells carrying sLe\(^x\). Furthermore, effective binding of selectins to primary human carcinoma specimens was observed (e.g., Ref. 12). It was shown that virtually all selectin-based interactions of carcinoma cells with platelets, leukocytes, and endothelium are possible in vitro as depicted in Fig. 2A. All of these interactions were dependent on the presence of carcinoma mucins. Interestingly, a purified carcinoma mucin alone was found to be capable of facilitating the interactions among leukocytes, platelets, and endothelium, thus stressing the potential of mucins in metastasis (12).

**Selectins as potential mediators of metastasis**

During hematogeneous metastasis, tumor cells carrying selectin ligands enter the circulation and may encounter vascular selectins; thus a possible role for selectins in cancer progression has been hypothesized (e.g., Ref. 10). Studies using mouse models and different carcinoma cell lines supported this hypothesis. The crucial step in the early phase of metastasis appears to be the interaction of tumor cells with the endothelium and its subsequent extravasation, which leads to the formation of new metastatic lesions (8). Initially, E-selectin was suggested to contribute to the seeding of tumor cells in distant organs, thus facilitating metastasis. The overexpression of E-selectin in the liver of a transgenic mouse model redirected the metastasis to this organ (2). Similarly, an intravenous injection of the E-selectin-blocking antibody led to...
attenuation of metastasis in another mouse model. Because E-selectin is only produced de novo by activated endothelium, it may not be of critical importance for the very early steps of tumor retention, due to its delay in cell-surface expression. In addition, this rather simplistic mechanism of the sole E-selectin involvement does not consider any roles for leukocytes and platelets in the early process of tumor emboli formation. More importantly, platelets and endothelia express P-selectin in a rapid fashion, making it one of the first cell adhesion molecules present upon activation. Therefore, we argued that P-selectin and/or L-selectin are also candidates for mediation of platelet-tumor cell-leukocyte-endothelium interactions as indicated by the microthrombi and microemboli characteristics of mucin-producing carcinomas (3, 4). Given the known function of platelets in facilitating metastasis, it seemed reasonable to concentrate on the role of platelet P-selectin in this process. Since metastasis was thought to ensue from tumor-platelet-leukocyte emboli formation, we investigated the role of selectins in the experimental metastasis model. For this purpose, P-selectin-deficient mice were bred into an immunodeficient background. In control mice (P-sel+/+), intravenous injection of human colon carcinoma cells has led to a pronounced metastasis to the lung. By contrast in P-selectin-deficient mice (P-sel−/−), attenuation of metastasis was observed (3). Immunohistochemical analysis of the lung microvasculature from P-sel−/− mice injected with tumor cells revealed a marked reduction in platelet aggregation around tumor cells, which lasted for another 6 h after injection. When tumor cells, from which mucins have been enzymatically removed, were intravenously injected, a similar reduction of platelet adhesion was observed, thus proving the interaction of platelets with tumor cells to be both P-selectin and carcinoma mucin dependent (3). Mice injected with mucin-deprived carcinoma cells also showed a marked reduction of metastasis, similar to the one observed in the P-sel−/− mice. In all of these conditions of temporally reduced or absent platelet-tumor cell interactions, an enhanced presence of Mac-1-positive monocytes (macrophage precursors) could be observed. In a different mouse model, the reduction of platelets by immunodepletion led to a reduction of metastasis, which was shown to be associated with an enhanced clearance of tumor cells by NK cells (14). This apparent protective effect of platelets for tumor cell survival seemed to be mostly due to the shielding from NK cells and not because of another direct prometastatic role of platelets. Interestingly, the number of tumor cells detected in the lung vasculature was always lower in the P-sel−/− mice (3). This observation suggests that either platelet-mediated embolus formation leads to an enhanced retention of tumor cells in this organ or that the absence of platelets enables a more efficient clearance of circulating tumor cells. Consequently, platelet P-selectin is part of the molecular mechanism that mediates platelet-tumor cell interaction and facilitates metastasis by protecting tumor cells from immune response.

Involvement of leukocytes in metastasis

Although the participation of leukocytes in platelet-tumor cell aggregates is well established, the importance of leukocyte presence in these complexes as well as the underlying molecular mechanism for their formation remains less clear. Virtually all subpopulations of leukocytes constitutively express cell surface L-selectin, which makes it a candidate for an interaction with circulating tumor cells. Their possible involvement was studied in two different mouse models in an L-selectin-deficient background (L-sel−/−) (4). Attenuation of metastasis in the absence of L-selectin was observed, hence directly implicating L-selectins (therefore leukocytes) in this process. Since this rather surprising result was also observed in the immunodeficient mice, which lack matured T and B cells, facilitation of metastasis by other leukocyte subsets (neutrophils, monocytes, or NK cells) could be expected (4). However, the subset of leukocytes remains to be identified. The contributions of P-selectin and L-selectin to the metastatic process were also evaluated in immunocompetent mice with a double deficiency in P-and L-selectin (PL-sel−/−) (4). Metastasis of an otherwise aggressive tumor was dramatically reduced in the PL-sel−/− mice, suggesting a synergistic action of P-and L-selectin in this process.

A recent review on inflammation and cancer discussed the role of a tumor microenvironment, which is largely organized by inflammatory cells and is an indispensable part of the malignant process (7). The connection between inflammation and cancer was first observed by Virchow in 1893 and has been regaining support in recent years (1). Tumor cells were shown to co-opt some of the signaling molecules of the innate immune system, including selectins, chemokines, and their receptors, for invasion and metastasis. Although most of the studies were focused on the tumorigenesis and its dependence on local inflammation, the contribution of the same machinery to the metastatic process could be anticipated. In this context, the observed role of L-selectin represents one possible mechanism for leukocytes to be recruited to tumor emboli and to facilitate metastasis. These findings suggest that targeting of P- and L-selectins by a specific blocker might be a valuable therapy for treatment of malignancies.

Heparin attenuates metastasis by inhibiting selectins

Heparin is a complex mixture of glycosaminoglycans, which have been used in the clinic as an anticoagulant for more than 50 years. Recent studies provide further insights into its specific structural requirements for a variety of biological functions (9). The anticoagulant activity of heparin is defined by a pentasaccharide, which binds to antithrombin and accelerates the interaction with thrombin and factor Xa. Despite no obvious structural similarity to the natural ligands of selectins, heparin was shown to efficiently bind P- and L-
The biological activity of heparin as a selectin ligand lies in the dense cluster of multiple negatively charged carboxylates and sulfates, but the precise structure recognized by selectins is not known. Notably, the unfractionated heparin was shown to effectively inhibit P- and L-selectin binding to its natural ligands (sLex) at the concentration currently therapeutically used for anticoagulation (13). Due to the common association of cancer with the venous thromboembolic complication (discussed above), heparin has been used as a standard initial anticoagulant treatment (16). Beneficial effects of heparin or low-molecular-weight heparin (LMW heparin) on attenuation of metastasis in humans were seen in several clinical trials (e.g., Ref. 6). On the other hand, the use of a vitamin K antagonist, which achieves anticoagulation by a different mechanism from those of heparin, did not lead to a general beneficial effect for the cancer patients. Notably, patients with Trousseau syndrome have also shown superiority for heparin treatment over treatment with vitamin K inhibitors. A recent thorough review (16) of heparin’s effect on cancer in experimental metastasis models concluded that there is some beneficial effect in most of the mice studies, albeit the effects are not based solely on anticoagulant activity.

The observed reduction of metastasis in the absence of P- and/or L-selectin prompted studies in mouse models, where heparin was used as a selectin inhibitor (3, 4). A single dose of heparin intravenously injected before tumor cells led to a significant reduction of platelet-tumor cell aggregate formations as observed in the lung vasculature. Although the effect of heparin on platelet-tumor cell association lasted only for 6–8 h, a dramatic reduction in lung colonization by metastasis was observed 4–6 wk later (3, 4). The metastatic load after the heparin injection was similar in P-sel–/– mice and the wild-type mice injected with mucin-depleted tumor cells. No additional effects of heparin on metastasis could be observed in the P-sel–/– mice, suggesting that a single heparin treatment affected mostly platelet P-selectin. Conversely, a single injection of heparin in the L-sel–/– mice led to a further reduction of metastasis to the levels seen in the PL-sel–/–/ mice (4). The additional effect of heparin in L-sel–/– mice indicates that leukocytes promote metastasis following the early phase of tumor emboli formation facilitated by platelets. Thus the initial platelet’s P-selectin-mediated interaction with mucins on tumor cells is crucial for a metastatic progression and can be blocked by heparin.

Heparin, a clinical anticoagulant, is a large, complex molecule, only a specific portion of which has antithrombin-binding activity. Other biological activities are associated with distinct subsets of heparin glycosaminoglycans, which interact in vivo with growth factors, enzymes, and cell adhesion molecules, thus effecting angiogenesis or inhibiting tumor heparinase function. Although all of these mechanisms can apply to some or all cancer situations, during metastasis the initial platelet-tumor cell blockade seems to lie upstream of all other potential mechanisms affected by heparin. The evidence obtained from experimental models together with the meta-analysis of cancer patients strongly suggests that the effect of heparin on cancer and metastasis is not associated only with its anticoagulant activity (6, 16).

Conclusions and therapeutic implications

These findings propose a new connection between seemingly disparate observations of poor prognosis for the mucin-producing carcinomas and a possible beneficial effect of heparin on malignancy. Platelets adhere to the mucinous carcinomas in a P-selectin-dependent manner and facilitate tumor emboli formation. In addition, the platelet “coat” protects tumor cells from the innate immune system. Thus the benefit of the heparin treatment observed in some patients in the past could have been due to selectin blockade rather than mere anticoagulant activity. More importantly, heparin treatment can affect not only P-selectin but also L-selectin-mediated interactions (Fig. 2B). A possibility that heparin inhibit the probable later L-selectin interactions raises an important clinical question: when should patients treated with heparin? On the basis of the new paradigm of its action in metastasis, heparin therapy of human cancers should be reevaluated. Heparin could be effective for treatment of malignancies when tumor cells are still in circulation. It is also known that circulating tumor cells are extremely vulnerable to a variety of host-defense mechanisms and only a few of the malignant cells that enter the bloodstream eventually metastatize and lead to a fatal outcome. Thus a therapeutic intervention aimed at the elimination of potential metastatic cells by heparin represents a way to reduce the likelihood for malignancy.

However, several questions still remain open. Not all cancer types express selectin ligands; thus other mechanisms for metastasis to occur. Although most colon carcinoma cell lines and primary tumor specimens stained positively for selectin ligands, the extent of selectin ligand expression on other carcinomas remains to be determined. Heparin in its unfractionated form is known to carry several biological activities; therefore, the effect on selectin inhibition might not be the only mechanism of its antimetastatic action. Consequently, the use of LMW heparin with more defined biological activities would be preferable. Indeed, a trend toward treatment with LMW heparin in clinic can be observed. Since the two commercially available LMW heparins were previously found to be less effective in selectin inhibition measured in vitro (13), rigorous testing for the blocking activity would be necessary. The advantage of a higher in vivo bioavailability of LMW heparins as well as the easier management of this therapy might compensate for the lower selectin inhibitory activity.

For blocking of selectins, specific inhibitors would be required. But defining such specific P- and/or L-selectin inhibitors might take years before clinical accessibility was
achieved. On the other hand, meta-analysis of patients who were treated with LMW or unfractionated heparin for other reasons has shown a better survival outcome for the ones suffering from cancer and suggests a great potential for the use of heparin in cancer treatment (6). Recently, a draft for a clinical study on heparin treatment of cancer has been proposed (17). Patients with newly identified adenocarcinoma tumors would immediately start to receive heparin treatment until surgical removal and would continue to receive heparin for several days after surgery. This approach should prevent blood-borne metastasis while taking advantage of the abundant experience with heparin therapy collected over the years.

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References