Non-Anticoagulant Effects of Heparin in Carcinoma Metastasis and Trousseau's Syndrome

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Several lines of evidence indicate that a process of tumorigenesis consists of multiple steps, which reflect genetic alterations driving the transformation of normal cells into malignant derivatives. The characteristic of carcinoma malignancy is associated with enhanced migration, enhanced degradation of extracellular matrix, remodeling and/or alterations of integrins or cell adhesion molecules and finally, altered cell-surface glycosylation [1, 2]. Hematogenous metastasis of cancer cells is a cascade of events involving many factors which allow the 'fittest' tumor cells to intravasate into the bloodstream, to evade innate immune surveillance, to interact with blood cells and vascular endothelial cells of distant organs, and to extravasate into such tissues. Classic studies from 70 and 80's have shown that blood platelets interact with tumor cells leading to formation of tumor cell emboli. The formation of tumor microemboli with platelets and leukocytes has been proposed to be the mechanism by which tumor cells evade the host immune responses and thereby facilitates metastasis. Clear evidence for the involvement of platelets in metastasis was obtained in a mouse model where the experimentally reduced platelet counts led to attenuation of metastasis [3]. It has been shown that within minutes of intravenous injection are tumor cells arrested in capillary beds and 'coated' by platelets and fibrin [4, 5]. However, the molecular mechanism of platelet-tumor cell emboli formation has remained unclear.

Cancer progression is often accompanied with a high incidence of thrombotic events. In initial studies cancer patients were treated with heparin and some beneficial effect could be observed [6]. The antimetastatic effect of heparin could be seen also in experimental models [7]. Since it was suggested that the improvement in patient's survival rate was achieved by the anticoagulant activity of heparin, a switch to more easily manageable therapies with other anticoagulants was initiated. However, the trials with a vitamin K antagonist-Coumadin, which works by entirely different mechanism than heparin, could not show the beneficial antimetastatic effects as observed with the heparin. The beneficial effects of vitamin K inhibitor were observed only in a few cancer cases or in the studies where patients with deep vein thrombosis were treated with Coumadin [8].

In apparently unrelated studies, a strong association of carcinoma metastasis with an enhanced production of alternatively glycosylated mucins was observed. Epithelial cells are coated by mucins and they also secrete large amounts of soluble mucins to the apical surfaces in respective organs. During malignancy, carcinoma mucins contain altered glycosylation of the terminal structures with an accumulation of certain carcinoma specific epitopes. The cell surface glycans on epithelial cells are modified by addition of terminal saccharides like fucose and sialic acid. Many studies have shown that the elevated presence of sialylated and fucosylated structures on

surfaces of various human cancers correlates with a poorer prognosis for patients due to metastasis. Among a rather limited variety of terminal glycosylation observed in cancer, a presence of terminal tetrasaccharide sialyl Lewis^{X/A} is one of the most abundant and can be detected also in the sera of patients.

The selectins are adhesion molecules, which comprise three structurally related glycoproteins that are involved in the initial events of leukocyte adhesion. The lectin part and the epidermal growth factor-like domain of selectins are involved in recognizing sialylated and fucosylated structures like sialyl Lewis^{X/A}. In addition, L- and P-selectins require additional sulfate groups either on sialyl Lewis^{X/A} or in it's close proximity. In this regard, the commonly used anticoagulant drug heparin was shown to carry ligands for P- and L-selectin [9]. Furthermore, currently used clinical doses of heparin can efficiently block selectins [9]. Several studies with carcinoma cells have shown that selectins bind carcinoma cells in a mucin-dependent manner (e.g. 10). We and others have demonstrated selectin-based interaction among cancer cells and platelets (P-selectin), leukocytes (L-selectin) and endothelium (P- and/or E-selectin) in vitro and that most of them occur also in vivo. In addition, we have shown that purified carcinoma mucins can elicit selectin dependent interactions. In this respect it was interesting to note that patients with mucin-rich carcinomas often suffer from accompanying thromboembolism, suggesting that mucin might be actively involved in this process. A typical clinical manifestation by carcinoma malignancies is a disseminated intravascular coagulation (DIC) known as *Thrombophlebitis migrans* or Trousseau's syndrome, which is associated with platelet-rich microthrombi formation.

Based on the previous observations we tested the following hypothesis: (1) Selectins are involved in the cell-cell interactions mediated by selectins and can facilitate metastasis; (2) Heparin can effectively block selectin based interactions, thus lead to attenuation of metastasis; (3) Secreted carcinoma mucins act as templates for P-selectin dependent platelet aggregation as observed in microangiopathic presentation of Trousseau's syndrome.

We have shown, that the commonly observed platelet-tumor cell aggregation is P-selectin dependent in some tumor model systems. An attenuation of metastasis in P-selectin deficient mice was observed [11]. In addition, removal of carcinoma mucins from tumor cells prior to injection has also lead to attenuation of metastasis [5]. We tested whether a single dose of heparin, which blocks P-selectin mediated interactions, affects metastasis when injected prior to tumor cells. Although, the inhibition of platelet-tumor cell interactions with heparin lasted only for

several hours, significant attenuation of metastasis was observed [5]. Furthermore, we provide evidence that leukocytes actively participate in the metastatic progression in an L-selectin dependent manner. Attenuation of metastasis in the absence of L-selectin was detected. In contrast to a rapid P-selectin mediated platelet-tumor cell interactions, the involvement of L-selectin should be expected at the later timepoints. Due to almost complete reduction of metastasis in double P- and L-selectin deficient mice, the action of P- and L-selectin was shown to be synergistic. Indeed, a single injection of heparin prior to tumor cells in L-selectin deficient mice led to further attenuation of metastasis, indicating that the single early heparin injection primarily affects P-selectin platelet tumor cell interaction.

Next we tested whether carcinoma mucins can serve as templates for aggregation of activated platelets as observed in Trousseau's syndrome. We purified a clean preparation of human carcinoma mucin fragments, devoid of any procoagulant activity. Intravenous injection of these mucins led to aggregation of platelets in vivo. Surprisingly, platelet aggregation was not only P-selectin dependent but also L-selectin dependent, indicating a role of leukocytes in this process. We have shown that tissue factor activity is not required for a mucin to serve as a template for platelet aggregation. Rather an essential role of L-selectin action is required for the initial activation and aggregation of platelets. Further platelet aggregation occurred even in the setting of thrombin blockade by hirudin, and heparin could inhibit the aggregation via a mechanism independent of its anti-thrombin potentiating effect. Taken together, these indicate that activation of fluid-phase coagulation is not the proximal event leading to platelet aggregation based upon carcinoma mucin. Thus, the carcinoma mucins can generate thrombin-independent, P- and L-selectin dependent, heparin sensitive platelet rich microthrombi in mice (Trousseau's syndrome).

Conclusion and Perspectives

Based on the proposed paradigm, heparin can act as a functional inhibitor of selectins mediated cell-cell interactions leading to metastasis. Results from our studies are in an agreement with retrospective clinical observations, where heparin treatment (an effective inhibitor of P- and L-selectin mediated interactions) proved beneficial for cancer patients, while other inhibitors of fluid phase coagulations are often ineffective. Thus the use of heparin, an FDA approved drug, for treatment of cancer should be revisited. Finally, the use of low molecular weight heparin for treating cancer patients with the focus on selectin inhibition should be explored in the future.

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