The Role of Thomsen-Friedenreich Antigen in Adhesion of Human Breast and Prostate Cancer Cells to the Endothelium

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ABSTRACT

Interactions of metastatic cancer cells with vascular endothelium are critical during early stages of cancer metastasis. Understanding the molecular underpinnings of these interactions is essential for the development of new efficacious cancer therapies. Here we demonstrate that cancer-associated carbohydrate T antigen plays a leading role in docking breast and prostate cancer cells onto endothelium by specifically interacting with endothelium-expressed β-galactoside-binding protein, galectin-3. Importantly, T antigen-bearing glycoproteins are also capable of mobilizing galectin-3 to the surface of endothelial cells, thus priming them for harboring metastatic cancer cells. The T antigen-mediated, tumor-endothelial cell interactions could be efficiently disrupted using synthetic compounds either mimicking or masking this carbohydrate structure. High efficiency of T antigen-mimicking and T antigen-masking inhibitors of tumor cell adhesion warrants their further development into antiadhesive cancer therapeutics.

INTRODUCTION

Metastasis is a major fatal complication associated with human malignant disorders. Recent findings demonstrate that hematogenous cancer metastases originate from intravascular growth of endothelium-attached rather than extravasated cancer cells (1), highlighting the key role of tumor-endothelial cell interactions in cancer metastasis. Understanding the molecular mechanisms of these interactions is crucial for the development of new efficacious cancer therapies. A broad array of adhesion molecules, such as carbohydrates, lectins, cadherins, and integrins, have been implicated in the adhesion of tumor cells to the vasculatory endothelium. This reflects a complexity of the adhesion process. Moreover, different adhesion molecules have been shown to participate at distinct stages in a multistep binding process (2). For example, selectins were shown to contribute to the initial contact of circulating cells with endothelium by inducing their rolling (3), whereas galectin-3 has been proposed to participate in docking of cancer cells on capillary endothelium (4), and integrins were demonstrated to play a role in the development of more stable attachment involving protein-protein interactions (3).

Among all of the variety of adhesion molecules, cell surface carbohydrate structures have been a focus of many investigative efforts into their roles in cancer cell adhesion and metastasis. Aberrations in cell surface carbohydrates, emblematic of malignant transformation, have been shown to facilitate tumor cell colonization and metastasis by effecting normal cell-cell interactions (5, 6). One of the most widely distributed cancer-associated cell surface carbohydrate moieties is the pancarcinoma T antigen (7). T antigen is a simple mucin-type disaccharide (8), βGalβ1–3GalNAc, expressed on the outer cell surfaces of T-cell lymphomas and most human carcinomas (7–9), including breast and prostate (10). The role for T antigen in tumor cell adhesion and metastasis has been proposed based on the existence of T antigen-mediated adhesion of highly metastatic murine lymphoma cells and hepatocytes (9). Recently, we demonstrated the participation of T antigen in human breast carcinoma cell adhesion to the endothelium (11). We also reported that a 15-amino acid T antigen-specific peptide, P-30 (HGRFILPWWYAFSPS), selected from a bacteriophage display library (12, 13), specifically and significantly (>50%) inhibited adhesion of human breast cancer cells to the endothelium (11). These results underscored the importance of T antigen-mediated interactions in breast cancer metastasis. However, the molecular mechanisms of T antigen-mediated adhesion as well as cognate physiological receptors for T antigen have not been identified.

On the basis of the fact that the terminal residue of T antigen is β-galactose, we investigated its potential interactions with galectin-3 (11), a M, ~30,000 member of a family of soluble β-galactoside-specific lectins (14, 15). Although the physiological role of this carbohydrate-binding protein is still greatly debated, galectin-3 has been implicated in several distinct fundamental cellular processes such as pre-mRNA splicing (16), cell growth and differentiation (17), regulation of apoptosis (18, 19), and cell-cell recognition and adhesion (19–21). In several experimental systems, the expression of galectin-3 in cancer cells was associated with increased malignant and metastatic phenotype (22, 23). Moreover, preferential adhesion of PC-3 human prostate cancer cells to bone marrow endothelial cells was found to be at least in part galectin-3-dependent (4). Recently, we demonstrated the involvement of the endothelium-expressed galectin-3 in breast carcinoma-endothelial cell adhesion (11) supportive of a possible interaction of this β-galactoside-binding lectin with T antigen.

We hypothesized that if T antigen is indeed interacting with galectin-3 during tumor cell docking onto the endothelium, it could be a common molecular mechanism of cancer cell adhesion pertinent to a metastatic dissemination of a variety of T antigen-expressing human malignancies. Carbohydrate-lectin interactions are believed to take place during an initial reversible phase of cell adhesion that determines cell-cell recognition specificity (24, 25). The efficient specific blockage of these early binding events may significantly modify the outcome of the adhesion and affect the metastatic process in whole.

In this study, we demonstrate that two synthetic inhibitors of T antigen-mediated adhesion, the T antigen-binding P-30 peptide, and a sugar-based T antigen mimetic, lactulosyl-l-leucine, efficiently (43–56%) inhibit the adhesion of breast and prostate carcinoma cells to
HUVEC monolayer. Both compounds exhibit an even more pronounced effect on cancer cell adhesion to human bone marrow microvascular endothelium (74–82% and 69–70% inhibition for breast and prostate carcinoma cells, respectively). These results strongly suggest possible involvement of T antigen-mediated adhesive interactions in breast and prostate cancer metastatic bone disease. Both T antigen-masking P-30 and T antigen-mimicking lactulosyl-l-leucine display the same maximal inhibitory effect on adhesion of breast and prostate cancer cells as a highly specific anti-T antigen monoclonal antibody does. The results of inhibition ELISA experiments confirm the specificity of both synthetic inhibitors and demonstrate direct binding of the recombinant human galectin-3 to the protein-linked T antigen. Remarkably, endothelial cells exhibit rapid and marked increase in cell surface galectin-3 expression when treated with T antigen-bearing glycoproteins. This observation suggests a novel function for circulating T antigen-expressing glycoproteins such as cancer-associated mucin, which may act by priming capillary endothelium for harboring cancer cells.

The results presented in this paper define T antigen as one of the leading factors during early stages of breast and prostate cancer-endothelial cell interactions. We demonstrate that T antigen is acting both as a major cell surface carbohydrate ligand for galectin-3 on breast and prostate cancer cells and as a factor causing mobilization of galectin-3 to the outer membrane on endothelial cells. The significance of T antigen-mediated adhesion in breast and prostate cancer identifies T antigen-galectin-3 interactions as a valid target for development of new antiadhesive therapies of cancer metastases.

MATERIALS AND METHODS

Antibodies, Chemicals, and Reagents. The monoclonal anti-T antigen antibody was developed as described (26). The TIB-166 hybridoma, producing rat monoclonal anti-galectin-3 (anti-Mac-2) was purchased from American Type Culture Collection (Rockville, MD). The monoclonal antibody to irrelevant plant protein was kindly provided by the Cell and Immunobiology Core (University of Missouri, Columbia, MO). The Escherichia coli strains, expressing human galectin-3 from pet5 vector, were kindly provided by Dr. A. Raz (Wayne State University, Detroit, MI) and Dr. H. Leffler (Lund University, Lund, Sweden). Recombinant human galectin-3 was isolated and affinity purified as described (27). DiI was from Molecular Probes (Eugene, OR). Biotinylated T-PAA was from Glycotech Corp. (Rockville, MD). T-HSA was from Dextra Laboratories (Reading, United Kingdom). T antigen binding peptide P-30 (HGRFILPWYYSFPS) and control peptide (RRLL-FYKYVYKKRYKQRG) were chemically synthesized using FMOC-based chemistry and purified to homogeneity on a C-18 reverse-phase HPLC column (ISCO Corp.). All other chemicals and reagents, unless otherwise specified, were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Lines and Cultures. The MDA-MB-435 human breast carcinoma cell line was kindly provided by Dr. J. Price (M. D. Anderson Cancer Center, Houston, TX). The DU-145 human prostate carcinoma cells were obtained from American Type Culture Collection (Rockville, MD). B16-F10, a highly metastatic variant of B16-F1 mouse melanoma, was kindly provided by Dr. I. Razky (Wayne State University, Detroit, MI) and Dr. H. Leffler (Lund University, Lund, Sweden). B16-F10, a highly metastatic variant of B16-F1 mouse melanoma, was kindly provided by Dr. I. Razky (Wayne State University, Detroit, MI) and Dr. H. Leffler (Lund University, Lund, Sweden). Recombinant human galectin-3 was isolated and affinity purified as described (27). DiI was from Molecular Probes (Eugene, OR). Biotinylated T-PAA was from Glycotech Corp. (Rockville, MD). T-HSA was from Dextra Laboratories (Reading, United Kingdom). T antigen binding peptide P-30 (HGRFILPWYYSFPS) and control peptide (RRLL-FYKYVYKKRYKQRG) were chemically synthesized using FMOC-based chemistry and purified to homogeneity on a C-18 reverse-phase HPLC column (ISCO Corp.). All other chemicals and reagents, unless otherwise specified, were purchased from Sigma Chemical Co. (St. Louis, MO).

To evaluate cell surface galectin-3 expression, live, nonfixed, and nonpermeabilized endothelial cells, grown to confluence directly on microscope slides, were incubated for 45 min at 37°C in complete medium supplemented
with rat anti-galectin-3 antibody without (control) or with 1 mg/ml (final concentration) of bovine fetuin or ASF, or 0.5 mg/ml (final concentration) of HSA or T-HSA. Slides were washed three times with PBS and incubated for 1 h with goat Texas Red-conjugated antirat antibody. After additional washes, the samples were mounted under coverglass and analyzed on a Bio-Rad MRC 600 confocal system using accumulation of four scans. The four-well slides (NalgeNunc) were used, which allowed simultaneous processing of samples to be compared. The exact same conditions, such as iris settings, laser intensity, gain, magnification, and number of scan accumulations, were used for all samples.

RESULTS AND DISCUSSION

T Antigen Expression. The expression of T antigen in breast cancer tissues is well documented and is associated with tumor progression and metastasis (29). However, this carbohydrate structure is not specific only to breast cancer. Rather, it is characteristic of a vast majority of human adenocarcinomas. Similarly, T antigen is often detectable in prostate cancer lesions (Fig. 1A). Furthermore, in patients with prostate carcinoma, the expression of T antigen has also been found to correlate with tumor grade and metastasis (10, 30). Both breast (MDA-MB-435) and prostate (DU-145) cancer cell lines used in this study express T antigen on their surfaces. We demonstrated previously the presence of T antigen on MDA-MB-435 human breast carcinoma cells (13). This cell line, originally isolated from the pleural effusion of a patient with breast cancer, has been shown to be highly metastatic in nude mice (31, 32). The fact that adhesion of MDA-MB-435 cells to the endothelium could be efficiently (2-fold) inhibited by the T antigen-binding P-30 peptide (11) is indicative of the importance of T antigen in breast cancer-endothelial cell interactions. The DU-145 human prostate carcinoma cell line, chosen for our experiments, was also originally isolated from a metastatic lesion (33). Importantly, this cell line retains its metastatic potential, as reflected in the ability of DU-145 cells to develop metastasis in nude mice (34). In this study, we show that similar to MDA-MB-435 metastatic breast cancer cells, the DU-145 human prostate carcinoma cells also express T antigen on their surfaces (Fig. 1B). This observation suggests that T antigen might likewise participate in prostate cancer cell adhesion to the endothelium. To address this question, we investigated whether compounds, capable of specifically masking T antigen, would interfere with prostate cancer-endothelial cell interactions.

Inhibition of Cancer Cell Adhesion to HUVECs by Masking T Antigen. The T antigen-specific P-30 peptide (HGRFILPWWYAF-SPS) was originally isolated from a bacteriophage display library (12). The synthetic P-30 binds with high affinity and specificity to both free T antigen disaccharide in solution and T antigen-bearing glycoproteins (13). It is also capable of specifically recognizing T antigen-expressing cancer cells of different origin (12, 13) and efficiently inhibiting ASF-mediated cancer cell aggregation (13) and breast carcinoma cell adhesion to the endothelium (11). Our previous results suggest that the peptide is masking T antigen epitopes on cancer cells, thus preventing interactions with their cognate ligands. In this study, we used the T antigen-masking P-30 peptide to investigate whether T antigen participates in prostate cancer cell adhesion to the endothelium. We studied the effect of different concentrations (0–0.1 mg/ml) of synthetic T antigen-specific P-30 on adhesion of DU-145 human prostate carcinoma cells to a monolayer of HUVECs. The results of these experiments (Fig. 2, A–H) showed that P-30 inhibited the adhesion of both Dil-labeled (Fig. 2A) and acridine orange-labeled (Fig. 2, B–G) DU-145 cells to endothelial cells in a dose-dependent manner, whereas the control peptide (RRLLFYKYVKYRAG-KQBG) failed to inhibit adhesion (Fig. 2H). To assess the efficiency of T antigen masking by P-30 peptide, we compared the maximal inhibitory effects on breast and prostate cancer cell binding to the
endothelium, achievable with P-30 and an anti-T antigen antibody. A monoclonal antibody to irrelevant plant protein was used as a control in these experiments. We found that the control antibody did not inhibit attachment of tumor cells to HUVECs (Fig. 2, I and J), whereas both anti-T antigen antibody and P-30 exhibited almost identical maximal inhibitory effect on breast (Fig. 2F) and prostate (Fig. 2J) cancer cell adhesion. The results of these experiments demonstrated that synthetic P-30 peptide was masking T antigen as efficiently as the highly specific monoclonal anti-T antigen antibody. However, neither P-30 nor anti-T antigen antibody inhibited tumor cell adhesion to the endothelium completely. The inhibition efficiency (ranging from 43 to 56% in different experiments) displayed by these two compounds most likely reflected the impact of T antigen-mediated interactions in our experimental system. Apparently, different adhesion molecules such as integrins might also contribute to the binding. It has been suggested that circulating metastatic cells interact with endothelium in two distinct stages (24): the initial reversible docking stage, mediated by carbohydrate-lectin interactions; and the second stabilizing integrin-mediated locking stage, which requires more prolonged cell contact (25). In our adhesion experiments, cancer and endothelial cells were in physical contact for 1 h, allowing sufficient time for integrin-mediated binding events to occur. Supportive of this suggestion are results reported by Lehr and Pienta (4) using a similar experimental design. They demonstrated that antibodies to different members of the integrin family, such as CD11a, CD18, and leukocyte function antigen-1, inhibited adhesion of PC-3 prostate cancer cells to endothelium from 20 to 55%, suggesting the importance of integrins in stabilizing adhesion of tumor cells docked onto the endothelium. In contrast, interactions between T antigen and the corresponding carbohydrate-binding lectin(s) preceding integrin-mediated adhesion most likely are crucial during an initial docking stage. Supportive of this idea are the results of experiments using in-flow experimental systems,4 showing that under conditions of flow, anti-integrin antibodies do not affect the adhesion of MDA-MB-435 breast carcinoma cells to the endothelium, whereas the synthetic T antigen mimetic lactulosyl-L-leucine inhibits it up to 80%. These results imply that T antigen, but not integrin-mediated interactions, plays a significant role in initiating cancer cell binding to the endothelium.

Inhibition of Cancer Cell Adhesion to HUVECs by Mimicking T Antigen. An independent approach to the study of T antigen-mediated adhesion would be to use compounds capable of mimicking T antigen. One such compound is a synthetic carbohydrate-amino acid conjugate (glycoamine), lactulosyl-L-leucine. Lactulosyl-L-leucine is a T antigen. One such compound is a synthetic carbohydrate-amino acid conjugate (glycoamine), lactulosyl-L-leucine. Lactulosyl-L-leucine is a T antigen mimetic lactulosyl-L-leucine inhibits it up to 80%. These results imply that T antigen, but not integrin-mediated interactions, plays a significant role in initiating cancer cell binding to the endothelium. Inhibition of Cancer Cell Adhesion to HUVECs by Mimicking T Antigen. An independent approach to the study of T antigen-mediated adhesion would be to use compounds capable of mimicking T antigen. One such compound is a synthetic carbohydrate-amino acid conjugate (glycoamine), lactulosyl-L-leucine. Lactulosyl-L-leucine is a T antigen mimetic lactulosyl-L-leucine inhibits it up to 80%. These results imply that T antigen, but not integrin-mediated interactions, plays a significant role in initiating cancer cell binding to the endothelium.

\[ \text{T antigen-specific PNA lectin to ASF (Fig. 3D, iv and v). Interestingly, the substitution of lactulosyl for lactitol completely abolishes the ability of the resulting compound, lactitol-l-leucine, to both mimic T antigen (Fig. 3D, vi) and inhibit tumor cell adhesion to the endothelium (Fig. 3G). These results demonstrate that the antiadhesive properties of lactulosyl-l-leucine strictly depend on the carbohydrate moiety and aptitude of the compound to mimic T antigen. Importantly, this T antigen-mimicking compound was already shown to inhibit up to 75% both the incidence and number of spontaneous breast cancer lung metastases in nude mice experiments (36). This finding highlights the critical role of T antigen-mediated interactions in the metastatic process. In patients, however, both breast and prostate tumors most often metastasize to bone (37). Therefore, it will be very important to investigate whether T antigen is relevant to breast and prostate cancer metastatic bone disease.} \]

\[ \text{Inhibition of Breast and Prostate Cancer Cell Adhesion to Human Bone Marrow Microvascular Endothelium. The results of postmortem examination demonstrate that ~70% of patients who die from carcinomas of the breast and prostate have evidence of bone metastases (37). Lately, it was suggested that specific tumor cell adhesion interactions with bone marrow endothelium could be an important factor in the predilection of prostate cancer metastases to the skeleton (4, 38). This hypothesis is strongly supported by the results presented recently by Lehr and Pienta (4). They demonstrated that prostate cancer cells adhere better to the bone marrow-derived endothelium than to the endothelium from other anatomical sites.} \]

\[ \text{To further delineate the role of T antigen in breast and prostate cancer metastasis, we investigated whether T antigen participates in adhesion of MDA-MB-435 breast carcinoma and DU-145 prostate carcinoma cells to the monolayer of HBMEC-60 human bone marrow} \]
endothelial cells. The results of these experiments (Fig. 4) show that both T antigen-masking (P-30 peptide and anti-T antigen antibody) and T antigen-mimicking (lactulosyl-L-leucine) compounds inhibit cancer cell adhesion to bone marrow endothelium even greater than to HUVECs (74–82% and 69–70% inhibition for breast and prostate carcinoma cells, respectively). These data indicate that T antigen-mediated interactions may play an important role in tumor cell adhesion to bone marrow microvasculature; however, the molecular basis of these interactions is still poorly understood. The identification of the molecules acting as T antigen receptors in cancer-endothelial cell adhesion will be of utmost interest.

Interaction of T Antigen with Galectin-3. Because the terminal sugar of T antigen is β-galactose, the involvement of β-galactoside-binding lectins (galectins) in T antigen-mediated adhesion was investigated. Two of nine currently known galectins, i.e., galectin-1 and galectin-3, were found previously to be most prominently expressed in cancer and endothelial cells and were implicated in cancer cell adhesion (39–41). On the basis of the fact that human galectin-3 exhibits 200-fold higher specific activity toward Galβ1–3GalNAc disaccharide than galectin-1 (14), we proposed previously the interaction of T antigen with galectin-3 during cancer cell adhesion to the endothelium (11). Supportive of this interaction is the observation reported by Bresalier et al. (42) that colon cancer mucin, a glycoprotein most often decorated with multiple T antigen epitopes, serves as a specific ligand for galectin-3. Moreover, they also demonstrated that fully glycosylated mucin binds >40-fold more galectin-3 than mucin from the cells in which oligosaccharide synthesis is blocked at the stage of addition of β3-linked galactose to the N-acetylgalactosamine (Tn antigen) to form T antigen. We reasoned that if T antigen is in fact interacting with galectin-3, then both T antigen-masking and T antigen-mimicking compounds should specifically inhibit this interaction. To test this hypothesis, we performed inhibition ELISA experiments in which T antigen conjugated to HSA was preabsorbed on plastic in 96-well plates and incubated with purified recombinant human galectin-3 in the presence of different concentrations of the P-30 peptide or lactulosyl-L-leucine. We found that both compounds inhibited galactin-3 binding to T antigen-HSA conjugate in a dose-dependent manner (Fig. 5). Although the effect of both compounds was saturable, neither one reached 100% inhibition. We attribute the remaining binding (~30%) to cooperative interactions characteristic of galectin-3 (17). To the best of our knowledge, this is the first observation that directly demonstrated galectin-3 binding to protein-linked T antigen. These data also confirm the specificity of both inhibitors tested, T antigen-masking P-30 peptide and T antigen-mimicking lactulosyl-L-leucine.

The role of galectin-3 in prostate cancer cell adhesion was challenged recently, however, by the results reported by Ellerhorst et al. (2). They demonstrated that galectin-3 is not present on the cell surface in any of the four prostate cancer lines they tested, including DU-145, which was used in our experiments, and PC-3, which was shown by another group to bind to the endothelium in a galectin-3-dependent manner (4). To clarify this issue, we investigated cellular distribution of the galectin-3 during cancer-endothelial cell interaction.

Cellular Distribution of Galectin-3 during Cancer Cell Adhesion to the Endothelium. Laser scanning confocal microscopy was used to study the localization of the galectin-3 in both cancer and endothelial cells upon their interaction. This technique allowed us to analyze the orthogonal plans (X-Z and Y-Z sections), which included both cancer and endothelial cells, as well as the sites of their contact. The results of these experiments (Fig. 6, A and B) revealed remarkably similar patterns of galectin-3 distribution during DU-145 prostate carcinoma (Fig. 6A) and MDA-MB-435 breast carcinoma (Fig. 6B) cell adhesion to HUVECs. In both cancer cell lines, galectin-3, although expressed at higher levels than in endothelial cells, remained predominantly intracellular. This result is in agreement with observations of Ellerhorst et al. (2) and suggests that galectin-3 of cancer cells does not actively participate in their adhesion to the endothelium. In contrast, on endothelial cells we observed the mobilization and clustering of this carbohydrate-binding protein toward the cancer-endothelial cell contacts. This phenomenon is consistent with the interaction of endothelium-expressed galectin-3 with T antigen displayed on the surface of cancer cells and is indicative of its active role in cancer-endothelial cell adhesion. These results also provide a feasible
Thus, we decided to investigate whether T antigen was a factor in the changes in galectin-3 surface expression on endothelial cells after treatment with T antigen-bearing glycoproteins, ASF and T-HSA. Both ASF and T-HSA display multiple immunoactive T antigen epitopes. Two other proteins, bovine fetuin and HSA were used as corresponding negative controls in these experiments. Bovine fetuin is identical to ASF and also contains multiple T antigen antennas, which are, however, covalently masked with sialic (neuraminic) acid and nonimmunoactive. HSA does not contain T antigen in any form. These experiments revealed that treatment with either ASF (Fig. 6C) or T-HSA (Fig. 6D) resulted in a rapid and significant increase in galectin-3 expression on a surface of endothelial cells, whereas neither fetuin nor HSA effected this expression. These results demonstrate that T antigen is acting not only as a ligand for galectin-3 but also as a factor causing the mobilization of this carbohydrate-binding protein to the cell surface. The latter observation in turn suggests an important novel function for the circulating cancer mucin, which is often found in the serum of patients with adenocarcinomas of different origin (42, 43). Circulating cancer mucin, similar to ASF and T-HSA, bears multiple T antigen moieties and may likewise induce the increase in galectin-3 surface expression on endothelial cells, thus priming vascular endothelium for binding the circulating metastatic tumor cells. Importantly, this phenomenon could be observed when highly metastatic T antigen-expressing MDA-MB-435 cells interact with microvascular endothelium under conditions of flow. In contrast, the MDA-MB-468 cells, which are deficient in T antigen expression and are nonmetastatic, do not induce galectin-3 surface mobilization. This observation implies that T antigen-expressing circulating metastatic cancer cells could themselves change endothelium adhesiveness.

The fact that T antigen can actually modify the adhesive properties of the endothelium by mobilizing cell surface adhesion molecules such as galectin-3 adds new and important insights into our understanding of tumor-endothelial cell interactions. It suggests the existence of an additional priming stage in adhesion of circulating metastatic static cells to the endothelium, which precedes the docking (initial reversible adhesion) and locking (permanent irreversible adhesion) stages (Fig. 7).

The results presented in this report identify T antigen as a major cancer cell surface carbohydrate ligand for galectin-3. Although both the former and the latter may also interact with other cognate ligands, the T antigen-galectin-3 interactions are likely to play a leading role in the initial stages of cancer cell adhesion to the endothelium. The efficient intervention with cancer cell endothelium interactions could result in the development of new antiadhesive cancer therapies (reviewed in Ref. 44), the concepts of which were brought about by early pioneering works of R. Kerbel et al. (45, 46) and Inohara and Raz et al. (45, 46) and Inohara and Raz.

**Fig. 6.** A and B, pseudocolored X-Z sections showing cellular distribution of galectin-3 upon adhesion of DU-145 prostate (A) and MDA-MB-435 breast (B) cancer cells to the endothelium. Different colors represent different concentrations of galectin-3 (purple, blue, green, yellow, red, and white, from lowest to highest). Note the accumulation and clustering of galectin-3 toward cell contacts on endothelial cells indicated by arrows, whereas in cancer cells the protein remains predominantly intracellular. C and D, the effect of ASF and T-HSA on galectin-3 surface expression on endothelial cells. Note the increase in galectin-3 surface representation upon treatment with ASF (C) or T-HSA (D) but not with fetuin or HSA, compared with control. In both C and D, the top panel shows a corresponding transmitted light photomicrograph of the same field.

**Fig. 7.** Schematic representation of T antigen participation in adhesion of metastatic cancer cells to the vasculatory endothelium. Circulating T antigen-bearing cancer-associated glycoproteins and metastatic tumor cells induce the mobilization of the galectin-3 to the surface of endothelial cells (priming stage). T antigen-expressing malignant cells bind to the endothelium through T antigen-galectin-3 interactions (Docking) allowing sufficient time for the stabilizing integrin-mediated adhesion events to take place (Locking stage).
The significant inhibition of breast and prostate cancer cell adhesion to the endothelium by T antigen masking and T antigen mimicking compounds suggests their high potential to be developed into efficacious anti metastatic agents.

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REFERENCES

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