Glycoconjugate Journal (1997) **14:** 577–584

Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer

Reiji Kannagi

Molecular Pathology, Aichi Cancer Center, 1-1 Kanokoden, Nagoya, 464, Japan

The carbohydrate determinants, sialyl Lewis A and sialyl Lewis X, which are frequently expressed on human cancer cells, serve as ligands for a cell adhesion molecule of the selectin family, E-selectin, which is expressed on vascular endothelial cells. These carbohydrate determinants are involved in the adhesion of cancer cells to vascular endothelium and thus contribute to hematogenous metastasis of cancer. The initial adhesion mediated by these molecules triggers activation of integrin molecules through the action of several cytokines and leads to the extravasation of cancer cells. Cancer cells also produce humoral factors that facilitate E-selectin expression on endothelial cells. The degree of expression of the carbohydrate ligands at the surface of cancer cells is well correlated with the frequency of hematogenous metastasis and prognostic outcome of patients with cancers. The alteration of glycosyltransferase activities that leads to the enhanced expression of these carbohydrate ligands on cancer cell surface are currently being investigated.

***Keywords*: Sialyl Lewis A, sialyl Lewis X, selectin, metastasis, cell adhesion, cancer**

Introduction

Hematogenous metastasis of cancer is a complicated process consisting of multiple steps. The process starts with the intravasation of cancer cells into the blood stream in the primary tumor lesion. The cancer cells then travel in the blood stream, where they interact with various blood cells, and finally they adhere to endothelial cells somewhere in the peripheral vessel walls. After extravasation, they enter the connective tissue and form a new metastatic lesion.

In the adhesion of cancer cells to the peripheral vessel walls, cancer cells having a higher affinity for endothelial cells have a higher chance of developing into a metastasis. This step is very important in cancer metastasis, and indeed sometimes appears rate limiting. Carbohydrate-mediated interactions are involved in the adhesion of cancer cells of endothelial cells.

E-selectin mediated adhesion of cancer cells to vascular endothelial cells in monolayer cell adhesion assay

When we started to look at the adhesion of cancer cells to endothelial cells in the monolayer cell adhesion assay system using human cancer cells in early 1990, what impressed us first was the very strong adhesion of human cancer cells to the IL-1β-treated endothelial cells, The number of cancer cells attached to the endothelial cells was almost similar to that observed with the adhesion of leukocytes. Upper panels of Figure 1 show typical examples of such cell adhesion experiments to illustrate how strongly cancer cells adhere to endothelial cells. In these experiments, cultured human colon or lung cancer cells were used as cancer cells, and human umbilical vein endothelial cells (HUVECs) for the endothelial cell monolayers.

IL-1β activated endothelial cells are known to express three major cell adhesion molecules, that is, ICAM-1, E-selectin and VCAM-1. We thought it highly probable that these molecules are involved in the adhesion of cancer cells to endothelial cells. So, we asked which of these three cell adhesion molecules is chiefly involved in the adhesion of cancer cells to endothelial cells, by conducting inhibition experiments using neutralizing monoclonal antibodies specific to these molecules [1]. As shown in the middle panels of Figure 1, the antibody against E-selectin (ELAM-1, endothelial-leukocyte adhesion molecule-1) strongly inhibited the adhesion of cancer cells, while other antibodies had no effect. This finding indicated that mainly E-selectin is involved in this cell adhesion.

Later this type of study was expanded to a wide variety of epithelial cancer cells, including those originating in the colon, stomach, pancreas, liver, lung and ovary [2]. Adhesion of most of the tested cancer cells was shown to be significantly inhibited by the anti-E-selectin antibody, confirming again that the E-selectin-carbohydrate interaction plays an important role in the adhesion of cancer cells to endothelial cells, We therefore concluded that E-selectin,

0282–0080

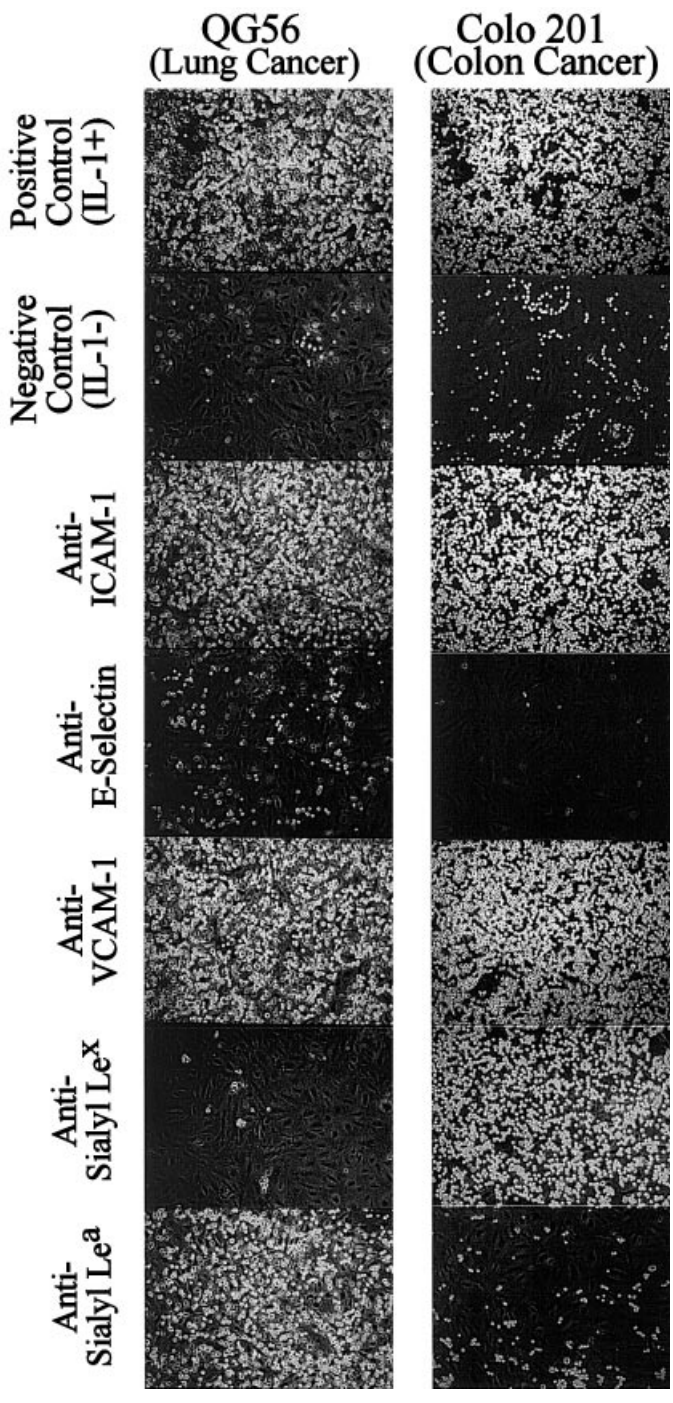

Figure 1. Monolayer cell adhesion assay using human cancer cells and typical inhibition patterns of the adhesion to recombinant IL-1β-activated HUVECs by treatment with anti-VCAM-1, anti-E-selectin, anti-ICAM-1, anti-sLe^x, and anti-sLe^a antibodies. HUVECs or cancer cells were treated with the respective antibodies (20 μg ml^{-1}) prior to the adhesion experiment for 30 min. Results for QG-56 (lung cancer), and Colo201 (colon cancer) cells are shown. Adhesion of both cell lines was inhibited with anti-E-selectin antibody, but not with anti-VCAM-1 and anti-ICAM-1 antibodies. Note that adhesion of QG-56 is inhibited by anti-sLe^x antibody, while that of Colo201 cells is inhibited by anti-sLe^a antibody. Adopted from reference [2].

but not ICAM-1 or VCAM-1, plays major roles in the adhesion of most cancer cells to endothelial cells.

Of course there were some exceptions. For instance, most leukemic cells adhered to endothelial cells *via* ICAM-1 and/or VCAM-1. Only a few leukemic cell lines were found to utilize E-selectin in their adhesion to endothelial cells. Adhesion of some neuroblastoma, melanoma, and other sarcoma cells was also frequently E-selectin-independent, their adherence to endothelial cells mainly being mediated by VCAM-1. However, the interaction of most of the typical epithelial cancer cells with endothelial cells was E-selectin dependent, and this has been repeatedly supported by work from other laboratories as well [3–5].

Sialyl Lewis A and sialyl Lewis X as ligands for E-selectin

E-selectin is expressed almost exclusively on endothelial cells, and the physiological significance of E-selectin was at that time thought to be in the recruitment of leukocytes to stimulated vascular endothelium in inflammation. The lectin-like domain of E-selectin is known to recognize sialyl Lewis X (sLe^x) expressed on leukocytes [6–8]. Sialyl Lewis X was expressed on some cancer cells that adhered to endothelial cells in the monolayer cell adhesion assay as described above. The sLe^x antigen expressed on such cancer cells may thus be involved in the process of hematogenous metastasis. However, there were a number of human cancer cells that did not express sLe^x but were still strongly adherent to E-selectin expressed on IL 1β-treated HUVECs.

Which carbohydrate ligand is involved in the adhesion is easily ascertained by the same type of cell adhesion inhibition experiments employing specific monoclonal antibodies against the carbohydrate determinants. Soon we and other investigators found that another well-known cancer-associated carbohydrate antigen, sialyl Lewis A (sLe^a), also serves as a ligand for E-selectin [1, 9, 10]. Findings in support of this include: (i) several cultured human colon cancer cell lines, for instance Colo201, exhibit clear E-selectin-dependent adhesion to recombinant IL-1β-activated HUVECs [1, 2, 9]; (ii) this adhesion is significantly inhibited by treatment with several anti-sLe^a, but not anti-sLe^x antibodies (typical examples are shown in lower panels of Figure 1) [1, 2, 9]; (iii) pretreatment of HUVECs with either purified sLe^x or sLe^a glycolipid results in nearly complete inhibition of adhesion [1]; and (iv) synthetic sLe^a-protein conjugates can bind to E-selectin [10, 11].

Distribution of sLe^a and sLe^x in human cancers

Adhesion of some human cancer cells to endothelial cells was found to be almost completely inhibited by the anti-sLe^x antibody, indicating an involvement of sLe^x in the adhesion process. This was the case for ovary, lung, liver and stomach cancer cells. Adhesion of some other human

cancer cells, including mostly cancers of the digestive oragans, such as the colon and pancreas, was not inhibited by anti-sLe^x antibody, but rather by anti-sLe^a antibody, indicating that the involved carbohydrate ligand here is sLe^a.

sLe^a and sLe^x have long been used as serum markers in the diagnosis of cancer in several countries including Japan, and many clinical reports are available on their statistical distribution and frequency in various human cancer tissues [12–16]. It is well known that the sLe^x is preferentially expressed on cancers of the lung, ovary, liver, kidney and breast. In these cases, we can expect sLe^x to play a major role in hematogenous metastasis. On the other hand, sLe^a is known to be much more dominantly expressed than sLe^x in colon, pancreas, and biliary tract cancers, and therefore we assume that sLe^a plays a major role in their hematogenous metastasis.

This distribution pattern of selectin ligand carbohydrates among human cancers has also been confirmed by other investigators. Dejana *et al.* reported adherence of a cultured colon cancer cell line to endothelial cells *via* sLe^a and Iwai *et al.* [17] showed that several pancreatic cancer cell lines adhere to endothelial cells through sLe^a. Leukocytes and leukemic cells usually do not express sLe^a, and their adhesion to endothelial cells is considered to be mediated by sLe^x [18–20]. sLe^a has so far been known to be involved only in the adhesion of epithelial cancer cells to the endothelium.

Expression of carbohydrate ligands on cancer cells and prognosis of patients

If the E-selectin-carbohydrate interaction truly plays a major role in hematogenous metastasis, patients with cancer cells that strongly express such carbohydrate ligands may be at greater risk of metastasis, and would therefore be expected to have a poor prognosis.

Some clinical statistics support this hypothesis [21–28]. In an early report describing the prognosis of patients with colon cancer, Kiriyama *et al.* reported that those patients with cancer tissues expressing sLe^a strongly or moderately had a significantly reduced postoperative survival when compared to patients with cancer cells that did not express or only weakly expressed sLe^a (upper panel of Figure 2). The statistics covered 88 patients at Nagoya University Hospital, and the difference was statistically significant at $p < 0.05$ [24, 25]. This was recently confirmed by more detailed studies by Shimono *et al.* (Kyushu University) and Nakayama *et al.* (Keio University), who reported a similar correlation observed in two larger series of patients with colon cancer ($n = 149$ and 309 respectively) with even greater statistical significance ($p < 0.01$ and $p < 0.001$, respectively, middle and lower panels of Figure 2) [26, 27]. Thus, a link between sLe^a expression and a poor prognosis has been confirmed in a total of more than 500 patients with colon cancer in Japan.

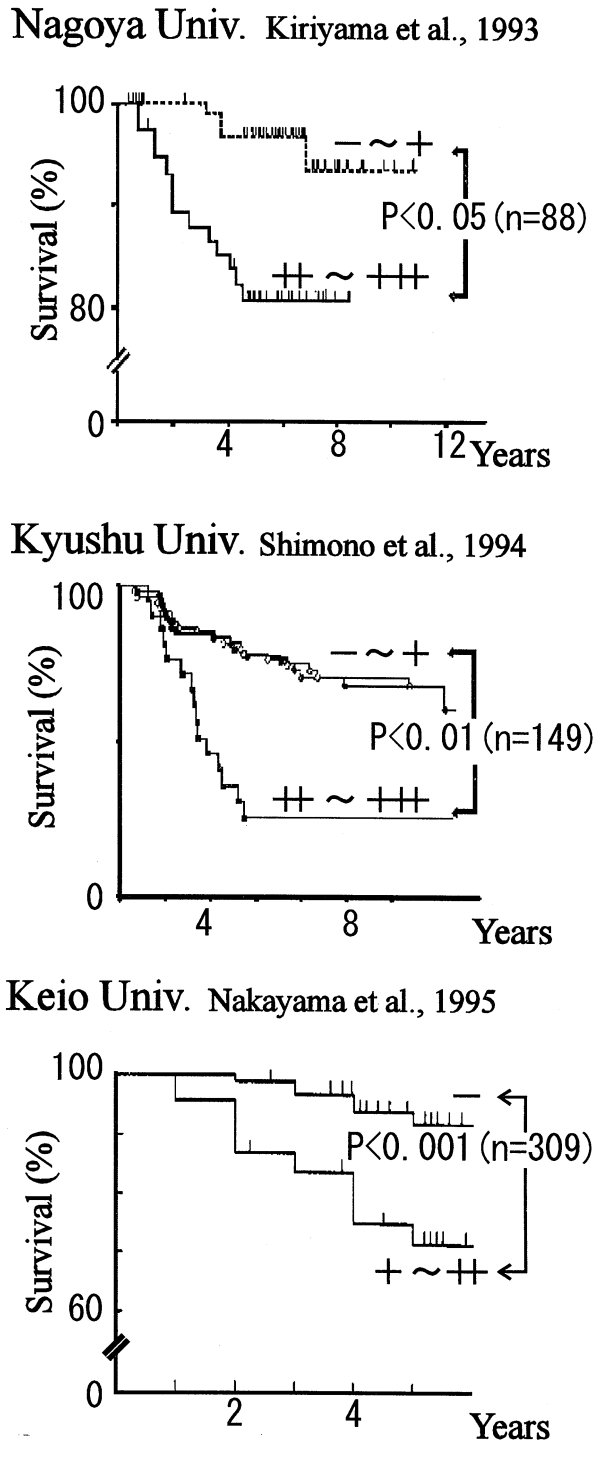

Figure 2. Correlation between the prognosis of patients with colon cancer, and the degree of expression of sLe^a determinant in cancer tissues. Adopted from references [24–27].

In the case of breast cancer, it is reported that the degree of expression of sLe^x, but not sLe^a, is correlated with patient prognosis [28]. This is consistent with the fact that breast cancer cells more frequently express sLe^x than sLe^a.

We expect that the prognosis of patients with colon, pancreas and biliary tract cancers would correlate with the degree of expression of sLe^a on their cancer tissues, while patient prognosis would be a function of sLe^x expression in the case of breast, ovary, lung, and liver cancers.

E-selection expression in the vasculature of patients with cancer

E-selectin is not always expressed on every vessel wall. Inflammatory cytokines such as IL-1β or TNF-α induce expression of E-selectin on human endothelial cells. Its expression is transient under *in vitro* conditions; it appears 4 h after cytokine stimulation of resting HUVECs, and disappears about 24 h later. TGF-β and IL-4 are known to be capable of inhibiting the induction of E-selectin expression. This suggests that the levels of E-selectin in patients could fluctuate.

This implies that the degree of E-selectin expression on the blood vessels might be another factor in the risk of development of metastasis, in addition to the strength of expression of the carbohydrate ligands on cancer cells. It might be of importance, therefore to evaluate the degree of E-selectin expression in the blood vessels of patients. The expression of E-selectin in a patient's body could be indirectly evaluated by estimating the serum E-selectin level. The results from this type of study indicate that some patients with cancers have normal levels of serum E-selectin, while some others have much higher levels [29]. Normal level of E-selectin expression on the vasculature of patients with cancer would eventually support the adhesion of cancer cells, while elevated expression of E-selectin would facilitate the adhesion of cancer cells to the endothelium.

Some cancer cells have an ability to induce expression of E-selectin on endothelial cells. The blood vessels near cancer nests frequently express E-selectin in immunohistochemical experiments [29], and many cultured human cancer cell lines produce humoral factors that induce the expression of E-selectin on endothelial cells, including IL-1α and another unknown factor that stimulated blood leukocytes to secrete IL-1β [30].

Cytokines and integrin molecular species involved in the second stage of adhesion

One of the characteristics of selectin-mediated cell adhesion is that it is quite resistant to shear-force. This is an important aspect when considering cell adhesion in the blood stream. Cell adhesion mediated by other molecules such as integrins is quite shear-force labile and is readily inhibited by appropriate rotation or stirring of the culture dishes, even in *in vitro* experiments. This led us to suggest that the first step in the adhesion of malignant cells in blood stream should be mediated by a more shear-force resistant cell adhesion system involving selectins rather than integrins. The integrins are able to participate in the secondary step of the cell adhesion process, when the tumour cells are already in contact with the vascular endothelial cells.

During the course of the initial adhesion of cancer cells to endothelia, cancer cells are stimulated by cytokines that are present on the surface of endothelial cells, and this leads to the activation of integrins on cancer cells. For instance, a prominent activation was observed with the $\alpha_2\beta_1$-integrin, when the human liver cancer cells, HepG2, were used in experiments [31]. This activation of the $\alpha_2\beta_1$-integrin was blocked by the addition of a neutralizing anti-HGF (hepatocyte growth factor) antibody to the culture medium, indicating that HGF on endothelial cells was one of the candidate cytokines that affected the integrin expression on the cancer cells. The addition of recombinant HGF to the culture of these cancer cells also produced a similar level of activation of $\alpha_2\beta_1$-integrin expression (Figure 3, left panel). The HepG2 cells expressing activated $\alpha_2\beta_1$-integrin had an increased adhesive activity to collagen-coated plates (Figure 3, right panel), and accelerated transmigratory activity through ECM gel in double-chamber chemoinvasion experiments [31].

Later we found that HB-EGF (heparin-binding epidermal growth factor) also activated integrin expression on other types of human cancer cells [32, 33]. In this case, the $\alpha_2\beta_1$- and $\alpha_3\beta_1$-integrins on human cultured breast or oesophageal cancer cells were activated by the addition of recombinant HB-EGF.

We predict that many other cytokines associated with human endothelial cells would have a similar activating effect on cancer cell integrins. The prerequisites for such a cytokine are: (i) They should be produced by the endothelial cells or at least adsorbed at the surface of endothelial cells, so that the cancer cells can be stimulated when they attach to the endothelial cells; (ii) the target cells of such cytokines should be epithelial cells (*ie* HGF, HB-EGF, KGF etc. would be better candidates than MIP-1β and

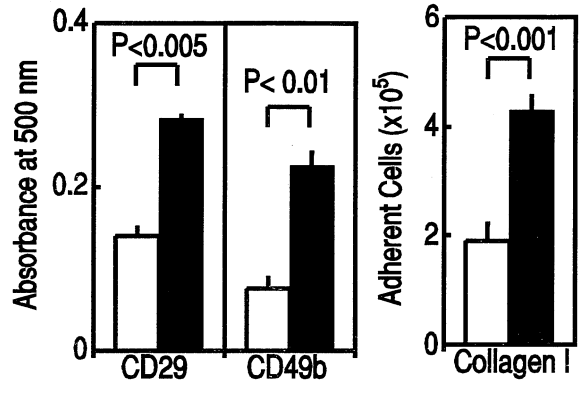

Figure 3. Increased expression of $\alpha_2\beta_1$-integrins (left panel) and accelerated adhesion to collagen (right panel) of HGF-treated human hepatocellular cancer HepG2 cells. ■, HGF(+); □, HGF(–). Adopted from reference [31].

other Rantes family cytokines), and (iii) the cancer cells should possess the functional receptor for the cytokines, which is the *c-met* product in the case of HGF, EGF-R in the case of HB-EGF, and K-*sam-II* in the case of KGF.

The HGF-mediated enhancement of integrin expression would be limited to the cancer cells that express *c-met* oncogene product. The *c-met* oncogene product is known to be expressed mainly on liver cancer cells and partly on gastric cancer cells. Similarly, the HB-EGF-mediated enhancement of integrin expression would be limited to the cancer cells that express EGF-receptor, which is frequently detectable on oesophageal cancer cells. This would indicate that the sets of cytokines and integrins involved in the second stage of cancer cell adhesion to endothelium is highly dependent on the lineage of cancer cells. This is in clear contrast to the molecules involved in the first step of cancer cell adhesion, where the carbohydrate ligands are expressed on a wide variety of cancer cells, and the selectin-mediated adhesion is commonly observed with epithelial cancer cells of various origins.

Concerted action of selectins and integrins in cancer metastasis

We postulate that there are two distinguishable steps in the process of cancer cell adhesion to endothelial monolayers, as schematically shown in Figure 4. The first step is the initial cell adhesion step mediated by selectin and its carbohydrate ligands. The second step is the process of implantation of cancer cells into the monolayer, which seems to be mediated mainly by β_1-integrins. The efficiency of the initial adhesion step seems to greatly affect the overall efficiency of cancer cell invasion to endothelial monolayers. This could well explain why the initial adhesion step mediated by selectin and its carbohydrate ligands appears to affect the total efficiency of hematogenous metastasis in clinical statistics. RGD peptides or other blocking agents for integrins would still be usefull in preventing hematogenous metastasis, since they block metastasis at the second step of cell adhesion. The tissue specificity of the hematogenous metastasis observed in some cancers such as breast and lung cancers can be partly explained by the molecular species of cytokines provided by endothelial cells and their receptors as well as integrin species expressed on particular cancer cells.

Possible contribution of P-selectin

The selectin family is composed of three members, E-, P- and L-selectins. E-selectin is known to be expressed on activated endothelial cells, and is assumed to play a principal role in the adhesion of cancer cells to the endothelium. P-selectin, first described in activated platelets, is known to be also expressed on endothelial cells. The role played by P-selectin is rather hard to evaluate in *in vitro* cell adhesion experiments using HUVECs, since HUVECs seem to lose P-selectin expression soon after isolation and initial passage. In the inflammation research area, however, good evidence exists indicating that the P-selectin on the endothelial cells is profoundly involved in the recruitment of leukocytes in various experimental models. Recombinant

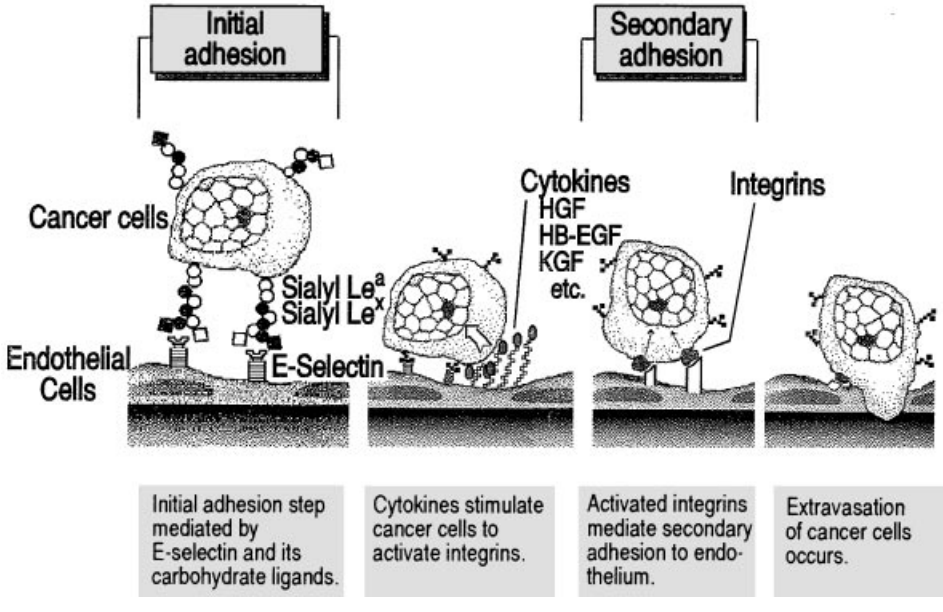

Figure 4. Schematic representation of the process of extravasation of cancer cells at vessel walls indicating involvement of cytokines and sequential activation of cell adhesion molecules.

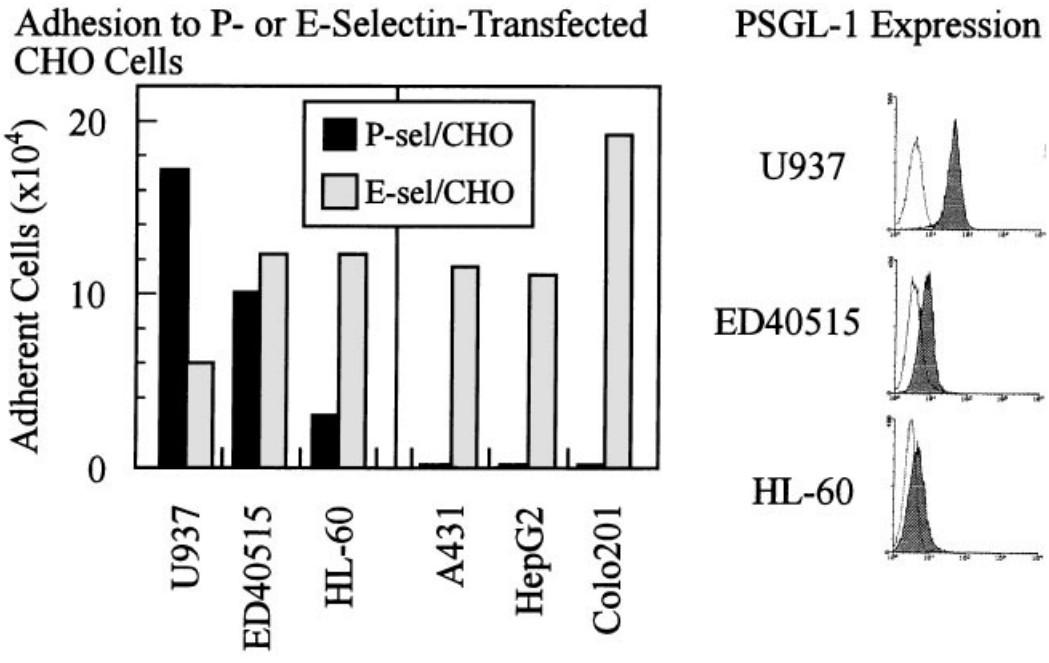

Figure 5. Comparison of E- and P-selectin-mediated adhesion of human cancer and leukemia cells and its relation to the PSGL-1 expression on the cell surface. Note that human leukemia cell lines (U937, ED40515 and HL-60) adhere to both E- and P-selectin-transfected CHO cells, while cancer cell lines (A431, HepG2 and Colo 201) adhere mainly to E-selectin-transfected CHO cells (left panel). These leukemia cell lines significantly expressed PSGL-1 (right panel), while cancer cell lines were negative for PSGL-1 (not shown).

P-selectin is known to bind various cancer cells more frequently and strongly than E-selectin, and this has been interpreted as evidence suggesting that P-selectin is more important than E-selectin in cancer metastasis [34].

P-selectin mediated adhesion of platelets to cancer cells in the blood stream would facilitate the implantation of the latter into the vessel wall. It has long been suggested that platelets play important roles in the hematogenous metastasis of cancer; with classical animal experiment models indicating that the frequency of metastatic foci is markedly reduced in platelet-poor animals. Platelets reportedly secrete an unknown soluble factor that induces E-selectin expression on endothelial cells, which would also facilitate metastasis [35].

However, there are some discrepancies regarding the importance of P-selectin in cancer metastasis. Recombinant P-selectin reportedly binds strongly to cancer cells [36], while a recent study [37] and our experiences (Figure 5) indicate that the cancer cells do not always adhere strongly to cells transfected with P-selectin. With regard to leukocytes, it is suggested that P-selectin specifically recognizes sLe^x presented by the PSGL-1, while E-selectin recognizes sLe^x and does not require PSGL-1 [38–41]. Most cancer cells fail to express PSGL-1 and do not strongly adhere to CHO cells expressing P-selectin. A recent finding that transfection of PSGL-1 cDNA to such cancer cells made them adherent to P-selectin-CHO cells [37] also indicates that cancer cells would need 'PSGL-1-like' molecules to adhere.

Glycosyltransferases involved in the accelerated expression of carbohydrate ligands for selectin on cancer cells

SLe^a and sLe^x are synthesized by the concerted action of several glycosyltransferases including sialyl- and fucosyltransferases. Hitherto, genes for five isoenzymes of human $\alpha 1 \rightarrow 3/4$ fucosyltransferases have been isolated [42–49]. Fuc-T III, first isolated from A431 epidermoid carcinoma cells [42], was shown to correspond to the Lewis type fucosyltransferase as has been characterized enzymologically. This enzyme is known to be capable of transferring fucose residues onto both type 1 and type 2 polylactosamine chains, thus contributing to the synthesis of both sLe^x and sLe^a determinants [42]. The Fuc-T IV, isolated from HL-60, is also known as ELFT (ELAM-1 ligand fucosyltransferase), and corresponds to the myeloid type enzyme [43, 44]. This isoenzyme is known to contribute to the synthesis of mainly Le^x and Le^y determinants [44]. Fuc-T V and VI have an ability to synthesize Le^x and sLe^x and the latter is proposed to correspond to the plasma type enzyme [45–47]. Recently cloned Fuc-T VII is unique in that this enzyme can synthesize only sLe^x and is distributed mainly on leukocytes and endothelial cells [48, 49]. Alteration of the message of these

five fucosyltransferases in human cancer tissues has not been well studied, except for several cultured cancer cell lines [50–52].

It has been shown that cancer cells expressing sLe^a on their surface express Fuc-T III mRNA unexceptionally [50]. Fuc-T III is the only fucosyltransferase that is capable of synthesizing sLe^a, and it must be involved in the synthetic process of sLe^a in cancer tissues. However, its mRNA level is not significantly increased in malignant tissues when compared to non-malignant tissues, for example in colorectal cancers; and cannot explain the accelerated synthesis of sLe^a in cancer tissues [53]. Even fucosyltransferase activity assayed enzymatically does not correlate with the expression of sLe^a determinants on the cell surface. Dohi *et al.* [54] studied $\alpha1 \rightarrow 3/4$ fucosyltransferase enzyme activities in gastrointestinal mucosa, and failed to detect any significant difference in the activities between malignant and non-malignant colorectal tissues.

On the other hand, Akamatsu *et al.* [55] compared $\alpha2 \rightarrow 3$ sialyltransferase and $\alpha1 \rightarrow 4$ fucosyltransferase activities in colorectal cancer tissues and adjacent non-malignant colonic epithelia by an enzymological technique, and concluded that the $\alpha2 \rightarrow 3$ sialyltransferase activity was significantly higher in cancer tissue than in non-malignant tissue ($p < 0.01$), but $\alpha1 \rightarrow 4$ fucosyltransferase activity showed no significant difference. Kemmner *et al.* [56] detected a significant increase of sialyltransferase enzyme activity in colon cancer tissues. Results of a more recent study [52] indicated the presence of a high level of ST-3O sialyltransferase message in two cultured human colon cancer cell lines, and suggested its relation to the increased synthesis of E-selectin ligand carbohydrates including sLe^a. Results of our recent study also indicated that the message of ST-3O, one of the sialyltransferase isoenzymes, was prominently increased in colorectal cancer as compared to non-malignant colonic epithelium among the five fucosyl- and three sialyltransferases tested [53]. These findings suggest, at least in colon cancer tissues, the increase in sialyltransferase activity rather than fucosyltransferase activity is involved in the enhanced expression of sLe^a. However, the sialyltransferase isoenzyme ST-3O lacked an ability to synthesize sLe^x, and could not explain the increased expression of sLe^x in colonic cancer tissues. Obviously further study is needed to elucidate the mechanism that leads to increased expression of carbohydrate ligands for selectin on cancer cells.

References

1 Takada A, Ohmori K, Takahashi N, Tsuyuoka K, Yago K, Zenita K, Hasegawa A, Kannagi R (1991) *Biochem Biophys Res Commun* **179**: 713–19.

2 Takada A, Ohmori K, Yoneda T, Tsuyuoka K, Hasegawa A, Kiso M, Kannagi R (1993) *Cancer Res* **53**: 354–61.

3 Majuri M-L, Mattila P, Renkonen R (1992) *Biochem Biophys Res Commun* **182**: 1376–82.

4 Merwin JR, Madri JA, Lynch M (1992) *Biochem Biophys Res Commun* **189**: 315–23.

5 Zaifert K, Cohen MC (1993) *Clin Immunol Immunopathol* **68**: 51–56.

6 Lowe JB, Stoolman LM, Nair RP, Larsen RD, Berhend TL, Marks RM (1990) *Cell* **63**: 475–84.

7 Phillips ML, Nudelman E, Gaeta FCA, Perez M, Singhal AK, Hakomori S, Paulson JC (1990) *Science* **250**: 1130–32.

8 Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B (1990) *Science* **250**: 1132–35.

9 Tyrrell D, James P, Rao N, Foxall C, Abbas S, Dasgupta F, Nashed M, Hasegawa A, Kiso M, Asa D, Kidd J, Brandley BK (1991) *Proc Natl Acad Sci USA* **88**: 10372–76.

10 Berg EL, Robinson MK, Mansson O, Butcher EC, Magnani JL (1991) *J Biol Chem* **266**: 14869–72.

11 Dejana E, Martin Padura I, Lauri D, Bernasconi S, Bani MR, Garofalo A, Giavazzi R, Magnani J, Mantovani A, Menard S (1992) *Lab Invest* **66**: 324–30.

12 Kannagi R, Kitahara A, Itai S, Zenita K, Shigeta K, Tachikawa T, Noda A, Hirano H, Abe M, Shin S, Fukushi Y, Hakomori S, Imura H (1988) *Cancer Res* **48**: 3856–63.

13 Itai S, Nishikata J, Yoneda T, Ohmori K, Tsunekawa S, Hiraiwa N, Yamabe H, Arii S, Tobe T, Kannagi R (1991) *Cancer* **67**: 1576–87.

14 Miyake M, Zenita K, Tanaka O, Okada Y, Kannagi R (1988) *Cancer Res* **48**: 715–58.

15 Zenita K, Kirihata Y, Kitahara A, Shigeta K, Higuchi K, Hirashima K, Murachi T, Miyaka M, Takeda T, Kannagi R (1988) *Int J Cancer* **41**: 344–49.

16 Kannagi R, Fukushi Y, Tachikawa T, Noda A, Shin S, Shigeta K, Hiraiwa N, Fukuda Y, Inamoto T, Hakomori S, Imura H (1986) *Cancer Res* **46**: 2619–26.

17 Iwai K, Ishikura H, Kaji M, Sugiura H, Ishizu A, Takahashi C, Kato H, Tanabe T, Yoshiki T (1993) *Int J Cancer* **54**: 972–77.

18 Ohmori K, Takada A, Ohwaki I, Takahashi N, Furukawa Y, Maeda M, Kiso M, Hasegawa A, Kannagi M, Kannagi R (1993) *Blood* **82(9)**: 2797–805.

19 Furukawa Y, Tara M, Ohmori K, Kannagi R (1994) *Cancer Res* **54**: 6533–38.

20 Kannagi R, Magnani JL (1995) in *Leukocyte Typing V* (Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder TF, Todd RF, eds) pp 1529–1531. Oxford: Oxford University Press.

21 Matsushita Y, Cleary KR, Ota DM, Hoff SD, Irimura T (1990) *Lab Invest* **63**: 780–91.

22 Shirahama T, Ikoma M, Muramastu T, Ohi Y (1992) *J Urol* **148**: 1319–22.

23 Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Kabuto T, Iwanaga T, Matsushita Y, Irimura T (1993) *Cancer Res* **53**: 3632–37.

24 Kiriyama K, Watanabe T, Sakamoto J, Ito K, Akiyama S, Yamauchi M, Takagi H (1991) *Nippon Geka Gakkai Zasshi* **92**: 320–30 (in Japanese).

25 Kiriyama K, Watanabe T (1994) in *Complex Carbohydrates* (Kannagi R, ed.) pp 67–76. Tokyo: Medical View Inc. (in Japanese).

26 Shimono R, Mori M, Akazawa K, Adachi Y, Sugimachi K (1994) *Am J Gastroenterol* **89**: 101–5.

27 Nakayama T, Watanabe M, Katsumata T, Teramoto T, Kitajima M (1995) *Cancer* **75**: 2051–56.
28 Narita T, Funahashi H, Satoh Y, Watanabe T, Sakamoto J, Takagi H (1993) *Cancer* **71**: 3044–53.
29 Ye C, Kiriyama K, Mitsuoka C, Kannagi R, Ito K, Watanabe T, Kondo K, Akiyama S, Takagi H (1995) *Int J Cancer* **61**: 455–60.
30 Narita T, Kawakami-Kimura N, Kasai Y, Hosono J, Nakashio T, Matsuura N, Sato M, Kannagi R (1996) *J Gastroenterol* **31**: 299–301.
31 Kawakami-Kimura N, Narita T, Ohmori K, Yoneda T, Matsumoto K, Nakamura T, Kannagi R (1996) *Br J Cancer* **75**: 47–53.
32 Sato M, Narita T, Kawakami-Kimura N, Higashiyama S, Taniguchi N, Akiyama S, Hashimoto T, Manabe T, Kannagi R (1996) *Cancer Lett* **102**: 183–191.
33 Narita T, Kawakami-Kimura N, Sato M, Matsuura N, Higashiyama S, Taniguchi N, Kannagi R (1996) *Oncology* (in press).
34 Aruffo A, Dietsch MT, Wan H, Hellstrom KE, Hellstrom I (1992) *Proc Natl Acad Sci USA* **89**: 2292–96.
35 Hakomori S (1994) *Biochem Biophys Res Commun* **203**: 1605–13.
36 Mannori G, Crottet P, Cecconi O, Hanasaki K, Aruffo A, Nelson RM, Varki A, Bevilacqua MP (1995) *Cancer Res* **55**: 4425–31.
37 Handa K, White T, Ito K, Fang H, Wang S-S, Hakomori S-I (1995) *Int J Oncol* **6**: 773–81.
38 Moore KL, Eaton SF, Lyons DE, Lichenstein HS, Cummings RD, McEver RP (1994) *J Biol Chem* **269**: 23318–27.
39 Asa D, Raycroft L, Ma L, Aeed PA, Kaytes PS, Elhammer AP, Geng J-G (1995) *J Biol Chem* **270**: 11662–70.
40 Moore KL, Patel KD, Bruehl RE, Fugang L, Johnson DA, Lichenstein HS, Cummings RD, Bainton DF, McEver RP (1995) *J Cell Biol* **128**: 661–71.
41 Ma L, Raycroft L, Asa D, Anderson DC, Geng J-G (1994) *J Biol Chem* **269**: 27739–46.
42 Kukowska-Latallo JF, Larsen RD, Nair RP, Lowe JB (1990) *Genes Devel* **4**, 1288–303.
43 Goelz SE, Hession C, Goff D, Griffiths B, Tizard R, Newman B, Chi Rosso G, Lobb R (1990) *Cell* **63**: 1349–56.
44 Lowe JB, Kukowska-Latallo JF, Nair RP, Larsen RD, Marks RM, Macher BA, Kelly RJ, Ernst LK (1991) *J Biol Chem* **266**: 17467–77.
45 Koszdin KL, Bowen BR (1992) *Biochem Biophys Res Commun* **187**: 152–57.
46 Weston BW, Nair RP, Larsen RD, Lowe JB (1992) *J Biol Chem* **267**: 4152–60.
47 Weston BW, Smith PL, Kelly RJ, Lowe JB (1992) *J Biol Chem* **267**: 24575–84.
48 Sasaki K, Kurata K, Funayama K, Nagata M, Watanabe E, Ohta S, Hanai N, Nishi T (1994) *J Biol Chem* **269**: 14730–37.
49 Natsuka S, Gersten KM, Zenita K, Kannagi R, Lowe JB (1994) *J Biol Chem* **269**: 16789–94.
50 Yago K, Zenita K, Ginya H, Sawada M, Ohmori K, Okuma M, Kannagi R, Lowe JB (1993) *Cancer Res* **53**: 5559–65.
51 Dall'Olio F, Chiricolo M, Lollini P, Lau JTY (1995) *Biochem Biophys Res Commun* **211**: 554–61.
52 Majuri ML, Niemelä R, Tiisala S, Renkonen O, Renkonen R (1995) *Int J Cancer* **63**: 551–59.
53 Ho H, Hiraiwa N, Sawada-Kasugai M, Akamatsu S, Tachikawa T, Kasai Y, Akiyama S, Ho K, Takagi H, Kannagi R (1997) *Int J Cancer* (in press).
54 Dohi T, Hashiguchi M, Yamamoto S,Morita H, Oshima M (1994) *Cancer* **73**: 1552–61.
55 Akamatsu S, Yazawa S, Tachikawa T, Furuta T, Nakamura J, Asao T, Nagamachi Y (1996) *Cancer* **77** Suppl: 1694–1700.
56 Kemmner W, Krueck D, Schlag P (1994) *Clin Exp Metastasis* **12**: 245–54.

Received 1 October 1996, accepted 27 February 1997