Acta Biochimica Polonica

Vol. 44 No. 2/1997

343–358

QUARTERLY

Minireview

Tumor cell N-glycans in metastasis*

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Received: 20 December, 1996; accepted: 24 May, 1997

Key words: metastasis, N-glycans, integrins, cadherins, Ig-SF, galectins, selectins

Metastasis accounts for most of deaths caused by cancer. The increasing body of evidence suggests that changes in N-glycosylation of tumor cell proteins such as increased branching, increased sialylation, polysialylation, decreased fucosylation, enhanced formation of Lewis X and sialyl Lewis X antigens are among important factors determining metastatic potential of tumor cell. Most of the adhesion proteins, e.g., integrins, members of immunoglobulin superfamily, and cadherins are heavily N-glycosylated. The other proteins involved in adhesion, like galectins and type-C selectins, recognize N-glycans as a part of their specific ligands. In this review we focus on recent reports concerning the contribution of N-glycosylation of tumor cell adhesion molecules and some selected membrane proteins in the tumor invasion and metastasis.

Despite tremendous efforts made over recent decades to combat cancer, and the indisputable success achieved, this disease still presents a great challenge to scientists and physicians. Progress expressed in terms of decreased cancer incidence and mortality is still unsatisfactory even in the most developed countries [1, 2].

So far, it has been well established that metastasis accounts for the majority of deaths caused by cancer [2–4]. Therefore, attempts have been made to understand this very complex phenomenon including the mechanisms operating at the molecular level [5].

Metastasis is a multistep cascade of events in the course of which some of the tumor cells:

Presented at the V Symposium on Glycoconjugates, XXXII Meeting of the Polish Biochemical Society, Kraków (Poland), September 17–20, 1996

*This work was in part supported by the State Committee for Scientific Research (Collegium Medicum, Jagiellonian University, 501/Pk/3/L and Institute of Zoology, Jagiellonian University, DS/IZ/FZ/96).

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Abbreviations: Ig-SF, immunoglobulin superfamily; ECM, extracellular matrix; SLe^x, sialyl Lewis X determinant; Le^x, Lewis X determinant; L-PHA, leucoagglutinin; Lamp-1 and -2, lysosome associated membrane glycoprotein; CEA, carcinoembryonic antigen.

- 1, invade the surrounding tissue, degrade and penetrate the basal membrane, and finally detach themselves from the primary lesion;
- 2, enter the vascular or lymphatic system, formed during vascularization of the tumor mass, where they try to survive being exposed to mechanical stress and the activity of immune system;
- 3, attach themselves to the endothelium at some distant site(s) as a result of interaction with endothelial cells in which platelets are also involved;
- 4, penetrate the subendothelial basement membrane at the site of attachement, enter the surrounding tissue and colonize it forming a metastatic site [3, 4, 6].

Tumor cells which show the ability to metastasize should therefore exhibit characteristic features differing not only from normal, but also other tumor cells found in a primary lesion. An increase in the number of mutations in genes the products of which are crucial for normal cell functioning, may lead to dramatic changes in cell behavior. In addition to the ability of unrestricted growth, loss of contact inhibition and growth factor independence, invasive tumor cells show multiple degradative activities, enhanced cell motility and changes in the properties of cell and matrix adhesion molecules. These features of such cells allow for their rotation and increase their mobility leading to invasion of surrounding tissue, extracellular matrix and basement membrane involving solubilizing activities of various hydrolases. In particular, changes in the ratio of matrix metalloproteinases (MMPs) to their naturally occurring tissue inhibitors (TIMPs) are critical for tumor invasion. Growing number of blood capillaries formed due to parallel, tumor promoted angiogenesis, favors penetration of endothelium and entry of some invasive cells into vasculature. This creates the chances for tumor metastasis. The major factors responsible for successful colonization of target tissue and formation of a metastatic site are the ability of disseminating cells to move in response to autocrine motility stimulating factors and tissue specific chemotactic and haptotactic factors, as well as changes in the adhesive properties of some tumor cells. The latter properties should be considered with respect to interactions with other cells and substrata of the connective tissue surrounding the primary lesion as well as the vasculature and target tissue. The changes in interactions between various adhesion molecules are thought to be crucial for tumor invasion and metastasis [4–11].

All the adhesion molecules are either N-glycoproteins which are often heavily glycosylated, e.g. integrins, cadherins and Ig-SF members, or they recognize N-glycans at least as a part of their specific ligands. Galectin-3 and type-C selectins are examples of the latter group. Therefore it seems that the structure of N-glycans which are present on cancer cells, as well as of those exposed on target cells is an important factor which is involved in interactions with other cells and ECM proteins.

N-GLYCANS OF TUMOR CELLS

N-glycosylation is one of the posttranslational modifications of proteins. N-glycans are widely distributed in soluble and membrane-bound glycoproteins, and their structure is often cell-, tissue- and species-specific. All N-glycans have the penta-saccharide $Man_3GlcNAc_2$ as a common "core structure". According to their structure and location of extra sugar residues attached to the core, N-glycans are further divided into different types [12–16]:

- a high mannose-type which contains only mannosyl (Man) residues attached to the core;
- a complex-type which has "antennae" or branches attached to the core. The antennae are composed of *N*-acetylglucosamine (GlcNAc), galactose (Gal), fucose (Fuc), sialic acid (NeuNAc), *N*-acetylgalactosamine (GalNAc) and sulfate. The number of antennae in mammals ranges from two (biantennary) to four (tetraantennary);
- a hybrid-type which has only mannose residues on the Man α 6 arm and one or two antennae on the Man α 1-3 arm;
- a poly-*N*-acetyllactosamine type which contains repeating units of (Galβ1-

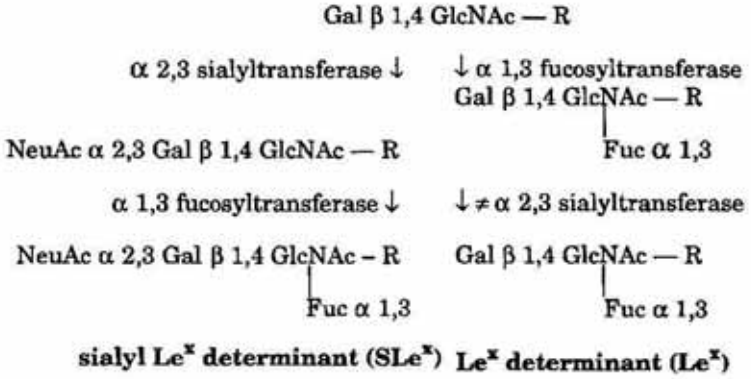
4GlcNAcβ1-3), attached to the core. This repeating structure may be branched.

All the N-glycans except for those of the high mannose-type may have the bisecting GlcNAc linked β 1-4 to the trimannosyl core.

Oligosaccharides differ from proteins and nucleic acids in a few characteristics: they are usually highly branched and their monomeric units are connected to one another by different types of linkages. Due to the branching and numerous linkage types, oligosaccharides are able to carry more information than other biological molecules [17]. The biosynthesis of sugar chains is not controlled by a template and is less rigid than that of proteins, which indicates that sugar chains can be altered by the physiological conditions of cells due to changes in the activity of processing enzymes [15]. The recently proposed name "glycocode" signifies that the structure of an oligosaccharide is specifically recognized by its receptor [18]. Any change in this structure would affect its interactions and recognition. To the most conclusive evidences supporting such an opinion belong significant changes in the oligosaccharide structure observed in such diseases as cancer [19–21], metastasis [22–30] and leukemia [8, 31, 32].

A well documented phenotypic alteration of the transformed cells is a rise in the molecular mass of cell surface complex-type N-glycans due to the increased branching on the trimannosyl core, increased poly-*N*-acetyllactosaminoglycan chain formation, and also enhanced sialylation. Such alterations have often been associated with a reduced cellular adhesion to extracellular matrices, and they are highly correlated with an increase in the invasive and metastatic potential [30, 33–42].

Linear poly-*N*-acetyllactosamine chains are composed of galactose and *N*-acetyl-glucosamine residues which are linked together by the alternating actions of a β 1,4 galactosyltransferase (β 1,4 Gal-Tn) and β 1,3 *N*-acetylglucosaminyltranferase (β 1,3 GlcNAc-Tn) [12, 13, 15, 43]. Several lines of evidence suggest that polylactosaminic sequences are preferentially added to the β 1,6-linked GlcNAc residues [35, 36, 44] as a result of action of β 1,6 *N*-acetylglucosaminyltranferase (β 1,6 GlcNAc -TnV), hence the expression of poly-*N*-acetyllactosaminic chains seems to be controlled by the degree of β 1,6 branching [16, 35, 36, 44]. The terminal lactosamine unit (Gal β 1,4 GlcNAc β 1,3] of a poly-*N*-acetyllactosamine chain may be terminated with sialic acid (NeuNAc) moieties in either α 2, 6 or α 2, 3 linkage, *via* the action of sialyltransferases, and with an α 1,3-linked fucose residue by the action of α 1,3 fucosyltransferases [15, 43, 45]. It is believed that fucosylation is a terminal event in the biosynthesis of these molecules; some α 1,3 fucosyltransferases can fucosylate α 2,3 sialylated polylactosamine moieties, whereas it has not been possible as yet to demonstrate α 2,3 sialylation of an α 1,3 fucosylated glycan [43, 45, 46] (Scheme 1).



Scheme 1.

The oligosaccharide determinant formed according to the scheme presented above is known as a sialyl Lewis X (SLe^X) moiety, and the analogous neutral form is known as a Lewis X (Le^X).

It has been observed in several experimental tumor models [30, 33, 35, 36] that the increased 1,6 β branching of N-linked chains, rather than simple transformation, is one of the most important cancer related changes, associated with acquisition of an invasive and metastatic potential [35]. For example, SP1, a tumorogenic but nonmetastatic mouse mammary carcinoma cell line, expressed very few β 1,6-branched oligosaccharides. Following transformation of SP1 with the activated *H-ras*, tumor cells showed enhanced expression of the GTnV activity, increased affinity to L-PHA, a lectin specific for N-linked glycans containing a β 1,6-linked antenna, as well as increased metastatic potential [30, 35]. L-PHA reactive oligosaccharides are preferentially expressed by the lysosome-associated membrane glycoproteins 1 (Lamp-1). It has also been suggested that the presence of β 1,6-branched N-linked chains facilitates the invasion of a basement membrane [30, 33–36].

Studies on human breast and colon neoplasia have shown that the amount of L-PHA reactive β 1,6-branched N-linked oligosaccharides is consistently increased in neoplasia [34]. Similar results have been reported for human melanoma [34].

A comparison of Asn-linked oligosaccharides attached to lysosomal membrane glycoproteins (Lamp-1, Lamp-2) from undifferentiated and differentiated HL-60 cells drew attention to the fact that the amount of N-linked oligosaccharides that contain poly-*N*-acetyllactosaminyl units, the number of poly-*N*-acetyllactosaminyl branches per glycopeptide and their length increase dramatically during differentiation [38]. Studies on N-linked oligosaccharides from different colonic carcinoma cells revealed that the highly metastatic cells express more of poly-*N*-acetyllactosaminyl side chains than cells with a low metastatic potential. In addition, they are more sialylated and less fucosylated [24]. It may thus be concluded that tumor cells, in particular metastatic ones, are enriched in tetraantennary N-glycans containing poly-*N*-acetyllactosamine repeats [24, 30, 33, 47].

As each branch may be potentially terminated by an NeuAc residue, the increased branching would yield an increased number of sialic acid residues per glycoprotein molecule [48]. Such a mechanism may also lead to an overall increase in sialic acid density on the cell surface, a phenomenon that is often found to be associated with a malignant transformation and invasive or metastatic potential [30, 33]. It should be stressed, however, that the shift to the expression of these phenotypically malignant structure types is quantitative rather than qualitative. If specific carbohydrate structures play indeed a role in malignant processes, it is likely that a certain cell-surface density has to be reached before an effect is exerted [36].

It has been discovered that the amount of SLe^x on colorectal carcinoma cells is correlated well with liver metastasis. The prognosis for patients with a higher expression of this antigen on the cell surface is much poorer than for those with a low expression of SLe^x [49]. A similar observation was made by Irimura *et al.* [50]. They showed the human colorectal carcinomas with an increased metastatic potential and poor prognosis are characterized by a high content of SLe^x antigens, and concluded that the SLe^x antigen is a unique molecular phenotype which determines colorectal cancer metastasis [50].

As yet no metastasis-specific oligosacharide has been identified; metastatic cells exhibit quantitative differences in the carbohydrate structure [23], but it is evident that many of the changes observed in the behavior of malignant cells are due to alterations in cell surface N-glycans which participate in the adhesion processes [24, 48, 51, 52].

N-GLYCANS AND ADHESION MOLECULES

Every cell within a multicellular organism is equipped with a number of adhesion proteins which determine its interactions with the surrounding tissue. The most important of them in respect of metastasis are integrins, proteins belonging to the immunoglobulin superfamily (Ig-SF), cadherins and galectins. The expression of these adhesion molecules influences the properties of a particular tumor cell with respect to its interaction with other cells and substrata such as laminin,

fibronection, vitronectin and others. Another set of adhesion proteins are encounter type-C selectins. These proteins are not present on cancer cells, but — being expressed on platelets, leukocytes and endothelial cells — are crucial to the attachment of a moving tumor cell to the endothelium during later phases of metastasis.

Integrins

The largest group of adhesion molecules are integrins, which are noncovalent heterodimers containing at least one out of 15 α and one out of 8 β-type subunits. Both α and β subunits are transmembrane glycoproteins. Extracellular domains of the αβ complexes participate in bivalent ion-dependent interactions with various extracellular matrix proteins and other numerous cell receptors belonging predominantly to the immunoglobulin superfamily [3, 6, 8, 53]. The expression of integrins depends on the tissue of origin and degree of its differentiation [54]. It has been shown that many, but not all, integrins require an arginine-aspartate-glycine sequence in their ligands [55–57]. The presence of such an amino-acid sequence has been ascertained in, e.g., fibronectin, fibrinogen, vitronectin, and collagen I. It is, however, still unclear whether this is the only signal recognized by integrins, since the requirement for a synergistic signal has also been reported [56]. In the case of human laminin, the recognition signal is still unknown [58]. In the majority of cells, $\alpha_6\beta_1$ and $\alpha_5\beta_1$ integrins are specific receptors for laminin and fibronectin, respectively. Studies on the role of integrin glycans in their expression in the cell surface, as well as on the structure and function of integrins have shown that the presence of mature, high mannose, complex bi-, tri- and tetraantennary glycans is not necessary for the formation of αβ heterodimers and their expression in the cell membrane. However, these N-glycans are indispensable to the interaction between integrins and fibronectin and laminin [55, 56, 59, 60]. Oz *et al.* [61] and Kawano *et al.* [62] characterized N-glycans of β_1 integrins, synthesized in the highly metastatic B16 melanoma cell line and its poorly metastatic, wheat germ agglutinin resistant glycosylation mutant Wa4b1. Their results showed that mutant β_1 integrin N-glycans containing the Lewis X-antigenic (Le^X) determinant with a reduced sialic acid content and an increased amount of the GlcNAc β 1-3 bound fucose unit, attenuated the interaction and spreading of β_1 type integrins ($\alpha_5\beta_1$, $\alpha_6\beta_1$) on endothelial basement membrane proteins. The presence of highly sialylated, tetraantennary N-glycans of integrins influences their adhesive properties and in consequence their interactions with endothelial fibronectin; furthermore, they seem to affect the metastatic potential of melanoma cells. Integrin $\alpha_6\beta_1$ of the mouse melanoma B16-F10 cell line possesses mainly branched, multiantennary N-glycans. An analysis of the role of $\alpha_6\beta_1$ glycans by Chammas *et al.* [63] has shown that the binding to laminin depends on the presence of terminal α-galactose of the α type chain, while the spreading on laminin depends on branched, complex N-glycans of the β type chains. However, the authors did not state that the presence of sialic acid significantly affected the interaction between melanoma cells and laminin. The results of studies with the subfamily of α_v integrins, in particular the $\alpha_v\beta_5$ and $\alpha_v\beta_6$ members of the colon adenocarcinoma cell line, indicate a crucial role of α_v, β_5 and β_6 N-glycans in the interaction between vitronectin and fibronectin, and the lack of any effect on the expression of either integrin in cancer cell membranes [64]. The latter observation is at variance with the bulk of evidence indicating that in some cases inhibition of N-glycosylation leads to a decreased expression of integrins in the cell membrane. However, even if such integrins are expressed, they lose their ability to bind to vitronectin or fibronectin and occasionally laminin due to lack of their N-glycans in a mature form. It is difficult to reach a clear-cut conclusion about the role of sialic acid in the interaction between $\alpha_v\beta_5$ and $\alpha_v\beta_6$ integrins and substrata. These and other results indicate that, in the case of the colon adenocarcinoma cell line studied, sialic acid does not play an important role [8, 64].

Immunoglobulin superfamily

Among the adhesion molecules worth mentioning there are some proteins belonging to an immunoglobulin superfamily — Ig-SF [4, 6]. Members of this widely spread group of proteins possess a common structural motif, an immunoglobulin fold composed of about 70–110 amino acids which form 7–9 β pleated sheets stabilized by disulphide bonds. Most of the members of this superfamily participate in the cell-cell recognition and in immunological processes. They are proteins of the major histocompatibility complex (MHC), T cell receptor, CD4, CD8, N-CAM, VCAM, ICAM-1 receptors. Some of them are found on almost every cell, especially on those of the immunological system and vascular endothelium which participates in metastasis.

The effect of changes in the N-glycosylation of N-CAM, an immunoglobulin superfamily Ca^{2+}-independent adhesion protein, on the metastatic potential of certain cells is an example of a close relationship between the function of a heavily N-glycosylated adhesion molecule and the structure of its N-glycans.

Changes in the glycosylation of this protein exemplify the expression of N-glycans in the form prevailing during embryonic development of the nervous tissue. N-CAM of adults usually contains not more than 2–3 sialic acid residues in the form of α 2,8-bound polysialic acid. During embryonic life, the number of sialic acid residues is considerably higher, up to ten units, and N-CAM is a major carrier of this structure in some migrating neural cells. In some types of cancer, mainly of a neuroendocrine origin, such as small-cell lung cancer or mesodermal Wilms renal tumor, a large amount of the N-CAM bound polysialic acid was found [65, 66].

The studies carried out on weakly metastatic E-2 and highly metastatic F-3 sublines, derived from small-cell lung cancer and containing a small and a large amount of polysialic acid, respectively, showed a good correlation between the presence of N-CAM-bound polysialic acid and its ability to induce metastasis in nude mice and to form colonies in soft-agar, on the one hand, and the strength of cell-cell interactions on the other [66]. Fukuda [66] suggested also that polysialic acid attenuated homotypic interactions between N-CAM molecules and, in consequence, prevented static adhesion and allowed the cells to move effectively. The results of these studies show that masking homo- and heterotypic cellular N-CAM interactions with polysialic acid help the mutant cells to leave the primary lesion.

ICAM-1 is another protein representing Ig-SF, whose expression plays an important role in the behavior of some tumor cells. ICAM-1, a N-glycoprotein present in leukocytic cell membranes, participates in interactions of leukocytes with targets *via* not sufficiently recognized integrins. ICAM-1 is not normally present on epithelial cells, but it is expressed in cancer cells. The presence of ICAM-1 in the membrane of, e.g., kidney cancer cells is usually a positive prognostic sign, perhaps due to its stimulation of interactions of cancer cells with immune system cells through $\alpha_L\beta_2$ and $\alpha_M\beta_2$ integrin receptors [4, 6].

It has been shown that, in the case of certain types of melanoma, ICAM-1 is present in the cell membrane and correlates well with the vertical phase of tumor growth. The reason for such different effects of the presence of ICAM-1 on the cell membrane is still obscure. On the ground of some preliminary observations it may be assumed that, in the case when ICAM-1 correlates with tumor progression, changes in its adhesive properties toward the ligands may result from alterations in the structure of ICAM-1 N-glycans. This, in turn, could enhance the metastatic ability of cancer cells [4].

The carcinoembryonic antigen (CEA) is another protein representing Ig-SF. It is expressed in the apical membrane of normal and tumor human colonic cells. CEA possesses 28 potential N-glycosylation sites, and N-glycans constitute even up to 50% of its molecular mass [67]. Garcia *et al.* [67] found CEA in the membrane of a normal colon phenotype as a 200-kDa/130-kDa protein, and as a 170-kDa single molecular form in the colon cancer phenotype. The observed change was due to modification of the N-glycosylation pattern of CEA [67, 68]. Similarly Sanders *et al.* [68] detected variable glycosylation of CEA present in primary and metastatic melanomas. Recently Li *et al.* [69] re-

ported an increased branching of N-glycans of CEA, expressed by a highly invasive form of colon adenocarcinoma. The role of a carcinoembryonic antigen in metastasis has not been well recognized so far, but it seems to represent a true oncofetal marker as in the case of polysialylated N-CAM [6].

Cadherins

Cadherins are a group of Ca^{2+}-dependent adhesion molecules that are also involved in tumor progression and metastasis [70–72]. Members of the cadherins family are N-cadherin in the neural and muscle tissue, P-cadherin in the placenta and epithelium, E-cadherin in the epithelium and L-cadherin in the liver [73, 74]. They have been divided into more than ten subclasses, but their list is supposedly still incomplete. Being typical transmembrane proteins, they are composed of a C-terminal cytoplasmic domain, a membrane spanning region and an N-terminal extracellular motif containing three repeated domains. Cadherins appear as a single polypeptide chain of different length (about 730 amino acids) and molecular mass, but with a high degree of homology (up to 60%). Cadherins are considered to be important regulators in the process of morphogenesis and development. Additionally, E-cadherin has been shown to play an important role in the process of metastasis. The C-terminal domain of E-cadherin interacts with cytoplasmic proteins α-, β-, and γ-catenin, or with proteins of the cytoskeleton, which seems to be a key event in the interaction between extracellular domains of E-cadherin and the analogous domain of E-cadherin present in the membrane of an other cell, e.g. a tumor one. All the cell types that form a solid tissue express these molecules displaying a homophilic cell-cell interaction. E-cadherins are N-glycoproteins but the structure and function of the oligosaccharide component have not been well recognized so far [73, 74].

There is strong evidence emergeing from studies of Boubelik *et al.* [75] suggesting that surface carbohydrates are involved in cadherin-mediated cell sorting during embryogenesis. Studies on the contribution of Le^x hapten, an oligosaccharide structure carried by embryoglycan, and of E-cadherin to intercellular adhesion have shown that the absence of Le^x has no effect on homotypic cell aggregation, while pretreatment of these cells with an E-cadherin specific antibody reduces the homotypic aggregation. Analysis of crystal structures has suggested that the cadherin-mediated cell adhesion is achieved *via* formation of a multimeric cadherin superstructure providing a cooperative mechanism in which attractive or repulsive carbohydrate-mediated forces play an important role.

The role of E-cadherin N-glycans in the cell-cell adhesion requires further detailed studies, since Yoshimura *et al.* [76] reported recently that introduction of glycosyltransferase III gene to highly metastatic murine B16 myeloma cells suppress metastasis.

Galectins

Several lines of evidence have demonstrated that the galectins — S-type lectins — participate in cell-cell and cell-matrix interactions by recognizing and binding the poly-*N*-acetyllactosamine moieties, and thus play a crucial role in various normal and pathological processes. Polymerized *N*-acetyllactosamine units are often found in extracellular matrix glycoproteins such as fibronectin, laminin etc. The S-type lectins are, in general soluble proteins, their carbohydrate-binding activities are cation-independent and occasionally thiol-dependent. Galectins are not glycosylated, in which they differ from other proteins that participate in cellular adhesion. Galectins have an unusual dual localization, both inside the cytoplasm and nucleus and outside the cell in soluble and membrane adsorbed forms [18, 77, 78]. Eight different galectins have been found in mammals. The best known is galectin-3 (Gal-3), sometimes called deadhesion molecule, a 31-kDa poly-*N*-acetyllactosamine binding protein. It is strongly expressed by some metastatic and oncogenically transformed cells [79–81]. The results of a search for its physiological ligands indicate that the only compounds which so far have been found to play this role, are members of Lamp-1 and Lamp-2, Mac-2-BP and laminin [81]. The most im-

portant interactions are perhaps those between Gal-3 and laminin, a major component of the basement membrane. Laminin is also a heavily N-glycosylated molecule. Up to 27% of its mass is provided by carbohydrate structures linked to its 71 potential N-glycosylation sites [77, 82]. The substantial role of the structure of laminin oligosaccharides in adhesion processes has not been elucidated as yet but treatment of laminin with some plant lectins (wheat germ agglutinin and *Griffonia simplicifolia* agglutinin) inhibits the binding of murine melanoma B16 cells to this protein [77]. It has also been shown recently that human laminin synthesized by cancer cells differs from normal laminin in glycosylation pattern [83]. It is therefore assumed that the Gal-3 expression in metastatic cells weakens the interaction between the cell and ECM by binding soluble Gal-3 to laminin poly-*N*-acetyllactosamine residues. This may in consequence stimulate the secretion of metalloproteinases, and may finally lead to degradation of the basement membrane [81].

Selectins

One of the most important biological functions of carbohydrates consists in their recognition by lectins, a family of proteins which are classified on the basis of similarities in specificity of their carbohydrate-recognition domains. The class of adhesion molecules that are involved in the binding of tumor cells to the vascular endothelium are called selectins. Three members of the selectin family have been reported to date, i.e. L — lymphocyte, P — platelet, and E — endothelium selectin. All three of them are N-glycoproteins with a calcium-dependent carbohydrate recognition domain at their N-termini, followed by a single epidermal growth factor-like domain, a variable number of complement-regulatory domains, a single *trans*-membrane polypeptide, and carboxy-terminal cytoplasmic domain. E-selectin (endothelial leukocyte adhesion molecule-1, ELAM-1) is an inducible protein expressed on the surface of endothelial cells. L-selectin is expressed constitutively on all leukocytes, and P-selectin — in addition to its presence on platelets — is also expressed on endothelial cells [48, 84–87].

Selectins mediate the cell-cell contact by binding — through their lectin domain — to a carbohydrate-containing counter-receptor on target cells. The action of selectins is essential for the rolling of leukocytes on the surface of the activated endothelium within an inflamed tissue. Subsequent steps lead to extravasation of leukocytes into tissue [43, 88, 89]. The process of tumor metastasis is reminiscent of that of leukocytes adhesion to inflammatory sites. On the basis of the above observation selectins have been suggested to promote attachment of a tumor cell to the endothelium, and thus to facilitate metastasis of certain types of tumors [90].

A variety of studies, e.g. those on the use of soluble carbohydrates as competitive ligands and anti-carbohydrate antibodies, and on induction of a selectin-dependent adhesion, identified the fucosylated tetrasaccharide, sialyl Lewis X, and its isomer, sialyl Lewis A, as ligands for all three selectins [86, 88, 91]. Both sialic acid and fucose linkages are essential for the binding [43, 84]. A comparative analysis of the carbohydrates derived from the library of E-selectin binding cell lines was used to identify endogenous protein-associated oligosaccharide ligands for this lectin. Three structures were identified; they all are tetra-antennary N-linked glycans with a NeuNAc α2,3 Gal β1,4 (Fuc α1,3) GlcNAc β1,3 Gal β1,4 (Fuc α1,3) GlcNAc lactosaminoglycan (sialyl-di-Lewis X, S-diLe^x) extension on the arm linked through the C4 residue on the mannose. E-selectin-agarose affinity chromatography confirmed that the S-diLe^x containing structures are high-affinity endogenous ligands for E-selectin [92].

As it was already discussed, N-linked oligosaccharides from different tumor cells, in particular metastatic ones, are enriched in tetraantennary N-glycans containing poly-*N*-acetyllactosamine repeats. It is possible to speculate that poly-*N*-acetyllactosamines are important for tumorogenicity because they provide a perfect backbone for SLe^x formation. An increase in the number of poly-*N*-acetyllactosamine units leads to an in-

creased amount of SLe^x structures. In fact, many carcinoma cells have been found to have an increased amount of SLe^x and SLe^a structures [24, 49, 66, 90]. Such presentation of carbohydrate ligands makes them more accessible for proteins, like e.g. selectins, than SLe^x in short side chains.

The results of numerous studies strongly suggest that one of the key factors of the metastatic spread is the amount, on tumor cells, of the SLe^x structure, a ligand of E-, and P-selectin [49, 50, 66, 93, 94]. It is therefore possible that blood-borne metastatic tumor cells are first bound to platelets *via* an interaction between tumor cell carbohydrate and platelet P-selectin. Such aggregates can be trapped in capillary veins, where tumor cells release some of the cytokines that activate endothelial cells. Once endothelial cells are activated, tumor cells are bound to E-selectin expressed on endothelial cells. Such binding to selectin may lead to a firm attachment to endothelial cells with additional participation of the cancer cell integrin VLA4 ($\alpha_4\beta_1$) and final extravasation [5, 90].

When twelve human melanoma lines were screened for the surface expression of carbohydrate ligands typical for the E-selectin binding [95], eleven of them were positive for S-diLe^x and seven were positive for SLe^a, but none of them exhibited any E-selectin dependent adhesion to activated human umbilical vein endothelial cells. The above-cited results showed that the majority of SLe^x/SLe^a type glycans produced endogenously by human melanoma cells, are not protein-associated nor do they mediate the E-selectin-dependent adhesion. These results support the hypothesis that the E-selectin-dependent adhesion requires presentation of SLe^x-type moieties on the appropriate N- and/or O-glycoproteins [95].

The cloning of selectin ligands permits a search for the structural requirements for selectin-ligand interactions. It is still not thoroughly known which structural motif on the ligand determines the recognition by selectin. Varki [96] suggests that, in the case of sialomucin type ligands of L-selectin, a cluster of common O-linked carbohydrate side-chains generates epitopes which are unique in character. The binding of ESL-1 to E-selectin is based on different structural requirements. ESL-1 is a 150-kDa N-glycoprotein with five potential N-glycosylation sites and no O-linked carbohydrates; it is identified by direct affinity isolation as a physiological E-selectin ligand. It has thus been shown that N-linked carbohydrates, sialic acid and fucose are essential for the binding to E-selectin [97]. A number of ligands have been described for E-selectin, of which only two were identified by direct affinity isolation. They are a 250-kDa N-glycoprotein of bovine peripheral γ/δ T-cells and ESL-1 [98, 99]. Other ligands defined by a reaction with antibodies are also N-glycoproteins; a subpopulation of β_2 integrins [100] and Lamp-1 lysosomal proteins on the surface of carcinoma cells [101]. At present it is still impossible to decide which of the known selectin ligands is most likely to be of physiological relevance [97].

CONCLUDING REMARKS

The following conclusions concerning the contribution of N-glycans to metastasis can be made:

- comparison of the structure and properties of N-linked oligosaccharides of various integrins in highly metastatic cells and their poorly metastatic counterparts showed that changes of N-glycans clearly influenced the interactions between tumor cells and basement membrane proteins although some controversies with respect to the role of sialic acid in these interactions still remain;
- polysialylation of N-glycans of Ca^{2+}-independent adhesion protein (N-CAM) and increased branching of N-glycans of carcinoembryonic antigen (CEA) were shown to increase the metastatic potential of some cancer cells. Changes in N-glycosylation of another adhesion protein of the immunoglobulin superfamily — ICAM-1 — may be considered as affecting its binding to the immune system cells;
- expression of the "deadhesion molecule"— galectin-3 — in metastatic cells and binding of its soluble form to poly-*N*-acetyllactosamine residues of laminin N-glycans is

supposed to weaken cancer cell — ECM interactions and may stimulate secretion of metalloproteinases and, in consequence, degradation of basement membrane;

- recent observations concerning N-glycans of E-cadherin, a Ca^{2+}-dependent adhesion molecule, and their role in E-cadherin mediated homotypic cell-cell interactions, suggest that N-linked oligosaccharides may also contribute to properties of metastatic cells;
- interactions between metastatic tumor cells and platelets as well as endothelial cells are doubtlessly mediated by tumor cell N-glycans due to their binding to P- and E-selectins. The well documented changes in structure and properties of N-glycans of lysosomal membrane proteins (Lamp I and Lamp II) and presumably of some other membrane proteins of tumor cell — increased branching, increased sialylation, decreased fucosylation, enhanced formation of Lewis X and sialyl Lewis X antigens, contribute to survival of metastatic cells in blood and help them to attach to endothelium and colonize new locations.

Despite many as yet unanswered questions as to the mechanisms of the metastasis, the increasing body of evidence suggests that changes in N-glycans of cancer cell adhesion molecules and some cell membrane proteins are an extremely important factor facilitating spreading of cancer cells and thus metastasis.

The authors are very grateful to Magdalena Pogonowska MD of 'Medicus' Polish American Educational Foundation for subscribtion of professional literature for the Institute of Medical Biochemistry of Jagiellonian University College of Medicine in Cracow.

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