Acta Biochimica Polonica

Vol. 49 No. 2/2002

303–311

QUARTERLY

*Review*

# Sialyl Lewis$^a$: a tumor-associated carbohydrate antigen involved in adhesion and metastatic potential of cancer cells★

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Received: 14 December, 2001; revised: 24 April, 2002; accepted: 22 May, 2002

**Key words:** sialyl Lewis$^a$, tumor-associated carbohydrate antigen, adhesion, selectin E, metastasis

**Neoplastic transformation is often associated with characteristic changes in the expression of the sialyl Lewis$^a$ and sialyl Lewis$^x$ antigens, representing typical tumor-associated carbohydrate antigens. High amounts of sialyl Lewis$^a$ are present in human adenocarcinomas of the colon, pancreas and stomach. A growing amount of data suggests that this carbohydrate structure is the ligand for E-selectin. Sialylated Lewis structures present on the surface of tumor cells are carried by the carbohydrate chains of glycoproteins and glycolipids. There are several lines of evidence showing that sialyl Lewis$^a$ is responsible for the adhesion of human cancer cells to endothelium. E-selectin present on endothelial cells mediates these interactions. Selectins and their carbohydrate ligands can thus play an important role in the selective homing of tumor cells during metastasis. However, the presence of sialyl Lewis$^a$ antigen on the surface of tumor cells and their adhesion to E-selectin-expressing cells in *in vitro* adhesion assay by itself can not be directly related to metastatic properties of all cancer cells.**

## SIALYL LEWIS$^a$ AS A TUMOR-ASSOCIATED CARBOHYDRATE ANTIGEN (TACA)

Lewis blood group antigens are biosynthetically and structurally related carbohydrate structures used as markers of cell differentiation and embryonic development (Feizi & Childs, 1985). In adults, their presence is not limited to erytrocytes, but they can be found in different tissues and organs. It has been shown that neoplastic transformation is

★Presented at the XXXVII Meeting of the Polish Biochemical Society, Toruń, Poland, September, 10–14, 2001.

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**Abbreviations:** d,l-*threo*-PPPP, 1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol·HCl; GalNAc-$\alpha$-O-benzyl, benzyl 2-acetamido-2-deoxy-$\alpha$-D-galactopyranoside; O-SGP, O-sialoglycoprotease.

often associated with characteristic changes in the expression of these blood group oligosaccharides, and their amounts usually increase during tumor progression and acquiring of malignant phenotype (Hakomori, 1996). Typical tumor-associated carbohydrate antigens are two carbohydrate structures named sialyl $Le^a$ and sialyl $Le^x$. Their structures are shown in Fig. 1.

of the colon, stomach, pancreas and gall-bladder (Koprowski *et al.*, 1979; Atkinson *et al.*, 1982; Sakamoto *et al.*, 1989). Low amounts of sialyl $Le^a$ antigen are also present on normal epithelial cells (Atkinson *et al.*, 1982; Arends *et al.*, 1983). In the human fetus, this carbohydrate epitope is expressed in epithelial cells of different organs, mainly of endodermal origin (Olding *et al.*, 1984).

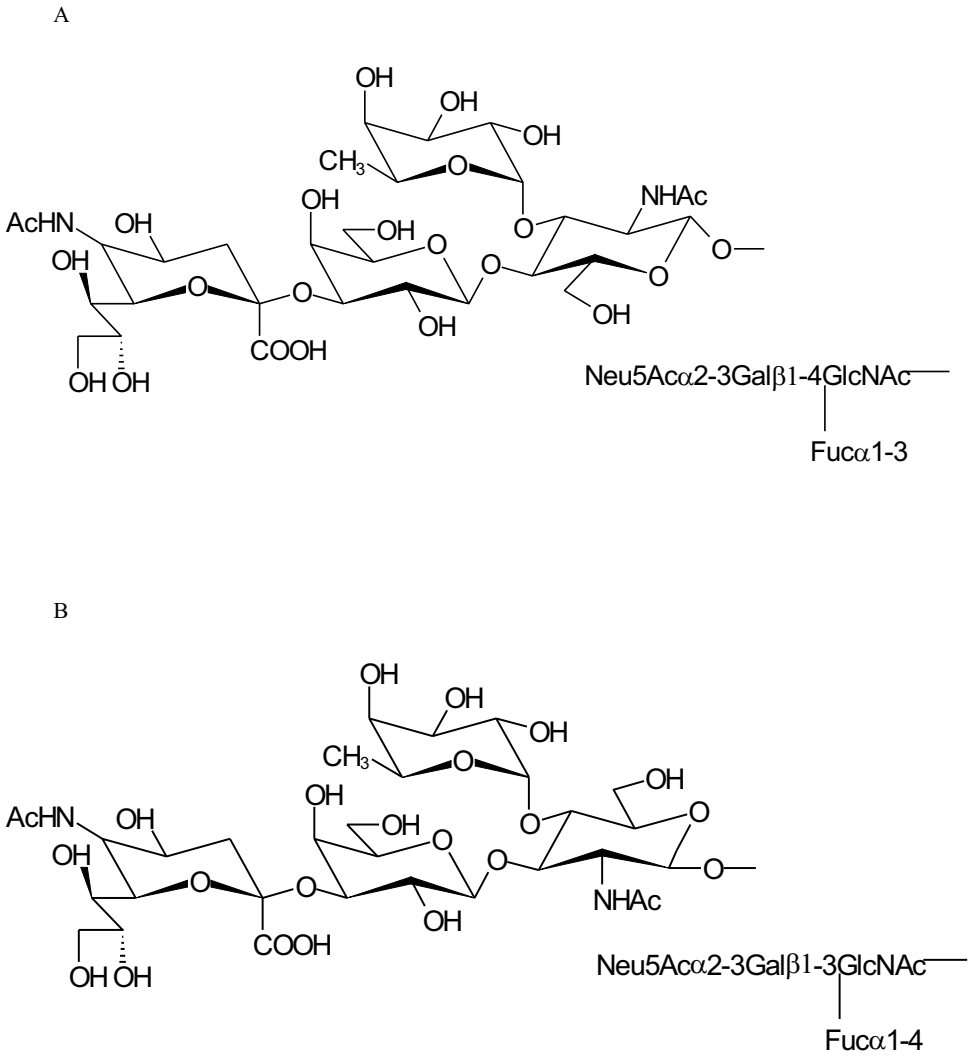


**Figure 1. Structures of sialyl Lewis$^a$ and sialyl Lewis$^x$ antigens.**

The sialyl $Le^a$ antigen was discovered by Koprowski *et al.* (1979) with the use of monoclonal antibody 19-9. It was found in high amounts on the surface of established *in vitro* human pancreatic, colon and gastric cancer cell lines (Falk *et al.*, 1983; Magnani *et al.*, 1982), as well as in human adenocarcinomas

The expression of sialyl Lewis$^a$ antigen is associated with tumor progression. A gradual increase in the amount of sialyl Lewis$^a$ was found in colon and rectum during neoplastic transformation and progression (Gong *et al.*, 1985). It has been shown that cells derived from human squamous lung cancer were

tumorigenic in nude mice only when this carbohydrate structure was present on the cell surface (Pettijohn *et al.*, 1988). Ugorski *et al.* (1990) demonstrated that the presence of sialyl Le[a] was restricted to tumorigenic and invasive human urothelial cell lines. Clinicopathological studies revealed that the expression level of sialyl Le[a] is a prognostic factor for colorectal carcinoma, and is associated with high incidence of recurrence and the length of survival time (Shimono *et al.*, 1994; Nakayama *et al.*, 1995; Yamada *et al.*, 1997). Multivariate analysis demonstrated that expression of this antigen in colorectal cancer is also significantly related with the presence of hepatic metastases (Isozaki *et al.*, 1998). In the case of gastric carcinoma, high level of sialyl Le[a] was associated with tumor location, gross appearance, depth of invasion and was recognized prognostic factor (Nakamori *et al.*, 1997).

## INVOLVEMENT OF SIALYL LEWIS[a] ANTIGEN IN ADHESION OF CANCER CELLS

The formation of metastases, called metastatic cascade, is a complex phenomenon involving several discrete steps: migration of cancer cells from the primary tumor, their intravasation, circulation with blood, interaction with endothelium, extravasation and establishment of a microenvironment and metastasis (Poste & Fidler, 1980). It is now generally accepted that every step of the metastatic cascade is dependent on specific adhesive interactions of cancer cells with other cells and components of the extracellular matrix (Honn & Tang, 1992). These interactions are mediated by different families of adhesion molecules including cadherins, integrins, members of the immunoglobulin superfamily, and selectins and their carbohydrate ligands – sialyl Le[a] and sialyl Lewis[x]. Several lines of evidence suggest that sialyl Lewis[a] oligosaccharides are involved in the adhesion of several types of cancer cells to E-selectin present on the surface of endothelial cells. This concept is based on the following facts: (i) various cancer cells with high expression of sialyl Lewis[a] antigen adhere strongly to E-selectin (Kłopocki *et al.*, 1996; Majuri *et al.*, 1992; Takada *et al.*, 1991; Takada *et al.*, 1993); (ii) these interactions are inhibited by monoclonal antibodies directed against sialyl Lewis[a], liposomes containing sialyl Lewis[a] gangliosides and mucins carrying sialyl Le[a] structures (Iwai *et al.*, 1993; Sawada *et al.*, 1994; Zhang *et al.*, 1996; Zhang *et al.*, 1994); (iii) specific inhibition of sialyl Lewis [a] expression in colon cancer cells by transfection with antisense FT III cDNA completely abolished their binding to E-selectin (Kłopocki *et al.*, 1998). According to Takada *et al.* (1993) (Takada *et al.*, 1993) sialyl Lewis[a] antigen is mainly responsible for adhesion of human colon, pancreas and gastric cancer cells to the endothelium, whereas binding of lung, liver and ovarian cancer cells is mediated by sialyl Lewis[x]. Our studies showed that adhesion of human urinary bladder cancer Hu 1703He cells to E-selectin-expressing CHO cells was also mediated by sialyl Lewis[a] oligosaccharides (Kłopocki et al., 1996).

## MUCINS AND GANGLIOSIDES CARRYING SIALYL LEWIS[a] STRUCTURES AS LIGANDS FOR E-SELECTIN

Carbohydrate structures, sialyl Lewis[a] and sialyl Lewis[x], are not true physiological ligands for selectins. In the case of leukocyte specific glycoproteins, most of them belonging to O-glycosylated mucin-type glycoproteins, were described as components responsible for mediating such functions (McEver *et al.*, 1995). Much less is known about sialyl Lewis[a]- and sialyl Lewis[x]-carrying glycoconjugates mediating the binding of pancreatic and colon cancer cells to E-selectin.

In adenocarcinomas of colon, stomach and pancreas sialyl Lewis$^a$ antigen was detected as monosialoganglioside (Magnani *et al.*, 1982). A highly elevated level of glycoproteins bearing this carbohydrate structure is found in sera of cancer patients, especially those with pancreatic tumors. In colon carcinoma COLO 205 cells sialyl Lewis$^a$ is present on two mucin-types glycoproteins (Baeckström *et al.*, 1991). The larger mucin (600–800 kDa), named H-CanAg, is a membrane protein, and its apoprotein was identified as MUC-1. The smaller glycoprotein, named L-CanAg, is secreted by the cells, and its apoprotein is identical with leukosialin (Baeckström *et al.*, 1991; Baeckström *et al.*, 1995). In a human pancreatic cell line, sialylated Lewis antigens are associated with MUC-1 (Ho *et al.*, 1995). It was suggested that increased level of sialyl Lewis$^a$ bearing glycoproteins of lower molecular mass is associated with a higher metastatic potential of tumor cells (Takabayashi *et al.*, 1993). The mucin-type glycoproteins as well as gangliosides were found to be carriers of sialyl Lewis$^a$ in human colon cancer CX-1 cells and human urinary bladder cancer Hu 1703He cells (Kłopocki *et al.*, 1996; 1998).

The involvement of mucin-type glycoproteins carrying sialylated Lewis structures in adhesion to E-selectin was also supported by studies using GalNAc-$\alpha$-O-benzyl, an inhibitor of O-glycosylation, and the enzyme O-sialoglycoprotease (O-SGP), which specifically cleaves mucin-type glycoproteins. It was shown that adhesion of colon and pancreatic cancer cells to endothelium was highly decreased after incubation with GalNAc-$\alpha$-O-benzyl (Sawada *et al.*, 1994; Izumi *et al.*, 1995; Kojima *et al.*, 1992), and that several human colon cancer cells treated with O-SGP lost their ability to interact with P- and E-selectins (Mannori *et al.*, 1995). In one study, it was shown that N-glycans carrying sialyl Lewis$^a$ structure mediate the adhesion of colon cancer cells to E-selectin (Sawada *et al.*, 1993).

In addition to glycoproteins, experimental data exist that support the thesis that sialylated Lewis gangliosides can be also ligands for selectins. When O-SGP was used, the enzyme treated HL-60 cells and neutrophils lost their ability to bind to P-selectin but not to E-selectin (Steininger *et al.*, 1992). This enzyme had also little effect on the binding of COLO 205, HT-29 and CX-1 colon cancer cells and Hu 1703He human bladder cancer cells to E-selectin (Kłopocki *et al.*, 1996; Mannori *et al.*, 1995), so the role of gangliosides carrying sialyl Lewis$^x$ and sialyl Lewis$^a$ structures in adhesion of colon and urothelial cancer cells was pointed out by the authors. Direct evidence for binding of selectins to gangliosides came from studies with isolated natural glycolipids and neoglycolipids. Interactions of E-, P-, and L-selectins with neoglycolipids carrying sialyl Lewis$^a$ and sialyl Lewis$^x$ were observed in TLC immunostaining assays, ELISA assays, or after incorporation into liposomes (Takada *et al.*, 1991; Foxall *et al.*, 1992; Larkin *et al.*, 1992; Tyrrell *et al.*, 1991; Phillips *et al.*, 1990). The involvement of glycolipids carrying sialyl Lewis$^x$ and synthetic glycolipids bearing sialyl Lewis$^a$ tetrasaccharide in cell tethering and rolling was confirmed under shear flow (Alon *et al.*, 1995; Burdick *et al.*, 2001).

However, the question remained whether sialyl Lewis$^a$ and/or sialyl Lewis$^x$ gangliosides present on the surface of cancer cells can be bound directly by selectins, when mucins are present on these same cells. Using inhibitors of glycosphingolipid (d,l-*threo*-PPPP) and O-glycan (GalNAc-$\alpha$-O-benzyl) biosynthesis and electron microscopic analysis we showed that the majority of sialyl Lewis$^a$ oligosaccharides involved directly in binding to E-selectin-expressing cells are carried by mucin-type oligosaccharides (Laskowska *et al.*, 2001). Lipid-bound sialyl Lewis$^a$ is probably not accessible for the interactions with selectins and antibodies, when the cell surface is covered with mucins. However, sialyl

Lewis$^a$ gangliosides became involved in binding when such glycoprotein coat is removed from the cells. Based on those and other data (Kłopocki *et al.*, 1996; 1998), we propose that in contrast to leukocytes there is no specific ligand for selectins present on the surface of cancer cells. We hypothesise that adhesion of cancer cells to E-selectin can be mediated by glycoproteins as well as glycolipids carrying sialyl Lewis$^a$ structures, as long as the carbohydrate determinants are properly exposed and present in a sufficiently high density on the cell surface.

## INVOLVEMENT OF SIALYL LEWIS$^a$ IN TUMOR PROGRESSION

There are several lines of evidence showing that sialyl Lewis$^a$ and sialyl Lewis$^x$ may be involved in formation of metastases by human pancreatic and colon cancer cells. Higher amounts of dimeric sialyl Lewis$^x$ were detected on human lung adenocarcinoma cell sublines with high lung colonisation potential in nude mice (Inofusa *et al.*, 1991). Izumi *et al.* (1995) found that cell variants of human colon carcinoma line KM 12C with high expression of dimeric sialyl Le$^x$ and/or sialyl Le$^x$ adhere to a greater degree to endothelium, and are more efficient in colonisation of liver after intrasplenic injection. A correlation between increased expression of sialyl Lewis$^a$, higher binding to human and murine vascular endothelium, and high metastatic potential in nude mice was described for human colon carcinoma cells obtained either by repeated injections of cancer cells into the spleen (Yamada *et al.*, 1997), or by the limiting dilution method (Sato *et al.*, 1997). Monoclonal antibodies directed against sialyl Lewis$^a$ decrease the number of hepatic colonies after implantation of cancer cells into athymic nu/nu mice (Sato *et al.*, 1997; Kishimoto *et al.*, 1996). We obtained similar results with human colon cancer cells transfected with an antisense expression vector containing a fragment of cDNA for FT III, which treatment suppressed the expression of sialyl Lewis$^a$ in these cells (Kłopocki *et al.*, 1998). The number of liver metastases was markedly lower in nu/nu mice after orthotopic implantation of such sialyl Lewis$^a$-negative colon carcinoma cells than in the case of mice inoculated with parental cells (Opolski *et al.*, 1998a; 1998b).

However, we showed recently that the presence of sialyl Lewis$^a$ antigen on the surface of tumor cells and their adhesion to E-selectin-expressing cells in an *in vitro* adhesion assay can not be directly related to the metastatic properties of all cancer cells (Wietrzyk *et al.*, 2000). Two bladder cancer cell lines, HCV 29T and Hu 1703He, were transplanted into NCr nu/nu mice. Hu 1703He cells express high amount of sialyl Lewis$^a$ and adhere strongly to E-selectin, while HCV 29T cells are sialyl Lewis$^a$-negative and do not adhere to E-selectin (Kłopocki *et al.*, 1996). Both cell lines were found to form tumor colonies mainly in the liver and lung, independently of the route of implantation, i.e. intrasplenic, intravenous and sub-renal capsule inoculation. Our data suggest that the mechanisms of uroepithelial cancer metastasis are at least partially different from those of colon and pancreas cancer cells and the formation of liver metastases dependent on sialyl Lewis$^a$ is limited to these latter cells.

## SUMMARY

Sialyl Lewis$^a$ tetrasaccharide, after its discovery, was first described as a tumor-associated carbohydrate antigen and used as tumor marker in colon and pancreatic cancer. When the selectin family of adhesion molecules was described, it was found that sialyl Lewis$^a$, along with sialyl Lewis$^x$ antigen, is a ligand for E-selectin, responsible for adhesion of several types of human carcinoma cells to endothelium. A growing number of results suggests that such interactions play an impor-

tant role in the formation of metastases by colon and pancreatic cancer cells.

Enormous progress in the field of adhesion research has opened new possibilities in the treatment of malignant diseases by interfering with cell–cell and cell–substratum interactions. Anti-adhesion therapy directed to block the binding between sialyl Lewis$^a$ and E-selectin, molecules responsible for the adhesion between tumor cells and endothelium, is an interesting possibility to treat neoplastic diseases.

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