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Staining tumor cells with biotinylated ACL-I, a lectin isolated from the marine sponge, *Axinella corrugata*

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Abstract

Axinella corrugata lectin 1 (ACL-1) was purified from aqueous extracts of the marine sponge, *Axinella corrugata*. ACL-1 strongly agglutinates native rabbit erythrocytes. The hemagglutination is inhibited by *N*-acetyl derivatives, particularly *N*, *N*′, *N*″-triacetylchitotriose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine and *N*-acetyl-D-galactosamine. We investigated the capacity of biotinylated ACL-1 to stain several transformed cell lines including breast (T-47D, MCF7), colon (HT-29), lung (H460), ovary (OVCAR-3) and bladder (T24). ACL-I may bind to both monosaccharides and oligosaccharides of tumor cells, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine glycan types. The lectins are useful, not only as markers and diagnostic parameters, but also for tissue mapping in suspicious neoplasms. In addition, they provide a better understanding of neoplasms at the cytological and molecular levels. Furthermore, the use of potential metastatic markers such as lectins is crucial for developing successful tools for therapy against cancer. We observed that biotinylated ACL-I stains tumor cells and may hold potential as a probe for identifying transformed cells and for studying glycan structures synthesized by such cells.

Key words: *Axinella corrugata*, cancer, carbohydrate, lectin, marine sponge, transformed cells

The carbohydrate portions of glycoproteins act as cellular receptors for hormones and growth factors (Carvalho 1990, Kaltner and Stierstorfer 1998). There is greater potential for coding information in the structures of glycans than in proteins and nucleic acids. The glycans constitute recognition molecules owing to the wide variety of combinations possible from a few simple carbohydrates. Glycocode synthesis constitutes a code for the developmental plan of an organism, and selective unions of lectins and glycans can decipher the code (Gabius et al. 1996, Sol et al. 2006, Gemeiner et al. 2009).

Lectins show affinity for defined carbohydrate sequences and therefore can be employed as specific probes for both cell surface glycoconjugates and intracytoplasmic compartments that can be used for normal histology and histopathology. Because some carbohydrates do not elicit antibody production owing to their less complex structure, the importance of lectins increases further for histochemical studies of carbohydrates and membrane glycoconjugates (Danguy et al. 1988, Carvalho 1990, Grün et al. 2006).

Aub et al. (1963), who studied wheat germ agglutinin in mouse lymphoma, discovered the capacity of lectins to stain cancerous cells. Hirszfeld et al.

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(1929) and Thomsen (1930) demonstrated biochemical changes in tumors that later were shown to be aberrant tumor-associated glycosylation (Aub et al. 1965, Burger and Goldberg 1967, Inbar and Sachs 1969). These investigations provided early evidence that alterations of carbohydrate synthesis on the cell surface are associated with cancer and led to the hypothesis that lectins are susceptible to agglutination, although this property is not shared by all cancerous cells (Sharon and Lis 1972, 2004, Brown and Hunt 1978, Sharon 2007).

Transformed cells present a wide qualitative and quantitative variation in the glycosylation pattern of the membrane and the formation of carbohydrate clusters that occupy restricted areas of the cell surface, which allows their interaction with lectins. By contrast, this distribution is homogeneous in normal cells (Sharon and Lis 1972, Carvalho 1990).

Lectins may be important tools for obtaining information regarding the development, differentiation stage, malignancy level, and capacity for metastasis of tumor cells (Mody et al. 1995, Mitchell and Schumacher 1999, Wu et al. 2009), all of which affect prognosis for cancers including breast, stomach, esophagus, colon, thyroid, prostate and lung (Brooks and Wilkinson 2003). Furthermore, lectins have great potential as cancer markers and anticancer agents. Lectins already are used as probes for histopathological diagnosis of primary tumors and their metastasis in clinical situations (Mody et al. 1995) and for non-histopathological diagnosis of cancer (Leerapun et al. 2007, Zhao et al. 2007).

Our goal was to analyze the staining of different transformed cell lines by biotinylated *Axinella corrugata* lectin I (ACL-I) and to study the carbohydrate structural binding aspects of this lectin bound to tumor cells.

Materials and methods

Material

ACL-I was purified as described earlier (Dresch et al. 2008). *N*-hydroxy-succinimidobiotin, streptavidin-peroxidase, RPMI, orcinol, bovine serum albumin (BSA), fetal bovine serum (FBS), poly-L-lysine, GlcNAc, and pyruvate were obtained from Sigma-Aldrich (St. Louis, MO). DMEM, penicillin, streptomycin, L-glutamine, trypsin, HEPES, fungizone, garamycine and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY). *N*,*N*-dimethyl formamide, silica gel 60, glycerol and 4-chloro-1-naphthol were obtained from Merck (Darmstadt, Germany). Polyisobutylmethacrylate was purchased from Rohm & Haas (Darmstadt, Germany) and Alexa 488-streptavidin was obtained from Molecular Probes (Eugene, OR). All other chemicals were of reagent grade. All solutions were prepared with Milli-Q purified water (Millipore system, Bedford, MA), designated as water in the text.

Establishment of culture

The cell lines cultures were obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and were maintained in our laboratories.

The human glioblastoma, U138-MG, derived from spontaneously occurring human malignant gliomas, and C6 rat glioma cells, derived from *N*-nitrosomethylurea-induced glioma in rat, were grown and maintained in DMEM containing penicillin and streptomycin, 0.5 IU/ml supplemented with 5% (C6) or 15% (U138-MG) (v/v) FBS. Cells were seeded in 24-well plates at a density of 1×10^4 cells/well in 500 μl of medium per well. Cultured cells were maintained in 5% CO_2 in air at 37° C. The T24 human bladder cancer cell line was grown in culture flasks in RPMI 1640 medium 10% (v/v) in FCS. The H460 lung cancer and OVCAR-3 epithelial adenocarcinoma human ovary cells were grown in culture flasks in RPMI 1640 medium in 10% (v/v) FBS containing 200 IU/ml penicillin and 100 μg/ml streptomycin. The HT-29 colorectal adenocarcinoma, MCF7 mammary epithelial adenocarcinoma and T-47D ductal carcinoma mammary human cells were grown in culture flasks in DMEM in 10% (v/v) FBS and 1 mM pyruvate with 200 IU/ml penicillin and 100 μg/ml streptomycin. All other culture conditions were the same as was used for glioma cells.

The V79-4 Chinese hamster lung fibroblasts were cultured in DMEM supplemented with 10% heat-inactivated FBS, 0.2 mg/ml L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were maintained in culture flasks at 37° C in a humidified atmosphere containing 5% CO_2 in air and were harvested by treatment with 0.15% trypsin and 0.08% EDTA in PBS. Cells (3×10^6) were seeded in 5 ml of complete medium in a 25 cm^2 flask and grown for 2 days until they reached confluence.

Primary rat astrocyte cultures were prepared as described previously (Wink et al. 2006). All animal procedures were approved by the local Animal Care Committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Healthy male Wistar rats were obtained from our own breeding colonies at the Departamento de Bioquímica da Universidade Federal do Rio Grande do Sul (Porto Alegre, Brazil). Animals were housed in cages under conditions of optimal light

(12:12 h light:dark cycle), temperature (22 ± 1° C), and humidity (50–60%). Cortex of newborn rats (1-2 days old) was removed, and dissociated mechanically in a Ca^{2+}- and Mg^{2+}-free balanced salt solution, pH 7.4, that contained 137 mM NaCl, 5.36 mM KCl, 0.27 mM Na_2HPO_4, 1.1 mM KH_2PO_4 and 6.1 mM glucose. After centrifugation at 1000 × g for 5 min, the pellet was re-suspended in culture medium (pH 7.6) containing 10% DMEM, 8.39 mM HEPES, (pH 7.6), 23.8 mM $NaHCO_3$, 0.1% fungizone, 0.032% garamicine and 10% FBS. The cells were plated at a density of 1.5 × 10^5 cells/cm^2 in 24-well plates pre-treated with poly-L-lysine. Cultures were maintained in 5% CO_2 and air at 37° C, and grown to confluence and used at 21–28 days in vitro. Medium was changed every 3–4 days.

Biotinylation of ACL-I

Purified ACL-I was biotinylated according to Jorgensen et al. (2000) using a solution of 200 μg/ml lectin in phosphate-buffered saline (PBS). After biotinylation, the material was centrifuged at 20000 x g for 10 min at 4° C and freeze-dried until use. After processing, the biotinylated lectin retained its hemagglutinating activity and its specificity toward *N*-acetylated carbohydrates.

Biotinylation was verified using 0.2 μg biotinylated lectin and a lectin sample that was not biotinylated. Both were applied to a nitrocellulose membrane that was treated for 15 min with PBS containing 0.1% Tween® 20. The material was washed three times with PBS, then incubated for 30 min with a solution containing 1 μg/ml streptavidin-peroxidase in water and glycerol (1:1, v/v) diluted with PBS (1:1000, v/v) containing 0.05% Tween® 20. The material was washed again with PBS and developed in the dark for 3 min with 20 mM Tris, pH 7.5, that contained 0.015% 4-chloro-1-naphthol and 0.03% hydrogen peroxide. The formation of a blue-violet to ash color indicated successful biotinylation. Biotinylated BSA was used as a positive control.

High performance thin layer chromatography (HPTLC)-lectin staining

Glycolipids (N4 = GalNAcβ1,4GlcNAcβ1,3mannoseβ1,4glucoseβ-ceramide, N5a = GalNAcα1,4GalNAcβ1,4GlcNAcβ1,3mannoseβ1,4glucoseβ-ceramide, N5b = galactoseβ1,3GalNAcβ1,4GlcNAcβ1,3mannoseβ1,4glucoseβ-ceramide and N7 = GlcNAcβ1,3galactoseβ1,3GalNAcα1,4GalNAcβ1,4GlcNAcβ1,3mannoseβ1,4glucoseβ-ceramide) were separated using HPTLC silica gel 60 in a running solvent of chloroform: methanol:aqueous 0.2% $CaCl_2$ (45:45:10) using a tank to obtain reproducible chromatograms. Plates were air dried for 15 min, coated by dipping in a 0.5% solution of polyisobutylmethacrylate (Plexigum P28) in hexane:chloroform (9:1) for 1 min, air dried for 10 min, washed with PBS with 0.1% Tween® 20, and incubated with 2 μg/ml biotin-ACL-I overnight at 4° C with agitation. After washing with PBS, the plates were incubated with 1 μg/ml streptavidin-peroxidase in PBS with 0.05% Tween® 20 for 60 min at room temperature in the dark and washed three times with PBS. The color reaction was developed using 20 mM Tris, 0.015% 4-chloro-1-naphthol and 0.03% hydrogen peroxide in methanol:PBS (1:29) for 20 min, and stopped by washing with water. As a positive control, after development of the chromatogram, the glycolipids were visualized chemically using 0.5% orcinol-3 N sulfuric acid reagent at 100° C for 10 min (Irazoqui et al. 2005).

Fluorescence staining of cells using ACL-I as probe

Cells were grown on glass slides in medium to approximately 60–70% confluence as recommended by the ATCC. Cells were washed with PBS and fixed in ice-cold acetone for 5 min, incubated with 1% BSA in PBS for 1 h at room temperature followed by incubation in 200 μg/ml biotin-ACL-I diluted 1:10 with 1% BSA in PBS for 2 h at room temperature. The material was washed again with PBS and incubated with Alexa 488-streptavidin diluted 1:1000 (v/v) in PBS with 0.05% Tween® 20, for 35 min in the dark (Irazoqui et al. 2005). The cells then were washed rapidly with PBS and mounted on a slide with Canada balsam and coverslipped. Stained fluorescent cells were examined with a Carl Zeiss Axioplan fluorescence microscope (Göttingen, Germany) and Nikon Eclipse TE300 (Tokyo, Japan).

Inhibition of lectin activity was assayed by prior incubation of biotinylated ACL-I with GlcNAc (200 mM) for 30 min at room temperature, which then was added to the cells. Cellular material blocked with 1% BSA in PBS without incubation with biotinylated lectin was used as a negative control. All experiments were carried out in triplicate.

Results

HPTLC-lectin staining

Biotinylated ACL-I interacted with high affinity for all glycolipids used in the HPTLC-lectin staining assay: N4, N5a, N5b and N7 (Fig. 1).

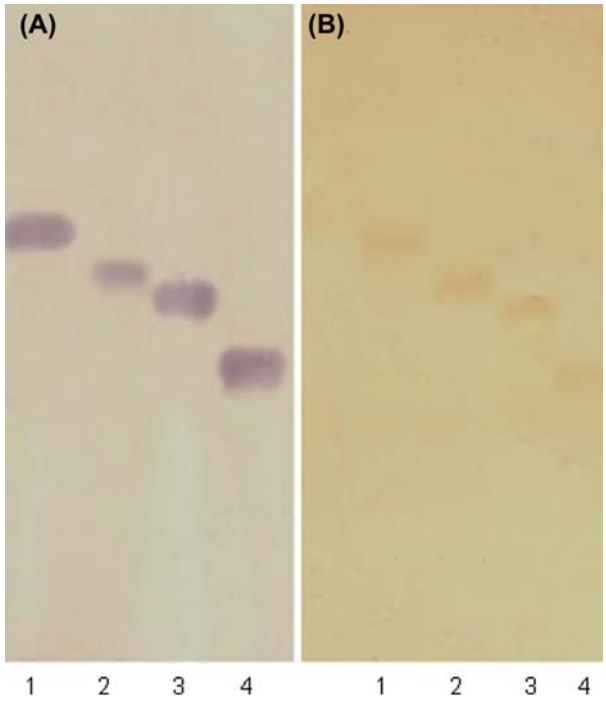

Fig. 1. A) Glycolipids visualized by lectin interaction by incubation with 2 μg/ml biotin-ACL-I. The color reaction was developed using 20 mM Tris, 0.15 mg/ml 4-chloro-1-naphthol and 0.03% hydrogen peroxide in methanol–PBS (1:29) for 20 min. B) Glycolipids visualized using orcinol-sulfuric acid reagent for 10 min at 100° C as positive control.

Fluorescence staining of cell lines

Biotinylated ACL-I was used to stain eight transformed cell lines in addition to Chinese hamster lung fibroblasts and primary astrocyte cultures (Table 1). Biotinylated ACL-I stained HT-29, T-47D, OVCAR-3, H460 and T24 lines; it stained the MCF7 cells with the greatest intensity. By contrast, biotinylated ACL-I did not stain the V79-4 cell, a line that is not cancerous (Figs. 2 and 3).

There was no labeling of astrocyte cells or the C6 cell line, both of which are derived from the central nervous system. Similarly, no labeling of the U138-MG line occurred (results not shown).

Discussion

Carbohydrates exhibit a great diversity of oligosaccharide configurations derived from a small number of monomers and linkages. Glycosidic linkages, for example, can be α- or β-anomeric and different hydroxyl C-positions on the adjacent monosaccharide occur. Furthermore, oligosaccharide chains can be branched with connections between two portions of carbohydrate through 1–2, 1–3, 1–4 or 1–6 linkages. *N*- or *O*-linked glycosylation may occur, as well and glycosidic linkages are flexible. All of these factors produce a wide variety of carbohydrate topologies for recognition by lectins that are fundamentally different from the ADN–RNA–protein information pathway, whose information is strictly linear. Consequently, the formation of complexes with distinct stoichiometry is considered a prerequisite for initiation of ensuing signaling events that can trigger a variety of post-binding events (Gabius et al. 1996, Kaltner and Stierstorfer 1998, Loris 2002, Irazoqui et al. 2005).

The monosaccharides that occur predominantly in membrane glycoconjugates are D-mannose, D-galactose, L-fucose, D-glucosamine, D-galactosamine and their acetylated derivatives, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid (Smets and Beek 1983, Wu et al. 2009). Alterations of cellular glycosylation produce altered cell behavior that is associated with metastatic potential and poor prognosis in various human adenocarcinomas (Singh et al. 1999, Brooks and Carter 2001, Brooks and Hall 2002). Increased synthesis of carbohydrates by human cells has been reported in melanoma, leukemic cells, uterine cervical tumors, and lung, stomach,

Table 1. Results of cells stained by biotinylated ACL-I

Cell lines	Denomination	Origin	Staining
–	Primary rat astrocyte cultures (*Rattus norvegicus*)	–	Negative
V79-4	Chinese hamster lung fibroblast (*Cricetulus griseus*)	ATCC (CCL-93)	Negative
C6	Rat glioblastoma (*Rattus norvegicus*)	ATCC (CCL-107)	Negative
U138-MG	Human glioblastoma	ATCC (HTB-16)	Negative
H460	Human lung cancer	ATCC (HTB-177)	Positive
HT-29	Human colorectal adenocarcinoma	ATCC (HTB-38)	Positive
MCF7	Human epithelial adenocarcinoma mammary	ATCC (HTB-22)	Positive
T-47D	Human ductal carcinoma mammary	ATCC (HTB-133)	Positive
OVCAR-3	Human epithelial adenocarcinoma ovary	ATCC (HTB-161)	Positive
T24	Human bladder cancer	ATCC (HTB-4)	Positive

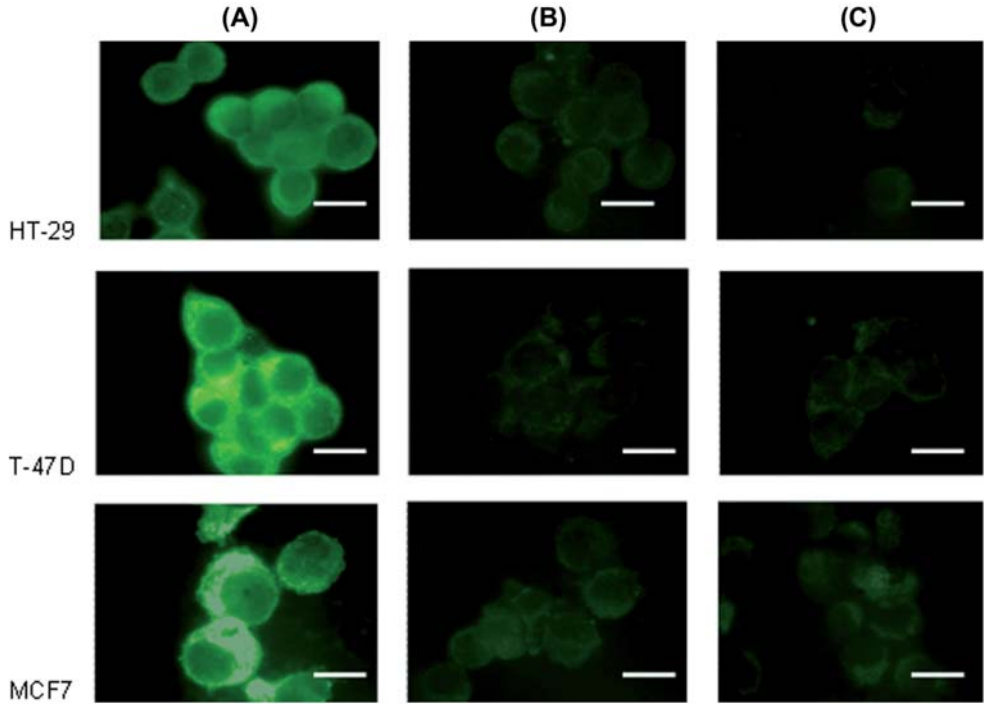

Fig. 2. Fluorescence staining of cells using biotinylated ACL-I as probe. Lectin staining was assayed without (C) or with (A) 200 μg/ml biotin-ACL-I in 1% BSA in PBS. For inhibition of cell-lectin staining, the biotin-ACL-I sample was incubated previously with GlcNAc (200 mM) in PBS (B). Samples were washed with PBS and incubated with Alexa 488-streptavidin (1 μg/ml) for 35 min in the dark followed by a rapid wash with PBS. Stained fluorescent cells were photographed with a Carl Zeiss Axioplan fluorescence microscope equipped with a 75W xenon lamp using epi-illumination. Scale bar = 12 μm.

breast, colon, liver and prostate neoplasms (Smets and Beek 1983, Cheresh et al. 1984, Fernandes et al. 1991, David et al. 1992, Ogawa et al. 1992, Brockhausen et al. 1995, Jorgensen et al. 1995, Whitehouse et al. 1997, Burchell et al. 1999, Irimura et al. 1999, Singh et al. 1999, 2001, Thies et al. 2001a,b, Peracaula et al. 2003, Pal et al. 2004, Gisbergen et al. 2005, Sakuma et al. 2006, Lescar et al. 2007). Another possible mechanism is the attachment of carbohydrates from tumor cells through the endothelium by the binding of selectins; this also represents an early stage of the metastatic process (Brockhausen et al. 1998).

The recognition of carbohydrates by lectins is complex and selective, because their preferred and natural binding ligands often are far more complex than mono- or disaccharides. The three-dimensional spatial arrangement of glycans may be critical for this recognition. Lectins with the same preferred carbohydrate binding partner may recognize different arrays of complex, naturally occurring oligosaccharide structures (Brooks and Carter 2001). Because ACL-I recognizes GlcNAc and GalNAc, it could be a useful tool for studying changes in the pattern of cellular glycosylation. Brooks and Carter (2001) verified that *Helix pomatia* agglutinin (HPA) GalNAc-binding, and *Griffonia simplicifolia* agglutinin II (GSA II) GlcNAc binding labeled sections of breast cancer. Furthermore, studies by Brooks et al. (2001) revealed intense staining of cells of the MCF7 line by HPA. Thus, it is possible that biotinylated ACL-I, which also possesses affinity for GalNAc, stained the T-47D and MCF7 lines via recognition of GalNAc.

Results comparable to those of our study were obtained by Mitchell et al. (1998) who studied T-47D and MCF7 lines using *Phaseolus vulgaris* leucoagglutinin (PHA-L) lectin specific for GlcNAc oligosaccharides and HPA. Both lectins stained the metastatic breast cell lines. The intense labeling of biotinylated ACL-I in the MCF7 line indicates the possibility of recognition of other carbohydrates besides GalNAc, such as GlcNAc, because the MCF7 cell membrane synthesizes related carbohydrates including Tn (GalNAcα1-*O*-serine/threonine), T (galactoseβ1,3GalNAcα1-*O*-serine/threonine), sialyl-T (sialylα2,3-galactoseβ1,3GalNAcα1-*O*-serine/threonine) and *O*-glycan core-2 antigens (β1,3-linked galactose and β1,6-linked GlcNAc) (Devine et al. 1992, Brockhausen et al. 1995). Furthermore,

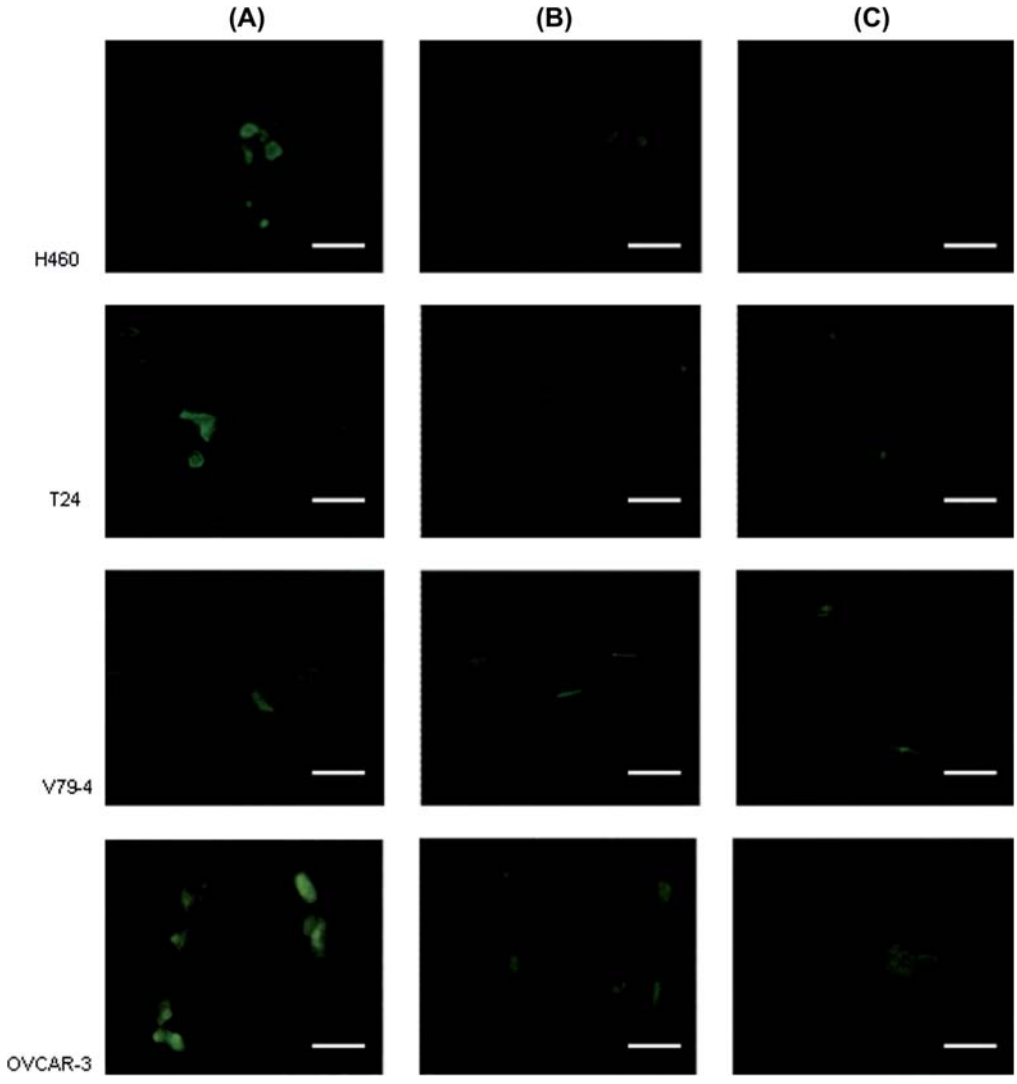

Fig. 3. Fluorescence staining of cells using biotinylated ACL-I as probe. Lectin staining was assayed without (C) or with (A) 200 μg/ml biotin-ACL-I in 1% BSA in PBS. For inhibition of cell-lectin staining, the biotin-ACL-I sample was incubated previously with 200 mM GlcNAc in PBS (B). Samples were washed with PBS and incubated with Alexa 488-streptavidin (1 μg/ml) for 35 min in the dark followed by a rapid wash with PBS. Stained fluorescent cells were photographed with a Nikon Eclipse TE300 fluorescence microscope. Scale bar = 60 μm.

compared to MCF7, the *O*-glycan core-2 is the only antigen that is not synthesized by the T-47D line.

The capacity of lectins for labeling cancerous cells with overexpressed GalNAc glycoconjugates also was investigated by Irazoqui et al. (2005) using *Euphorbia milii* lectin (biotin-EML), which stained the HT-29 and T-47D lines. Moreover, Gabor et al. (1998) demonstrated that WGA and *Solanum tuberosum* lectin (STL) conjugated with fluorescein, both *N*-acetylglucosamine-specific lectins, stained the HT-29 line intensively, as was the case for ACL-I. Sakuma et al. (2009) prepared peanut agglutinin (PNA)-immobilized fluorescent nanospheres with surface poly-*N*-vinylacetamide (PNVA) chains and observed intense staining of T-47D and HT-29 lines, as observed with biotinylated ACL-I.

From previous studies, it may be inferred that ACL-I recognizes GalNAc and GlcNAc carbohydrates in the HT-29 line, because these cells synthesize related glycoconjugates in their membranes, such as Tn, T, sialyl-T, sialyl-Tn, sialyl Lewis x (sialylα2,3galactoseβ1,4(fucoseα1,3)GlcNAc), sialyl Lewis a (sialylα2,3galactoseβ1,3(fucoseα1,4)GlcNAc) and sialyl dimeric Lewis x (sialylα2,3galactoseβ1,4GlcNAc (fucoseα1,3)β1,3Galactoseβ1,4GlcNAc(fucoseα1,3)) antigens (Majuri et al. 1995, Huet et al. 1998, Singh et al. 2001, Lescar et al. 2007).

Biotinylated ACL-I also labeled cells of the H460 and T24 lines. Binding was more intense in T24 cells

in our study than that obtained with HPA in two lines of bladder carcinoma (T24 and RT-4) (Hammarström 1973); however, we found no reports in the literature that other lectins labeled H460 cells.

The OVCAR-3 line also was stained by biotinylated ACL-I, which probably recognized GlcNAc and GalNAc derivatives, because OVCAR-3 cells synthesize T and Tn antigens related to GalNAc (Avichezer et al. 1997), and MX35 antigen, a glycoprotein containing GlcNAc (Welshinger et al. 1997).

Biotinylated ACL-I did not stain any cells of the central nervous system, because the carbohydrate, *N*-acetyl-D-lactosamine (LacNAc), is synthesized on the cell membrane of these cells (Sasaki et al. 2002) and is a weak inhibitor of ACL-I. Thus, the LacNAc glycans (Gal1,4GlcNAc), characteristic of gliomas, are not labeled by ACL-I.

Few reports describe lectins isolated from marine sponges for labeling transformed cells. Opric et al. (1996) studied the labeling of sections from human breast, thyroid and melanoma tumors with *Chondrilla nucula* and *Geodia cydonium* lectins. In both cases, the binding was neither sufficiently specific nor consistent. *Chondrilla nucula* and *Geodia cydonium* lectins stained only a few sections of benign breast cancer and malignant lesions.

Neutral glycosphingolipid assays confirmed the results obtained from transformed cell binding. Biotinylated ACL-I interacts with N5a, which contain a GalNAc as the terminal nonreducing monosaccharide; this is present also in the Tn antigen, which is synthesized in HT-29, MCF7, OVCAR-3 and T-47D cells (Devine et al. 1992, Brockhausen et al. 1995, Avichezer et al. 1997, Lescar et al. 2007), all of which are stained by biotinylated ACL-I. Furthermore, biotinylated ACL-I recognizes N7 glycosphingolipids that contain the subterminal Galβ1,3GalNAcα disaccharide that corresponds to the T antigen and also is synthesized in both cells (HT-29, MCF7, OVCAR-3 and T-47D cells) (Devine et al. 1992, Brockhausen et al. 1995, Avichezer et al. 1997, Singh et al. 2001). Moreover, the glycosphingolipids, N4, N5a and N5b, possess terminal or subterminal GalNAcβ1,4GlcNAc in their structure, which is another possible binding site for ACL-I. Lectin assays with glycosphingolipids also have been carried out by Irazoqui et al. (2005) using EML. EML interacted with high affinity to N4 and N5a, which possess GalNAc as their terminal nonreducing monosaccharide; these results are similar to those obtained with biotinylated ACL-I.

We also isolated *Arundo donax* (ADL) lectin from rhizomes of *Arundo donax* using affinity chromatography on a rabbit stroma-polyacrylamide gel column. This lectin was biotinylated and stained HT-29 and T24 lines (unpublished results), as was the case with biotinylated ACL-I. Furthermore, ADL is inhibited by *N*, *N*′, *N*″- triacetylchitotriose and GlcNAc carbohydrates, which also inhibited ACL-I.

It is possible that ACL-I may label glycan tumor cell structures containing terminal GalNAc and GlcNAc monosaccharides or oligosaccharides such as GlcNAcβ1,4 (monomeric units of *N*, *N*′, *N*″- triacetylchitotriose, the best inhibitor tested for ACL-I according to Dresch et al. 2008), GlcNAcβ1,6 branching, GalNAcβ1,4GlcNAc, Galβ1,3GalNAc and Galβ1,3GlcNAc.

Our study revealed that ACL-I potentially may be used as a probe for identifying transformed cells. Studies of sections of human carcinoma tissues should be undertaken with ACL-I to verify its prognostic value for estimating metastatic probability. Furthermore, the capacity of ACL-I to bind tumor cells (HT-29, MCF7, T-47D, OVCAR-3, H460 and T24) means that it potentially is a useful tool for studying some over-synthesized glycoconjugates.

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