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Galectin-1 Induces Cell Adhesion to the Extracellular Matrix and Apoptosis of Non-Adherent Human Colon Cancer Colo201 Cells

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To isolate cDNAs for molecules involved in cell adhesion to the extracellular matrix, expression cloning with non-adherent colon cancer Colo201 cells was carried out. Four positive clones were isolated and, when sequenced, one was found to be galectin-1, a β-galactoside–binding protein. When cultured on fibronectin-, laminin-, and collagen-coated and non-coated dishes, the adherent galectin-1 cDNA-transfected Colo201 cells increased and spread somewhat. Immunofluorescence staining revealed that galectin-1 was expressed inside and outside of Colo201 cells. The adhesion was dependent on the carbohydrate-recognition domain of galectin-1 since lactose inhibited the adhesion and exogenously-added galectin-1 caused the adhesion. PD58059, an inhibitor of mitogen-activated protein kinase, or LY294002, a phosphoinositide 3-OH kinase inhibitor, decreased the adhesion. Furthermore, the expression of galectin-1 in Colo201 cells induced apoptotic cell death, while exogenously-added galectin-1 did not cause apoptosis. These results indicate that galectin-1 plays a role in both cell–matrix interactions and the inhibition of Colo201 cell proliferation, and suggest that galectin-1 expressed in cells could be associated with apoptosis.

Key words: apoptosis, cell adhesion, Colo201 cells, expression cloning, galectin-1.

Abbreviations: ECM, extracellular matrix; CRD, carbohydrate recognition domain; kDa, kilodalton(s); FCS, fetal calf serum; SDS-PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PCR, polymerase chain reaction; PVDF, poly(vinylidene difluoride); GSH, glutathione; GST, glutathione S-transferase.

Extracellular matrix (ECM) components have marked effects on cellular morphology, cell migration, growth and differentiation, suggesting the existence of transmembrane linkages and signal transduction pathways that can relay information from the ECM to the nucleus (*1*). Cells in culture adhere to ECM components and a large number of cytoplasmic proteins, some of which are likely to play a role in an intracellular response to extracellular signals (*1*, *2*). However, the necessity of generating diverse junctions on individual cells must require highly interconnected signaling cascades that can modulate responses between various pathways.

Galectin-1 is an endogenous β-galactoside binding protein (galectin family), and is produced by a variety of normal and neoplastic cells (*3*). The galectin family share jelly roll–like folding and a common topology of the carbohydrate recognition domain (CRD) with high-sequence homology among mammals (*3*, *4*). Galectins are secreted *via* a non-classical pathway, and interact with the extracellular glycans of cell-surface components including laminins, fibronectins and integrins (*3*, *4*). Galectin-1 plays a role in several biological and pathological processes, including cell growth regulation, cell–matrix interactions and T-cell apoptosis (*5*). In cells, galectins are located in the cytosol and nucleus, interacting with several signal proteins (*6*). With respect to colon tumor biology, the presence of galectin-1 and galectin-3 has so far been investigated histopathologically using different methods, the results being divergent (*7*). Namely, tumors express different levels and kinds of galectin. It is considered that galectins may be attractive targets for the development of new therapeutic strategies since most glycans are located outside cells where cell–cell or cell–matrix interactions occur and play a role in the normal maintenance of multi-cellular organisms (*4*, *7*).

Colo201 cells, which were isolated from the ascites fluid of a patient with an adenocarcinoma of the colon, are part of a unique cell line that has lost its epithelial appearance and exhibits a non-adherent morphology (*8*). Colo201 cells are a useful tool for clarifying the mechanisms involved in cell adhesion and the metastatic mobility of adenocarcinoma cells. We have screened cell adhesion–related molecules with non-adherent colon cancer Colo201 cells and found that they acquired the characteristics of adherent cells when treated with retinoic acid (*9*) and protein kinase inhibitors (*10*). The expression of constitutively active forms of GTP-binding proteins, Rac1 and Cdc42, resulted in selective increases in α6β4 integrin expression, leading to the induction of adhesion and membrane ruffles of Colo201 cells (*11*). Thus, Colo201 cells possess cytoplasmic signaling pathways that regulate integrin activation and have the ability to

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become adherent cells. In a search for the molecular mechanisms responsible for the abnormal adherent properties of Colo201 cells, we carried out expression cloning to obtain factors promoting the adhesion of Colo201 cells, and isolated galectin-1 as an adhesion molecule. We here report that galectin-1 not only caused the adhesion of Colo201 cells *via* sugar-dependent interactions with extracellular proteins but also led to apoptotic cell death.

MATERIALS AND METHODS

Materials—The mammalian expression HeLa cell cDNA library (pcDNA3) was a product of Invitrogen (Carlsbad, CA). Restriction endonucleases and DNA modifying enzymes were obtained from Takara (Kyoto) and Toyobo (Tokyo). Anti–c-Myc (clone: 9E10) monoclonal and anti-caspase 3 antibodies were purchased from Sigma (St. Louis, MI) and BD Biosciences (San Jose, CA), respectively. FluoroLink-Cy2 labeled anti-mouse IgG was from Amersham-Pharmacia (Buckinghamshire, UK). Tissue culture dishes coated with collagen (type I), laminin and fibronectin were the products of BD Bioscience Co. PD58059 and LY294002 were purchased from Calbiochem-Novabiochem (San Diego, CA). Other reagents used were of analytical grade.

Cell Culture and DNA Transfection—Human colon cancer cell line Colo201, obtained from the Japan Cell Bank (Tsukuba), was grown in RPMI 1640 medium supplemented with 10% FCS (Invitrogen, Carlsbad, CA), 100 U/ml penicillin and 50 μg/ml streptomycin (*9*). The cells were transfected using SuperFector reagent (B-Bridge, San Jose, CA). Plasmids carrying various cDNAs were also transfected into Colo201 cells, by electroporation. Namely, aliquots of 10^7 cells were electroporated at 330V and 1,000 μF with 10 μg of DNA containing various amounts of appropriate plasmids, as indicated, and the pUC18 vector as a mock DNA, using an Invitrogen electroporator II according to the manufacturer's instructions.

Screening of Cell Adhesion–Related Molecules—To isolate molecules causing cell adhesion, Colo201 cells were transfected with 40 μg of the plasmid-containing cDNAs (pcDNA3) by electroporation. After incubation for 16 h, the cells were treated with 0.05% trypsin/0.2% EDTA to disperse them. The cells were then washed three times with RPMI 1640 medium, re-suspended in RPMI 1640 medium containing 10% FCS and incubated for 2 h. The medium was removed and the adherent cells were washed three times with RPMI 1640 medium to separate non-adherent cells. The adherent cells were lysed with 10 mM Tris-HCl containing 2% SDS and 10 mM EDTA (*12*). The solubilized solution was treated with phenol/chloroform/isoamylalcohol (25/24/1, v/v/v), followed by treatment with chloroform/isoamylalcohol (24/1, v/v). DNA was precipitated with ethanol. After washing with 70% ethanol, the DNA was resolved in 10 μl of sterile water, and then transformed into *E. coli* XL-1 Blue by electroporation to amplify the plasmid. These screening treatments were repeated three times. At the final screening step, plasmids were isolated from several clones. The DNA sequences obtained were analyzed by means of a computer-assisted Blast-nucleotide sequence search.

Plasmids—To replace the vector of pcDNA3-galectin-1 with pcDNA3(c-myc), pcDNA3-galectin-1 was digested with *Eco*RI and *Hin*dIII, and then the insert was ligated into *Eco*RI–*Hin*dIII–digested pcDNA3.1(–)/Myc-His B (Invitrogen). The resulting plasmid [pcDNA3(c-myc)-galectin-1] was transformed into *E. coli* XL1-Blue. To construct GST fusion protein expression plasmids, portions of human galectin-1 cDNA were amplified by PCR and the resulting fragments were ligated into the pGEX-4T vector (Amersham-Pharmacia Biotech). The primers used were AAGGATCCTGTGGTCTGGTCGCC and AAGTCGACTCAAAAGGCCACACATTTGAT. The plasmid thus constructed was pGEX/galectin-1 encoding GST-galectin-1, *i.e.* galectin-1 fused with GST. The protein was expressed in *E. coli* (strain: DH-5α) with 0.3 mM isopropyl-1-thio-β-D-galactoside at 37°C for 2 h.

Adhesion of Cells to the Cell Matrix—Bacteria expressing GST-galectin-1 were re-suspended in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween 20, 10% glycerol, 1mM DTT, and 0.1 mM PMSF), and then lysed by brief sonication. The GST fusion proteins were purified with GSH-Sepharose 4B beads in accordance with the manufacturer's instructions (*13*). To assay the binding of Colo201 cells to the matrix, GST and GST-galectin-1 protein were added to RPMI 1640 medium containing 1% FCS. Cells on collagen-coated dishes were incubated at 37°C for 2 h, after which they were washed five times with RPMI 1640 medium. After the final wash, the cells were treated with 0.02% trypsin and then counted.

Immunostaining—For immunostaining, adherent cells were fixed with 4% paraformaldehyde in PBS containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ [PBS(+)] for 20 min, and permeabilized with 0.1% Triton-X100 in PBS (+) for 30 min. The cells were then incubated with anti–c-Myc as described (*13*). After washing with PBS (+), they were incubated with fluorolink Cy2-labeled anti-mouse IgG (Amersham Pharmacia Biotech.). To stain the cell-surface, the treatment with Triton X-100 was omitted. Fluorescence microscopy was performed with a Carl-Zeiss LSM510 microscope (Tokyo).

Immunoblotting—The lysates of Colo201 cells transfected with the empty vector pcDNA3 and pcDNA3 (c-myc)-galectin-1, respectively, were also subjected to SDS-PAGE and electroblotted onto a PVDF membrane. Immunoblotting was performed with anti–c-myc-tag or anti–caspase 3. The protein concentration was estimated by Bradford's method, using bovine serum albumin as the Standard (*14*).

TUNEL Assay—To detect DNA fragmentation in apoptotic cells by direct end-labeling of cellular genomic DNA with fluorescein-conjugated dUTP using terminal deoxynucleotidyltransferase enzyme, TUNEL assays were performed with an in situ apoptosis detection kit (Takara) (*15*).

Cell-Viability Assay—After transfection of the plasmid, the cells were cultured. At indicated times, aliquots of the cell suspension were withdrawn, and the live and dead cells were counted by means of trypan blue exclusion. The percentage of dead cells relative to the control was determined.

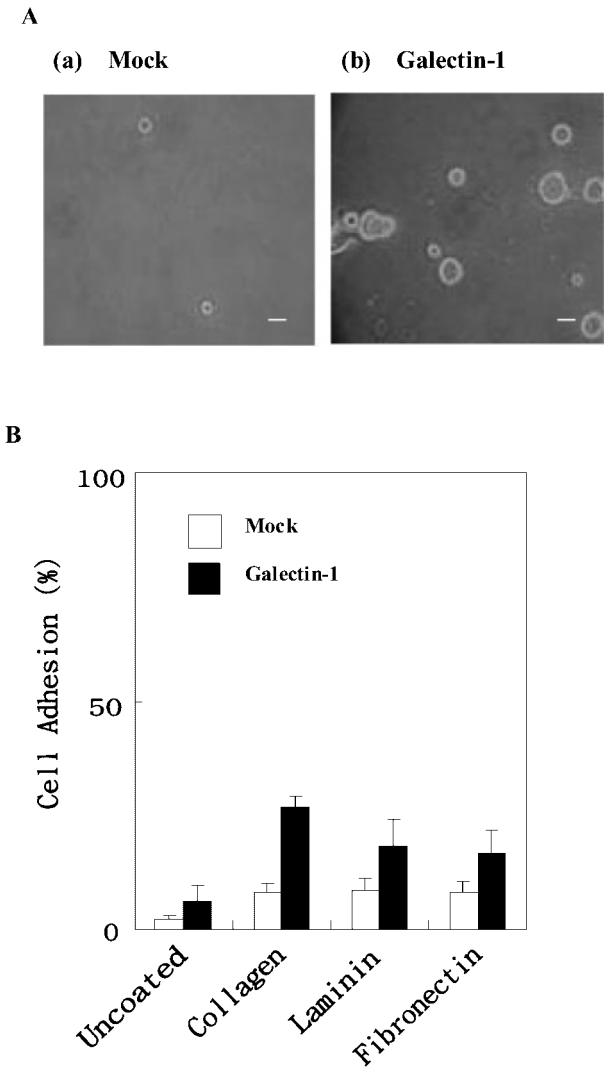

Fig. 1. **A: Morphology of adherent Colo201 cells expressing galectin-1.** Cells were transfected with 20 μg of galectin-1 (a) and mock (b) cDNA. After 16 h-incubation, the cells were washed and adherent cells were photographed at ×100. Bars = 20 mμ. **B: The cell adhesion of Colo201 cells to ECM on transfection with galectin-1 cDNA.** Galectin-1–transfected cells, seeded on uncoated, and collagen-, laminin-, and fibronectin-coated dishes, were incubated for 2 h. After the cells had been washed five times with RPMI 1640 medium, the adherent cells were counted.

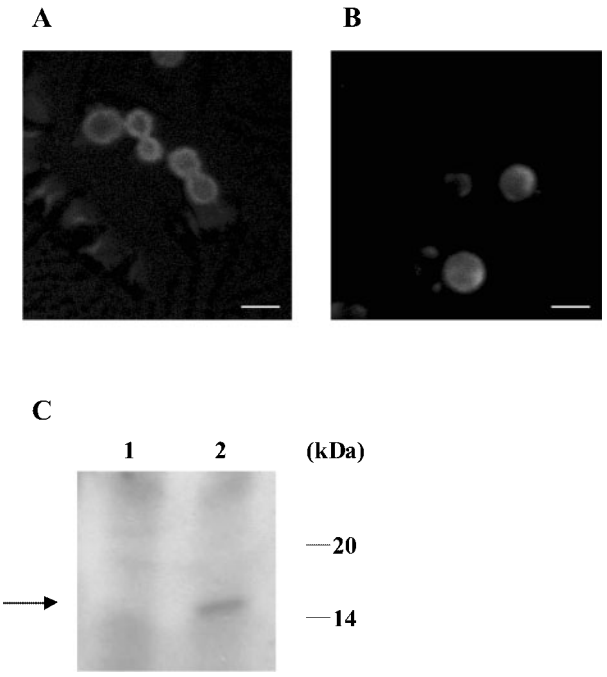

Fig. 2. **Expression of galectin-1 in Colo201 cells.** Cells were transfected with galectin-1 cDNA and the incubated for 16 h. After washing the cells, they were fixed, permeabilized and then stained with a monoclonal antibody for c-myc (A). Bars=20 mμ. The cells were also stained without permeabilization (B). (C) The cells were lysed and immunoblotting with anti–c-myc was performed after the cellular proteins had been analyzed by SDS-PAGE. The cells were transfected with mock (lane 1) and galectin-1 (lane 2) cDNA.

RESULTS

Isolation of Cell Adhesion Molecules with Non-Adherent Colon Cancer Colo201 Cells.—To isolate adhesion molecules, expression cloning was carried out by means of transfection of pcDNA3 vectors carrying human HeLa cell cDNAs into human colon cancer Colo201 cells, based on ability of adhesion of a single transfected cell to the matrix. The plasmids from pools of cDNA clones (10^4 clones) were transiently transfected into Colo201 cells. After 16h-incubation, the medium and non-adherent cells were removed, and the remaining adherent cells were treated with trypsin. After washing the cells twice with the medium, they were cultured for 2 h in RPMI 1640 medium containing 10% FCS. The plasmids from the adherent cells were recovered and amplified in *E. coli*. After three repeated screenings with 3×10^5 independent clones, four positive clones causing cell adhesion of Colo201 cells were obtained, and the DNA was isolated and sequenced. A computer-assisted blast search with the DNA sequences revealed that the four clones corresponded to galectin-1, RhoG, and ribosomal proteins L18 and 27. The cDNAs for ribosomal proteins did not contain the entire protein-coding region, but galectin-1 and RhoG did contain the entire protein-coding region. We (*11*) previously reported that the active forms of Rac-1 and Cdc42 promote the adhesion and spreading of Colo201 cells. Analogous to these GTP-binding proteins, another GTP-binding protein, RhoG, is considered to play a role in the adhesion. Here we were interested in galectin-1 cDNA as the adhesion molecule and thus further examined its properties in detail. Human galectin-1 consists of 125 amino acid residues with the CRD in the central region of the protein. Galectins are known to interact with cell-surface proteins and matrix proteins *via* the CRD (*3*, *4*). Figure 1A shows the changes in the morphology of Colo201 cells on the expression of galectin-1. When cultured on laminin-coated dishes, the cells adhered and spread somewhat. Galectin-1 cDNA was transfected into Colo201 cells, and the cells were cultured in fibronectin-, laminin-, and collagen-coated flasks. As shown in Fig. 1B, on the non-coated dishes, the numbers of adherent cells increased about 3-fold with the expression of galectin-1. On laminin-, fibronectin-, and collagen-coated dishes, the

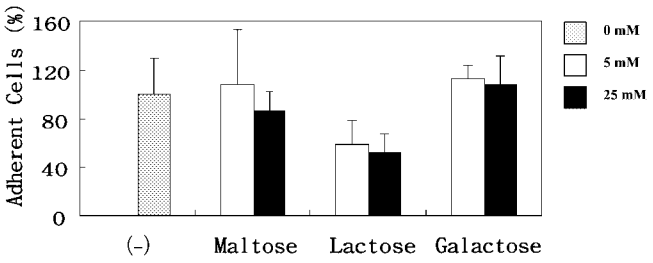

Fig. 3. **Effects of carbohydrates on the adhesion of Colo201 cells expressing galectin-1.** Cells transfected with galectin-1 cDNA were incubated for 16 h. After the cells had been collected and washed three times with RPMI 1640 medium, they were seeded onto laminin-coated dishes in RPMI 1640 medium containing the indicated concentrations of lactose, maltose and galactose, and then incubated for 2 h. The cells were washed five times with RPMI 1640 medium and then the adherent cells were counted. The concentrations of sugars used were 5 mM (open bars) and 25 mM (solid bars).

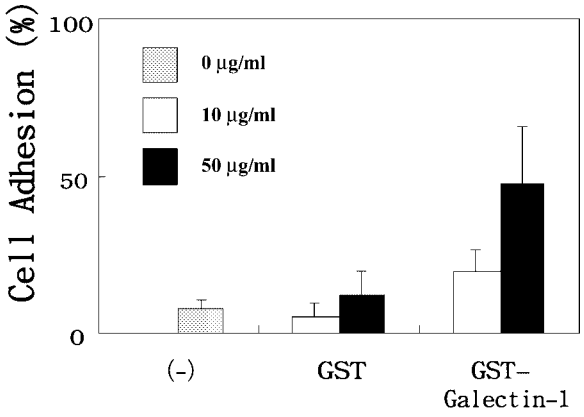

Fig. 4. **Effect of exogenously-added GST-galectin-1 fusion protein on the adhesion of Colo201 cells.** Colo201 cells were incubated in RPMI 1640 medium containing the indicated concentrations of GST or GST-galectin-1. After washing the cells with the medium, the adherent cells were counted.

numbers of adherent cells of galectin-1 cDNA transfected cells were 5- to 6-fold more than those of mock DNA transfected cells. Immunofluorescence staining revealed that galectin-1 was mainly expressed in the cytosol (Fig. 2A). Localization of galectin-1 on the cell-surface was also observed (Fig. 2B). When immunoblotting with a monoclonal antibody for anti–c-myc was performed, a specific band corresponding to a molecular mass of 17 kDa was observed for the pcDNA3-c-myc-galectin-1 cDNA transfected cells (Fig. 2C).

Involvement of Galectin-1 Expressed Inside and on the Cell Surface of Colo201 Cells in Cell Adhesion—To determine whether or not galectin-1 on the cell surface is involved in cell adhesion, the effect of various sugars on galectin-1–induced adhesion were examined. As shown in Fig. 3, of the carbohydrates tested, only lactose inhibited the adhesion in a dose-dependent manner, suggesting that the galactoside terminus of the oligosaccharide is required for the adhesion. Furthermore, to determine if exogenously-added galectin-1 induces the adhesion of Colo201 cells, the GST-galectin-1 fusion protein was synthesized in *E. coli* and purified. Figure 4 shows the

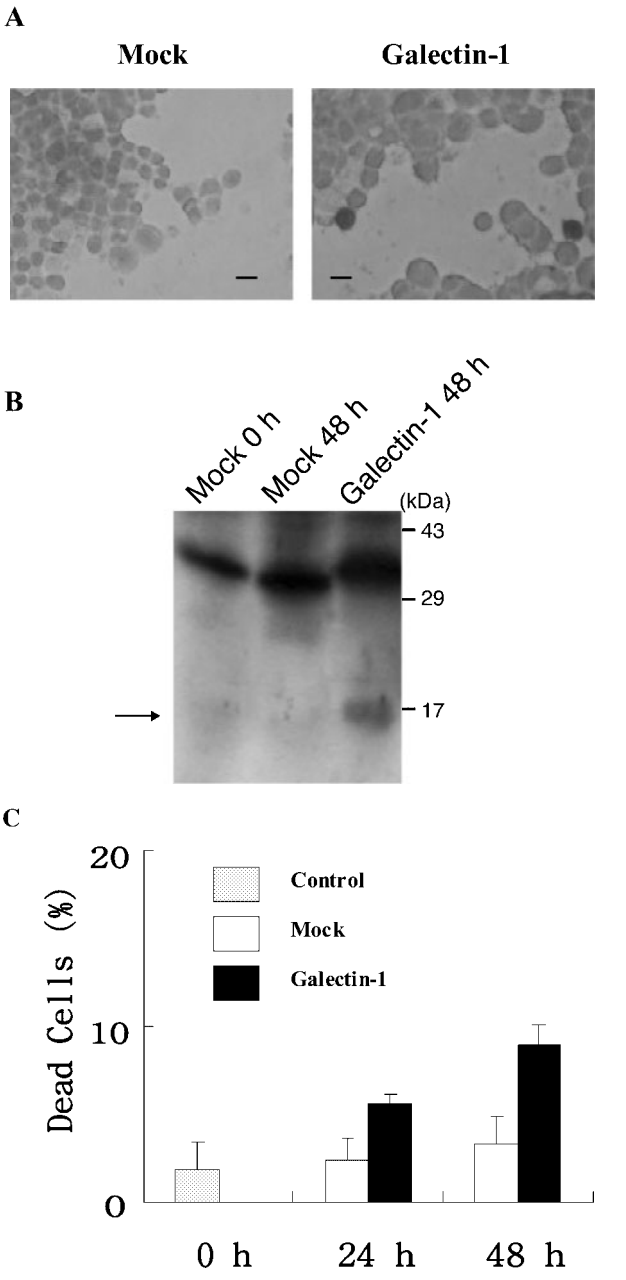

Fig. 5. **Apoptosis induced by the expression of galectin-1.** A: TUNEL assaying to detect apoptosis. Colo201 cells were transfected with pcDNA3 (c-myc)-galectin-1, and after 48 h they were assayed for DNA fragmentation by TUNEL staining. B: Immunoblotting analysis of caspase-3. Cells at 48 h after transfection were lysed, and then the cellular proteins were analyzed by SDS-PAGE. Immunoblotting with anti–caspase-3 was carried out. The arrow indicates the position of the activated caspase-3. C: Quantitative analysis of cell death. Cells after transfection with mock (open) and galectin-1 (solid) cDNA were incubated for 48 and 72 h. The total proportion of dead cells was determined by trypan blue exclusion.

changes in the adhesion of Colo201 cells on the addition of the GST-galectin-1 fusion protein. By increasing the amount of GST-galectin-1 in the medium, an increase in adherent cells was observed, while GST had no effect. It has been reported that galectin undergoes sugar-independent interactions with other proteins within cells,

suggesting that it may play a role in cell adhesion (*16*, *17*). We then examined the effects of protein kinase inhibitors on the induction of cell adhesion by galectin-1. PD58059 (100 μM), an inhibitor of mitogen-activated protein kinase, decreased the cell adhesion to 25%. LY294002 (30 μM), a phosphoinositide 3-OH kinase (PI3K) inhibitor, completely abolished the adhesion. These results indicated that galectin-1 expressed both inside and outside of Colo201 cells is involved in the induction of cell adhesion.

Induction of Apoptosis by Galectin-1—Galectins have been proposed to play a role in the regulation of cellular differentiation and proliferation. Thus, we finally examined the ability of the expression of galectin-1 to induce apoptosis in Colo201 cells. Figure 5A shows the results of TUNEL assaying of apoptotic gactectin-1 cDNA-transfected Colo201 cells. At 42 h after galectin cDNA transfection, TUNEL-positive cells were observed on transfection with galectin-1 cDNA, while no apoptosis was observed in the control. When the apoptosis was examined by immunoblotting with anti-caspase 3, cleavage of the active fragment of caspase 3 in galectin-1 expressing cells was observed (Fig. 5B). To quantify the apoptotic cells, cell death was assessed by means of a dye-exclusion assay. As shown in Fig. 5C, the number of surviving cells after transfection with galectin-1 cDNA was lower than that of mock DNA-transfected cells. In separate experiments, Colo201 cells were cultured with the addition of GST-galectin-1 fusion protein (10 μg/ml), and then the effect of exogenously-added galectin-1 on apoptosis was examined by TUNEL assaying and immunoblotting with anti-caspase 3. Apoptosis was not induced on incubation with GST-galectin-1 at up to 64 h (data not shown). Thus, the function of galectin-1 in the cells was separated from those via sugar-dependent interactions outside the cells.

DISCUSSION

We isolated galectin-1 as an adhesion molecule of non-adherent colon cancer Colo201 cells by expression cloning. Increased adhesion of galectin-1–expressing Colo201 cells occurred on non-coated plates, and markedly increased adhesion was observed on collagen-, laminin-, and fibronectin-coated plates. Adhesion to the ECM is CRD-dependent since lactose inhibits the binding. Furthermore, the addition of GST-galectin-1 protein to the culture medium of Colo201 cells led to an increase in the adhesion of Colo201 cells. The expression of galectin-1 in Colo201 cells resulted in cellular apoptosis while exogenously-added galectin-1 did not cause apoptosis. These results indicated that over-expressed galectin-1 in Colo201 cells caused the inhibition of cell proliferation while only extracellular galectin was involved in adhesion.

Galectin-1, an endogenous β-galactoside–binding protein, has emerged as a potent immunomodulatory protein (*3*, *5*). By virtue of its ability to interact with specific glycoconjugates, this protein has been implicated in some biological processes, including cell proliferation, cell adhesion, apoptosis, metastasis and immunoregulation (*3*, *4*). Galectin-1 induced apoptosis in the activated form of mature T cells and a specific subset of immature thymocytes (*5*, *18*). Due to its ability to inhibit the effector function of T cells, galectin-1 must have potent anti-flammatory and immunoregulatory effects *in vivo*. On the other hand, Van den Brule *et al*. (*19*) reported that a human melanoma cell line expresses galectin-1 in both the cytosol and plasma membrane. As galectin-1 on the cell-surface can bind preferentially to laminin to interact specifically with poly-*N*-acetyllactosamine residues (*3*), this endolectin could be a potential modulator of tumor cell-laminin interactions. These observations demonstrate that galectin-1 modulates the adhesion of melanoma cells to laminin and, therefore, could be a modulator of invasion and metastasis (*19*, *20*). Colo201 cells belong to a metastatic phenotype of colon cancer (*8*). Since the expression of galectin-1 is correlated with the acquisition of the metastatic phenotype in several types of tumors, the increase in the binding of Colo201 cells caused by the cell-surface galectin-1 with CRD recognition to the ECM may reflect the invasive potential.

In addition to the well-documented function of cell-surface–located galectin-1 as a β-galactoside–binding protein, intracellular galectin-1 functions as a regulator of pre-mRNA splicing. Furthermore, it has been demonstrated that galectin-1 interacts with H-Ras and activates this small GTP-binding protein, resulting in the promotion of membrane anchoring of H-Ras (*16*). We (*11*) previously reported that when the active form of Rac1 was expressed in Colo201 cells, the adhesion and spreading of cells occurred. The expression of the constitutively-active form of Cdc42 in Colo201 cells led to the formation of lamellipodia and membrane ruffles, accompanied by actin polymerization. In this study, an increase in adherent Colo201 cells was observed when RhoG was over-expressed. We had no direct evidence of the interaction of galectin-1 with GTP-binding proteins, but the adhesion is not fully dependent on the interaction of extracellular galectin-1 with sugar chains since the adhesion caused by galectin-1 occurred on uncoated and collagen-coated plates. Collagen is not a major glycoprotein, distinct from laminin and fibronectin, which are ligands of the galectin family. The adhesion of Colo201 cells to collagen (type I) caused by galectin-1 on the cell-surface appeared to be a CRD-independent event occurring *via* an effect inside the cells. The interaction of interacellular galectin-1 with some GTP-binding proteins may trigger adhesion and spreading. Alternatively, galectin-1 may have the ability to enhance the potential of some adhesion molecules.

We previously showed that the rapid activation of PI3K or p21-activated kinase, downstream effectors of Cdc42 and Rac1, is required for the adhesion of Colo201 cells (*11*). A rapid decrease in adhesion caused by inhibitors of PI3K was also observed in Rac 1 or Cdc42-expressing Colo201 cells (*11*), suggesting that the PI3K-mediated transduction pathway involves Rac 1 and regulates the surface expression of integrin molecules. The treatment of galectin-1–expressing Colo201 cells with LY294002, a specific inhibitor of PI3K, led to the complete abolition of adhesive Colo201 cells. This study showed that PD98059, an inhibitor of MAP kinase, also inhibited the galectin-1–dependent adhesion, consistent with the observation by Paz *et al*. (*16*) that the expression of galectin-1 in 293T cells resulted in the activation of extracellular signal–regulated kinase (ERK). These results indicated that the interaction of intracellular galectin-1 with some GTP-binding proteins is linked to

both ERK and PI3K or ERK to PI3K signal transduction pathways.

In colon cancer, the expression of galectin-1 and galectin-3 remains controversial. Lotz *et al*. (*21*) and Sanjuan *et al*. (*22*) observed decreased expression of galectin-3 with the neoplastic progression of colon carcinomas. Galectin-1 in cultured colon cancer cells increased when the cells were induced to differentiate by treatment with sodium butyrate (*23*). This study showed that the overexpression of galectin-1 induced apoptosis. In contrast, marked expression of galectin-1 in epithelial tissues has been reported (*3*, *4*). Irimura *et al*. (*24*) reported increased endogenous galectin-3 expression in high-grade human colorectal carcinomas. Furthermore, it has also been reported that the expression of cytoplasmic galectin-1 and -3 markedly increased during disease progression in the human colon (*25*). Thus, malignant and dysplastic colon cancers exhibit significantly higher galectin-1 and -3 levels than normal colon tissues. In summary, changes in the expression of galectins may be due to the heterogeneity of neoplasm and cancer cell lines or their malignant and metastatic potentials. Galectins are key factors in the development of new therapeutic strategies for a wide range of analyse, including cystic diseases and metastasis. Judging from the phenotype of Colo201 cells, *i.e.* a metastatic type of colon cancer, and on the observation that galectin-1 induced apoptosis of the cells, galectin-1 may function as an inhibitory factor for the growth of metastatic colon cancers.

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REFERENCES

1. Ruoslahti, E. and Obrink, B. (1996). Common principles in cell adhesion. *Exp. Cell Res.* **159**, 263–273
2. Ridley, A.J., Paterson, H.F., Johnston, C.F., Diekmann, D., and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401–410
3. Yang, R.Y. and Liu, F.T. (2003) Galectins in cell growth and apoptosis. *Cell Mol. Life Sci.* **60**, 267–276
4. Liu, F.T., Patterson, R.J., and Wang, J.L. (2002) Intracellular functions of galectins. *Biochim. Biophys. Acta.* **1572**, 263–273
5. Perillo, N.L., Pace, K.E., Seilhamer, J.J., and Baum, L.G. (1995) Apoptosis of T cells mediated by galectin-1. *Nature* **378**, 736–739
6. Rabinovich, G.A. (1999) Galectins: an evolutionarily conserved family of animal lectins with multifunctional properties; a trip-from the gene to clinical therapy. *Cell Death Differ.* **6**, 711–721
7. Lahm, H., Andre, S., Hoeflich, A., Fischer, J.R., Sordat, B., Kaltner, H., Wolf, E., and Gabius, H.J. (2001) Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. *J. Cancer Res. Clin. Oncol.* **127**, 375–386
8. Semple, T.U., Quinn, L.A., Woods, L.K., and Moore, G.E. (1978) Tumor and lymphoid cell lines from a patient with carcinoma of the colon for a cytotoxicity model. *Cancer Res.* **38**, 1345–1355
9. Nakagawa, K., Sogo, S., Hioki, K., Tokunaga, R., and Taketani, S. (1998). Acquisition of cell adhesion and induction of focal adhesion kinase of human colon cancer Colo 201 cells by retinoic acid-induced differentiation. *Differentiation* **62**, 249–257
10. Mohri, T., Kameshita, I., Suzuki, S., Hioki, K., Tokunaga, R., and Taketani, S. (1998). Rapid adhesion and spread of non-adherent colon cancer Colo201 cells induced by the protein kinase inhibitors, K252a and KT5720 and suppression of the adhesion by the immunosuppressants FK506 and cyclosporin A. *Cell Strutc. Funct.* **23**, 255–264
11. Mohri, T., Adachi, Y., Ikehara, S., Hioki, K., Tokunaga, R., and Taketani, S. (1999) Activated Rac1 selectively up-regulates the expression of integrin α6β4 and induces cell adhesion and membrane ruffles of nonadherent colon cancer Colo201 cells. *Exp. Cell Res.* **253**, 533–540
12. Naglich, J.G., Metherall, J.E., Russell, D.W., and Eidels, L. (1992) Expression cloning of a diphtheria toxin receptor: identity with a heparin-binding EGF-like growth factor precursor. *Cell* **69**, 1051–1061
13. Taketani, S., Kakimoto, K., Ueta, H., Masaki, R., and Furukawa, T. (2003) Involvement of ABC7 in the biosynthesis of heme in erythroid cells: interaction of ABC7 with ferrochelatase. *Blood* **101**, 3274–3280
14. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
15. Mizutani, A., Furukawa, T., Adachi, Y., Ikehara, S., and Taketani, S. (2002) A zinc-finger protein, PLAGL2, induces the expression of a proapoptotic protein Nip3, leading to cellular apoptosis. *J. Biol. Chem.* **277**, 15851–15858
16. Paz, A., Haklai, R., Elad-Sfadia, G., Ballan, E., and Kloog, Y. (2001) Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation. *Oncogene* **20**, 7486–7493
17. Lin, H.M., Pestell, R.G., Raz, A., and Kim, H.R. (2002) Galectin-3 enhances cyclin D(1) promoter activity through SP1 and a cAMP-responsive element in human breast epithelial cells. *Oncogene* **21**, 8001–8010
18. Perillo, N.L. and Uittenbogaart, C.H., Nguyen, J.T., and Baum, L.G. (1997) Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J. Exp. Med.* **185**, 1851–1858
19. Van den Brule, F.A., Buicu, C., Baldet, M., Sobel, M.E., Cooper, D.N., Marschal, P., and Castronovo, V. (1995) Galectin-1 modulates human melanoma cell adhesion to laminin. *Biochem. Biophys. Res. Commun.* **209**, 760–767
20. Rye, P.D., Fodstad, O., Emilsen, E., and Bryne, M. (1998) Invasion potential and N-acetylgalactosamine expression in a human melanoma model. *Int. J. Cancer.* **75**, 609–614
21. Lotz, M.M., Andrews, C.W. Jr., Korzelius, C.A., Lee, E.C., Steele, G.D. Jr., Clarke, A., and Mercurio, A.M. (1993) Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma. *Proc. Natl. Acad. Sci. USA* **90**, 3466–3470
22. Sanjuan, X., Fernandez, P.L., Castells, A., Castronovo, V., Van den Brule, F., Liu, F.T., Cardesa, A., and Campo, E. (1997) Differential expression of galectin 3 and galectin 1 in colorectal cancer progression. *Gastroenterology* **113**, 1906–1915
23. Ohannesian, D.W., Lotan, D., and Lotan, R. (1994) Concomitant increases in galectin-1 and its glycoconjugate ligands (carcinoembryonic antigen, lamp-1, and lamp-2) in cultured human colon carcinoma cells by sodium butyrate. *Cancer Res.* **54**, 5992–6000
24. Irimura, T., Matsushita, Y., Sutton, R.C., Carralero, D., Ohannesian, D.W., Cleary, K.R., Ota, D.M., Nicolson, G.L., and Lotan, R. (1991) Increased content of an endogenous lactose-binding lectin in human colorectal carcinoma progressed to metastatic stages. *Cancer Res.* **51**, 387–393
25. Hittelet, A., Legendre, H., Nagy, N., Bronckart, Y., Pector, J.C., Salmon, I., Yeaton, P., Gabius, H.J., Kiss, R., and Camby, I. (2003) Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. *Int. J. Cancer* **103**, 370–379