Thesis

Analysis of Galectin-1 Expression in Human Placenta Trophoblasts with Gal3D11, a Novel Monoclonal Anti-Galectin-1 Antibody

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BACKGROUND: Galectin-1 belongs to the galectin family of lectins, and is expressed in a variety of tissues including placenta, liver, skeletal and smooth muscle. Galectin-1 is thought to be a versatile modulator of cell function, but the biological significance of galectin-1 in human placenta is unclear. The galectin family comprises 14 proteins on the basis of amino acid sequence. **METHODS:** I raised a novel monoclonal antibody, Gal3D11, against human galectin-1, and characterized the epitope of the antibody using immunoblotting, high performance liquid chromatography, and mass spectrometry. **RESULTS:** The epitope of Gal3D11 comprises amino acids 29-48 of the galectin-1 protein. In this study, I used Gal3D11 to clarify the expression of galectin-1 at various stages of placental development. I found that galectin-1 was expressed specifically and ubiquitously in placenta trophoblasts, especially in syncytiotrophoblasts, during the first, second, and third trimester. **CONCLUSIONS:** The widespread expression of galectin-1 in placenta suggests that this protein plays an important role during the development of the placenta. In addition, Gal3D11 would appear to be an indispensable monoclonal antibody with which to further study the function of galectin-1 in different tissues.

INTRODUCTION

During its growth and maturation, the placenta undergoes cell proliferation, particularly during the first trimester. The placenta has two components in terms of its origin: the fetal portion comprises chorionic tissue, and the maternal portion comprises uterine decidual tissue. As the interface of fetomaternal interaction, the placenta plays a crucial role in maintaining the fetal environment and supporting development of the fetus[1]. To maintain pregnancy, the placenta secretes endocrine and paracrine hormones and cytokines, in addition to autocrine substances that support the development of the placenta[2]. Consequently, any abnormality in the functioning of the placenta has a profoundly negative effect on the fetus.

Galectins are a family of β-galactoside-binding lectins[3-6]. Galectins are present in a variety of species, including sponges, nematodes, and humans, and are found in different types of tissue, including placenta[7, 8], skeletal and smooth muscle, thymus, lymph nodes, prostate, testes, liver, lung, skin, spleen, peripheral nerves, heart, and developing brain[9-12].

Galectin-1 is concentrated in the cytoplasm and extracellular compartments in the cells of many embryonic and adult tissues[13], and is believed to be exported from the cytoplasm to the extracellular space by a non-classical secretory mechanism[14, 15]. Galectin-1, a prototype galectin, consists of 134 amino acids[7]. Under physiological conditions, galectin-1 forms non-covalently associated homodimers with conserved carbohydrate recognition domains (CRDs), which preferentially recognize Type-I and Type II *N*-acetyllactosamine residues on all complex N-linked and many *O*-linked glycoproteins[16, 17]. Galectin-1 is thought to be a versatile modulator of cell function[6], and has been implicated in axonal growth and/or guidance[18], cell adhesion[19], cell migration[20, 21], cell proliferation[22-24], embryogenesis[25], proinflammatory reactions[26], spliceosome assembly[27], gliomas malignancy[28], metastasis[29], and apoptosis[30-33]. Several receptors for galectin-1 have been identified, including ganglioside GM1[19], glycoprotein 90K/MAC-2BP[34],

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laminin[35], H-Ras[36], and pre-B cell receptor[37]. Galectin-1 binds lectin only when galectin-1 is in a reduced form; once galectin-1 has been oxidized, it loses its ability to bind lectin[38]. Hori and colleagues have demonstrated that the oxidized form of galectin-1 promotes axonal regeneration after axotomy[38-40]. Galectin-1 has also been shown to be present in human placental tissue through the use of polyclonal antibodies that were raised against galectin-1[8, 41, 42]. Galectin-1 from ovine placenta has been reported to be involved in T-cell death[43]. However, mice that lack the gene that encodes galectin-1 were viable, and failed to show any reproductive failure[44, 45]. Therefore, the biological significance of galectin-1 in placental tissue remains to be elucidated. In addition, the lack of suitable anti-galectin-1 antibodies has been a limiting factor in examining the biological significance of galectin-1. Consequently, I raised monoclonal antibodies against human galectin-1. Here, I show that one such monoclonal antibody, Gal3D11, could be useful for investigating the expression and function of galectin-1 in the development of human placenta.

MATERIALS AND METHODS

Placental tissue

First and second trimester placentas were obtained from women who were undergoing elective pregnancy termination by suction curettage (vacuum aspiration) at 7-11 and 12-24 weeks of gestation(n=3). Term placentas were obtained from women during elective Caesarean sections at 37-39 weeks of gestation(n=3). All the patients were healthy with singleton pregnancies, and there were no known complications or fetal abnormalities. The study was approved by the human research and ethics committee of the Saitama Medical School, and informed written consent was obtained from each patient.

Estimation of protein quantity

An aliquot of homogenized placental tissue from each patient was used to estimate the amount of protein using a bicinchoninic acid kit (BCA kit; Pierce, Rockford, IL, USA), according to the manufacturer's protocol.

Cell lines

A mouse myeloma cell line, namely P3/NS-1/Ag-4 (referred to hereafter as NS-1), as Ill as COS1 cells (derived from monkey kidney) were purchased from Dainippon Seiyaku (Tokyo, Japan). NS-1 cells and COS1 were maintained in RPMI 1640 and DMEM, respectively, supplemented with 10 % fetal calf serum (Tissue Culture Biologicals, Tulture, CA, USA), 2 mM sodium pyruvate, (Wako, Tokyo, Japan) and 50 μg/ml Gentamycin sulfate (Gibco, Tokyo, Japan).

Culture of trophoblasts

Portions of placental tissue were dissected free, washed twice with ice-cold phosphate-buffered saline (PBS), and incubated with 0.25 % trypsin in saline for 10 min to dissociate the cells. Thereafter, RPMI 1640 medium (supplemented as described above) was added to the dissociated cells, and the cell suspension was then filtered through a stainless steel mesh (150 mm) before being centrifuged at 1,000 rpm for 7 min. The supernatant was discarded, and the cells were re-suspended in the aforementioned medium before being cultured in a humidified incubator (95% O_2/5% CO_2, 37℃).

Antibodies

To identify chorionic cells, I used rabbit anti-human placental lactogen (hPL) antibody (Dako, Carpinteria, CA, USA). Peroxidase-conjugated affiniPure Donkey anti-mouse IgM (μchain-specific) was purchased from Jackson ImmunoReseach Laboratories (Ist Grove, PA, USA). Fluorescein-5-isothiocyanate (FITC)-conjugated goat anti-mouse IgM(μchain-specific)was purchased from Sigma (Saint Louis, MO, USA). Alexa 560-conjugated goat anti-rabbit IgG was purchased from Molecular Probes (Eugene, OR, USA).

Purification and preparation of recombinant human oxidized galectin-1

Recombinant human oxidized galectin-1 (rhGal-1/ox) was prepared as described previously [38, 39]. Briefly, rhGAL-1 was expressed in *E. coli* that had been transformed with a plasmid for bacterial expression of human galectin-1. Thereafter, rhGAL-1 was purified from the supernatant of the sonicated *E. coli* by O-(diethylaminoethyl) anion-exchange (DEAE) high-performance liquid chromatography (HPLC). The oxidized form of rhGAL-1, rhGal-1/ox, was prepared by the air oxidation method using $CuSO_4$ as a catalyst, and rhGal-1/ox was purified by reversed-phase HPLC.

Production of monoclonal antibody

The methods that I used to produce the monoclonal antibody Gal3D11 have been described elsewhere[46]. Purified rhGal-1/ox (20 μg) was dissolved in PBS, mixed with an equal volume of Freund's complete adjuvant, and was then used to immunize 6-8-Iek-old female Balb/c mice. The screening of hybridomas was carried out by enzyme-linked immunoadsorbent assay (ELISA) using rhGal-1/ox (1 μg/ml), as described previously by Yoshimura and colleagues[46].

Typing of monoclonal antibody

Typing of the monoclonal antibody Gal3D11 was

carried out using a mouse monoclonal antibody isotyping kit (Amersham Bioscience, San Francisco, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed as previously described[46]. Briefly, ninety-six-Ill microtiter plates (Corning International, Tokyo, Japan) Ire coated with 1 μg/ml rhGal-1/ox and reduced rhGal in 0.1 m NaHCO3 at 4℃ overnight and blocked with 5 % bovine serum albumin (Sigma) in PSB, for 1h at room temperature. The plates Ire washed three times with PBST (PBS containing 0.05 % Tween 20) and incubated with hybiridoma supernatants for 1 h. After another washing step, plates Ire incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse Ig (whole) (Amersham Biosciences; diluted 1:5000 in blocking buffer). Antibody binding was visualized by using soluble TMB substrate (Pierce; Rockford, IL, USA) and 2 M sulfuric acid. Absorbance (ABS) was recorded at a wavelength of 405 nm with an ELISA plate reader MR5000 (Dynatech Lab Inc, Alexandria, VA, USA).

Immunohistochemistry

Immunohistochemical staining of placental tissue was performed according to the methods of Yoshimura and colleagues[47]. For indirect immunofluorescence, placental tissue was washed thoroughly with PBS, fixed overnight in PBS containing 4 % paraformaldehyde, placed in a 30 % sucrose solution, and embedded in OCT compound (Miles-Sankyo, Tokyo, Japan). A cryostat (Leica, Tokyo, Japan) was then used to prepare 8 mm-thick frozen sections. PBS containing 5 % normal goat serum with 0.05 % Triton X-100 (blocking solution A) was used to block nonspecific binding for 30 min. Culture supernatant containing mouse anti-human galectin-1 was used with no dilution; rabbit anti-hPL antibody was diluted 1:200 in blocking solution A, and then allowed to react with the tissue sections for 1h. Each section was then washed with PBS. FITC-conjugated goat anti-mouse IgM (diluted 1:100 in blocking solution A) was then allowed to react with sections for 1h. Alexa 560-conjugated goat anti-rabbit IgG (diluted 1:100 in blocking solution A) was then allowed to react with sections for 1h. Sections were then washed with PBS, and observed under a fluorescence microscope (Axiophot, Zeiss, Germany) that was equipped with a charge-coupled device (CCD) camera (Sensys, IPLab spectrum, Tokyo, Japan). For histological examination of the tissue, frozen sections were stained with hematoxylin-eosin (HE). Negative controls were performed routinely. Omission of the primary antibody or the use of non-immune rabbit serum in place of specific rabbit antiserum resulted in a complete absence of immunostaining. The monoclonal antibody Gal3D11 was used at a concentration of 5-10 μ g/ml, as determined by ELISA (Quantitation Kit for mouse IgM; Bethyl Laboratories, Montgomery, TX, USA).

Immunocytochemistry

Immunocytochemical staining of cultured cells was performed as described previously (Yoshimura et al., 2001). After two days in culture (see details above), COS1 cells and trophoblasts were washed twice with PBS, fixed with 4 % paraformaldehyde in PBS for 20 min at room temperature, and then washed with PBS. Immunofluorescence staining of COS1 cells and trophoblasts was carried out using mouse monoclonal antibody Gal3D11 and FITC-conjugated goat anti-mouse IgM. The supernatant of cultured NS-1 myeloma cells was used instead of primary antibody, as a negative control. For double-immunofluorescence staining, species-specific secondary antibodies were used as described above.

Western blot

Western blot was carried out as previously described[47]. Briefly, fresh placental tissues (all trimesters) were washed twice with ice-cold PBS, homogenized in Laemmli's sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) buffer[48], and then centrifuged at 10,000 x *g* for 20 min at room temperature. To determine the concentration of protein, the supernatant was collected into aliquots to which 2-mercaptoethanol was added (2 % final concentration) and then held at 99℃ for 5 min before being applied to SDS-PAGE gel electrophoresis. For gel electrophoresis, 60 μg of protein was added to each lane of a 10 % SDS-PAGE gel (ATTO, Tokyo, Japan). After electrophoresis, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (BioRad, Richmond, CA, USA) by semi-dry blotting (Horizblot AE-6677; ATTO, Tokyo, Japan). After a 1-h incubation in blocking solution B (3 % skim milk in TTBS (20 mM Tris-HCl at pH 7.4, 0.05 % Tween 20)), the membrane was incubated for 1h with the supernatant of cell cultures that contained the monoclonal antibody. After washing three times with PBS, bound antibody was detected by goat anti-mouse IgM conjugated to peroxidase (1:100,000 dilution in blocking solution B). Immunoblots were visualized on Hyperfilm (Amersham Bioscience) with SuperSignal west Dura Extended Duration Substrate (Pierce).

Determination of epitope of Gal3D11

Galectin-1 was digested with endoproteinase Arg-C (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's protocol. The digests were separated by reverse-phase HPLC, and fractionated. After dot blotting, Gal3D11-immunoreactive peptide was analyzed with an amino acid sequencer (ABI 492H) and an LC mass spectrometer (LCQ Advantage, Yokohama, Japan).

Multiple sequence alignment analysis

Multiple sequence alignment analysis was carried out using the CLUSTAL W Multiple Sequence Alignment Program[49].

Homology analysis of the Gal3D11 epitope with 13 other members of galectin family of proteins

Homology analsysis of the Gal3D11 epitope with 13 other members of galectin family of proteins was performed with Blast program (http://www.ncbi.nlm.nih.gov/blast/)[50].

RESULTS

Production of the monoclonal antibody Gal3D11

Hybridoma cell clones were screened using ELISA. Several hybridomas produced monoclonal antibodies that were reactive to galectin-1. I screened out one clone that exhibited relatively strong reactivity to both the oxidized and reduced form of galectin-1 (Fig. 1). I named this antibody Gal3D11, and used it to investigate the expression of galectin-1 in placental tissue (see below).

Isotyping of antibody Gal3D11

Isotyping of the novel anti-galectin-1 monoclonal antibody, Gal3D11, revealed that the immunoglobulin class of Gal3D11 was IgM (k) (data not shown).

Confirmation of specificity of Gal3D11

Gal3D11 bound both the reduced and oxidized forms of galectin-1 in Western blot (Fig. 2). Immunoreactivity against the reduced form of galectin-1 was stronger than that of the oxidized form of the same protein (Fig. 2).

To determine whether Gal3D11 would bind galectin-1 in cells, I used Gal3D11 to immunostain COS1 cells, which produce galectin-1[38]. As expected, Gal3D11 produced positive immunostaining in the COS1 cells (Fig. 3). Additional analysis of Gal3D11 immunostaining of COS1 cells and placental tissues by Western blot analysis suggested that Gal3D11 reacted with a 14-kDa band that corresponded to galectin-1. There was no cross-reactivity with galectin-3 (MW=31 kDa).

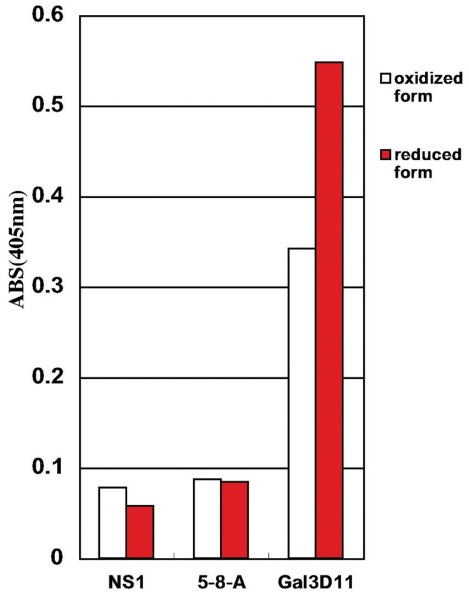

Fig. 1. Immunoreactivity of recombinant human galectin-1 in an enzyme-linked immunoadsorbent assay. NS1 and 5-8-A were used as negative controls. For NS1, culture supernatant from mouse myeloma cells that was used instead of the primary antibody. 5-8-A indicates the monoclonal antibody that reacted specifically with peripheral nerve myelin P0 protein. The symbols open and closed (red) bar indicate the oxidized and reduced forms of galectin-1, respectively.

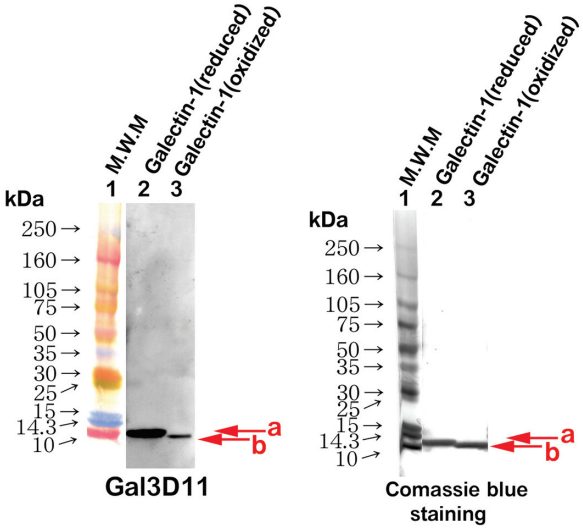

Fig. 2. Immunoreactivity of the novel monoclonal anti-galectin-1 antibody, Gal3D11. Left panel: Western blot analysis of binding of reduced and oxidized recombinant human galectin-1 by Gal3D11. Right panel: Coomassie blue staining of recombinant human galectin-1. Arrows at a and b indicate the reduced and oxidized forms of galectin-1, respectively. Lanes 1-3: molecular weight markers; reduced galectin-1; oxidized galectin-1.

Determination of the epitope of Gal3D11

The amino acid sequence of the Gal3D11-immunoreactive peptide was SFVLNLGKDSNNLCLHFNPR; this corresponds to the amino acid residues 29 to 48 of galectin-1, i.e., Gal3D11 recognizes an epitope that is localized within the amino acid sequence of galectin-1 (Fig. 4). This result was confirmed by mass spectrometry. In addition, alignment of amino acid sequences of several different species revealed that the epitope that Gal3D11 recognizes is common to mouse, rat, and human (Table 1).

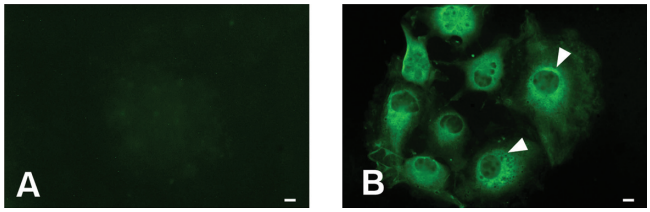

Fig. 3. Immunolocalization of galectin-1 in COS1 cells. Immunocytochemical staining of cultured COS1 cells with the monoclonal anti-galectin-1 antibody, Gal3D11. Galectin-1-immunoreactivity was located primarily within the perinuclear region. Inset: arrowhead indicates the typical pattern of galectin-1-immunoreactivity perinuclear region. A: Control. B: Gal3D11 immunoreactivity. Scale bar=10 mm.

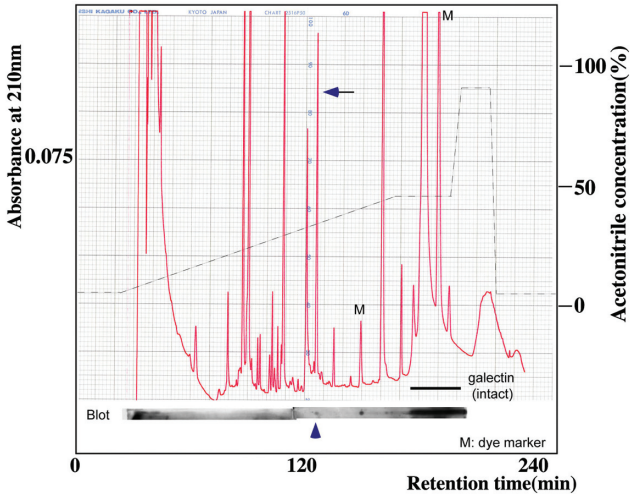

Fig. 4. Determination of the epitope of Gal3D11. The arrow and arrowhead indicate a peak and a spot, respectively. M represents an internal marker.

Table 1. Alignment of amino acid sequence of the epitope of the novel monoclonal anti-galectin-1 antibody, Gal3D11, from different species

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                1                   29                ▼ ▼48  ▼       60
Human           ACGLVASNLNLKPGECLRVRGEVAPDAKSFVLNLGKDSNNLCLHFNPRFNAHGDANTIVC
Mouse           ACGLVASNLNLKPGECLKVRGEVASDAKSFVLNLGKDSNNLCLHFNPRFNAHGDANTIVC
Rat             ACGLVASNLNLKPGECLKVRGELAPDAKSFVLNLGKDSNNLCLHFNPRFNAHGDANTIVC
Cow             ACGLVASNLNLKPGECLRVRGEVAADAKSFLLNLGKDDNNLCLHFNPRFNAHGDVNTIVC
Sheep           ACGLVASNLNLKPGECLRVRGEVAADAKSFSLNLGKDDNNLCLHFNPRFNAHGDINTIVC
Chicken         EQGLVVTQLDVQPGECVKVKGKILSDAKGFSVNVGKDSSTLMLHFNPRFDCHGDVNTVVC
Frog            AAGMVMNNFSLKQGHCLELKGFIPKDAKSFAINLGKDSSNYVIHFNPRFDHEGDTNKIIC
                  *:* .::.:: *.*:.::* :  ***.* :*:***...  :******: .** *.::*
                ▼  65  ▼  ▼▼                                              120
Human           NSKDGGAWGTEQREAVFPFQPGSVAEVCITFDQANLTVKLPDGYEFKFPNRLNLEAINYM
Mouse           NTKEDGTWGTEHREPAFPFQPGSIIEVCITFDQADLTIKLPDGHEFKFPNRLNMEAINYM
Rat             NSKDDGTWGTEQRETAFPFQPGSITEVCITFDQADLTIKLPDGHEFKFPNRLNMEAINYM
Cow             NSKDAGAWGAEQRESAFPFQPGSVVEVCISFNQTDLTIKLPDGYEFKFPNRLNLEAINYL
Sheep           NSKDGGAWGAEQRETAFPFQPGSVAEVCISFNQTDLTIKLPDGYEFKFPNRLNLEAINYL
Chicken         NSKEDGTWGEEDRKADFPFQQGDKVEICISFDAAEVKVKVP-EVEFEFPNRLGMEKIQYL
Frog            NSKEENSWGTEQRENVFPFQQGAETSICFEYQADHLKVKLSDGQEFNFPIRMPLDTITFL
                *:*: .:** *.*:  **** *   .:*: ::  .:.:*:.   **:** *: :: * ::
               121         134
Human           AADGDFKIKCVAFD
Mouse           AADGDFKIKCVAFE
Rat             AADGDFKIKCVAFE
Cow             SAGGDFKIKCVAFE
Sheep           SAGGDFKIKCVAFE
Chicken         AVEGDFKVKAIKFS
Frog            SMDG-IELKAISLH
                :  * :::*.: :
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*Positions in which there is a single, fully conserved residue. Colon (:) indicates that one of the following 'strong' groups is fully conserved: STA NEQK NHQK NDEQ QHRK MILV MILF HY FYW. Period (.) indicates that one of the following 'Iaker' groups is fully conserved: CSA ATV SAG STNK STPA SGND SNDEQK NDEQHK NEQHRK FVLIM HFY. Red letters indicate amino acids that are within the epitope of the novel monoclonal anti-galectin-1 antibody, Gal3D11. ▼: Carbohydrate recognition domain.

Western blot analysis of placental tissue

Western blot analysis of placental tissue from various stages of pregnancy revealed that galectin-1 was expressed in each trimester (Fig. 5), although the level of expression was variable. Two independent replications of this experiment produced the same result.

Immunohistochemistry of placental tissue

Gal3D11 recognized galectin-1 in placental tissue from each trimester. Gal3D11 appeared to bind syncytiotrophoblasts specifically in the outer layer of the placenta (Fig. 6 and 7), although villous stroma was also immunopositive to Gal3D11 (Fig. 6).

Immunocytochemistry of cultured trophoblasts

Double-immunofluorescence staining of cultured human trophoblasts with Gal3D11 and anti-human hPL antibody revealed that Gal3D11 was immunolocalized to putative syncytiotrophoblasts (Fig. 8).

DISCUSSION

Cytokines and adhesion molecules, including lectins, are involved in various aspects of placental function[2]. Galectin-1 is a lectin that is expressed throughout the body, and plays a role in cell adhesion[51], cell proliferation[22, 52, 53], inflammation[53], invasion of cancer[28, 54], and apoptosisin various cell types including lymphocytes, thymocytes, and vascular cells[6, 30, 31]. Although galectin-1 is expressed in placental tissue, where it might be involved in immunomodulation[41-43, 55, 56], a detailed analysis of the pattern of galectin-1 expression in placenta has been attempted in relatively few studies[8, 41, 42].

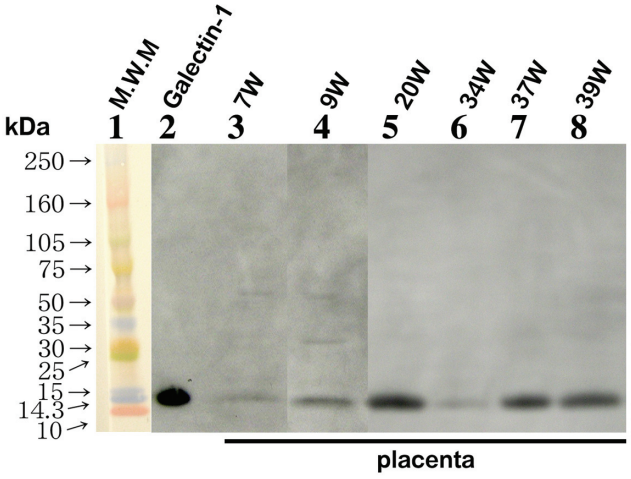

Fig. 5. Western blot analysis of Gal3D11 binding in human placental tissue from different trimesters. Upper panel: Western blot for Gal3D11. Left lane: molecular weight markers. Lane 1: recombinant human galectin-1. Lanes 2-8 contain samples of placental tissue at weeks 7, 9, 9, 20, 34, 37, and 39 of gestation, respectively.

The production and characterization of Gal3D11, a monoclonal antibody that is specific to galectin-1

To investigate the expression of galectin-1 in placenta, I produced a monoclonal antibody, Gal3D11, that reacted strongly with galectin-1. The results of the present study indicate that this monoclonal antibody binds galectin-1 specifically. Gal3D11 recognized amino acids 29-48 of a peptide digest of galectin-1, and multiple sequence alignment from different species showed that the sequence of the Gal3D11 epitope is common in human, mouse, and rat (Table 1). Homology analysis of the Gal3D11 epitope with 13 other members of the galectin family of proteins revealed that FVLNG (corresponding to amino acid 30-35) has some homology with the corresponding amino acid sequence of galectin-2. But the staining pattern of epithelial cells of the intestine using Gal3D11 was different from that using anti-galectin-2 antibody previously reported (data not shown)[57]. HFNPR belongs to the CRD that is common to galectin-1, galectin-2, and galectin-3 (Table 2). It would appear that Gal3D11 does not recognize the CRD: Western blot analysis showed that Gal3D11 reacted specifically with galectin-1, but not with a closely related protein (galectin-3; see Fig. 5). Therefore, the epitope of Gal3D11 is likely within the sequence of amino acid 36-43, which is distinct from all of the remaining 13 members of galectin family. Therefore, Gal3D11 will be useful for investigating the expression of galectin-1 in human, mouse, and rat tissues.

Expression of galectin-1 in syncytiotrophoblasts

Several investigators have reported that galectin-1 is expressed in human placenta[41-43, 55, 56]. However, there has been no study of the expression of galectin-1 at different stages of pregnancy. I addressed this issue using the novel anti-galectin-1 antibody, Gal3D11. Double-immunostaining with Gal3D11 and anti-hPL antibody revealed that galectin-1 was expressed in syncytiotrophoblasts (Fig. 7). Vicovac and colleagues used a polyclonal antibody to describe the expression of galectin-1 in syncytiotrophoblasts in first trimester placental tissue[42]. In the present study, Western blot analysis and immunohistochemistry revealed that galectin-1 was expressed in syncytiotrophoblasts throughout pregnancy (Fig. 5-7). I was also able to confirm the expression of galectin-1 in cultured trophoblasts (Fig. 8). Syncytiotrophoblasts arise from the syncytial layer that covers the villus as a result of the fusion of cells, and is a part of villi that comprise the

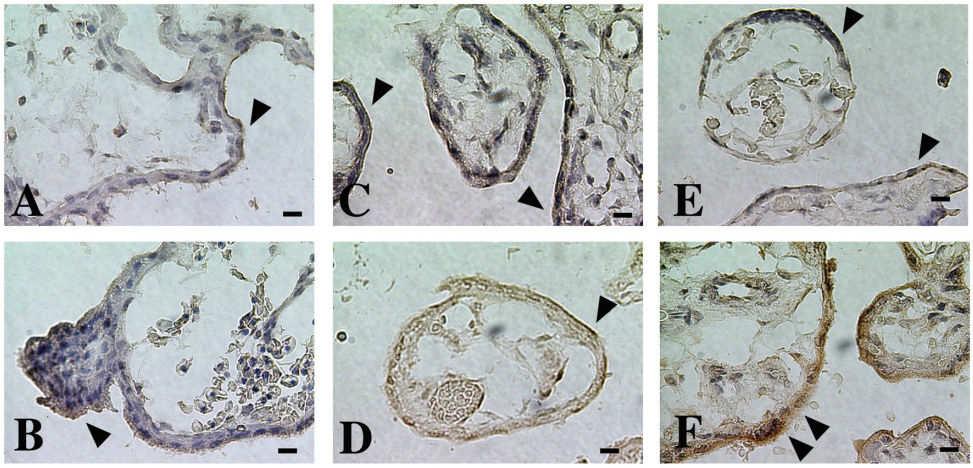

Fig. 6. Immunolocalization of galectin-1 in human placental tissue from different trimesters. All immunostaining obtained using the anti-galectin-1 antibody, Gal3D11. Note that the galectin-1 was located primarily within syncytiotrophoblasts in the outermost fetal portion. Dark brown color represents positive Gal3D11 immunoreactivity. Arrowheads indicate galectin-1-immunopositive syncytiotrophoblasts. A-F: representative samples of placental tissue at weeks 7, 9, 18, 20, 34, and 37 of gestation, respectively. Nuclei (dark blue) were stained with hematoxylin-eosin.

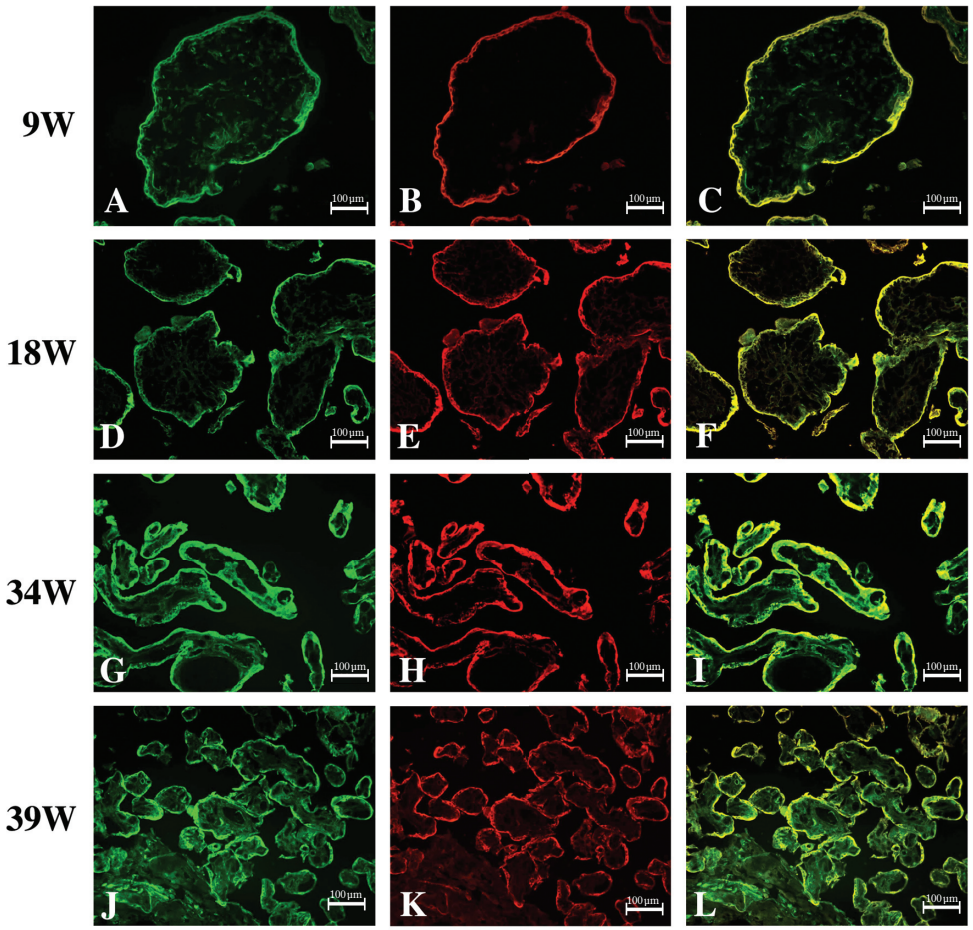

Fig. 7. Double-immunohistochemical staining of villous trophoblasts using Gal3D11 anti-galectin-1 antibody and polyclonal anti-human placental lactogen antibody. Both antibodies were intensely immunoreactive in syncytiotrophoblasts (arrowheads). A-C: Placental tissue from week 9 of gestation. D-F: Placental tissue from week 18 of gestation. G-I: Placental tissue from week 34 of gestation. J-L: Placental tissue from week 39 of gestation. A, D, G, J: Gal3D11 immunoreactivity. B, E, H, K: human placental lactogen (hPL) immunoreactivity. C, F, I, L: Merged images from A and B, D and E, G and H, and J and K, respectively. Scale bar=100 mm.

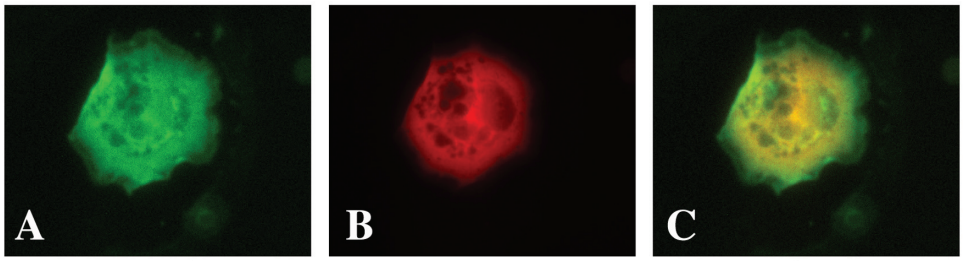

Fig. 8. Double-immunohistochemical staining of cultured trophoblasts using Gal3D11 anti-galectin-1 antibody and polyclonal anti-human placental lactogen antibody. Putative syncytiotrophoblasts were immunopositive for both antibodies.

Table 2. Comparison of amino acid sequences of amino acid residues 29-48 of human galectin-1, galectin-2, and galectin-3.

Protein	Amino acid sequence
Galectin-1	S*FVLNLG*KDSNNLCL**HF**N**PR**
Galectin-2	G*FVINLG*QGTDKLNL**HF**N**PR**
Galectin-3	NRIALDFQRGNDVAF**HF**N**PR**

Bold letters indicate residues that comprise the carbohydrate recognition domain that is common to all known members of the galectin family of proteins. Italic letters indicate highly homologous amino acid sequences.

fetal portion of the placenta. Syncytiotrophoblasts are bathed in maternal blood, and function as an interface for gas and nutrient exchange for the developing embryo or fetus. The organization of the placenta is thought to recruit adhesion molecules such as the cadherins and integrins during development[58, 59], and galectin-1 is an additional candidate adhesion molecule. Perillo and colleagues reported that galectin-1 that was extracted from placenta could induce apoptosis of activated maternal T-cells, and suggested that galectin-1 might have a role in immunomodulation[6, 30].

The results of the present study reveal that galectin-1 is expressed in the outer layer of the placenta throughout pregnancy. This finding also implicates galectin-1 in syncytiotrophoblasts in playing a role in cell adhesion (to act as a barrier), acting as a modulator of the immune system (specifically, T-cells), or promoting cell proliferation. Because cultured trophoblasts produce extracellular matrix, including laminin[60], the expression of galectin-1 in syncytiotrophoblasts in placenta throughout pregnancy is suggestive of a role for this protein in the organization of the extracellular matrix. The fact that mice that lack galectin-1 have normal syncytiotrophoblasts supports the hypothesis that galectin-1 plays a supporting role in various aspects of cell function, and in addition suggests that other members of the galectin family are able to carry out the same functions as galectin-1[25, 61]. Further study is required to clarify the function of galectin-1.

In conclusion, the ubiquitous expression of galectin-1 in trophoblasts in human placental tissue suggests that this protein has an important role during the development of the placenta. In addition, our novel monoclonal anti-galectin-1 antibody, Gal3D11, will be valuable for clarifying the function of galectin-1 in various tissues.

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