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Molecular forms of galectin-1 from human placenta and trophoblast cells

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Abstract: Galectin-1 (gal-1) is the best-studied member of the galectin family of the human placenta, which is assumed to play important roles in pregnancy. Standard isolation of gal-1 from human placenta using lactose extraction and affinity chromatography in the presence of a reducing agent produced several known forms of gal-1, which were compared to the recombinant human gal-1 (rhgal-1) and oxidized recombinant human gal-1 (Ox-gal-1). The isolated placental gal-1 retained lectin-binding activity, evidenced by hemagglutination and dot blot lectin assays. Characterization of the forms present by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI–TOF MS), based on hydrophilic interactions or immunorecognition, provided a sensitive tool for the detection of the fine differences among the diverse molecular forms. The forms detected included previously established biologically active oxidized gal-1 and reduced gal-1, as well as some other currently uncharacterized (less investigated forms).

Keywords: galectin-1; placenta; molecular form; SELDI-TOF MS.

INTRODUCTION

Galectin-1 (gal-1) is a member of the animal lectin family with binding capacity for *β*-galactoside residues of glycoconjugates. It exists as a non-covalent dimer composed of identical ≈14.6 kDa subunits, with evident carbohydrate-binding activity in the reduced state only.[1,2] Although gal-1 shows characteristics of typical cytoplasmic proteins, it can be found on the extracellular side of cell membranes and in the extracellular matrices of various normal and pathological tissues.[3] Its localization indicates that gal-1 could act intracellularly through protein–protein interactions and extracellularly via sugar-dependent interactions.[4,5]

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Most of the gal-1 isolation protocols are performed in the presence of a reducing agent in order to prevent oxidation and maintain carbohydrate-binding activity.[6–8] The relatively high content of cysteine residues in the gal-1 molecule, *i.e.*, six cysteine residues per monomer, implicates gal-1 sensitivity to oxidative inactivation and loss of lectin activity.[9,10] For example, during purification of gal-1 from bovine heart, the lectin activity was lost upon oxidation, suggesting that this isolation protocol results in the presence of both reduced and oxidized lectin.[9] In addition, gal-1 from a transfected cell line COS1, was isolated and the relevance of oxidation for functional characteristics shown.[11] In nerve regeneration models *in vivo* and *in vitro* oxidized recombinant human gal-1 (Ox-rhgal-1) was recognized as a factor that promotes Schwann cell migration followed by axonal growth.[11,12] This form of human gal-1 obtained from bacterially expressed recombinant gal-1 by the air oxidation with $CuSO_4$ as a catalyst is a protein with a molecular mass of 14.579 kDa and is characterized by reduced or no lectin activity.[12,13] Experimental strategies involving construction of diverse gal-1 mutants have been devised in order to elucidate relevance/contribution of specific domains to gal-1 interaction with potential ligands and function.[10,14] In this context, a stabilized gal-1 form (CS-gal-1) in which six cysteine residues were replaced with serine, expressed in and purified from *E. coli*, functions like a lectin with molecular mass of 14.886 kDa.[12]

It is well known that gal-1 is abundantly expressed in human placenta and in trophoblast derived cell lines.[15–17] Gal-1 was the first isolated and purified galectin from the human placenta.[6,18] More recently, other members of the galectin family were localized in human and non-human placenta.[19,20] Functionally, gal-1 acts as an important stimulator of the trophoblast invasion *in vitro*.[13] Furthermore, it was shown that CS–gal-1 was more potent than Ox-rhgal-1 in inducing trophoblast invasion,[13] confirming a previous finding of a molecular form dependent effect in an axonal regeneration model.[12] No direct data are yet, however, available regarding the presence of different gal-1 forms in trophoblast. Therefore, the present study was aimed at investigating whether gal-1 isolated from the human placenta or present in extravillous trophoblast cell line HTR-8/SVneo is homogenous or comprised of different molecular forms.

EXPERIMENTAL

Reagents

RPMI 1640, antibiotic/antimycotic solution and fetal calf serum (FCS) were obtained from PAA Laboratories (Linz, Austria). Matrigel, Collagen Type I (Col I) and serum fibronectin (sFN) were purchased from BD Biosciences (Bedford, MA, USA). Acrylamide, *N*,*N*′-methylenebisacrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Ponceau S, glycine, lactose and asialofetuin (ASF) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein standards and silver stain kit were from Bio-Rad, Inc. (Hercules, CA, USA). The oxidized form of recombinant

human gal-1 and rabbit anti-rhgal-1 with gal-1 neutralizing activity were obtained from Kirin Brewery (Tokyo, Japan). Recombinant human gal-1 was from Acris Antibodies GmbH (Hiddehausen, Germany). Biotinylated goat anti-rabbit, avidin-biotinylated peroxidase complex (ABC) and diaminobenzidine (DAB) substrate kit for peroxidase were obtained from Vector Laboratories (Burlingame, CA, USA). Anti-rabbit IgG HRP-linked antibody was from Cell Signaling Technology, Inc. (Danvers, MA, USA).

All other chemicals were of *p.a*. grade.

Isolation of gal-1 from human placenta

Galectin-1 was isolated from three fresh term human placentas by lactose extraction with phosphate buffered saline (PBS), 0.05 mol L^{-1}, pH 7.2 containing 0.01 mol L^{-1} 2-mercapto-ethanol (ME) and 0.002 mol L^{-1} ethylenediaminetetraacetic acid (EDTA) (EDTA–MEPBS) and purified by affinity chromatography on a lactosyl-Sepharose 4B column.[6] The obtained preparations were not pooled and produced identical results. Bound protein was then eluted with 0.1 mol L^{-1} Gly–HCl, pH 2.5 and concentrated.[21] The purity and homogeneity of the isolated protein was tested by silver staining and Western blot analysis after SDS–PAGE under reducing conditions. After isolation, gal-1 was extensively dialyzed against PBS in order to remove ME and stored in PBS at –20 °C.

Assays for lectin activity

The lectin activity of the isolated placental gal-1 was determined by hemagglutination and dot blot analysis. Agglutination assays were performed using trypsinized rabbit erythrocytes and purified placental gal-1. Lectin solutions prepared in PBS containing 0.005 mol L^{-1} dithiothreitol (DTT) as a reducing agent were gently mixed with a 2 % suspension of erythrocytes without or with 0.1 mol L^{-1} lactose. Results were read after 1 h incubation at room temperature (RT).

To assay the binding of placental gal-1 to different glycoproteins, dot blot analysis was performed. Asialofetuin, sFN, Col I and matrigel (containing 3 μg protein each) were spotted on a nitrocellulose membrane and dried at RT. Unspecific binding sites were blocked with 3 % bovine serum albumin (BSA) for 2 h at RT. Solutions of isolated gal-1 (6 μg mL^{-1}) were pre-incubated without or with different lactose concentrations (0.025, 0.05, 0.1 or 0.2 mol L^{-1}) in EDTA–MEPBS for 1 h at RT, and then used for incubation with individual strips overnight at 4 °C. Strips were further incubated with anti-gal-1 antibodies (1 μg mL^{-1}) for 2 h at RT, followed by incubation with biotinylated anti-rabbit IgG for 30 min and with ABC for another 30 min. The reaction was visualized using DAB as the chromogen.

SDS-PAGE, silver stain and Western blot

Isolated placental gal-1 was subjected to SDS-PAGE on 5–20 % gradient polyacrylamide gel under reducing conditions, and analyzed using a Bio-Rad Silver Stain Kit, according to manufacturer's instructions. Recombinant human gal-1, ox-rhgal-1, placental gal-1, HTR-8/SVneo cell lysate and culture media were resolved by SDS–PAGE under reducing conditions on 12.5 % polyacrylamide gel and 4 % stacking gel and subsequently transferred to nitrocellulose membranes. The membranes were incubated with rabbit anti-gal-1 antibody (1μg mL^{-1}) overnight at 4 °C, with constant shaking. After incubation with anti-rabbit IgG HRP-linked antibody, a gal-1 band was detected using Pierce ECL Western blotting substrate. The membranes were scanned with an ImageScanner from Amersham Biosciences, Inc. (Piscataway, NJ, USA) and densitometric analysis was performed using the ImageMaster Total-Lab v2.01 program (Amersham Bioscience).

Cell culture

Human extravillous trophoblast cell line HTR-8/SVneo was cultured and propagated as previously described.[13,22] For lectin affinity chromatography and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI–TOF MS), the HTR-8/SVneo cells were grown in complete media for 48 h, lysed in 0.02 mol L^{-1} 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) with 1 % Triton X-100, pH 8. The cell culture media were collected, centrifuged at 700 g for 5 min, and then analyzed using SDS--PAGE and SELDI–TOF MS. For SDS-PAGE, trypsinized HTR-8/SVneo cells were washed with PBS pH 7.2, lysed in a sample buffer containing a protease inhibitor cocktail (6×10^6 cells mL^{-1}), centrifuged (1600 g for 5 min at 4 °C) and the supernatant reserved. Cell lysates were heated for 5 min in boiling water and subjected to SDS-PAGE.

Lectin affinity chromatography

Asialofetuin was covalently linked to CNBr-activated Sepharose (Pharmacia, Sweden) at 1 mg mL^{-1} of gel according to the manufacturer's instructions. HTR-8/SVneo cell lysate (6×10^6 cells mL^{-1}) prepared in 0.02 mol L^{-1} HEPES with 1 % Triton X-100, pH 8, was applied to ASF column under reducing or non-reducing conditions. Non-bound material was eluted with PBS or EDTA–PBSME. Under non-reducing conditions, the elution was performed with 0.1 mol L^{-1} Gly–HCl, pH 2.5, while under reducing conditions PBS containing 0.1 mol L^{-1} lactose was used for elution. The collected fractions were pooled and concentrated prior to SDS–PAGE analysis.

Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry

In this study, gal-1 was analyzed by SELDI–TOF MS. Protein chip arrays were processed according to manufacturer's instructions. Briefly, 5 μL of sample (placental gal-1, rhgal-1 or ox-rhgal-1) per spot was applied to normal phase NP20 chip array, left to air-dry at RT and rinsed three times with PBS. The pre-activated-surface protein chip array PS20 was coated with anti-gal-1 antibody (5 μL per spot) in a humid chamber overnight at 4 °C. After rinsing with PBS, the spots were blocked with 0.5 mol L^{-1} Tris–HCl, pH 8 for 1 h in the humid chamber at RT. After another washing step with PBS, 5 μL of sample (HTR-8/SVneo whole cell lysate, conditioned medium or placental gal-1) was loaded on each spot and incubated for 2 h in a humid atmosphere at RT. Unbound material was removed from the spot by intensive rinsing with PBS, followed by deionized water. Both NP20 and PS20 protein chip arrays were left to completely air-dry, before the energy-absorbing matrix (EAM) was added. Sinapinic acid (SPA) dissolved in 50 % acetonitrile/0.5 % trifluoroacetic acid was used as the EAM. To each spot, 1 μL of 50 % SPA solution was applied twice, and after drying, the protein arrays were ready for processing. The protein chips were read in ProteinChip Reader, Series 4000, Personal edition (Bio-Rad Laboratories, Inc., Hercules, CA, USA). External calibration was realized using ProteinChip all-in-one protein standards II. Mass analysis was performed at a laser energy of 6000 nJ, with 8815 laser shots per spot. The spectra were acquired in 25 kV positive ion acquisition, in the mass range between 2.5 and 200 kDa, with the focus mass at 15 kDa. The spectra were analyzed using Ciphergen Express Software 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The peaks were normalized according to the total ion current between 2500 and 70000 *m*/*z* and detected automatically for signal to noise ratio >3.

RESULTS AND DISCUSSION

In the present study, gal-1 isolated from human term placenta was characterized and compared to two different forms of recombinant gal-1, as well as the gal-1 from the trophoblast cell line HTR-8/SVneo. Most of the commonly used procedures for gal-1 isolation and purification are based on lactose extraction from tissue and lactose affinity column, in the presence of a reducing agent, which is critical for the assessment of lectin activity of gal-1.[2,23] In the absence of a reducing agent, gal-1 from placental tissue extract lost lectin activity and was unable to bind to a lactose affinity column (Fig. 1A). Therefore, a procedure with 2-mercaptoethanol was used for the isolation of placental gal-1 (Fig. 1A). The presence of gal-1 was confirmed by dot blot in fractions eluted with 0.1 mol L^{-1} Gly–HCl, pH 2.5, and gal-1 was further analyzed by SDS-PAGE and immunoblot using polyclonal rabbit antibody raised against human gal-1 (Fig. 1B). The production, characterization and specificity of the polyclonal anti-gal-1 was previously described.[11] Both analyses showed the presence of a single prominent band

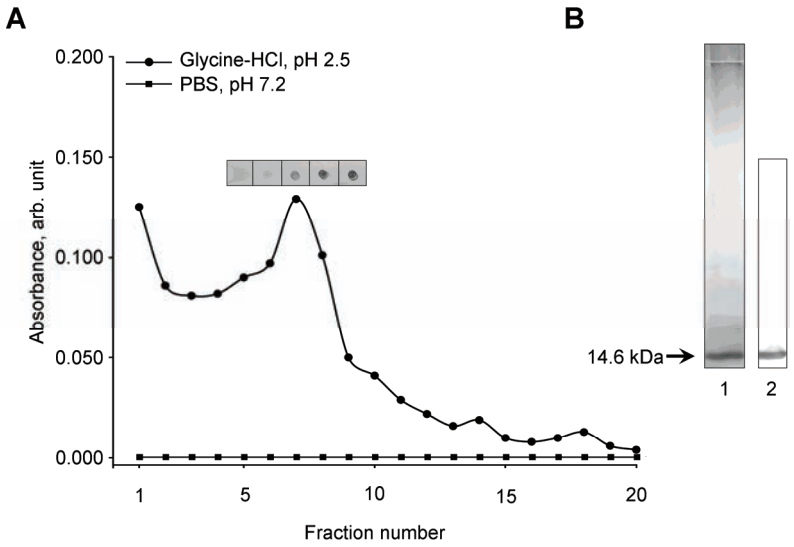

Fig. 1. Isolation and identification of gal-1 from human term placenta. A) Preparative affinity chromatography was performed under reducing conditions. Unbound material was washed with EDTA–MEPBS buffer pH 7.2. Gal-1 was eluted from lactosyl-Sepharose 4B column with Gly–HCl pH 2.5 (circles) under 3 mL min^{-1} flow rate. The absorbance was measured at 280 nm and additionally, the presence of gal-1 in fractions 5–9 was confirmed by dot blot using anti-gal-1 antibodies. In the absence of reducing agent, gal-1 was not detected in the eluted fractions (squares). B) SDS-PAGE and Western blot of isolated gal-1. 1) Purity of isolated gal-1 was confirmed using silver staining after SDS-PAGE electrophoresis under reducing conditions. A band of ≈14.6 kDa was observed by silver staining. 2) Western blot analysis using anti-gal-1 antibodies confirmed that the ≈14.6 kDa band was gal-1.

of ≈14.6 kDa, which was consistent with the molecular mass of gal-1. Although other galectin family members are known to be present in human placenta and to share binding characteristics with gal-1,[13,24] only gal-1 is isolated from term placenta using lactose extraction and affinity purification, most likely, due to the specific cell type composition of the starting material (containing low amounts of cytotrophoblast expressing gal-3 and gal-8). One of the most characteristic features of gal-1 is high affinity binding of *β*-galactoside containing glycoconjugates, which is commonly inhibited by lactose for experimental purposes.[25,26] The lectin activity of gal-1 was determined by hemagglutination and binding to several glycoproteins or their mixture Matrigel (Fig. 2A and B). Placental gal-1 induced agglutination in the presence of DTT as a reducing agent. As expected, lectin-mediated agglutination was inhibited in the presence of lactose (Fig. 2A). Binding of the placental gal-1 to different glycoproteins was tested by the dot blot assay in the absence or presence of lactose. Some of the *β*-galacto-

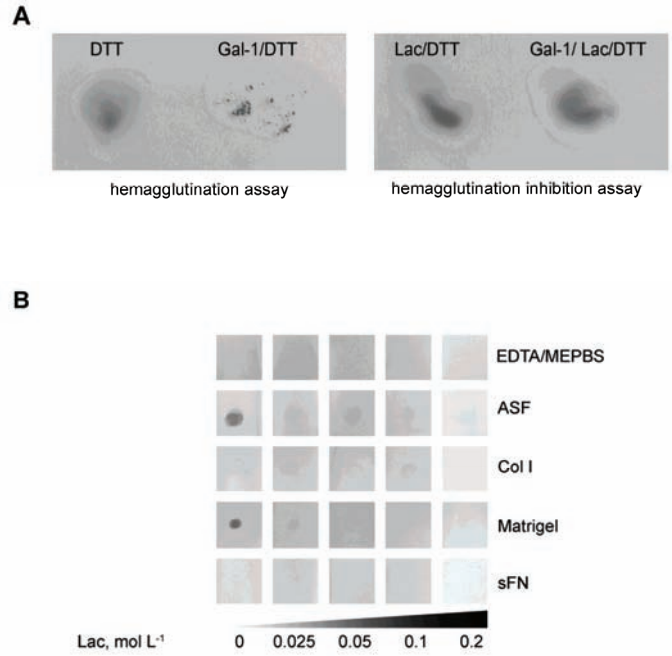

Fig. 2. Lectin properties of gal-1 from the human placenta. A) Hemagglutination activity of placental gal-1 under reducing conditions (in the presence of DTT). Addition of lactose inhibited hemagglutination, confirming that the isolated placental gal-1 had lectin activity. DTT or DTT/lactose served as the negative controls. B) Binding of placental gal-1. Gal-1 ligands (ASF and Matrigel), negative control (sFN, Col I) and blank probe (EDTA–MEPBS) were spotted onto nitrocellulose membranes. The membranes were incubated with gal-1 pre-incubated without or with different concentrations of lactose (0.025–0.2 mol L^{-1}) in EDTA–MEPBS. Bound gal-1 was detected using anti-gal-1 antibodies.

side-containing ligands that are bound by gal-1 are found in trophoblast, including heavily glycosylated proteins of the extracellular matrix (laminin and cell fibronectin),[27] as well as the cell membrane glycoproteins, integrins[28] and mucins.[29] In this study placental gal-1 was shown to bind ASF, the commercial basement membrane protein preparation Matrigel that contains laminin, collagen type IV and heparan sulfate proteoglycan, but not sFN or Col I (Fig. 2B). The binding of gal-1 to ASF or Matrigel was reduced by lactose (0.025–0.2 mol L^{-1}), which confirms that gal-1 binds *via* carbohydrate recognition. In different tissues and cell types, gal-1 is implicated in many biological processes, such as cell adhesion, invasion, differentiation, and most of these effects are supposed to be mediated through binding to *β*-galactoside cell membrane glycoconjugates participating in signal transduction.[30,31] In this study, HTR-8/SVneo derived gal-1 monomer was shown to differently bind ASF in the presence or absence of a reducing agent (Fig. 3A), resulting in a different proportion of bound/unbound gal-1 (Fig. 3B). A similar finding was reported for the recombinant and mutant forms

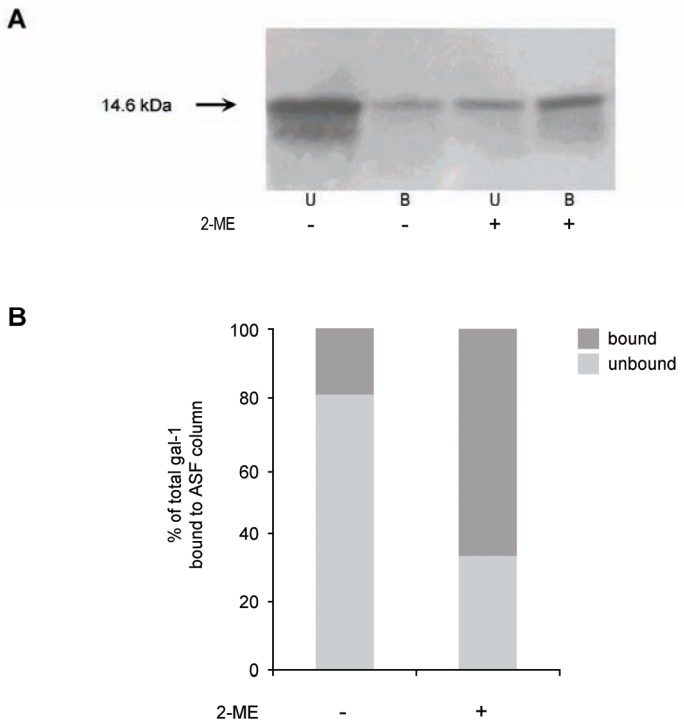

Fig. 3. Binding of gal-1 from HTR-8/SVneo cell lysates to ASF in the absence or presence of 2-mercaptoethanol (ME). A) Western blot analysis of unbound (U) or bound (B) gal-1 containing fractions eluted in the absence (–) or presence (+) of the reducing agent. B) Relative proportion of unbound/bound gal-1 as detected by Western blot and densitometric analysis.

of gal-1 binding to lactose,[12,14] showing that redox status influences the structure and binding properties of gal-1.

Placental gal-1 was compared to rhgal-1 and ox-rhgal-1. All gal-1 samples were trapped on NP20 arrays due to hydrophilic interactions, and the resulting proteomic spectra were analyzed by SELDI–TOF MS (Fig. 4A). Although the

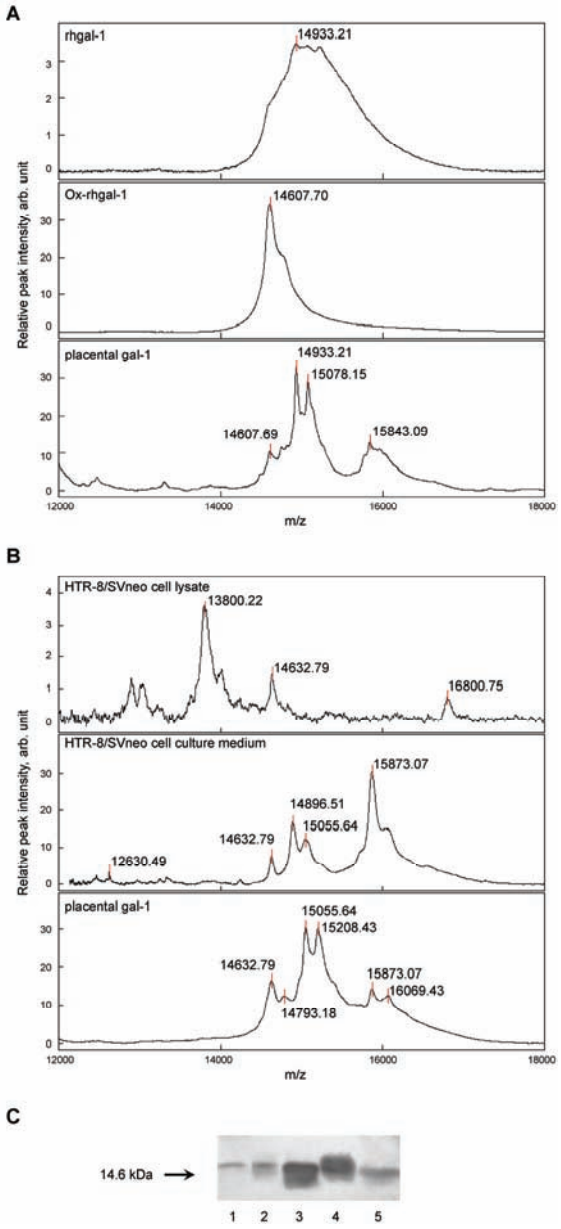

Fig. 4. SELDI–TOF and Western blot analysis of gal-1 in different samples. A) Mass spectra on NP20 protein chip array of isolated placental gal-1 compared to rhgal-1 and ox-rhgal-1. B) Mass spectra of isolated placental gal-1 and gal-1 from HTR-8/SVneo cell line (whole cell lysate and secreted) identified on a PS20 protein chip array, using anti-gal-1 antibodies as bait. Spectra (A and B) are represented as relative peak intensity *vs.* mass to charge ratio. Protein peaks are identified automatically, for signal to noise ration (S/N) > 3. C) Western blot analysis of gal-1 from different sources: HTR-8/SVneo cell lysate (1) and the corresponding medium (2), ox-rhgal-1 (3), rhgal-1 (4), gal-1 from human term placenta (5).

obtained protein profiles of gal-1 were similar, some subtle differences were noted. The dominant gal-1 forms in trophoblast were of 14.6, 14.933, 15.078 and 15.843 kDa. In the sample of ox-rhgal-1, a 14.6 kDa protein was detected, in keeping with the molecular mass of 14.579 kDa previously determined by MALDI–TOF MS.[12] A prominent peak at 14.9 kDa was detected in the sample of rhgal-1 used in the present study, the molecular mass of which is slightly higher, compared to the size of the mutant CS-gal-1 and reduced form of rhgal-1 measured by MALDI–TOF MS.[12] Four relatively high intensities peaks at 14.6 kDa, 14.933, 15.078 and 15.843 kDa were observed in the preparation of placental gal-1, two of which, at 14.6 and 14.933 kDa, coincided with the ox-rhgal-1 and rhgal-1 peaks (Fig. 4A).

The analysis based on the antibody binding (immobilized to a PS20 array) of gal-1 from different biological sources also revealed multiple forms of gal-1 (Fig. 4B). All the preparations, including cell lysate, and secreted gal-1, as well as the gal-1 isolated from placenta, contained a form of 14.6 kDa consistent with the oxidized gal-1. There was considerable overlap in the presence of other forms, while the 13.8 kDa form was specific to HTR-8/SVneo cell lysate. In addition, differences in the mobility between the same gal-1 containing preparations were detected by Western blot (Fig. 4C). A similar molecular form (of 14.2 kDa) was previously identified by MALDI-TOF[32] in a gal-1 form lacking the first six amino acids, but also in other human cell lines (of 13.5 kDa, by Western blot).[33] The molecular nature and biological function of this form is not clear at present. On the other hand, higher molecular forms were detected in other cell models and might be related to the unconventional secretion and membrane targeting of gal-1.[26] The obtained data showed that the placental tissue at term contains multiple molecular forms of gal-1, and among them all of the known biologically active ones.

CONCLUSION

Isolation of gal-1 from the human placenta using lactose and affinity column produced several known forms of gal-1. The system devised for the characterization of the forms present, based on either hydrophilic interactions or immunorecognition on chips followed by SELDI–TOF MS, provided a sensitive tool for the detection of fine differences among the diverse molecular forms. The forms detected contained previously established biologically active entities, but also some the potential significance of which remains to be investigated in the future.

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ИЗВОД

МОЛЕКУЛСКЕ ФОРМЕ ГАЛЕКТИНА-1 ИЗ ПЛАЦЕНТЕ ЧОВЕКА И ЋЕЛИЈА ТРОФОБЛАСТА

ДАНИЦА ЋУЈИЋ[1], ЖАНКА БОЈИЋ-ТРБОЈЕВИЋ[1], НИКОЛА КОЛУНЏИЈА[1], TOSHINIKO KADOYA[2] и ЉИЉАНА ВИЋОВАЦ[1]

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Галектин-1 (gal-1) је највише изучаван члан фамилије галектина у ћелијама трофобласта хумане плаценте. Применом стандардне процедуре изоловања gal-1 лактозном екстракцијом и афинитетном хроматографијом у присуству редукујућег средства, добијено је неколико познатих форми gal-1 које су упоређене са рекомбинантним хуманим gal-1 (rhgal-1) и оксидованим рекомбинантним хуманим gal-1 (ox-rhgal-1). Изоловани плацентни gal-1 је био лектински активан, што је показано хемаглутинацијом и лектинским тестом на чврстој фази. Карактеризација форми применом SELDI–TOF масене спектрометрије, засноване на хемијском или имунолошком препознавању, омогућила је детекцију дискретних разлика између молекулских форми плацентног gal-1. У изолованом gal-1 биле су присутне форме оксидованог и редукованог gal-1, чија је биолошка активност претходно показана. Поред ових, уочене су и мање испитиване форме gal-1.

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REFERENCES

1. L. B. Clerch, P. Whitney, M. Hass, K. Brew, T. Miller, R. Werner, D. Massaro, *Biochemistry* **27** (1988) 692
2. W. M. Abbott, T. Feizi, *J. Biol. Chem.* **266** (1991) 5552
3. I. Camby, M. Le Mercier, F. Lefranc, R. Kiss, *Glycobiology* **16** (2006) 137R
4. R. C. Hughes, *Biochim. Biophys. Acta* **1473** (1999) 172
5. D. N. Cooper, S. H. Barondes, *J. Cell Biol.* **110** (1990) 1681
6. J. Hirabayashi, K. Kasai, *Biochem. Biophys. Res. Commun.* **122** (1984) 938
7. C. P. Sparrow, H. Leffler, S. H. Barondes, *J. Biol. Chem.* **262 (**1987) 7383
8. V. I. Teichberg, I. Silman, D. D. Beitsch, G. Resheff, *Proc. Natl. Acad. Sci. USA* **72** (1975) 1383
9. B. M. Tracey, T. Feizi, W. M. Abbott, R. Carruthere, B. N. Green, A. M. Lawson, *J. Biol. Chem.* **267** (1992) 10342
10. M. F. López-Lucendo, D. Solís, S. André, J. Hirabayashi, K. Kasai, H. Kaltner, H. J. Gabius, A. Romero, *J. Mol. Biol.* **343** (2004) 957
11. H. Horie, Y. Inagaki, Y. Sohma, R. Nozawa, K. Okawa, M. Hasegawa, N. Muramatsu, H. Kawano, M. Horie, H. Koyama, I. Sakai, K. Takeshita. Y. Kowada, M. Takano, T. Kadoya, *J. Neurosci.* **19** (1999) 9964
12. Y. Inagaki, Y. Sohma, H. Horie, R. Nozawa, T. Kadoya, *Eur. J. Biochem.* **267** (2000) 2955
13. N. Kolundžić, Ž. Bojić-Trbojević, T. Kovačević, I. Stefanoska, T. Kadoya, Lj. Vićovac, *PLoS ONE* **6** (2011) e28514
14. C. M. Guardia, J. J. Caramelo, M. Trujillo, S. P. Méndez-Huergo, R. Radi, D. A. Estrin, G. A. Rabinovich. *Glycobiology* **24** 428
15. E. Maqui, F. A. van den Brule, V. Castronovo, J. M. Foidart, *Placenta* **18** (1997) 433
16. Lj. Vićovac, M. Janković, M. Čuperlović, *Hum. Reprod.* **13** (1998) 730
17. Ž. Bojić-Trbojević. M. Božić, Lj. Vićovac, *Arch. Biol. Sci.* **60** (2008) 11

18. J. Hirabayashi, H. Kawasaki, K. Suzuki, K. Kasai, *J. Biochem.* **101** (1987) 987
19. N. G. Than, R. Romero, C. J. Kim, M. R. McGowen, Z. Papp, D. E. Wildman, *Trends Endocrinol. Metab.* **23** (2012) 23
20. R. Froehlich, N. Hambruch, J. D. Haeger, M. Dilly, H. Kaltner, H. J. Gabius, C. Pfarrer, *Placenta* **33** (2012) 195
21. T. Lekishvili, S. Hesketh, M. W. Brazier, D. R. Brown, *Eur. J. Neurosci.* **24** (2006) 3017
22. D. Ćujić, Ž. Bojić-Trbojević, N. Tošić, S. Pavlović, Lj. Vićovac, *Arch. Biol. Sci.* **65** (2013) 1331
23. P. L. Whitney, J. T. Powell, G. L. Sandford, *Biochem. J.* **238** (1986) 683
24. H. Leffler, S. Carlsson, M. Hedlund, Y. Quian, F. Poirier, *Glycoconjugate J.* **19** (2004) 433
25. Q. Zhou, R. D. Cummings, *Arch. Biochem. Biophys.* **300** (1993) 6
26. E. P. Moiseeva, Q. Javed, E. L. Spring, D. P. de Bono, *Cardiovasc. Res.* **45** (2000) 493
27. Y. Ozeki, T. Matsui, Y. Yamamoto, M. Funahashi, J. Hamako, K. Titani, *Glycobiology* **5** (1995) 255
28. E. P. Moiseeva, B. Williams, A. H. Goodall, N. J. Samani, *Biochem. Biophys. Res. Commun.* **310** (2003) 1010
29. Ž. Bojić-Trbojević, M. Jovanović Krivokuća, N. Kolundžić, M. Petronijević, S. Vrzić-Petronijević, S. Golubović, Lj. Vićovac, *Histochem. Cell Biol.* **142** (2014) 541
30. Y.-L. Hsu, C.-Y. Wu, J.-Y. Hung, Y.-S. Lin, M.-S. Huang, P.-L. Kuo, *Carcinogenesis* **34** (2013) 1370
31. I. Fischer, S. Shulze, C. Kuhn, K. Friese, H. Walzel, U. R. Markert, U. Jeschke, *Placenta* **30** (2009) 1078
32. T. Miura, M. Takahashi, H. Horie, H. Kurushima, D. Tsuchimoto. K. Sakumi, Y. Nakabeppu, *Cell Death Differ*. **11** (2004) 1076
33. A. Satelli, P. S. Pao, P. K. Gupta, P. R. Lockman, K. S. Srivenugopal, U. Subrahmanyeswara Rao, *Oncol. Rep.* **19** (2008) 587.