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The chimera-type galectin-3 is a positive modulator of trophoblast functions with dysregulated expression in gestational diabetes mellitus

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Abstract

Problem: From conception, a delicate regulation of galectins, a family of carbohydrate-binding proteins, is established to ensure maternal immune tolerance in pregnancy. Though galectin-3 (gal-3), the only chimera-type galectin, is abundantly expressed at the feto-maternal interface; the physiological role of this lectin during pregnancy remains to be fully elucidated and requires further investigation.

Method of study: In this study, we analyzed serum gal-3 levels during the course of healthy gestation. Trophoblast functions were evaluated upon gal-3 exogenous stimulation using trophoblastic cell lines (e.g. , HIPEC65, SGHPL-4, and BeWo cells). Finally, we investigated variations in peripheral gal-3 levels associated with the development of spontaneous abortion and gestational diabetes mellitus (GDM).

Results: Gal-3 circulating levels increased as normal pregnancy progressed. In vitro experiments showed that exogenous gal-3 positively regulated trophoblast functions inducing invasion, tube formation, and fusion. Compared with normal pregnant

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women, circulating gal-3 levels were significantly decreased in patients who developed GDM.

Conclusion: Our results reveal a physiological role for gal-3 during pregnancy, promoting proper trophoblast functions associated with healthy gestation. GDM is associated with a failure to increase circulating gal-3 levels late in gestation. Thus, dysregulation of gal-3 may indicate a contribution of the chimera-type lectin to this adverse pregnancy outcome.

KEYWORDS

gal-3, pathological pregnancy, placenta, trophoblast

1 | INTRODUCTION

Pregnancy constitutes a major challenge to the maternal immune system because it requires tolerance of fetal alloantigens encoded by paternal genes. Local factors at the maternal-fetal interface are required to maintain such tolerance and ensure normal development of the semiallogeneic conceptus.[1] Galectins are a family of at least 15 galactoside-binding proteins that share conserved carbohydrate recognition domains (CRD).[2] Several members of this family are emerging as key regulators of the three pillars in pregnancy-associated processes: maternal immune responses, angiogenesis, and placentation.[3]

Galectin-3 (gal-3) is the only chimera-type galectin, with a C-terminal domain containing the carbohydrate recognition domain (CRD) displaying the lectin activity linked to the N-terminal domain via a repetitive collagen-like sequence.[4] Extracellular gal-3 interacts with β-galactoside residues of several glycoproteins via the CRD and through their N-terminal domain gal-3 monomers form pentamers and are able to cross-link carbohydrates.[5] During the menstrual cycle, gal-3 is—together with gal-1—the predominant member of this lectin family in the human endometrium.[6] Progesterone and estradiol regulate its expression[7] with an increase during the secretory phase. The increase in gal-3 expression is attributed to glandular epithelial cells whereas the expression in stromal cells and leukocytes remains unchanged. Moreover, gal-3 expression peaks in intensity in the regressing corpus luteum.[8] During the first trimester, cytotrophoblast (CTB) stem cells in the placental villi express gal-3 while the syncytiotrophoblast (STB) overlying the CTB stem cells are negative for gal-3.[9,10] This lectin is also abundant in CTB cell columns, which anchor the placental villi to the maternal decidua. Both interstitial and endovascular extravillous CTB (EVT) that leave the cell columns, invade the decidua and remodel maternal spiral arteries express gal-3.[10] However, Bozic et al reported no gal-3 expression in EVT.[11] In the decidua itself, stromal and glandular cells strongly express gal-3.[10,12] Toward term pregnancy, low levels of gal-3 are expressed in the decidua, STB, and EVT.[13] Under pathological conditions such as gestational trophoblastic disease, placental gal-3 is up-regulated.[11] In line with this, gal-3 expression is increased in EVT of preeclamptic and HELLP patients but not in women with IUGR.[13] Furthermore, small-for-gestational neonates showed higher gal-3 levels in their cord blood than appropriate-for-gestational age infants, which might result from elevated inflammatory signals.[14,15] In concordance, treatment of cord blood samples with a Group B streptococcus sepsis strain induces gal-3 in vitro.[14] Although gal-3 expression has been reported in human pathological gestation, its kinetics during normal pregnancy remains to be elucidated.

In the current study, we analyzed the expression of gal-3 during normal and pathological pregnancies to gain insight into its possible function during human gestation. We show that normal progression of pregnancy is associated with an increase in systemic gal-3 levels. Using various human trophoblast cell lines, we demonstrate that gal-3 influences the invasive of EVT cell lines properties and tube formation capacity of the cells, revealing the importance of gal-3 in trophoblast functions associated with placental vascularization. Furthermore, maternal circulating gal-3 decreased upon onset of gestational diabetes mellitus (GDM). These observations provide prospects for the development of complementary diagnostic tools that target gal-3 in routine gynecologic analysis.

2 | MATERIALS AND METHODS

2.1 | Study populations

Three human cohorts were part of this study. For measurement of circulating gal-3 levels during normal pregnancy, blood samples were collected from healthy pregnant women in the first, second, and third trimester of pregnancy at their planned visits to the Department of Obstetrics and Gynecology, Umeå University Hospital, Sweden, and to the Polyclinic Maternity Care Units for control of pregnancy progression as described.[16] All the patients involved in this work were properly informed about the purpose of our research and gave their written consent before the sampling. The study was approved by the ethics committee of the Umeå University Hospital. The characteristics of the recruited participants are summarized in (Table 1). At recruitment, blood samples were taken by venous puncture and serum was harvested after centrifugation (1500 x *g*/20 min) and stored at −80°C until further use.

For measurement of circulating gal-3 levels during the first trimester of normal pregnancies and spontaneous abortion (SA),

TABLE 1 Characteristics of the recruited participants at Umeå University Hospital, Sweden

Parameters	Non-pregnant (n = 20)	First trimester (n = 30)	Second trimester (n = 20)	Third trimester (n = 20)
Age (y)	28.9 ± 5.9	28.6 ± 3.04	30.0 ± 4.5	30.1 ± 4.4
GA (wk)	–	9 - 12	18 – 12	37 –41
IUD	5	–	–	–
OC	7	–	–	–
Other methods	8	–	–	–

Note: Exclusion criteria: pregnant women with underlying conditions such as obesity, diabetes mellitus type I or type II, cardiovascular diseases including high blood pressure, autoimmune diseases, hormonal disorders, previous history of recurrent abortions or infertility, chronic diseases, any permanent medication or a smoking habit, pathological pregnancy progression such as an intrauterine growth retardation, preeclampsia, intrauterine infections, premature labor, placenta praevia, bleedings and other placental or fetal abnormalities.

Abbreviations: IUD, intrauterine device; GA, gestational age in weeks; OC, oral contraception.

samples from a prospective cohort study conducted by the Departments of Internal Medicine, Psychosomatics and Obstetrics at the Charité, University Medicine Berlin, Germany were used.[17] Written informed consent was obtained from all the women, and the study was approved by the ethics committee of the local and Charité- Medicine University of Berlin (renewed EA2/030/06). The recruited participants' characteristics are summarized in (Table 2).

For analyses of gal-3 levels during normal pregnancies and GDM, blood samples were collected from healthy and GDM pregnant women in the first, second, and third trimester of pregnancy at their planned visits to the Department of Obstetrics, Sao Paulo Federal University (UNIFESP), Brazil. All patients involved in this work were properly informed about the purpose of our research and gave their written consent before the sampling. The study was approved by the ethics committee of Sao Paulo Federal University (UNIFESP). Characteristics of the recruited participants are summarized in Table 3. Diagnosis of GDM was based on the criteria proposed by the World Health Organization: fasting glucose ≥126 mg/dL and/or ≥140 mg/dL 2 hours after the ingestion of 75 g of glucose (OGTT). Control population consisted of 155 healthy pregnant women without any maternal or fetal disorders. Groups were matched by ethnicity (self-referred). Inclusion criteria for both groups were as follows: singleton pregnancy with living fetus and gestational age between 6 and 36 weeks. Exclusion criteria for both groups were as follows: autoimmune diseases, pre-existing diabetes, uterine malformation, pregnancy resulting from in vitro fertilization, placental abruption, infection, cancer, or any other systemic disease, including pre-existing hypertension. We also excluded women with solid organ transplantation and in the use of steroids, antibiotics, immunosuppressants, antihistamines, or anti-inflammatory medication.

TABLE 2 Characteristics of the recruited participants at Charité—Universitätsmedizin Berlin, Germany

Parameters	Normally progressing pregnancy (n = 80)	Subsequent spontaneous abortion (n = 55)
Age (y)	29.7 ± 2.8	30.5 ± 3.5
GA (wk)	4-12	4-12

Note: Exclusion criteria: fertility treatment, hepatitis B/C or HIV infection; signs of an imminent miscarriage such as vaginal bleeding, low β-hCG, missing embryonic/fetal heart rate during ultrasound screening. Exclusion criteria for the subsequent spontaneous abortion group: molar pregnancy, abnormal fetal karyotype or infection-induced abortion.

Abbreviation: GA, gestational age in weeks.

2.2 | Galectin-3 staining in human samples

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded placental tissues as previously described.[18] Briefly, 4 μm sections derived from the first trimester and term placenta biopsies derived from normal pregnancy were dewaxed and rehydrated through graded alcohols. Antigen retrieval was performed at 99°C for 20 min in a pH9 retrieval solution, and slides were incubated in Sequenza racks with primary antibody gal-3 (0.5 μg/mL; Santa Cruz Biotechnology sc-32790), cytokeratin 7 (0.09 μg/mL; Abcam ab68459), mouse IgG1 isotype control (Dako) or rabbit monoclonal antibody (Cell Signaling Technology) at equivalent concentrations at 4°C for 18 hours. Anti-HLA-G immunohistochemistry was performed as previously described.[19] Staining was visualized using the NovaRed peroxidase HRP substrate kit (Vector Laboratories) and counterstained using Mayer's hematoxylin (Merck Millipore). The sections were imaged using a NanoZoomer-SQ Digital Slide Scanner (Hamamatsu) and NanoZoomer Digital Pathology software at 200× magnification, and antibody staining was quantitated using ImageJ.

2.3 | Purification of CTB and EVT cells

Placental tissue was obtained from patients undergoing a legal abortion during the first trimester (8-12 weeks of gestation) or at delivery. Informed written consent was obtained from all the patients before their inclusion in the study, for which approval was obtained from the

TABLE 3 Characteristics of the recruited participants at Sao Paulo Federal University (UNIFESP), Brazil

Parameters	Controls (n = 40)		Gestational diabetes mellitus (n = 40)	
Age (y)	30.3 ± 6.1	32.0 ± 6.1	31.5 ± 4.1	33.2 ± 5.3
GA (wk)	39.8 ± 1.54	39.75 ± 1.16	39.67 ± 1.30	39.83 ± 1.40
BMI (pre-pregnancy)	21.92 ± 3.97	25.04 ± 7.90	29.38 ± 8.03	26.96 ± 4.73
Birthweight (g)	3339.8 ± 568	3294 ± 440	3662.1 ± 562	3635.9 ± 661
Umbilical artery pH	7.28 ± 0.10	7.30 ± 0.08	7.30 ± 0.07	7.30 ± 0.10
Child gender	Male	Female	Male	Female

Note: Diagnosis of GDM was based on the criteria proposed by the World Health Organization: fasting glucose ≥126 mg/dL and/or ≥140 mg/dL 2 h after the ingestion of 75 g of glucose (OGTT). Inclusion criteria: singleton pregnancy with living fetus; gestational age between 6 and 36 wk. Exclusion criteria: autoimmune diseases, pre-existing diabetes, uterine malformation, pregnancy resulting from in vitro fertilization, placental abruption, infection, cancer, or any other systemic disease, including pre-existing hypertension, solid organ transplantation, use of steroids, antibiotics, immunosuppressants, antihistamines or anti-inflammatory medication.

Abbreviation: GA, gestational age in weeks at birth.

local ethics committee of Geneva University Hospital, Switzerland. Trophoblast cells were isolated as previously described.[16] In brief, fresh tissue specimens were isolated and washed several times in sterile Hanks balanced salt solution. Tissue was then enzymatically digested five times for 20 minutes at 37°C (0.25% trypsin, 0.25 mg/mL Dnase I; Roche, Diagnostics GmbH). After incubation, the trypsin cocktail was neutralized with fetal bovine serum (FBS), and the cells resuspended in Dulbecco's modified Eagle's medium (DMEM; Invitrogen). This cell suspension was filtered through a 50-μm mesh laid onto a Percoll gradient (70-5% Percoll diluted with HBSS) and centrifuged for 25 minutes at 1200 *g*. The 30%-45% percoll layer containing trophoblast cells was collected, the cells washed and resuspended in DMEM (Invitrogen). The cells were then immunopurified with immobilized anti-CD45 antibodies. Ninety-five percent of cells were positive for cytokeratin 7 and negative for vimentin. To obtain extravillous cytotrophoblast (EVT) cells, the cells were seeded on Petri dishes for 15 minutes. Supernatants containing EVT were centrifuged, and the cells were resuspended in culture medium and seeded in 6-well plates (4×10^6 cells/well) and in 96-well plates (1×10^5 cells/well). Ninety-five percent of 24 hours cultured cells were positive for cytokeratin 7 and HLA-G and negative for vimentin.

2.4 | Invasion in vitro assay

The human invasive, proliferative extravillous cytotrophoblast HIPEC-65 cell line (a generous gift from Prof. D. Evain-Brion, Paris[20]) was grown in DMEM high glucose containing 10% FBS (Oxoid AG) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin; Invitrogen) at 37°C in a humidified, 5% CO_2 atmosphere. 4×10^5 HIPEC-65 cells were seeded in a six-well plate. The following day, cells were serum-starved for 24 hours. Invasion assay was performed in an invasion chamber as described elsewhere.[21] Briefly, 3×10^4 HIPEC-65 cells in 100 μL were added to the upper compartment of the transwell chambers and 400 μL of culture medium was added in the lower chamber. Cells were then treated with different concentrations (0, 0.5, 1, and 2 μg/mL) of human recombinant gal-3 (R&D Systems) in serum-free medium for 24 hours at 37°C in a CO_2 (5%) incubator. After incubation, viable cells that invaded collagen were stained with crystal violet and measured at 560 nm. Each experiment was run in triplicate. Data were expressed as the percentage of treated cells that invaded the collagen-coated membrane relative to the untreated (control) cells.

2.5 | Tube formation in vitro assay

SGHPL-4 cells were a kind gift from Judith E. Cartwright (St George's University of London, UK). SGHPL-4 cells are derived from primary human first-trimester extravillous trophoblasts (EVT) transfected with the early region of SV40 (previously known as MC418) and retain features of normal EVT,[22] including similar invasive potential. Tube formation was done in growth factor–reduced Matrigel (BD Bioscience) in μ-slides (Ibidi). SGHPL-4 cells in serum-free media were seeded onto the Matrigel-coated wells (10 000 cells per well) and were treated with Placental Growth Factor (PLGF, R&D systems 264-PGB/CF, 100 ng/mL), human recombinant gal-3 (R&D Systems 1154-GA 2 μg/mL) or gal-3C (10 μg/mL). After 48 hours incubation (37°C), tube formation was assessed through an inverted phase-contrast microscope at ×5 (Zeiss). Quantification was done with the WimTube software (Wimasis).

2.6 | Detection of cell fusion by cell-labeling

BeWo choriocarcinoma cells (ECACC) were treated as previously described.[23] Briefly, 2.5×10^5 BeWo cells were labeled with 8 μg/mL DiO (1,1′-dioctadecyl-indocarbocyanine perchlorate) fluorescent cell-labeling

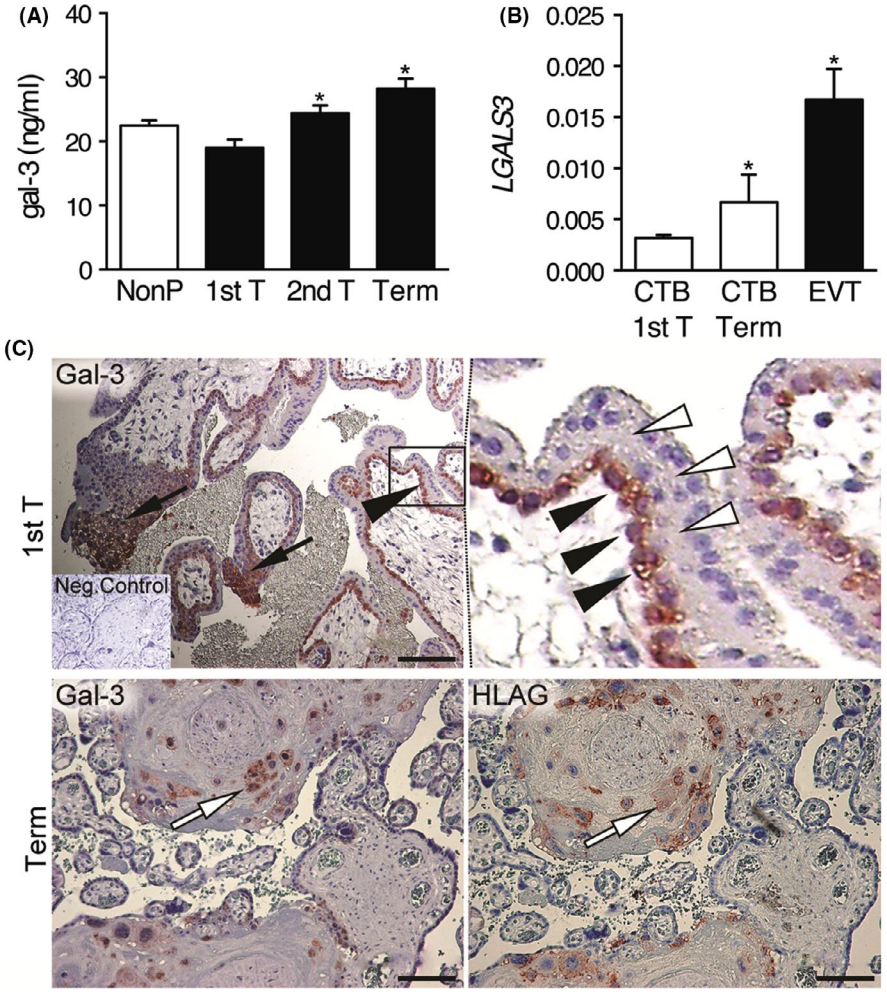

FIGURE 1 Local and peripheral gal-3 expression during normal pregnancy. A, Circulating gal-3 levels were measured with ELISA in non-pregnant (Non-P) and pregnant women during the first, second, and third trimester. B, Placental *LGALS3* expression in isolated extravillous trophoblasts (EVT) and cytotrophoblasts (CTB) from first trimester and term pregnancy fresh placenta as analyzed by quantitative real-time PCR. Data are expressed as mean ± SEM. $^*P < .05$ using one-way ANOVA and Tukey's post-test. C, Representative gal-3 immunostaining of normal first trimester and term placenta paraffin-embedded serial sections. Arrowheads in the upper right panel indicate negative syncytiotrophoblast (STB) and positive cytotrophoblast (CTB) stained for gal-3 expression. At term, extravillous cytotrophoblast (EVT) stained positive for gal-3/HLA-G (arrows). Bars = 250 μm

solution (Vybrant Cell-Labeling Solutions, Molecular Probes) in serum-free DMEM medium for 20 minutes at 37°C without CO_2. Further 2.5×10^5 BeWo cells were labeled with 4 μg/mL DiI (1,1′-dioctadecyl-3,3,3′-tetramethylindocarbocyanine perchlorate) fluorescent cell-labeling solution. Cells were washed with serum-free DMEM medium three times. After washing, DiO and DiI labeled cell suspensions were mixed in one well of a 24-well plate. Finally, cells were incubated with 10 ng/mL gal-3 (R&D Systems 1154-GA) for 48 hours at 37°C. Cell suspensions without application of gal-3 were used as control. Three independent experiments for BeWo cells were performed. The amount of cell fusion was evaluated in ten randomly chosen fields of each well using Zeiss Axiovert 40 CFL fluorescent microscope (Zeiss). Images were obtained with a digital camera system (Power Shot A620; Canon).

2.7 | Galectin-3 ELISA

Gal-3 concentrations in the serum of pregnant patients were determined by ELISA as described previously.[24] The paired antibodies for gal-3 ELISA assay are anti-human gal-3 (AF1154) and biotin-conjugated anti-human gal-3 (BAF1154) from R&D system. Each reported value is the mean of triplicate assays.

2.8 | Statistical analyses

All data are presented as mean ± standard error, except where indicated. Results were analyzed with GraphPad Prism 8.0 (GraphPad Software Inc). Comparisons were performed with non-parametric Mann-Whitney U test or one-way ANOVA and Tukey's post-test. A *P* value <.05 was considered as significant.

3 | RESULTS

3.1 | Normal pregnancy progression implies up-regulation of gal-3 systemic levels and trophoblast lineage-specific gal-3 expression

To determine if variable gal-3 levels occur in human pregnancy, we first analyzed circulating gal-3 levels by ELISA in a cohort of patients coursing healthy pregnancies during the first, second, and third trimester. We observed a steady, significant increase of circulating gal-3 levels from the first to the third trimester and when compared to non-pregnant women (Figure 1A). In addition, as shown in Figure 1B, analyses of lineage-specific *LGALS3* expression revealed higher mRNA levels in

EVT derived from first-trimester chorionic villi compared with villous CTB from the first and third trimesters. When comparing CTB from the first and third trimester the *LGALS3* mRNA levels increased only slightly with pregnancy progression (Figure 1B). Localization of gal-3 in human placenta was characterized using immunohistochemistry on formalin-fixed paraffin-embedded sections. Gal-3 localized to the progenitor cytotrophoblast cells of the chorionic villi (Figure 1C) and the trophoblast cell columns in an increasing gradient of expression toward the distal invasive edge, but was not detected in the STB (Figure 1C, upper panels). In third-trimester placenta, gal-3 was found in the EVT (Figure 1C, bottom panels). To confirm identity of the EVT, HLA-G immunohistochemistry was performed on serial sections (Figure 1C).

3.2 | Gal-3 promotes trophoblast functions associated with placental vascularization in vitro

Since the EVT is responsible for uterine artery remodeling and gal-3 is highly expressed in EVT during normal gestation, we next investigated the influence of gal-3 on trophoblast invasion using the human EVT cell line HIPEC-65. We found that gal-3 dose-dependently increased the relative cell invasion in vitro (Figure 2A). To provide further insights into the mechanism by which gal-3 regulates EVT function, we treated the EVT-derived cell line (SGHPL-4) with human recombinant (hr) gal-3. As depicted in Figure 2B, the number of networks and total length of capillaries was significantly increased in hrgal-3 treated SGHPL-4 cells compared with untreated cells and similarly to PLGF-treated positive controls. Accordingly, we found that treatment with a truncated form of gal-3 (gal-3C, a dominant-negative inhibitor of gal-3[25]) decreased the number of networks and total length of capillaries of SGHPL-4 cells (Figure 2B). In a third model, we found that treatment of BeWo trophoblast cells with hrgal-3 resulted in an increase in cell fusion (Figure 2C).

3.3 | Onset of GDM is associated with a decrease of gal-3 maternal serum levels

In order to define if gal-3 is dysregulated during adverse pregnancy outcome entities, we next analyzed circulating levels of gal-3 in pregnancies affected by spontaneous abortion and gestational diabetes mellitus (GDM). In our prospective cohort, first-trimester gal-3 serum levels did not differ between healthy pregnant women and women who subsequently suffered from spontaneous abortion (Figure 3A). In

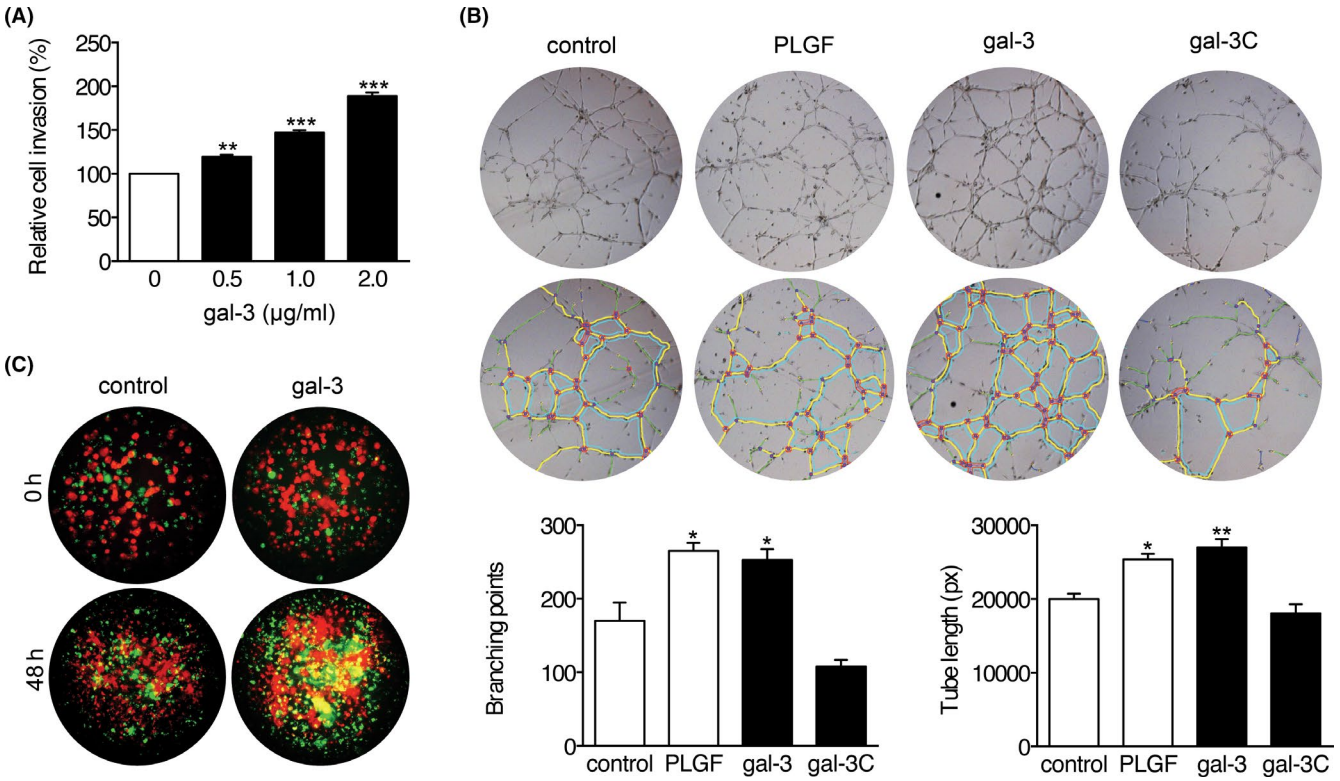

FIGURE 2 Exogenous gal-3 influences trophoblast cell properties in vitro. A, Relative cell invasion of the extravillous trophoblast (EVT) cell line HIPEC-65 when treated with hrgal-3 (n = 4). B, Effect of gal-3 treatment on capillary-like network formation by SGHPL-4 cells (n = 3-5). Treatment of SGHPL-4 cells with gal-3 significantly increased the formation of the capillary-like networks, as scored by the number of branch points and total length formed following 24 h of treatment. In addition, the inhibitor gal-3C blocked the formation of capillary-like networks by SGHPL-4 cells. C, BeWo cells were stained with DiO (green) or Dil (red) and mixed. Both cell populations are seen in 0 h cell culture in vitro. Fusion BeWo cells stimulated with gal-3 appear in yellow, 48h. All treatments were performed in triplicate in at least three independent experiments. In all figures, data are plotted as mean ± SEM. *$P < .05$, **$P < .01$, and ***$P < .001$, using one-way ANOVA with Tukey's multiple comparisons test

contrast, placenta of spontaneous abortion in late first and early second trimester displayed reduced expression of gal-3 (Figure 3B), which in gestational-age matched controls was mainly confined to CTB and EVT of the trophoblast cell columns and cell islands (Figure 3B). CK7 green fluorescent labeling used to identify the trophoblasts shows that gal-3 is expressed throughout the cell column whereas CK7 expression is detected only more distally (Figure 3B).

When analyzing the circulating gal-3 levels during the development of GDM, we did not observe any significant differences between normal and GDM pregnancies during the first and second trimesters (Figure 3C). However, during the third-trimester GDM was associated with a significant decrease in systemic gal-3 levels (Figure 3D). In GDM placenta, gal-3 expression was observed in a pattern similar to normal third-trimester pregnancies (Figure 3D) with CK7 employed in serial sections to identify trophoblast.

4 | DISCUSSION

Gal-3 has been recognized as an important modulator of biological processes and an emerging player in the pathogenesis of several diseases including metabolic and immune/inflammatory disorders. However, scarce attention has been paid to the role of gal-3 in normal pregnancy progression and onset of pregnancy complications. In the present study, we demonstrated that gal-3 increased in maternal circulation with progression of uneventful pregnancy. Moreover, we showed that gal-3 is mainly expressed in EVT during the first trimester promoting critical trophoblast functions (e.g., invasion and tube formation) which influence healthy placental development. Finally, our findings suggest that progression of GDM is associated with changes in maternal gal-3 levels, highlighting a novel role during impaired glucose homeostasis.

The expression of gal-3 significantly increases in the secretory phase endometrium and shows a specific pattern within the decidua and placenta during the first trimester of pregnancy.[9,10,12] In the present study, we show that normal progression of pregnancy is associated with increased circulating levels of gal-3 during the second and third trimesters. The increase in maternal peripheral levels coincides with the period of placental growth during the second and third trimesters, suggesting that trophoblast cells could be one of the sources of the circulating chimera lectin. Supporting this, we have shown that gal-3 expression in the CTB increases from the first to the third trimester in healthy placental tissue. In addition, our analysis of EVT gal-3 expression revealed that the chimera lectin is highly expressed in the EVT layer of the human placenta. This observation is in agreement with previous studies showing that both interstitial and endovascular EVT are main sources of gal-3[10] and with the recently established role of gal-3 as a component of the human trophoblast invasion machinery.[26] The functional studies reported here have confirmed that gal-3 participates in trophoblast cell migration/invasion. Furthermore, the tube formation ability of trophoblasts (angiogenesis) was stimulated by gal-3 and reduced in presence of gal-3C. Because

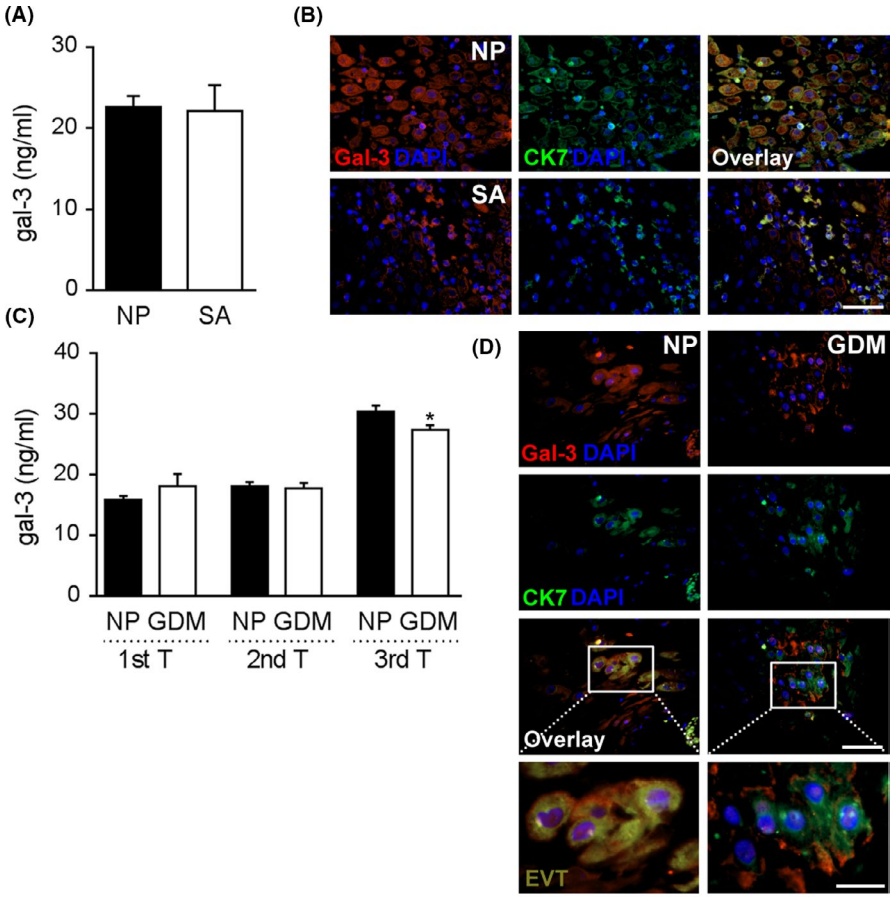

FIGURE 3 Local and peripheral gal-3 expression during pathological pregnancies. A, Circulating gal-3 levels during the first trimester were analyzed with ELISA in normal pregnant women (NP) and in patients with spontaneous abortions (SA). B, Immunofluorescence double staining with gal-3 (red) and CK7 (green) expression in a NP and SA trophoblast sample. Nuclei are labeled with DAPI (blue), Bar: 50 μm. C, Gal-3 levels in the circulation of normal pregnant (NP) women and patients with gestational diabetes mellitus (GDM) were measured with ELISA in the first, second, and third trimester. D, Immunofluorescent double labeling of gal-3 (red) and CK7 (green) expression in NP and GDM term placenta (Bars: 50 μm upper panel and 120 μm lower panel). In (A) and (C), data are expressed as mean ± SEM and analyzed with Mann-Whitney *t* test and one-way ANOVA with Tukey's post-test (*$P < .05$)

trophoblast growth and function play a critical role in determining fetal growth, our results showing that gal-3 promotes Bewo syncytium formation together with its localization to villous CTB cells in the first-trimester placenta, indicate that the chimera lectin is necessary for placental health. Indeed, those EVT lineages that subsequently invade the decidua display the highest gal-3 expression, implying that this chimera lectin might be a major trigger for the process of trophoblast cell differentiation and also STB fusion. The syncytial surface is a critical component of physiological repair and differentiation of the placental villous tree, its alteration has been suggested to reduce nutrient flow between mother and fetus resulting in poor neonatal outcomes.[27]

While several reports have highlighted dysregulation of gal-3 placental expression during poor pregnancy outcomes,[11,13,14] their association with variations of maternal gal-3 circulating levels remains elusive. Our study provides the first evidence regarding systemic levels of maternal gal-3 during the first trimester in women who subsequently suffered from spontaneous abortion. Although we did not find any differences in the maternal levels of circulating gal-3 compared with normally progressing pregnancy, our findings demonstrate the need to incorporate more members of the galectin family to the panel of diagnostic markers defining the galectin signature that characterizes each pregnancy complication. Of note, we previously found that circulating gal-1 levels were down-regulated in SA using the same cohort of patients.[16] In addition, our results show that the kinetics of peripheral gal-3 differs from gal-1 as circulating levels of the prototype lectin significantly decreased during the first trimester,[16] even when β-hCG values were within the normal range. In this regard, it must also be noted that gal-3 expression during early gestation is under regulation of β-hCG.[28] Thus, the absence of changes in circulating gal-3 in early pregnancy may be related to its kinetics itself but not be predictive of the development of spontaneous abortion. In support of this notion, it has been shown that maternal gal-3 circulating levels are decreased after the onset of missed abortion.[29] In addition, dysregulation of gal-3 in placental villi has also been described for patients with missed abortion and may explain the observed dysregulation of peripheral gal-3 levels.[30]

An additional aim of this study was to determine the kinetics of the circulating maternal gal-3 throughout gestation, evaluating both uneventful pregnancies and development of GDM. We report here that serum gal-3 levels were reduced in patients that developed GDM. The differential peripheral gal-3 kinetics observed in GDM pregnant women was only evidenced during the third trimester, suggesting that gal-3 is sensitive to the hormonal and metabolic changes that characterize GDM. Although gal-3 has both pro- and anti-inflammatory effects,[31] in the context of chronic inflammation disorders as GDM, the chimera lectin exerts anti-inflammatory effects including stimulation of T-cell apoptosis, inhibition of T-cell growth and Th1 differentiation limiting further tissue injury.[32,33] Therefore, it is conceivable that reduced peripheral levels of gal-3 would contribute to the pro-inflammatory response (eg, TNF-α, IL-6, and adipocytokines) in GDM patients.[34] In addition, several studies have reported inflammation in association with altered glucose homeostasis in gal-3 deficient mice fed a high-fat diet, suggesting that gal-3 decreases immunity to overnutrition and protects against the obesity-associated type 2 diabetes.[35,36] The results reported here provide new insights in the relation between metabolic alterations during GDM and gal-3 and point the need of further investigation on the effect of gal-3 and glucose homeostasis during gestation.

In summary, this study reveals that the course of normal pregnancy requires the up-regulation of gal-3 expression, highlighting its requirement for proper EVT functions and reinforcing the concept that unique functional properties in support of healthy pregnancy are specific to each of the different members of the placental galectin network.

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CONFLICT OF INTEREST

Constance M. John is CEO and founder of MandalMed, Inc

AUTHOR CONTRIBUTIONS

S.M.B. conceived, designed the research, and secured grant funding. N.F., I.T-G., M.C., D.G-W., G.B., U.J., and S.M.B. performed research and analyzed data. L.M-N., S.D., and C.M.J. contributed essential reagents and human cohort's data. N.F. and G.B. gave input on writing the manuscript. S.M.B. wrote the paper. All authors read and approved the final manuscript.

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