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High Glucose Alters Proteoglycan Expression and the Glycosaminoglycan Composition in Placentas of Women with Gestational Diabetes Mellitus and in Cultured Trophoblasts

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Abstract

Impaired glucose metabolism with diabetes may alter the expressions of proteoglycans (PGs), which may impair the biological functions of placenta. In this study, we investigated the expression of PGs and their conjugated glycosaminoglycan (GAG) composition in the placentas of mothers with gestational diabetes mellitus (GDM) and trophoblasts cultured in a high-glucose condition. The PGs by guanidine/HCl extraction and DEAE Sepharose fractionation followed by GAG degradation enzyme digestion analyses showed that the expression of chondroitin sulfate and/or dermatan sulfate (CS/DS) PGs was increased whereas the heparan sulfate (HS) PG was decreased in GDM placentas compared to controls. Western blot analyses demonstrated that the increased CS/DS PGs in GDM placentas were predominantly the small leucine-rich proteoglycans (SLRPs), decorin and biglycan. Increased mRNA expression level was consistently shown by quantitative real-time PCR. Immunohistochemistry indicated intensive staining of decorin and biglycan in the diabetic placenta with different localizations. Additionally, the basement membrane HSPG, perlecan was found to contain both CS/DS and HS in GDM placentas and plain HS in controls. Similar findings of PG alterations induced by hyperglycemia were observed in cultured trophoblast in a high-glucose condition. This study demonstrated that hyperglycemia induced not only the gene expressions of PGs but also alterations in the carried GAG type and composition.

Keywords: Biglycan; Decorin; Gestational diabetes mellitus; Glycosaminoglycan; Perlecan; Proteoglycan

1. Introduction

Diabetes mellitus during pregnancy may have adverse effects in the placenta including increased placental weight [1,2], oxidative stress [3], and nutrient transport and filtration problems [4]. Pathological studies revealed patchy syncytial necrosis, dilated rough endoplasmic reticulum, cytotrophoblastic hyperplasia, narrowing of the small vessels, focal thickening of the basement membranes, and related extracellular matrix alterations in the diabetic placenta [2–6].

Proteoglycan is a complex macromolecule composed of a core protein and one or more negatively charged polysaccharide chains called glycosaminoglycans (GAGs), which are covalently attached to the protein core [7,8]. The GAG is made up of repeating disaccharide units, classified into the four common types of heparin/heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS). In the placenta, proteoglycans are distributed in the endothelium, cell basement membranes, vessel walls, and villous stroma

Abbreviations: PG, proteoglycan; ECM, extracellular matrix; GDM, gestational diabetes mellitus; CS, chondroitin sulfate; DS, dermatan sulfate; SLRP, small leucine-rich proteoglycan; HS, heparan sulfate; GAG, glycosaminoglycan; KS, keratan sulfate; Hepase, heparintinase; Chase ABC, chondroitinase ABC; Chase B, chondroitinase B; CHAPS, 3-[(3)-cholamidopropyl-dimethyl-ammonio]-1-propanesulfonate; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; CPC, 1-hexadecyl pyridinium chloride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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[9–13]. Detailed characterizations indicate the placental basement membrane tissue predominantly contains heparan sulfate proteoglycan (HSPG), whereas chondroitin sulfate and/or dermatan sulfate proteoglycans (CS/DS PGs) are mainly located in the intervillous space of the ECM [14]. Others and ourselves have demonstrated a placental HSPG, perlecan, to be present in the mesenchymal villi and cytotrophoblastic cell islands and cell columns, which consists in extravillous trophoblasts [9,15] and co-localizes with other basement membrane proteins, such as laminin and collagen type IV [9]. The HSPG, perlecan, composed of a 400–470 kDa core protein with three potential GAG attachment sites [16,17], is abundant in the extracellular basement membrane of vascularized tissues. It was implied that perlecan expression might be essential to placental vascularization during early gestation [15]. The other major PG in the placenta is CS/DS PG, which includes the small leucine-rich proteoglycans (SLRPs), decorin and biglycan. Decorin usually has one CS/DS GAG, whereas biglycan has two; they are the most-closely related molecules in the SLRP superfamily. It was shown that decorin and biglycan are associated with collagen type VI in placental stroma [10,18]. Immunocytochemistry and electron microscopic analyses suggested that SLRPs might be involved in the assembly of the ECM structure of the placenta through their regulation of collagen fibrillogenesis [19,20]. We showed that the expression of perlecan was significantly higher in placentas of mothers with GDM, and hyperglycemia induced the production of perlecan by trophoblasts [21]. Previous reports revealed that perlecan is involved in various diseases resulting from hyperglycemia-induced basement membrane alterations and vasculopathies [21–28]. It remains unclear whether hyperglycemia induces PG, especially decorin and biglycan expressions and changes in their conjugated glycosaminoglycan composition. Thus, in the present study, we investigated alterations in proteoglycans (PGs) and their conjugated glycosaminoglycan (GAG) composition in the GDM placenta. In addition, an in vitro culture system for trophoblasts exposed to high- and low-glucose conditions was also set up to demonstrate the hyperglycemic effect on the expressions of proteoglycans of interest and their GAG composition. These studies provide further information on alterations of proteoglycans induced by hyperglycemia that may help to understand the correlation of these molecules with complications in the diabetic placenta.

2. Materials and methods

2.1. Materials

Sixteen (eight normal as the controls and eight with GDM) third trimester placentas were obtained from pregnant women at gestational ages 36–40 weeks after a caesarean section or vaginal delivery. The diagnosis of GDM was based on a 100 g oral glucose load with two or more venous plasma glucose levels meeting the following criteria: fasting, $\geq$105 mg/L; 1 h, $\geq$190 mg/L; 2 h, $\geq$165 mg/L; or 3 h, $\geq$145 mg/L [29]. According to the clinical characteristics, women with GDM and poor glycemic control, including those clinically managed by diet alone ($n = 3$) or by diet and regular insulin ($n = 5$), were selected for the GDM group in this study. There were no significant differences in the clinical characteristics of patients with GDM and controls except for gravidity, 3.3 ± 1.8 vs. 1.3 ± 0.5; $p = 0.01$ and HbA1c, 5.8 ± 0.6 vs. 5.1 ± 0.3; $p = 0.013$ after undergoing dietary treatment or dietary with insulin treatment during pregnancy. The GDM group had a poor obstetric history with more instances of prior spontaneous abortions. The fetal birth weight (3421.3 ± 233.7 vs. 2952.4 ± 107.5 g) and placenta weight (672 ± 175.2 vs. 500 ± 35.4 g) in the GDM group were higher than those of controls but did not significantly differ ($p > 0.05$). Approval for this study was obtained from Mackay Memorial Hospital and informed consent was obtained from each participating subject.

Heparintinase (Hepase) from *Flavobacterium heparinum* was purchased from Seikagaku, chondroitinase ABC (Chase ABC) from *Proteus vulgaris* and chondroitinase B (Chase B) from *F. heparinum* were purchased from Sigma. Monoclonal antibodies against human perlecan for immunoprecipitation were obtained from Chemicon (clone A7L6). Monoclonal antibodies against human perlecan for Western blot analysis were purchased from Zymed (clone 7B5). Antibodies against human decorin (LF136) and biglycan (LF51) were kindly provided by Dr. Larry W. Fisher from the National Institute of Health (Bethesda, MD, USA). All chemicals were purchased from Sigma (St. Louis, MO, USA) unless specifically stated otherwise.

2.2. Isolation of proteoglycans from the placenta

The protocol for extraction of PGs from the placenta followed that previously described by Yang et al. [9]. Placental tissues were minced and washed with phosphate-buffered saline (PBS) (0.15 M NaCl, pH 7.4, 1:20, w/v). Subsequently, the tissue slices were re-suspended in 4 M guanidine/HCl (15:20, w/v) containing 0.5% 3-[(3)-cholamidopropyl-dimethyl-ammonio]-1-propanesulfonate (CHAPS), 2% β-mercaptoethanol, or 10 mM dithiothreitol, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM diisopropyl fluorophosphates in propanol) and incubated end-over-end for at least 12 h at 4 °C, followed by centrifugation at $16{,}000 \times g$ for 60 min at 4 °C. The supernatant was then dialyzed to 7 M urea in 10 mM Tris–HCl (pH 8.3) at 4 °C with three buffer changes. The proteoglycan extract was stored at −20 °C for further analysis.

2.3. Fractionation of the placental proteoglycan extract

The placental PG extract in 7 M urea in 10 mM Tris–HCl (pH 8.0) was diluted with buffer A (10 mM Tris–HCl; pH 8.3) (1:1, v/v) and subjected to a DEAE Sepharose Fast Flow column (HR 5/10, Amersham Biosciences, Piscataway, NJ, USA), which had been pre-equilibrated with buffer A in a liquid chromatographic system (AKTA Basic 10, Amersham Biosciences, Piscataway, NJ, USA). After extensive washing with buffer A to remove any unbound material, the absorbed material was eluted by a salt gradient from 0 to 1 M NaCl in buffer A. Fractions comprising five and seven major peaks according to the positive absorbance at OD 280 and OD 214 were pooled for collection. The fractions in the individual peaks and their eluted salt concentrations were P1, fractions 1–5 (unbound material); P2, fractions 7–9 (0–0.08 M NaCl); P3, fractions 10–15 (0.08–0.2 M NaCl); P4, fractions 16–21 (0.3–0.35 M NaCl); P5, fractions 23–29 (0.35–0.48 M NaCl); P6, fractions 31–35 (0.6–0.7 M NaCl); P7, fractions 39–49 (0.8–1.0 M NaCl). The fractionated samples were stored at −20 °C until use. The protein concentration was determined by a protein assay kit (Bio-Rad, D*c* protein assay, Hercules, CA, USA).

2.4. Proteoglycan sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Proteoglycans (PGs) were separated by SDS-PAGE as described by Laemmli [30]. After fixation with 40% ethanol and 10% acetic acid, the gel was stained with alcian blue solution (0.5% alcian blue in 3% acetic acid) for the observation of intact PGs and subsequently stained with Coomassie blue G25 staining for the core protein.