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Technical note

Decorin expression is decreased in first trimester placental tissue from pregnancies with small for gestation age infants at birth

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ABSTRACT

Fetal growth restriction (FGR) is a leading cause of perinatal morbidity and mortality. FGR pregnancies are often associated with histological evidence of placental vascular thrombosis. The proteoglycans are important components and regulators of vascular homeostasis. Previous studies from our laboratory highlighted mRNA and protein expression differences in placental proteoglycan decorin (*DCN*), within a clinically well-characterised cohort of third-trimester idiopathic FGR compared with gestation-matched uncomplicated control pregnancies. We also showed that decorin contributes to abnormal angiogenesis and increased thrombin generation *in vitro*. These observations suggest that *DCN* gene expression may contribute to the etiology of FGR. Small for gestational age (SGA) is frequently used as a proxy for FGR and is defined as a birth weight below the 10th percentile of a birth weight curve. We therefore made use of a unique resource of first trimester tissues obtained *via* chorionic villus sampling during the first trimester to investigate the temporal relationship between altered DCN expression and any subsequent development of SGA. We hypothesized that placental *DCN* expression is decreased early in gestation in SGA pregnancies. Surplus chorionic villus specimens from 15 women subsequently diagnosed with FGR and 50 from women with uncomplicated pregnancies were collected. *DCN* mRNA and DCN protein were determined using real-time PCR and immunoblotting, respectively. Both *DCN* mRNA and protein were significantly decreased in placentae from first-trimester SGA-pregnancies compared with controls ($p < 0.05$). This is the first study to report a temporal relationship between altered placental *DCN* expression and subsequent development of SGA.

1. Introduction

Fetal growth restriction (FGR) is a significant pregnancy disorder that has major consequences for the fetus and neonate as well as an increased risk of long-term morbidity extending into adulthood. Small for gestational age (SGA) is often used as a surrogate for FGR and is defined as a birth weight below the 10th percentile on a birth weight curve [1]. While a number of maternal and fetal factors which contribute to FGR have been identified, the etiologies of the majority of cases remain uncertain [1]. FGR pregnancies are often associated with histological evidence of placental vascular thrombosis, more specifically microvascular thrombosis within the placenta [2–4].

Recent studies from our laboratory reported that altered placental proteoglycan expression and function may contribute to the coagulation disturbance that leads to maternal-placental vascular thrombosis in third trimester FGR pregnancies. Proteoglycans are macromolecules located within vessel walls that contain a core protein to which sulphated glycosaminoglycan (GAG) chains are covalently linked. There are four types of GAG chains located in the blood vessel wall; chondroitin sulphate, dermatan sulphate, heparan sulphate, and hyaluronan [5]. However,

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only chondroitin sulphate, dermatan sulphate, and heparan sulphate are covalently linked to a core protein [5]. The placenta contains two major types of proteoglycans; those containing heparan sulphate and those containing chondroitin sulphate or dermatan sulphate [6–9]. Decorin (DCN) usually has one chondroitin sulphate or dermatan sulphate GAG attached to its core protein.

We previously demonstrated that DCN protein is localised to the stroma surrounding fetal blood vessels of the third trimester placental villi and its expression is decreased in placentae obtained from third trimester FGR-affected pregnancies [10]. Analysis of mice with targeted deletion of the *Dcn* gene provided conclusive evidence for a role of the *Dcn* gene in the causal link to preterm birth and reduced birth weight in the offspring [11]. However, determining the cause of human FGR remains a major challenge in human pregnancy research. Uncertainties still exist as to whether the decreased placental *DCN* expression observed in third trimester human FGR-affected pregnancies is truly causative or rather reflects a response to an altered growth process.

We therefore made use of a unique resource of first trimester tissues obtained *via* chorionic villus sampling during the first trimester to investigate the temporal relationship between altered decorin expression and any subsequent development of SGA. We hypothesized that placental *DCN* expression is decreased early in gestation in SGA pregnancies. Early gestation sampling can be accomplished by chorionic villus sampling (CVS), a procedure generally performed between 10 and 14 weeks' gestation. Differentiation of normal from pathological groups is possible, as the eventual maternal and fetal outcomes of ongoing pregnancies are determinable [12,13]. The aim of this study was to quantify *DCN* gene expression using real-time PCR in placental tissues collected during CVS performed at first trimester (10–12 weeks' gestation) from on-going pregnancies with known clinical outcomes, i.e. SGA or uncomplicated control pregnancies.

2. Materials and methods

2.1. Surplus CVS tissue samples

First-trimester placental villous tissue was obtained from surplus tissue at CVS, which was performed vaginally between 10 and 12 weeks' gestation for maternal age or serum screening related risk for aneuploidy [14]. CVS was performed at the University Medical Centre of Groningen, The Netherlands. Surplus CVS tissue samples were collected from pregnant women with informed consent and in accordance to the guidelines of the Federation of Dutch Medical Scientific Societies regarding surplus material not needed for diagnostics.

Patient demographics including follow-up of pregnancy outcome details were collected *via* a questionnaire returned by the patient postpartum. Pregnancies later complicated by SGA were selected from the database, and controls were selected matched on maternal age, parity and gestational age at the time of sampling. SGA cases were selected based on the birth weight below the 10th percentile according to Dutch population charts from the Stichting Perinatale Registratie Nederland [15]. Patient identification was removed, the samples coded and subsequently snap frozen for RNA and protein analysis.

The exclusion criteria for both control and SGA pregnancies were chromosomal abnormalities, congenital anomalies, gestational diabetes, preeclampsia, maternal hypertension, maternal chemical dependency and multiple gestations. The control group was selected based on the crown-rump-length (CRL) of each SGA fetus at the time of CVS. A total of 65 pregnancies were selected based on the RNA quality; n = 15 were from SGA pregnancies and n = 50 were from control pregnancies (Table 1).

2.2. Real-time PCR

Total placental RNA was extracted and purified using the Macherey-Nagel NucleoSpin® RNA kit according to the manufacturer's instructions (Macherey-Nagel Inc. Bethlehem, USA). cDNA was prepared from 500 ng total RNA using Qiagen QuantiTect Reverse Transcription Kit according to the manufacturer's instructions (Qiagen, USA). Decorin mRNA expression was determined using validated assays that consisted of a TaqMan® FAM™ labelled MGB probe (*DCN*, Hs00754870_s1, Thermo Fisher Scientific, USA) on an ABI Prism 7500 (Thermo Fisher Scientific, USA). Gene expression quantitation was performed as the second step in a two-step Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) protocol according to the manufacturer's instructions. Gene expression quantitation for the housekeeping gene *18S* rRNA (VIC-labelled probe, Thermo Fisher Scientific, USA) was performed in the same reaction as described previously [10,16]. Levels of gene expression relative to *18S* rRNA were calculated according to the $2^{-\Delta\Delta CT}$ method [17].

2.3. Western immunoblotting

Total placental protein was extracted using the Macherey-Nagel NucleoSpin® protein kit according to the manufacturer's instructions (Macherey-Nagel Inc. Bethlehem, USA). Placental protein concentration was determined using a Pierce protein assay kit (Thermo Fisher Scientific, USA). Immunoblotting was performed as previously described [10,16]. Briefly, 25 μg of total placental protein from three groups of pooled control (n = 50) and three groups of pooled SGA (n = 15) samples was electrophoresed on a pre-cast Criterion gel (10% Tris-HCl; Bio-Rad Laboratories, USA) under non-reducing conditions. Immunoreactivity was detected using anti-human mouse monoclonal DCN antibody (0.1 μg/mL, ab137508, Abcam, Australia) or rabbit monoclonal Glyceraldehyde 3-phosphate dehydrogenase (0.2 μg/mL, GAPDH; Imgenex, USA). Secondary antibodies used were rabbit anti-mouse IgG (H+L) horse-radish peroxidise (HRP) conjugate (750 ng/mL; Invitrogen/Life Technologies, USA) or goat anti-rabbit IgG (H+L) HRP conjugate (500 ng/mL; Life Technologies, USA). The enhanced chemiluminescence (ECL) prime Western blotting detection reagent (GE Healthcare, UK) was used to detect immunoreactive protein on an ImageQuant LAS-4000 (GE Healthcare, UK). The level of DCN immunoreactive protein relative to GAPDH was determined semi-quantitatively using the scanning densitometry software, ImageJ 1.44p (Wayne Rasband, National Institute of Health).

2.4. Data analysis

All data were analysed using GraphPad Prism (version 7). The student *t*-test was used to evaluate differences between groups (Table 1) and the Mann-Whitney *U* test was used for differences in *DCN* mRNA, while un-paired *t*-test was used for differences in decorin protein between SGA and control groups. A probability value of <0.05 was considered to be statistically significant and 95% confidence intervals (CI) were given where appropriate.

3. Results and discussion

In this study we used a unique collection of placental tissues to study the expression of *DCN* proteoglycan in early pregnancy. These placental tissues were collected during routine CVS between 10 and 12 weeks of pregnancy, from ongoing pregnancies that later developed SGA at term, as well as controls. Table 1 depicts the demographic data collected at delivery for both SGA and control pregnancies used in this study. A significant decrease in birth