Placenta 36 (2015) 1218–1224

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Placental expression of heparan sulfate 3-O-sulfotransferase-3A1 in normotensive and pre-eclamptic pregnancies

F. Amraoui [a, *], H. Hassani Lahsinoui [b, c], S. Boussata [c], R. Keijser [c], G.J.M. Veenboer [c], S. Middeldorp [a], J.A.M. van der Post [b], C. Ris-Stalpers [b, c], G.B. Afink [c], B.J.H. van den Born [a]

[a] Department of Vascular Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
[b] Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, The Netherlands
[c] Reproductive Biology Laboratory, Academic Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form
13 September 2015
Accepted 14 September 2015

Keywords:
Pre-eclampsia
Heparan sulfate
3-O-Sulfotransferase-3A1
Glycocalyx
Blood pressure
Birth weight

ABSTRACT

Introduction: The endothelial glycocalyx, consisting of membrane-bound proteoglycans and attached glycosaminoglycans plays an important role in vascular homeostasis. We aimed to assess whether glycocalyx mRNA transcripts are differentially expressed in placental tissue of pre-eclamptic and normotensive women.

Methods: We evaluated the expression of transcripts encoding for proteins involved in glycocalyx synthesis and degradation using a microarray analysis of placental mRNA obtained from pre-eclamptic and normotensive women. Participants were recruited from the department of obstetrics at a university hospital in Amsterdam, The Netherlands. The most prominent differentially expressed transcript was validated by qPCR on 112 additional placenta samples.

Results: Of 78 preselected genes involved in glycocalyx synthesis and degradation, only *HS3ST3A1* mRNA was differentially expressed in placental tissue obtained from pre-eclamptic women (N = 12) compared to normotensive women (N = 12, fold change = 0.61, $p = 0.02$). Validation with qPCR in additional placental samples of 64 normotensive and 48 pre-eclamptic women confirmed that normalized mRNA expression of *HS3ST3A1* was decreased by 27% (95% CI 14%–41%) in placental tissue obtained from pre-eclamptic compared to normotensive women ($p < 0.001$). *HS3ST3A1* expression was positively correlated with neonatal birth weight in normotensive women ($r = 0.35$, $p < 0.01$) and inversely correlated with mean arterial pressure of women with pre-eclampsia ($r = 0.32$, $p = 0.02$).

Conclusions: The mRNA expression of *HS3ST3A1*, which encodes for a 3-O sulfating enzyme of heparan sulfate (3-OST-3A1), is decreased in pre-eclamptic placental tissue. Expression of this glycocalyx synthesis transcript is correlated with maternal blood pressure and neonatal birth weight, suggesting a possible role in pre-eclampsia-associated placental dysfunction.

1. Introduction

Pre-eclampsia is a hypertensive emergency characterized by new onset hypertension and proteinuria in the second half of pregnancy and is a leading cause of maternal and fetal mortality and morbidity worldwide [1]. Placental dysfunction is critical in the pathophysiology of pre-eclampsia and its fetal sequelae including growth retardation and preterm birth [2]. Inadequate placental perfusion is currently considered a critical step in the pathophysiology of pre-eclampsia [3]. Incomplete spiral artery remodelling and cytotrophoblast differentiation from an epithelial origin to an endothelium-like anticoagulant phenotype may compromise placental blood flow [2,4]. This process of pseudo-vasculogenesis appears to be disturbed in pre-eclampsia and may contribute to local thrombosis and attenuation of placental perfusion [5,6].

The endothelial glycocalyx is a layer of complex sugars

* Corresponding author. Department of Vascular Medicine, Academic Medical Center, Meibergdreef 9, Room F4-139, PO Box 22660, 1100 DD Amsterdam, The Netherlands.
E-mail addresses: f.amraoui@amc.nl (F. Amraoui), h.hassanilahsinoui@amc.nl (H. Hassani Lahsinoui), s.boussata@hotmail.com (S. Boussata), r.keijser@amc.nl (R. Keijser), g.j.veenboer@amc.nl (G.J.M. Veenboer), s.middeldorp@amc.nl (S. Middeldorp), j.a.vanderpost@amc.nl (J.A.M. van der Post), c.ris@amc.nl (C. Ris-Stalpers), g.b.afink@amc.nl (G.B. Afink), b.j.vandenborn@amc.nl (B.J.H. van den Born).

http://dx.doi.org/10.1016/j.placenta.2015.09.008
0143-4004/

consisting of membrane-bound proteoglycans and attached glycosaminoglycan chains lining the intraluminal vessel wall. The endothelial glycocalyx is essential for endothelial function and thus organ perfusion by regulating its anticoagulant and anti-inflammatory properties [7,8]. Heparan sulfate is the major constituent within the glycocalyx and essential for the interaction between vascular endothelial growth factor (VEGF) and VEGFR1, the membrane bound FLT-1 surface receptor [9,10]. Scavenging of VEGF by the soluble form of FLT-1 has been shown to play a critical role in pre-eclampsia [11].

We hypothesize that analogous to the protective role of the glycocalyx in the systemic circulation, perturbation of the placental glycocalyx may jeopardize placental perfusion in pre-eclampsia. In the present study, we compared mRNA expression of enzymes involved in glycocalyx synthesis and degradation in placental tissue obtained from pre-eclamptic and normotensive pregnant women.

2. Methods

2.1. Study population

For this study, clinical data and biosamples were obtained from participants in the Pre-eclampsia And Non pre-eclampsia DAtabase (PANDA) program of the department of obstetrics of the Academic Medical Center in Amsterdam, The Netherlands. Definitions and clinical criteria used in the biobank program have been published previously [12,13]. Briefly, pre-eclampsia was defined by systolic blood pressure (BP) $\geq$140 mmHg or diastolic BP $\geq$ 90 mmHg recorded on two occasions at least 4 h apart, after 20 weeks' gestation in a previously normotensive woman combined with new-onset proteinuria with urinary protein excretion $\geq$ 300 mg/24-h or >1 + on protein dipstick. BP was measured manually in the sitting position at the right upper arm using an aneroid sphygmomanometer. Diastolic BP was determined at Korotkoff sound V. Birth weight percentiles were assessed according to the local Dutch birth weight percentiles. The appropriate chart was chosen based on parity and gender of the baby. (http://www.perinatreg.nl/). HELLP syndrome was defined by lactate dehydrogenase $\geq$600 U/L or haptoglobin <0.2 g/L, aspartate or alanine aminotransferase $\geq$70 U/L, and platelet count <100 $*10^9$/L. Experiments were carried out in accordance with the declaration of Helsinki after informed consent from the participants was obtained. Experiments were approved by an independent ethics committee.

2.2. Microarray analysis, tissue preparation and quantitative real-time PCR

Genes involved in glycocalyx synthesis and degradation were selected from the human GLYCOv4 oligonucleotide array, a custom AffymetrixGeneChip designed for the Consortium for Functional Glycomics which is freely available online (see http://glycomics.scripps.edu/coreE/glycogenelistv4Human.xls). Differential expression of this subset of genes was assessed in 12 normotensive and 12 pre-eclamptic placenta samples using previously obtained RNA microarray data from (NCBI Gene Expression Omnibus GSE54618) [12].

For quantitative real-time PCR (qPCR), placental biopsies from a macroscopically viable (non-infarcted) central cotyledon from the maternal side were obtained immediately after delivery and stored in RNAlater (Ambion) at −80 °C until use. Isolation of mRNA was carried out using the MagNA Pure LC mRNA HS Kit (Roche) and reverse transcription was performed with random hexamers using AMV First Strand cDNA Synthesis Kit (Roche). Q-PCR was performed on a LightCycler 480 system (Roche) according to the manufacturer's protocol, with cDNA reaction mixtures containing 0.4 μmol/L of each primer (Invitrogen), 100 nmol/L UPL probe (Roche) and 5 μl Absolute qPCR mix (Thermo Scientific) in a total volume of 10 μl. Primers/probes were designed using the Roche Universal ProbeLibrary Assay Design Center: *HS3ST3A1*, AGGCCATCATCATCGGAGT/CTGCGGTCGAAGAAGTGG, UPL probe# 67; *PSMD4*, GGCAAGATCACCTTCTGCAC/CTTCCCACAAAGGCAATGAT, UPL probe# 21. Data were analyzed and quantified, using the second derivative maximum for Cp determination, with the LightCycler 480 software 1.5.0 (Roche). *HS3ST3A1* copy numbers were normalized to *PSMD4* (proteasome 26S subunit, non-ATPase 4) mRNA copy numbers.

To determine tissue specificity of *HS3ST3A1* expression, 10 μg total RNA from 20 normal human tissues (thymus, stomach, trachea, uterus, spinal cord, skeletal muscle, spleen, testis, liver, lung, placenta, prostate, fetal brain, fetal liver, heart, kidney, colon, small intestine, brain (whole), mammary gland) were reverse transcribed and investigated by the real-time qPCR method described above. The Human Total RNA Master Panel was purchased from Clontech (Mountain View, CA, USA). Total RNA was pooled from Caucasian males and females with a wide age range. The number of sources for the RNA pool was variable and ranged from 1 (stomach) to 64 (thyroid) per tissue and is provided in the product description by Clontech (catalog No: 636643).

2.3. In situ hybridisation

In situ hybridisation was performed as described previously [14]. Briefly, placental tissue was fixed in 4% paraformaldehyde, dehydrated in a graded alcohol series, and embedded in paraplast. Sections with a thickness of 10 μm were mounted onto aminoalkylsilane-coated slides. Probe binding was observed using NBT/BCIP, according to the manufacturer's protocol (Roche). After color development, sections were rinsed in double-distilled water, dehydrated in a graded ethanol series, treated by xylene and embedded in Entellan (Merck, Darmstadt, Germany). Probes correspond to nucleotides 232 to 926 of *HS3ST3A1* mRNA, (Genbank NM_006042.2).

2.4. Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) for variables with a skewed distribution. Categorical data are expressed as number and percentages. Between group differences were assessed by t-test for parametric and Mann–Whitney U test for non-parametric distributions. One-way ANOVA was used to assess differences in three groups. Chi-square statistics were used for categorical variables. Linear regression analysis was used to assess the correlation of *HS3ST3A1* mRNA expression with neonatal birth and maternal blood pressure. For statistical analyses, SPSS software was used (Statistical Package for the Social Sciences, version 19.0, Inc. Chicago, Illinois, USA). *P*-values were considered to indicate a significant difference if $p < 0.05$.

3. Results

3.1. Microarray analysis

All of the 78 preselected transcripts relating to glycocalyx synthesis and degradation present on the Functional Glycomics AffymetrixGeneChip were also present on the Illumina HumanHT-12v.4 Expression BeadChips. Of this subset, only expression of *HS3ST3A1* was significantly different in placental tissue of pre-eclamptic women compared to placental tissue of normotensive women (fold change 0.61, $p = 0.02$, Supplemental Table). Expression of