Experimental and Molecular Pathology 100 (2016) 26–30

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Preeclampsia transforms membrane N-glycome in human placenta

Dragana Robajac [a,*], Valerie Vanhooren [b,c,1], Romana Masnikosa [a,2], Željko Miković [d], Vesna Mandić [d], Claude Libert [b,c], Olgica Nedić [a]

[a] Institute for the Application of Nuclear Energy — INEP, University of Belgrade, Belgrade 381, Serbia
[b] Department for Molecular Biomedical Research, VIB, Ghent 32, Belgium
[c] Department of Biomedical Molecular Biology, Ghent University, Ghent 32, Belgium
[d] Department of High-risk Pregnancies, Clinic of Gynaecology and Obstetrics "Narodni Front", University of Belgrade, Belgrade 381, Serbia

ARTICLE INFO

Article history:
Received 5 August 2015
Accepted 30 November 2015
Available online 2 December 2015

Keywords:
Preeclampsia
IUGR
Glycosylation
Placental membrane
DSA-FACE
IR
IGF1R

ABSTRACT

Posttranslational modifications (PTM) which accompany pathological conditions affect protein structure, characteristics and modulate its activity. Glycosylation is one of the most frequent PTM influencing protein folding, localisation and function. Hypertension is a common gestational complication, which can lead to foetal growth restriction (IUGR) and even to foetal or maternal death. In this work we focused on the impact of preeclampsia complicated with IUGR on placental membrane N-glycome. Results have shown that preeclampsia reduced fucosylation of placental glycans, increased the appearance of paucimannosidic and mannosidic structures with lower number of mannose residues and decreased the amount of glycans with more mannose residues. Since preeclampsia is tightly connected to IUGR, glycosylation changes were investigated also on the functional membrane receptors responsible for growth: insulin receptor and the type 1 insulin-like growth factor receptor (IR and IGF1R). It was found that IR present in the IUGR placenta contained significantly less α2,6-Sia. Therefore, glycans on placental membranes alter due to preeclampsia, but changes seen at the level of the entire N-glycome may be different from the changes detected at the level of a specific glycoprotein. The difference recorded due to pathology in one membrane molecule (IR) was not found in another homologous molecule (IGF1R). Thus, besides studying the glycosylation pattern of the entire placental membrane due to preeclampsia, it is inevitable to study directly glycoprotein of interest, as no general assumptions or extrapolations can be made.

1. Introduction

Posttranslational modifications (PTMs) are biochemical reactions in which aminoacid residues are covalently modified (Prabakaran et al., 2012). PTMs greatly contribute to protein diversity and influence protein folding, localisation, function and activity (Gamblin et al., 2008). Numerous PTMs have been reported, and glycosylation, phosphorylation, oxidation, proteolysis and ubiquitination are among those which occur most often (Walsh et al., 2005). Besides physiological modifications, structural changes in proteins have been detected in relation to various pathologies, such as cancer, diabetes, and arthritis (Dall'Olio et al., 2013).

Hypertension is one of the most common gestational complications, with 5–10% incidence, and it is the second main cause of maternal death in developed countries (Vest and Cho, 2014). It is related to high risk of intracerebral bleeding, placental abruption, intrauterine growth retardation (IUGR), early birth and intrauterine foetal death (Vest and Cho, 2014). IUGR is defined as inability of foetus to fulfil its genetically predestined intrauterine growth (Monk and Moore, 2004) and it is usually combined with preeclampsia. Data indicate that preeclampsia is genetically determined (Roberts and Gammill, 2005) and there are numerous data indicating that IUGR is also genetically influenced (Monk and Moore, 2004; Yamada et al., 2005). It is assumed that a key factor for preeclampsia and IUGR is low invasion of endovascular trophoblasts and impaired spiral artery remodelling (Pijnenborg et al., 2006).

In our previous work, placental membrane N-glycome was investigated using DNA-sequencer-aided fluorophore-assisted carbohydrate electrophoresis (DSA-FACE) (Robajac et al., 2014) and it was shown that it was altered along the gestation (from first to the third trimester of pregnancy). We reported that specific glycosylation pattern was associated with specific event characteristic for these two trimesters. In another study, increased carbonylation of placental proteins, including insulin receptor (IR) and type 1 insulin-like growth factor receptor (IGF1R), was detected in mothers with preeclampsia, suggesting susceptibility of receptors to modification due to preeclampsia (Robajac et al., 2015). Taking into account these findings, it seemed relevant to

* Corresponding author at: INEP — Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia.
E-mail address: draganar@inep.co.rs (D. Robajac).
[1] Present address: Ablynx, Ghent 32, Belgium.
[2] Present address: Laboratory for Physical Chemistry, Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia.

http://dx.doi.org/10.1016/j.yexmp.2015.11.029
0014-4800/

study the effect of preeclampsia on total placental N-glycome, as well as glycosylation pattern of receptors crucial for foetal growth, IR and IGF1R (Forbes and Westwood, 2008). DSA-FACE, lectin blot and lectin affinity chromatography were employed to investigate N-glycans on membrane proteins isolated from third trimester placentas obtained from mothers with severe preeclampsia complicated with IUGR.

2. Materials and methods

2.1. Sample collection and isolation of membrane proteins (solubilisates)

Placentas were collected from the Clinic of Gynaecology and Obstetrics (CGO) "Narodni Front", Belgrade, Serbia. The study was approved by the Institutional review board of the CGO "Narodni Front" and the Ethical committee of INEP. Placentas were obtained from healthy mothers ($n = 30$) and mothers diagnosed with severe preeclampsia (Bolte et al., 2001) with IUGR ($n = 14$). The gestational age of newborns from healthy mothers was 37–40 weeks and from mothers with preeclampsia 32–35 weeks. The birth weight of babies was 2.80–4.00 kg in the first group and 0.88–2.05 kg in the second. Placentas were collected within 2 h after delivery, membrane proteins (solubilisate) were isolated and their concentration determined by bicinhoninic protein assay kit as previously described (Robajac et al., 2015).

2.2. DSA-FACE

N-glycan analysis was performed using DSA-FACE method (Robajac et al., 2014). N-glycans on membrane glycoproteins (10 mg/ml of proteins) were released by digestion with peptide N-glycosidase F (New England Biolabs, Ipswich, MA, USA). Desialylated N-glycans were labelled with 8-amino-1,3,6-pyrentrisulfonic acid (APTS, Molecular Probes, Eugene, OR, USA) and separated on ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Ribonuclease B from bovine pancreas (RNase B, Sigma-Aldrich, St. Louis, MO, USA) and human serum were used as a glycoprotein standards. Gene Mapper software v. 3.7 (Applied Biosystems, Foster City, CA, USA) was used to estimate peak areas and calculate relative abundance of each glycan (in %). The presence of fucose was confirmed after digestion of APTS-labelled desialylated N-glycans using bovine kidney α-L-fucosidase (Prozyme, San Leandro, CA, USA) and DSA-FACE analysis.

2.3. Electrophoresis, lectin and immunoblotting

Membrane proteins (1.5 mg/ml proteins) were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on 8% gels under reducing conditions. They were transferred to nitrocellulose membranes (Sigma-Aldrich, Steinheim, Germany), blocked overnight in 0.01 M TBST pH 7.4 containing 3% BSA and incubated for 1 h at room temperature with 0.2–1 μg/ml of biotinylated lectins (Vector Laboratories, Burlingame, CA, USA): *Lens culinaris* agglutinin (LCA), *Phaseolus vulgaris* erythroagglutinin (PHA-E), *P. vulgaris* leukoagglutinin (PHA-L), *Maackia amurensis* II (MAL-II) and *Sambucus nigra* agglutinin (SNA). Biotinylated lectins were selected taking into account previous findings on glycans present on placental membrane glycoproteins (Robajac et al., 2014). Membranes were washed in TBST, incubated with 0.05 μg/ml HRP-avidin D (Vector Laboratories, Burlingame, CA, USA) for 1 h, washed again and incubated with luminol substrate within enhanced chemiluminescence (ECL) kit (Thermo Fisher Scientific, Rockford, IL, USA). Lectin-reactive glycoproteins were detected using X-ray films (Kodak, Paris, France).

For immunoblotting, membranes were incubated with mouse monoclonal anti-IR (1:3000, Calbiochem, Darmstadt, Germany) or anti-IGF1R antibody (1:400, Biosource International, Camarillo, CA, USA), followed by goat anti-mouse secondary antibody (1:5000, Biosource International, Camarillo, CA, USA) and ECL detection.

Lectin and immunoblots were subjected to densitometric evaluation using ImageMaster TotalLab software v. 2.01 (Amersham BioSciences, Buckenghamshire, UK).

2.4. Lectin affinity chromatography

Lectin affinity chromatography was performed using mini spin columns containing 0.2 ml of agarose bound lectins (Vector Laboratories, Burlingame, USA): LCA, PHA-E, PHA-L and SNA, under conditions suggested by the producer, with the addition of detergent Triton X-100 in all solutions (to avoid aggregation of solubilised proteins).

Each solubilisate (0.05 mg/ml proteins) was incubated with radiolabelled ligand ^{125}I-insulin or ^{125}I-desIGF-I (3×10^4 cpm) at 4 °C overnight and applied to all lectin columns. After 1 h of recirculation, the unbound material containing flow through and the washing fraction (6×0.5 ml) was collected as the total unbound radioactivity, whereas eluted material (5×0.2 ml) was collected as the total bound fraction (bound radioactivity). Radioactivity of all fractions was measured on a γ-counter and the specific binding of each ligand expressed in % of the total applied radioactivity. Ligands, human recombinant insulin (Novo, Copenhagen, Denmark) and desIGF-I (GroPep, Adelaide, Australia), were labelled with Na^{125}I (Isotope, Budapest, Hungary) using chloramine T method (Hunter and Greenwood, 1962), to the specific activity of approximately 100 μCi/μg.

2.5. Statistical analysis

Data obtained in DSA-FACE, lectin blot, immunoblot and lectin affinity chromatography experiments were statistically analysed and the differences between two groups of placentas (from healthy and mothers with preeclampsia) assessed by Student's t-test. Statistically significant differences were considered for $p < 0.05$.

3. Results

3.1. DSA-FACE

Placental membrane solubilisates were subjected to DSA-FACE and typical desialylated N-glycan profiles originating from mothers with preeclampsia (PE) and healthy mothers (H) are shown in Fig. 1.

Membrane proteins from two groups of placentas contained the same eleven N-glycan peaks, suggesting conservation of all major carbohydrate residues in mothers with preeclampsia. The connection between retention times (t_r) and the identity of glycan structures was explained in the previous work (Robajac et al., 2014). The relative abundance of each peak (expressed in %) was calculated and compared between the groups (Fig. 2).

As shown in Fig. 2 (also in Fig. 1), the most abundant N-glycan in both groups was NA2F (peak 7). Peak 1, corresponding to paucimannosidic structure, was significantly more abundant in PE compared to H samples, as well as peak 2, corresponding to M5 from RNAse B and 4, whose structure could not be directly assumed. On the other hand, peak 5 (NA2) was less abundant in PE compared to H samples, as well as peak 6, corresponding to M8 from RNAse B and 10 (NA3Fb).

When further analysis was performed, two groups of glycan subtypes emerged. In the first group were glycans eluted in peaks 3, 7, 8, 9, 10 and 11 which were fucosylated, those in peaks 3, 7, 8 and 10 contained core-fucose and glycan in peak 8 contained additional bisecting GlcNAc. In the second group were glycans eluted in peaks 9, 10 and 11, which were branched. The relative abundance of each of these groups (expressed in %) is shown in Fig. 3 (A and B).

No significant difference was found between PE and H samples when glycan subtypes were analysed, although slightly lower degree of fucosylation and branching was noted in PE membrane glycoproteins. This result was confirmed by lectin blotting using fucose-binding lectin LCA (Fig. 3A) and lectins PHA-E and PHA-L specific for highly branched