Placenta 36 (2015) 191–198

Contents lists available at ScienceDirect

Placenta

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

Galectin-13/PP-13 expression in term placentas of gestational diabetes mellitus pregnancies

L. Unverdorben [a], R. Hüttenbrenner [a], J. Knabl [b], U. Jeschke [a, *], S. Hutter [a]

[a] Department of Gynecology and Obstetrics, Ludwig Maximilians University of Munich, Maistraße 11, 80337 Munich, Germany
[b] Department of Gynecology and Obstetrics, Klinik Hallerwiese, Diakonie Neuendettelsau, St.-Johannis-Mühlgasse 19, 90419 Nuremberg, Germany

ARTICLE INFO

Article history:
Accepted 25 November 2014

Keywords:
Galectin-13
PP-13
Gestational diabetes mellitus (GDM)
Trophoblast

ABSTRACT

Introduction: Gestational diabetes mellitus (GDM) is an increasing harm in pregnancy. Inflammatory processes in the placenta seem to have an influence on pathogenesis besides known factors like maternal BMI. Galectin-13 (gal-13) is an immunoregulatory protein, which is suspected to play a role in development of GDM in the placenta.
Methods: A total of 40 placentas were obtained from women treated for gestational diabetes mellitus. Placental tissue for control group was obtained from 40 women with normal pregnancy. We investigated the protein expression of gal-13 in term placentas with immunohistochemistry and immunofluorescence. Immunohistochemical staining was analyzed with the semi-quantified IRS score.
Gal-13 serum levels were performed with ELISA on a total of 20 probes from women with GDM and healthy control pregnancies in the third trimester.
Results: Gal-13 was found in syncytiotrophoblast, in nuclei of syncytiotrophoblast and trophoblast cells as well in extravillous trophoblast cells of normal placentas. In GDM placentas, gal-13 expression was significantly decreased in all of these examined cell types (syncytiotrophoblast $p = 0.003$, nuclei of syncytiotrophoblast $p = 0.007$; extravillous trophoblast cells $p = 0.001$). The ELISA showed a significant lower gal-13 serum level in blood from pregnant women with GDM in comparison to healthy controls.
Discussion: As gal-13 with its anti-inflammatory functions plays a role in regulation of maternal immune system, a lack of gal-13 may contribute to an imbalance in inflammation processes in the placenta during pregnancy and therefore influences development of GDM.

1. Introduction

The prevalence of gestational diabetes mellitus (GDM) is increasing steadily [1,2]. Therefore associated complications during pregnancy and labour become more frequent. Pregnant women suffering from GDM have a higher risk of pregnancy and delivery complications [3–5]. For the Fetus, the risk of placental malfunction and diabetic fetopathy, mostly including macrosomia, is increased. Postpartal fetal hypoglycemia and higher incidence of acute respiratory distress syndrome may also occur [4,6]. Pregnant women with GDM have a higher long-term risk of diabetes mellitus Type 2 or cardiovascular diseases [7–9].

Gestational diabetes mellitus is defined as impaired glucose tolerance with onset in or first recognition during pregnancy [10,11]. The pathophysiology is believed to be similar to the development of type-2 diabetes mellitus and also based on the same risk factors like obesity, genetic disposition and lack of physical activity [8,12]. Insulin resistance, which is associated with hypercholesterinaemia and hypertension in pregnancy [13], is known, to occur also in normal pregnancies. This is probably caused i.e. by increase of triglycerides, cholesterol, leptin and tumour necrosis factor (TNF)α in normal pregnancies [7,14]. Leptin is produced in adipocytes, but also in trophoblast cells and is induced by insulin [15]. An association between inflammatory components like TNFα and interleukin-6 or C-reactive protein and insulin resistance could be shown, even in normal pregnancies [12,16]. More extensively some adipokines may play a role in development of GDM and preeclampsia [17]. Additionally, there are further factors influencing development of GDM like i.e. Hypoxia inducible factor HIF-1α or Peroxisome-proliferator-activating receptor (PPAR), which

* Corresponding author. Department of Gynaecology and Obstetrics, Campus Innenstadt, Ludwig-Maximilian-University Munich, Maistrasse 11, 80337 Munich, Germany. Tel.: +49 89 4400 54240; fax: +49 89 4400 54169.
E-mail address: udo.jeschke@med.uni-muenchen.de (U. Jeschke).

http://dx.doi.org/10.1016/j.placenta.2014.11.019
0143-4004/

Table 1
Clinical data of specimens.

	GDM		Control		*p*-value
	Male	Female	Male	Female	
Number of samples	20	20	20	20	
Mean maternal BMI in kg/m^2	30.26	26.28	21.92	25.0	0.073
Mean maternal age (years)	32.05	33.6	30.3	32	0.019
Mean birthweight in g	3680.26	3542.5	3339.75	3294	0.099
Gestational age (weeks)	39.75	39.95	39.8	39.75	

appears to be a promising target for different pharmacological therapies [10,18].

Galectins are β-galactoside-binding lectins with characteristic amino acid sequences recognizing Galβ1-4GlcNAc sequences of cell surface oligosaccharides [19–21]. Galectins have modulating functions in cell growth, differentiation, cell death and on immune reaction [22,23]. They are known in various human tissues, for example in the intestinal tract and cells of the immune system, but also in the endometrium and placenta and endothelial tissue. Therefore they play important roles in tumorogenesis and metastasis growth [23–26]. The former described Placental Protein-13 (PP-13) has a homologous structure to the galectin family and is now described as galectin-13 (gal-13) [27,28]. Gal-13/PP-13 serum levels especially in the first trimester seem to correlate with the incidence of preeclampsia [29,30]. As the other galectins, we suspect gal-13 to effect cell differentiation and inflammatory processes in the placenta with or without gestational diabetes mellitus. In this study, we examined the expression of gal-13 in term placentas, especially in comparison to GDM-placentas and evaluated the correlation of gal-13 and gestational diabetes mellitus.

2. Material and methods

2.1. Tissue samples

A total of 40 placentas were obtained from women treated for gestational diabetes mellitus in the department of Obstetrics and Gynaecology of Ludwig-Maximilians-University Munich. Placental tissue for control group was obtained from 40 women with normal pregnancy. For further demographical and clinical characteristics see Table 1.

Inclusion criteria for placental tissue were diagnosed gestational diabetes mellitus of the mother in pregnancy after WHO-definition with at least one or no pathologic OGTT parameter for GDM-group or control group respectively [11]. All women were primigravidae without signs of infection at the time of delivery. Exclusion criteria were intrauterine growth restriction (IUGR), fetal malformation, premature delivery and pre-existing Diabetes mellitus Type I or II. All the patients were in medical care by their obstetrician during pregnancy and delivery. From GDM-group, 30 women were under insulin therapy, 7 had no insulin therapy, while in the case of 3 women we had no information about therapy with insulin.

Placentas were obtained after term vaginal delivery or caesarean section. The tissue was obtained from the central part of the placenta in every case directly after delivery. One part of taken placental tissue was frozen immediately and stored at −80 °C for double immunofluorescence with cryosections. The other part was fixed in 4% buffered formalin for 20–24 h and embedded in paraffin for use in immunohistology [31].

Serum for the ELISA was obtained from women suffering from gestational diabetes mellitus in the third trimester and from normal healthy control pregnancies.

A total of 40 blood sera from women in the third trimester were performed with ELISA.

The study was approved by the ethical committee of the University of Munich and informed consent was obtained from each patient in written form. Samples and clinical information were anonymized for statistical workup.

2.2. Immunohistochemistry

For immunostaining, a monoclonal anti-gal-13 antibody was used (Table 2).

Paraffin-embedded slides were dewaxed in xylol and washed in 100% ethanol. For endogen peroxidase inhibition, tissue samples were put to methanol with 3% H_2O_2 and rehydrated in a descending series of alcohol. The samples were heated in sodium citrate buffer (pH 6.0), containing 0.1 M citric acid and 0.1 M sodium citrate in distilled water for five minutes and after cooling washed in Aqua dest. and PBS (phosphate buffered saline solution).

The slides were incubated with blocking solution (Reagent 1, Zytochem-Plus HRP-Polymer-system (mouse/rabbit), Zytomed, Berlin, Germany) for 20 min to reduce non-specific staining in background.

After washing with PBS, the slides were incubated with the antibody against gal-13 (Table 2), diluted 1:50 in PBS, for 16 h at 4 °C over night. Then post-block reagent (reagent 2, Zytochem-Plus HRP-Polymer-system (mouse/rabbit), Zytomed) for 20 min and HRP-Polymer (reagent 3, Zytochem-Plus HRP-Polymer-system (mouse/rabbit), Zytomed) for 30 min were applied. In between, washing steps were carried out with PBS.

Immunostaining was visualized with substrate buffer and chromogen 3,3′-diaminobenzidine (DAB; Dako, Carpinteria, USA) for 2 min. Counterstaining was performed with Meyer's acid Hemalaun for 2 min and blueing in tab water for 5 min. Slides were dehydrated in an ascending series of alcohol. After treatment with xylol slides were cover-slipped with Consul-Mount™ medium (ThermoSherton, Pittsburgh, USA).

For positive control an appropriate tissue was used to perform immunohistochemical staining (Table 2). Positive cells show a brownish colour and the negative control, as well as unstained cells and acid cell structures like DNA, appeared blue [32]. Negative and positive controls are shown in Fig. 4.

For analyzing of immunostaining the semiquantitative IRS score was used by two-blinded examiners. In Decidua, two populations of cells with different staining were found, so they were scored each. In the syncytiotrophoblast as well in the decidua, cytoplasm and nuclei were both immunoscored with IRS. The IRS is the product of optical staining intensity (grades: 0 = no, 1 = weak, 2 = moderate and 3 = strong staining) and the estimated percentage of positive stained cells (0 = no staining, 1 ≤ 10% of the cells, 2 = 11–50% of the cells, 3 = 51–80% of the cells and 4 ≥ 81% of the cells) in 4 fields per slide in 40× lens. Calculation was (mean staining intensity) × (percentage) = IRS (minimum = 0, maximum = 12). A positive staining was determined above an IRS ≥2 according to recommendation of IRS score on mamma-Ca [33].

2.3. Double immunofluorescence staining

Cryosections of the frozen tissue samples of GDM and control placentas were examined for the characterization of galectin-13-expressing cells in decidua. After thawing, samples were fixed in acetone for 5 min. Cytokeratin (CK) 7 (Novocastra, Berlin, Germany) (Table 2) was used as a specific marker for trophoblast cells [34].

First, slides were blocked with Ultra V Block for 15 min to avoid non-specific staining. Then, they were incubated with gal-13 antibody (diluted 1:50 in PBS; Table 2) over night and with CK7 (1:30 diluted in Dako diluting medium, Dako, Carpinteria, USA) for 60 min at room temperature.

After washing steps with PBS in between each step before, Cy2-and Cy3-labelled antibodies were applied as second antibodies. For gal-13 a Cy3-labelled goat-anti-rabbit IgG antibody (dilution 1:500 in Dako diluting medium; Table 2) appearing in red and for CK7 a Cy2-labelled goat-anti-mouse IgG (dilution 1:100 in Dako diluting medium; Table 2) for a green appearance were used. They both were incubated for 30 min. After washing and drying in the dark, the sections were finally embedded in mounting buffer containing 4′,6-diamino-2-phenylindole (DAPI) resulting blue staining of the nucleus [35]. Sections were examined with a Zeiss (Jena, Germany) Axiophot photomicroscope. Images were obtained with a digital camera system (Axiocam; Zeiss CF20DXC; KAPPA Messtechnik, Gleichen, Germany) and saved on a computer.

2.4. ELISA

For ELISA analysis, we used the Human PP13 (Placental Protein13-Gal13)-ELISA-Array (My Bio Source; Nr. MBS2508612) according to manufacturer's instructions.

Table 2
Antibodies used in study.

Antigen	Type of antibody	Dilution	Manufacturer	Positive control
Galectin-13	Rabbit IgG polyclonal concentration 0.1 mg/ml	1:50 in Dako diluting medium	Novus Biologicals NBP1-91922	Placenta
CK7	Mouse IgG_1 monoclonal	1:30 in Dako diluting medium	Novocastra NCL-L-CK7-OVTL	Syncytiotrophoblast of placenta
Goat-anti-rabbit IgG Cy3	Goat IgG	1:500 in Dako diluting medium	Dianova 111-115-144	
Goat-anti-mouse IgG Cy2	Goat IgG	1:100 in Dako diluting medium	Dianova 115-226-062	