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Gestation-associated changes in the glycosylation of placental insulin and insulin-like growth factor receptors

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ABSTRACT

Introduction: Insulin receptor (IR) and type 1 and type 2 insulin-like growth factor receptors (IGF1R and IGF2R) play important roles in regulation of placental and foetal growth. All three receptors are abundantly glycosylated. N-glycosylation significantly affects protein conformation and may alter its function. We have recently found that the N-glycome of placental membrane proteins alters during gestation. The aim of the study presented herein was to investigate whether there were gestation-related changes in N-glycan profiles of placental IR and IGFRs.
Methods: Placentas from healthy women at first (FTP) and third trimester (TTP) of pregnancy were collected, membrane proteins isolated, solubilised and used as the source of IR and IGFRs. Reactivity of glycoforms of IR and IGFRs with lectins was monitored by measuring radioactivity of ^{125}I-ligands-receptors complexes.
Results: Significant differences in the binding pattern of all three receptors to the lectins were observed between FTP and TTP, which suggest gestational changes in N-glycans bound to receptors. These changes include decrease in total fucosylated, core-fucosylated biantennary N-glycan (NA2F) and α2,6-sialo-N-glycans (for IR); decrease in total fucosylated and α2,6-sialo-N-glycans and an increase in NA2F N-glycans (for IGF1R) and an increase in the total fucosylation and NA2F N-glycans (for IGF2R).
Discussion: The gestational alterations in N-glycans attached to IR and IGFRs may represent a mechanism by which these receptors acquire new/additional roles as gestation progresses.

1. Introduction

The insulin-like growth factor (IGF) system plays an important role in regulation of placental and foetal growth [1]. The system includes ligands (IGF-I and IGF-II), IGF receptors (IGF1R and IGF2R), insulin receptor (IR) and IGF-binding proteins (IGFBP). The signals of insulin and IGF-I are transduced through the IR and IGF1R, respectively. The receptors share structural similarity [2,3]. IGF-II exerts many of its actions via binding to the IGF1R [4], but it also binds to the structurally distinct IGF2R. The role of the IGF2R in signal transduction has been a matter of debate, and it was proposed to serve just as a clearance route for IGF-II [5]. However, independent signalling roles for IGF2R in the human placenta were reported [6].

IR contains 18 potential N-glycosylation sites [7], of which 16 are occupied, making IR heavily glycosylated [8]. IGF1R possesses 16 potential N-glycosylation sites, while IGF2R possesses 19 sites, with the latter carrying at least two N-glycans [9,10].

Most cell surface proteins are N-glycosylated [11]. N-glycosylation is the only posttranslational modification that profoundly affects protein conformation, which may greatly alter its function. For many glycoproteins alternative glycosylation has important regulatory roles [12]. N-glycans play essential roles in growth factor signalling [13]. Modification of N-glycan attached to the nerve growth factor (NGF) receptor disrupts its signal transduction in response to NGF treatment [14]. N-glycosylation affects membrane interactions and structural arrangement of the ligand-bi ding ectodomain of the epidermal growth factor receptor [15]. N-glycans borne by β-subunit of IR play a role in its enzymatic activation and signal transduction [16]. Complex N-glycans on the IGF1R modulate its targeting and function [17]. Complex, oligomannose or bisecting

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type N-glycans on membrane proteins contain information for localisation of these proteins to various regions of the membrane in both a glycan-specific and protein-specific manner [18]. Though we know that virtually all interactions at the cell surface are modulated by N-glycans, they are often ignored [12].

Analysis of N-glycans attached to growth factor receptors is key for understanding their functions, which are vital to the development and survival of organisms. Studies of the N-glycosylation profile of IGFRs and IR, thus, are of great physiological significance. However, such data are not available in the literature, except one detailed N-glycan analysis of the IR from CHO-K1 cell line [8].

We were the first to profile the most abundant types of N-glycans on human placental IR and IGFRs in placentas taken at term [19]. However, we have recently found that the N-glycome of placental membrane proteins alters during gestation [20]. The aim of the study presented herein was to investigate whether there were gestation-related changes in N-glycan profiles of placental IR and IGFRs. To this end, we studied the composition of specific N-glycans borne by IR and IGFRs originating from first and third trimester pregnancy placentas.

2. Materials and methods

2.1. Human placentas

Placentas were obtained from the Clinic of Gynaecology and Obstetrics "Narodni Front", Belgrade, Serbia. The study was approved by the Institutional Review Board of the Clinic "Narodni Front" and the Ethical committee of INEP. All women gave oral consent. The first trimester placentas (FTP) were collected as anonymous left-overs of elective termination of healthy pregnancies between 7th and 12th week of gestation (n = 160, mean age of mothers 30.8 years). Third trimester placentas (TTP) were obtained after full-term deliveries of healthy mothers (n = 30, mean age of mothers 33.5 years).

2.2. Preparation of solubilised placental cell membranes

Placentas were collected within 1 h after extraction or delivery and placed immediately on ice. They were extensively washed in ice cold 0.1 M phosphate buffered saline (pH 7.4), frozen in liquid nitrogen and kept at −80 °C until used. Solubilised placental cell membranes (solubilisates) were prepared exactly as previously described [20,21]. The minute amounts of FTP samples were first grouped into seven pools, from which seven FTP solubilisates were prepared. 30 TTP solubilisates were prepared from 30 individual TTP samples. 7 TTP pools were formed by mixing equal amounts of TTP solubilisates. Finally, two total pools were formed by mixing the solubilisates obtained from placentas of the same gestational age: tFTP consisted of 7 FTP solubilisates, tTTP consisted of 30 TTP solubilisates.

2.3. ^{125}I-ligands

Human recombinant insulin (Novo, Denmark), desIGF-I and IGF-II (GroPep, Australia) were ^{125}I-labelled using the chloramine T method [22]. Apart from binding to the IGF1R, IGF-I binds with a high-affinity to the soluble IGFBP-3 [23], which is N-glycosylated [24], present in human placenta [25] and which can also be membrane-bound [26]. DesIGF-I binds to the IGF1R with an affinity similar to that of IGF-I, but its binding to IGFBP-3 is much reduced, hence the formation of ^{125}I-IGF-I-IGFBP-3 complexes was insignificant [27].

2.4. Western immunoblotting (WIB)

FTP and TTP pools of solubilisates (seven of each) were diluted to the same protein concentration and subjected to non-reducing SDS-PAGE and WIB. The placental IR, IGF1R and IGF2R were detected using mouse monoclonal anti-IR (GroPep Limited, Australia), anti-IGF1R (BioSource International, USA) and anti-IGF2R antibodies (Calbiochem, Germany). The receptors were visualised using goat anti-mouse HRP-conjugated antibodies (BioSource International, USA), chemiluminescence kit (Pierce, USA) and autoradiography, as described [28].

2.5. Lectin-affinity chromatography

Lectin chromatography was performed using seven different agarose-immobilised lectins (Vector Laboratories, USA): AAL, LCA, UEA I, PHA-L, Con A, GNL and SNA (Fig. 4). The samples were: seven FTP and 30 TTP solubilisates. Samples (0.5 mg/ml) were

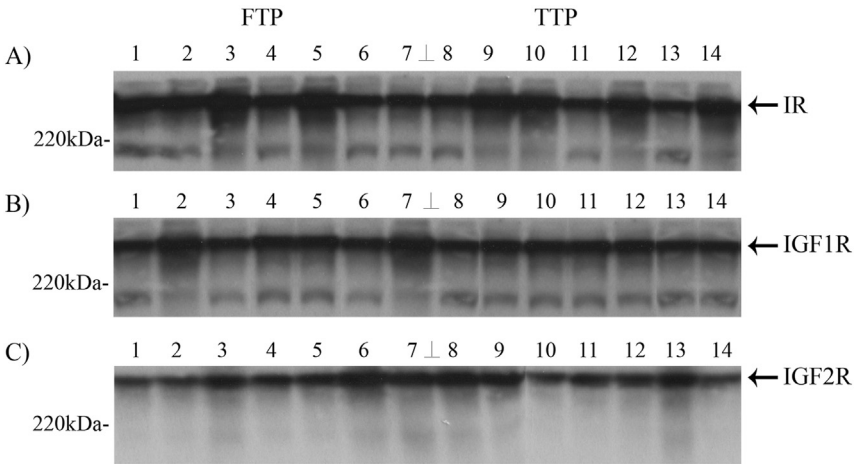

Fig. 1. Insulin receptor (IR), IGF-I receptor (IGF1R) and IGF-II receptor (IGF2R) in FTP and TTP solubilisates. Placental membrane solubilisates were resolved by non-reducing SDS-PAGE and transferred to nitrocellulose membranes for immunoblotting with anti-IR (a), anti-IGF1R (b) or anti-IGF2R antibody (c). The lanes are as follows: 1–7, first trimester placental solubilisates (FTP); 8–14: third trimester solubilisates (TTP). Protein mass markers are indicated on the left and the positions of receptors on the right. Two identical gels were run in parallel in each case, one was subjected to the immunoblotting, while its replica was Coommassie stained to check uniformness of protein distribution. Nitrocellulose membranes were stained with Ponceau S to check uniformness of protein transfer.