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Placental gene expression patterns of epidermal growth factor in intrauterine growth restriction

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ABSTRACT

Objective: In this study, we compared human placental gene expression patterns of epidermal growth factor (EGF) in pregnancies with intrauterine growth restriction (IUGR) vs. normal pregnancies as control.

Study design: Gene expression of EGF was determined from human placental samples collected from all pregnancies presenting with IUGR at our institution during the study period January 1, 2010–January 1, 2011. Multiple clinical variables were also assessed including maternal age, gestational weight gain, increase of BMI during pregnancy and fetal gender.

Results: A total of 241 samples were obtained (101 in the IUGR pregnancy group, 140 in the normal pregnancy group). EGF was found to be underexpressed in the IUGR group compared to normal pregnancy ($Ln2^{\alpha}$: −1.54; $p < 0.04$). Within the IUGR group no fetal gender-dependent difference was seen in EGF gene expression ($Ln2^{\alpha}$: 0.44; $p < 0.06$). Similarly, no significant difference in EGF expression was noted in cases with more vs. less severe forms of IUGR ($Ln2^{\alpha}$: −0.08; $p = 0.05$). IUGR pregnancies were significantly more common in the maternal age group 35–44 years compared to other age groups. Gestational weight gain and gestational BMI increase were significantly lower in IUGR pregnancies compared to controls.

Conclusions: Placental expression of EGF was found to be reduced in IUGR pregnancies vs. normal pregnancies. This may partly explain the smaller placental size and placental dysfunction commonly seen with IUGR. An increased incidence of IUGR was observed with maternal age exceeding 35 years. The probability of IUGR correlated with lower gestational weight gain and lower BMI increase during pregnancy.

1. Introduction

Intrauterine growth restriction (IUGR) is defined as fetal birthweight below the tenth percentile for sex and gestational age [1] (though it must be remarked that the fifth and third percentiles as a borderline of IUGR are also used in obstetrics). IUGR may result from placental dysfunction, fetal malformation, intrauterine infection or maternal factors. Although the most common etiology for IUGR is thought to be placental dysfunction, its pathology at molecular level remains largely unknown [2,3].

During human gestation, maternal serum levels of multiple growth factors rise. Among these, insulin-like growth factor 1 and 2 (IGF-1, IGF-2) appear to be especially important in the pathogenesis of both IUGR and premature delivery [4,5]. Epidermal growth factor (EGF) has been found to play a role in stimulating placental growth [6].

Structurally, human EGF is a polypeptide consisting of 53 amino acids. Its precursor is substantially larger, consisting of a 1207 amino acid polypeptide chain [7,8]. In all tissues, EGF appears to

Abbreviations: IUGR, intrauterine growth restriction; IGF-1, insulin-like growth factor 1; IGF-2, insulin-like growth factor 2; VEGF-A, vascular endothelial growth factor A; TGF-β, transforming growth factor beta; EGF, epidermal growth factor; TGF-α, transforming growth factor alfa; EGFR, EGF-receptor; ErbB-1-4, erythroblastic leukemia viral oncogene homolog 1–4; AC, abdominal circumference; BMI, body mass index; LIF, leukemia inhibiting factor.

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have mitogenic activity [9]. Moreover, EGF also seems to play a role in placental growth and in the regulation of physiological changes of placental function during intrauterine fetal development [9–13].

The physiologic activity of EGF is mediated through the EGF receptor (EGFR) also known as erythroblastic leukemia viral oncogene homolog (ErbB-1). After binding to its receptor, EGF acts in the initiation of cell division. During human gestation this mechanism appears to be important primarily in promoting placental growth [14–16]. Other members of the EGF protein family may activate ErbB receptors 1–4 [17]. While ErbB receptors 2–4 can be identified in both villous and extravillous trophoblasts, EGFR (ErbB-1) only occurs in villous trophoblasts [18,19].

Changes in EGFR receptor distribution have been observed in both IUGR and other medical conditions associated with increased risk during pregnancy, such as smoking. Quantitative changes in EGFR distribution may be associated either with alterations in EGF secretion or with changes in placental expression of the EGF gene [20,21].

Our primary objective in this study was to identify and characterize alterations in placental EGF gene expression patterns in IUGR pregnancies compared to normal pregnancies. We believed that clarifying these alterations would contribute to a better understanding of the role played by EGF in placental growth. Our secondary aim was to identify gender-related alterations of EGF gene expression in IUGR. We also investigated the relationship between the degree of growth restriction in IUGR (fetal birthweight 0–5 percentile vs. 5–10 percentile) and placental EGF expression.

2. Materials and methods

We obtained 101 placental samples for characterization of EGF expression from all patients treated for IUGR in our clinic at the Second Department of Gynecology and Obstetrics, Semmelweis University, Budapest, in the study period between January 1, 2010 and January 1, 2011, as well as 140 placental samples from cases of normal pregnancy used as controls during the same time period. Maternal age, gestational weight gain and BMI increase during pregnancy were also evaluated. IUGR was diagnosed per standard criteria as fetal birthweight below the tenth percentile for fetal sex and gestational age. (In certain cases the fifth or 3rd percentile are also used to diagnose IUGR, but in the majority of cases the tenth percentile is the marked borderline.) We have taken into consideration those cases of IUGR in which the growth restriction was diagnosed prenatally by ultrasound, and the measurement of the birthweight confirmed the diagnosis postnatally. The IUGR group was subdivided into two groups by the degree of growth restriction as below: less severe growth restriction defined as birth weight of 5–10 percentiles vs. more severe growth restriction (0–5 percentile). Abdominal circumference (AC) determined through ultrasonography was also considered when establishing the clinical diagnosis of IUGR. Abdominal circumference values in cases with a clinical diagnosis of IUGR were compared to cases with similar gestational age in the normal pregnancy group.

Only those placentas were included in the study where IUGR was likely to be due to placental dysfunction after the exclusion of intrauterine infections, chromosomal abnormalities, fetal malformations, developmental disorders, maternal malnutrition, multiple pregnancy and structural abnormalities in the placenta [22,25]. We also excluded cases of IUGR caused by maternal preeclampsia, because it would have strongly influenced the correct evaluation of the etiological role of EGF in the background of intrauterine growth restriction.

Delivery was either vaginal or by cesarean section based on clinical decision. In the final analysis of data, no distinction was made with respect to the type of delivery.

Placental tissue samples were taken in a uniform manner with approximate dimensions of 2 cm × 2 cm × 2 cm (8 cm^3), which were then kept at −70 °C for genetic expression testing. The sampling of each placenta was random, so all areas of each placenta had an equal chance of being sampled. (In 10 cases of IUGR and 10 control cases the sampling of the placental tissue was performed from four different points of the placental tissue, and the gene expression values did not differ.)

Maternal demographics and relevant clinical data during pregnancy or the postnatal period were collected including maternal and paternal age, obstetric history, genetic history, general medical history, maternal birthweight, gestational age, fetal gender, weight gain and BMI increase during pregnancy, pregnancy-related pathology including disorders of carbohydrate metabolism, neonatal weight and Apgar score. Consent was obtained in each case from the mother (signatures on file).

Whole placental RNA content was isolated with Quick RNA microprep kit (Zymo Research). RNA concentration was determined using NanoDrop spectophotometer (NanoDrop). Reverse transcription was performed in 20 μl target volume using 5 μg whole RNS, 75 pmol random hexamer primer, 10 mM dNTP (Invitrogen), 20 U M-MuLV Reverse Transcriptase enzyme (MBI Fermentas) and 1×-es buffer (MBI Fermentas). The reaction mix was incubated for 2 h at 42 °C. Subsequently, the enzyme was inactivated at 70 °C for 15 min.

The reverse transcriptase reaction solution was diluted threefold with nuclease-free water. For the real-time PCR assay, 1 μl diluted cDNS (approximately15 ng RNA-equivalent) and 1× SYBR Green Master Mixet (Applied Biosystems) were used. Primers were designed using Primer Express Software (Applied Biosystems). Primer sequences are detailed in Table 1. Real-time PCR was performed in 20 μl target volume using 1 μl cDNA, 1 pmol, gene-specific Forward and Reverse primer and 1 x SYBR Green PCR Master mix. All real-time PCR were performed using the MX3000 Real-time PCR (Stratagen) system with the following settings: 40 cycles at 95 °C, denaturing process for 15 s, annealing at 60 °C, chain elongation and detection for 60 s. For each gene, relative expression was normalized using the human *β-actin* gene as standard.

For gene expression studies of the EGF gene in the IUGR vs. normal pregnancy groups two-sample t-test was used with 95% confidence interval. Determination of degree of freedom was performed using the Welch–Satterthwaite correction. Values of gene expression testing were interpreted in the following manner: (1) overexpression = Ln value >1, $p < 0.05$; (2) underexpression = Ln value <-1, $p < 0.05$; (3) normal expression = Ln value <1, >-1, $p < 0.05$. GraphPad Prism 3.0 (GraphPad Software Inc.) software was used in all statistical analytic procedures.

Demographics and clinical data were analyzed with SPSS software. Logistic regression was used for dichotomous outcomes

Table 1
Primers and sequences in real-time PCR.

Gene name and code	Forward primer	Reverse primer
EGF (NM_001963)	5′-AATACCGTTAAGATACAGTGTAGGCACTTTA-3′	5′-ATCACAACTCATTTTGGCAAAATC-3′
β-Actin (M10277)	5′-GGCACCCAGCACAATGAAG-3	5′-GCCGATCCACACGGAGTACT-3′