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Hypoxia-Inducible Factor 1α Expression in Chorionic Tissue and Decidua of Women with Spontaneous Abortion at the First Trimester of Pregnancy

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Abstract: Oxygen-regulated genes expression has important role in pre-implantation embryonic metabolism regulation. Hypoxia Inducible Factor (HIF) regulated by hypoxia oxygen tension is crucial for placenta development. But the data about its role in spontaneous abortion is very poor. Thus, we aimed to determine an expression level of *HIF-1α* in chorionic tissue and decidua at pregnancy. Samples of chorionic tissue and decidua were taken after surgical termination of normally progressing pregnancies in 5-9 week of gestation (n = 8) and spontaneous abortion in 5-9 week of gestation (n = 9). *HIF-1α* expression was analyzed using semi-quantitative reverse transcription-polymerase chain reaction. Compared with decidual tissue, the expression of *HIF-1α* was increased in chorionic tissue in condition of normally progressing pregnancy. *HIF-1α* expression in samples of both tissues is equal in spontaneous abortion. In same time the expression of *HIF-1α* was decreased (1,5 fold) in chorionic tissue for spontaneous abortion compared with control group. The results demonstrated that low *HIF-1α* expression level in chorionic tissue can be associated with spontaneous abortion in first trimester of pregnancy.

Keywords: Spontaneous Abortion, HIF-1, Gene Expression, Chorionic Tissue

Introduction

About 15% of all human pregnancies end in spontaneous abortion before 12 weeks of gestation. The pathophysiology of pregnancy loss is complicated and poorly understood. Major part of the pregnancy loss causes remains unexplained after comprehensive study. Immunity, angiogenesis, apoptosis-related genes are involved in pathogenesis. The aberrant maternal inflammation associated with spontaneous abortion is closely linked to deficient placental perfusion (Renaud *et al*., 2011).

Early stages of the mammalian placenta development are regulated by oxygen tension and the hypoxic uterine environment (Giaccia *et al*., 2004). A hypoxic environment is essential for proper embryonic development. Low oxygen appears to prevent trophoblast differentiation into an invasive phenotype. This physiological switch in oxygen tension is a prerequisite for proper placental development (Patel *et al*., 2010). Low oxygen tension induces embryo development up to the blastocyst stage (Kind *et al*., 2005; Harvey *et al*., 2007). Vascular development during embryonic and fetal growth in utero is triggered by hypoxia (Simon and Keith, 2008).

Oxygen-regulated genes expression plays an important role in pre-implantation embryonic metabolism regulation. Hypoxia Inducible Factor (HIF) regulated by hypoxia oxygen tension is crucial for placenta development. This factor is up-regulated under hypoxic conditions that take place during implantation, fetal placentation, organogenesis, angiogenesis and embryo growth (Adelman *et al*., 2000). On the other hand, HIF-1α protein expression can also be induced by other stimuli, for example hormones, cytokines and growth factors (Pringle *et al*., 2010).

HIF-1 modulates gene transcription by binding to a specific DNA sequence (Hypoxic Response Element (HRE)). HIF-1 is a heterodimer composed of HIF-1α and HIF-2α subunits. HIF-1α and HIF-2α activate a number of common genes. But HIF-1α exclusively induces the hypoxic transcription of glycolytic genes such as phosphoglycerate kinase I, aldolase (Wang *et al*., 2005; Covello *et al*., 2006).

HIF is the primary molecular sensor which responds to oxygen tension changes (Adelman *et al*., 1999;

Maltepe *et al*., 2005). HIF as transcription factor regulates many cellular processes, for example angiogenesis, invasion, erythropoiesis and cell survival (Semenza, 2000; Bruick, 2003; Covello and Simon, 2004; Cowden Dahl *et al*., 2005a). But the data about its role in spontaneous abortion is very poor.

To further investigate the role of HIF-1a in spontaneous abortion, we measured the *HIF-1a* gene expression in chorionic tissue and deciduas.

Material and Methods

Prior to inclusion in the study, all subjects underwent a standard diagnostic work-up. The women were examined using transvaginal ultrasonography for the absence of uterine abnormalities and polycystic ovary syndrome. Women with previously diagnosed arterial hypertension, diabetes, thyroid diseases, autoimmune pathology and infections during pregnancy were excluded from studied population. Women contacting with exogenous risk factors, such as alcohol, electromagnetic radiation, industrial noise, vibration, chemical pollutants were also excluded. The study was approved by the Southern Federal University Bioethics Committee. The participants willingly signed the informed consent. After approval by institutional review board, 9 women (mean age 29) with spontaneous abortion and 8 women (mean age 29) with normally progressing pregnancies were studied.

Samples of chorionic and decidual tissues were taken after surgical termination by curettage of normally progressing pregnancies in 5-9 week of gestation (n = 8) and spontaneous abortion in 5-9 week of gestation (n = 9). Villous samples from the control group were obtained from women undergoing elective abortion for social reasons. Samples were stored at -80°C in aliquots for RNA isolation and thawed only once to avoid degradation.

Total RNA isolation was extracted by the acid guanidinium thiocyanate phenol method (Chomczynski and Sacchi, 1987). Upon isolation, RNA was immediately treated with DNAse I (Syntol, Russia). RNA integrity was assessed using non-denaturing 1,5% agarose gel electrophoresis. Clear 18S and 28S bands were observed with no signs of RNA degradation. The RNA was reverse transcribed immediately following the RNA isolation and the DNAse treatment using the "RT kit" (Syntol, Russia) with the template denaturation step and the oligo (dT) primer. Reverse transcription (with M-MLV enzyme) was performed during 50 min incubation at 42°C for 50 minutes, followed by duration of 92°C for 10 min. cDNA samples were stored at -20°C.

Polymerase Chain Reaction (PCR) was performed with commercially available reagents by Syntol (Russia). Sequences of the *HIF-1α*-specific primers were: forward 5`-ATCTCGGCGAAGTAAAGAATCTG-3`; and reverse 5`-GTCACCATCATCTGTGAGAACC-3`. Human *β-Actin* gene was used as a reference gene. Sequences of the *β-Actin*-specific primers were: 5`-CTTCTACAATGAGCTGGGTG-3`; and 5`-TCATGAGGTAGTCAGTCAGG-3`. PCR was performed according to the protocol for TerCyc thermocycler (DNK Technologiya, Russia). Cycling parameters for *HIF-1α* were the following: 1 cycle: 94°C for 10 c; 35 cycles: 94°C for 15 c, 64°C for 30 c and 72°C for 30 c; final elongation: 72°C for 2 min.

The PCR products were analyzed by 2% agarose gel electrophoresis. Gel images were captured using GelDoc XR system (Bio-Rad, USA). Densitometry was performed using ImageJ (NIH, USA). The background was subtracted with the rolling ball radius of 50 pixels.

The intensities of the bands of the target gene (*HIF-1α*) was normalized to that of *β-Actin*. All experiments were conducted in duplicate. Data were analyzed with MedCalc 11.4.2 software using the appropriate non-parametric Mann–Whitney test. P-value <0.05 was considered statistically significant.

Results

The expression of *HIF*-1 differs for chorionic and decidual tissues in condition of normal gestation. Compared with decidual tissue, the expression of *HIF-1α* was statistically increased in chorionic tissue in condition of normally progressing pregnancy (P = 0.016) (Fig. 1).

HIF-1α expression in samples of both tissues in spontaneous abortion is equal (Fig. 2). Thus the expression of *HIF-1α* in chorionic tissue in case of spontaneous abortion does not match for normal gestation condition.

There wasn`t any difference in the level of *HIF-1α* expression in decidua in condition of normal pregnancy compared to spontaneous abortion.

Compared with control group, the expression of *HIF-1α* was decreased (1.5 fold) in chorionic tissue (P = 0.057) in case of spontaneous abortion (Fig. 3).

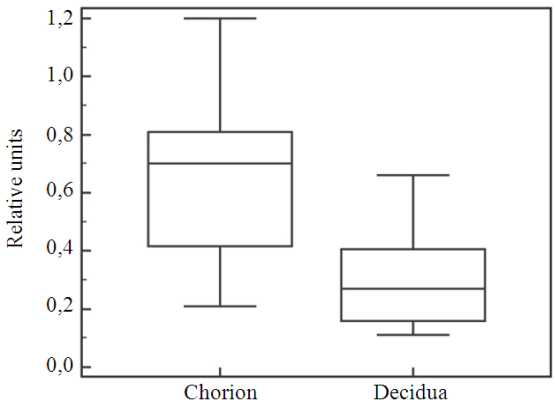

Fig. 1. *HIF-1α* expression level in chorionic tissue and decidua in condition of normally progressing pregnancy. Gene expression is provided in the same scale in relative units. The mid-lines are medians and the box lines are 25th and 75th percentiles

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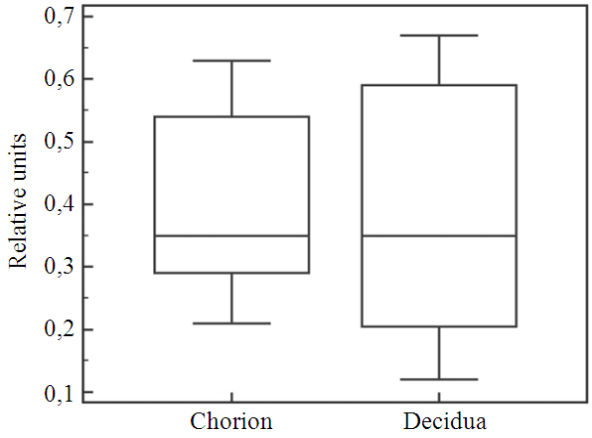

Fig. 2. *HIF-1α* expression level in chorionic tissue and decidua in condition of spontaneous abortion. Gene expression is provided in the same scale in relative units. The mid-lines are medians and the box lines are 25th and 75th percentiles

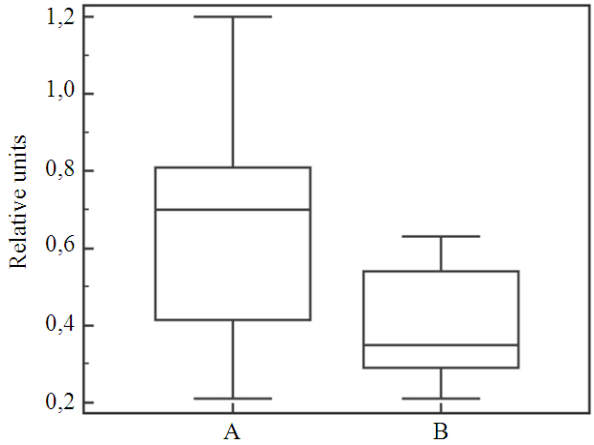

Fig. 3. *HIF-1α* expression level in control group (A) and spontaneous abortion (B) in chorionic tissue. Gene expression is provided in the same scale in relative units. The mid-lines are medians and the box lines are 25th and 75th percentiles

Thus, the high expression level of the *HIF-1α* gene in chorionic tissue is characteristic for a normal pregnancy. The decrease in the expression level of *HIF-1α* gene in chorionic tissue may be associated with miscarriage in the first trimester.

Discussion

Our study shows, that during normally progressing pregnancy *HIF-1α* expression level in chorionic tissue is significantly increased compared to decidua. In spontaneous abortion *HIF-1α* expression in chorionic tissue decreases and reaches values typical for decidua. This may result in trophoblast differentiation alteration, implantation changes, or altered angiogenesis in the forming placenta. Furthermore, as a transcription factor, low levels of HIF may have negative effect on organogenesis and embryo growth. On the other hand, low level of *HIF* expression may reflect changes in hypoxic environment and active oxygen radicals level increase, which leads to lipid peroxidation intensification, cell membrane damage and cell death. This statement demands further investigation.

During the first trimester of pregnancy placental oxygen remains low. It appears to be necessary for placental metabolic activity and for protecting placental and fetal tissues against toxic oxygen metabolites (Illsley *et al*., 2010). The invasion of trophoblast cells is regulated by major different factors including signaling of the adhesion and growth factors regulated by the interactions of decidua and trophoblast (Flaminio and Antczak, 2005; Harris, 2010). Hypoxic conditions are the typical factor that regulates the invasion of trophoblast cells which migrate and invade the surrounding blood vessels of the endometrium in the maternal uterus in persisting hypoxic conditions. Hypoxia induces alteration of various genes including integrin, MMP and TIMP (Luo *et al*., 2011; Onogi *et al*., 2011; Na *et al*., 2012). It was found that the invasion ability of trophoblast is regulated by the expression of *HIF-1α* (Dubinsky *et al*., 2010). The invasive ability of trophoblast cells decreases according to the inhibition of *HIF-1α* expression by siRNA (Choi *et al*., 2012).

HIF-1α is expressed in syncytiotrophoblast and in villous cytotrpophoblast (Rajakumar, 2000). HIF-1α mRNA and protein peaked at 7-10 weeks of gestation (Ietta *et al*., 2006). HIF in hypoxia condition provides a potent stimulus for VEGF synthesis and is essential for development of maternal and placental vasculation in early human pregnancy (Cowden Dahl *et al*., 2005a; Nau *et al*., 2002; De Marco and Caniggia, 2002; Daikoku *et al*., 2003; Qian *et al*., 2004; Zhang *et al*., 2009; Arjamaa *et al*., 2009). *HIF* expression changes exceeding optimal level lead to pathological processes. There is increase level of *HIF* expression in choriocarcinoma and other trophoblastic diseases (Bolat *et al*., 2010).

Defects in HIF are often responsible for early termination of pregnancy (Goldman-Wohl and Yagel, 2002; Sibai *et al*., 2005). Complete disruption of HIF signaling results in improper placental development (Fryer and Simon, 2006). Homozygosity for a null allele at the mouse *Hif1α* locus results in embryonic lethality attributable to failed vascularization (Iyer *et al*., 1998). Cowden Dahl *et al*. (2005b) reported that *HIF-1α- HIF-2α* knockout mice displayed a 17% reduction in trophoblast invasion compared with wild type placenta. Several pro- and anti-invasive factors expressed by either the trophoblasts or the decidua were HIF target genes (Cowden Dahl *et al*., 2005b). These studies

suggest that HIF appear to act as a key mediator in regulation of placental differentiation, growth and function during early pregnancy.

Conclusion

Our findings show that a low *HIF-1α* expression level in chorionic tissue (close to values, typical for decidua) can be associated with spontaneous abortion in first trimester of pregnancy.

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Author's Contributions

E.V. Mashkina: Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article.

K.A. Kovalenko: Analysis and interpretation of data, Contributed unpublished essential data or reagents.

E.V. Butenko: Conception and design, Analysis and interpretation of data, Drafting or revising the article.

Ethics

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. Each author confirms the manuscript represents honest work. All authors have approved the manuscript. Each author agrees with the order in which his name appears on the title page. Study design and methods were approved by Ethics Committee of Southern Federal University.

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