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Expression of Long Non-Coding RNAs in Placentas of Intrauterine Growth Restriction (IUGR) Pregnancies

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Abstract

Background: Intrauterine growth restriction (IUGR), a pathologic diminution of the rate of fetal growth, has been associated with alterations in expression of several genes. However, the role of long non-coding RNAs (lncRNAs) in its pathogenesis has not been studied.

Methods: In this study we evaluated the expression of four lncRNAs namely, nuclear paraspeckle assembly transcript (*NEAT1*), taurine up-regulated 1 (*TUG1*), p21-associated ncRNA DNA damage-activated (*PANDA*), and metastasis-associated lung adenocarcinoma transcript-1 (*MALAT1*) in placenta samples obtained from IUGR and normal pregnancies to determine their possible contributions in the pathogenesis of IUGR.

Results: We found no significant differences in expression levels between cases and controls. We also found no correlation between expression and clinical data of study participants; however, we found significant correlations between expression levels of all the assessed lncRNAs in both cases and controls.

Conclusions: These results imply the existence of a possible shared regulatory mechanism for the expression of these transcripts in placenta. Future studies are needed to perform such evaluations in larger sample sizes or in animal models in earlier stages of pregnancy.

Keywords: IUGR, *NEAT1*, *MALAT1*, *PANDA*, Placenta, *TUG1*.

Introduction

Intrauterine growth restriction (IUGR), a pathologic diminution of fetal growth rate, affects 3-7% of births and is associated with a substantial increase in perinatal mortality (1). Several maternal, placental, and fetal factors are involved in the pathogenesis of IUGR (2). Unsuccessful placentation is regarded as an important factor in this condition (3). Histopathological examinations of placentas in IUGR pregnancies have shown lower placental weights and greater frequencies of infarction, intervillous fibrin deposition, stromal fibrosis, syncytial knotting, basement membrane thickening, and cytotrophoblastic hyperplasia than seen in placentas from normal pregnancies (4). Moreover, gene expression patterns in placentas of IUGR pregnancies have been shown to be different from those of normal pregnancies. For example, Sitras et al. showed different expression of genes associated

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with chemokine and cytokine signaling pathways and glucocorticoid metabolism in IUGR placentas than that seen in normal placentas (5). Rab et al. demonstrated lower placental expression of epidermal growth factor in IUGR pregnancies than in those from normal pregnancies (6). Szentpéteri et al. reported greater placental expression of endoglin in IUGR placentas than in normal placentas and suggested this alteration as the underlying cause of vascular dysfunction and prolonged fetal hypoxia in these pregnancies (7). Others have focused on long non-coding RNA (lncRNA) expression profiles in IUGR. This proportion of the human transcriptome has fundamental regulatory roles in many physiological aspects including angiogenesis, apoptosis, cell proliferation and migration, inflammation, and gametogenesis (8), so alterations in their expression might be involved in the pathogenesis of IUGR or other pregnancy complications. Supporting evidence for this hypothesis has been provided by the observed down-regulation of the lncRNA maternally expressed gene 3 (*MEG3*) in a significant proportion of placental samples obtained from preeclamptic patients compared with samples obtained from normotensive pregnancies (9). Also, Gremlich et al. demonstrated greater expression of the lncRNA nuclear paraspeckle assembly transcript 1 (*NEAT1*) in the fetal part of IUGR placentas than in normal placentas (10).

In the present study, we assessed expression of four lncRNAs, namely, *NEAT1*, taurine up-regulated 1 (*TUG1*), p21-associated ncRNA DNA damage-activated (*PANDA*), and metastasis-associated lung adenocarcinoma transcript-1 (*MALAT1*) in placenta samples obtained from IUGR and normal pregnancies to find their possible contributions in the pathogenesis of IUGR. These lncRNAs were selected based on their involvement in apoptosis and cell proliferation, migration, and invasion (11, 12).

Materials and methods

Study participants

We recruited 33 healthy women with uncomplicated pregnancies and 33 women with IUGR in Shafa Surgery Center, Zahedan, Iran during June to December 2016. All participants had single pregnancies and were followed until delivery. The maternal and fetal data and medical histories were prospectively assessed and recorded. Intrauterine growth restriction was diagnosed based on fetal birth weight below the tenth percentile for fetal sex and gestational age. Controls were chosen from pregnancies with birth weights between the tenth and ninetieth percentiles. Birth weight was assessed both prenatally by ultrasound and postnatally. Gestational age was determined by ultrasound during the first trimester. Cases with known intrauterine infections, chromosomal abnormalities, congenital malformations, maternal malnutrition, cigarette smoking, multiple pregnancy, preeclampsia, or structural abnormalities in the placenta were excluded from the study. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, and informed written consent was obtained from all the participants.

Sampling

Placental tissue samples were obtained from placental fetal sites with rough dimensions of 2 cm × 2 cm × 2 cm (8 cm^3). Tissue samples were stored at -70°C until they were used for gene expression analyses.

Quantitative real time PCR

Total RNA was isolated from placenta samples using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I to remove DNA contamination. The quantity and quality of the extracted RNA was assessed by Nanodrop (Thermo Scientific) and gel electrophoresis. cDNA was produced using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit. The relative expression of the lncRNAs was assessed in the Rotor Gene 6000 Corbett Real-Time PCR System using Applied Biosystems TaqMan® Universal PCR Master Mix. Hypoxanthine phosphoribosyltransferase 1 (*HPRT1)* was used as the reference gene. The PCR program included a denaturation step at 95 °C for 10 min followed by 40 cycles of 95 °C for 10 sec and 60 °C for 60 sec, with a final extension step at 72 °C for 5 min.

The primers and probes used in this study are shown in Table 1.

Table 1. Primers and probes used in this study.

Gene name	Nucleotide sequences	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC	18	
	R: CACAGAACTAGAACATTGATA	21	88
	FAM - CATCTGGAGTCCTATTGACATCGC- TAMRA	24	
NEAT1	F: CCAGTGTGAGTCCTAGCATTGC	20	
	R: CCTGGAAACAGAACATTGGAGAAC	22	78
	FAM - ACCCTGGAGGAGAGAGCCCGCC - TAMRA	23	
TUG1	F: ACCGGAGGAGCCATCTTGTC	24	
	R: GAAAGAGCCGCCAACCGATC	24	149
	FAM - ACCGCACGCCCGTTCCTTCGC - TAMRA	24	
PANDA	F: GTTTTCCTGTTCGTCGATTCTGG	24	
	R: GGAAAGCTGAGAGAGACTTTGAAC	23	81
	FAM - CTGGACCACCTCTGAAGGCAGGCA - TAMRA	24	
MALAT1	F: CGCTAACGATTTGGTGGTGAAG	22	
	R: GGATCCTCTACGCACAACGC	20	135
	FAM - CTCGCCTCCTCCGTGTGGTTGCC -TAMRA	23	

Statistical analysis

Statistics were analyzed using R & R Stan based using Bayesian Regression Models brms) and Quantile Regression Estimation and inference methods quantreg packages with iteration = 5000 and warmup = 1000. Bayesian Multilevel and Quantile regression models were used. RHat and WIAC parameters were used to assess convergence of models.

Results

General data of study participants

The current study was performed on placenta samples obtained from 33 IUGR pregnancies (case group) and 33 normal pregnancies (control group). The ages (mean ± standard deviation) of study participants were 27.6 ± 6.8 and 32.9 ± 5.9 in the case and control groups, respectively. Birth weight values (mean ± standard deviation) were 2000.6 ± 493.5 and 3117.5 ± 405 gm in the case and control groups, respectively.

Table 2. General demographic and clinical data of study participants.

	Category	Control n (%)	Case n (%)
Pregnancy age (Weeks)	37	4 (12.1)	12 (36.4)
	38	22 (66.7)	18 (54.5)
	≥39	7 (21.2)	3 (9.1)
Abortion History	Yes	5 (15.2)	7 (21.2)
	No	28 (84.8)	26 (78.8)
Parity	1	5 (15.2)	7 (21.2)
	2	8 (24.2)	9 (27.3)
	3	10 (30.3)	9 (27.3)
	≥4	10 (30.3)	8 (24.2)

Relative expression of lncRNAs in IUGR vs. normal placentas

No significant differences in lncRNA expression were found between placenta samples from IUGR cases and normal controls (Table 3).

Table 3. The results of expression analysis obtained through Bayesian generalized multilevel model by controlling the effects of confounding variables (SE: standard error, 95% CIr: credible intervals).

LncRNAs	Expression ratio in IUGR samples compared with normal samples	Estimate LN (Efficiency^-Delta CT)	SE	P-value	95% CIr
*NEAT*1	0.8288	1.22	0.98	0.245	[-0.75, 3.1]
PANDA	1.6425	-0.27	0.94	0.584	[-2.1, 1.58]
TUG1	0.8660	-0.9	1.2	0.298	[-3.24, 1.4]
MALAT1	0.9361	0.75	0.87	0.356	[-1.04, 2.4]

Correlations between relative expression of lncRNAs and demographic and clinical data of study participants

No significant correlation was found between expression levels of lncRNAs and age of study participants or the pregnancy age.

Table 4. Spearman correlation analysis between expression of lncRNAs and participants' data.

		Spearman coefficient	Correlation
		Age	Pregnancy Age
NEAT1	Case	0.016	-0.047
	Control	0.001	-0.033
PANDA	Case	-0.110	0.035
	Control	-0.091	0.051
TUG1	Case	-0.112	0.012
	Control	0.116	-0.1
MALAT1	Case	-0.005	-0.063
	Control	0.027	-0.082

Correlation between relative expression of genes

We found significant correlations between expression levels of all assessed lncRNAs in both cases and controls (Table 5).

Table 5. Correlation coefficients between expression levels of studied genes in IUGR and control samples.

		PANDA	*TUG1*	*MALAT1*
NEAT1	Cases	0.814**	0.725**	0.878**
	Controls	0.659**	0.627**	0.824**
MALAT1	Cases	0.75**	0.667**	
	Controls	0.76**	0.62**	
TUG1	Cases	0.488**		

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

Discussion

In this study, we evaluated expression of four lncRNAs in placental samples from IUGR and normal pregnancies. The placenta is a vital temporary organ that enables connection and nutrient transfer between the mother and fetus.

The differentiation and function of trophoblast cells, such as invasion and the construction of the maternal/fetal interface, have essential roles in these processes (13). Evidence from human cancer studies have shown that lncRNAs can regulate apoptosis, cell differentiation, and invasive capacities of cells (14). Placentation shares remarkable features with tumorigenesis

including construction of an immune-privileged microenvironment, high angiogenic and proliferative index, and invasive properties (15). Consequently, lncRNAs are expected to exert parallel roles in placentation and tumorigenesis. Two lines of evidence support participation of lncRNAs in IUGR; first, a single nucleotide polymorphism within the *H19* lncRNA in the mother, offspring, or both, has been associated with fetal growth (16, 17); second, greater lncRNA *NEAT1* expression has been demonstrated in the fetal part of IUGR placentas than in normal placentas (10). The role of *NEAT1* has been well documented in the context of cancer. Its abnormal up-regulation and its effect on patient survival have been demonstrated in different kinds of solid tumors, including lung, esophageal, and colorectal cancers, and hepatocellular carcinoma. On the other hand, *NEAT1* expression is decreased in acute promyelocytic leukemia where it enhances leucocyte differentiation (18). Contrary to Gremlich et al. (10), we found no significant difference in *NEAT1* expression between cases and controls. Such inconsistency might be due to differences in sample size (12 *vs.* 38 samples in each group), different ethnicities, or different ages of study participants.

We also found no significant differences between cases and controls in expression of the other three lncRNAs. *PANDA*, an evolutionarily-conserved lncRNA whose expression is triggered by DNA damage in a p53-dependent mode, exerts anti-apoptotic effects (19). It restrains cells in their standing proliferative situation, and alteration of its expression is an important indicator of senescence (20). Biron et al. reported increased senescence in IUGR placentas as demonstrated by a greater proportion of senescence-associated heterochromatin foci in IUGR than in controls (21). Based on these studies, we expected different *PANDA* expression patterns between cases and controls. The lack of this expected difference may be due to the relatively small number of subjects or the sampling method (sampling from fetal side of the placenta). Future studies can assess its expression in the maternal side of placentas to find any difference in its expression between IUGR and normal pregnancies. Taurine up-regulated 1 has an essential role in cell proliferation, cancer cell migration, and induction of epithelial-mesenchymal transition (EMT) via modulation of expression of matrix metalloproteinases, E-cadherin, TGF-β, and TGF-β receptor (22). The role of TGF-β signaling has been demonstrated in some IUGR-induced pathologic conditions (23). Moreover, a kind of EMT is involved in differentiation of villous cytotrophoblasts into extravillous cytotrophoblasts and acquisition of the migration and invasion capabilities (24). Finally, Tseng et al. demonstrated over-expression of *MALAT1* in placenta previa increta/percreta. They also showed the effect of *MALAT1* silencing in suppression of trophoblast-like cell invasion in vitro (25). In addition, *MALAT1* has been demonstrated to participate in the regulation of proliferation, cell cycle, apoptosis, migration, and invasion of trophoblast cells, and its down-regulation during initial placentation contributes to the pathogenesis of preeclampsia (26). The lack of difference in *MALAT1* expression between cases and controls in the current study might be due to the time of sampling (at delivery), so we did not exclude the possibility of its altered expression during early phases of placentation.

We found no significant correlation between lncRNA expression and study subject age or pregnancy age. However, we did find significant correlations between expression levels of all assessed lncRNAs in both cases and controls, which implies the existence of a possible shared regulatory mechanism for their expression in placenta.

In brief, as we demonstrated no significant difference in the expression of *MALAT1*, *PANDA*, *TUG1*, or *NEAT1* between IUGR and normal placentas, we suggest future studies to perform such evaluations in larger sample sizes or in animal models in earlier stages of pregnancy.

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