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First-trimester trophoblast cell model gene response to hypoxia

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KEY WORDS
Microarray
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Objective: Trophoblast invasion, which sets the stage for placentation and pregnancy outcome, likely occurs in a hypoxic environment. We used microarray technology in a trophoblast cell line to identify hypoxia-responsive genes that may impact placentation.

Study design: An immortalized extravillous cytotrophoblast cell line, HTR-8/SVneo, was exposed to normoxia (20% oxygen) or hypoxia (1% oxygen) for 6 hours. Total RNA was harvested and prepared for microarray study. Quantitative reverse transcriptase polymerase chain reaction was performed for array confirmation.

Results: We confirmed the up- and down-regulation of 10 hypoxia-responsive genes using quantitative reverse transcriptase polymerase chain reaction. Ontologic gene categories that were found to be hypoxia-responsive included motility/migration, angiogenesis, and apoptosis.

Conclusion: Specific genes that were found to be up-regulated in this first-trimester array (such as plasminogen activator inhibitor-1 and tissue inhibitor of metalloproteinase 3) have been described in preeclampsia. The hypoxia-responsive genes that we identified may be physiologic in early pregnancy. However, up-regulation of these same genes in later pregnancy augurs poorly.

Oxygen is critical to human life. However, some processes that involve cellular invasion (growth of tumors, blastocyst implantation, and placental development) actually occur in a hypoxic environment.[1]

The early human placenta develops in a physiologically low oxygen environment, compared with the surrounding endometrium.[2] The early placenta and fetus are vulnerable to toxic oxygen metabolites, which include free-radical superoxide anions.[3] Early hypoxia therefore is critical because active antioxidant enzymes (a main defense against these toxic metabolites) are virtually absent in the syncytiotrophoblast during the first 2 months of gestation. Antioxidant activity increases progressively after 9 weeks of gestation.[4]

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Table I Primer sequences used for quantitative RT-PCR confirmation

Gene	Accession	Primer sequence	Product size
PAI-1–forward	NM_002575	CAGATGAAATTGCCGATGTG	97
PAI-1–reverse		GCCATTTTGTCTTTGCTGGT	
VLDLR-forward	L22431	ATGGGCCATTCTTCCTCTCt	69
VLDLR-reverse		CAATTCCGCCACATCAAGTA	
TIMP3-forward	NM_000362	CCTGGGTTGTAACTGCAAgA	55
TIMP3-reverse		AGTCACAAAGCAAGGCAGGT	
FGF5-forward	AF171928	AGTCAATGGATCCCACGAAG	69
FGF5-reverse		ACAATCCCCTGAGACACAGC	
IGFBP3-forward	NM_000598	CTCTGCGTCAACGCTAGTGC	95
IGFBP3-reverse		CGGTCTTCCTCCGACTCACT	
SMAD4-forward	NM_005359	CACCTGGAGATGCTGTTCAT	109
SMAD4-reverse		CGATGACACTGACGCAAATC	
TAF6L-forward	NM_006473	CAGGTCAAAGCAGATGGACA	75
TAF6L-reverse		GGCCTTCATCTTCAGCAGTC	
ZCWCC1-forward	NM_014941	AGAAGCTGCAGAAGCTGAGG	97
ZCWCC1-reverse		ATGTAGGCGTCCAGCTCATC	
RAMP1-forward	NM_005855	GAGACGCTGTGGTGTGACTG	51
RAMP1-reverse		CAGCTCCCTGTAGCTCcTGA	
GPR161-forward	NM_007369	GACACTGGCTTCAGCTGCTC	85
GPR161-reverse		GAGAGGGAGGGTTGTCATCA	

Although hypoxia may be the physiologic uterine environment before 10 weeks of gestation, significantly elevated Po_2 has been documented by 12 to 13 weeks of gestation.[2] Around this time, the second wave of trophoblast invasion is thought to begin, which converts the maternal spiral arteries into large-caliber, low-resistance channels.[5] Trophoblast invasion is crucial to placental development. Abnormalities of the second wave of trophoblast invasion are associated with complications such as intrauterine growth restriction and preeclampsia.[6,7]

In addition to epidermal growth factor,[8] tumor necrosis factor alpha,[9] and vascular endothelial growth factor,[10] oxygen is thought to play a pivotal role in extravillous trophoblast invasion.[11] Moreover, a variety of processes are required for a successful trophoblast invasion.[12] These processes include differentiation, motility/migration, cell adhesion, enzymatic activity, angiogenesis, cytokine activation, apoptosis, and growth. Given the likely impact of hypoxia on trophoblastic invasion, we hypothesized that hypoxia-responsive genes could be identified with a first-trimester trophoblast cell model and oligonucleotide microarrays.

Material and methods

Cell culture

HTR-8/SVneo cells are an established cell line after transformation of explant cultures of human first-trimester placentas (8-10 weeks of gestation) with SV40 large T antigen. The HTR-8/SVneo cells were grown to confluence in a 20% oxygen atmosphere at 37°C. The culture medium (RPMI-1640 [Invitrogen, Carlsbad, CA] supplemented with 5% heat-inactivated fetal bovine serum [Hyclone, Logan, UT], 1% glutamine, and 1% [100 U] of penicillin/streptomycin [Invitrogen]) was changed every 24 hours.

The cells were maintained under normoxic (20% oxygen) or hypoxic (0-1% oxygen) conditions for 6 hours at 37°C, as previously described.[13] Hypoxia was achieved by the infusion of calibrated nitrogen gas into both the incubator and the glove box that was designed by the Geoffrey Gurtner laboratory at the New York University School of Medicine, New York, New York.

Microarray analysis

After being washed with cold phosphate-buffered saline solution, total RNA was prepared with TRIzol reagent (Invitrogen). According to the manufacturer's instructions, total RNA was purified and precipitated with the RNeasy column (Qiagen, Valencia, CA). Synthesis of double-stranded complementary DNA (cDNA) and fragmented complementary RNA, hybridization, washing, and scanning were completed at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. Three separate microarray experiments were performed on 3 independent samples for both normoxic and hypoxic samples; 6 total microarray chips were used.

Microarray statistics

Raw data without normalization were generated with the Affymetric GeneChip Operating software (version 1.2 [GCOS 1.2]; Affymetrix, Santa Clara, CA). GeneSpring software (version 7.2; Agilent Technologies-Silicon Genetics, Redwood City, CA) was the statistical