Placenta 45 (2016) 24–31

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Regulation of hypoxia-inducible factor-1α, regulated in development and DNA damage response-1 and mammalian target of rapamycin in human placental BeWo cells under hypoxia

F. Zhou [a, b], L.B. Guan [c], P. Yu [c], X.D. Wang [a, b], Y.Y. Hu [a, *]

[a] Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China
[b] Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, Sichuan, China
[c] Laboratory of Cell and Gene Therapy, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China

A R T I C L E I N F O

Article history:
Received 15 April 2016
Received in revised form
4 July 2016
Accepted 12 July 2016

Keywords:
Hypoxia-inducible factor-1
Regulated in development and DNA damage response-1
Mammalian target of rapamycin
Hypoxia
Trophoblast
BeWo cells
Regulation

A B S T R A C T

Introduction: Hypoxia-inducible factor-1 (HIF-1), regulated in development and DNA damage response-1 (REDD1) and mammalian target of rapamycin (mTOR) are crucial mediators of many metabolic processes in various cell types under hypoxia. The involvement and regulation of these three factors underlying trophoblasts' response to hypoxia remains to be determined.
Methods: Specific siRNAs were applied to inhibit the expression of the corresponding genes and to investigate the roles of HIF-1α in modulating REDD1/mTOR and REDD1 in regulating mTOR/HIF-1α in the human choriocarcinoma cell line BeWo under normoxia and hypoxia.
Results: Exposure of BeWo cells to 1% oxygen (compared with 21% oxygen) led to a remarkable increase of both HIF-1α and REDD1 and an obvious decrease of mTOR at both the mRNA and protein levels. Interference of HIF-1α expression by siRNA resulted in an apparent reduction of REDD1 parallel with that of HIF-1α during normoxia and hypoxia. Additionally, the hypoxia-induced REDD1 increase was blocked through loss of HIF-1α, and the downregulation of mTOR in hypoxia could be partly obstructed by HIF-1α-siRNA transfection. Separately, the modulation effect of REDD1 was confirmed in an experiment demonstrating that the hypoxia-induced decrease of mTOR was inhibited by REDD1 knockdown, which was measured by changes in both the mRNA and protein levels. The disruption of REDD1 expression also led to increased accumulation of HIF-1α under both normoxia and hypoxia.
Discussion: The HIF-1α-REDD1-mTOR pathway was involved in the response to hypoxia in BeWo cells. Hypoxia-induced REDD1 upregulation is mediated by a HIF-1α-dependent pathway. Disruption of REDD1 blocked the effects of hypoxia on suppressing mTOR and resulted in additional accumulation of HIF-1α in BeWo cells.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Preeclampsia (PE) and intrauterine growth restriction (IUGR) are pregnancy-specific disorders that are responsible for a certain proportion of maternal-perinatal morbidity and mortality [1,2]. PE and IUGR are complicated by suboptimal placental perfusion or even placental hypoxia that are caused by multiple reasons, including increased uterine artery resistance and/or impaired placenta development [3,4]. Placental trophoblasts play a decisive role in these processes. They are able to invade the decidualized endometrium and transform muscular spiral arteries into flaccid vessels with low resistance and thin walls; thus, remodeling the vessel conductivity of utero-placenta to supply adequate oxygenated blood for the growing placenta and fetus [5–7]. However, the potential involvement and functional relationships of many mediators underlying trophoblasts in response to hypoxia have not been fully elucidated.

One of the crucial mediators that responds to compromised oxygen during physiological or pathological processes in multiple cells is hypoxia-inducible factor-1 (HIF-1) [8]. HIF-1 serves as a key DNA-binding transcription factor and triggers various adaptive

* Corresponding author. Department of Obstetrics and Gynecology, West China Second University Hospital, No. 20, Renmin South Road, Section 3, Chengdu, 610041, China.
E-mail address: yayi.hu@163.com (Y.Y. Hu).

http://dx.doi.org/10.1016/j.placenta.2016.07.003
0143-4004/© 2016 Elsevier Ltd. All rights reserved.

responses required for cell survival from hypoxic stress by trans-activating a myriad of genes [9]. It is comprised of two subunits, hypoxia-induced HIF-1α and constitutively expressed HIF-1β. HIF-1α is capable of inducing the formation of the active HIF-1 complex after being stabilized by hypoxia. It also participates in many physiological and pathological processes in the placenta [10–12]. Preliminary evidence showed that HIF-1α was expressed in the placental cytotrophoblasts and was decreased with advanced gestational weeks in normal pregnancy [10]. HIF-1α can mediate the differentiation of human placental trophoblasts based on the oxygen availability [11,12]. High levels of placental HIF-1α were detected in the early gestational stage [11]. Moreover, accumulating evidence suggests that HIF-1α is involved in the potential pathological mechanisms of many pregnancy complications [11–17]. Increased stabilization of HIF-1α was observed in placentas from PE and intrahepatic cholestasis of pregnancy (ICP), although a recent study suggested that this u-regulation in PE might be hypoxia independent [13].

Another key role of HIF-1α in response to low oxygen tension is regulation of the downstream genes regulated in development and DNA damage response-1 (REDD1) and mammalian target of rapamycin (mTOR) [17,18]. REDD1 was identified as an essential mediator of the anti-apoptotic effect of HIF-1α under hypoxia. REDD1 also contributes to DNA damage and apoptosis during cell stress [19,20]. It could negatively regulate mTOR [21], which is one of the intracellular signaling molecules that belongs to the phosphoinositide kinase-related kinase (PIKK) family. mTOR is essential for protein synthesis, cell growth and cell proliferation after integration with the oxygen and energy statuses, which are crucial for cells to respond to and survive hypoxia [22–24]. In addition, placental mTOR also plays important roles in regulating the placental amino acid transporter and fetal growth [25]. Our previous studies [26,27], together with others [11,13,14,28], have demonstrated that high levels of REDD1 were detected in placentas from an early gestational stage, PE and ICP, and abnormal expression of mTOR was detected in placentas from PE, IUGR and ICP.

Although HIF-1α, REDD1 and mTOR were demonstrated to be crucial mediators of many metabolic processes in various cell types under hypoxia, as well as participate in many physiological and pathological processes in placenta, the involvement and regulation of HIF-1α, REDD1 and mTOR in trophoblasts in response to hypoxia remains to be determined. Our study investigated the changes of HIF-1α, REDD1 and mTOR levels in BeWo cells after inhibiting either HIF-1α or REDD1 gene expression by siRNA transfection under both hypoxia and normoxia.

2. Methods

2.1. Cell culture

BeWo cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Gibco® RPMI 1640 medium (ThermoFisher Scientific, Shanghai, China) supplemented with 10% fetal bovine serum (Invitrogen, Sunnyvale, CA, USA), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C with 21% oxygen. Exponential-phase cells were subcultured into flat bottom plates for 24 h, with each well containing 1×10^5 cells. Then, cells in siRNA groups were transfected with siRNA for 72 h, and control groups were cultured without transfection in parallel. Next, half of the plates were moved to a Bio-bag (BD, Shanghai, China) with 1% oxygen, and the other half were kept under 21% oxygen. Then, cells were collected at different time points (1 h, 2 h, 4 h and 8 h; each with 3 parallel wells).

2.2. Transfection

The target sequence, sense and antisense sequences are shown in Table 1. All siRNA duplexes were synthesized by RIBOBIO CO., LTD (Guangzhou, China). Cells were transfected with HIF-1α siRNA or REDD1 siRNA (100 nM) using the RiboFECT™ CP reagent (ThermoFisher Scientific, Shanghai, China) according to the manufacturer's guidelines. Briefly, a mixture consisting of 2.5 μl of 20 μM siRNA, 60 μl of 1 × riboFECT CP Buffer and 6 μl of riboFECT CP reagent was incubated for 15 min at room temperature before 931.5 μl medium was added.

2.3. RNA isolation, reverse transcription and real-time PCR

Total RNA was extracted using TRIzol (Invitrogen Life Technologies, Shanghai, China). RNA (500 ng) was reverse-transcripted using the PrimeScriptTM RT reagent Kit (TaKaRa, Dalian, China) in a final volume of 10 μl that contained 2 μl of 5 × PrimeScript Buffer, 0.5 μl of PrimeScript RT Enzyme Mix I, 0.5 μl of oligo-dT primer (50 μM), 0.5 μl of random 6-mers (100 μM), 2 μl of total RNA and 4.5 μl of RNase free dH_2O. Then, the reaction was incubated at 37 °C for 15 min and inactivated at 85 °C for 5 s. Next, the cDNA was amplified in a mixture containing 4.5 μl of 2.5 × RealMaster Mix/ 20 × SYBR Solution (TIANGEN, Beijing, China), 0.5 μl of primer mix (10 μM each), 2 μl of cDNA, 3 μl of RNase free dH_2O. The primer sequences are shown in Table 2. Amplification was performed as follows: 95 °C for 10 min; then 94 °C for 10 s, 56 °C for 10 s, and 68 °C for 20 s (35 cycles); and finally, 68 °C for 5 min. The expression levels of mRNA were standardized against those of β-actin.

2.4. Western blotting

After being washed twice with PBS, the cells were detached using a trypsin-EDTA solution (2 ml) and centrifuged at 800g for 10 min. Next, the liquid was removed and 150 μl lysis buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 1% Triton X-100) was added to the cells with occasional rocking. Then, the cell lysate was resolved on 8% SDS-PAGE, and the membranes were incubated in a blocking solution (0.1% TBS-Tween 20 with 5% milk) at room temperature for 1 h. Then, the protein (30 μg) was separated on a 10% bis-tris acrylamide gradient gel (Invitrogen Life Technologies, Shanghai, China) and transferred onto a nitrocellulose membrane. Next, the membranes were immunoblotted with monoclonal antibodies separately: anti-HIF-1α (1:300, Abcam, Shanghai, China), anti-REDD1 (1:300, Proteintech Group, IL, USA) and anti-mTOR (1:500, Cell Signaling Technology, MA, USA). After being washed 3 times with TBS-T, the membranes were incubated with HRP-conjugated secondary antibody (1:10, 000, Gene C Ltd., Beijing, China) at room temperature for 2 h. The protein expression was quantified using the Molecular Analyst Software (Bio-Rad Laboratories, CA, USA). The intensity ratios of β-actin were obtained to quantify the relative protein levels.

Table 1
Target sequence and sense/antisense sequences for HIF-1α siRNA and REDD1 siRNA.

	Sequence
HIF-1α siRNA target	CTGATGACCAGCAACTTGA
sense	CUGAUGACCAGCAACUUGAdTdT
antisense	UCAAGUUGCUGGUCAUCAGTdTd
REDD1 siRNA target	CTCGGAACAGCTGCTCATT
sense	CUCGGAACAGCUGCUCAUUdTdT
antisense	AAUGAGCAGCUGUUCCGAGTdTd

Download English Version:

https://daneshyari.com/en/article/2788271

Download Persian Version:

https://daneshyari.com/article/2788271

Daneshyari.com