Available online at www.sciencedirect.com
ScienceDirect
PLACENTA
Placenta 29 (2008) 324–331

Hypoxia Inducible Factor 1 Alpha Regulates Matrigel-induced Endovascular Differentiation under Normoxia in a Human Extravillous Trophoblast Cell Line

K. Fukushima [a,*], M. Murata [a], M. Hachisuga [a], K. Tsukimori [a], H. Seki [b], S. Takeda [c], K. Asanoma [a], N. Wake [a]

[a] Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Obstetrics and Gynecology, Saitama Medical Center, Saitama Medical University, Saitama, Japan
[c] Department of Obstetrics and Gynecology, Juntendo University, Tokyo, Japan

Accepted 19 January 2008

Abstract

Extravillous trophoblast (EVT) cells mimic endothelial cells during angiogenesis, inducing remodeling of the spiral arteries that increases blood flow toward the intravillous space. We have previously shown that signals involving the vascular endothelial growth factor (VEGF) axis are essential for endovascular differentiation through integrin signaling from the extracellular matrix: This was accomplished with use of the human EVT cell line TCL1, which shows tube formation that specifically recalls morphological changes in endothelial cells. To investigate endovascular differentiation in EVT further, we investigated the role of hypoxia inducible factor (HIF)1A, a subunit of *HIF1* transcription factor that regulates not only adaptive responses to hypoxia, but also many cellular functions under normoxia, which was up-regulated in DNA microarray analysis during matrigel-induced endovascular differentiation under normoxia. *HIF1A* induces VEGF and ITGAV/ITGB3 aggregation, actions known to be important for cellular survival and endovascular differentiation in EVT. Inhibition of *HIF1A* up-regulation using siRNA introduction or chemical inhibition suppressed hypoxia-responsive element transcriptional activity, VEGF induction, ITGAV/ITGB3 aggregation accompanied by the inhibition of tube formation in TCL1 cells. These results suggest that *HIF1A* has a crucial role in regulating EVT behavior including matrigel-induced endovascular differentiation under normoxia.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: Hypoxia inducible factor 1 alpha; Endovascular differentiation; Extravillous trophoblast

1. Introduction

Differentiation of extravillous cytotrophoblast (EVT) is essential to successful organization of the feto-maternal interface during early development. Interstitial invasion promotes placental anchorage and is accompanied by endovascular differentiation, wherein EVT finally replaces and remodels the spiral arteries, resulting in increased blood flow toward the intravillous space [1,2].

Integrin subunits expressed in EVT are dramatically altered during interstitial and endovascular differentiation [3,4]. The significance of integrin subunit conversion is shown by the fact that in preeclampsia, in which both interstitial and endovascular invasion are abnormally shallow, EVT cells show significant defects in differentiation [5,6]. Therefore, it is assumed that expression and conversion of integrin subunits is involved in the regulation of normal EVT differentiation that is essential for establishment and maintenance of pregnancy [5,6]. Tumor necrosis factor alpha (TNF) can switch integrin expression and induce apoptosis in a human EVT cell line, with apoptosis

* Corresponding author. Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5395; fax: +81 92 642 5414.
E-mail address: kfuku@gynob.med.kyushu-u.ac.jp (K. Fukushima).

0143-4004/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2008.01.006

suppressed by signals via ITGB1 [7]. Vascular endothelial growth factor (VEGF) strengthens ITGAV/ITGB3 adhesion [8]. Thus, it is likely that TNF and VEGF, together with extracellular matrix (ECM), collaboratively regulate biological behavior of EVT through integrin signaling in early human pregnancy. In another words, the microenvironment that surrounds EVT might regulate its differentiation as a physiological adaptation.

Hypoxia inducible factor (HIF)1 is a transcription factor that regulates adaptive transcriptional responses to hypoxia by inducing expression of genes involved in glycolysis, red blood cell production and angiogenesis including VEGF [9,10]. HIF1 is composed of two subunits, HIF1A whose expression is regulated by various stresses, and constitutively expressed subunit HIF1B [11,12]. In addition to the effect of HIF1 transcriptional activity in a hypoxic environment, it also seems to be involved in determination of cell fate in a normoxic environment [13]. HIF1 expression under normoxia results in activation of factors associated with ECM degradation and invasion of glioma cells [14]. Cytokines and growth factors, as well as hypoxia in some cell types, can activate the signaling pathways involving MAPK and PI3K, both of which promote cell proliferation/survival as well as contribute to HIF1 activity [15]. An HIF1-dependent mechanism activates of ILK/Akt and mTOR pathways via integrin receptor activity [16]. In this context, *HIF1A* might be involved in regulation of cellular differentiation in EVT.

In the present study, to clarify mechanisms regulating endovascular differentiation in EVT, we investigated the role of HIF1A, which was up-regulated during matrigel-induced endovascular differentiation under normoxia.

2. Materials and methods

2.1. Reagents

For ECM, poly-L-lysine was purchased from Sigma Chemical Co. (St Louis, MO) and growth factor-reduced matrigel was purchased from Becton Dickinson Labware (Bedford, MA).

Anti-HIF1A antibody was purchased from BD Transduction Laboratories (Bedford, MA). Anti-VEGF antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antimycin A, an inhibitor of mitochondrial electron transport and *HIF1A* [17], was purchased from Alexis Biochemicals (Lausen, Switzerland). FITC or rhodamine-conjugated anti-mouse IgG (Molecular Probes Invitrogen, Carlsbad, CA) was used as the secondary antibody in immunofluorescence experiments.

2.2. Cell lines and cell culture

TCL1 cells were established from mixed primary cultures of cells isolated from chorionic membrane obtained from elective, pre-term caesarean sections [18,19]. Primary cultures contained 8–10% EVT. Isolated cells were immortalized by retroviral expression of SV40 large antigen; single-cell cloning revealed that cells with an epithelial morphology were the only type present after six months of culture. Cells showed no tumorigenicity either *in vitro* or *in vivo*. The cloned population expressed human chorionic gonadotropin, alpha, beta, and colony stimulating factor 1, but they did not express markers for decidualized endometrial cells, macrophages, or natural killer (NK) cells. TCL1 cells were positive for cytokeratin, whereas none stained for vimentin. In addition, TCL1 cells constitutively expressed gelatinase-A, but gelatinase-B was expressed only when cells were cultured in the presence of ECM, which specifically restricted phenotype of invading cytotrophoblast (CT) [7,8,18–20]. Cells were cultured in RPMI1640 (Nipro, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS, Gibco Invitrogen) or conditioned serum in a humidified atmosphere containing 95% air −5% CO_2 at 37 °C.

2.3. DNA microarray

DNA microarray analysis was performed using FilgenArray Human35k commercial service (Filgen Inc., Nagoya, Japan). Data analysis was performed with Array-Pro Analyzer Ver4.5 (Media Cybernetics, Inc., Silver Spring, MD) to determine signal intensity of each spot and its local background on microarrays. We calculated net intensity by subtracting mean intensity of all pixels within the local background area from mean intensity of all pixels within the spot area. Software normalized biases in net intensity between fluorescent dye channels in a microarray by global normalization. Analyzed data were selected using MicroArray Data Analysis Tool (Filgen).

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total cellular RNA prepared with TRIZOL (Invitrogen) was reverse transcribed using the first-strand cDNA synthesis kit (Amersham Biosciences, Piscataway, NJ) as recommended. Transcribed cDNAs were amplified with KOD polymerase (Toyobo, Osaka, Japan). PCR reactions for HIF1A were carried out with the following primers: sense primer 5′-TGG ACT CTG ATC ATC TGA CC-3′ and antisense primer 5′-CTC AAG TTG CTG GTC ATC AG-3′. Primers for the internal control, GAPDH (glyceraldehyde-3-phosphate dehydrogenase), were included in each reaction: sense primer 5′-GAG TCA ACG GAT TTG GTC GT-3′ and antisense primer 5′-GTT GTC ATG GAT GAC CTT GG A-3′. PCR was carried out for 30 cycles, each at 94 °C for 45 s, at the appropriate annealing temperature for 45 s, and at 72 °C for 1 min.

The gel was visualized with ethidium bromide and photographed.

2.5. siRNA expression and colony isolation

Transient expression of small interfering (si) RNA was performed using lipofectoamine 2000 (Invitrogen). Briefly, 50% confluent TCL1 cells were seeded and incubated overnight. The siRNA against *HIF1A* (Invitrogen) was introduced as recommended by the supplier. After 24 h of incubation, cells were prepared for each assay.

For isolation of a permanent expression clone, we constructed siRNA expression vector using a commercial service (TaKaRa BIO Inc.). DNA transfection was performed using lipofectoamine 2000 as recommended by the supplier. After two weeks of incubation with selection media containing 800 μg/mL G418 (Invitrogen), several single colonies were isolated. From these clones, we chose Clone 41, which did not express HIF1A (see text), for further investigation.

2.6. Tube formation assay

Growth factor-reduced matrigel (Becton Dickinson) was added (300 μL) to each well of a 24-well plate and allowed to polymerize for one hour at 37 °C. A total of 2×10^5 TCL1 cells, pre-incubated with serum-free media for one hour, were seeded. Cells were incubated at 37 °C at room air, viewed (magnification ×40–×400), and photographed using an Olympus IX71 microscope (Olympus, Tokyo, Japan). The number of tube formations was measured by counting the number of tube like structures formed by connected capillary bridge (see Fig. 1). At least three fields per well were examined; each experimental condition was tested in triplicate [21].

2.7. Immunofluorescence

A total of 2×10^5 exponentially growing cells were seeded on coverslips. After incubation, cells were fixed with 4% paraformaldehyde for 10 min and permeabilized with phosphate buffered saline (PBS) containing 0.5% Triton-X. After blocking with 3% bovine serum albumin for 30 min, cells were

Download English Version:

https://daneshyari.com/en/article/2789833

Download Persian Version:

https://daneshyari.com/article/2789833

Daneshyari.com