RBM ONLINE

ELSEVIER

Article

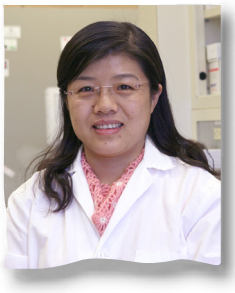
Nicotine promotes vascular endothelial growth factor secretion by human trophoblast cells under hypoxic conditions and improves the proliferation and tube formation capacity of human umbilical endothelial cells

Hongbo Zhao [a,*], *Lanxiang Wu* [b], *Yahui Wang* [a], *Jiayi Zhou* [a], *Ruixia Li* [a], *Jiabing Zhou* [a], *Zehua Wang* [a], *Congjian Xu* [a,c,*]

[a] *Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Hospital and Institute of Obstetrics and Gynecology, Fudan University, Shanghai, China*

[b] *Institute of Life Sciences, Chongqing Medical Universtiy, Chongqing, China*

[c] *Department of Obstetrics and Gynecology of Shanghai Medical School, Fudan University, Shanghai, 200032, China*



Hongbo Zhao received her Bachelor's degree and Master's degree from Dalian Medical University, China in 2001 and 2004, respectively. She obtained her PhD degree from Fudan University in 2007 and began to work at the Obstetrics and Gynecology Hospital of Fudan University. Between 2012 and 2014 she received postdoctoral training at the MD Anderson Cancer Center. Her research mainly focuses on trophoblastic disease and embryonic development.

KEY MESSAGE

Despite the significant advances in antenatal and perinatal care, pre-eclampsia remains a life-threatening condition for pregnant women and fetuses. Clarification of the underlying mechanisms by which smoking reduces the risk of pre-eclampsia may enhance our understanding of the pathogenesis of pre-eclampsia and contribute to the development of potential prevention strategies.

A B S T R A C T

Pre-eclampsia, characterized as defective uteroplacental vascularization, remains the major cause of maternal and fetal mortality and morbidity. Previous epidemiological studies demonstrated that cigarette smoking reduced the risk of pre-eclampsia. However, the molecular mechanism remains elusive. In the present study, it is demonstrated that a low dose of nicotine decreased soluble vascular endothelial growth factor receptor 1 (sFlt1) secretion in human trophoblast cells under hypoxic conditions. Nicotine was then observed to promote vascular endothelial growth factor (VEGF) secretion by reducing sFlt1 secretion and increasing VEGF mRNA transcription. Further data showed that nicotine enhanced hypoxia-mediated hypoxia-inducible factor-1α (HIF-1α) expression and HIF-1α small interfering RNA abrogated nicotine-induced VEGF secretion, indicating that HIF-1α may be responsible for nicotine-mediated VEGF transcription under hypoxic conditions. Moreover, conditioned medium from human trophoblast cells treated with nicotine under hypoxic conditions promoted the proliferation and tube formation capacity of human umbilical endothelial cells (HUVEC) by promoting VEGF secretion. These findings indicate that nicotine may promote VEGF secretion in human trophoblast cells under hypoxic conditions by reducing sFlt1 secretion and up-regulating VEGF transcription and improve the proliferation and tube formation of HUVEC cells, which may contribute to elucidate the protective effect of cigarette smoking against pre-eclampsia.

* Corresponding authors.

E-mail addresses: xucongjian@gmail.com (C. Xu); zhaohongbo01@sina.com (H. Zhao).

http://dx.doi.org/10.1016/j.rbmo.2016.12.014

1472-6483/

Please cite this article in press as: Hongbo Zhao, et al., Nicotine promotes vascular endothelial growth factor secretion by human trophoblast cells under hypoxic conditions and improves the proliferation and tube formation capacity of human umbilical endothelial cells, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2016.12.014

Introduction

Pre-eclampsia, as a leading cause of maternal mortality and morbidity, is characterized by compromised placental artery remodelling and vascular dysfunction. Although a series of risk factors have been identified, however, the pathogenesis of pre-eclampsia remains poorly understood. The main pathophysiology of pre-eclampsia is the insufficient trophoblast invasion and the defective angiogenesis and vascular transformation (Guzin et al., 2005). Vascular endothelial growth factor (VEGF) is the key factor for endothelial cell proliferation and placental vasculogenesis (Carmeliet et al., 1996; Fan et al., 2014; Ferrara et al., 1996). Serum-free VEGF concentrations in women with pre-eclampsia are frequently decreased, as compared with VEGF concentrations observed in normal pregnancy women. The reduction of free VEGF is mainly due to the overproduction of soluble fms-like tyrosine kinase-1 (VEGFR1/sFlt1), which can bind to VEGF with high affinity and act as an endogenous antagonist of VEGF (Fan et al., 2014; Rios et al., 2015). Notably, VEGF is one of the targets of hypoxia-inducible factor-1 (HIF-1), which consists of HIF-1α and ARNT subunits and activates many genes involved in the cellular response to low oxygen tension (Adelman et al., 2000).

Cigarette smoking is a major cause of cardiovascular disease and cancer. Despite its harmful effects, a series of epidemiological studies have demonstrated that cigarette smoking before and during pregnancy is correlated with a reduced risk of pre-eclampsia, indicating the possible protective effect of cigarette smoking against pre-eclampsia (Hammoud et al., 2005; Lisonkova and Joseph, 2013; Perni et al., 2012; Stone et al., 2007; Wei et al., 2015). The main component of cigarette smoke is nicotine, low doses of which have been shown to promote the proliferation and tube formation capacity of endothelial cells. However, the regulation of nicotine on human trophoblast cells remains largely unknown (Heeschen et al., 2001; Mimura et al., 2010).

The present study aims to characterize the effect of nicotine on VEGF secretion of human trophoblast cells and its role in the interaction between human trophoblast cells and human umbilical vein endothelial cell line (HUVEC) cells, in an effort to expand our understanding of the protective effect of nicotine against pre-eclampsia.

Materials and methods

Cell culture

HUVEC was obtained from the cell bank at the Chinese Academy of Sciences (Shanghai, China). The human choriocarcinoma cell line BeWo was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). BeWo and HUVEC cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 complete medium and DMEM-high glucose complete medium supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 at 37°C, respectively. BeWo cells were cultured either under normal cell culture conditions (21% O_2, 5% CO_2, at 37°C) in a humidified incubator (Heal Force, HF 100) or under hypoxic conditions (1% O_2, 5% CO_2, 94% N_2 at 37°C) in a humidified incubator (Heal Force, HF 100). HUVEC cells were cultured under normal cell culture conditions (21% O_2, 5% CO_2, at 37°C) in a humidified incubator (Heal Force, HF 100).

Table 1 – Primer sequences used for real-time quantitative reverse transcription polymerase chain reaction.

Product	Gene sequence	Size (base pairs)
VEGF	Forward: 5-ACGGTCCCTCTTGGAATTGG-3 Reverse: 5-CGGCCGCGGTGTGTCTA-3	112
sFlt1	Forward: 5-ACAATCAGAGGTGAGCACTGCAA-3 Reverse: 5-TCCGAGCCTGAAAGTTAGCAA-3	180
α-tubulin	Forward: 5'-CCTGTTGGGAGCTTTACTGC-3' Reverse: 5'-AAGGTGTTGAAGGCATCGTC-3'	100

sFlt1 = soluble vascular endothelial growth factor receptor 1; VEGF = vascular endothelial growth factor.

Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted using the Trizol reagent (Invitrogen Life technologies, Carlsbad, CA, USA) according to the manufacturer's guidelines, followed by reverse transcription (RT) using PrimeScript RT kit (Takara) to generate cDNA. Quantitative RT-PCR was performed using the SYBR Premix Ex TaqII (Perfect Real Time, Takara, Dalian, China) with the ABI 7900 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The primers used for the detection of VEGF, soluble vascular endothelial growth factor receptor 1 (sFlt1) and α-tubulin are indicated in **Table 1**.

Western blotting

BeWo cells were lysed in 1 × sodium dodecyl sulphate (SDS) lysis buffer (50 mmol/l Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 1 mmol/l phenylmethylsulphonyl fluoride (PMSF), and 1 mmol/l Na_3VO4) as previously described (Zhao et al., 2012). The bicinchoninic acid (BCA) assay was used to determine the protein concentration of the lysates. An equal amount of total protein was loaded on an SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). Following blocking with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (containing 0.05% Tween 20), the membrane was incubated with HIF-1α or α-tubulin primary antibody (Abcam, Cambridge, UK) and followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (Kang-Chen Biotech, Shanghai, China). The protein bands of interest were visualized by fluorography by using an enhanced chemiluminescence (ECL) detection system (Thermo Scientific, Rockford, IL).

VEGF and sFlt1 quantification by ELISA

BeWo cells were seeded in 6-well plates at a density of 2×10^5/well, treated with nicotine (10^{-7}mol/l) (Sigma-Aldrich, St Louis, MO, USA), or sFlt-1 neutralizing antibody (R and D Systems, Minneapolis, MN), or HIF-1α siRNA and maintained in 1% O_2 for 48 h. The supernatants were collected and the amounts of VEGF and sFlt1 were determined utilizing ELISA kits (R and D systems) according to the manufacturer's instructions.

Please cite this article in press as: Hongbo Zhao, et al., Nicotine promotes vascular endothelial growth factor secretion by human trophoblast cells under hypoxic conditions and improves the proliferation and tube formation capacity of human umbilical endothelial cells, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2016.12.014