Molecular and Cellular Endocrinology 442 (2017) 90–97

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

1,25-Dihydroxy vitamin D_3 stimulates system A amino acid transport in primary human trophoblast cells

Yi-Yung Chen [a, c, *], Theresa L. Powell [a, b], Thomas Jansson [a]

[a] Division of Reproductive Science, Department of Obstetrics & Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
[b] Section of Neonatology, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
[c] Division of High-risk Pregnancy, Department of Obstetrics & Gynecology, Mackay Memorial Hospital, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form
8 December 2016
Accepted 8 December 2016
Available online 9 December 2016

Keywords:
Placenta
Nutrient transport
Fetal growth
Maternal-fetal exchange

ABSTRACT

Vitamin D deficiency during pregnancy is linked to adverse perinatal outcomes such as small for gestational age infants. Recent evidence suggests that changes in placental amino acid transport contribute to altered fetal growth. We tested the hypothesis that 1,25-dihydroxy vitamin D_3 increases the gene expression of System A and L amino acid transporter isoforms and stimulates placental amino acid transport activity in cultured primary human trophoblast cells mediated by mTOR signaling. Treatment with 1,25-dihydroxy vitamin D_3 significantly increased mRNA expression of the System A isoform SNAT2 and System A activity, but had no effect on System L and did not affect mTOR signaling. siRNA silencing of the vitamin D receptor prevented 1,25-dihydroxy vitamin D_3-stimulated System A transport. In conclusion, 1,25-dihydroxy vitamin D_3 regulates System A activity through increased mRNA expression of SNAT2 transporters. Effects on placental amino acid transport may be the mechanism underlying the association between maternal vitamin D status and fetal growth.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Vitamin D deficiency has emerged as a global public health issue (Holick and Chen, 2008) due to inadequate sunlight exposure and intake. In the United States, the incidence of vitamin D deficiency continues to rise and women of reproductive age are particularly at risk of deficiency (Looker et al., 2011). Besides the well-established function of vitamin D in calcium homeostasis and bone mineralization, vitamin D deficiency during pregnancy is associated with a range of poor perinatal outcomes, including preterm birth, preeclampsia, small-for-gestational-age infants, insulin resistance and gestational diabetes mellitus (Bodnar et al., 2014; Gernand et al., 2014). The mechanisms underlying these associations are not fully understood, however the placenta, which is the interface between maternal and fetal circulations, is likely to be involved.

The bioactive form of vitamin D, 1,25-dihydroxy vitamin D_3, exerts its function via genomic and non-genomic pathways. Transcriptional regulation is initiated through the vitamin D binding receptor (VDR) to alter gene expression. The rapid non-transcriptional responses involve stimulation of secondary messenger systems (Ca^{2+} and cyclic AMP) and activation of signaling molecules, such as phospholipase C, phospholipase A_2, protein kinase C and mitogen-activated protein kinases (Haussler et al., 2011; Hii and Ferrante, 2016; Omdahl et al., 2002). The placenta is the primary site for the exchange of nutrients, gases and waste products and in concert with decidua has an important role in vitamin D metabolism during pregnancy (Murthi et al., 2016). The human placenta not only expresses all components of vitamin D signaling, including VDR, retinoid X receptor (RXR), and vitamin D hydroxylase but also synthesizes 1,25-dihydroxy vitamin D_3 (Shin et al., 2010; Weisman et al., 1979), suggesting a potential link between maternal vitamin D levels and placental function.

Placental amino acid transfer is pivotal for fetal growth and more than 20 amino acid transport systems have been identified in human placenta (Cleal and Lewis, 2008; Jansson, 2001). System A amino acid transporters mediate sodium-dependent uptake of non-essential amino acids such as alanine, serine and glutamine (Mackenzie and Erickson, 2004). The system A isoforms expressed in the human placenta are Sodium-coupled Neutral Amino acid Transporter 1 (SNAT1), SNAT2 and SNAT4 which are encoded by the genes Slc38a1, Slc38a2 and Slc38a4 respectively (Broer, 2014).

* Corresponding author. Department of Obstetrics & Gynecology, University of Colorado Anschutz Medical Campus, Research Complex-2; Mail Stop 8613, 12700 East 19th Avenue, Aurora, CO, 80045, USA.
E-mail address: yhyy@mmh.org.tw (Y.-Y. Chen).

http://dx.doi.org/10.1016/j.mce.2016.12.009
0303-7207/© 2016 Elsevier Ireland Ltd. All rights reserved.

System L is a Na^+-independent transporter mediating the transport of essential amino acids such as leucine across the placenta (Jansson, 2001). It is a heterodimer consisting of a light chain, typically L-type amino acid transporter 1 (LAT1) or LAT2, covalently attached to a heavy chain (CD98/4F2hc) (Jansson, 2001). Both LAT1 and LAT2 mRNA are highly expressed in the placenta (Gaccioli et al., 2015; Pineda et al., 1999; Prasad et al., 1999).

Recently, a study from the Southampton Women's survey demonstrated that maternal 25-hydroxyvitamin D and vitamin D binding protein levels were positively associated with placental expression of several amino acid transporter genes, suggesting placental amino acid transport may be regulated by maternal vitamin D and/or vitamin D-binding protein (Cleal et al., 2015). Previous studies in murine skeletal myotubes have shown that vitamin D enhances protein synthesis through mechanistic target of rapamycin (mTOR) signaling pathway (Salles et al., 2013) and we have reported that mTOR signaling is a positive regulator of amino acid transport in cultured primary human trophoblast (PHT) cells (Rosario et al., 2013). We therefore hypothesized that 1,25-dihydroxy vitamin D_3 increases the gene expression of system A and L amino acid transporter isoforms and stimulates placental amino acid transport activity in cultured PHT cells mediated by mTOR signaling.

2. Materials and methods

2.1. Study subjects and tissue collection

Term placental tissue was collected after written informed consent from thirteen healthy pregnant women undergoing elective Cesarean section at 37–40 weeks of gestation. Samples and medical information were added to a tissue repository approved by the Colorado Multiple Institutional Review Board (COMIRB-14-1073) and subsequently study personnel were provided anonymized placental tissue and clinical information used in this study.

2.2. Primary human trophoblast cell culture and treatments

Placental tissue was collected within 15 min of delivery and processed immediately. PHT cells were isolated as originally described (Kliman et al., 1986) with modifications (Aye et al., 2013, 2014; Roos et al., 2009). Briefly, approximately 35 g of villous tissue was dissected free of decidua and blood vessels and washed in warm phosphate buffered saline (PBS) to remove excess blood. Cells were transferred to digestion buffer with trypsin (0.25%, Invitrogen, Carlsbad, CA) and Deoxyribonuclease I (Sigma-Aldrich, St. Louis, MO). Cytotrophoblast cells were separated and collected through a discontinuous 10–70% Percoll gradient centrifugation. Cells were then cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) and Ham's F-12 nutrient mixture (Invitrogen) containing 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA), 50 μg/ml gentamicin, 60 μg/ml benzyl penicillin and 100 μg/ml streptomycin (Sigma-Aldrich), and incubated in a 5% CO_2 humidified atmosphere at 37 °C. Cells were plated in 35 mm dishes at 1.2 million cells per well for amino acid uptake or at 2.75 million per well for RNA and protein analyses. Following 18 h of incubation, attached PHT cells were washed twice with warmed Dulbecco's PBS and culture media was changed daily.

At 66 h (total culture time), PHT cells were treated with increasing concentrations of 1,25-dihydroxy vitamin D_3 (0, 0.1, 1 and 10 nM, Sigma-Aldrich) in culture media containing 1% FBS. All experiments were terminated at 90 h of culture. At this time, cell lysates were processed for RNA extraction or protein lysates, and amino acid uptake or cell viability assays were performed. The viability and differentiation of PHTs following any treatment was determined by daily human chorionic gonadotropin (hCG) secretion in the culture media (from 18 to 90 h of culture) and hCG secretion was not altered by any treatment (Supplemental Fig. 1). In addition, syncytin protein expression increased over the culture period (18–90 h, Supplemental Fig. 2), confirming syncytialization.

2.3. Small interfering RNA (siRNA) transfection

After 18 h of culture, PHT cells were transfected with 100 nM siRNA targeting VDR (1,25-dihydroxy vitamin D_3 receptor, ThermoFisher, AM51331) or non-targeting Scrambled (Scr) siRNA (SIC001, Sigma-Aldrich) using Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol and as previously reported (Aye et al., 2015).

2.4. Measurement of amino acid transport activity

System A and L amino acid transport activity were determined by measuring Na^+-dependent uptake of ^{14}C-methyl-amino-isobutyric acid (MeAIB) and 2-amino-2-norbornane-carboxylic acid (BCH)-inhibitable uptake of ^{3}H-leucine (Leu), respectively, as previously described (Roos et al., 2009). Following treatment of PHT cells as indicated above, cells plated in triplicate were washed 3 times with 4 ml 37 °C Tyrode's salt solution with or without Na^+ (iso-osmotic choline replacement) and then incubated with Tyrode's salt solution (Na^+ or Na^+-free with addition of 1 mM BCH) containing ^{14}C-MeAIB (final concentration 20 μM) and ^{3}H-Leu (final concentration 12.5 nM) for 8 min, a time point on the initial linear phase of uptake (Rosario et al., 2013). The uptake was terminated by washing cells 3 times with ice-cold Na^+ free Tyrode's salt solution. Cells were then lysed for 2 h in distilled water and the water was counted in a liquid scintillation counter. Protein content of lysed cells was determined using the Lowry method (Lowry et al., 1951). Transporter-mediated uptakes were calculated by subtracting uptake in Na^+-free/BCH buffer (non-mediated uptake) from uptake in Na^+-containing buffer (total uptake) and transport activity is expressed as pmol per mg of protein per minute (pmol/mg/min).

2.5. Reverse transcription and quantitative polymerase chain reaction (Q-PCR)

Extraction of total RNA was performed using TRIzol Reagent (Thermo Fisher Scientific) and followed by cDNA synthesis using the High-Capacity RNA-to-cDNA kit (Thermo Fisher Scientific). Q-PCR for SNAT1, SNAT2, SNAT4, LAT1, LAT2, SDHA and TBP was performed in triplicate with 0.2 μg of total RNA reverse transcribed into cDNA using SYBR Select Master Mix (Thermo Fisher Scientific). PCR amplification and detection were performed on a QuantStudio 6 Flex Real-Time PCR system (Thermo Fisher Scientific) using the primers as shown in supplemental table. Amplification of a single product was confirmed by melting curve analysis. The amplified transcripts were quantified using the relative standard curve method and normalized to the geometric mean of SDHA and TBP.

2.6. Western blot analyses

Cells were harvested in radioimmunoprecipitation (RIPA) buffer (50 mM Tris HCl, pH 7.4; 150 mM NaCl; 0.1% SDS; 0.5% Na-deoxycholate and 1% Triton X100) containing protease inhibitors and phosphatase inhibitor cocktail 1 and 2 (1:100, Sigma-Aldrich). Protein concentrations were determined using the bicinchoninic acid assay, as per manufacturer's instructions using bovine serum albumin as the standard (Thermo Fisher).

Protein (2 μg total protein/well) was separated on Any KD Mini-

Download English Version:

https://daneshyari.com/en/article/5534084

Download Persian Version:

https://daneshyari.com/article/5534084

Daneshyari.com