

OBSTETRICS

Placental expression of aminopeptidase-Q (laeverin) and its role in the pathophysiology of preeclampsia

Mona Nystad, MSc; Vasilis Sitras, MD, PhD; Merethe Larsen, MSc; Ganesh Acharya, MD, PhD

OBJECTIVE: The purpose of this study was to investigate the expression and subcellular localization of laeverin, a placenta-specific membrane-bound aminopeptidase, in preeclamptic placentas and its role in trophoblast cell migration and invasion.

STUDY DESIGN: Expression of laeverin was investigated in 6 normal and 6 preeclamptic placentas with the use of immunofluorescence, sodium dodecylsulfate-polyacrylamide gel electrophoresis with Western blot analysis and immunoelectron microscopy. The role of laeverin in trophoblast migration and invasion was studied with the use of the xCelligence system and Boyden chambers with Matrigel in HTR-8/SVneo cells. The effect of laeverin gene-silencing on selected genes that are involved in cell transformation and tumorigenesis was evaluated by polymerase chain reaction array. The Student *t* test, Mann-Whitney *U* test, χ^2 test, or F-test was used to compare groups as appropriate.

RESULTS: Laeverin was expressed in the cell membrane of villous trophoblasts in third-trimester healthy placentas; in preeclamptic placentas, it was expressed ectopically in the cytoplasm, especially in

microvesicles. Immunoelectron microscopy showed laeverin leakage into the fetal capillaries and abundant expression in microvesicles in preeclamptic placentas. Migration and invasion of HTR-8/SVneo cells were reduced by 11.5% ($P = .023$) and 56.7% ($P = .001$), respectively, by laeverin gene-silencing. Analysis of downstream pathways affected by laeverin-silencing demonstrated significant down-regulation of integrin A2 (39-fold), integrin B3 (5-fold), and matrix metalloprotease 1 (36-fold).

CONCLUSION: Expression of laeverin protein is altered in preeclamptic placentas. Its ectopic expression in the cytoplasm and microvesicles, rather than the cell membrane and leakage into the fetal capillaries, may have a role in the pathophysiologic condition of preeclampsia. Laeverin gene appears to be involved in trophoblast cell migration and invasion through interaction with integrins and matrix metalloprotease 1.

Key words: aminopeptidase, laeverin, microvesicle, placenta, preeclampsia

Cite this article as: Nystad M, Sitras V, Larsen M, et al. Placental expression of aminopeptidase-Q (laeverin) and its role in the pathophysiology of preeclampsia. *Am J Obstet Gynecol* 2014;211:686.e1-31.

Preeclampsia complicates 5-10% of pregnancies and is a major cause of maternal mortality worldwide.¹ Although it is clearly a placenta-specific disorder, its pathogenesis is not understood fully. Therefore, its prediction, timely diagnosis, and appropriate management remain challenging.

Laeverin, a membrane-bound aminopeptidase, was first reported to be expressed by human trophoblast cells

in 2004 by Fujiwara et al² and has been suggested to cooperate with the chemokine system in the regulation of human placentation.³ The same group recently presented some molecular evidence suggesting that laeverin is important for extravillous trophoblast invasion.⁴ Laeverin is a trophoblast-specific protein; however, it has been reported to be expressed in other tissues in some inflammatory diseases, such as

rheumatoid arthritis.⁵ In a previous study, comparing global placental gene expression profile between preeclamptic and healthy pregnancies, we found 16 genes that were able to predict preeclampsia phenotype in our study population.⁶ Laeverin was among those genes, and it was up-regulated significantly in the preeclamptic placentas. Therefore, we hypothesized that the deregulation of laeverin protein may lead

From the Department of Clinical Medicine, Women's Health and Perinatology Research Group, Faculty of Health Sciences, University of Tromsø, Norway (Ms Nystad and Drs Sitras and Acharya), and Departments of Obstetrics and Gynecology (Ms Nystad and Drs Sitras and Acharya) and Occupational and Environmental Medicine (Ms Larsen), University Hospital of North Norway, Tromsø, Norway; Department of Obstetrics, Oslo University Hospital—Rikshospitalet, Oslo, Norway (Dr Sitras); and Department of Clinical Sciences, Intervention, and Technology, Karolinska Institute, Stockholm, Sweden (Dr Acharya).

Received March 14, 2014; revised May 26, 2014; accepted June 18, 2014.

Supported by Northern Norway Regional Health Authority grant numbers 12032 and 12101 and by the Division of Child and Adolescent Health, University Hospital of North Norway, through its 2012 Research Fund.

The authors report no conflict of interest.

Corresponding author: Mona Nystad, MSc. mona.nystad@unn.no

0002-9378/\$36.00 • © 2014 Elsevier Inc. All rights reserved. • <http://dx.doi.org/10.1016/j.ajog.2014.06.047>

to abnormal trophoblast function and have a role in the pathophysiologic condition of preeclampsia.

In the present study, we investigated the expression of laeverin protein and its subcellular localization in healthy and preeclamptic placentas. Additionally, we investigated the role of laeverin in trophoblast cell migration and invasion.

MATERIALS AND METHODS

The study was approved by the Regional Committee for Medical and Health Research Ethics-North Norway (REK Nord reference no. 2010/2058-4), and informed written consent was obtained from all the participants.

Placental samples from a total of 12 pregnant white European women (6 healthy and 6 with severe preeclampsia) were collected. They were matched for maternal age, parity, onset of labor (spontaneous or induced), and the mode of delivery (vaginal or cesarean), and the results were compared. *Severe preeclampsia* was defined as blood pressure $\geq 160/110$ mm Hg and proteinuria ≥ 300 mg/24-hour urine or $\geq 2+$ in spot urine after 20 weeks of gestation in previously normotensive women.⁷ Women with preexisting medical conditions that may have affected the course and outcome of pregnancy were excluded. Doppler ultrasonography was performed at ≤ 48 hours before delivery in each case to assess uteroplacental and umbilical circulation.

Collection of maternal blood and placental samples

Maternal venous blood samples were taken at ≤ 48 hours before delivery for the analysis of hemoglobin, hematocrit, liver function, and renal function. None of the women were in labor when blood samples were taken. Placental tissue samples were obtained immediately after delivery, as described previously.⁶

Cell line

The immortalized HTR-8/SVneo trophoblast cell line, which was obtained from primary cultures of human trophoblast cells,⁸ was used for migration, invasion, and polymerase chain reaction (PCR) array studies. Cells were maintained in RPMI Medium 1640

supplemented with 5% fetal bovine serum (FBS; GIBCO, Invitrogen, Carlsbad, CA) in a 37°C water-jacketed incubator (Forma Scientific, Marietta, OH) with 5% CO₂. Trypsin-ethylenediamine tetraacetic acid (Sigma Chemical Co, St. Louis, MO) was used for harvesting and for the subculturing of cells.

Laeverin antibodies

Polyclonal antibodies against laeverin were raised (Eurogentec, Seraing, Belgium). Rabbits were immunized with synthetic oligo-peptides that contained 2 predicted epitopes (EP073418:CRV-HANLQTIKNENLK and EP073419: CERAEVGRPLSPGTG). Peptide sequences for these epitopes were chosen from the amino acid sequence of laeverin (Q0P5U8; <http://www.ncbi.nlm.nih.gov/protein/121946569>). Immunogenic epitopes of the exposed amino acids of the laeverin 3-dimensional structure were chosen for peptide synthesis with software that was provided by Sigma Chemical Co.

A commercially available goat polyclonal antibody of laeverin was used as control (Santa Cruz Biotechnology Inc, Santa Cruz, CA).

Immunofluorescence

Tissue samples from 3 preeclamptic placentas and 3 normal healthy controls were fixed in formalin, embedded in paraffin blocks, cut (4–6 μ sections), and mounted on glass slides. Immunofluorescence cell staining was performed⁹ with our laeverin antibody (2.1 μ g/mL) and secondary goat anti-rabbit immunoglobulin G-fluorescein isothiocyanate (2.5 μ g/mL; Santa Cruz Biotechnology Inc). Slides were counterstained with DAPI (4',6-diamidino-2-phenylindole) II (Vysis; Abbott Diagnostics, Lake Forest, IL). Images were obtained with CytoVision digital system (Applied Imaging, Grand Rapids, MI) that was equipped with a charge-coupled device camera (Cohu Inc, Poway, CA). A total of ≥ 200 cells were inspected on each slide. Experiments were run in triplicate.

Protein isolation

Placental tissue was cut in small pieces, and proteins were isolated with the

use of T-PER (Pierce Chemical Co, Rockford, IL) with Complete Mini ethylenediamine tetraacetic acid-free protease inhibitor cocktail in combination MagNA Lyser Green Beads for homogenizing on MagNA Lyser (Roche, Indianapolis, IN). Protein concentration was measured with the use of the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) in a ThermoMax Microplate Reader (Molecular Devices, Downingtown, PA).

Sodium dodecylsulfate-polyacrylamide gel electrophoresis and Western blot analysis

Reduced and denatured proteins (5 μ g) that had been isolated from 8 placentas (4 preeclamptic and 4 normal) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 4–12% NuPAGE (Invitrogen). Electrophoresis and blotting (polyvinylidene difluoride nylon membrane, pore size 0.45 μ m; Invitrogen) were run on Novex Mini Cell XCell Sure Lock (Invitrogen). Blots were cut under a 49-kDa protein band to provide 2 blots; 1 for laeverin and another for the housekeeping protein actin. Labeling was done with primary antibodies against laeverin (our antibody [0.42 μ g/mL] and commercial antibody [1 μ g/mL; Santa Cruz Biotechnology Inc] or actin [1 μ g/mL; Santa Cruz Biotechnology Inc]). Detection was performed with goat anti-mouse immunoglobulin G-alkaline phosphatase-conjugated antibody (0.2 μ g/mL; Santa Cruz Biotechnology Inc) and CDP-Star (Roche). Pictures were taken on ImageQuant LAS 4000 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Experiments were run in triplicate.

Immunoelectron microscopy

Immunoelectron microscopy was performed on ultrathin tissue sections of 2 healthy placentas and 2 placentas that were obtained from women with severe preeclampsia. All experiments were run in triplicates.

Fresh placental tissue samples were dissected, mounted in membrane carriers, and frozen at high pressure (EMPACT 2 HPF; Leica Microsystems,

Download English Version:

<https://daneshyari.com/en/article/6145417>

Download Persian Version:

<https://daneshyari.com/article/6145417>

[Daneshyari.com](https://daneshyari.com)