DOI 10.1007/s10517-019-04352-z
Bulletin of Experimental Biology and Medicine, Vol. 166, No. 3, January, 2019 GENETICS

Oxyquinoline-Dependent Changes in Claudin-Encoding Genes Contribute to Impairment of the Barrier Function of the Trophoblast Monolayer

E. N. Knyazev[1], V. A. Petrov[1], I. N. Gazizov[1], T. N. Gerasimenko[1], I. M. Tsypina[1,2], A. G. Tonevitsky[1,2], and G. T. Sukhikh[3]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 166, No. 9, pp. 342-346, September, 2018
Original article submitted July 26, 2018

Natural response to hypoxia critically depends on rapid stabilization of hypoxia-inducible factor (HIF). Under normoxic conditions, HIF-prolyl hydroxylases mark α-subunits of HIF for degradation, while hypoxia results in stabilization of HIF-α. Oxyquinoline derivatives suppress activity of HIF-prolyl hydroxylases leading to HIF activation in the cell. Here we show that 24-h incubation of BeWo b30 choriocarcinoma cells (a model of trophoblast in the placental barrier) with oxyquinoline derivative leads to a decrease in transepithelial electrical resistance (TEER) of the cell monolayer, while the permeability of the monolayer for FITC-dextran (70 kDa) remains unchanged. These findings suggest that the overall barrier function is preserved, while the structure of intercellular tight junctions can undergo minor changes. Using Affymetrix Human Transcriptome Array 2.0, we showed that the treatment with oxyquinoline derivative was followed by a decrease in the expression of claudins 6 and 7 (*CLDN6*, *CLDN7*), occludin (*OCLN*), contact adhesion molecule 3 (*JAM3*), and angiomotin-like protein 1 (*AMOTL1*).

Key Words: *BeWo b30; placenta; hypoxia; oxyquinoline; barrier*

Trophoblast is a critical structure of the placental barrier that takes part in the transfer of various substances between the circulations of the mother and developing fetus. Damage or loss of this barrier function is observed in preeclampsia and other pregnancy complications [15]. Trophoblast cells in the placental barrier are joined together by tight junctions consisting of transmembrane and additional membrane-associated proteins. Among transmembrane proteins, claudins and occludin forming homodimeric bridges between the neighboring cells should be noted. Tight junctions play a role of a barrier for ions and molecules regulating cell—cell transport between the apical and basolateral space of the cells [11]. Tight junctions are the main barrier in epithelial tissues; they support the electroosmotic gradient and provide electrical resistance of the cell barrier. Relatively high expression of many tight junction components, including ZO1 proteins and CLDN1, 4, 5, 16 claudins was detected in the placental trophoblast [15].

Normal functioning of the placenta depends on tight junctions. Defects in their structure contribute to abnormal placental permeability accompanying various pathological conditions, *e.g.* preeclampsia. During preeclampsia, a decrease in the expression of a number of claudin proteins was found in the placenta. In the cell model of the placenta consisting of trophoblast cells, violation of barrier permeability was also observed under hypoxic conditions simulating preeclampsia [15].

Oxygen level is a critical factor for the development of the placenta and maintenance of normal pregnancy. Hypoxia of the fetoplacental barrier is a typical

[1]BioClinicum Research Center; [2]National Research University Higher School of Economics; [3]V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology, and Perinatology, Moscow, Russia. ***Address for correspondence:*** tonevitsky@mail.ru. A. G. Tonevitsky

pathological characteristic of preeclampsia. The pathogenesis of preeclampsia is associated, among other things, with the increase in the level of HIF-1α factor associated with hypoxia leading to abnormalities in the placental barrier. However, the mechanisms underlying impairment of the permeability caused by hypoxia remain unclear. Studies of the placental barrier have demonstrated the role of hypoxia in modulation of the expression of tight-junction proteins mediated by activation of transcription factor HIF-1 (under conditions of hypoxia modeled by cobalt chloride(II) application) [15]. We assumed that a similar link can be observed in BeWo b30 cells simulating placental trophoblast after chemical induction of the response to hypoxia modeled by treatment with oxyquinoline derivatives.

It have been previously found that oxyquinoline derivatives with a branched tail can mimic hypoxic response in cells by inhibiting HIF-prolyl hydroxylases and stabilizing the HIF-1 complex [7,8].

Here we studied the effect of oxyquinoline derivative on the BeWo b30 cell monolayer as a model of placental trophoblast, including changes in transepithelial electrical resistance and permeability for labeled substrates, and modification of the expression profile of genes encoding tight-junction components.

MATERIALS AND METHODS

A clone of BeWo b30 choriocarcinoma cells was obtained from Prof. Christiana Albrecht (University of Bern, Switzerland) with permission of Dr. Alan Schwartz (Washington University in St. Louis, USA). The cells were cultured in standard 75 cm^2 culture flasks in DMEM with high glucose content (4.5 g/liter), without L-glutamine and pyruvate (Gibco) supplemented with 10% One Shot fetal calf serum (Gibco), 2 mM sterile L-glutamine (PanEco), 1% MEM Non-essential Amino Acid Solution 100× (Gibco) and penicillin/streptomycin solution (Gibco) in final concentrations of 100 U/ml and 100 μg/ml, respectively, at 37°C and 5% CO_2 in a cell culture CO_2 incubator. The cells were subcultured every 2-3 days.

For studying the effect of hypoxia induction, BeWo b30 cells were seeded into 96 well plates with Transwell membrane inserts (0.143 cm^2, 30,000 cells per insert, ~2.1×10^5 cells/cm^2). The medium was daily replaced in recommended volumes after measuring transepithelial electrical resistance (TEER) with an EVOM2 instrument and a STX100C96 electrode (World Precision Instruments). In 42 h after cell seeding in Transwell inserts, when the cell monolayer reached confluence, the medium in the upper and lower chambers of the Transwell inserts was changed to a fresh portion containing oxyquinoline derivative (hereinafter oxyquinoline) in DMSO; the final concentration of oxyquinoline in the medium was 10 μM (1.5 μl of the solution in DMSO per 75 μl medium). In the control, the cells were incubated in a medium with 1.5 μl DMSO per 75 μl medium (without oxyquinoline). TEER was measured in 6- and 24-h incubation with oxyquinoline.

After 24-h incubation with oxyquinoline, the permeability of the monolayer for FITC-dextran with a molecular weight of kDa was analyzed. To this end, the medium in the upper and lower chambers of the Transwell system was changed (75 and 235 μl, respectively); the medium in the upper chamber contained 1 g/liter FITC-dextran with a molecular weight of 70 kDa. In 2 h, the medium was sampled and the level of FITC-dextran was analyzed using SpectraMax i3 plate reader (Molecular Devices) at λ_{ex}=485 and λ_{em}=535 nm. The concentration of FITC-dextran was calculated using a calibration curve. The permeability was expressed as the percentage of the substance penetrated through BeWo b30 monolayer within 2 h.

After measuring monolayer permeability for FITC-dextran, the cells were lysed in Qiazol lysis buffer (Qiagen) supplemented with 15 mg/ml GlycoBlue Coprecipitant (Thermo Fisher Scientific) to a final concentration of 50 ng/μl in order to increase the total RNA yield when isolated by a miRNeasy Micro Kit (Qiagen) according to a protocol for a small expected amount of RNA [1,4,13] with intermediate processing of the column with DNase from RNase-Free DNase I Set (Qiagen). RNA concentration was measured on a NanoDrop ND-1000 instrument (Thermo Fisher Scientific) [9]. The quality of the isolated RNA was assessed on a Bioanalyzer 2100 using RNA 6000 Nano Kit (Agilent), all isolated RNA samples had RIN quality score >9.5.

The isolated total RNA (500 ng) was used for transcriptome analysis using Human Transcriptome Array 2.0 microarrays (Affymetrix) [5,12]. Three microarrays with biological replicates of the experiments with cell exposure to oxyquinoline+ DMSO or DMSO alone for 24 h where processed. The comparison of gene expression was carried out by the Transcriptome Analysis Console version 4.0.1.36 (Thermo Fisher Scientific) using eBayes statistical method. It was assumed that significant changes in expression should be no less than 1.5 times and FDR corrected *p*-value should be <0.05. The distribution of TEER values was tested for normality using the Kolmogorov—Smirnov method. The distribution did not differ significantly from the normal distribution; therefore, standard Student's *t* test was used to compare TEER values.

RESULTS

In 42 h after the start of the experiment with BeWo b30 cells on Transwell inserts, TEER values reached

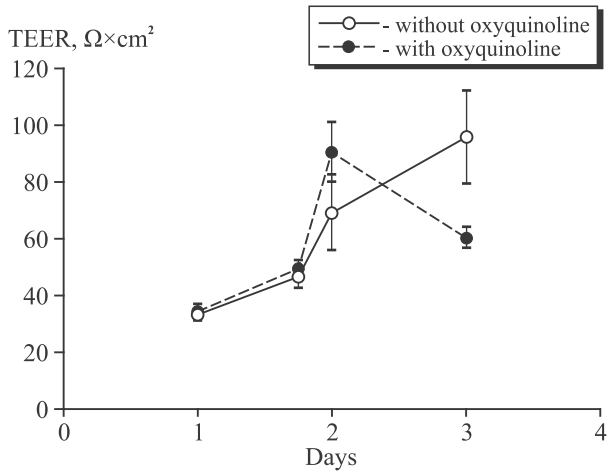

Fig. 1. Changes in TEER of BeWo b30 cells during incubation with and without oxyquinoline.

about 50 Ω×cm², which indicates the achievement of the confluent state. In BeWo b30 cell monolayer incubated in the presence of 10 μM oxyquinoline, TEER increased in comparison with the control (p<0.01) over the first 6 h, but after 24 h this parameter significantly decreases (p<0.01, Fig. 1).

The decrease in TEER values reflected an increase in cell monolayer permeability charged molecules that provide electrical current between the electrodes of the TEER measuring device. These changes can be explained by modification tight junction configuration with the preservation of the barrier function for large molecules, but they can also be associated with cell death and destruction of tight junctions with the loss of the barrier function. In order to determine the cause of the decrease in TEER under the action of the oxyquinoline derivative, an experiment was performed with an analysis of the monolayer permeability for FITC-dextran with a molecular weight of 70 kDa. Cell monolayer both under control conditions and after 24-h incubation with oxyquinoline demonstrated similar permeability for large molecules such as FITC-dextran, which indicates the preservation of tight junctions between the cells: passage of FITC-dextran with a molecular weight of 70 kDa from the upper to the lower chamber of Transwell through the monolayer of BeWo b30 cells exposed and not exposed to oxyquinoline was 1.9±0.8 and 1.9±1.6%, respectively, over 2 h. This fact suggests that changes in TEER are determined by minor changes in the structure of tight junctions. Transcriptomic analysis of BeWo b30 cells was performed both after the incubation with oxyquinoline and under control conditions to determine the changes in the expression of the genes involved in the formation of tight junctions.

A decrease in the expression of a number of proteins of cell—cell junction was noted in BeWo b30 cells exposed to oxyquinoline, in particular, the expression of claudins 6 and 7 decreased by 1.7 times and expression of occludin increased by 1.5 times. It was previously demonstrated that claudins 1, 3, 4, and 5 are expressed in BeWo cells, while the formation of syncytiotrophoblast under the influence of forskolin is associated with a slight decrease in the expression of claudin 5 [2]. The expression of occludin and claudin-7 was detected on the lateral membranes of amniotic epithelial cells during gestation week 35, and by week 37, the expression was mainly detected in the cytoplasm, which can be due to an increase in the level of glucocorticoids in the amniotic fluid [6]. The expression of claudin 7 was detected in the luminal and glandular cells of mouse endometrium during the preimplantation period, whereas during invasion of trophoblast cells, the expression of this claudin was not detected, which can indicate destruction of tight junctions during the trophoblast invasion [10].

Moreover, in BeWo b30 cells exposed to oxyquinoline, a pronounced decrease (by 3.2 times) in the expression of *JAM3* gene encoding the protein involved in the formation of tight junctions was observed. Previously, it has been shown that expression of this protein is typical of the placenta. The expression of another protein of this family, JAM2, in mouse placental structures was studied in detail; together with JAM3, this protein participates in the formation of tight junctions in some layers of mouse trophoblast, although the expression of these genes is more typical of endothelial cells [14]. The observed changes in the expression of JAM2 in BeWo b30 cells under conditions of modeled hypoxia was not previously reported in trophoblast studies.

We detected a 1.6-fold decrease in the expression of *AMOTL1* gene encoding angiomotin-like protein 1; the function of this protein in trophoblast cells has not previously been studied. This protein is a component of tight junctions participating in pericellular permeability and maintenance of cell polarity. Moreover, AMOTL1 is involved in the regulation of actin cytoskeleton, adhesion and migration of cells, and maintenance of cell polarity in the epithelial layers [3]. The decrease in its expression can also contribute to TEER decrease upon HIF-1 activation in BeWo b30 cells exposed to oxyquinoline.

Thus, chemical induction of the hypoxic response in BeWo b30 cells achieved by 24-h incubation with oxyquinoline derivative leads to TEER decrease in comparison with the control, but this does not result in an increase in permeability of the monolayer for large FITC-dextran molecules (70 kDa). These observations can indicate reorganization of tight junctions without any loss of barrier properties of the monolayer. The analysis of the expression of tight-junction genes revealed a decrease in the expression of genes encoding

claudins 6 and 7 (*CLDN6*, *CLDN7*), occludin (*OCLN*), contact adhesion molecule 3 (*JAM3*) and angiomotin-like protein 1 (*AMOTL1*), which can explain the observed TEER changes in response to oxyquinoline.

The study was supported by the Federal Targeted Programme for Research and Development in Priority Areas of Advancement of the Russian Scientific and Technological Complex for 2014-2020 (RFMEFI58817X0007).

REFERENCES

1. Fomicheva KA, Osip'yants AI, Knyazev EN, Samatov TR, Shkurnikov MY. Detection of potential Metastatic prostate cancer circulating biomarkers by comparison of miRNA profiles in DU145 cells and culture medium. Bull. Exp. Biol. Med. 2017;162(6):792-796.
2. Harada M, Kondoh M, Masuyama A, Fujii M, Nakanishi T, Utoguchi N, Yagi K, Watanabe Y. Effect of forskolin on the expression of claudin-5 in human trophoblast BeWo cells. Pharmazie. 2007;62(4):291-294.
3. Huang T, Zhou Y, Zhang J, Cheng ASL, Yu J, To KF, Kang W. The physiological role of Motin family and its dysregulation in tumorigenesis. J. Transl. Med. 2018;16(1):98.
4. Knyazev EN, Fomicheva KA, Mikhailenko DS, Nyushko KM, Samatov TR, Alekseev BY, Shkurnikov M.Y. Plasma levels of hsa-miR-619-5p and hsa-miR-1184 differ in prostatic benign hyperplasia and cancer. Bull. Exp. Biol. Med. 2016;161(1):108-111.
5. Knyazev EN, Nyushko KM, Alekseev BY, Samatov TR, Shkurnikov MY. Suppression of ITGB4 gene expression in PC-3 cells with short interfering RNA induces changes in the expression of β-integrins associated with RGD-receptors. Bull. Exp. Biol. Med. 2015;159(4):541-545.
6. Kobayashi K, Kadohira I, Tanaka M, Yoshimura Y, Ikeda K, Yasui M. Expression and distribution of tight junction proteins in human amnion during late pregnancy. Placenta. 2010; 31(2):158-162.
7. Osipyants AI, Smirnova NA, Khristichenko AY, Hushpulian DM, Nikulin SV, Chubar TA, Zakhariants AA, Tishkov VI, Gazaryan IG, Poloznikov AA. Enzyme-substrate reporters for evaluation of substrate specificity of HIF prolyl hydroxylase isoforms. Biochemistry (Mosc). 2017;82(10):1207-1214.
8. Poloznikov AA, Zakhariants AA, Nikulin SV, Smirnova NA, Hushpulian DM, Gaisina IN, Tonevitsky AG, Tishkov VI, Gazaryan IG. Structure-activity relationship for branched oxyquinoline HIF activators: Effect of modifications to phenylacetamide "tail". Biochimie. 2017;133:74-79.
9. Rudimov EG, Knjazev EN, Khaustova NA, Grigorieva OV, Buravkova LB. Transcriptomic changes in human umbilical cord blood endothelial cells under simulated microgravity. Dokl. Biochem. Biophys. 2017;472(1):1-4.
10. Schumann S, Buck VU, Classen-Linke I, Wennemuth G, Grümmer R. Claudin-3, claudin-7, and claudin-10 show different distribution patterns during decidualization and trophoblast invasion in mouse and human. Histochem. Cell Biol. 2015;144(6):571-585.
11. Shen L. Tight junctions on the move: molecular mechanisms for epithelial barrier regulation. Ann. N.Y. Acad. Sci. 2012;1258:9-18.
12. Shkurnikov MY, Knyazev EN, Wicklein D, Schumacher U, Samatov TR, Tonevitskii AG. Role of L1CAM in the Regulation of the canonical Wnt pathway and class I MAGE genes. Bull. Exp. Biol. Med. 2016;160(6):807-810.
13. Skurnikov MY, Makarova YA, Knyazev EN, Fomicheva KA, Nyushko KM, Saribekyan EK, Alekseev BY, Kaprin AD. Profile of microRNA in blood plasma of healthy humans. Bull. Exp. Biol. Med. 2016;160(5):632-634.
14. Stelzer IA, Mori M, DeMayo F, Lydon J, Arck PC, Solano ME. Differential mouse-strain specific expression of Junctional Adhesion Molecule (JAM)-B in placental structures. Cell Adh. Migr. 2016;10(1-2):2-17.
15. Zhang Y, Zhao HJ, Xia XR, Diao FY, Ma X, Wang J, Gao L, Liu J, Gao C, Cui YG, Liu JY. Hypoxia-induced and HIF1a-VEGF-mediated tight junction dysfunction in choriocarcinoma cells: Implications for preeclampsia. Clin. Chim. Acta. 2017. Dec 6. pii: S0009-8981(17)30495-3. doi: 10.1016/j.cca. 2017.12.010.