SHORT COMMUNICATION

Reduced Expression of Hydrogen Sulfide—Generating Enzymes Down-Regulates 15-Hydroxyprostaglandin Dehydrogenase in Chorion during Term and Preterm Labor

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Chorionic NAD-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) plays a pivotal role in controlling the amount of prostaglandins in the uterus and has been implicated in the process of labor. Prior studies identified hydrogen sulfide—generating enzymes cystathionine- β -synthetase (CBS) and cystathionine- γ -lyase (CSE) in fetal membranes. We investigated whether hydrogen sulfide is involved in the regulation of PGDH expression in the chorion during labor. The chorionic tissues were obtained from pregnant women at preterm in labor and at term in labor or not in labor at term. Levels of CSE and CBS and hydrogen sulfide production rate were down-regulated in term in labor and preterm in labor groups compared with not in labor at term group. The CBS level correlated to PGDH expression in the chorion. Hydrogen sulfide donor NaHS and precursor L-cysteine dose-dependently stimulated PGDH expression and activity in cultured chorionic trophoblasts. The effect of L-cysteine was blocked by CBS inhibitor and CBS siRNA but not by CSE inhibitor and CSE siRNA. Hydrogen sulfide treatment suppressed miR-26b and miR-199a expression in chorionic trophoblasts. miR-26b and miR-199a mimics blocked hydrogen sulfide upregulation of PGDH expression. Our results indicate that hydrogen sulfide plays pivotal roles in maintenance of PGDH expression in the chorion during human pregnancy. Reduced expression of hydrogen sulfide—generating enzymes contributes to an increased amount of prostaglandins in the uterus during labor. (*Am J Pathol* 2018, 188: 63–71; <https://doi.org/10.1016/j.ajpath.2017.09.006>)

Prostaglandins (PGs) play important roles in all of the physiological processes of parturition, including ripening of the cervix, membrane rupture, and induction of uterine contraction.^{1–3} Elevated production of PGs by gestational tissues contributes to the initiation of parturition in both of term and preterm birth.^{1–5} Thus, the regulatory mechanisms governing PG synthesis and metabolism in gestational tissues become of considerable interest.

PGs are formed from arachidonic acid by the action of PG H synthase.^{3–5} PG metabolism starts in oxidation of the 15-hydroxyl group by the NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH).^{6,7} This reaction inactivates PGs to form 15-keto metabolites, which constitutes the key step in PG metabolism. In fetal

membranes, the chorion laeve, situated between the amnion and the decidua, not only produces PGs, but also controls the intrauterine concentration of active PGs by its high PGDH activity.^{8,9} Several studies have demonstrated that the expression and activity of PGDH are significantly down-regulated in the chorion with term labor.^{9–13} In infection-associated preterm labor, a further decrease in enzyme activity and protein expression of PGDH was reported.^{11,13}

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However, the mechanisms of regulation of PGDH in the chorion remain largely unknown.

Hydrogen sulfide, one of the important endogenous gaseous signaling transmitters, has been implicated in many physiological and pathologic processes, including vasodilation,¹⁴ angiogenesis,¹⁵ and inflammation.¹⁶ Hydrogen sulfide is synthesized from L-cysteine mainly through the activity of the enzymes cystathionine- γ -lyase (CSE; enzyme system number 4.4.1.1) and cystathionine- β -synthetase (CBS; enzyme system number 4.2.1.22).¹⁷ Several studies have demonstrated that human gestational tissues, including placenta, fetal membrane, and myometrium, express hydrogen sulfide-generating enzymes CBS and CSE.^{18,19} Recently, we found that hydrogen sulfide suppresses contractions in human myometrial strips *in vitro*²⁰ and prolongs lipopolysaccharide-induced preterm birth via inhibition of inflammation in intrauterine tissues,²¹ which suggests that hydrogen sulfide is involved in the labor process. However, the mechanism underlying hydrogen sulfide regulation of pregnancy and labor remains largely unknown.

Although CBS and CSE protein has been identified in human and rat fetal membranes,¹⁸ the localization of CSE and CBS in fetal membranes and whether hydrogen sulfide modulates PG metabolism in fetal membrane have not been investigated. Herein, we determined the localization of CBS and CSE in the human chorion and expression levels of these proteins during labor at term and preterm and explored the effects of hydrogen sulfide on PGDH expression and the possible mechanisms in a model of cultured chorionic trophoblasts.

Materials and Methods

Tissue Collection

The present study was approved by the specialty committee on ethics of biomedicine research, Second Military Medical University (Shanghai, China). Human fetal membranes were collected from the following groups of women with singleton pregnancy: term no labor (TNL; $n = 24$), term labor (TL; $n = 24$), and preterm labor (PTL; $n = 21$). Written informed consent was obtained from all patients. TNL tissues were collected at elective cesarean section. The fetal membranes of TL and PTL were collected from women with uncomplicated pregnancies, who went into natural labor and delivered vaginally. The patients who had evidence of underlying disease (eg, hypertension, diabetes, preeclampsia, or intrauterine growth restriction) were not included in this study.

The chorion laeve was separated, and the uniform removal of decidual tissue was monitored by using an inverted phase contrast microscope. Chorion tissues were snap frozen in liquid nitrogen and stored at -80°C .

Immunohistochemistry

Paraffin sections (5 μm thick) were cut, rehydrated, and microwaved in citric acid buffer to retrieve antigens. After inhibition of endogenous peroxidases with 3% H_2O_2 , unspecific

antibody binding was blocked with 10% rabbit serum. The sections were then incubated with specific antibodies against human CSE (sc101924; Santa Cruz Biotechnology, Dallas, TX) and CBS (sc46830; Santa Cruz Biotechnology) overnight at 4°C . The bound antibodies were detected with the biotin-streptavidin-peroxidase system (UltraSensitive-SP-kit; MaiXin Biotechnology, Fuzhou, China) using diaminobenzidine (Sigma-Aldrich, St. Louis, MO) as chromogen. Counterstaining was performed with hematoxylin. Negative controls were performed by substituting primary antibody with IgG.

Chorionic Trophoblast Culture

Chorionic trophoblasts were isolated and cultured, according to the modified method of Kliman et al,²² as described previously.²³ Briefly, approximately 40 g of fresh chorion obtained from TNL patients was minced and then was dispersed with 0.125% trypsin (Invitrogen Corp., Carlsbad, CA) and 0.02% collagenase (Invitrogen Corp.) in Dulbecco's modified Eagle's medium (Sigma-Aldrich) containing 0.1% bovine serum albumin for three times. A purified fraction of cytotrophoblasts was obtained following Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation. Cells were then seeded into 12-well plates at a density of 1×10^6 /well and grown in phenol red-free Dulbecco's modified Eagle's medium containing 10% fetal calf serum at 37°C in 5% CO_2 -95% air. Cell purity was assessed by immunostaining for cytokeratin and vimentin using primary antibodies (Dako, Inc., Carpinteria, CA), as described previously.²³

After 72 hours of incubation, cells were then treated with various concentrations of NaHS and L-cysteine (Sigma-Aldrich) in the presence or absence of DL-propargylglycine (Sigma-Aldrich) and aminooxyacetic acid hemihydrochloride (Sigma-Aldrich) for 4 to 24 hours. In some cases, the cells were treated with miR-199a and miR-26b mimics for 24 hours. Then, the cells were harvested and stored at -80°C .

Total RNA Extraction and Quantitative Real-Time RT-PCR

Total RNA was prepared from chorionic tissues and cells by using TRIzol reagent (Invitrogen Corp.). RNA (2 μg) was reverse transcribed with oligo(dT)18 primer using the M-MLV Reverse Transcriptase (Promega, Madison, WI) and then stored at -20°C . Specific primers for the amplification of PGDH were as follows: sense, 5'-TTGCACAGCAGCCGG-TTTAT-3'; and antisense, 5'-TTGGCAATCAATGGTGG-GTC-3' (<https://www.ncbi.nlm.nih.gov/nuccore>; accession number NM_000860). Quantitative real-time PCR was performed using Rotor-Gene 3000 (Corbett Research, Mortlake, NSW, Australia). The reaction solution consisted of 2.0 μL diluted cDNA, 0.2 $\mu\text{mol/L}$ of each paired primer, 200 $\mu\text{mol/L}$ deoxynucleotide triphosphates, and $1 \times$ PCR Master Mix (TaKaRa, Otsu, Japan). SYBR Green (BMA, Rockland, ME) was used as detection dye. The annealing temperature was set at 60°C , and amplification cycles were set at 40

cycles. The temperature range to detect the melting temperature of the PCR product was set from 60°C to 95°C. Each sample was normalized on the basis of its β -actin mRNA content. The relative expression of PGDH was determined by using the comparative threshold cycle (C_T) method.²⁴

Total miRNAs of chorionic tissues and cells were extracted using miRcute miRNA Isolation Kit (TIANGEN, Beijing, China), according to the manufacturer's instructions. The first-strand cDNA synthesis of total miRNAs was synthesized using miRcute miRNA First-Strand cDNA Synthesis Kit (TIANGEN). miRNAs were quantified by miRcute miRNA qPCR Detection kit (TIANGEN). Specific primers for the amplification of miR-26b and miR-199a were 5'-UCAA-GUAAUUCAGGAUAGGU-3' (forward) and 5'-CCCA-GUGUUCAGACUACCUGUUC-3' (forward), respectively. Amplification of the housekeeping gene U6 was measured for each sample as an internal control for sample loading and normalization. The temperature range to detect the melting temperature of the PCR product was set from 60°C to 95°C. The comparative C_T method with arithmetic formulas ($2^{-\Delta\Delta C_T}$) was used to determine the relative quantitation of miRNA expression.²⁴

Western Blotting Analysis

Chorionic tissues or cells were homogenized in radio-immunoprecipitation assay lysis buffer containing phosphatase inhibitor cocktail (Roche, Indianapolis, IN). Proteins (50 μ g) were denatured and separated by 12% SDS-PAGE and subsequently transferred to nitrocellulose membranes by electroblotting. The membranes were incubated in blocking buffer, and then with specific antibodies against CBS (Abcam, Cambridge, UK), CSE (Abcam), and PGDH (Cayman Chemical, Ann Arbor, MI) at 4°C overnight; finally, they were incubated with a secondary horseradish peroxidase-conjugated IgG (1:1000). Immunoreactive proteins were visualized using the enhanced chemiluminescence Western blotting detection system (Santa Cruz Biotechnology). The intensities of light-emitting bands were detected and quantified using Sygene Bio Image system (Synoptics Ltd, Cambridge, UK). To control sampling errors, the ratio of band intensities/ β -actin was obtained to quantify the relative protein expression level.

PGDH Activity Assay

PGDH activity was assayed by measuring 13,14-dihydro-15-keto PG F2, the stable metabolite of PG F2. 13,14-Dihydro-15-keto PG F2 content in culture medium was determined by using a commercial 13,14-dihydro-15-keto PG F2 enzyme immunoassay kit (Cayman Chemical), as described previously.²⁵ The standard curve ranged from 1000 to 7.81 pg/mL.

RNA Interferences

The siRNAs for CSE and CBS were designed and synthesized by GenePharma Corp. (Shanghai, China). Control siRNA

was a scrambled sequence without any specific target. The sequences for targeting human CSE and CBS are as follows: CSE sense, 5'-GGCCUUUGCUUCAGGUUUATT-3'; and CBS sense, 5'-CGGAACUACAUGACCAAGUTT-3'. Primary chorionic trophoblasts were transfected with CSE siRNA, CBS siRNA, and control siRNA using Lipofectamine 2000 (Invitrogen Corp.). The chorionic cells were then treated with 10^{-3} mol/L L-cysteine.

Real-Time Hydrogen Sulfide Production Measurement

Approximately 100 mg of placental tissues was placed in phosphate-buffered saline containing protease inhibitor (2 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, and 10 μ g/mL aprotinin) on ice. Tissues were homogenized and centrifuged at 4°C, and supernatants were then collected. Real-time kinetics of hydrogen sulfide production were determined by using a miniaturized hydrogen sulfide microrespiration sensor (model H₂S-MRCh; Unisense, Aarhus, Denmark) coupled to a Unisense PA2000 amplifier.²⁰ Briefly, measurement was made in a temperature-controlled microrespiration chamber (Unisense). After the sensor signals stabilized, L-cysteine (1 mmol/L) and pyridoxal-5'-phosphate (1 mmol/L), a cofactor for generation of hydrogen sulfide, were added. Hydrogen sulfide production rates were then determined at the initial steepest slopes of each trace. In some cases, 10^{-3} mol/L CSE inhibitor DL-propargylglycine and 10^{-3} mol/L CBS inhibitor aminooxyacetic acid hemihydrochloride were added.

Verification of the Targets of miRNAs

The 3'-untranslated region (UTR) fragments of PGDH (600 bp) containing miR-199 and miR-26 binding sites and the mutant PGDH 3'-UTR fragment were inserted into Psi-CHECK2 vector (Promega). Chorionic trophoblasts were cotransfected with the above plasmids in the absence or presence of miR-199a, miR-26a, or miR-26b mimics (GenePharma Corp.) using Lipofectamine 2000. After 24 hours of transfection, both firefly and Renilla luciferase activities were measured using a dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

Statistical Analysis

All of the results are presented as means $\pm$ SEM. In some cases, the results are presented as percentage control $\pm$ SEM for illustrative purposes. All of data were tested for homogeneity of variance by Kolmogorov-Smirnov test. One-way analysis of variance, followed by the Student-Newman-Keuls test, was used to assess significant differences between groups. Pearson correlation was used to examine the correlation between CSE or CSE and PGDH, CBS, or PGDH, and miR-26b or miR-199a. Significance was set at $P < 0.05$.

Figure 1 Localization of cystathionine- γ -lyase (CSE) and cystathionine- β -synthetase (CBS) in human fetal membranes. **A** and **B**: Immunohistochemical analysis shows representative sections for positive staining for CSE (**A**) and CBS (**B**). **C** and **D**: The negative control sections that were obtained using the primary antibodies are pre-absorbed with excess corresponding peptide. **Arrows** indicate positive staining. Original magnification, $\times 200$ (**A–D**). AE, amnion epithelia; CT, chorionic trophoblast; DC, decidua.

Results

Localization of CSE and CBS in Fetal Membranes

Both CSE and CBS were expressed in human fetal membranes (**Figure 1**). The epithelia of amnion and trophoblasts in chorion were positively stained by both CBS and CSE antibodies. The immunoreactivity was abolished when the antibody was preabsorbed with excess CBS or CSE peptide. In addition, positive immunostaining of CSE and CBS was also found in endothelial cells of blood vessels and the decidua.

The Expression of CBS and CSE and the Hydrogen Sulfide Production Rate in Chorionic Tissues Decrease during Term Labor and Preterm Labor

Western blotting analysis showed that the expression level of CBS and CSE was decreased in the TL group compared with the TNL group (**Figure 2**, A and B). CBS and CSE expression levels were also lower in PTL groups compared with the TNL group.

The homogenate of the chorionic tissue was able to produce hydrogen sulfide after addition of L-cysteine. This effect was blocked by either CSE inhibitor DL-propargylglycine or CBS inhibitor aminooxyacetic acid hemihydrochloride (**Figure 2D**). The production rate of hydrogen sulfide was lower in the TL and PTL groups than that in the TNL group (**Figure 2E**).

As expected, the level of PGDH was down-regulated in the TL and PTL groups (**Figure 2C**). Correlation analysis

showed that CBS, but not CSE, level correlated to PGDH expression in chorionic tissues (**Figure 2**, F and G).

Hydrogen Sulfide Upregulates PGDH Expression and Activity in Cultured Chorionic Trophoblasts

Whether hydrogen sulfide affects PGDH expression and activity in chorionic trophoblasts was then examined. Treatment of cultured chorionic trophoblasts with increasing concentration of hydrogen sulfide donor NaHS (10^{-6} to 10^{-5} mol/L), for 24 hours, resulted in an increase in expression of PGDH mRNA and protein in a dose-dependent manner (**Figure 3**, A–I). The highest effect of NaHS occurred at 5×10^{-5} mol/L. NaHS treatment dose-dependently increased PGDH activity. The highest effect was reached by the dosage of 5×10^{-5} mol/L. Hydrogen sulfide precursor L-cysteine (10^{-4} to 10^{-3} mol/L) also increased the PGDH expression and activity in a dose-dependent manner. The effects of L-cysteine (10^{-3} mol/L) can be blocked by CBS inhibitor aminooxyacetic acid hemihydrochloride (10^{-3} mol/L) but not by CSE inhibitor DL-propargylglycine (10^{-3} mol/L).

To further confirm which hydrogen sulfide-generating enzyme is responsible for the role of hydrogen sulfide, the siRNA targeting CSE and CBS approach was applied. Transfection of CBS and CSE siRNA into cells resulted in 83% and 88% reductions in CBS and CSE, respectively (**Figure 3**, J and K). L-cysteine (10^{-3} mol/L) treatment did not affect PGDH expression in the cells with CBS siRNA transfection. However, the effect of L-cysteine (10^{-3} mol/L) on PGDH expression remained in the cells transfected with CSE siRNA (**Figure 3L**).

Figure 2 Cystathionine-γ-lyase (CSE) and cystathionine-β-synthetase (CBS) expression, hydrogen sulfide-producing rate, and the correlation between 15-hydroxyprostaglandin dehydrogenase (PGDH) and CSE or CBS in term labor (TL), not in labor at term (TNL), and in preterm labor (PTL) chorionic tissues. Chorionic tissues were collected from the women with singleton pregnancy at TNL ($n = 24$), TL ($n = 24$), or PTL ($n = 21$). **A–C:** The protein expression of CSE (**A**), CBS (**B**), and PGDH (**C**) in TNL, TL, and PTL chorionic tissues. Representative protein bands were presented on the top of corresponding histograms. **D:** Representative traces of hydrogen sulfide production in the homogenate of chorionic tissues. **E:** Hydrogen sulfide production rate in TNL, TL, and PTL chorionic tissues. **F** and **G:** The correlation between PGDH and CSE or CBS in chorionic tissues. Data are expressed as means $\pm$ SEM (**A–C** and **E**). $^{**}P < 0.01$ versus TNL. AOAA, aminooxyacetic acid hemihydrochloride; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PAG, DL-propargylglycine; PLP, pyridoxal-5'-phosphate.

miR-26b and miR-199a Modulate Hydrogen Sulfide Regulation of PGDH Expression in Chorionic Trophoblasts

miRNAs modulate post-transcriptional regulation of gene expression.²⁶ Recently, Montenegro et al²⁷ have shown that several miRNAs are regulated in fetal membranes with onset of labor and implicated that miRNAs play roles in pregnancy and labor. In addition, our previous study has shown that hydrogen sulfide can modulate the expression of miRNAs in placental trophoblasts.²⁸ We explored whether hydrogen sulfide regulates PGDH expression via modulation of the miRNAs targeting the PGDH gene.

Bioinformatics analysis shows that miRNAs targeting the PGDH gene include miR-199a, miR-26a, and miR-26b. At first, it was examined whether PGDH is a direct target of miR-199a, miR-26a, and miR-26b by using PGDH-3'-UTR-luciferase reporter assay. Both miR-199a and miR-26b mimic suppressed PGDH-3'-UTR reporter activity, whereas they did not affect mutant PGDH-3'-UTR reporter activity (Figure 4, A and B). In contrast, miR-26a mimics had no effect on PGDH-3'-UTR reporter activity. Moreover,

we also examined the effects of miR-199a and miR-26b mimics or inhibitors on PGDH protein expression. Both miR-199a and miR-26b mimic decreased PGDH protein expression, whereas miR-199a and miR-26b mimic increased PGDH expression (Figure 4C).

The role of miR-199a and miR-26b in hydrogen sulfide regulation of PGDH expression was then explored. NaHS and L-cysteine suppressed miR-199a and miR-26b expression in chorionic trophoblasts (Figure 4, D–G). The mimics of miR-199a and miR-26b reversed upregulation of PGDH by NaHS and L-cysteine.

miR-26b and miR-199a Expression Is Upregulated in Chorion during Term Labor and Preterm Labor

The expression level of miR-26b and miR-199a was significantly increased in TL and PTL chorion compared with that in the TNL group ($P < 0.01$) (Figure 4, H and I). The level of CBS did not significantly correlate to miR-26b or miR-199a. No significant correlation was found between PGDH and miR-26b or miR-199a either (data not shown).

Figure 3 Effects of hydrogen sulfide on 15-hydroxyprostaglandin dehydrogenase (PGDH) expression and activity in cultured chorionic trophoblast cells. **A–C:** The effect of NaHS on PGDH mRNA (**A**) and protein (**B**) expression as well as activity (**C**). Cells were treated with an increasing concentration of NaHS (10^{-5} to 10^{-4} mol/L) for 24 hours. **D–F:** The effects of L-cysteine on PGDH mRNA (**D**) and protein (**E**) expression as well as activity (**F**) in chorionic cells. Cells were treated with increasing concentration of L-cysteine (10^{-4} to 10^{-3} mol/L) for 24 hours. **G–I:** The effects of DL-propargylglycine (PAG) and aminoxyacetic acid hemihydrochloride (AOAA) on L-cysteine stimulation of PGDH mRNA (**G**) and protein (**H**) expression as well as activity (**I**). Cells were treated with L-cysteine (10^{-3} mol/L) in the presence or absence of PAG and AOAA for 24 hours. **J–L:** The role of cystathionine- β -synthetase (CBS) and cystathionine- γ -lyase (CSE) in L-cysteine stimulation of PGDH expression. Cells were transfected with CSE siRNA, CBS siRNA, or scramble siRNA, and then treated with 10^{-3} mol/L L-cysteine for 24 hours. The representative expression of CBS and CSE in cells transfected with CSE siRNA (**J**) and CBS siRNA (**K**). The effect of L-cysteine on PGDH expression in cells with transfection of CSE siRNA and CBS siRNA (**L**). PGDH mRNA and protein expression and activity were determined by real-time RT-PCR, Western blot analysis, and conversion assay, respectively. Representative protein bands are presented on the top of corresponding histogram. Data are expressed as means $\pm$ SEM percentage control (**A–I** and **L**). $n = 5$ cultures performed in triplicate (**A–I** and **L**). * $P < 0.05$, ** $P < 0.01$ versus vehicle control; †† $P < 0.01$ versus L-cysteine. L-cys, L-cysteine; PGFM, prostaglandin F₂.

Discussion

The present study has demonstrated, for the first time, that fetal chorion is able to produce hydrogen sulfide, and hydrogen sulfide-generating enzymes CBS and CSE are localized in chorionic trophoblasts. CBS and CSE expression and hydrogen sulfide production rate are down-regulated in term and preterm labor. miR-199a and miR-26b are involved in endogenous hydrogen sulfide stimulation of PGDH expression and activity in chorionic trophoblasts.

Various factors, including steroid hormones and cytokines, have been reported to be involved in the regulation of chorionic PGDH expression. It has been shown that tumor necrosis factor- α suppresses PGDH expression in cultured chorionic trophoblasts.²⁵ However, progesterone and cortisol exhibit the opposite effect on PGDH expression.²⁹ Our previous studies have shown that corticotropin-releasing hormone and peroxisome proliferator-activated receptors play pivotal roles in the regulation of PGDH in the chorion during pregnancy.^{23,30} Moreover, we have also demonstrated that reduced PGDH expression is associated

Figure 4 miR-26b and miR-199a are involved in hydrogen sulfide regulation of 15-hydroxyprostaglandin dehydrogenase (PGDH) expression and the expression of miR-26b and miR-199a in chorion. **A** and **B**: Luciferase assay of the reporters containing the 3'-untranslated regions (UTRs) of PGDH and their mutant nucleotides in the 3'-UTRs. Chorionic trophoblasts were transfected with the above reporters in the absence or presence of the mimics of miR-26a, miR-26b, and miR-199b. Luciferase activity was measured in cell lysates using a dual-luciferase reporter assay system. **C**: The effect of miR-26b and miR-199a inhibitors and mimics on PGDH expression. Chorionic trophoblasts were treated with miR-26b and miR-199a inhibitors and mimics for 24 hours. **D** and **E**: The effects of hydrogen sulfide on miR-199a and miR-26b expression. Cells were treated with L-cysteine and NaHS for 24 hours. The levels of miR-199a (**D**) and miR-26 (**E**) were determined by real-time RT-PCR. **F** and **G**: The effects of miR-26b and miR-199a mimics on hydrogen sulfide regulation of PGDH expression. Cells were treated with L-cysteine and NaHS in the absence or presence of miR-26b (**F**) and miR-199a (**G**) mimics for 24 hours. PGDH protein expression was determined by Western blot analysis. **H** and **I**: The levels of miR-26b and miR-199a in the chorion of not in labor at term (TNL), term in labor (TL), and preterm in labor (PTL) groups. The expression of miR-26b (**H**) and miR-199a (**I**) was determined by real-time RT-PCR. Data are expressed as means $\pm$ SEM percentage control (**F** and **G**); means $\pm$ SEM (**H** and **I**). $n = 5$ cultures performed in triplicate (**F** and **G**). * $P < 0.05$, ** $P < 0.01$ versus vehicle control; †† $P < 0.01$ versus TNL.

with decreased expression of peroxisome proliferator-activated receptor- γ and increased expression of glucocorticoid receptor and progesterone receptor A.^{30,31} Herein, hydrogen sulfide produced locally increased PGDH expression in the chorion, and PGDH expression correlated with the expression level of hydrogen sulfide-generating enzymes in the chorion. Taken together, this suggests that the PGDH expression level is controlled by a molecular network within the chorion. Furthermore, it might be implicated that an imbalanced molecular network causes upregulated or down-regulated PGDH expression. To support this theory, the level of hydrogen sulfide-generating enzymes was reduced in the chorion of PTL patients, which might suggest that decreased hydrogen sulfide generation contributes to preterm labor.

As mentioned, human gestational tissues, including placenta, fetal membrane, and myometrium, express

hydrogen sulfide-generating enzymes CBS and CSE.^{18,19} We previously showed that CSE and CBS expression in myometrium is down-regulated during labor.²⁰ Consistently, the expression levels of CBS and CSE in the chorion are significantly decreased after the onset of labor. In the model of myometrial strips and the model of cultured myometrial cells, we have demonstrated that hydrogen sulfide suppresses myometrial contractions²⁰ and inhibits the expression of contraction-associated proteins, including oxytocin receptors, connexin-43, and PGH synthase-2.³² Interestingly, the present study implicated that hydrogen sulfide plays an important role in controlling the amount of active PG reaching the myometrium through stimulation of PGDH expression in the chorion. Together, it indicates that hydrogen sulfide is one of the key factors in the maintenance of uterine quiescence during pregnancy.

Herein, hydrogen sulfide was predominantly produced by CBS because CBS inhibitor and siRNA reversed the effect of L-cysteine; in contrast, CSE inhibitor and siRNA did not reverse this effect. Consistently, CBS, but not CSE, level correlated to PGDH expression in the chorion. The mechanisms responsible for the differential effect of hydrogen sulfide produced by CBS and CSE on PGDH expression are unknown. However, CBS and CSE can produce different products; besides, they can generate hydrogen sulfide. Thus, the effect of other products of CSE and CBS on PGDH expression cannot be excluded. Interestingly, Ning et al³³ recently showed that CBS and CSE have a differential role in oviductal embryo transport, although the hydrogen sulfide donor shows the regulatory effect on oviduct contraction.

Our previous study has shown that hydrogen sulfide may modulate the expression of various miRNAs in placental trophoblasts, including miR-20a, miR-20b, miR-200c, and miR-133b.^{28,34} Hydrogen sulfide exerts differential effects on the expression of various miRNAs. Hydrogen sulfide suppresses the expression of miR-20a, miR-20b, and miR-200c, whereas it stimulates miR-133b expression in the placenta. The present study showed that hydrogen sulfide inhibited miR-26b and miR-199a expression in chorionic trophoblasts, suggesting that hydrogen sulfide is one of the factors that modulate miR-26b and miR-199a expression. The molecular mechanisms underlying the biological actions of hydrogen sulfide remain largely unknown. It has been implicated that hydrogen sulfide can induce S-sulfhydration of cysteine residue and disulfide bond of two cysteine residues in various proteins.³⁵ Thus, it is of interest to investigate whether hydrogen sulfide induces the functional changes of the proteins that control the expression of the miRNAs. No significant correlation between CBS or PGDH and miR-26b or miR-199a was found in the chorion. However, the data do not indicate that miR-26b and miR-199a are not the key factors mediating hydrogen sulfide modulation of PGDH expression *in vivo*. PGDH may be one of the targets of these miRNAs because each miRNA has multiple targets. Moreover, our previous studies have shown that PGDH expression is modulated by various factors.^{30,31} Thus, the regulatory mechanisms responsible for PGDH expression remain to be further elucidated.

In conclusion, endogenous hydrogen sulfide produced locally enhances PGDH expression in human chorionic trophoblasts. Hydrogen sulfide is one of the key factors in maintenance of PGDH expression in the chorion during pregnancy. Decreased hydrogen sulfide production contributes to reduced PGDH expression in the chorion during labor.

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