Supplemental Materials for

Collagen I induces preeclampsia-like symptoms by suppressing proliferation and invasion of trophoblasts

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Expanded Materials & Methods

Placental histological analysis hematoxylin eosin (H&E), Masson-trichrome and
**Sirius red staining**

After fixation in 4% paraformaldehyde of placenta, the slices were embedded in paraffin. Section of 3μm were stained by structural identification, with Masson’s trichrome staining for collagen fiber observation, and with Sirius red stain for collagen identification.

**Masson-trichrome staining**

For Masson trichrome staining, the sections were stained using a Masson’s trichrome staining kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol. The slides were incubated in Weigert's iron hematoxylin (5 min), Biebrich Scarlet-Acid Fuchsin Solution (15 min), Phosphomolybdic- Acid Solution (15 min) and Aniline Blue Solution (10 min), all at room temperature. The slides were visualized under the light microscope. Collagen fiber were stained blue, while cytoplasm and red blood cells were stained red and nucleus blue and brown. Fibrosis area% was calculated in μm digitally using the software NDP.view2 (Hamamatsu Corp, Japan). The area of tubulointerstitial fibrosis was measured in 5 random fields under ×200 magnification.

**Sirius red staining**

For Sirius Red staining, the Picrosirius Red stain kit (Abcam, Cambridge, UK) was utilized. Sections were stained with Weigert's iron Sumu essence dye for 15 min, rinsed for 5 min, and then washed with distilled water. The sections were covered with 200 μl Sirius red dye for 1 h. Each analyzed field was chosen randomly and the positive red-stained areas and red-yellow density were quantified using computerized image
Western blotting

Placenta tissue and in vitro-treated cells was homogenized and protein extracted as described below.

Homogenized by a Qiacube machine (Qiagen, Courtaboeuf, France) in RIPA lysis buffer (Thermo, Rockford, USA) on ice. The supernatants were collected after centrifugation at 12000×g at 4°C for 20 min. Protein concentration was determined using a BCA protein assay kit (Thermo, Rockford, USA), and whole lysates were mixed with 5×SDS loading buffer (Coolaber, Shanghai, China) at a ratio of 1:4. Protein samples were heated at 98°C for 5 min and were separated on SDS-polyacrylamide gels (Biodragon, Guangzhou, China). The separated proteins were then transferred to a PVDF membrane (Dogesce, Shanghai, China). The membrane blots were first probed with a primary antibody. After incubation with horseradish peroxidase-conjugated second antibody, autoradiograms were prepared using the enhanced chemiluminescent system to visualize the protein antigen. The signals were recorded using SYNGENE BIO IAMGING (GENE GNOME, Shanghai, China). Primary antibodies for Western Blot are rat anti-collagen I, anti-MMP9, anti-, anti-vimentin, anti-E-cadherin, anti-N-cadherin, anti-β-catenin, anti-ERK, anti-p-ERK and rabbit anti-GAPDH (Cell Signaling, San Jose, CA, USA). GAPDH was used as a protein loading control. The secondary antibody was HRP-conjugated anti-rabbit (Cell Signaling, San Jose, CA, USA). Images shown in the figures were representative of 5 individuals. ImageJ software (https://imagej.nih.gov/ij) was used for image acquisition and densitometric
Quantitative PCR (RT-qPCR)

Total RNA was extracted with Trizol reagent (Invitrogen, Corporation, USA) according to the manufacturer’s instructions. The reverse transcription reaction was carried out with reverse transcription enzyme (Toyobo, Shanghai, China). Quantitative real-time PCR was carried out on an LightCycler96 real-time PCR system (Roche, Basel, Switzerland) and the specific primers for quantitative PCR are shown below (IGE, Guangzhou, China).

**Collagen I**

Forward: 5′-CCAAGACGAAGACATCCCACCA-3′

Reverse: 5′-CCGTTGTCGCAGACGCAGAT-3′

**CCK-8**

HTR-8/SVneo cells were seeded in 96-well plates at the density of 10000 cells per well in 250μl of complete culture medium. After treatments, three methods were utilized for cell proliferation analysis. Cell Counting Kit-8 (Beyotime, Guangzhou, China) analysis: 10 ul of CCK-8 was added to each well. The culture plates were shaken for 90 min and the optical density (OD) values were read at 450 nm.

**Cell cycle**

After treatment of collagen I, HTR-8SV/neo cells were harvested by trypsinization and washed with cold PBS for two times. Then, cells were fixed with 75% alcohol for 12 h at 4°C. After washing with cold PBS, cells were treated with 50 ug/mL RNase (MULTI SCIENCES, Shanghai, China) for 30 min at 37 °C, then stained with 50 μg/mL Propidium iodide (MULTI SCIENCES, Shanghai, China) for 30 min at 4 °C in the
dark before being analyzed using a FACS Calibur flow cytometer (BD, CA, USA). Cells (1×10^6) were detected for each sample. Cell cycle was analyzed by ModFit software (Verity Software House, ME, USA).

**Transcriptome sequencing**

**Sample collection and preparation**

HTR-8SV/neo cells were cultured in the medium described above in plates precoated with 100 μg/mL of collagen I for 48 hours. Total RNA was extracted using Trizol. After total RNA was extracted, potential RNA degradation and contamination was monitored on 1% agarose gel. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

**Library preparation for Transcriptome sequencing**

A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, Massachusetts, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each sample.

Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse
Transcriptase (RNase H-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3’ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 250~300 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min in followed by 5 min at 95°C before PCR. Then PCR was performed with Phusion High -Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

**Clustering and sequencing**

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia, CA, USA) according to the manufacturer’s instructions. After cluster generation, the library preparations were sequenced on an Illumina Novaseq platform and 150 bp paired-end reads were generated.
Supplemental Materials On line table

Table S1 Comparison of clinical data in two study group

| Characteristic                              | Normal         | Preeclampsia | p-value |
|---------------------------------------------|----------------|--------------|---------|
| N                                           | 10             | 10           |         |
| Age(y)                                      | 30.40 ± 0.96   | 33.10 ± 2.17 | p=0.27  |
| SBP (second trimester, mmHg)                | 120.9 ± 2.82   | 158.6 ± 3.29 | p<0.01  |
| DBP (second trimester, mmHg)                | 73.90 ± 1.73   | 99.70 ± 3.25 | p<0.01  |
| MAP (second trimester, mmHg)                | 89.56 ± 2.09   | 118.73 ± 3.26| p<0.01  |
| Gestational weeks, (w)                      | 39.53 ± 0.28   | 35.66 ± 0.86 | p<0.01  |
| Body Mass Index (kg/cm2)                    | 27.48 ± 0.94   | 29.68 ± 1.59 | p=0.39  |
| ALT (third trimester, U/L)                  | 15.30 ± 3.43   | 11.20 ± 1.54 | p=0.29  |
| AST (third trimester, U/L)                  | 18.80 ± 1.02   | 18.80 ± 2.21 | p=1     |
| albumin (third trimester, g/L)              | 35.58 ± 1.034  | 38.55 ± 0.3859| p<0.05  |
| Total protein (third trimester, g/L)        | 64.96 ± 1.349  | 63.12 ± 1.075| p=0.30  |
| Hb (third trimester, g/L)                   | 108.2 ± 2.01   | 121.4 ± 3.43 | p<0.01  |
| PLT (third trimester, *10^9NAl)             | 222.7 ± 17.81  | 242.3 ± 21.15| p=0.49  |
| Cr (third trimester, μmol/L)                | 47.20 ± 2.78   | 56.79 ± 3.71 | p=0.05  |
| Bun (third trimester, mmol/mL)              | 3.54 ± 0.26    | 4.16 ± 0.52  | p=0.30  |
| D-Dimer (third trimester, ug/mL)            | 0.75 ± 0.19    | 0.52 ± 0.11  | p=0.32  |
| Proteinuria(0 ~ ++++)                       | < +            | ++          | p<0.01  |
| Intrapartum hemorrhage (volume, mL)         | 249.0±20.96    | 264.0±39.53 | p=0.74  |
| Neonatal weight (g)                         | 3276 ± 0.15    | 2730 ± 0.28  | p=0.11  |
| Cesarean delivery, n (%) | 30% | 60% |
|-------------------------|-----|-----|
| Primiparous, n (%)      | 40% | 50% |

**Note:** Differences in characteristics between PE and Normotension group were evaluated, using Student’s t-test.

**Abbreviations:** N, sample size; Normal, normotensive pregnant women; PE, preeclampsia; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, Mean arterial pressure; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; Cr, creatinine; Bun, urea nitrogen; Hb, hemoglobin; PLT, blood platelet.

Evaluation of proteinuria was estimated by examination of fresh urine using multistix (Bayer, Auckland, New Zealand) on a scale of 0 to 4 +, where 0/trace = negative, 1+ = 30, 2+ = 100, 3+ = 300, and 4+ = over 2000 mg/dl.
Table S2 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis

| ID       | Description                                           | GeneRatio | BgRatio | pvalue     | p.adjust   | qvalue     | Count | group          |
|----------|-------------------------------------------------------|-----------|---------|------------|------------|------------|-------|----------------|
| hsa03010 | Ribosome                                              | 48/576    | 158/8076 | 8.24E-19   | 2.47E-16   | 2.07E-16   | 48    | All-DEGs       |
| hsa05016 | Huntington disease                                    | 45/576    | 306/8076 | 2.11E-06   | 0.000317   | 0.00266    | 45    | All-DEGs       |
| hsa05205 | Proteoglycans in cancer                              | 31/576    | 205/8076 | 4.86E-05   | 0.004864   | 0.004079   | 31    | All-DEGs       |
| hsa04141 | Protein processing in endoplasmic reticulum           | 27/576    | 171/8076 | 6.99E-05   | 0.00524    | 0.004395   | 27    | All-DEGs       |
| hsa00920 | Sulfur metabolism                                    | 5/576     | 10/8076  | 0.000338   | 0.017164   | 0.014394   | 5     | All-DEGs       |
| hsa05014 | Amis                                                  | 44/576    | 364/8076 | 0.000343   | 0.017164   | 0.014394   | 44    | All-DEGs       |
| hsa04110 | Cell cycle                                            | 20/576    | 124/8076 | 0.000449   | 0.017533   | 0.014703   | 20    | All-DEGs       |
| hsa05219 | Bladder cancer                                        | 10/576    | 41/8076  | 0.000468   | 0.017533   | 0.014703   | 10    | All-DEGs       |
| hsa05022 | Pathways of neurodegeneration - multiple diseases    | 53/576    | 475/8076 | 0.000637   | 0.019157   | 0.016065   | 53    | All-DEGs       |
| hsa05165 | Human papillomavirus infection                        | 40/576    | 331/8076 | 0.000639   | 0.019157   | 0.016065   | 40    | All-DEGs       |
| hsa04370 | VEGF signaling pathway                               | 12/576    | 59/8076  | 0.000777   | 0.021197   | 0.017776   | 12    | All-DEGs       |
| hsa01522 | Endocrine resistance                                 | 16/576    | 98/8076  | 0.001425   | 0.035201   | 0.029519   | 16    | All-DEGs       |
| hsa04910 | Insulin signaling pathway                            | 20/576    | 137/8076 | 0.00162    | 0.035201   | 0.029519   | 20    | All-DEGs       |
| hsa04810 | Regulation of actin cytoskeleton                      | 28/576    | 218/8076 | 0.001643   | 0.035201   | 0.029519   | 28    | All-DEGs       |
| hsa05225 | Hepatocellular carcinoma                            | 23/576    | 168/8076 | 0.00181    | 0.035874   | 0.030084   | 23    | All-DEGs       |
| hsa04144 | Endocytosis                                           | 31/576    | 252/8076 | 0.001913   | 0.035874   | 0.030084   | 31    | All-DEGs       |
| hsa05163 | Human cytomegalovirus infection                       | 28/576    | 225/8076 | 0.00263    | 0.046406   | 0.038916   | 28    | All-DEGs       |
| hsa03010 | Ribosome                                              | 48/350    | 158/8076 | 2.15E-28   | 5.86E-26   | 5.49E-26   | 48    | Up-regulation  |
| hsa05016 | Huntington disease                                    | 35/350    | 306/8076 | 1.11E-07   | 1.51E-05   | 1.42E-05   | 35    | Up-regulation  |
| hsa05022 | Pathways of neurodegeneration - multiple diseases    | 40/350    | 475/8076 | 3.47E-05   | 0.002842   | 0.002663   | 40    | Up-regulation  |
| hsa05014 | Amyotrophic lateral sclerosis                        | 33/350    | 364/8076 | 4.16E-05   | 0.002842   | 0.002663   | 33    | Up-regulation  |
| geneID  | disease                                      | DEG1   | DEG2   | padj1  | padj2  | padj3  | pvalue1 | pvalue2 | pvalue3 | FoldChange | Regulation |
|---------|----------------------------------------------|--------|--------|--------|--------|--------|---------|---------|---------|------------|------------|
| hsa05012 | Parkinson disease                            | 25/350 | 249/8076 | 7.25E-05 | 0.003957 | 0.003708 | 25       | Up-regulation |
| hsa04714 | Thermogenesis                                | 23/350 | 231/8076 | 0.00016  | 0.007271 | 0.006813 | 23       | Up-regulation |
| hsa00190 | Oxidative phosphorylation                    | 16/350 | 133/8076 | 0.000192 | 0.007485 | 0.007013 | 16       | Up-regulation |
| hsa05020 | Prion disease                                | 25/350 | 273/8076 | 0.000311 | 0.01062  | 0.009951 | 25       | Up-regulation |
| hsa00240 | Pyrimidine metabolism                         | 9/350  | 57/8076  | 0.000693 | 0.021017 | 0.019692 | 9        | Up-regulation |
| hsa00330 | Arginine and proline metabolism              | 8/350  | 51/8076  | 0.001428 | 0.038988 | 0.03653  | 8        | Up-regulation |
| hsa05205 | Proteoglycans in cancer                       | 20/226 | 205/8076 | -1.03E-06 | 0.000253 | 0.00002  | 20       | Down-regulation |
| hsa04810 | Regulation of actin cytoskeleton              | 20/226 | 218/8076 | 2.72E-06 | 0.000331 | 0.000263 | 20       | Down-regulation |
| hsa04919 | Thyroid hormone signaling pathway            | 14/226 | 121/8076 | 6.43E-06 | 0.000523 | 0.000415 | 14       | Down-regulation |
| hsa04390 | Hippo signaling pathway                       | 14/226 | 157/8076 | 0.00122  | 0.004942 | 0.003923 | 14       | Down-regulation |
| hsa04360 | Axon guidance                                | 15/226 | 181/8076 | 0.000159 | 0.005548 | 0.004404 | 15       | Down-regulation |
| hsa05100 | Bacterial invasion of epithelial cells        | 9/226  | 77/8076  | 0.000277 | 0.008101 | 0.00643  | 9        | Down-regulation |
| hsa04141 | Protein processing in endoplasmic reticulum  | 14/226 | 171/8076 | 0.000299 | 0.008101 | 0.00643  | 14       | Down-regulation |
| hsa04151 | PI3K-Akt signaling pathway                    | 22/226 | 354/8076 | 0.000358 | 0.008729 | 0.006929 | 22       | Down-regulation |
| hsa04110 | Cell cycle                                   | 11/226 | 124/8076 | 0.000679 | 0.015057 | 0.011952 | 11       | Down-regulation |
| hsa04730 | Long-term depression                          | 7/226  | 60/8076  | 0.001339 | 0.027231 | 0.021616 | 7        | Down-regulation |
| hsa04611 | Platelet activation                           | 10/226 | 124/8076 | 0.002429 | 0.04529  | 0.03595  | 10       | Down-regulation |
| hsa05225 | Hepatocellular carcinoma                     | 12/226 | 168/8076 | 0.002599 | 0.04529  | 0.03595  | 12       | Down-regulation |
| hsa03010 | Ribosome                                     | 12/137 | 158/8076 | 1.4E-05  | 0.002992 | 0.002455 | 12       | PE_related-DEGs |
| hsa05225 | Hepatocellular carcinoma                     | 12/137 | 168/8076 | 2.59E-05 | 0.002992 | 0.002455 | 12       | PE_related-DEGs |
| hsa05016 | Huntington disease                            | 14/137 | 306/8076 | 0.00066  | 0.040278 | 0.033038 | 14       | PE_related-DEGs |
| hsa05205 | Proteoglycans in cancer                       | 11/137 | 205/8076 | 0.000697 | 0.040278 | 0.033038 | 11       | PE_related-DEGs |
| hsa04151 | PI3K-Akt signaling pathway                    | 15/137 | 354/8076 | 0.000935 | 0.043187 | 0.035423 | 15       | PE_related-DEGs |