Depletion of CTCF disrupts PSG gene expression in the human trophoblast cell line Swan 71

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Pregnancy-specific glycoproteins (PSGs) are fetal proteins secreted by the placenta during pregnancy. The PSG level in maternal serum is an indicator of risk for pregnancy complications. However, little is known about the molecular mechanisms underlying PSG gene expression. Recently, the importance of epigenetic regulation of placental genes has been emphasized in the study of developmental defects and placental disease. In this study, the role of the CCCTC-binding factor (CTCF) in regulation of PSG expression was investigated to better understand the epigenetic regulatory mechanisms of the PSG genes. Inhibition of CTCF expression disturbed transcription of several PSG genes: PSG1, PSG2, PSG4, PSG5, PSG8, and PSG9 were upregulated and PSG6 and PSG11 were downregulated. These transcriptional changes were correlated with decreased CTCF binding and changes in histone modification at the PSG promoters. Our data demonstrate that CTCF is a potential mediator in the regulation of PSG gene expression.

Pregnancy-specific glycoproteins (PSGs) are the members of the carcinoembryonic antigen family, one of immunoglobulin super families. PSG genes are clustered on chromosome 19q13.2 encoding 10 genes in humans and chromosome 7 comprising 17 genes in mice [1]. Human PSGs are expressed in the syncytiotrophoblast of the placenta and secreted into the maternal bloodstream. PSGs are among the most abundant fetal proteins detected in the maternal circulation produced by the placenta during pregnancy [2]. The known functions of PSG during pregnancy are implicated in immune regulation, angiogenesis, and platelet regulation, which are important for normal placental development [3–5]. Defects in placental development are directly associated with the disruption of the maintenance of pregnancy [6]. In particular, several case–control studies have shown that low levels of PSG are associated with complicated pregnancy outcomes such as intrauterine growth retardation (IUGR), preterm labor, and pre-eclampsia (PE) [7–9]. These reports imply the pivotal roles of PSG in successful pregnancy outcomes; however, the molecular mechanism underlying the regulation of PSG gene expression is not well understood.

During development, epigenetic changes modulate the accessibility of transcription factors to the DNA and consequently affect gene expression. Epigenetic mechanisms during development include DNA methylation, histone modifications, the action of non-coding RNAs, and 3D structural changes in the chromatin, all of which affect cell phenotype and function and contribute to the associated pathological conditions

Abbreviations
CEA, carcinoembryonic antigen; ChIP, chromatin immunoprecipitation; CTCF, CCCTC-binding factor; H1-hESC, H1 human embryonic stem cell; HUVEC, human umbilical vein endothelial cell; HVMF, human villous mesenchymal fibroblast; IUGR, intrauterine growth retardation; PE, pre-eclampsia; PSGs, pregnancy-specific glycoproteins; UCSC, University of California Santa Cruz.
Many recent studies have shown that epigenetic modifications are related to placental development and disease during pregnancy [11–14]. Appropriate placenta- tion and fetal environment are not only important for the intrauterine growth and survival of the fetus but also affect neurodevelopmental disease and metabolism in the postnatal phase through a process called fetal reprogramming [15]. Under pathologic conditions such as PE, IUGR, and small for gestational age, epigenetic dysregulation correlates with abnormal placental gene expression [16]. It is, therefore, imperative to understand the expression pattern of placental genes and their epigenetic regulatory mechanisms to elucidate the causal relationship between placental development and disease during pregnancy and for the development of preventive measures or treatment regimens.

Among the epigenetic modifiers, the CCCTC-binding factor (CTCF) is a highly conserved, and ubiquitously expressed DNA-binding protein. CTCF regulates gene transcription as an activator, a repressor, or an insulator and serves as a core chromatin organizer by contributing to establishing the three-dimensional structure of the genome [17]. Abnormal CTCF binding to the DNA-binding sites may perturb appropriate long-range chromatin interactions, thereby regulating genomic instability [18]. Many studies have shown that abnormal CTCF binding may cause disturbances in the transcriptional regulation of tumor suppressors or oncogenic genes in many human cancers. CTCF also regulates embryonic development by affecting the expression of several genes that influence cell survival and proliferation (reviewed in [19]). Specifically, CTCF has been demonstrated to regulate the clustered gene families such as HOX, protocadherin, and β-globin through the organization of chromatin structures [20–22]. DNA methylation, which is involved in a wide range of gene expression during development and disease, is known to affect the CTCF-DNA binding occupancy [23]. A study showing the disruption of endothelial vascular development in the placenta of CTCF-deficient mice demonstrates the importance of CTCF for adequate placentation to facilitate normal fetal development [24].

To date, no study has reported the association between CTCF and regulation of PSG gene expression. In the present study, we investigated whether CTCF binding, which is highly conserved across the PSG loci, contributes to the regulation of PSG gene expression in trophoblast cells. Further understanding of the mechanisms underlying PSG gene regulation will lay the scientific foundation for the development of diagnostics and therapeutics for placental disease.

Materials and methods

Cell culture and transfection

The human extravillous trophoblast cell line Swan 71 was kindly provided by J.-Y. Kwon (Institute of Women’s Life Medical Science, Yonsei University Health System, Seoul, Korea). These cells were cultured in Dulbecco’s modified Eagle’s medium (WELGENE Inc., Daegu, Korea) supplemented with 10% FBS (WELGENE Inc.), 1× antibiotic-antimyocotic solution (WELGENE Inc.), 1× HEPES buffer solution (WELGENE Inc.), and 1× non-essential amino acid solution (WELGENE Inc.) in humidified air at 37 °C and 5% CO₂. For gene knockdown (KD) study, Swan 71 cells were seeded at 1 × 10⁶ cells in 100 mm culture plate and were transfected with 10 nM of siRNA using G-fectin (Genolution, Seoul, Korea) for 48 h. ON-TARGET plus CTCF siRNA (L-020165-00-0005) were purchased from Dharmacon (Cambridge, UK), which utilizes a patented dual-strand modification to reduce off-target effects. Negative control siRNA (sense sequence, 5'-CCUCUGUCC-GUUUCAUCAGGUAGUU-3'; antisense sequence, 5'-CUACCUUGGAACGGCACGAGGUU-3') was provided by Genolution Inc.

Total RNA isolation and reverse-transcription PCR

Total RNA from cultured cells was extracted using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer’s instructions. cDNA synthesis was performed with 1 μg of total RNA using ImProm-II Reverse Transcriptase (Promega, Madison, WI, USA) and RNase inhibitor (Promega). Since the nucleotide sequence of all PSG genes is very similar, the primer design process and PCR conditioning were carefully adjusted to guard against off-target priming. PCR conditions for amplification of PSG cDNAs were as follows. An initial denaturation step was done at 95 °C for 10 min, followed by 30–42 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 20 s, and extension at 72 °C for 30 s. The final extension step was done at 95 °C for 5 min. After confirming that the PCR products of all PSG genes appeared as a single band with the correct size, RT-qPCR was performed. For RT-qPCR, cDNA was amplified using SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) on an ABI7300 real-time PCR system (Applied Biosystems). PCR primers are listed in Table 1.

ChIP assay

Swan 71 cells were harvested at 80–90% confluency with 0.7 × 10⁶ cells per antibody. The cells were cross-linked with 1% formaldehyde using 37% formaldehyde for 15 min at room temperature. To quenching the reaction, 2.5 mM glycine was added to make 125 mM for 10 min at
room temperature. Then, cells were washed twice with 1× cold PBS and lysed in lysis buffer for 10 min on ice. Lysis buffer consisting of 1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate, 10 mM EDTA, 50 mM Tris–HCl (pH 8.0), and a protease inhibitor (Merck, Darmstadt, Germany) was added. Sonication sheared 500–1000 bp DNA fragments, which is confirmed by loading with 1% agarose gel. Then, the lysates were incubated with Protein A/G PLUS-Agarose (Santa Cruz, Dallas, TX, USA) in a rotator for 1 h at 4°C. For DNA-protein complexes, precleared DNA is incubated with antibody overnight at 4°C. ChIP-grade anti-CTCF antibody (Cell Signaling Technology, Beverly, CA, USA), anti-Pol II (Santa Cruz), anti-histone H3 trimethyl K4 (Abcam, Cambridge, UK), and anti-histone H3 trimethyl K27 (Abcam) were used.

**ChIP-seq database**

Bioinformatic analyses for CTCF binding sites (CBSs) were performed using the University of California Santa Cruz (UCSC) genome browser (https://genome.ucsc.edu/). The ChIP-seq data for four cell lines represented in Fig. 1A can be accessed using the following codes: HeLa-S3, GSM749729; h1 human embryonic stem cell (H1-hESC), GSM733672; human umbilical vein endothelial cell (HUVEC), GSM749749, and human villous mesenchymal fibroblast (HVMF), GSM1022630.

**Table 1. Primer sequences for RT-PCR and ChIP-PCR.**

| Primer sequences for qRT-PCR | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|-----------------------------|------------------------|-----------------------|
| PSG1                        | GAG CTT GAG AAT TGG TCC | GAG GGG CTT GAG GGG TTC |
| PSG2                        | CCA CCC ATT AGC CTC GGA  | CCA GGA CCC CTC TCC ATT TGA TGT |
| PSG3                        | GGC AGA CAG TGG CTT CTC GCA  | TTC TGG GGC ACT TAG GCA GC |
| PSG4                        | CAT GTG AGC CAC TCA GAA CTC A  | TAA GAG GGG TGG GAG CCT TA |
| PSG5                        | CTC GGA GAG AGG AGG CTC AGC A  | CAG GGC TTC AAT GCT GAC TTG |
| PSG6                        | ACT TAA ACC CCA GGG AGA AGA  | GAT TCC ATG GCA GGG ACC A |
| PSG7                        | TCC GTG ACA GTG AGA GTC TCA  | CTG ACT TAT AGG GCT TCT GG |
| PSG8                        | CAA GCC CTA CAT CAC CAT CAA C  | GAT GCC ACC ATA TTG GTC CCT T |
| PSG9                        | TCC TGC ACA CAG CTC ATC  | GGT GTA GGT TCC TGC ATC CT |
| PSG11                       | GTC TCA GGG CAG AAG GAG G  | CCA TTT GAT GGT CTC TGT GC |
| β-actin                     | CAT GTT TGA GAC CTT CAA CAC CCC  | GCC ATC TCC TGC TCG AAG TCT AG |

**Table 1. Primer sequences for ChIP-PCR.**

| Primer sequences for ChIP-PCR | Forward primer (5′-3′) | Reverse primer (5′-3′) | Position from TSS |
|-----------------------------|------------------------|-----------------------|------------------|
| PSG1-up                     | TGG AAT TGC TGC CTG CTT CAC  | TCA CGA GGG GAA AGC ACC CG  | 1427 to 1751 |
| PSG2-up                     | GAG GTC AGG AGG GGA GAA AG  | GTG TGA AGT GAG CAC ACT CAC A  | 1361 to 1755 |
| PSG3-up                     | GCT GGA AGG TGA AGG AGC TA  | TTA GGG AGC AGT GAC AAG AC  | 1354 to 1827 |
| PSG4-up                     | AGG TCA CGA GGG GAA AGC GT  | GTC ACA CAG ACT GGG ACT CAA  | 1279 to 1737 |
| PSG5-up                     | AGG AGG TGT GTG GTC GGG CT  | CTT GTG CAG TGG GCA GAT CCA TA  | 1464 to 1777 |
| PSG6-up                     | GAG CTA CCA CTA GCA GAC GC  | CTG CTT TGG GGA CAT CTG AC  | 1493 to 1900 |
| PSG7-up                     | CAG CTC TAG CTC TCA CCT GC  | ACA CTT CCC TCC TCT TCC TAC A  | 1344 to 1776 |
| PSG8-up                     | CAC GAG GGG AAA GCC CCT  | CTT GTG AAG TGT GCA GAT CCA T  | 1461 to 1850 |
| PSG9-up                     | GAC CTC GTG TGG CAA GAA ATA A  | GGA AGG ACC AGG TGG CTG GG  | 1486 to 1793 |
| PSG11-up                    | AGG AAC TAC CTG TAG GGG GA  | GTG AAG TGT GCA GAT CCA AGT A  | 1520 to 1811 |
| Gene desert                 | TGT TTT TGG AGT TAC TAG ACT GGG  | TT CCC CTG CTT TTA TGT GG  |                  |

**Statistical analysis**

Data were obtained from three separate experiments and represented as mean ± standard error of the mean (SEM). The Student’s t-test was used for comparison between siCTCF-treated cells and control. Differences were statistically significant at P < 0.05.

**Results and Discussion**

The binding of CTCF to the DNA is determined by the recognition of the consensus sequence; some binding sites exhibit differential CTCF binding patterns depending on the tissues, while others consistently show strong binding to CTCF in most tissues. CBSs distribute all throughout the genome, that is, intergenic region, gene bodies, and promoters, and its role is determined by where the CTCF binds [25]. Before
exploring the role of CTCF as a PSG gene regulator in trophoblast cells, we first examined the CTCF binding patterns across the PSG gene loci using publicly available data. Among the experimental models from which CTCF ChIP-seq data deposited in ENCODE were derived, HVMF is the only placenta-related cell line. A view of chromosome 19 containing PSG genes (chr19: 43 076 96–43 873 383) shows numerous CTCF binding signals in many regions of the PSG gene body, as well as in the upstream and downstream regions (Fig. 1A). RNA-seq data show that several PSG genes are expressed in the placental HVMF cell line. In the enlarged genomic map, three strong CTCF binding peaks are common to all PSG genes, except PSG3 and PSG8, two of which are in the upstream region of each gene (Fig. 1B). In addition to HVMF, these peaks are highly conserved in various cell types, including HUVEC, embryonic stem cells, and Hela cells. One is located 1.6–1.8 kb upstream, and the other is located 5.5–5.7 kb upstream from the transcription start site (TSS) of each PSG gene. In the case of PSG3 and PSG8, only one peak close to the TSS is observed. Therefore, we focused on the proximal peak close to the TSS observed in common in all PSG genes and studied the location, orientation, and conservation status of CBSs. As a result, we found three consensus binding sequences for all 10 PSG genes, two being forward orientation motifs and one being reverse orientation motif.

To investigate the role of CTCF in the regulation of PSG gene expression, we needed an appropriate cell line model in which all PSG genes were expressed, so
first analyzed the mRNA expression of 10 PSGs (PSG1-PSG9, PSG11) from two different human trophoblast cell lines, Swan 71 and JEG-3, by RT-PCR. Unlike JEG3, the Swan71 cells were found to express the entire set of PSG genes under normal conditions (Fig. 2A), indicative of their applicability as an excellent model system to study the role of specific factors affecting gene expression at the site of PSG gene. Although the expression of several PSG genes has been previously identified in other trophoblast cell line and primary placental cells [26,27], no studies have demonstrated the expression of all human PSG genes in a particular cell line or tissue as shown in this study. On the other hand, CTCF, known as a ubiquitously expressed architectural protein, is well expressed in both trophoblast cell lines, JEG-3 and Swan 71 (Fig. 2B,C). Therefore, the ChIP experiment to examine CTCF occupancy in the PSG gene locus was performed using Swan 71 cells in consideration of the expression pattern of the PSG genes. Based on the consensus sequence motif shown in Fig. 1B, we designed amplicons for ChIP-PCR to cover three binding sites in the upstream region of each PSG gene. PCR primer sequences used to amplify each amplicon site are listed in Table 1. ChIP-PCR results showed that CTCF binds to the upstream region of all PSG genes from PSG1 to PSG11 throughout the locus (Fig. 2D).

To gain further evidence on whether CTCF contributes to the transcription of the PSG gene cluster, the mRNA level of each PSG gene was measured following the silencing of CTCF expression. The siRNA-mediated KD of CTCF significantly downregulated CTCF expression (Fig. 3A). In this condition, the mRNA levels of PSG genes were affected; some PSG genes, such as PSG1, PSG2, PSG4, PSG5, PSG8, and PSG9, were upregulated, while PSG6 and PSG11 genes were downregulated (Fig. 3B). Under the CTCF KD condition, CTCF binding was significantly reduced at all PSG gene loci tested (Fig. 3C). These results suggest that CTCF binding to the upstream region of PSG genes is involved in the transcriptional regulation of PSG genes. Our data also show that CTCF binding exerts different effects on individual PSG genes. In other words, the loss of CTCF binding upregulates the expression of some PSG genes, while downregulating the expression of others.

Fig. 2. The expression of PSG and CTCF in placental cells. (A) Comparison of PSG gene expression in Swan 71 and JEG-3 cells by RT-PCR. β-Actin was used as an internal control for RT-PCR. (B) mRNA levels of CTCF in human placental cell lines. The number of each sample indicates two biological replicates. (C) Protein levels of CTCF in human placental cell lines. (D) ChIP-PCR results for CTCF binding at the designed amplicon sites in Swan 71 cells, which express the entire set of PSG genes. Primers for gene desert regions (chr20:57 828 313–57 828 465) were used as negative control for ChIP-PCR.
Next, to examine whether the CTCF KD-mediated variation in PSG expression accompanies epigenetic changes, we analyzed the modification patterns of histone proteins enriched in the upstream regions of PSG genes. As the promoter region of PSG genes is not clearly defined from the current literature, we tried to narrow down the scope of the interesting region by evaluating the polymerase II (Pol2) binding position. Three amplicon sites were generated by dividing the 1 kb upstream region from the TSS site of each PSG.
gene. PSG8 is presented as a representative gene (Fig. 4A). PCR was performed using DNA obtained by performing ChIP experiment with Pol2 antibody. Of these sites, the P3 site showed the strongest binding to Pol2 (Fig. 4B). Therefore, we analyzed for H3K4me3 and H3K27me3 enrichment, targeting approximately the same region in several other PSG genes, including PSG8. PSG2 and PSG8 were selected as the genes upregulated after CTCF KD, while PSG6 and PSG11 were chosen as the downregulated genes. The increase in the level of PSG2 and PSG8 transcripts after CTCF silencing was accompanied by an increase in the recruitment of active mark H3K4me3 and a decrease in the recruitment of repressive mark H3K27me3. The downregulation of PSG6 and PSG11 expression mediated by CTCF KD was consistent with the enrichment of H3K27me3 rather than reduction of H3K4me3 (Fig. 4C).

Together, our results provide evidence that CTCF may regulate PSG gene expression via epigenetic mechanisms. Keeping in mind that CTCF will define the topological boundary of chromatin, CTCF deficiency is expected to cause expression changes in the same direction for all genes in the cluster, but our data show that there are individual genes that are more particularly affected by CTCF deficiency. This suggests that CTCF is likely to cooperate or counteract with other transcription factors or cofactors bound to specific gene sites rather than acting alone when involved in gene transcription. Considering that functional changes are induced upon the manipulation of PSG expression, as shown in previous studies [28], PSG deregulation by CTCF shown in the present study may lead to dysfunction of PSG. On the other hand, apart from the effect of CTCF expression level, a change in DNA methylation pattern can affect the ability of CTCF to bind to the target gene. During development, the DNA methylation status of the placenta is the least methylated unlike other tissues [29–31]. Abnormal regulation of placental DNA methylation is associated with pregnancy disorders [32–35].

Thus, changes in DNA methylation patterns and dysregulated CTCF expression can both affect the expression of the PSG genes at developmental stages or especially in complicated pregnancy. As mentioned earlier, PSG is considered an important factor in maintaining pregnancy based on several previous studies showing its role in placental development, vascular morphogenesis, and immunomodulation. While the specific function of each PSG gene or its regulatory mechanism is not yet known, our results demonstrating the role of CTCF as a regulatory factor of PSG genes may take one step closer to revealing the unknown field.

The next step in understanding how CTCF contributes to structural changes in chromatin at the PSG locus will be to explore the promoter and enhancer regions involved in PSG gene transcription and further investigate the changes in looping structure by CTCF. Accumulation of relevant studies may answer whether the CTCF-mediated spatial chromatin organization contributes to the normal expression of the placental PSG clustered gene family during pregnancy.

![Fig. 4. Histone modification changes in the promoter of PSG genes in response to CTCF expression KD. (A) Genomic map of PSG8 and amplicons for the detection of Pol2 binding site. The 1 kb long-range putative promoter region was divided into three sites as follows: P1 (−688 to −897), P2 (−551 to −707), and P3 (−315 to −553). (B) ChIP-PCR result showing the strongest Pol2 binding at P3 site. Amplicon for gene desert region was identical to that used in Fig. 2D. (C) Changes in the enrichment of H3K4me3 active histone mark and H3K27me3 repressive mark at the PSG gene promoter following CTCF expression KD. To determine the binding pattern of each histone mark, three biological replicates were generated and analyzed.](image-url)
Conclusion
Our results showed that CTCF, which is known to regulate the transcription of several clustered genes, also may regulate the transcription of human PSG genes (PSG1-PSG9, PSG11) in trophoblast cells. Silencing of CTCF expression upregulated or down-regulated the expression of several PSG genes, and this effect was accompanied by epigenetic changes. H3K4me3 was enriched in the promoter region of the upregulated PSG genes, whereas H3K27me3 was recruited to the promoter region of the genes with reduced transcripts, such as PSG6 and PSG11. Further studies are warranted to determine whether CTCF mediates the communication between PSG genes and regulatory elements and induces any alterations in the chromatin structure. In conclusion, our findings suggest that CTCF may be involved in the transcriptional regulation of PSG genes once its expression is induced and maintained in the placenta during pregnancy.

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Data accessibility
Data will be available from the corresponding author upon reasonable request.

Author contributions
DSJ and J-YL designed the experiments, analyzed the data, and wrote the manuscript. MHK and J-YL supervised the study and finalized the manuscript.

Conflict of interest
The authors declare no conflict of interest.

References
1 Moore T and Dveksler GS (2014) Pregnancy-specific glycoproteins: complex gene families regulating maternal-fetal interactions. *Int J Dev Biol* 58, 273–280.
2 Lin TM, Halbert SP and Spellacy WN (1974) Measurement of pregnancy-associated plasma proteins during human gestation. *J Clin Invest* 54, 576–582.
3 Ha CT, Waterhouse R, Wessells J, Wu JA and Dveksler GS (2005) Binding of pregnancy-specific glycoprotein 17 to CD9 on macrophages induces secretion of IL-10, IL-6, PGE2, and TGF-beta1. *J Leukoc Biol* 77, 948–957.
4 Wu JA, Johnson BL, Chen Y, Ha CT and Dveksler GS (2008) Murine pregnancy-specific glycoprotein 23 induces the proangiogenic factors transforming-growth factor beta 1 and vascular endothelial growth factor a in cell types involved in vascular remodeling in pregnancy. *Biol Reprod* 79, 1054–1061.
5 Shanley DK, Kiely PA, Golla K, Allen S, Martin K, O’Riordan RT, Ball M, Aplin JD, Singer BB, Caplice N et al. (2013) Pregnancy-specific glycoproteins bind integrin alphaIIbbeta3 and inhibit the platelet-fibrinogen interaction. *PLoS One* 8, e57491.
6 Woods L, Perez-Garcia V and Hemberger M (2018) Regulation of placental development and its impact on fetal growth-new insights from mouse models. *Front Endocrinol (Lausanne)* 9, 570.
7 Pihl K, Larsen T, Laursen I, Krebs L and Christiansen M (2009) First trimester maternal serum pregnancy-specific beta-1-glycoprotein (SP1) as a marker of adverse pregnancy outcome. *Prenat Diagn* 29, 1256–1261.
8 Grudzinskas JG, Gordon YB, Menabawey M, Lee JN, Wadsworth J and Chard T (1983) Identification of high-risk pregnancy by the routine measurement of pregnancy-specific beta 1-glycoprotein. *Am J Obstet Gynecol* 147, 10–12.
9 Silver RM, Heyborne KD and Leslie KK (1993) Pregnancy specific beta 1 glycoprotein (SP-1) in maternal serum and amniotic fluid; pre-eclampsia, small for gestational age fetus and fetal distress. *Placenta* 14, 583–589.
10 Handy DE, Castro R and Loscalzo J (2011) Epigenetic Modifications. *Circulation* 123, 2145–2156.
11 Fogarty NM, Burton GJ and Ferguson-Smith AC (2015) Different epigenetic states define syncytiotrophoblast and cytotrophoblast nuclei in the trophoblast of the human placenta. *Placenta* 36, 796–802.
12 Bourque DK, Avila L, Penaherrera M, von Dadelszen P and Robinson WP (2010) Decreased placental methylation at the H19/IGF2 imprinting control region is associated with normotensive intrauterine growth restriction but not preeclampsia. *Placenta* 31, 197–202.
13 Li P, Guo W, Du L, Zhao J, Wang Y, Liu L, Hu Y and Hou Y (2013) microRNA-29b contributes to preeclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. *Clin Sci (Lond)* 124, 27–40.
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14 Kohan-Ghadr HR, Kadam L, Jain C, Armant DR and Drewlo S (2016) Potential role of epigenetic mechanisms in regulation of trophoblast differentiation, migration, and invasion in the human placenta. *Cell Adh Migr* **10**, 126–135.

15 McGowan PO and Matthews SG (2018) Prenatal stress, glucocorticoids, and developmental programming of the stress response. *Endocrinology* **159**, 69–82.

16 Mezouar S and Mege J-L (2018) Gene expression profiling of placenta from normal to pathological pregnancies in placenta. [https://doi.org/10.5772/intechopen.80551](https://doi.org/10.5772/intechopen.80551).

17 Herold M, Bartkuhn M and Renkawitz R (2012) CTCF: insights into insulator function during development. *Development* **139**, 1045–1057.

18 Lang F, Li X, Zheng W, Li Z, Lu D, Chen G, Gong D, Yang L, Fu J, Shi P et al. (2017) CTCF prevents genomic instability by promoting homologous recombination-directed DNA double-strand break repair. *Proc Natl Acad Sci USA* **114**, 10912–10917.

19 Arzate-Mejia RG, Recillas-Targa F and Corces VG (2018) Developing in 3D: the role of CTCF in cell differentiation. *Development* **145**, dev137729.

20 Xu M, Zhao GN, Lv X, Liu G, Wang LY, Hao DL, Wang J, Liu DP and Liang CC (2014) CTCF controls HOXA cluster silencing and mediates PRC2-repressive higher-order chromatin structure in NT2/D1 cells. *Mol Cell Biol* **34**, 3867–3879.

21 Golan-Mashiach M, Grunspan M, Emmanuel R, Gibbs-Bar L, Dikstein R and Shapiro E (2012) Identification of CTCF as a master regulator of the clustered protocadherin genes. *Nucleic Acids Res* **40**, 3378–3391.

22 Splinter E, Heath H, Kooren J, Palstra RJ, Klous P, Grosveld F, Galjart N and de Laat W (2006) CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. *Genes Dev* **20**, 2349–2354.

23 Engel N, Thorvaldsen JL and Bartolomei MS (2006) CTCF binding sites promote transcription initiation and prevent DNA methylation on the maternal allele at the imprinted H19/IGf2 locus. *Hum Mol Genet* **15**, 2945–2954.

24 Roy AR, Ahmed A, DiStefano PV, Chi L, Khryzha N, Galjart N, Wilson MD, Fish JE and Delgado-Olguin P (2018) The transcriptional regulator CCCTC-binding factor limits oxidative stress in endothelial cells. *J Biol Chem* **293**, 8449–8461.

25 Holwerda SJ and de Laat W (2013) CTCF: the protein, the binding partners, the binding sites and their chromatin loops. *Philos Trans R Soc Lond B Biol Sci* **368**, 20120369.

26 Camolotto S, Racca A, Rena V, Nores R, Patrìto LC, Genti-Raimondi S and Panzetta-Dutari GM (2010) Expression and transcriptional regulation of individual pregnancy-specific glycoprotein genes in differentiating trophoblast cells. *Placenta* **31**, 312–319.

27 Camolotto SA, Racca AC, Ridano ME, Genti-Raimondi S and Panzetta-Dutari GM (2013) PSG gene expression is up-regulated by lysine acetylation involving histone and nonhistone proteins. *PLoS One* **8**, e55992.

28 Blois SM, Tirado-Gonzalez I, Wu J, Barrientos G, Johnson B, Warren J, Freitag N, Klapp BF, Irmak S, Ergun S et al. (2012) Early expression of pregnancy-specific glycoprotein 22 (PSG22) by trophoblast cells modulates angiogenesis in mice. *Biol Reprod* **86**, 191.

29 Ehrlich M, Gama-Sosa MA, Huang LH, Midgett RM, Kuo KC, McCune RA and Gehrke C (1982) Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res* **10**, 2709–2721.

30 Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, Miyazaki T, Ogura C, Okazaki Y and Jinno Y (2004) Age related changes in 5-methylcytosine content in human peripheral leukocytes and placenta: an HPLC-based study. *Ann Hum Genet* **68**, 196–204.

31 Zhou Y, Liu S, Hu Y, Fang L, Gao Y, Xia H, Schroeder SG, Rosen BD, Connor EE, Li CJ et al. (2020) Comparative whole genome DNA methylation profiling across cattle tissues reveals global and tissue-specific methylation patterns. *BMC Biol* **18**, 85.

32 Martin E, Ray PD, Smeester L, Grace MR, Boggess K and Fry RC (2015) Epigenetics and preeclampsia: defining functional epimutations in the preeclamptic placenta related to the TGF-β pathway. *PLoS One* **10**, e0141294.

33 Ma M, Zhou QJ, Xiong Y, Li B and Li XT (2018) Preeclampsia is associated with hypermethylation of IGF-1 promoter mediated by DNMT1. *Am J Transl Res* **10**, 16–39.

34 Chen PY, Chu A, Liao WW, Rubbi L, Janzen C, Hsu FM, Thamotharan S, Ganguly A, Lam L, Montoya D et al. (2018) Prenatal growth patterns and birthweight are associated with differential DNA methylation and gene expression of cardiometabolic risk genes in human placentas: a discovery-based approach. *Reprod Sci* **25**, 523–539.

35 Schroeder DI, Schmidt RJ, Cray-Dooley FK, Walker CK, Ozonoff S, Tancredi DJ, Hertz-Picciotto I and LaSalle JM (2016) Placental methylome analysis from a prospective autism study. *Mol Autism* **7**, 51.