Genes & Diseases (2023) 10, 650–653

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Decreased expression of m6A demethylase ALKBH5 in decidua contributes to preeclampsia via m6A-CORIN-HuR pathway

Preeclampsia (PE) is a pregnancy-specific hypertensive disorder which poses a severe threat to maternal and fetal health.[1] Defective decidualization may contribute to PE.[2] N6-methyladenosine (m6A) is associated with various diseases. The regulatory mechanism of m6A in PE is not well established to date. We aimed to identify differentially expressed m6A and explore its regulatory role in the pathogenesis of PE. Here we showed that m6A demethylase alkB homolog 5 (ALKBH5) was lower expressed in the decidua of PE. Silencing ALKBH5 suppressed the decidualization of human endometrial stromal cells (hESCs) and the invasion of trophoblast (HTR-8) cells into hESCs. The meRIP-seq analysis revealed the altered expression of ALKBH5 target genes after ALKBH5 knocking down, including CORIN. CORIN is a cardiac protease that activates atrial natriuretic peptide (ANP). Mechanism study indicated that ALKBH5 demethylated CORIN transcripts, maintained CORIN stability and enhanced CORIN expression through HuR dependent pathway, which was essential for ALKBH5 to regulate the decidualization of hESCs and the invasion of HTR-8 cells. In conclusion, our data indicate that low ALKBH5 expression in PE decidua inhibits the decidualization of hESCs and trophoblast cell invasion through the regulation of CORIN and ANP via the m6A-HuR-dependent pathway.

The clinical information of 42 participants of the study is presented in Table S1. There was no statistically significant difference in age between severe preeclampsia (SPE) and normal pregnancy (NP) group. However, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the SPE group were significantly higher than those of the NP group. The SPE patients' proteinuria was at least 2^{+}, while that of the NP group was negative. On the contrary, the SPE group's newborn birth weight and gestational age were lower than those of the NP group.

To explore the expression of m6A-modifying enzymes in the SPE group's decidual tissue, we first examined their expression by RT-qPCR. The mRNA levels of *ALKBH1*, *ALKBH5*, and *ALKBH6* in SPE group were significantly lower than those in NP tissue, and other m6A "writer", "eraser" and "reader" levels in the two groups were not different (Fig. S1A, B). We then focused on ALKBH5 in the study. We found increased global m6A levels in SPE group (Fig. S1C), and the expression of *ALKBH5* mRNA was negatively correlated with global m6A levels (Fig. S1D). Similarly, the decrease of ALKBH5 protein level in SPE group could be found compared with those of NP group (Fig. 1A, B). Besides, immunohistochemistry of paraffin sections from decidual tissue of both groups showed that the expression of ALKBH5 was identical to the Western blot results (Fig. S1E). These results suggested that ALKBH5 expression was decreased in SPE decidual tissue compared with NP group.

To analyze the expression of ALKBH5 during the process of decidualization, we induced decidualization using cAMP and MPA. After decidualization induction for 6 d, the mRNA expression of *PRL*, *IGFBP1*, and *ALKBH5* was elevated obviously in hESCs and primary hESCs, indicating that the treated cells had undergone decidualization (Fig. S2A, B). To determine the effects of *ALKBH5* knockdown on stromal cells' function, we used siRNA to decrease *AKBH5* expression in hESCs, and found loss of ALKBH5 inhibited the expression of *PRL* and *IGFBP1*, indicating ALKBH5 was required for decidualization (Fig. 1C).

To understand the regulatory role of ALKBH5 in decidualization, we compared m6A distribution in control (Ctrl) and *ALKBH5* knockdown hESCs. We mapped the m6A methylomes of *siCtrl* and *siALKBH5* hESCs by m6A sequencing (meRIP-seq). The AGACUC motif was identified to be highly enriched

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.05.032

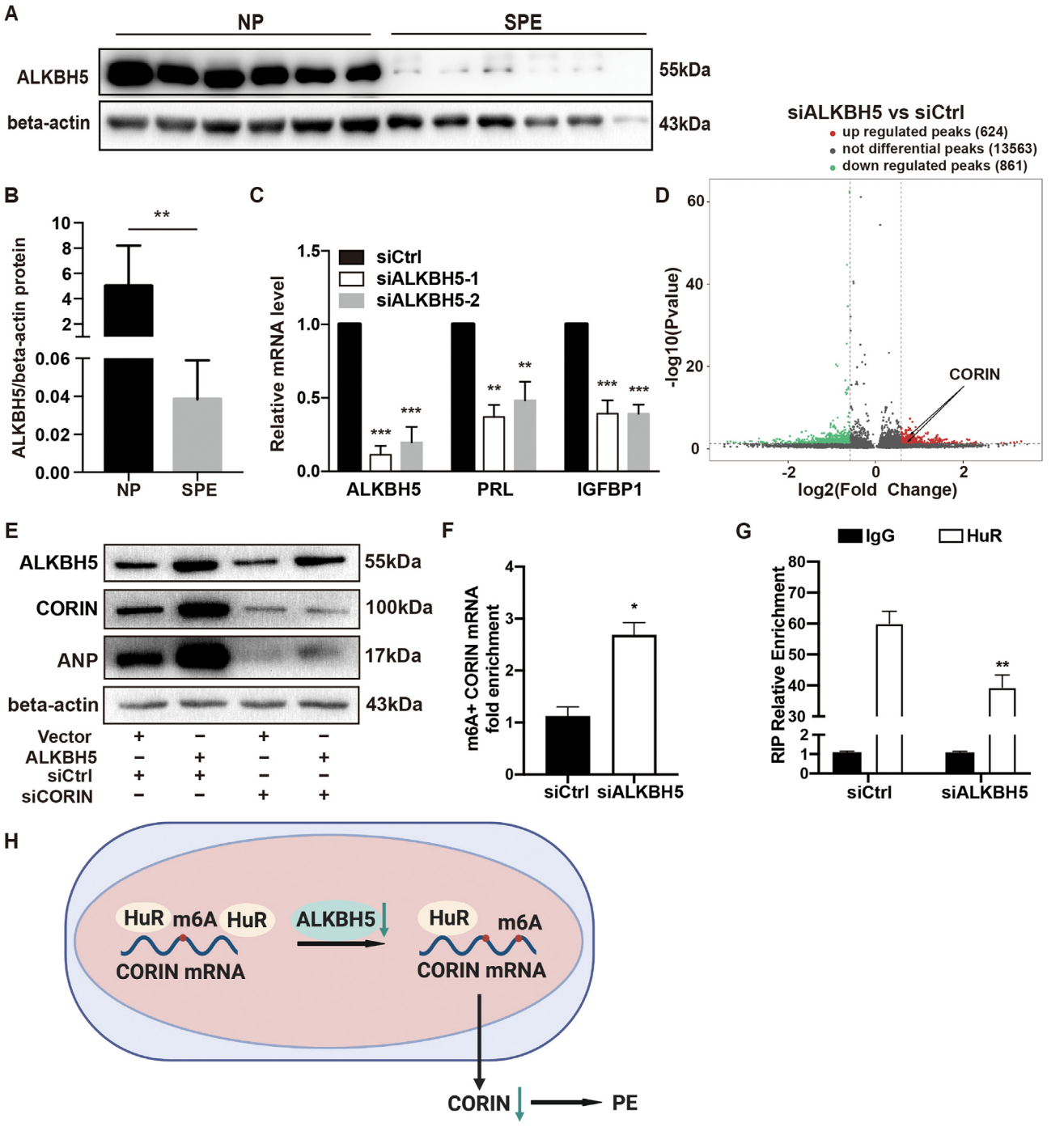

Figure 1 Decreased expression of ALKBH5 in SPE decidual tissue group contributes to the occurrence of PE. **(A, B)** Relative protein expression of in SPE and NP group. Beta-actin served as a loading control. **(C)** Expression of *PRL*, *IGFBP1*, and *ALKBH5* mRNA relative to *ACTB* after *ALKBH5* knockdown in hESCs. **(D)** Volcano plots displaying the distinct m6A peaks (fold change $\geq$1.5 and $P < 0.05$). *CORIN* was shown. **(E)** Vector and *ALKBH5* overexpressing cells were treated with si*Ctrl* or si*CORIN*; ALKBH5, CORIN, ANP protein levels were measured by Western blot in hESCs. **(F)** MeRIP-qPCR analysis of m6A levels of *CORIN* in hESCs with or without *ALKBH5* knockdown. **(G)** RIP-qPCR using anti-HuR antibody showed the affinity of *CORIN* RNA to HuR in hESCs with or without *ALKBH5* knockdown. **(H)** Schematic diagram showing our findings regarding the regulation of m6A directed by ALKHB5 on CORIN. Briefly, compared with the NP group, the expression of ALKBH5 in the decidual tissue of SPE was reduced. The deficiency of ALKBH5 increased the m6A level of *CORIN*, thereby reducing the binding of *CORIN* mRNA with HuR protein, consequently reducing the stability and expression of CORIN. Decreased CORIN damaged the decidualization of hESCs and the invasion of trophoblast cells into hESCs, thus promoted the development of PE. The data are expressed as the means $\pm$ SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; NC, negative control; NP, normal pregnancy; Scale bars = 100 μm; SPE, severe preeclampsia.

within m6A sites in hESCs (Fig. S2C). As Figure 1D displayed, compared with control, 624 significantly hypermethylated m6A peaks and 861 significantly hypomethylated m6A peaks were harvested in *ALKBH5*-deficient hESCs. Among these upregulated peaks, we selected CORIN, an important PE-related gene, as a candidate target of ALKBH5-mediated m6A modification for further investigation. From our meRIP-seq data, we identified two statistically significant m6A peaks of *CORIN* in si*ALKBH5* hESCs, the 660-size peak and the 390-size peak, and the 660-size peak contained the 390-size one (Fig. S2D). All mammalian members of the ELAV/Hu family, including HuR, regulate the stability and translation of their target mRNAs[3] We found HuR-CORIN binding site by CLIPdb, and then the binding site was transformed by LiftOver on the UCSC Genome Browser (http://genome.ucsc.edu). The results showed that the HuR binding site was close to the m6A modification site as revealed by our m6A-seq (Fig. S2D). In conclusion, CORIN was identified as an ALKBH5 downstream target.

Since *CORIN* could be a direct target gene of ALKBH5, we examined the regulatory mechanism of CORIN expression by ALKBH5. First, both hESCs and primary hESCs with *ALKBH5* knockdown displayed a lower *CORIN* mRNA expression (Fig. S3A). Consistently, *CORIN* mRNA increased when ALKBH5 was overexpressed (Fig. S3B). Furthermore, we demonstrated CORIN expression was downregulated in the decidua of SPE group compared with NP (Fig. S3C—E). Moreover, the number of invasive HTR-8 cells increased upon *ALKBH5* overexpression and decreased when *CORIN* was knocked down (Fig. S3F). To sum up, ALKBH5 regulates CORIN expression in hESCs and primary hESCs, which was essential for decidualization and trophoblast invasion.

To further support the notion that ALKBH5 targets *CORIN* mRNA through m6A modification, we validated the m6A-sequencing data set using meRIP-qPCR. Using specific primers to detect the peak region, we found m6A level was upregulated in this region in siALKBH5 cells (Fig. 1F). Then, we constructed luciferase reporters containing either the wild-type or mutant *CORIN* to address the effect of m6A modification on its expression. For the mutant CORIN, m6A modification was abrogated because of the replacement of adenosine base by cytosine in m6A consensus sequences (RRACH) (Fig. S4A). Luciferase reporter assay showed that the transcriptional level of wild-type *CORIN*, but not the mutation, significantly decreased or increased in the absence or overexpression of ALKBH5 (Fig. S4B, C), revealing the regulation of CORIN level was under the control of ALKBH5 associated m6A modification. To further explore the negative correlation between methylation and CORIN expression, we studied RNA-binding proteins. HuR has been shown to disfavor binding with m6A-modified RNA and to exert stabilizing effects on its bound RNA[4] RIP-qPCR using anti-HuR antibody showed dramatically reduced affinity of HuR to *CORIN* mRNA in *ALKBH5*-silenced hESCs (Fig. 1G). These data suggested that HuR played an essential role in the regulation of CORIN by ALKBH5, and it was the affinity of the reader to the target gene that affected decidualization in PE.

In the study, we verified that m6A demethylase ALKBH5 was decreased in the decidual tissue of PE patients, and ALKBH5 was essential to decidualization. By meRIP-seq and meRIP-qPCR, we found *ALKBH5* knockdown increased the m6A level of *CORIN* mRNA. The increase in m6A reduced the binding of HuR protein to *CORIN* mRNA, which decreased the stability of *CORIN* mRNA and protein. Downregulated CORIN reduced the production of ANP, suppressed the differentiation of endometrium stromal cells, subsequently jeopardized the invasion of trophoblasts into the decidua that is inadequately decidualized, all of which likely contributed to the occurrence of PE. The process is briefly depicted in Figure 1H.

In conclusion, we demonstrate that ALKBH5 deficiency may suppress the decidualization of human endometrium, and CORIN is a key component in mediating ALKBH5-dependent decidualization in PE. These findings might open up avenues for developing effective therapeutic strategies for PE treatment and indicate their potential value in PE prognosis.

Conflict of interests

The authors declared no conflict of interests.

Funding

This study was supported by grants from the National Key R&D Program of China (No. 2019YFA0802600) and NSFC (No. 32170863, 31871512) to C. Zhang. Support was also obtained from the Shanghai Commission of Science and Technology (No. 17DZ2271100) and Open Project of Shandong Provincial Key Laboratory of Reproductive Medicine (No. SDKL2017018), China.

Acknowledgements

The authors would like to thank all patients and their relatives for their participation and help during this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.032.

References

1. Gestational hypertension and preeclampsia: ACOG practice bulletin, number 222. *Obstet Gynecol*. 2020;135(6):e237—e260.
2. Zhang C, Large MJ, Duggavathi R, et al. Liver receptor homolog-1 is essential for pregnancy. *Nat Med*. 2013;19(8):1061—1066.
3. Mukherjee N, Corcoran DL, Nusbaum JD, et al. Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol Cell*. 2011;43(3):327—339.
4. Wang Y, Li Y, Toth JI, et al. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat Cell Biol*. 2014;16(2):191—198.

Jieqiong Yang [a,b,c,d], Jing Tong [b,d], Hongwanyu Li [b,d], Shijian Lv [b,d], Weiya Li [b,d], Cong Zhang [b,d,e,**], Wen Di [a,c,f,*]

[a] *Department of Obstetrics and Gynecology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China*

[b] *Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, China*
[c] *Shanghai Key Laboratory of Gynecologic Oncology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China*
[d] *Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, China*
[e] *Shandong Provincial Key Laboratory of Animal Resistance Biology, College of Life Sciences, Shandong Normal University, Shandong 250014, China*
[f] *State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China*

*Corresponding author. Department of Obstetrics and Gynecology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.

**Corresponding author. Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, China.
E-mail addresses: zhangxinyunlife@163.com (C. Zhang), diwen163@163.com (W. Di)

11 April 2022
Available online 10 June 2022