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# Loss of high-mobility group N5 contributes to the promotion of human endometrial stromal cell decidualization

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## Abstract

**Purpose**: High-mobility group N (HMGN) proteins are the only non-histone proteins that specifically bind within the nucleosome between core histones and DNA. Among them, HMGN5 is one of the candidates that could participate in mouse endometrial decidualization; however, the specific role of HMGN5 remains to be clarified in human endometrial stromal cells (HESCs).

**Methods**: Primary HESCs were isolated from hysterectomy specimens and incubated with or without 8-bromo-cyclic adenosine monophosphate (8-br-cAMP) and medroxyprogesterone acetate (MPA).

**Results**: We demonstrated that HMGN5 expression in decidualized HESCs stimulated by 8-br-cAMP and MPA decreased significantly. The inhibition of HMGN5 expression by small interfering RNA (siRNA) induced the major decidual marker genes expression, including *IGFBP1* (insulin-like growth factor binding protein 1) and *PRL* (prolactin). In addition, microRNA-542-3p (miR-542-3p), which was identified as a regulatory miRNA of *IGFBP1* during decidualization, was significantly suppressed by HMGN5 siRNA. However, the expression of HMGN5 was not alternated by miR-542-3p overexpression.

**Conclusions**: These findings suggest that the down-regulation of HMGN5 plays a role in the promotion of human endometrial stromal decidualization and acts upstream of miR-542-3p.

KEYWORDS

decidualization, endometrium, HMGN5, IGFBP-1, miR-542-3p

## 1 | INTRODUCTION

Decidualization is the differentiation process of endometrial stromal cells into specialized secretory decidual cells that regulates blastocyst implantation and subsequent placental formation.[1,2] This process denotes both a morphological and biochemical transformation that is driven by the post-ovulatory rise in circulating progesterone levels and increasing local cAMP production.[2,3] Decidualization is also essential to establish a functional feto-maternal interface because it regulates endovascular trophoblast invasion, tissue homeostasis, and could grant resistance to environmental oxidative stress.[4–6] Abnormalities in decidualization can be followed by various pregnancy complications, such as recurrent miscarriages, fetal growth restriction, preterm labor, and preeclampsia.[3] While there is some understanding of this process, it has not been fully elucidated what the key regulatory factors is contributing to differentiation of uterine stromal cells into decidual cells.

High-mobility group N (HMGN) proteins are ubiquitous nuclear proteins among the HMG superfamily that target chromatin and are the only nuclear proteins shown to specially bind to the nucleosome core particle, the building block of the chromatin fiber.[7] HMGN proteins influence epigenetic modifications and modulate the structure and function of chromatin; thus, they play an important role in transcription.[8] HMGN5 (also known as NSBP1) is a new member of the HMGN protein family and has a unique molecular structure compared to the other members of the HMGN protein family.[9,10] HMGN5 contains a typical HMGN nucleosome-binding domain and comprises a long C-terminal region.[9,11] When HMGN5 binds to core nucleosome particles, there is a reduction in the compacting of chromatin fiber, leading to altered transcription.[11] HMGN5 is broadly distributed in various tissues and is mainly located in the nucleus.[9,10] While transcription profiles indicate that HMGN5 affected the expression of more than 2000 genes,[11] the biological role of HMGN5 is not yet fully characterized.

Sakai et al. reported that structural changes of histones play an important role in human endometrium stromal cells (HESCs) during the decidualization process. Histone acetyltransferases and histone deacetylases (HDACs) can induce differentiation and have potential as enhancers of decidualization of HESCs.[12] In addition, it has been shown that HMGN5 regulates the endometrial decidualization process in mice. However, the specific role of HMGN5 in HESCs has not yet been clarified. In the last decade, several studies demonstrated microRNAs (miRNAs) had important roles for endometrial decidualization. In addition, we recently identified miR-542-3p as a negative regulatory miRNA of IGFBP1 which was well known as the decidual marker genes.[13] Therefore, this study was undertaken to investigate the expression, regulation, and function of HMGN5 through miR-542-3p during the decidualization of HESCs.

## 2 | MATERIALS AND METHODS

### 2.1 | Isolation of HESCs and cell culture

The Institutional Review Board of the Saitama Medical University Hospital approved this study, and informed consent was obtained before tissue collection from all patients. HESCs were obtained from uterine fibroids patients of normal menstrual cycle, and of not receiving hormonal treatment before operation. HESCs were isolated and cultured as previously described.[4,13–15] Decidualization treatments of HESCs were performed by 0.5 mM 8-bromo-cyclic adenosine monophosphate (8-br-cAMP; Sigma-Aldrich, St. Louis, MO, USA) and $10^{-6}$ M medroxyprogesterone acetate (MPA; Sigma-Aldrich, St. Louis, MO, USA) for each indicated time point.

### 2.2 | Prolactin measurement

The concentration of PRL in the HESC culture media was measured by the electrogenerated chemiluminescence immunoassay (ECLIA) method using ECLusys Prolactin III reagent and Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

### 2.3 | Transfection of siRNA and miRNA mimics

The siRNA targeting HMGN5 (HS0107792; Takara bio Inc., Japan) was transfected to HESCs using Xfect RNA Transfection Reagent (Takara Shuzo, Shiga, Japan). The mirVana™ miRNA mimic (#MC11340; Thermo Fisher Scientific, Waltham, MA, USA) or hsa-miR-542-3p was transfected to HESCs using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Control cultures were transfected with non-targeting siRNA or a control miRNA mimic. After 4 or 6 hours, the medium was changed to control medium or decidual medium.

### 2.4 | Total RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA from HESCs were extracted by miRNeasy Mini kit (Qiagen, Hilden, Germany). For analysis of mRNAs, the synthesis of cDNA from total RNA by reverse transcription was performed using BioScript reverse transcriptase (Bioline, London, UK) with oligo (dT) 18 primer. qRT-PCR was measured using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences for each gene were shown in Table 1. For analysis of miRNAs, cDNA was synthesized using the TaqMan microRNA RT kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was measured by TaqMan Universal Master Mix II with UNG (Thermo Fisher Scientific, Waltham, MA, USA). For miR-542-3p assay, TaqMan MicroRNA assays and the endogenous control U6 (Thermo Fisher Scientific, Waltham, MA, USA) were used for reverse transcription and amplification as previously reported.[13] Eash qRT-PCR determined using the PikoReal 96 real-time PCR system. The expression level of *HMGN5*, *IGFBP1*, *PRL*, and *WNT4* mRNA expression relative to *GAPDH* or miR-542-3p expression relative to U6 were calculated by the $2^{-\Delta\Delta ct}$ method.[16]

**TABLE 1** Primer sequence for qRT-PCR

| Primer name | Primer sequence (5′ to 3′) |
|---|---|
| *HMGN5*-forward | CTGCCTTCGGCTTTTTTTCTG |
| *HMGN5*-reverse | AGACAACCTGGCAGATCTTCTC |
| *GAPDH*-forward | CGACCACTTTGTCAAGCTCA |
| *GAPDH*-reverse | AGGGGTCTACATGGCAACTG |
| *IGFBP1*-forward | CTGCGTGCAGGAGTCTGA |
| *IGFBP1*-reverse | CCCAAAGGATGGAATGATCC |
| *PRL*-forward | CTACATCCATAACCTCTCCTCA |
| *PRL*-reverse | GGGCTTGCTCCTTGTCTTC |
| *WNT4*-forward | CATGCAACAAGACGTCCAAG |
| *WNT4*-reverse | AAGCAGCACCAGTGGAATTT |

### 2.5 | Immunofluorescent staining

HESCs were cultured on cover glass before confluence and treatment with or without decidualization medium 3 days. After 3 days, the cells were fixed by 4% paraformaldehyde and stained using primary HMGN5 antibody (Abcam, Cambridge, UK) and the secondary Alexa Fluor 488 antibody (Thermo Fisher Scientific, Waltham, MA, USA). The mounting media was used containing DAPI (Vector Laboratories, Burlingame, CA, USA) and visualized by a fluorescent microscope (Axiocam; Carl Zeiss, Oberkochen, Germany).

### 2.6 | Western blotting

Cells ware lysed by RIPA (Radio-Immunoprecipitation Assay) buffer (WAKO Pure Chemical, Osaka, Japan) with Protein inhibitor, cOmplete Tablets EDTA-free (Roche, Mannheim, Germany). The protein extracts were separated by SDS-polyacrylamide gel electrophoresis and transferred to Amersham Protran nitrocellulose blotting membrane (GE Healthcare, Buckinghamshire, UK). anti-HMGN5 rabbit polyclonal antibody (Abcam, Cambridge, UK) or anti-α-Tubulin rabbit polyclonal antibody (Protein Tech, Rosemont, USA) was used for primary antibody. The Western blots were probed with anti-Rabbit IgG, HRP-Linked Whole Ab Donkey (GE Healthcare, Buckinghamshire, UK). The recognized proteins were detected using ECL prime Western blotting detection kits (GE Healthcare, Buckinghamshire, UK) by c-Digit Blot Scanner (LI-COR, NE, USA).

### 2.7 | Statistic analyses

Each experiment was repeated more than three different cell culture preparations (n = 3-6). Statistical analyses were performed by two-tailed Student's *t*-test, and data were indicated by mean ± standard error. *P* value of <0.05 was considered significant.

## 3 | RESULTS

### 3.1 | *HMGN5* expression in HESCs decreased upon decidualization

The efficiency of decidualization of HESCs was confirmed by characteristic morphological changes and evaluating *IGFBP1* and *PRL* mRNA expression, which are widely used as biological decidual

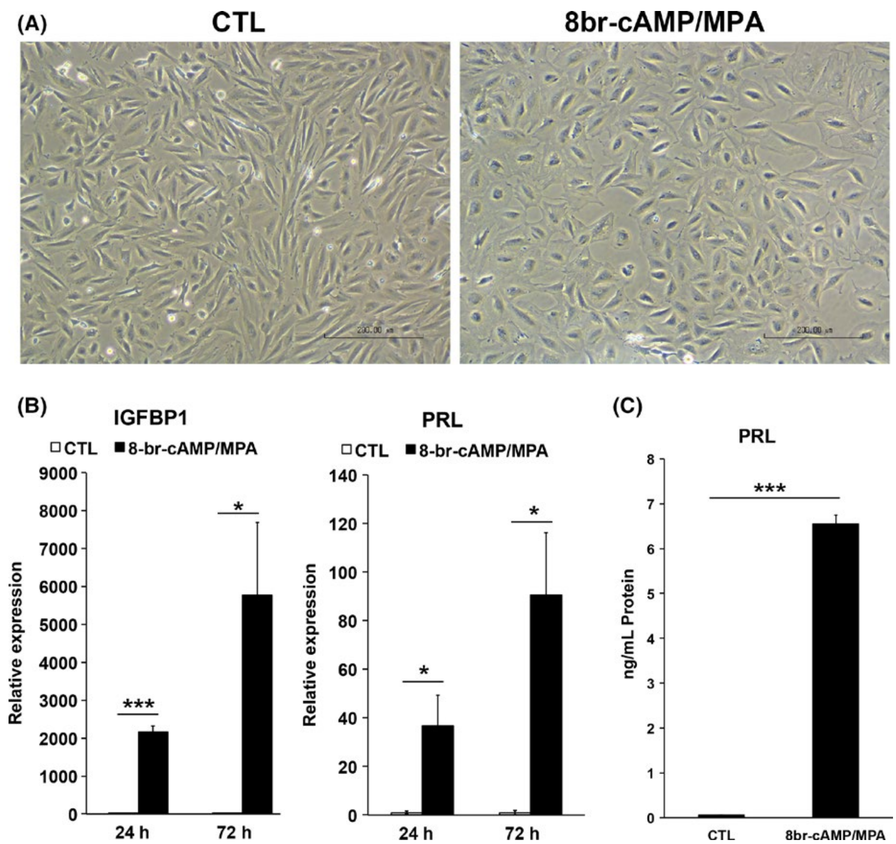


**FIGURE 1** Morphological transformation and *IGFBP1* and *PRL* expression in decidualizing HESCs. (A) Undifferentiated primary HESCs exhibit a fibroblastic spindle-shaped morphology (CTL). Primary HESCs treated with 8-br-cAMP (0.5 mM) and MPA ($10^{-6}$ M) (8-br-cAMP/MPA) for 3 days transform the spindle-shaped cells into cells with larger nuclei and abundant cytoplasm, which are the typical morphology of decidual cells. Scale bar indicates 200 µm. (B) Primary HESCs were stimulated in the absence (CTL) or presence of 8-br-cAMP and MPA (8-br-cAMP/MPA). Expression of *IGFBP1* and *PRL* mRNA, were measured by RT-qPCR and normalized to GAPDH (n = 4-6). (C) Confluent HESCs were treated with or without 8-br-cAMP and MPA for 3 days. The data represent the mean of PRL protein concentration in the supernatant (n = 6). The data represent mean ± standard error. *P < 0.05, ***P < 0.001

markers.[13,17–20] As reported previously,[13,17,20–22] HESCs appear as spindle-shaped fibroblast-like cells on light microscopy when cultured without treatment. Treatment with 8-br-cAMP and MPA induced the decidual phenotype, characterized by larger and rounder cells (Figure 1A), as well as significantly induced the expression of *IGFBP1* and *PRL* mRNA in a time-dependent manner (n = 4-6) (Figure 1B). Consistent with qRT-PCR analysis, PRL secretion in HESCs was significantly induced by treatment with 8-br-cAMP and MPA (n = 6) (Figure 1C). To explore the potential role of HMGN5 in the decidualization process of HESCs, the expression and localization patterns of HMGN5 in non-decidualized and decidualized HESCs were examined by qRT-PCR and immunofluorescent staining. The expression level of HMGN5 in 8-br-cAMP- and MPA-treated HESCs was significantly inhibited compared with control cells after 12 and 24 hours (Figure 2). However, this inhibitory effect of *HMGN5* mRNA expression in decidualized HESCs disappeared after 3 days of culture (n = 3-6). In agreement with the RNA analysis, confocal microscopy demonstrated that staining of HMGN5 protein in non-decidualized HESCs was localized in the nucleus, and the immunoreactivity decreased upon decidualization (Figure 3A). Furthermore, we conformed that HMGN5 protein expression was inhibited in HESCs decidualizaed with 8-br-cAMP and MPA (n = 3) (Figure 3B).

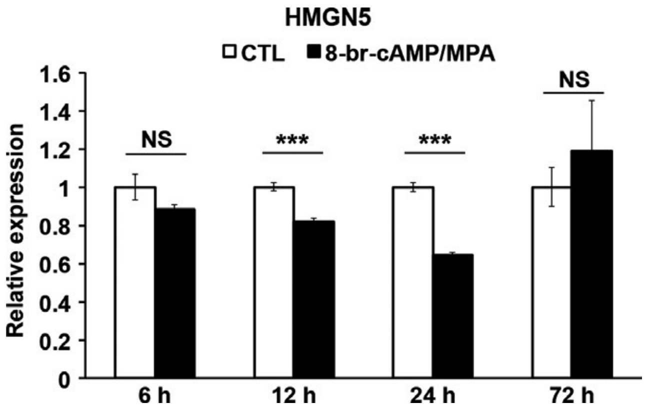


**FIGURE 2** *HMGN5* mRNA expression is decreased upon decidualization. *HMGN5* expression was determined at 6, 12, 24, and 72 hours by qRT-PCR. Primary HESCs were stimulated in the absence (CTL) or presence of 8-br-cAMP and MPA (8-br-cAMP/MPA). *HMGN5* mRNA expression levels were determined by RT-qPCR and normalized to GAPDH. *HMGN5* mRNA levels decreased significantly at 12 and 24 hours by decidualization (n = 3-6). The data represent mean ± standard error. *$P < 0.05$, ***$P < 0.001$

## 3.2 | Loss of HMGN5 promotes decidualization

Next, to determine the functional significance of HMGN5 expression during HESC differentiation, we transfected primary HESCs with either non-targeting siRNA or siRNA targeting *HMGN5*; we then cultured the cells in medium containing 8-br-cAMP and MPA for 3 days. To evaluate the transfection efficiency, qRT-PCR for *HMGN5* was performed. Compared with cells transfected with non-targeting siRNA, *HMGN5* expression in response to 8-br-cAMP and MPA treatment was markedly down-regulated by approximately 60% in cells transfected with siRNA targeting *HMGN5* (n = 3-4) (Figure 4A). The mRNA expression of the decidual marker genes, *IGFBP1* and *PRL*, but not *WNT4*, was significantly increased by siRNA targeting *HMGN5* (n = 3-4) (Figure 4B-4D).

## 3.3 | miR-542-3p is involved in the decreased expression of HMGN5

HMGN5 binds to core nucleosome particles and can affect transcription.[8–11] This study demonstrated that loss of HMGN5 expression induces the expression of decidual markers IGFBP-1 and PRL and, as a result, promotes the decidualization process; thus, there is a possibility of a factor responsible for the down-regulation seen between HMGN5 and the decidual marker genes. Recently, we demonstrated that the down-regulation of miR-542-3p promotes decidualization

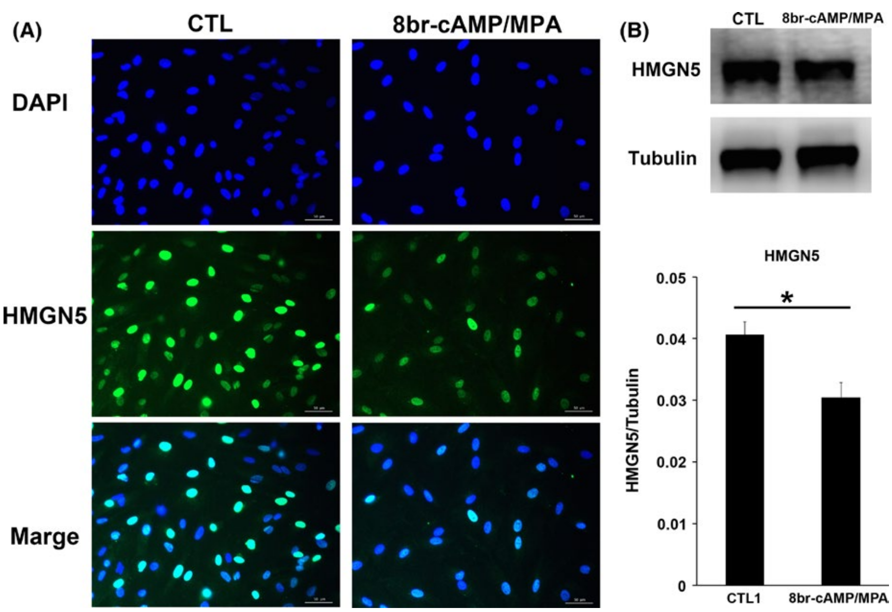


**FIGURE 3** Localization and expression of HMGN5 in undecidualized and decidualized HESCs. (A) The localization of HMGN5 expression in HESCs cultured in the absence (CTL) or presence of 8-br-cAMP and MPA (8-br-cAMP/MPA) at day 3 was visualized by fluorescent microscopy using the HMGN5/NSBP1 antibody (green; Alexa Fluor 488) and DAPI nuclear staining (blue). (B) Expression of HMGN5 proteins induced in HESCs by treatment with 8-br-cAMP and MPA for 3 days. α-Tubulin expression was used as loading control. The data represent mean ± standard error. *$P < 0.05$

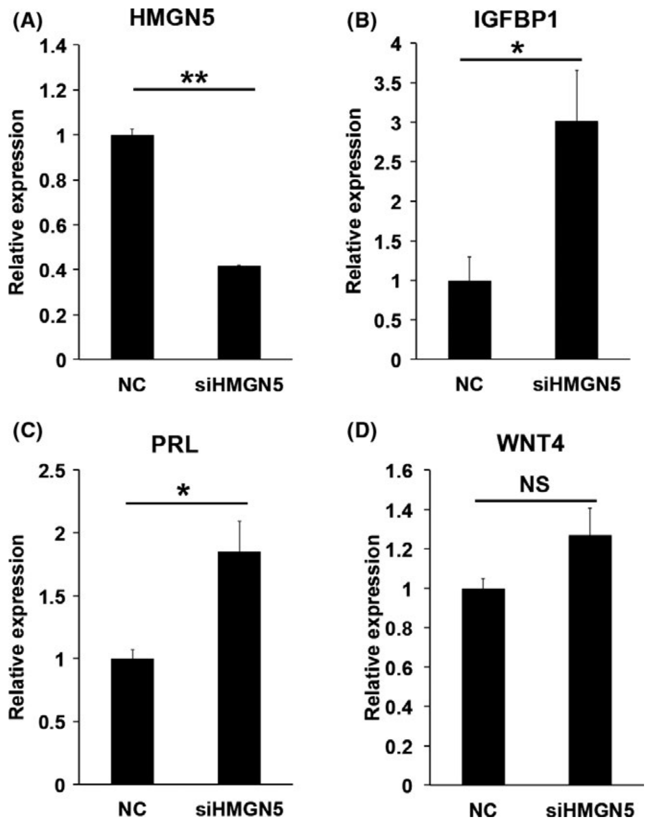


**FIGURE 4** Effect of HMGN5 knockdown by siRNA for decidualization. Small interfering RNA (siRNA) for HMGN5 was transfected into HESCs. (A) Efficiency of siRNA for HMGN5 knockdown (siHMGN5) was approximately 60% compared with cells transfected with non-targeting siRNA (NC) by qRT-PCR. The expressions of decidual marker genes (B) *IGFBP1*, (C) *PRL*, and (D) *WNT4* were also measured (n = 3-4). The data represent mean ± standard error. *$P$ < 0.05, **$P$ < 0.01

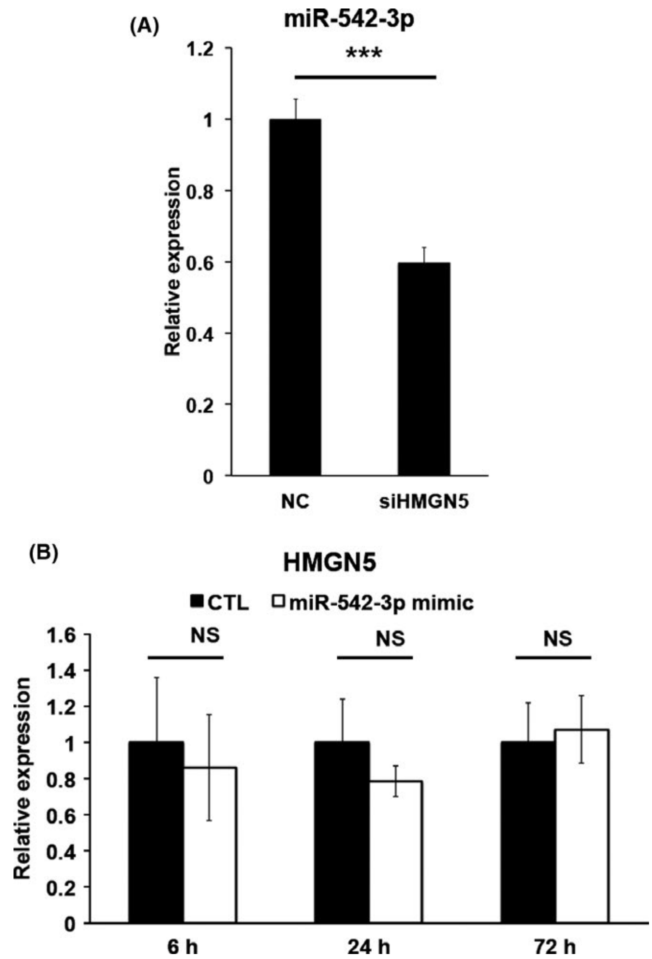


**FIGURE 5** miR-542-3p is involved in the decreased expression of *HMGN5*. (A) miR-542-3p expression in decidualized HESCs was decreased under the knockdown of *HMGN5* by siRNA compared with cells transfected with non-targeting siRNA by qRT-PCR (n = 6). (B) The expression of *HMGN5* under the miR-542-3p mimic in HESCs cultured in the absence (CTL) or presence of 8-br-cAMP and MPA (8-br-cAMP/MPA) was not significantly different at 6 hour, 24 hour, and day 3 by qRT-PCR (n = 5-6). The data represent mean ± standard error. ***$P$ < 0.001

by targeting *IGFBP1*.[15] Therefore, we postulated that HMGN5 may indirectly regulate *IGFBP1* expression through miR-542-3p. To elucidate this hypothesis, we investigated miR-542-3p expression under knockdown of HMGN5 in decidualized HESCs. HMGN5 knockdown was sufficient to inhibit miR-542-3p expression (n = 6) (Figure 5A). To examine whether HMGN5 is downstream of miR-542-3p, we investigated the alternation of HMGN5 expression under miR-542-3p overexpression using a miR-542-3p mimic in decidualized HESCs. miR-542-3p overexpression was insufficient to alternate HMGN5 expression during all culture periods (n = 5-6) (Figure 5B), suggesting that HMGN5 indirectly regulates *IGFBP1* expression through miR-542-3-p (Figure 6).

## 4 | DISCUSSION

Decidualization is characterized by the transformation of endometrial cells into specialized secretory cells, with a distinctive process of vascular remodeling and an influx of immune cells into the stroma, that is, exclusively uterine natural killer cells and macrophages.[3] This process is closely controlled by several factors, including ovarian steroids, growth factors, cytokines, and other signal molecules.[23,24] Abnormalities in decidualization result in various pregnancy complications, such as recurrent miscarriages, fetal growth restriction, preterm labor, and pre-eclampsia.[3] However, key regulatory factors contributing to the differentiation of uterine stromal cells have not been fully elucidated. Thus, it is worthwhile to clarify the precise mechanism underlying decidualization to facilitate the development of therapeutic strategies for these complications.

In this study, we demonstrated that *HMGN5* mRNA expression decreases upon decidualization. The inhibition of *HMGN5* expression by HMGN5 siRNA promoted the induction of major decidual marker genes, including *IGFBP1* and *PRL*. Moreover, miR-542-3p expression, which is a known regulator of *IGFBP1* expression during decidualization, was significantly suppressed by HMGN5 siRNA. These findings suggest that the down-regulation of HMGN5 expression contributes

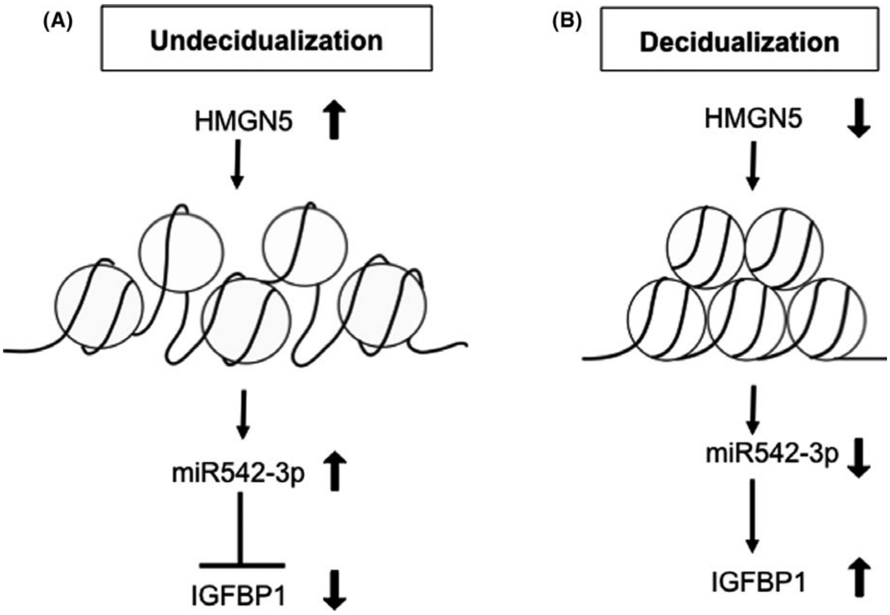


**FIGURE 6** Proposed mechanisms by which HMGN5 contributes to the promotion of human endometrial stromal cell decidualization. (A) In undecidualized HESCs, high levels of HMGN5 expression reduces the compacting of chromatin fiber and induces transcription level of miR542-3p, which in turn down-regulates IGFBP1 expression. (B) In decidualized HESCs, low expression level of HMGN5 induces the compacting of chromatin fiber and inhibits transcription level of miR542-3p. Then down regulation of miR542-3p induces IGFBP1 expression

to the promotion of human endometrial stromal decidualization, acting upstream of miR-542-3p (Figure 6A,B).

Shirakawa et al. demonstrated that HMGN5 is highly expressed in mouse trophoblasts, and its expression is related to placental formation.[25] It has recently been reported that HMGN5 expression is induced by decidual stimulation in a mouse in vitro system[26]; however, this finding contradicts the results of this study. This discrepancy may be due to differences in the species studied or the decidualization protocol used, that is, Li et al. adopted a combination of estradiol and progesterone to induce decidualization, which is a much weaker stimulus than treatment with a cAMP analog and MPA.[18,19] To the best of our knowledge, this study is the first report to demonstrate that HMGN5 regulates differentiation of HESCs through decidual gene targeting via miR-542-3p expression.

As small non-coding RNAs, miRNAs were identified as regulatory RNAs that act by silencing translation or reducing the stability of mRNA and have been reported to regulate various biological processes, including cell growth, proliferation, and differentiation.[27] It is well known that abnormal miRNA expression is related to various endometrial disorders, such as endometriosis, endometrial hyperplasia, and carcinoma.[28,29] To date, several studies have revealed that miRNA plays an important role during endometrial decidualization.[30,31] Recently, we reported that the down-regulation of miR-542-3p expression in HESCs enables the morphological and biological differentiation of the endometrium.[13,17] Initially, we hypothesized that HMGN5 directly regulates decidual gene expression; however, contrary to our expectation, a reciprocal relationship was found between the expression of HMGN5 and IGFBP-1 during decidualization in this study (Figure 2). This finding raised the possibility of a factor responsible for the down-regulation seen between HMGN5 and IGFBP-1. Therefore, we focused on miR-542-3p as a candidate.

In this study, we demonstrated that HMGN5 expression in decidualized HESCs was down-regulated early during the culture period compared with non-decidualized HESCs. Previous studies have suggested that decidual transformation is at least a biphasic process, characterized initially by an acute auto-inflammatory phase, followed by a profound anti-inflammatory response.[32,33] Therefore, HMGN5 may regulate genes associated with the acute auto-inflammatory phase. In addition, we showed that the mRNA expression of decidual marker genes, *IGFBP1* and *PRL*, but not *WNT4*, increased significantly by siRNA targeting *HMGN5*. However, we previously showed that the overexpression of miR-542-3p inhibited *IGFBP1*, *PRL*, and *WNT4* mRNA expressions in decidualized HESCs. Other unknown regulatory factors of *WNT4* may account for this finding, and further studies are required to resolve this discrepancy.

In conclusion, this study demonstrated that the down-regulation of HMGN5 promotes the differentiation of HESCs through miR-542-3p expression. Our results indicate that HMGN5 is involved in human endometrial decidualization by regulating miR-542-3p expression.

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## DISCLOSURE

*Conflict of interest*: The authors declare no conflict of interest. *Human Rights Statement and informed Consent*: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institution and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients included in the study. The

Institutional Review Board of the Saitama Medical University Hospital approved this study. *Animal Studies*: This article does not contain any studies with animal subjects performed by any of the authors.

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## REFERENCES

1. Kajihara T, Brosens JJ, Ishihara O. The role of FOXO1 in the decidual transformation of the endometrium and early pregnancy. *Med Mol Morphol*. 2013;46:61-68.
2. Gellersen B, Brosens J. Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *J Endocrinol*. 2003;178:357-372.
3. Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod*. 2007;25:445-453.
4. Kajihara T, Jones M, Fusi L, et al. Differential expression of FOXO1 and FOXO3a confers resistance to oxidative cell death upon endometrial decidualization. *Mol Endocrinol*. 2006;20:2444-2455.
5. Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG, Roberts CT. Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. *Hum Reprod Update*. 2010;16:415-431.
6. Schneider H. Oxygenation of the placental-fetal unit in humans. *Respir Physiol Neurobiol*. 2011;178:51-58.
7. Bustin M. Chromatin unfolding and activation by HMGN(*) chromosomal proteins. *Trends Biochem Sci*. 2001;26:431-437.
8. Hock R, Furusawa T, Ueda T, Bustin M. HMG chromosomal proteins in development and disease. *Trends Cell Biol*. 2007;17:72-79.
9. Shirakawa H, Landsman D, Postnikov YV, Bustin M. NBP-45, a novel nucleosomal binding protein with a tissue-specific and developmentally regulated expression. *J Biol Chem*. 2000;275:6368-6374.
10. King LM, Francomano CA. Characterization of a human gene encoding nucleosomal binding protein NSBP1. *Genomics*. 2001;71:163-173.
11. Rochman M, Postnikov Y, Correll S, et al. The interaction of NSBP1/HMGN5 with nucleosomes in euchromatin counteracts linker histone-mediated chromatin compaction and modulates transcription. *Mol Cell*. 2009;35:642-656.
12. Sakai N, Maruyama T, Sakurai R, et al. Involvement of histone acetylation in ovarian steroid-induced decidualization of human endometrial stromal cells. *J Biol Chem*. 2003;278:16675-16682.
13. Tochigi H, Kajihara T, Mizuno Y, et al. Loss of miR-542-3p enhances IGFBP-1 expression in decidualizing human endometrial stromal cells. *Sci Rep*. 2017;7:40001.
14. Christian M, Zhang X, Schneider-Merck T, et al. Cyclic AMP-induced forkhead transcription factor, FKHR, cooperates with CCAAT/enhancer-binding protein beta in differentiating human endometrial stromal cells. *J Biol Chem*. 2002;277:20825-20832.
15. Leitao B, Jones MC, Fusi L, et al. Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals. *FASEB J*. 2010;24:1541-1551.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25:402-408.
17. Sultana S, Kajihara T, Mizuno Y, et al. Overexpression of microRNA-542-3p attenuates the differentiating capacity of endometriotic stromal cells. *Reprod Med Biol*. 2017;16:170-178.
18. Cloke B, Huhtinen K, Fusi L, et al. The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. *Endocrinology*. 2008;149:4462-4474.
19. Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology*. 1999;140:4809-4820.
20. Grimaldi G, Christian M, Steel JH, Henriet P, Poutanen M, Brosens JJ. Down-regulation of the histone methyltransferase EZH2 contributes to the epigenetic programming of decidualizing human endometrial stromal cells. *Mol Endocrinol*. 2011;25:1892-1903.
21. Kajihara T, Tochigi H, Prechapanich J, et al. Androgen signaling in decidualizing human endometrial stromal cells enhances resistance to oxidative stress. *Fertil Steril*. 2012;97:185-191.
22. Kajihara T, Tanaka K, Oguro T, et al. Androgens modulate the morphological characteristics of human endometrial stromal cells decidualized in vitro. *Reprod Sci*. 2014;21:372-380.
23. Brosens JJ, Gellersen B. Death or survival–progesterone-dependent cell fate decisions in the human endometrial stroma. *J Mol Endocrinol*. 2006;36:389-398.
24. Brosens JJ, Pijnenborg R, Brosens IA. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol*. 2002;187:1416-1423.
25. Shirakawa H, Rochman M, Furusawa T, et al. The nucleosomal binding protein NSBP1 is highly expressed in the placenta and modulates the expression of differentiation markers in placental Rcho-1 cells. *J Cell Biochem*. 2009;106:651-658.
26. Li DD, Zhao SY, Yang ZQ, et al. Hmgn5 functions downstream of Hoxa10 to regulate uterine decidualization in mice. *Cell Cycle*. 2016;15:2792-2805.
27. Mizuno Y, Yagi K, Tokuzawa Y, et al. miR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. *Biochem Biophys Res Comm*. 2008;368:267-272.
28. Kuokkanen S, Chen B, Ojalvo L, Benard L, Santoro N, Pollard JW. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. *Biol Reprod*. 2010;82:791-801.
29. Santamaria X, Taylor H. MicroRNA and gynecological reproductive diseases. *Fertil Steril*. 2014;101:1545-1551.
30. Estella C, Herrer I, Moreno-Moya JM, et al. miRNA signature and Dicer requirement during human endometrial stromal decidualization *in vitro*. *PLoS ONE*. 2012;7:e41080.
31. Shah KM, Webber J, Carzaniga R, et al. Induction of microRNA resistance and secretion in differentiating human endometrial stromal cells. *J Mol Cell Biol*. 2013;5:67-70.
32. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev*. 2014;35:851-905.
33. Peter Durairaj RR, Aberkane A, Polanski L, et al. Deregulation of the endometrial stromal cell secretome precedes embryo implantation failure. *Mol Hum Reprod*. 2017;23:582.

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