miR-133b Reverses the Hydrosalpinx-induced Impairment of Embryo Attachment Through Down-regulation of SGK1

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Context: Hydrosalpinx impairs endometrial receptivity and embryo implantation. However, the exact underlying mechanism remains elusive.

Objective: This study aimed to explore how an miR-133b-mediated mechanism controls endometrial receptivity and embryo attachment in the endometrium of women with hydrosalpinx.

Design, Setting, Patients, and Interventions: Ishikawa cells were treated with hydrosalpinx fluid (HF) from infertile patients and cultured for in vitro analysis. The attachment rates of BeWo spheroids and mouse embryos to Ishikawa cells were assayed.

Primary Outcome Measure: miR-133b, serum and glucocorticoid-regulated kinase 1 (SGK1), and homeobox A10 (HOXA10) expression levels were evaluated by quantitative real-time PCR and Western blot assays.

Results: The expression of miR-133b and HOXA10 was significantly down-regulated, whereas the miR-133b target gene SGK1 was up-regulated in mid-secretory endometrial tissues of women with hydrosalpinx and in HF-treated Ishikawa cells. Moreover, hydrosalpinx inhibited miR-133b expression through the activation of nuclear factor \( \text{NF-} \beta \), and SGK1 decreased miR-133b-induced HOXA10 expression by phosphorylating cAMP responsive element binding protein in Ishikawa cells. Our results also showed that miR-133b and HOXA10 contributed to BeWo spheroid adhesiveness, whereas SGK1 inhibited BeWo spheroid attachment to Ishikawa cells. Importantly, miR-133b overexpression reversed the HF-mediated impairment of embryo attachment in vitro.

Conclusion: miR-133b directly targets SGK1 to reverse the hydrosalpinx-induced down-regulation of HOXA10 and to attenuate the impairment of embryo attachment in vitro. (J Clin Endocrinol Metab 101: 1478–1489, 2016)
compared with patients exhibiting tubal factors without hydrosalpinx (2, 3). Many explanations regarding the deleterious effects of hydrosalpinx fluid (HF) on reproduction have been proposed, including mechanical effects, embryonic toxicity, imbalanced intrauterine fluid formation, and hindered embryo implantation (4).

Successful implantation of a good-quality human embryo in the endometrium requires uterine transition into the receptive state, which is ensured by sex steroids, growth factors, cytokines, and adhesion molecules (5–8). Recently, reports have demonstrated that the expression levels of receptivity markers such as homeobox A10 (HOXA10), leukemia inhibitory factor, mucin 1, and increasing HOXA10 expression. Furthermore, we uncovered a potential prognostic biomarker and molecular target for the treatment of implantation failure.

MicroRNAs (miRNAs) are highly conserved, small, noncoding RNAs that diversely regulate gene expression at the post-transcriptional level by binding to complementary “seed sequences” on target transcripts (11). Emerging evidence suggests the important roles of miRNAs in endometrial receptivity and embryo implantation (12–14). For example, miR-135a and miR-135b impaired endometrial receptivity by repressing HOXA10 expression in the endometrium of patients with endometriosis (13), and miR-145 suppresses embryo-epithelial juxtacrine communication at implantation by modulating maternal IGF1R (14). Georgantas (15) et al demonstrated that inflammatory cytokines TNF-α suppress adult skeletal muscle differentiation by inhibition of miR-133b expression in inflammatory myopathy. Interestingly, another study found that miR-133b stimulates ovarian estradiol synthesis and regulates ovarian follicle development by targeting Foxl2 (16) and TAGLN2 (17), respectively, indicating its important roles in female reproduction. In this study, we found lower miR-133b expression in the mid-secretory endometrial tissues of women with hydrosalpinx. However, the regulatory mechanisms and functional relevance of this miRNA in human endometrium remain unknown.

In the present study, we identify miR-133b as an inflammatory microRNA that contributes to endometrial receptivity and embryo attachment by suppressing serum and glucocorticoid-regulated kinase 1 (SGK1) expression and increasing HOXA10 expression. Furthermore, we demonstrated that miR-133b could rescue the hydrosalpinx-induced impairment of embryo attachment to human endometrial epithelial cells in vitro. Our data highlight a functional role for miR-133b in implantation and uncover a potential prognostic biomarker and molecular target for the treatment of implantation failure.

Materials and Methods

Patients, samples, and HF collection

Midsecretory endometrial tissue samples obtained by Pipelle suction curettage were collected from 10 women with hydrosalpinx (detected at first by ultrasound and then confirmed by hysterosalpingography) and from nine disease-free women as controls; the age range of these women was 26–38 years old. Control patients were women with no evidence of hydrosalpinx or other benign gynecological disorders. HF was collected from 21 infertile patients with unilateral (n = 13) or bilateral hydrosalpinx (n = 8) undergoing surgical treatment before IVF treatment under an approved human investigation committee protocol and then diluted to a 25 or 50% concentration (vol/vol) in Dulbecco’s modified Eagle’s medium (Gibco). Only fluid from each woman with bilateral hydrosalpinx was individually pooled. All patients enrolled onto this study had regular menstruation without hormone treatment before surgery or collection. This study was approved by the Drum Tower Hospital Research and Ethics Committee, and signed informed consent was obtained from all patients. Essential patient demographics are presented in Supplemental Table 1.

Cell culture and transfection

The Ishikawa and BeWo cells were cultured in Dulbecco’s modified minimum essential medium containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (HyClone Laboratories). Ishikawa cells were cultured for the indicated durations under the treatments described in each figure legend. Treatments included human recombinant IL-1α, IL-1β, TNF-α, and TGF-β1 (Peprotech), as well as PDTC (pyrrolidine dithiocarbamate, an inhibitor of nuclear factor κB [NF-κB] activation, Sigma). For the cell transfection assay, the miR-133b inhibitor, a nonspecific miRNA inhibitor control, small-interfering RNAs (siRNAs) targeting cAMP responsive element binding protein (CREB), and siCONTROL nontargeting siRNAs were purchased from RiboBio Biotech Corporation and transfected into cells using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions.

Western blot analysis

The cells were washed with ice-cold PBS and lysed in whole-cell lysis buffer (50mM Tris-HCl [pH 7.6], 150mM NaCl, and 1.0% NP-40) containing a protease inhibitor cocktail and phosphatase inhibitor cocktails 2 and 3 (Sigma). The protein bands were separated on SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (Millipore). The membranes were blocked in Tris-buffered saline solution containing 5% nonfat milk for 1 hour and then exposed to the following primary antibodies: β-actin (1:5000; Bioworld), SGK1 (1:2000; Millipore), HOXA10 (1:1000; Santa Cruz Biotechnology), Flag-HP (1:3000; Sigma), p-p65 (1:2000; Santa Cruz Biotechnology), CREB (1:2000; Bioworld), and p-CREB (ser133) (1:2000; Bioworld). Immunodetection was accomplished using goat antirabbit (1:3000; Bio-Rad Laboratories) or donkey antigoat (1:10000; Santa Cruz Biotechnology) secondary antibodies and an enhanced chemiluminescence detection kit (Millipore).

Real-time quantitative PCR

Total RNA from cell lines and tissue samples was extracted using TRIzol reagent (Invitrogen). Purified total RNA (1 μg) was...
reverse-transcribed into cDNA using a PrimeScript RT reagent kit (Takara) according to the manufacturer’s instructions. Quantitative real-time PCR (qRT-PCR) was performed using a SYBR green PCR kit and a MyiQ Single Color Real-time PCR Detection System (Bio-Rad Laboratories). The specific primers that were used for quantitative PCR analysis were as follows: mir-133b, 5’-CACTCCAGCTGGGTTTGGTCCTCCTTCAAC-3’ and 5’-GTTCTGGATGCAGAATCCGTGACGT-3’; U6, 5’-CTGCTGGGAGCGCAAC-3’ and 5’-AACGCTTACAGGATGTGC-3’; HOX10, 5’-GCCGCCTTCCGAGAGCAGCACA-3’ and 5’-AGCTGAGAGCAGAACAGG-3’. The eluates were incubated with proteinase K, and DNA was purified by phenol: chloroform extraction and ethanol precipitation. Finally, the purified DNA fragments were used as a template for PCR amplification. The specific primers that were used for PCR amplification were as follows: mir-133b, 5’-CAATGTCTATGGCTGGGAGAA-3’ and 5’-CCCGGAGGTGGACGCTGCG-3’. Samples were run in duplicate using RNA preparations from three independent experiments. The fold change in the expression of each gene of interest was normalized to the expression of the endogenous control (U6/18S rRNA), and the data were analyzed using the 2^−ΔΔCT method.

Luciferase assays

Preconfluent (60%) Ishikawa cells in 12-well plates were transfected with the indicated plasmids using Lipofectamine 2000 reagent (Invitrogen). Cells were collected and luciferase activities were analyzed after 48 hours using the Dual-Luciferase Assay System (Promega). Luciferase activity was measured using a luminoscence counter (Centro XS3 LB 960, Berthold Technologies) according to the manufacturer’s instructions. Firefly luciferase activity was normalized for transfection efficiency to the corresponding Renilla luciferase activity.

Chromatin immunoprecipitation/PCR assay

Briefly, Ishikawa cells (70–80% confluence) were treated with HF (50% concentration) for 48 hours. Cells were then washed with PBS and crosslinked with 1% formaldehyde for 15 minutes at room temperature. Crosslinking was stopped with the addition of glycine (0.125M final concentration) for 10 minutes. Cells were washed twice with cold PBS, collected in lysis buffer (20mM Tris-HCl [pH 8.0], 85mM KCl, 1mM EDTA, 0.5mM EGTA, 0.5% Nonidet P40, and protease inhibitor cocktail [Sigma]), and pelleted by centrifugation. Cell pellets were then lysed in nuclear lysis buffer (50mM Tris-HCl [pH 8.0], 10mM EDTA, 1% SDS, and protease inhibitor cocktail) and sonicated on ice to yield genomic DNA fragments with sizes of approximately 500–2000 base pairs (bp). Next, precleared sonicates were immunoprecipitated using a specific antibody, anti-p65, and nonspecific IgG as a technical control. Beads were collected and washed extensively. Immune complexes were eluted by incubation with fresh elution buffer (1% SDS and 0.1M NaHCO_3) at 65°C for 30 minutes followed by incubation at room temperature for 15 minutes. Crosslinks were reversed by incubation with NaCl at a final concentration of 0.3M for 5 hours at 65°C. The eluates were incubated with proteinase K, and DNA was purified by phenol: chloroform extraction and ethanol precipitation. Finally, the purified DNA fragments were used as a template for PCR amplification. The specific primers that were used to amplify the mir-133b promoter DNA fragments containing a NF-κB binding sequence (GGGGAGTTCG) were 5’-CCCA GGGAGAAGGACAATT-3’ and 5’-CATTCCAGCCGGA AACAT-3’ (−1525 to −1234; expected size, 291 bp), and the specific primers that were used to amplify the mir-133b promoter DNA fragments without a NF-κB binding sequence were 5’-CAATGTCTATGGCTGGGAGAA-3’ and 5’-CCCGGAGGTGGACGCTGCG-3’. Samples were run in duplicate using RNA preparations from three independent experiments. The fold change in the expression of each gene of interest was normalized to the expression of the endogenous control (U6/18S rRNA), and the data were analyzed using the 2^−ΔΔCT method.

Statistical analysis

All values are reported as the mean ± SEM or median and interquartile range, as specified. Student t tests were used for comparisons of two groups, ANOVA was applied for experiments involving more than two groups, and Kruskal-Wallis with Dunn’s multiple comparison tests were used to test the stability of embryo attachment to Ishikawa cells. Correlation was tested by Pearson’s correlation coefficient (r). A value of P < .05 was considered statistically significant.

Results

Reduced expression of HOXA10 and miR-133b in women with hydrosalpinx

To assess the mechanisms by which hydrosalpinx affects implantation, we analyzed the expression of the en-
dometrial receptivity marker HOXA10 protein and an inflammation-related miRNA, miR-133b, in women with hydrosalpinx. qRT-PCR and Western blot analyses showed that the expression levels of HOXA10 and miR-133b decreased by more than 60% and 50%, respectively, in patients with hydrosalpinx (n = 10) compared with the controls (n = 9) (P < .01; Figure 1, A and B). Moreover, when Ishikawa cells were treated with 50% HF (n = 21) for 48 hours, miR-133b expression was reduced by approximately 50% compared with the untreated cells (Figure 1C). Simultaneously, HF also significantly decreased HOXA10 protein expression in these cells (Figure 1D).

miR-133b increases HOXA10 expression and promotes BeWo spheroid attachment to Ishikawa cells

We first demonstrated that HF is not cytotoxic to Ishikawa cells or BeWo cells using MTT assays (Supple-
mental Figure 1A). Subsequently, an appropriate trophoblast-epithelial cell interaction model confirmed that treatment of Ishikawa cells with 50% HF (n = 3), but not 25%, resulted in a significant reduction in BeWo spheroid adhesion (Supplemental Figure 1, B and C). Furthermore, HF from 21 patients exhibited significant inhibitory effects on BeWo spheroid adhesion to Ishikawa cells compared with the control group (24.27 ± 6.57 vs 37.44 ± 2.84%; P < .01; Figure 1E).

Next, we confirmed the enhanced expression of miR-133b adenovirus (Supplemental Figure 2A) in Ishikawa cells, and investigated the role of miR-133b in the process of embryo attachment. Ectopic expression of miR-133b led to increased HOXA10 mRNA and protein expression in a concentration-dependent manner (Figure 1, F and G) and promoted BeWo spheroid attachment, as well as adenovirus-mediated overexpression of HOXA10 (Figure 1H). In contrast, transfection of a miR-133b inhibitor decreased endogenous miR-133b expression (Supplemental Figure 2B) and inhibited HOXA10 expression in Ishikawa cells (Figure 1, I and J). These data suggest that miR-133b contributes to endometrial receptivity and embryo attachment.

**Hydrosalpinx represses miR-133b expression through activation of NF-κB/p65**

To determine the molecular mechanisms by which a hydrosalpinx affects miR-133b expression, Ishikawa cells were treated with cytokines (IL-1α, IL-1β, TNF-α, and TGF-β1) and HF for 1 hour; phosphorylated p65 expression increased (Figure 2, A and B) and miR-133b expression decreased after 48 hours of treatment (Figure 2, C and D). Moreover, when NF-κB activation was inhibited by PDTC pretreatment in Ishikawa cells, the repressive effect on miR-133b expression induced by IL-1β, TNF-α, TGF-β1 and HF was reversed (Figure 2, C and D).

A chromatin immunoprecipitation/PCR experiment further demonstrated that the miR-133b promoter, which contained potential NF-κB-binding sites (GGGGAGTTTC, −1420 to −1411), was effectively recovered from immunoprecipitates of NF-κB/p65 proteins in HF-treated Ishikawa cells, whereas a negative control region and IgG control immunoprecipitates were not recovered (Figure 2F). In addition, treatment of Ishikawa cells with HF markedly decreased the luciferase activity of the NF-κB-Luc and miR-133b-Luc construct with the NF-κB-binding site (Figure 2F). These results suggest that HF represses miR-133b expression by activation of NF-κB/p65.

**SGK1 is a target gene of miR-133b in Ishikawa cells**

Next, we used the TargetScan software (http://www.microrna.org, http://mirdb.org/miRDB/, http://targetscan.org/) to search for the direct mRNA targets of miR-133b. Among the hypothetical targets of miR-133b, SGK1 provoked our interest. As shown in Figure 3A, SGK1 mRNA contains a putative target site (seed sequence GGACCAA) in its 3’ Untranslated Regions (3’ UTR) that aligned with the sequence of miR-133b. Luciferase reporter assays showed that ectopic miR-133b expression in Ishikawa cells attenuated the luciferase activity of the wild-type 3’ UTR compared with the control with Ad-LacZ (P < .05; Figure 3B), whereas the mutant 3’ UTR showed no response to miR-133b (Figure 3B). In addition, SGK1 mRNA and protein levels decreased after miR-133b expression was enhanced (Figure 3, C and D) and increased upon miR-133b inhibitor treatment in a concentration-dependent manner in Ishikawa cells (Figure 3, E and F). Importantly, ectopic expression of SGK1 significantly impaired the miR-133b-induced BeWo spheroid attachment (P < .05; Figure 3G).

Analysis of SGK1 expression revealed that subjects with hydrosalpinx (n = 10) had 1.9-fold higher SGK1 protein expression relative to controls (n = 9) (P < .05; Figure 3H) and a significant negative correlation between miR-133b and SGK1 expression (r = −0.67; P = .0016; Figure 3I), consistent with the notion that miR-133b regulates SGK1 expression. Simultaneously, HF also markedly increased SGK1 protein expression in Ishikawa cells (Figure 3J).

**HOXA10 is a downstream target of the miR-133b/SGK1/CREB pathway**

To further investigate the mechanism by which the miR-133b/SGK1 axis modulates embryo attachment, we analyzed the effect of SGK1 on the expression of HOXA10, a key regulator of embryo implantation. In contrast with the miR-133b-induced increase in HOXA10 expression (Figure 1, F and G), SGK1 overexpression in Ishikawa cells significantly decreased the expression of HOXA10 in a concentration-dependent manner (Figure 4, A–C) and increased the expression of p-CREB (ser133), which is the active form of CREB (Figure 4C). Furthermore, reducing CREB expression via CREB-specific siRNAs attenuated p-CREB (ser133) expression in Ishikawa cells and relieved the SGK1-induced decrease in HOXA10 protein expression (Figure 4D). Importantly, ectopic miR-133b expression decreased SGK1, CREB, and p-CREB expression in Ishikawa cells (Figure 4E). The effect of miR-133b on CREB, p-CREB (ser133), and HOXA10 expression was also reversed by the simultaneous coexpression of SGK1 (Figure 4F). In addition, ectopic expression HOXA10 significantly reversed the SGK1-induced impairment of BeWo spheroid attachment (P < .05; Figure 4G). Taken together, these data suggest that miR-133b


up-regulates HOXA10 expression by repressing SGK1-mediated CREB activity.

**miR-133b is a novel modulator of the hydrosalpinx-induced impairment of embryo attachment in vitro**

Given that our data support a role for miR-133b/SGK1/HOXA10 signaling in regulating embryo attachment and dysregulated expression in women with hydrosalpinx, we next examined whether miR-133b functions in the hydrosalpinx-induced impairment of embryo attachment. As expected, forced overexpression of miR-133b attenuated HF-induced SGK1 protein expression and restored HF-repressed HOXA10 protein expression (Figure 5A). Importantly, miR-133b or HOXA10 overexpression also rescued the inhibitory effect of HF on BeWo spheroid attachment: 40.93 ± 6.23% (Ad-miR-133b) vs 23.93 ± 3.66% (Ad-LacZ; P < .01) and 45.93 ± 4.41% (Ad-HOXA10) vs 23.93 ± 3.66% (Ad-LacZ; P < .01) (Figure 5B).

Furthermore, an in vitro model of mouse embryo attachment assay (Figure 5C) showed that embryos inco-
bated with miR-133b-treated cells had high attachment scores of median [interquartile range] 3 [3–4] (and HF-treated cells had attachment scores of 2 [1–3], compared with scores of 3 [2–4] for Ad-LacZ-treated cells (Figure 5D). Ectopic miR-133b expression rescued the inhibitory effect of HF on the stability of mouse embryo attachment to Ishikawa cells (attachment scores of 3 [2–4]; * P < .05 vs control) (Figure 5D). All these data suggest that the miR-133b/SGK1/HOXA10 pathway regulates HF-impaired embryo attachment (Figure 6).

Discussion

Hydrosalpinx is associated with poor reproductive outcomes for patients, even with IVF embryo transplant assistance (2, 3). However, the exact mechanisms by which a hydrosalpinx affects implantation are unclear. In this study, we demonstrated that hydrosalpinx alters the expression of the endometrial receptivity marker gene HOXA10 and impairs embryo attachment by modulating a cytokine/NF-κB/miR-133b/SGK1/HOXA10 pathway in vitro and in vivo.
Embryo implantation is a dynamic process that includes blastocyst apposition and attachment to the receptive endometrium and invasion of the trophoblast cells of the conceptus into the endometrium to establish the placenta (20). Uterine receptivity is defined as a restricted time-related period in which uterine endometrium can establish a successful dialog with blastocysts. The development of endometrial receptivity is primarily coordinated by maternal estrogen and progesterone (21). Suboptimal endometrial receptivity is widely thought to be a major cause of implantation failure. In women with hydrosalpinx, the abundant presence of cytokines and growth factors in the HF flowing into the uterine cavity not only mechanically interferes with embryonic apposition but also may stimulate NF-κB production in the endometrium, leading to excessive inflammatory and immune responses in the endometrium that may reduce endometrial receptivity and embryo implantation (22). Although the level of NF-κB is increased in the endometrium of infertile patients with hydrosalpinx (23), the exact mechanisms by which the NF-κB pathway affects implantation in these patients require further investigation. Our present study demonstrated that HF decreased the expression of miR-133b in an NF-κB-dependent manner and consequently reduced the expression of the endometrial receptivity marker gene HOXA10 and the attachment of embryos in vitro. Considering that hydrosalpinx is a chronic condition of the fallopian tubes caused by recurrent pelvic infections (24), we provide a potential mechanism by which miR-133b may link this inflammation with the impaired endometrial receptivity caused by hydrosalpinx, suggesting therapeutic approaches for infertile women with hydrosalpinx.
Homeobox-containing transcription factor HOXA10, which is essential for endometrial cell differentiation and receptivity (18, 25, 26), regulates the expression of various downstream target genes that are also involved in implantation, such as integrinβ3 (18), matrix metalloproteinase-26 (MMP-26) (27), and empty spiracles homeobox 2 (28). Deficiencies in the expression of HOXA10 have been described in association with abnormal implantation (29). HOXA10-null mice are infertile primarily due to a failed attachment reaction as well as aberrant decidualization (26). Daftary et al (30) showed that expression of HOXA10 mRNA was significantly lower in infertile women with hydrosalpinx compared with fertile controls. Salpingectomy resulted in a 15-fold increase in endometrial HOXA10 expression (30). However, the detailed mechanism remains unknown. Several reports have examined the regulation of HOXA10 by miRNAs; for example, miR-135 was first identified as a post-transcriptional regulator of HOXA10 in endometrium from subjects with and without endometriosis (13), and miR-705 was shown to act as an inhibitor of mesenchymal stem cell osteoblast differentiation by targeting HOXA10 (31). However, bioinformatics-based analysis revealed the lack of a predicted seed sequence of miR-133b in the 3' UTR of HOXA10 genes. Our target analysis demonstrated that SGK1 is a functional target of miR-133b-mediated

Figure 5. miR-133b is a novel modulator of the hydrosalpinx-induced impairment of embryo attachment in vitro. A, Ishikawa cells transduced with Ad-LacZ or Ad-miR-133b were treated with 50% HF (n = 3) for 48 h, and the protein levels of SGK1 and HOXA10 were examined by Western blot. B, Ishikawa cells infected for 6 h with Ad-LacZ, Ad-miR-133b, or Ad-Flag-HOXA10 were treated with (n = 10) or without (n = 10) HF for 48 h. The attachment of BeWo spheroids to Ishikawa cells was assayed after 2 h of coculture. The results are expressed as the mean ± SEM of three independent experiments. **, P < .01 vs cells treated with Ad-LacZ alone; ##, P < .01 vs cells treated with Ad-LacZ, Ad-miR-133b, and HF; &&, P < .01 vs cells treated with Ad-LacZ, Ad-miR-133b, and HF. C, Images in the upper or lower planes of mouse embryos attached to Ishikawa cells show the stages of embryo attachment. D, Ishikawa cells infected for 6 h with Ad-LacZ or Ad-miR-133b were treated with (n = 10) or without (n = 10) HF for 48 h. The attachment of mouse embryos to Ishikawa cells was assayed after 24 h of coculture. The results are expressed as the median with the interquartile range of three independent experiments; boxes show quartiles, and whiskers show range. *, P < .05 vs cells treated with Ad-LacZ alone; #, P < .05 vs cells treated with Ad-miR-133b and HF; &&, P < .01 vs cells treated with Ad-LacZ and HF.
HOXA10 expression, consistent with a previous report that increased SGK1 activity in the endometrium decreases HOXA10 expression and interferes with embryo implantation (32).

SGK1, a serine-threonine protein kinase homologous to AKT (33) is rapidly induced in response to an increase in progesterone levels in both human and mouse endometrium (32, 34). SGK1 mRNA levels transiently decline in the luminal epithelium during the window of endometrial receptivity in mice (34). Loss of SGK1 activity in the luminal epithelium is essential for rendering the endometrium receptive to implantation (34); SGK1 also plays crucial roles in regulating the activation of several transcription factors including CREB (35). Cytokines IL-6 and TGF-β1 have been demonstrated to stimulate SGK1 protein expression via the p38 MAPK pathway and the 3-phosphoinositide-dependent kinase 1 (36, 37), suggesting that the cytokines present in HF may up-regulate SGK1 protein levels, leading to impaired endometrial receptivity. Our data showed that the expression of SGK1 was up-regulated in midsecretory endometrial tissues of women with hydrosalpinx and in HF-treated Ishikawa cells. Loss of CREB expression in Ishikawa cells relieved the SGK1-induced down-regulation of HOXA10 protein expression. Importantly, given our result that ectopic miR-133b expression not only modulated HF-induced SGK1 and SGK1-decreased HOXA10 expression but also attenuated the negative effects of hydrosalpinx on mouse embryo attachment in Ishikawa cells, these observations led us to conclude that miR-133b is a new endometrial receptivity regulatory miRNA and that the miR-133b/SGK1/CREB pathway is involved in regulating hydrosalpinx-induced reduction of HOXA10 expression in human endometrium.

To our knowledge, our study provides the first evidence that HF deregulates miR-133b expression result in impairment of endometrial receptivity and embryo attachment and defines a unique mechanism by miR-133b in embryo implantation may contribute to inflammation-related infertility. However, human miR-133 contains two mature isoforms, miR-133a and miR-133b, which differ at a single 3’ terminal base (38). Additional studies are required to investigate the functional role of miR-133a in endometrial receptivity and embryo implantation.

Figure 6. Schematic illustrating the pathway through which HF inhibits miR-133b expression via NF-κB and miR-133b promotes HOXA10 expression by targeting SGK1 in human Ishikawa cells.
Acknowledgments

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Author Contributions: H.S. and G.Y. were responsible for the conception and design of the study; C.K., L.S., M.Z., L.D., Q.Z., T.F., Z.D., Q.Y., H.Z., X.Z., and Y.H. were responsible for acquisition of data; C.K., L.S., M.Z., L.D., H.S., and G.Y. performed the data analysis and drafted the manuscript; H.S. and G.Y. revised and commented the draft; and C.K., L.S., M.Z., L.D., Q.Z., X.C., Q.Y., H.Z., T.F., X.Z., Y.H., H.S., and G.Y. read and approved the final version of the manuscript.

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