Journal of the Endocrine Society, 2024, **8**, 1–11
https://doi.org/10.1210/jendso/bvae001
Advance access publication 8 January 2024
Research Article

ENDOCRINE SOCIETY

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Kisspeptin Regulates Cell Invasion and Migration in Endometrial Cancer

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Abstract

Kisspeptin (a product of the KISS1 gene and its receptor) plays an important role in obstetrics, gynecology, and cancer cell metastasis and behavior. In hypothalamic-pituitary-gonadal axis and placentation, Kisspeptin/Kisspeptin receptor affects hormone release and represses trophoblast invasion into maternal deciduae. Endometrial cancer is one of the common gynecological cancers and is usually accompanied by metastasis, the risk factor that causes death. Recently, research has demonstrated that Kisspeptin/Kisspeptin receptor expression in aggressive-stage endometrial cancer tissues. However, the detailed mechanism of Kisspeptin/Kisspeptin receptor in regulating the motility of endometrial cancers is not well understood. In this study, we use endometrial cancer cell lines RL95-2, Ishikawa, HEC-1-A, and HEC-1-B as models to explore the molecular mechanism of Kisspeptin on cell motility. First, we discovered that Kisspeptin/Kisspeptin receptor was expressed in endometrial cancer cells, and Kisspeptin significantly regulated the migration and invasion of endometrial cancer cells. Furthermore, we explored the epithelial–mesenchymal transition marker expression and the underlying signals were regulated on Kisspeptin treatment. In conclusion, we suggest that Kisspeptin regulates endometrial cancer cell motility via FAK and Src expression and the ERK1/2, N-Cadherin, E-Cadherin, beta-Catenin, Twist, and matrix metalloproteinase signaling pathways. We expect these molecules could be candidates for the development of new approaches and therapeutic targets.

Key Words: Kisspeptin, endometrial cancer, invasion, migration, FAK, Src

Abbreviations: EMT, epithelial-to-mesenchymal transition; FAK, focal adhesion kinase; FBS, fetal bovine serum; GPR54, G protein-coupled receptor; KP10, Kisspeptin-10; MMP, matrix metalloproteinase; Src, steroid receptor coactivator.

Endometrial cancer is the most common gynecological malignancy in developed countries, and its incidence is increasing. Invasion from the endometrium into deep myometrium or lymphovascular space is the critical life-threatening risk factor. More than 25% of patients diagnosed with endometrial carcinoma have invasive primary cancer accompanied by metastasis. In endometrial cancer, most of the deaths result from chemotherapy-resistant metastases. The survival rate is approximately 20% for those patients with distant metastasis. Thus, interpretation of the molecular mechanisms of endometrial cancer metastasis could deliver insights for the development of improved therapies. Despite aggressive treatment, the 5-year survival rate is still less than 20% [1, 2]. The novel therapeutic approaches to prevent tumor invasion and metastasis would improve the prognosis of patients with advanced endometrial cancer. Numerous molecules associated with tumor migration and invasion have been investigated. However, the mechanisms involved in the pathogenesis of endometrial cancer remain poorly understood.

Kisspeptin, encoded by KISS1 gene that functions as its ligands for the G protein-coupled receptor (GPR54), plays an important role in reproductive function and cancer cell metastasis [3, 4]. Kisspeptin/Kisspeptin receptor regulates release of GnRH, which may induce trophoblast invasion into maternal deciduae for adequate placentation [5, 6]. Furthermore, Kisspeptin/Kisspeptin receptor acts as a tumor suppressor in melanoma, breast cancer, colorectal cancer, and endometrial cancer [7-9]. Research indicates that Kisspeptin/Kisspeptin receptor expression is low in aggressive stages of tumor tissues collected from patients with endometrial cancer [10]. However, the detailed molecular mechanism of Kisspeptin/Kisspeptin receptor in regulating the migration and invasion of endometrial cancers is still not well demonstrated. Focal adhesion kinase (FAK) and steroid receptor coactivator (Src) are intracellular tyrosine kinases that enhance cell migration and invasion [11, 12], although the mechanisms for this are not well understood. ERK 1/2 are important factors in hormone-mediated signaling pathways in many cell types [13-15]. N-cadherin is a member of the superfamily of fundamental membrane glycoproteins that controls cell motility and adhesion [16, 17]. N-cadherin has a critical role in stimulating invasion during cancer progression [18, 19]. Twist, a helix-loop-helix transcription factor, has been demonstrated to regulate the cancer cell invasion and tumor metastasis [20, 21]. Furthermore, Twist has been shown as a critical transcription factor that controls the expression of N-cadherin in cancer cells [22, 23]. Epithelial-to-mesenchymal transition (EMT) can work in many epithelial cell types and is an important process in embryogenesis [24]. In cancer biology, EMT has been considered in individual tumor cells as a critical

Received: 17 September 2023. **Editorial Decision:** 2 January 2024. **Corrected and Typeset:** 23 January 2024
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process of invasion and metastasis [25, 26]. Epithelial-to-mesenchymal transition has been widely investigated and has shown a complex framework for future research on EMT in cancer biology. In particular, epithelial cells express some different cell–cell junctional proteins, various cytoskeletal proteins, and distinct signaling pathways. The important role of these different EMT-related proteins still needs to be extensively studied. Some evidence shows the important role of EMT markers (N-cadherin, E-cadherin, beta-Catenin, and Twist) and matrix metalloproteinases (MMPs) in endometrial cancer progression and metastasis, via regulation of FAK, Src, and ERK1/2 expression and the subsequent cell migration and invasion [27-31].

Based on these studies, we hypothesize that Kisspeptin analogues regulate human endometrial cancer cell invasion and migration by the regulation of FAK and Src expression and the ERK1/2, Cadherin, Twist, and MMP signaling pathways. Our data suggest that Kisspeptin analogues might be a potential molecule for clinical treatment of human endometrial cancer.

Materials and Methods

Cell Lines and Cell Culture

The human endometrial cancer cell lines RL95-2 [32], Ishikawa [33], HEC-1-A, and HEC-1-B [34] were used in this study. They were derived from well-differentiated adenocarcinoma of the endometrium. These cell lines were obtained from the American Type Culture Collection (USA). The cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% antibiotics, in a humidified incubator at 37 °C with 5% CO_2. The cells were starved in DMEM without FBS and phenol red overnight before treatment with the Kisspeptin analogues. To determine the efficacious concentration, the reagents were applied in a dose-dependent manner at concentrations of 10 to 500 nM [35], with a maximal effect at 100 nM in these experiments. The 4 human endometrial cancer cell lines were treated with Kisspeptin-10 (KP10; 100 nM) and KP234 (100 nM) individually for 2 hours, followed by immunoblot study and cell migration and invasion assay. To explore the effects of FAK, Src, and ERK1/2 inhibitors in RL95-2 endometrial cancer cells, cells were pretreated with FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually for an hour, and then treated with KP234 for 2 hours.

Reagents

The Kisspeptin agonist, KP10 (Tocris Bioscience Cat#2570, RRID: AB_3076555), a synthetic peptide, and the Kisspeptin antagonist, KP234 (Tocris Bioscience Cat#3881, RRID: AB_3076556), capable of suppressing GPR54, were purchased from Tocris Bioscience (Bristol, UK). The FAK inhibitor PF573228 (Tocris Bioscience Cat#3239, RRID:AB_3076559), the Src inhibitor SU6656 (Tocris Bioscience Cat#6475, RRID: AB_3076557), and the ERK1/2 inhibitor U0126 (Sigma-Aldrich, Cat#U120, RRID: AB_3076557) were purchased from Tocris Bioscience (Bristol, UK) and Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Immunoblot Analysis

The cells were lysed in buffer containing 20 mM Tris, pH 7.4, 2 mM EGTA, 2 mM Na2VO3, 2 mM Na4P2O7, 2% Triton X-100, 2% SDS, 1 μM aprotinin, 1 μM leupeptin, and 1 mM PMSF. The protein concentration was determined with a protein assay kit using BSA standards according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). Equal amounts of cell lysate were separated by SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Hybond-C, Amersham Pharmacia Biotech Inc., Oakville, ON, Canada). Following blocking with Tris-buffered saline containing 5% nonfat dry milk for 1 hour, the membranes were incubated overnight at 4 °C with anti-Kisspeptin (molecular weight: 15 kDa) (Abcam Cat#ab19028, RRID:AB_444734), anti-Kisspeptin receptor (molecular weight: 43 kDa) (Abcam Cat#ab137483, RRID:AB_3076560), anti-Twist (molecular weight: 21 kDa) (Thermo Fisher Scientific Cat# MA5-17195, RRID:AB_2538666), anti-*β-catenin* (molecular weight: 92 kDa) (Santa Cruz Biotechnology Cat# sc-7199, RRID:AB_634603), anti-E-cadherin (molecular weight: 97 kDa)(Abcam Cat# ab40772, RRID:AB_731493), anti-N-cadherin (molecular weight: 140 kDa) (Millipore Cat# 05-915, RRID:AB_441927), anti-MMP-2 (molecular weight: 72 kDa) (Abcam Cat# ab37150, RRID:AB_881512), anti-MMP-9 (molecular weight: 92 kDa) (Abcam Cat# ab137867, RRID:AB_3076581), anti-phospho-FAK (molecular weight: 119 kDa) (Cell Signaling Technology Cat# 3284, RRID:AB_10831810), anti-FAK (Cell Signaling Technology Cat# 3285, RRID:AB_2269034), anti-phospho-Src (molecular weight: 60 kDa) (Cell Signaling Technology Cat# 2105, RRID:AB_331034), anti-Src (Cell Signaling Technology Cat# 2108, RRID:AB_331137), anti-phospho-ERK1/2 (molecular weight: 42/44 kDa) (Cell Signaling Technology Cat# 4370, RRID:AB_2315112), or anti-ERK1/2 (Cell Signaling Technology Cat# 9106, RRID:AB_33176) antibody, followed by incubation with a horseradish peroxidase-conjugated secondary antibody. The immunoreactive bands were detected with an enhanced chemiluminescence kit. The membrane was then stripped with stripping buffer (62.5 mM Tris, 10 mM DTT, and 2% SDS, pH 6.7) at 50 °C for 30 minutes and reprobed with an anti-GAPDH antibody (Santa Cruz Biotechnology Cat# sc-32233, RRID:AB_627679) as a loading control.

Migration and Invasion Assays

Migration and invasion assays were performed in Boyden chambers with minor modifications. Cell culture inserts (24-well, pore size 8 μm; BD Biosciences, Mississauga, ON) were seeded with 1×10^5 cells in 250 μL of medium with 0.1% FBS. Uncoated inserts were used for migration assays, whereas inserts precoated with growth factor-reduced Matrigel (40 μL, 1 mg/mL; BD Biosciences) were used for invasion assays. Medium with 10% FBS (750 μL) was added to the lower chamber and served as a chemotactic agent. After a 24-hour (migration) or 48-hour (invasion) incubation, nonmigrating/invading cells were wiped from the upper side of the membrane and cells on the lower side were fixed in cold methanol (−20 °C) and air dried. The cells that had not penetrated the filter were removed by wiping, and the cells that had invaded the lower surface of the filter were fixed with ice-cold methanol and stained with 0.5% crystal violet.

Statistical Analysis

The results are shown as mean ± SEM. Pairwise comparisons were done with the *t*-test for paired data. Multiple

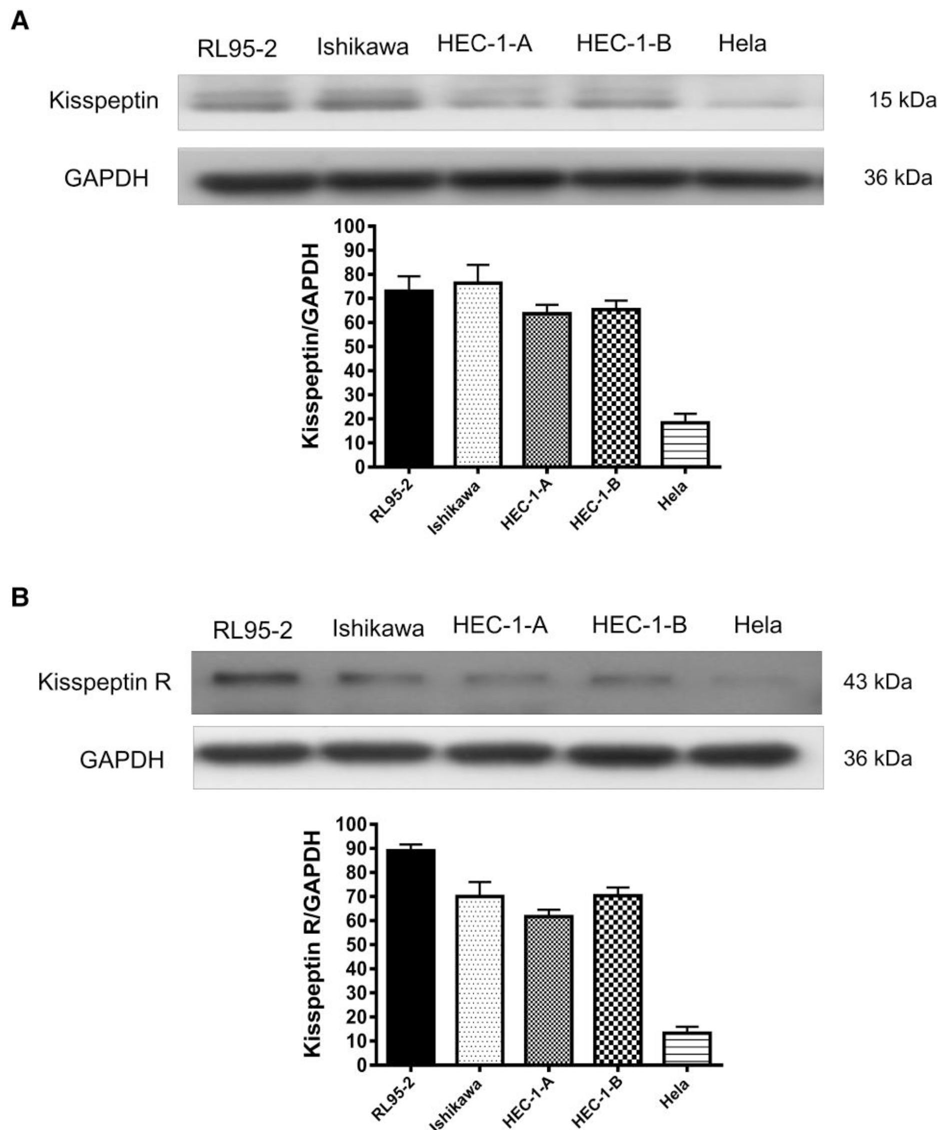

Figure 1. Kisspeptin and Kisspeptinancer cell lines. Endometrial cancer cell lines RL95-2, Ishikawa, HEC-1-A, HEC-1-B, and cervical cancer cell line Hela were cultured in DMEM without phenol-red and fetal bovine serum (FBS) overnight. Hela cells served as a negative control. The cell lysates were collected to analyze Kisspeptin (A) and Kisspeptin receptor (B) protein expression by western blot. Cells were cultured in no phenol-red DMEM with 10% FBS as control. The quantification of the signals was showed in lower columns.

comparisons were first analyzed by 1-way ANOVA, followed by Tukey multiple comparison tests. A significant difference was defined as $P < .05$.

Results

Kisspeptin and Kisspeptin Receptor Were Differentially Expressed in Endometrial Cancer Cell Lines

To study whether the expression of Kisspeptin and Kisspeptin receptor is associated with the motility of endometrial cancer cells, 4 endometrial cancer cell lines (RL95-2, Ishikawa, HEC-1-A, and HEC-1-B) were used. The Hela cervical cancer cell line, known to lack Kisspeptin and GPR54 expression, was used as a negative control [36, 37]. As shown in Fig. 1A and 1B, western blotting results confirmed the differential expression of Kisspeptin and Kisspeptin receptor in 4 endometrial cancer cell lines.

Kisspeptin Agonist-KP10 and Antagonist-KP234 Significantly Regulated the Motility of Endometrial Cancer Cell Lines

In cancer invasion and metastasis, an imbalanced regulation of cell motility and proteolysis appears to be a critical event [38]. To examine whether Kisspeptin agonist and antagonist are involved in regulation of human endometrial cancer cell migration and invasion, the 4 human endometrial cancer cell lines (RL95-2, Ishikawa, HEC-1-A, and HEC-1-B) were treated with Kisspeptin agonist KP10 (100 nM) and Kisspeptin antagonist KP234 (100 nM) individually. As shown in Fig. 2A, treatment with KP10 (100 nM) significantly inhibited the cell migration in all of the 4 cell lines. Furthermore, treatment with KP234 (100 nM) significantly promoted the cell migration in the 4 cell lines. In addition, as shown in Fig. 2B, invasion assay results revealed that treatment with KP10 (100 nM) inhibited the cell invasion in the 4 cell lines, and treatment with KP234 (100 nM) significantly

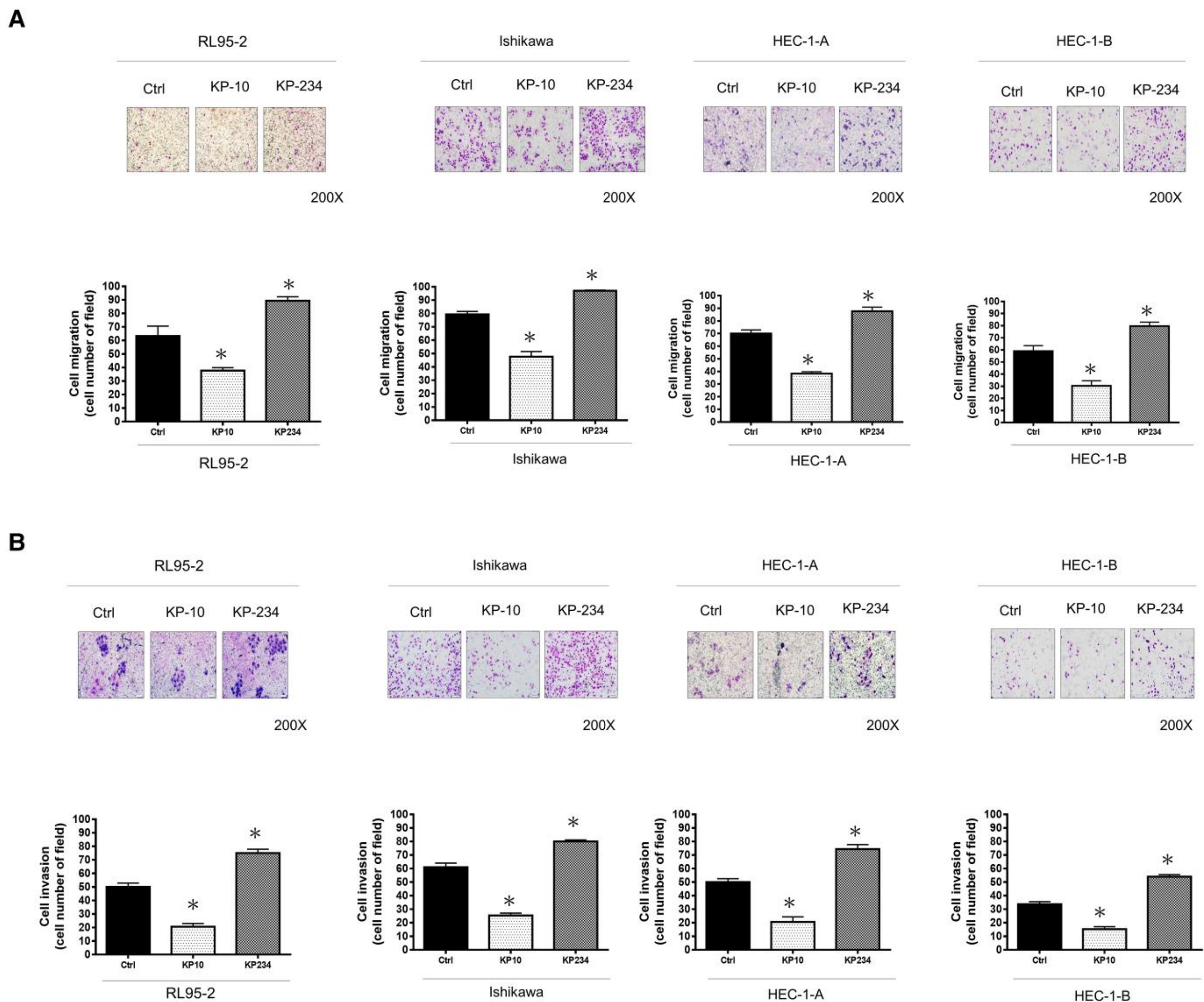

Figure 2. Kisspeptin agonist-KP10 and antagonist-KP234 significantly regulated the motility of endometrial cancer cell lines. (A) Migration, endometrial cancer cells were seeded on an uncoated porous filter in a transwell chamber containing 100 nMof KP10 or 100 nM of KP234 individually. (B) Invasion, endometrial cancer cells were seeded onto a Matrigel-precoated filter in Transwell chambers containing 100 nM of KP10 or 100 nM of KP234 individually. After 24 (migration, A) and 48 (invasion, B) hours of incubation, cells on the upper side of the filter were removed and the invaded or migrated cells on the lower surface were fixed, stained with crystal violet, and counted. The photomicrographs demonstrate characteristic results. KP10 inhibited the migration and invasion of endometrial cancer cells, and KP234 promoted the migration and invasion of endometrial cancer cells. Bar charts express the mean number of migrated or invaded cells of 4 fields of triplicate wells from 3 independent experiments ± SEM; **P* < .05 vs control.

stimulated the cell invasion in the 4 cell lines. Taken together, these results clearly indicated that KP10 exhibited an inhibitory effect on the cell migration and invasion in human endometrial cancer. Although KP10 acted as an agonist for Kisspeptin receptor, KP234, by contrast, showed the stimulated effects on the cell migration and invasion in human endometrial cancer.

The Expression of Junction Proteins (N-cadherin, E-cadherin, Beta-Catenin, and Twist) Following Kisspeptin Agonist-KP10 and Antagonist-KP234 Treatment in Endometrial Cancer Cell Lines (RL95-2, Ishikawa, HEC-1-A, and HEC-1-B)

We explored the epithelial–mesenchymal transition markers (N-cadherin, E-cadherin, beta-Catenin, and Twist) expression via immunoblot analysis. After treatment with KP10, E-cadherin expression was up-regulated and N-cadherin, beta-Catenin, and Twist levels were down-regulated in all 4 cell lines when compared with the normal control (Fig. 3). In addition, KP10 treatment inhibited endometrial cell lines migration and invasion. These results indicated that KP10 suppressed human endometrial cancer cell migration and invasion by down-regulating the expression of N-cadherin, beta-Catenin, and Twist and up-regulating the E-cadherin expression. As shown in Fig. 3, after treatment with KP234, the expression of N-cadherin, beta-Catenin, and Twist was up-regulated, and E-cadherin level was down-regulated in all 4 cell lines when compared with the normal control. Moreover, KP234 treatment promoted endometrial cell lines migration and invasion. These results implied that KP234 stimulated human endometrial cancer cell migration and invasion by up-regulating the expression of N-cadherin, beta-Catenin, and Twist and down-regulating the E-cadherin expression.

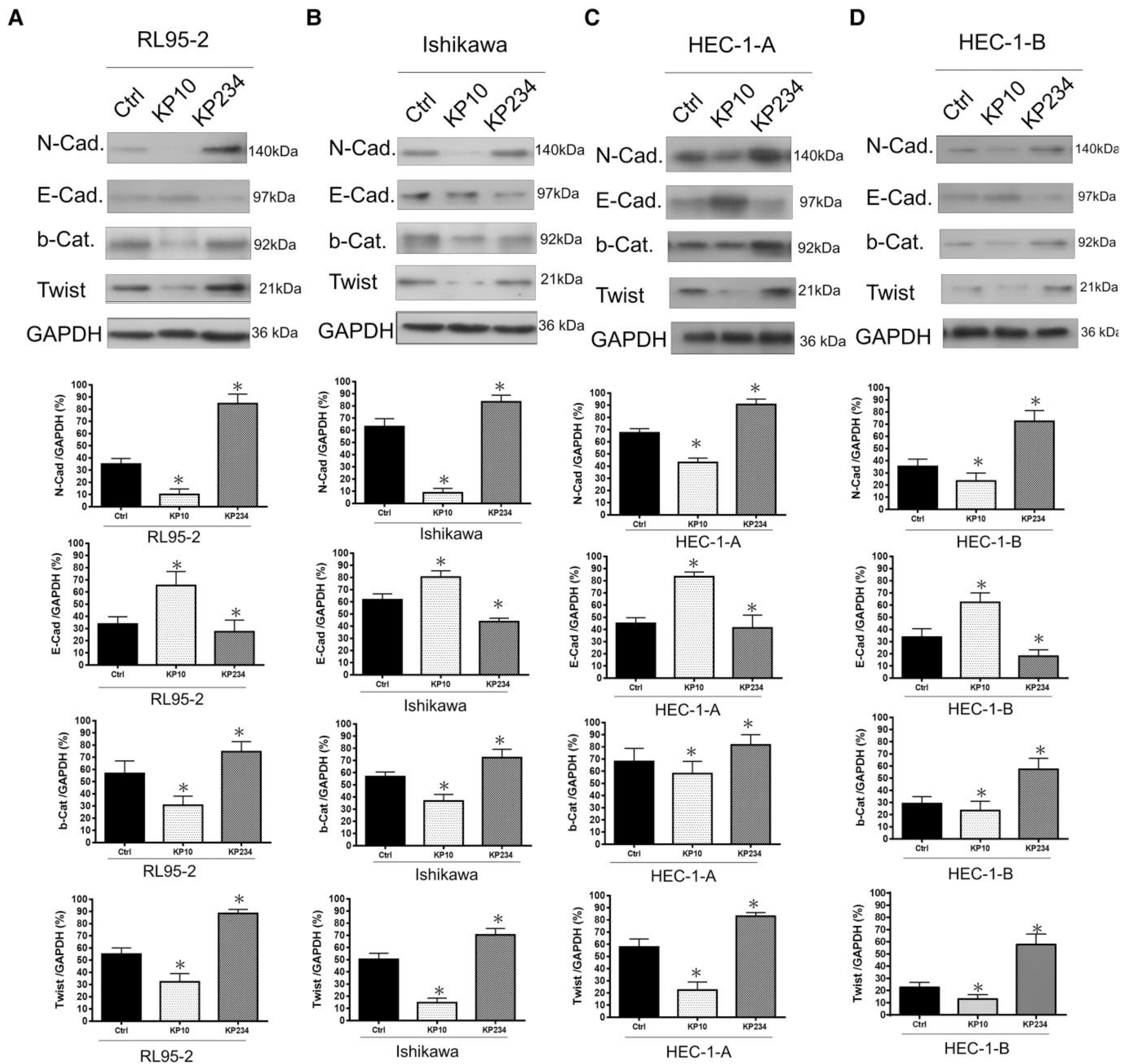

Figure 3. The expression of junction proteins (N-cadherin, E-cadherin, beta-Catenin, and Twist) following Kisspeptin agonist KP10 and antagonist KP234 treatment in endometrial cancer cell lines RL95-2 (A), Ishikawa (B), HEC-1-A (C), and HEC-1-B (D). Immunoblot analysis of epithelial–mesenchymal transition marker (N-cadherin, E-cadherin, beta-Catenin, and Twist) in endometrial cancer cells after treatment with KP10, the expression of E-cadherin (E-Cad) was up-regulated and the levels of N-cadherin (N-Cad), beta-Catenin (b-Cat), and Twist were down-regulated in RL95-2 (A), Ishikawa (B), HEC-1-A (C), and HEC-1-B (D) endometrial cancer cells when compared with the normal control. In contrast, treatment with KP234, the expression of E-cadherin (E-Cad) was down-regulated and the levels of N-cadherin (N-Cad), beta-Catenin (b-Cat), and Twist were up-regulated in RL95-2 (A), Ishikawa (B), HEC-1-A (C), and HEC-1-B (D) endometrial cancer cells when compared with the normal control. Absorbance values for N-cadherin, E-cadherin, beta-Catenin, and Twist proteins were standardized to GAPDH protein levels. The results are expressed as the mean ± SEM of 3 independent experiments. (* $P < .05$ vs control).

Kisspeptin Agonist—KP10 and Antagonist—KP234 Activates FAK, Src, and ERK1/2 Signaling Pathway in Endometrial Cancer Cell Lines

To investigate the molecular mechanism of Kisspeptin-induced cell migration and invasion in endometrial cancer cells, the activation of FAK, Src, and ERK1/2 signaling were examined with immunoblot analysis. In Fig. 4A (RL95-2) and 4B (HEC-1-A), FAK, Src, and ERK1/2 phosphorylation was up-regulated by KP234 treatment, and down-regulated by KP10 treatment in endometrial cancer cells, respectively. These results suggest that Kisspeptin agonist and antagonist regulate the migration and invasion of human endometrial cancer cells through the activation of the FAK, Src, and ERK1/2 signaling pathways.

Kisspeptin Agonist-KP10 and Antagonist-KP234 Regulated the Migration and Invasion of Endometrial Cancer Cells Through FAK, Src, and ERK1/2-mediated MMP-2/9 Expression

As shown in Fig. 5A, the treatment with KP10 significantly lowered MMP-2/9 protein expression in the RL95-2 endometrial cancer cell line compared with the normal control.

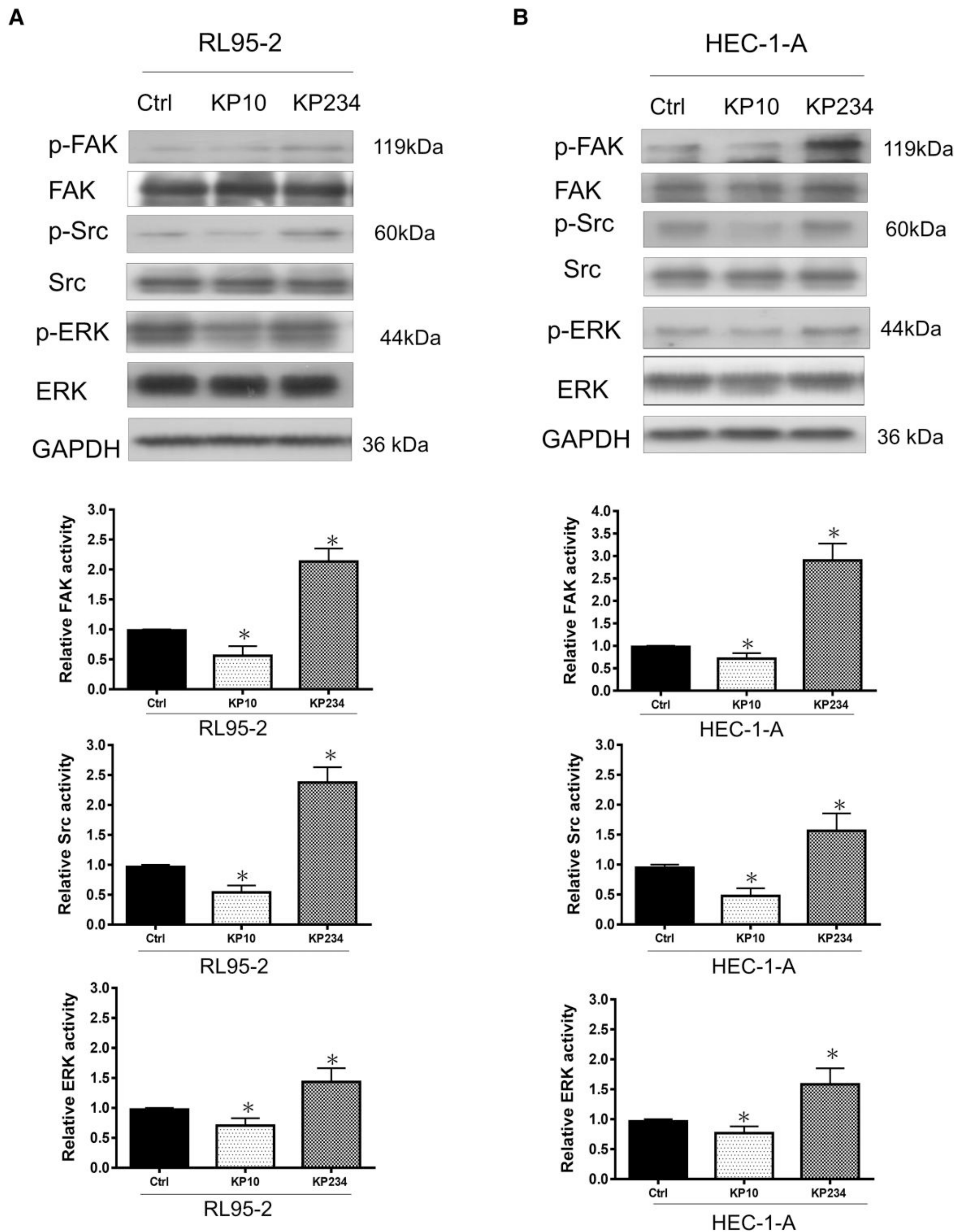

Figure 4. Kisspeptin agonist KP10 and antagonist KP234 activates FAK, Src, and ERK1/2 signaling pathway in endometrial cancer cell lines. The effects of Kisspeptin on FAK, Src, and ERK1/2 signaling activation. The RL95-2 (A) and HEC-1-A (B) endometrial cancer cell lines cells were exposed to Kisspeptin agonist (KP10) (100 nM) and Kisspeptin antagonist (KP234) (100 nM). The phosphorylated FAK (p-FAK), phosphorylated Src (p-Src), and phosphorylated ERK1/2 (p-ERK1/2) levels were analyzed by immunoblot analysis. Immunoblot analysis of p-FAK, p-Src, and p-ERK1/2 levels indicated increased expression following KP234 exposure, but a decreased expression following KP10 exposure. Results were expressed as mean ± SEM of 3 independent experiments. (* $P < .05$ vs control).

Furthermore, the challenge with KP234 significantly raised MMP-2/9 protein expression in the RL95-2 endometrial cancer cell line compared with the normal control. As shown in Fig. 5B, to explore the effects of FAK, Src, and ERK1/2 inhibitors in RL95-2 endometrial cancer cells, cells were pretreated with FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually, and then treated with KP234 for 2 hours. Cell lysates were collected for immunoblot analysis. The KP234-stimulated expressions of FAK, Src, and ERK1/2 were inhibited by FAK, Src, and ERK1/2 inhibitors individually. Furthermore, as shown in Fig. 5C, RL95-2 endometrial cancer cells were pretreated with FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually, and then treated with KP234

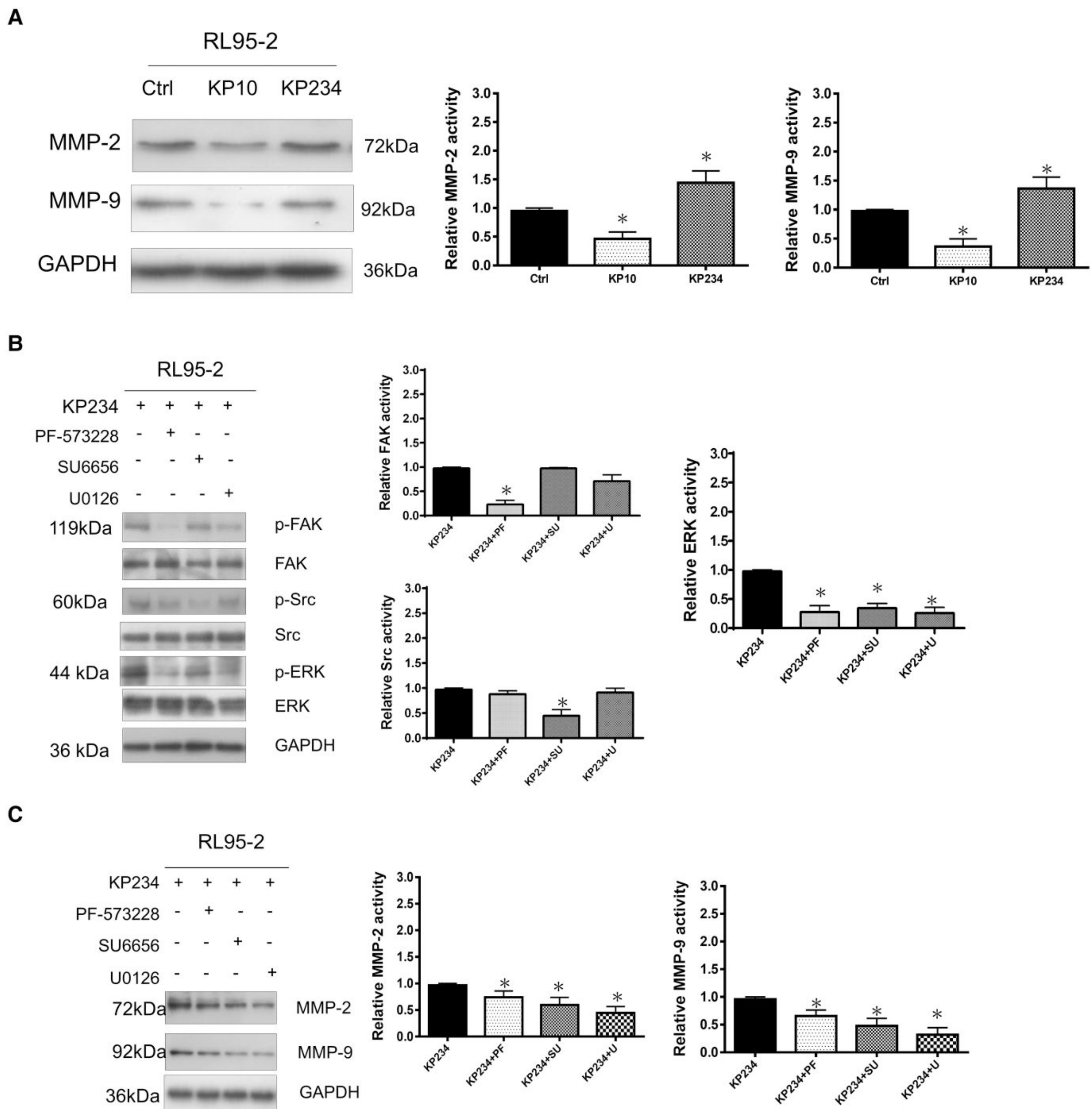

Figure 5. Kisspeptin agonist-KP10 and antagonist-KP234 significantly regulated the migration and invasion of endometrial cancer cells through FAK, Src, and ERK1/2-mediated MMP-2/9 expression. (A) The treatment with KP10 significantly decreased MMP-2/9 protein expression in RL95-2 endometrial cancer cell line when compared with the normal control. Meantime, the challenge with KP234 significantly increased MMP-2/9 protein expression in RL95-2 endometrial cancer cell line when compared with the normal control. (B) To verify the effects of FAK, Src, and ERK1/2 inhibitors in RL95-2 endometrial cancer cells, cells were pretreated with FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually, and then treated with KP234 for 2 hours. Cell lysates were collected for immunoblot analysis. The KP234-stimulated expressions of FAK, Src, and ERK1/2 were inhibited by FAK, Src, and ERK1/2 inhibitors individually. (C) RL95-2 endometrial cancer cells were pretreated with FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually, and then treated with KP234 for 2 hours. Cell lysates were collected for immunoblot analysis. The KP234-stimulated MMP-2/9 expression was weakened by FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually. Results are expressed as mean ± SEM of 3 independent experiments (* $P < .05$ vs control).

for 2 hours. Cell lysates were collected for immunoblot analysis. The KP234-stimulated MMP-2/9 expression was weakened by FAK inhibitor (PF573228), Src inhibitor (SU6656), and ERK1/2 inhibitor (U0126) individually. These results demonstrated that Kisspeptin agonist and antagonist regulated the migration and invasion of human endometrial cancer cells through the activation of the FAK and Src-mediated MMP-2 and MMP-9 signaling pathways. Results are expressed as mean ± SEM of three independent experiments ($P < .05$, vs control).

Discussion

The expression of Kisspeptin and Kisspeptin receptor in endometrium is still not conclusive [35, 39], nor has the function of Kisspeptin and Kisspeptin receptor axis in endometrial cancer been clarified. To date, the incidence of endometrial cancer in the world has significantly increased. However, the underlying molecular mechanisms involved in the pathogenesis of endometrial cancer are still not well known. In mammals, Kisspeptin is secreted by the hypothalamus. In the hypothalamus, Kisspeptin binds to its receptor, Kisspeptin receptor, to stimulate the synthesis and secretion of GnRH and the subsequent gonadotropins for hormone axis [40]. The concentration of Kisspeptin varies throughout the menstrual cycle. During the early follicular phase, Kisspeptin levels are low and sharply increase when the dominant follicle grows. Kisspeptin levels during the periovulatory period are then high and ultimately initiate the LH surge associated with ovulation [41]. Besides, kisspeptin concentrations are associated with factors such as age, parity, adiposity, insulin sensitivity, and hormonal contraception [42, 43]. Current reviews suggest that age impact on Kisspeptin and its receptor expression may be tissue-dependent and should not be correlated with the plasma level of kisspeptin [43]. It has been shown that Kisspeptin and Kisspeptin receptor can be found in many extrahypothalamic tissues and tumor cells [4, 44]. In human cancers, Kisspeptin and Kisspeptin receptor have been considered endocrine and autocrine regulatory factors regulating cell behavior. In a recent study, expression of Kisspeptin and Kisspeptin receptor was lower in serous, clear cell, and grade 3 endometrioid adenocarcinomas compared with that of low-grade patients [43]. In addition, loss of immunohistochemical expression of Kisspeptin and Kisspeptin receptor was significantly associated with invasion into deep myometrium and lymphovascular space [10, 43]. Metastasis represents the main cause of death for patients with endometrial cancer. This evidence strongly suggests that weak expression of Kisspeptin and Kisspeptin receptor is a poor prognostic factor in patients with endometrial cancer. In our study, a high level of Kisspeptin and Kisspeptin receptor expression was found in Ishikawa, RL95-2, HEC-1-A, and HEC-1-B, known as well-differentiated human endometrial cancer cell lines with good prognosis, indicating the possible potential role of Kisspeptin and Kisspeptin receptor in the molecular behavior of endometrial cancer cells.

Kisspeptin agonist has been shown to inhibit cell migration and invasion in multiple types of cancer cells [45-48]. In this study, we used KP10, a short peptide of 10 amino acids that is proteolytically processed from kisspeptin. KP10 is 10 times as active as Kisspeptin and is regarded as a candidate for clinical use [49]. The antitumor effects were confirmed in which migration and invasion of human endometrial cells were suppressed by KP10 treatment. Schmidt et al has established an in vitro study that KP10 treatment affects stromal-derived factor 1-induced invasion of endometrial cancer cells [9]. In contrast, pretreatment of Kisspeptin antagonist, kisspeptin-234 (KP234), promoted the effects of migration and invasion of endometrial cancer cells, indicating the potential development of different Kisspeptin analogues for therapeutics approach in endometrial cancer.

Epithelial-mesenchymal transition markers play a key role in tumor progression. The previous study demonstrated that Twist is frequently expressed in endometrial cancers and its high expression is significantly associated with poor prognosis [50]. Endometrial cancer with deep myometrial invasion or nodal metastasis was likely to have strong Twist expression. Moreover, up-regulated Twist was found in cancer cells concomitant with loss of E-cadherin expression, resulting in increased cell migration [51, 52]. This observation of opposite N-cadherin and E-cadherin expression during Twist-regulated differentiation and fusion of human cells is consistent with other reports of loss of N-cadherin and E-cadherin regulation during human cell differentiation and fusion [53, 54]. Disruption of N-cadherin and E-cadherin-mediated cell adhesion appears to be important in the EMT from noninvasive to invasive tumor cells [51, 55, 56]. Therefore, the up-expression of Twist seems to be associated with deep myometrial invasion by endometrial cancer cells and is concurrent with aberrant N-cadherin and E-cadherin expression [50].

As expected, the expression of EMT markers (N-cadherin, E-cadherin, beta-Catenin, and Twist) was correlated with Kisspeptin agonist and antagonist treatment [57]. In the present study, our results supported that down-regulated Twist, N-cadherin, beta-Catenin, and up-regulated E-cadherin after Kisspeptin agonist KP10 treatment in endometrial cancer cells provide less infiltrative phenotypes. In contrast, up-regulated Twist, N-cadherin, beta-Catenin, and down-regulated E-cadherin after Kisspeptin antagonist KP234 treatment in endometrial cancer cells showed more migration and invasion effects, indicating the development of Kisspeptin analogues for the future treatment of endometrial cancer.

MMPs have been known to play a critical role in cancer metastasis, and the up-regulation of MMP2 is associated with increased invasion and a poor prognosis in cancer [58-61]. MMPs are widely demonstrated in promoting angiogenesis and tumor metastasis [28, 29]. Some evidence shows a potential role in regulation of gynecologic tumor progression, such as metastasis, through the activation of MMPs and the following aggravation in cell migration and invasion [27]. In addition to their enzymatic activities, MMPs can also promote cancer cell migration by influencing cytoskeletal organization through their connection with different types of adhesion receptors [62]. In the present study, we demonstrated that Kisspeptin agonist suppressed the cell migration and invasion of endometrial cancer cells through the decreased expression and proteolytic activity of MMP-2 and MMP-9, which specifically degrade the basement membrane. On the other hand, Kisspeptin antagonist promoted the tumor progression via increasing expression and proteolytic activity of MMP-2 and MMP-9. Furthermore, in endometrial cancer cells, FAK/Src/ERK were considered to contribute to signaling pathways that mediate cellular response to different extracellular stimuli and thereby determine the cell's behavior. FAK and Src are shown to be a motility-promoting signaling complex by regulating adhesion through multiple signaling pathways [63, 64]. The expression of FAK and Src was essential for cell migration and invasion and is mediated through expression of MMPs [65]. FAK-stimulated phosphorylation of ERK1/2 can also promote transcription-associated increases in MMP expression [66]. ERK1/2 signaling pathways modulate cellular reaction to different extracellular impulses affecting cell function and behavior. With pretreatment of PF573228, SU6656, and U0126, respectively, the inhibitors significantly abolished the protein expression of MMP-2/9 induced by KP234, suggesting that the FAK, Scr, and ERK1/2 signaling pathway were strongly correlated with MMP-2/9 expression. In this

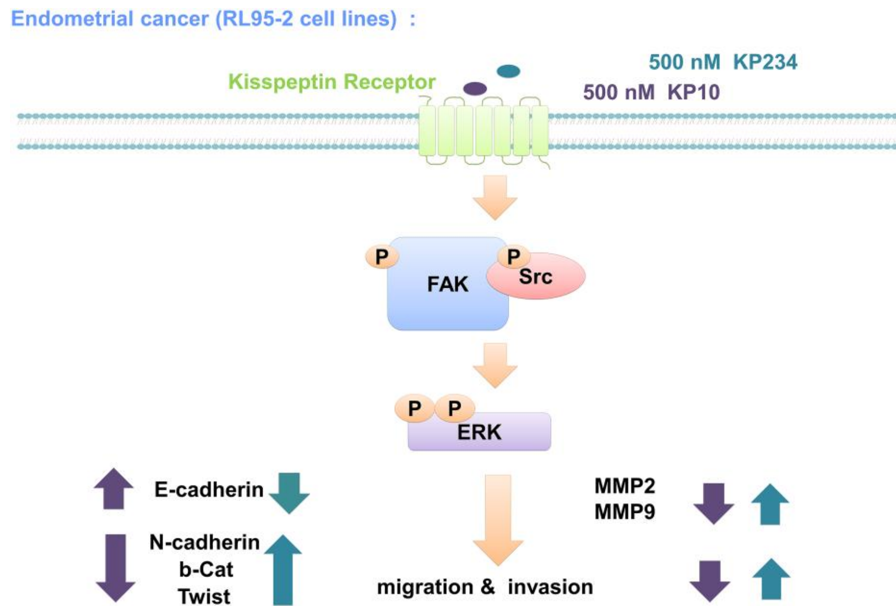

Figure 6. The proposed signaling pathways involved in Kisspeptin-modulated cell invasion and migration in endometrial cancer cells. KP234, a Kisspeptin antagonist, and KP10, a Kisspeptin agonist, bind to Kisspeptin receptors and regulate the invasion and migration of human endometrial cancer cells by enhancing or inhibiting, individually, FAK and Src expression and the following ERK1/2, Cadherin, Twist, and MMP signaling pathways.

study, we established for the first time that the Kisspeptin agonist, KP10, inhibited the cell migration and invasion of human endometrial cancer by down-regulating Twist, N-cadherin, beta-Catenin, and up-regulating E-cadherin expression in dependent of FAK, Src, and ERK1/2-mediated MMP-2/9 signaling pathway (Fig. 6). These results provide insights into the prospect of developing targeted therapy for human endometrial cancer to prevent cancer cells from metastasis.

In summary, our results reveal the potential role of Kisspeptin in regulation of human endometrial cancer cell migration and invasion. We demonstrate that KP10 suppressed endometrial cancer cell migration and invasion via MMP-2/9 expression in dependent of ERK signaling pathway, subsequent down-regulation the metastasis-related proteins Twist, N-cadherin, beta-Catenin, and up-regulation E-cadherin. This information provides a mechanistic rationale for the observed Kisspeptin receptor expression in endometrial cancer. Our findings suggest a new insight into the mechanism of Kisspeptin analogue-regulated cell motility in endometrial cancer and expect the previously mentioned molecular could be a candidate to development of newly approaches and therapeutic target in precision medicine.

Acknowledgments

The authors acknowledge F.W. Chen at the Gynecological and Obstetrics Laboratory, Chang Gung Memorial Hospital, for their technical assistance in this study.

Funding

This work was supported by grants from Chang Gung Medical Foundation (CMRPG3I0421, CMRPG3I0422, CMRPG3I0423, and CMRPG3M0871).

Author Contributions

H.-M.W. and W.-J. C. performed the experiments, interpreted the results, and prepared the manuscript. H.-M.W., C.-L.T., L.-H.C., and W.-J. C. contributed to the scientific discussion and the manuscript editing. H.-M.W. and C.-L.T. supervised in the design of the study and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

Disclosures

The authors declare that they have no conflict of interest.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

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