RESEARCH ARTICLE

Oxytocin promotes invasiveness in endometriotic cells via miR-34a and miR-145: Influence of matrix metalloproteinase-2

Yasmin Begum[1], Snehasikta Swarnakar[2*]

ABSTRACT

Background: Endometriosis, a benign gynecological disease causes pain and impaired fertility, and seldom culminates into ovarian cancer. While its cause remains unclear, hormonal dysregulation plays a key role. Oxytocin is upregulated in the disease besides altered matrix metalloproteinases (MMPs) and microRNAs (miRs) that contribute to cellular invasion. Altered levels of miR-34a/miR-145 are reported in endometriosis. A link between oxytocin and miRNAs has also been observed in human myometrium. ***Hypothesis:*** Increased oxytocin might be responsible for cellular invasiveness due to reduced levels of miR-34a/miR-145 which is linked to high levels of MMP-2 activity in endometriotic cell lines.

Materials and Methods: We used zymography and immunoblotting to assess MMP-2 expression and activity respectively in SK-OV-3 cells treated with mimics or inhibitors of miR-34a/miR-145 in the presence or absence of oxytocin. Invasion, migration, and EMT markers were also evaluated under similar treatments as well as oxytocin alone in SK-OV-3 and End1/E6E7 cells. ***Results:*** MMP-2 activity and expression slightly decreased with miR-34a and miR-145 treatment without oxytocin but increased with the addition of oxytocin. Oxytocin dose-dependently enhanced invasion and migration, while miRNA mimics reduced both, which were significantly induced by the addition of oxytocin. Oxytocin also enhanced N-cadherin and vimentin while reducing E-cadherin in the miRNA mimic group.

Conclusion: Our study demonstrates that oxytocin induces cellular invasion and migration via downregulating miR-34a and miR-145 during endometriosis progression.

Keywords: Oxytocin, miR-34a, miR-145, MMP-2, Cellular invasion, Endometriosis

Indian Journal of Physiology and Allied Sciences (2025); DOI: 10.55184/ijpas.v77i01.487 **ISSN:** 0367-8350 (Print)

INTRODUCTION

Endometriosis is a gynecological disease of reproductive women caused by the accumulation of endometrium-derived tissue in extra-uterine regions leading to excessive pain and impaired fertility. It is a hormone driven disorder that affects 10-15% of women globally (1). Although its exact cause is unknown, several theories exist. Sampson's theory of retrograde menstruation, the prevailing hypothesis, posits that menstrual blood containing endometrial cells flows in reverse through the fallopian tubes into the peritoneal cavity, where it establishes ectopic lesions (2). Another theory proposes that normal mesothelial cells transform into ectopic lesions via metaplasia. The pathogenesis of endometriosis involves factors like estrogen dependence, hormonal imbalance, oxidative stress, dysregulated matrix metalloproteinases (MMPs), and aberrant signaling pathways (3). Low estrogen and progesterone causes thinning and inactiveness of the endometrium, leading to amenorrhea, while excessive estrogen leads to hyperplasia and is strongly linked to endometriosis (4, 5). Additionally, oxytocin, known for its role in uterine contractions, also contributes to endometriosis progression (3). Oxytocin receptor (OTR), regulated by oxytocin levels, is a G-protein-coupled receptor that depends on cholesterol and magnesium (3). Estrogen upregulates OTR, while progesterone downregulates it, highlighting their opposing effects on OTR synthesis and degradation (6, 7). In endometriosis, excess estrogen disrupts OTR distribution, increasing circulating oxytocin levels and intense retrograde uterine contractions, causing endometrial fragments to spread into the peritoneal cavity and form lesions (3). High OTR levels are maintained via continuous receptor synthesis and are prominently expressed by smooth muscle cells in peritoneal, deep, and ovarian endometriotic lesions (8, 9). Oxytocin is found to reduce nitrogen oxide and activate the ERK signaling cascade in the physiological endometrium (10, 11). OTR activation triggers the Ca^{+2} signaling pathway by elevating intracellular Ca^{+2} levels

[1]Infectious Diseases & Immunology Division, Council of Scientific and Industrial Research-Indian Institute of Chemical Biology, Jadavpur, Kolkata-700032, India.

[2]Former Chief Scientist, Infectious Diseases & Immunology Division, Lab-254, CSIR-Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata-700032.

***Corresponding author:** Snehasikta Swarnakar, Former Chief Scientist, Infectious Diseases & Immunology Division, Lab-254, CSIR-Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata-700032. , Email: snehasiktaiicbidi@gmail.com

How to cite this article: Begum, Y., Swarnakar, S. Oxytocin promotes invasiveness in endometriotic cells via miR-34a and miR-145: Influence of matrix metalloproteinase-2. *Indian J Physiol Allied Sci* 2025;77(1):26-35.

Conflict of interest: None

Submitted: 24/11/2024 **Accepted:** 19/02/2025 **Published:** 30/03/2025

(1). Atosiban, an OTR-blocking agent, improves the rates of pregnancy and implantation in the cases of infertility associated to endometriosis and also shows efficacy in a rat model of endometriosis (12-14). However, more research is required to explore oxytocin's functional role and mechanisms in endometriosis.

Previous studies also suggested that dysregulation of MMPs is another key to endometriosis pathogenesis. MMPs, zinc-dependent proteolytic enzymes, are critical for extracellular matrix (ECM) remodeling and processes like invasion, migration, proliferation, and autophagy (15-17). The major focus of our lab is to evaluate the functions of MMP-2, -7, -3, and -13 in endometriosis, while studies from other groups identify roles of MMP-7, -9, and -3 in patients and mouse models (18, 19). Recently, MMP-2 has been shown to promote angiogenesis via pathways mediated by prostaglandin E2 in ovarian endometriosis (20).

Recently, a class of naturally occurring small non-coding RNAs (MicroRNAs), have gained prominence as molecular tools for diagnosing and treating various diseases. They typically exert their gene-silencing function by binding to their target genes 3′-untranslated region (3′-UTR) through imperfect base-pairing. This ability permits a single microRNA to target and regulate multiple protein-coding genes (21). As master gene regulators, microRNAs influence critical cellular pathways, including uncontrolled cell proliferation, metastasis, DNA damage repair, and the cell cycle (22). Dysregulation of microRNAs is well-documented in several cancers, where they are reported to act as oncogenes or tumor suppressors (21). The transcription factor p53 is known to induce many microRNAs, including the miR-34 family and miR-145 (21). miR-34a, recognized as a key tumor suppressor, is often deregulated in cancer and endometriosis. Previous studies have shown significantly reduced miR-34a and p53 levels in endometriosis patients (23-25). Furthermore, miR-145 has also been found to have an inhibitory effect on endometriotic cell proliferation, invasion, as well as stemness (26, 27).

Studies have shown a remote relationship between oxytocin and various miRNAs including miR-34a (28-30). While miR-34a and miR-145 have been associated with endometriosis and ovarian cancer, their regulation by oxytocin remains unexplored, creating a gap in understanding how hormonal signaling impacts disease progression. This research, therefore, seeks to delve into the complex functions of oxytocin, miR-34a, and miR-145, along with MMP-2, in endometriotic pathology.

MATERIALS AND METHODS

Chemicals and Reagents

RPMI-1640 Medium (Cat#31800022); Fetal Bovine Serum (FBS) (Cat#16140071); Penicillin-Streptomycin Solution (100x) (Cat#15140122); Trypsin-EDTA Solution (Cat#15400054); phosphate buffered saline (PBS) (Cat#10010023); 0.4% trypan blue solution (Cat#,15250061); and OptiMEM (Cat#31985070) are from Gibco, Thermo Fisher Scientific, USA; Matrigel (Corning, Merck, Cat#CLS356231, USA); MISSION hsa-miR-34a mimic (Cat#HMI0508); MISSION hsa-miR-34a-5p inhibitor (Cat#HSTUD0508); MISSION hsa-miR-145 mimic (Cat#HMI0224); MISSION hsa-miR-145 inhibitor (Cat#HSTUD0224); negative control (Cat#HMC0003) are from Sigma-Aldrich, St. Louis, MO, USA); Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Cat#11668019, USA);. [Ser4,Ile8]-Oxytocin (trifluoroacetate salt) (Cayman Chemical, Cat#24767, USA); Atosiban (Cayman Chemical, Cat#20952, USA); Gelatin (Cat#G2500); Triton X-100 (Cat#T9284); Coomassie blue (Cat#B0770); Protease inhibitor cocktail (Cat#P8340); Bradford reagent (Cat#B6916); BSA (Cat#A7030); Paraformaldehyde (Cat#158127); and Crystal Violet Solution (Cat#C6158) are from Sigma-Aldrich, St. Louis, MO, USA; RIPA Lysis Buffer (Cell Signaling Technology (CST), Cat#9806, USA); 3 color Prestained Protein Ladder, 10-250 kDa (Puregene, Cat#PG-PMT2922); PVDF Membrane (Merck, Immobilon-P, Cat#IPVH00010, USA); HRP Substrate Immobilon Western Chemiluminescent HRP (Merck, Cat#WBKLS0500, USA).

In Vitro Study

The epithelial cell lines End1/E6E7 (derived from endocervical tissue) and SK-OV-3 (originating from ovarian adenocarcinoma) from the American Type Culture Collection (Manassas, VA, USA) were subsequently maintained in standard culturing conditions i.e. in RPMI-1640 medium alongwith 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (100 U/ml), plus under 5% CO_2. Upon 80% confluency, one set of End1/E6E7 cells was treated with oxytocin (0, 1, and 5 µM conc.) post 8 hours incubation with atosiban, an oxytocin inhibitor, or PBS for wound-healing assay while from another set of oxytocin treated cells, the cells were collected post 48 hours of treatment and used for invasion and migration assay.

Transfection experiments were performed in SK-OV-3 cells with 10 nanomolar of either synthetic mimics/inhibitors specific to miR-34a or miR-145 or with a negative control using Lipofectamine 2000 and OptiMEM. 6 hours post-transfection, cells were replenished with newly prepared incomplete medium with or without the treatment of 5µM oxytocin. The gelatin zymography was done from the supernatant collected 48 hours post-transfection. The cells were counted post-trysinization, and used for invasion and migration assays. Additionally, transfection experiments with similar treatments were performed for wound healing assays and immunoblotting. Each analysis was biologically replicated a minimum of three times to ensure accuracy.

Gelatin Zymography

The MMP-2 activity assay was conducted by collecting the supernatant of the transfected cell (either with mimics/inhibitors specific to miR-34a or miR-145, or with a negative control), followed by electrophoresing 10µl of supernatant per lane in 8% SDS-polyacrylamide gel containing gelatin (1mg/ml) as a substrate in a non-reduced environment. The washing of the gels was done two times in 2.5% Triton X-100,

followed by incubating in a Ca^{+2} assay buffer (100mM Tris-HCl, pH 7.4; 150mM NaCl; 5mM $CaCl_2$) for 22 hours at 37°C. 0.1% Coomassie blue dye was then used for staining followed by destaining. The image was taken using ChemiDoc MP. The negative staining zones were quantified for the gelatinolytic activity by utilizing densitometric analysis connected to LabImage software (Kapelan Bio-Imaging, Leipzig, Germany), and images were processed in Adobe Photoshop 2021 (version 22.5.1.441; Adobe Systems, San Jose, CA, USA).

Transwell Invasion Assay

End1 E6/E7 and SK-OV-3 cells were subjected to invasion assays employing Millicell Transwell inserts (Merck, Cat#PTEP24H48, USA). 25×10^3 transfected cells (with the mimics/inhibitors of miR-34a or miR-145 with or without the treatment with oxytocin) were added to the transwell chamber's upper side (pre-coated with Matrigel) under serum-deprived situation whereas the below chamber was filled with the media containing 10% FBS. Cells were scraped off from the upper chamber post 24 hours, and the fixation, staining of the cells on the below part of the chamber was done using 4% paraformaldehyde and 0.5% crystal violet solution respectively. Cells were counted from the images taken from 4 random sites of each well from each replica. Cell imaging was executed in an Olympus microscope equipped with Olympus Camedia software (E-20P, 5.0-megapixel; Chicago, MI, USA). Images were processed in Adobe Photoshop 2021 (version 22.5.1.441; Adobe Systems, San Jose, CA, USA).

Transwell Migration Assay

End1 E6/E7 and SK-OV-3 cells were subjected to migration assays employing Millicell Transwell inserts similar to invasion assay excluding the coating with Matrigel, and the cells were counted from the images taken from 4 random sites of each well from each replica. Cell imaging was executed in an Olympus microscope equipped with Olympus Camedia software (E-20P, 5.0-megapixel; Chicago, MI, USA). Images were processed in Adobe Photoshop 2021 version 22.5.1.441 as mentioned above.

Wound-Healing Assay

End1 E6/E7 and SK-OV-3 cells were subjected to wound-healing assay by utilizing a scratch method. Seeding and culturing of cells (1×10^6) were done until a confluent monolayer was established, followed by creating a linear wound using a micropipette tip. The treatment of cells was done, post-scratch formation, with oxytocin +/- its inhibitor in one set, and with or without oxytocin treatment in mimics/inhibitors transfected cells in another set. Cell imaging was done in multiple intervals of time (0, 12, 24, and 48 hrs) to study the cellular migration using an Olympus microscope equipped with Olympus Camedia software (E-20P, 5.0-megapixel; Chicago, MI, USA). Images were processed in Adobe Photoshop 2021 (version 22.5.1.441 as mentioned above.

Immunoblotting

Cells were treated with miR mimics/inhibitors in the presence or absence of oxytocin and were directly lysed using RIPA buffer having a protease inhibitor cocktail, and the lysates were subsequently centrifuged at 12,000 × g for 15 minutes to remove cellular debris and protein estimation using the Bradford method. Whole-cell lysates (50 µg per lane) were resolved by 8% SDS-PAGE under reducing conditions followed by transfer onto PVDF membranes. After the blocking step, the membranes were subjected to overnight incubation at 4 °C with specific primary antibodies targeting N-cadherin (CST #13116, Cell Signaling Technology, Danvers, MA, USA), vimentin (CST #5741, Cell Signaling Technology, Danvers, MA, USA), E-cadherin (R&D #AF748, R&D Systems, Minneapolis, USA), and GAPDH (SC #SC-47724, Santa Cruz Biotechnology, Dallas, TX, USA), used at 1:1000 (R&D, CST) and 1:500 (Santa Cruz) dilutions. After washes, membranes were probed with HRP-conjugated secondary antibodies (anti-rabbit CST#7074, anti-mouse CST#7076 & anti-goat SC#2922). All the antibodies were diluted in TBS-T with 5% BSA. Protein bands were detected using the Immobilon HRP chemiluminescent substrate (Millipore) under a ChemiDoc MP and the quantification was done utilizing densitometric analysis connected to labimage software (Kapelan Bio-Imaging, Leipzig, Germany). Images were processed in Adobe Photoshop 2021 (version 22.5.1.441 as mentioned above.

Statistical Analysis

Statistical analyses were conducted using GraphPad Instat3 (GraphPad Software, Inc). Comparisons group were conducted via one-way or two-way ANOVA followed by Tukey's post hoc test. Experiments were performed thrice, and data were presented as mean ± SEM. The level chosen at a P-value of <0.05 was accepted significant.

RESULTS

Oxytocin regulates MMP-2 activity via miR-34a

To check the functional implications of oxytocin on MMP-2 by miR-34a and miR-145, gelatin zymography was performed. An elevated MMP-2 activity has been observed when treated with the inhibitor of miR-34a as compared to its mimic. Furthermore, the addition of oxytocin induces the MMP-2 activity in the mimic group as well and the level was elevated by ~1.6-fold (Figure_1a and b). Next, immunoblotting was performed with a similar treatment in SK-OV-3 cells and GAPDH was used as a normalization control for normalizing the expression of protein. Low levels of MMP-2 expression were found (~1.2-fold) in miR-34a mimic as compared to its inhibitor in the absence of oxytocin, however, with the addition of oxytocin the expression of MMP-2 increased in the miR-34a mimic group as well by ~1.2 and 1.7 times for active and pro-MMP-2 respectively. Furthermore, the change in the MMP-2 level is moderate and not significant ($p>0.05$). Additionally, miR-145 was not found to be associated with

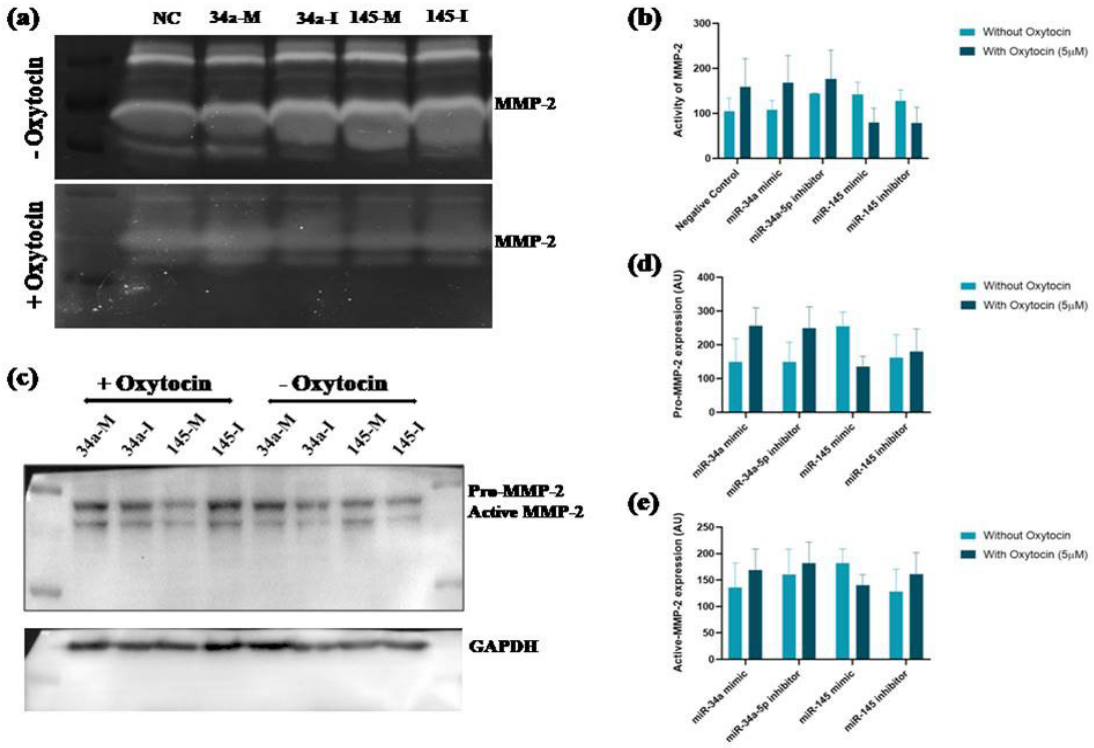

Figure 1: Activity and expression profile of MMPs. Zymographic technique was conducted to assess MMP-2 activity in SK-OV-3 cells. (a) Transfection with miR-34a/miR-145 shows non-significant changes in MMP-2 activity while oxytocin upregulates the activity of MMP-2 non-significantly in the miR-34a mimic group. (b) Quantitative analysis of MMP-2 activity. Immunodetection of MMP-2 shows an elevated expression of MMP-2 in the miR-34a inhibitor group as compared to mimic where it gets upregulated by the incorporation of oxytocin (c-e). Each assay was conducted three independent times, with data depicted as mean ±SEM.

MMP-2 since the expression and activity pattern of MMP-2 are not found to be consistent which proved that the MMP-2 might be regulated by oxytocin via miR-34a and not via miR-145 (Figure_1c-e).

Oxytocin promotes cellular invasion and migration in End1/E6E7 cell line

To elucidate the potential involvement of oxytocin in regulating cellular invasion, End1/E6E7 cells were subjected to a Transwell invasion assay. Cells treated with a low (1μM) and a high dose (5μM) of oxytocin and without oxytocin. Treatment with 5 μM oxytocin resulted in enhanced cell invasion relative to the control, as evidenced by a higher number of cells traversing the matrix to the lower chamber (Figure_2a and Figure_3a). This result shows the role of oxytocin in promoting the invasiveness of the endometriotic cells.

Similarly, the effect of oxytocin on cell migration was evaluated using Transwell migration assays in End1/E6E7 cells, revealing a significant increase in the number of cells migrating toward the lower chamber compared to the control, indicating a pro-migratory role of oxytocin (Figure_2b and Figure_3b).

Oxytocin's influence on migratory capacity was further confirmed on End1/E6E7 cells through the wound-healing experiment. Cells pre-treated with either atosiban or PBS

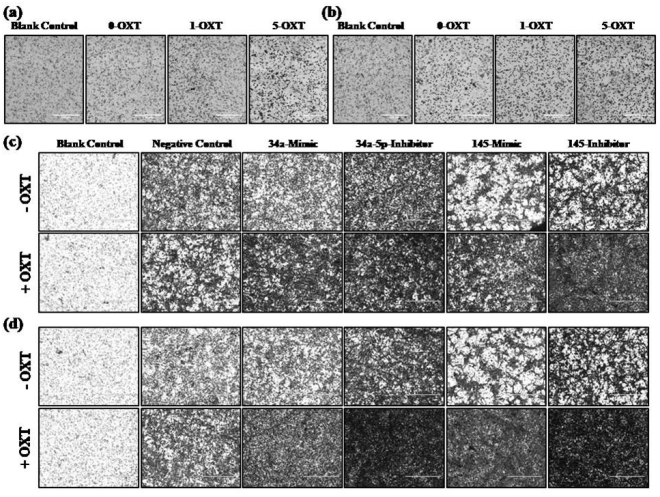

Figure 2: Invasion and migration assay performed in End1/E6E7 and SK-OV-3 cells. The cells showed a significant rise in invasion (a) as well as migration (b) upon oxytocin treatment dose-dependently in End1/E6E7 cells. (c and d) The miR-34a and miR-145 mimic groups show less invasiveness and migration which got reversed by the treatment with their inhibitors in SK-OV-3 cells. The addition of oxytocin significantly induced invasion (c) as well as migration (d) in the mimic groups as well. Each assay was conducted three independent times. Magnification 10X, scale bar = 100 μm, and 400μm.

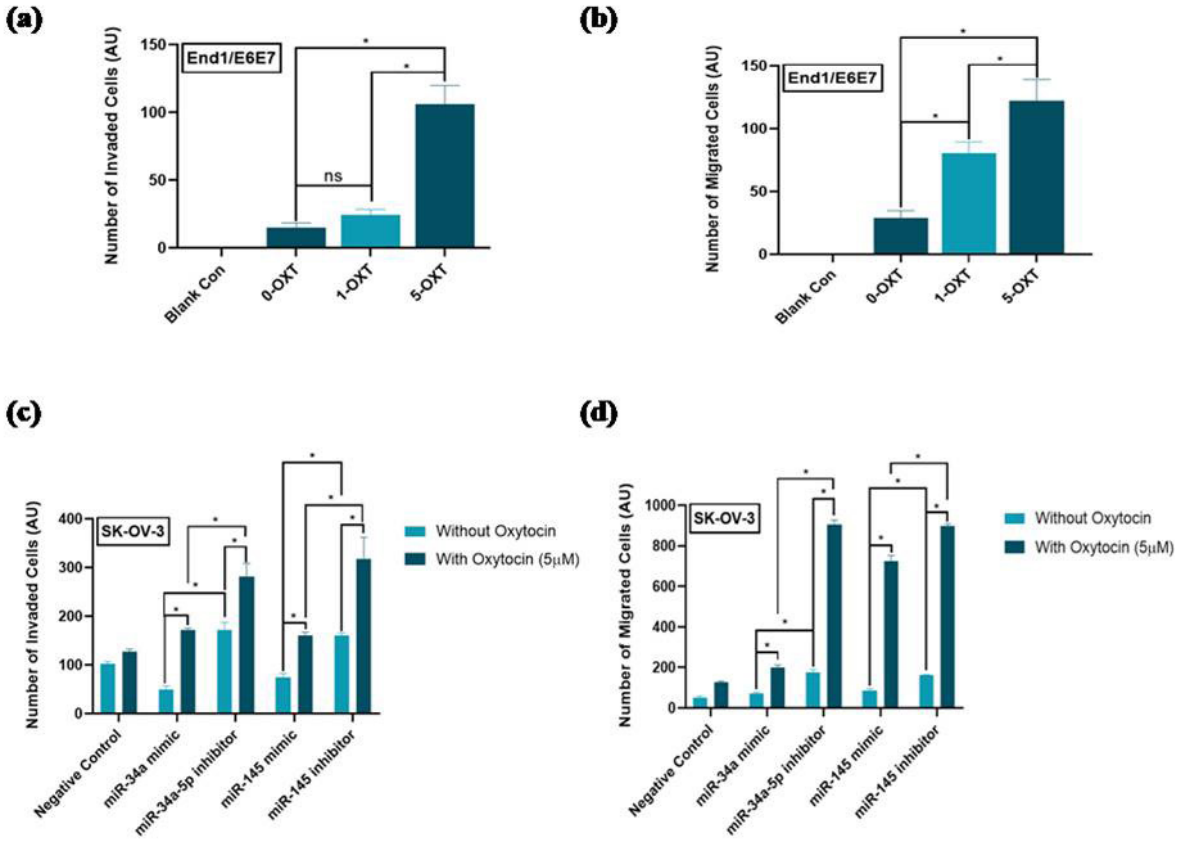

Figure 3: Quantitative analysis of invasion and migration assays. (a and b) Histogram showing the number of invaded and migrated cells respectively in End1/E6E7 cells upon oxytocin treatment. (c and d) Histogram showing the number of invaded and migrated cells respectively in miR transfected SK-OV-3 cells in the presence or absence of oxytocin.

were treated with oxytocin at a low (1μM) and a high dose (5μM) after a scratch was made with a microtip. The cellular migration were observed at successive periods of 0, 12, 24, and 48 hours. The cells in the oxytocin-treated group showed increased migration dose-dependently and the addition of its inhibitor reversed back its effect (Figure_4a and b).

Oxytocin regulates invasion and migration via miR-34a and miR-145

To understand if miR-34a/miR-145 have any relation to oxytocin, the transfected SK-OV-3 cells with their mimics/inhibitors were treated with or without oxytocin and their functions on the invasiveness were checked by a transwell assay using matrigel coating. Treatment with miR-34a and miR-145 mimics led to a reduction in invasive capacity compared to the negative control, which were noticeably higher in their inhibitor-treated group ($p<0.0001$ and $p<0.001$ for miR-34a and miR-145 respectively). Furthermore, the treatment with oxytocin resulted in a significantly higher number of invaded cells which is visible (~2 ($p<0.0001$) to 3.5-fold ($p<0.001$) for miR-34a and miR-145 respectively) across the matrix of the chambers's lower side in the mimic group as compared to the mimic-treated cells without oxytocin (Figure_2c and Figure_3c). This finding highlights oxytocin's regulatory effect on the inhibitory functions of miR-34a and miR-145 in suppressing cell invasiveness.

Similarly, the transwell migration assays were executed to investigate the regulatory effect of oxytocin on miR-34a and miR-145 mediated migration of SK-OV-3 cells and it was observed that both the mimics-treated cells resulted in decreased migration as compared to negative control while the migration is considerably higher in their inhibitor group in the absence of oxytocin ($p<0.0001$ and $p<0.001$ for miR-34a and miR-145 respectively) and the addition of oxytocin visibly led to a remarkable elevation in the numbers of migratory cell (~ 2.8 ($p<0.0001$) to 8-fold ($p<0.0001$) for miR-34a and miR-145 and ~ 5 to 5.5 ($p<0.0001$) in their inhibitors respectively) across the chamber's lower side in the mimic as well as inhibitor group as compared to the cells without oxytocin treatment (Figure_2d and Figure_3d). This result points to the regulatory role of oxytocin on miR-34a and miR-145 mediated migration of the cells.

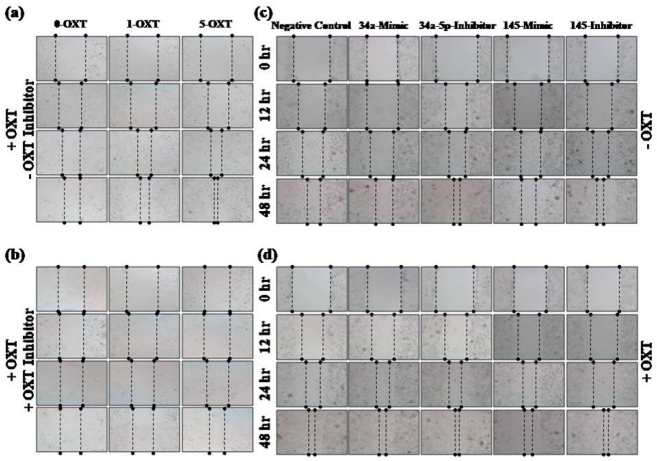

Figure 4: Wound-healing (scratch) assay. (a and b) End1/E6E7 cells with oxytocin treatment show increased migration of the cells with an increasing dose of oxytocin. The migration rate slows down by the treatment with its inhibitor. (c) The miR-34a and miR-145 mimic transfected SK-OV-3 cells show reduced migratory rate when compared to negative control in the absence of oxytocin and the results reverse back by the treatment of their inhibitors. (d) Upon the addition of oxytocin, the migration of the cells transfected with mimic increases significantly. Each assay was conducted three independent times and the migration rate is shown as dotted lines. Magnification 10X.

To revalidate the role of oxytocin in regulating the migratory properties via miR-34a and miR-145, SK-OV-3 cells were also subjected to a scratch assay to assess wound closure dynamics. After scratch formation on the cultured monolayer, miR mimic and inhibitor transfected cells were treated with or without oxytocin and the migratory rate was evaluated periodically at 0, 12, 24, and 48 hours. The cells with the miR-34a mimic show reduced migration in the absence of oxytocin as compared to its inhibitor. However, the addition of oxytocin in transfected cells resulted in a significantly higher rate of cell migration as compared to the group without oxytocin (Figure_4c and d). This result establishes the regulatory effect of oxytocin on miR-34a and miR-145.

Regulatory role of miR-34a and miR-145 on EMT upon oxytocin treatment in SK-OV-3 cell line

To further affirm the influence of oxytocin upon the regulatory role of miR-34a and miR-145 in epithelial-to-mesenchymal transition, the expression levels of N-cadherin, vimentin, and E-cadherin, markers of EMT, were assesed in SK-OV-3 cells upon similar treatment. Immunoblotting was performed using 50 µg of total protein from whole-cell lysates, and GAPDH was employed as the loading control for normalizing the protein expression levels. Results showed

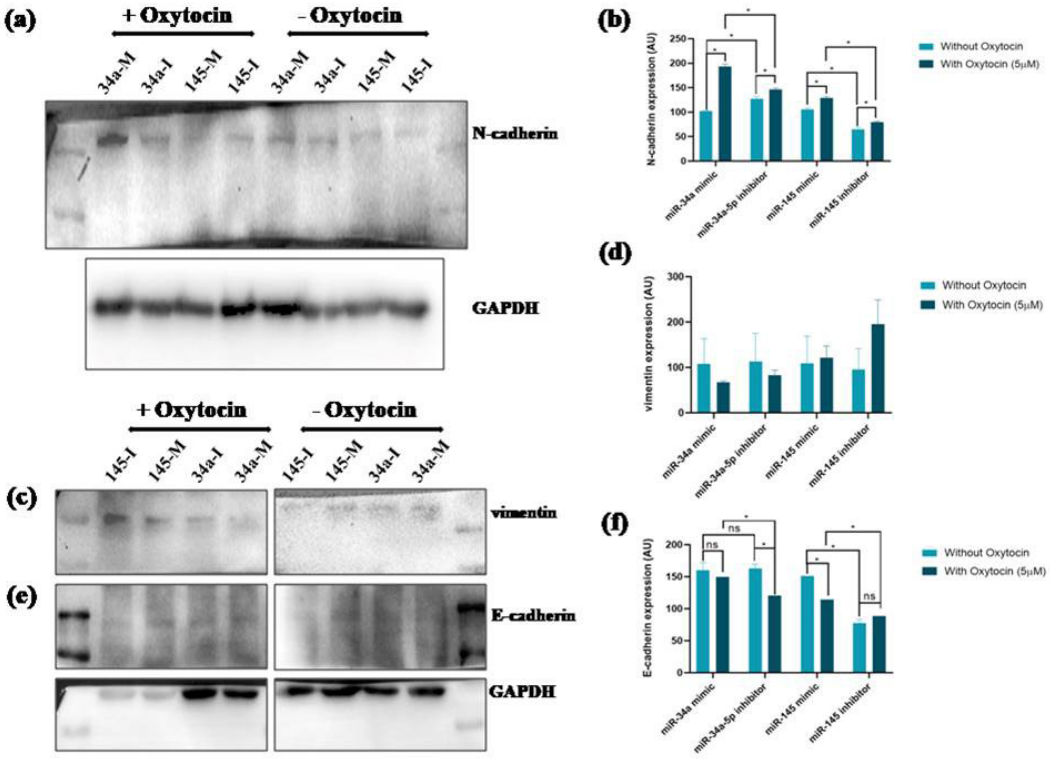

Figure 5: Immunodetection of epithelial-to-mesenchymal transition markers in SK-OV-3 cells. (a-f) The oxytocin treatment of cells transfected with miR mimic shows increased EMT as compared to the oxytocin untreated group. The expression profile of vimentin however show no significant changes. Each assay was conducted three independent times, with data depicted as mean ±SEM. A P-value of <0.05 is referred to as *, and non-significant as ns.

that oxytocin significantly upregulates N-cadherin (~1.9-fold, p<0.0001) expression, whereas inhibits E-cadherin levels (p <0.01) in the miR mimic group compared to the group without oxytocin. No significant changes were observed in vimentin levels (Figure_5). These results explained that the suppressive role of miR-34a and miR-145 on EMT is affected by the presence of oxytocin.

DISCUSSION

Endometriosis is a non-malignant, estrogen-driven disorder marked by the abnormal existence of functional endometrial glands and stroma in extrauterine sites (31). The migration and growth of refluxed endometrial cells are hindered by ECM cleavage and other contributing factors that contribute substantially to endometriosis development (32). Although benign, endometriosis constitutes a notable predisposing factor for ovarian carcinoma (33). Research has found that matrix metalloproteinases, including MMP-2, -9, -7, and -3, as being associated with this disease (18-20, 34). Endometrial cells have a tendency to revert to their mesenchymal phenotype through epithelial-to-mesenchymal transition due to retained mesenchymal traits. EMT has been found to contribute in the pathogenesis of endometriosis in prior studies, as evidenced by the loss of epithelial traits such as polarity and cell adhesion, alongside elevated motility and a shift in cadherin expression from E-cadherin to N-cadherin (35-37). Recent research highlights the role of EMT in deep-infiltrating endometriosis, supported by our previous findings on the involvement of MMP-7 in EMT (31, 35). Moreover, the dysregulated expression of microRNAs has been implicated in endometriosis and ovarian cancer, leading to disruptions in numerous biological processes (38-46).

MicroRNA-34a and miR-145 play critical roles in endometriosis, with miR-34a acting as a universal tumor suppressor and regulator of EMT, stemness, tumor growth, and drug resistance, while miR-145 inhibits proliferation, invasion, and stemness of endometriotic cells, showing potential as therapeutic targets (24, 26, 47-52). However, to date, no research has been conducted to explore the regulatory effect of oxytocin on miR-34a and miR-145 concerning EMT and MMP-2 in endometriosis. The present study shows a moderate upregulation of MMP-2 (not significant) in the cells transfected with the miR-34a-5p inhibitor transfected group as compared to mimic. We also found that oxytocin induced the MMP-2 expression and activity in the cells transfected with mimic as well. Previous studies with skeletal muscle and ovarian tissues including ovarian cancer tissues have shown a complex relationship between oxytocin and various miRNAs, including miR-34a (28-30, 53). Furthermore, we did not find much change in MMP-2 in the miR-145 transfected group. Additionally, oxytocin significantly induced the invasion and migration dose-dependantly in the endometriotic cell line which was reduced by the incorporation of atosiban, an oxytocin inhibitor. This is supported by the previously studied role of oxytocin on invasiveness in endothelial cells (54). The involvement of oxytocin receptors in various cancers including ovarian cancer has also been seen and silencing the oxytocin receptor inhibits cellular proliferation, invasion, and migration and induce cell cycle arrest (55). This study also revealed that oxytocin reduced the inhibitory function of miR-34a and miR-145 in the invasive and migratory potential of the endometrial cells. In addition to that, it also modulated the EMT markers in the mimic transfected groups which were reduced in the absence of oxytocin.

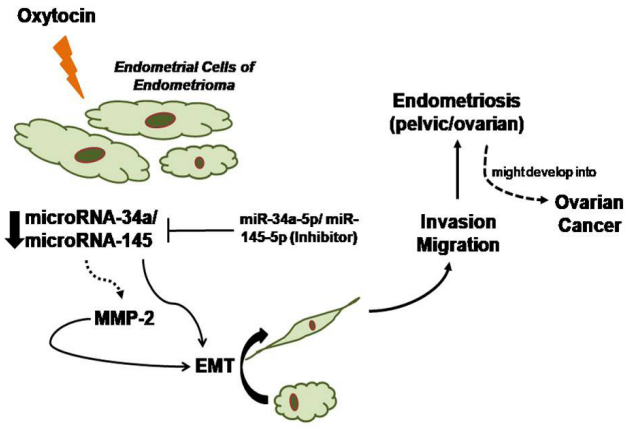

Figure 6: Conclusive illustration. Oxytocin negatively regulates miR-34a and miR-145 in the endometrial cells of endometrioma causing increased MMP-2 and EMT leading to increased migration and invasion which causes the endometrial cells to migrate and invade distant sites thereby leading to the growth of ectopic lesions and endometriosis progression.

Clinical diagnosis of endometriosis is often deferred as a result of the late onset of symptoms, resulting in extended patient discomfort. Based on the outcome of our study regarding miRNAs as potential biomarkers of the disease, the development of a miRNA-based diagnostic approach could facilitate earlier and more accurate detection of endometriosis, opening a new era of endometriosis diagnosis. However, this study focused on in vitro models, which may not fully replicate the complex microenvironment of endometriotic tissues. Additional in vivo studies are obligatory to validate the function of oxytocin in disease progression.

In summary, this study provides the first evidence that miR-34a-5p and miR-145 are regulated by the presence of oxytocin in endometriosis and that excess of local circulating oxytocin might cause the downregulation of miR-34a and miR-145 in endometriotic tissues. In vitro studies on epithelial cells unveiled that oxytocin might be an additional source of the increased MMP-2 activity in endometriosis owing to the ineffective downregulation of MMP-2 by miR-34a-5p. Oxytocin also induces N-cadherin while inhibiting E-cadherin in the mimic transfected groups which further supports that the decrease in miR-34a and miR-145 and increase in oxytocin level promotes endometriosis via the activation of EMT (Figure_6). Altogether, our present study

demonstrates that oxytocin promotes EMT and MMP-2 via reducing miR-34a and miR-145 thereby resulting in induced cellular invasion and migration in endometriosis aggravating disease progression.

AUTHORS CONTRIBUTIONS

YB performed conceptualization (supporting), formal analysis, investigation, methodology, validation, visualization, statistical analysis, writing original draft, reviewing, and editing; and SS did the conceptualization (lead), supervision (lead), reviewing, and editing (supporting). Both authors read and approved the final version of the paper.

ACKNOWLEDGEMENT

We greatly acknowledge Dr. Tapasi Roy for carefully reading and correcting the manuscript.

FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This study was supported by institutional grant no. P-07 from CSIR-Indian Institute of Chemical Biology, Kolkata, India.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Data availability statement

all data presented in the article is available with the author and can be presented upon reasonable request.

REFERENCES

1. Pandit A, Begum Y, Chatterjee K, Swarnakar S. Hormonal regulation of endometriosis and clinical significance. *Indian J Biochem Biophys*. 2018;55(5):351-60. Available at https://nopr.niscpr.res.in/handle/123456789/45076.
2. Swarnakar S, Paul S. Curcumin arrests endometriosis by downregulation of matrix metalloproteinase-9 activity. *Indian J Biochem Biophys*. 2009;46(1):59-65. Available at https://nopr.niscpr.res.in/handle/123456789/3329.
3. Acevedo-Rodriguez A, Mani SK, Handa RJ. Oxytocin and Estrogen Receptor beta in the Brain: An Overview. *Front Endocrinol (Lausanne)*. 2015;6:160. DOI: 10.3389/fendo.2015.00160.
4. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2005;3:28. DOI: 10.1186/1477-7827-3-28.
5. Wingfield M, Macpherson A, Healy DL, Rogers PA. Cell proliferation is increased in the endometrium of women with endometriosis. *Fertil Steril*. 1995;64(2):340-6. DOI: 10.1016/s0015-0282(16)57733-4
6. Quinones-Jenab V, Jenab S, Ogawa S, Adan RA, Burbach JP, Pfaff DW. Effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the uterus, pituitary, and forebrain of the female rat. *Neuroendocrinology*. 1997;65(1):9-17. DOI: 10.1159/000127160
7. Bishop CV. Progesterone inhibition of oxytocin signaling in endometrium. *Front Neurosci*. 2013;7:138. DOI: 10.3389/fnins.2013.00138.
8. Anaf V, Simon P, Fayt I, Noel J. Smooth muscles are frequent components of endometriotic lesions. *Hum Reprod*. 2000;15(4):767-71. DOI: 10.1093/humrep/15.4.767.
9. Huang M, Li X, Guo P, Yu Z, Xu Y, Wei Z. The abnormal expression of oxytocin receptors in the uterine junctional zone in women with endometriosis. *Reprod Biol Endocrinol*. 2017;15(1):1. DOI: 10.1186/s12958-016-0220-7.
10. Danylovychlu V. [Effect of steroid hormones and oxytocin on NO and H2O2 production in the endometrium]. *UkrBiokhimZh (1999)*. 2004;76(1):88-96. PMID: 15909422.
11. Zlatnik MG, Copland JA, Ives K, Soloff MS. Functional oxytocin receptors in a human endometrial cell line. *Am J Obstet Gynecol*. 2000;182(4):850-5. DOI: 10.1016/s0002-9378(00)70334-6.
12. Husslein P, Quartarolo JP. Review of clinical experience with atosiban and the Tractocile Efficacy Assessment Survey in Europe (TREASURE) study protocol. *Int J Clin Pract*. 2003;57(2):121-7. PMID: 12661796.
13. He Y, Wu H, He X, *et al*. Administration of atosiban in patients with endometriosis undergoing frozen-thawed embryo transfer: a prospective, randomized study. *Fertil Steril*. 2016;106(2):416-22. DOI: 10.1016/j.fertnstert.2016.04.019.
14. Simsek Y, Celik O, Karaer A, *et al*. Therapeutic efficiency of Atosiban, an oxytocin receptor blocking agent in the treatment of experimental endometriosis. *Arch Gynecol Obstet*. 2012;286(3):777-83. DOI: 10.1007/s00404-012-2390-7.
15. Salamonsen LA, Zhang J, Hampton A, Lathbury L. Regulation of matrix metalloproteinases in human endometrium. *Hum Reprod*. 2000;15(Suppl 3):112-9. DOI: 10.1093/humrep/15.suppl_3.112.
16. Sui X, Li Y, Sun Y, Li C, Li X, Zhang G. Expression and significance of autophagy genes LC3, Beclin1 and MMP-2 in endometriosis. *Exp Ther Med*. 2018;16(3):1958-62. DOI: 10.3892/etm.2018.6362.
17. Wang Y, Ren Y, Li N, Zhao J, Zhao S. Rab11a promotes the malignant progression of ovarian cancer by inducing autophagy. *Genes Genomics*. 2022;44(11):1375-84. DOI: 10.1007/s13258-022-01314-0.
18. Paul S, Sharma AV, Mahapatra PD, Bhattacharya P, Reiter RJ, Swarnakar S. Role of melatonin in regulating matrix metalloproteinase-9 via tissue inhibitors of metalloproteinase-1 during protection against endometriosis. *J Pineal Res*. 2008;44(4):439-49. DOI: 10.1111/j.1600-079X.2007.00547.x.
19. Jana S, Paul S, Swarnakar S. Curcumin as anti-endometriotic agent: implication of MMP-3 and intrinsic apoptotic pathway. *Biochem Pharmacol*. 2012;83(6):797-804. DOI: 10.1016/j.bcp.2011.12.030.
20. Jana S, Chatterjee K, Ray AK, DasMahapatra P, Swarnakar S. Regulation of Matrix Metalloproteinase-2 Activity by COX-2-PGE2-pAKT Axis Promotes Angiogenesis in Endometriosis. *PLoS One*. 2016;11(10):e0163540. DOI: 10.1371/journal.pone.0163540.
21. Xu M, Mo YY. The Akt-associated microRNAs. *Cell Mol Life Sci*. 2012;69(21):3601-12. DOI: 10.1007/s00018-012-1129-8.
22. Pandit A, Begum Y, Saha P, Srivastava AK, Swarnakar S. Approaches toward targeting matrix metalloproteases for prognosis and therapies in gynecological cancer: MicroRNAs as a molecular driver. *Front Oncol*. 2021;11:720622. DOI: 10.3389/fonc.2021.720622.
23. Misir S, Hepokur C, Oksasoglu B, Yildiz C, Yanik A, Aliyazicioglu Y. Circulating serum miR-200c and miR-34a-5p as diagnostic

biomarkers for endometriosis. *J GynecolObstet Hum Reprod*. 2021;50(4):102092. DOI: 10.1016/j.jogoh.2021.102092.
24. Rezk NA, Lashin MB, Sabbah NA. MiRNA 34-a regulate SIRT-1 and Foxo-1 expression in endometriosis. *Noncoding RNA Res*. 2021;6(1):35-41. DOI: 10.1016/j.ncrna.2021.02.002.
25. Kolanska K, Bendifallah S, Canlorbe G, *et al*. Role of miRNAs in normal endometrium and in endometrial disorders: Comprehensive review. *J Clin Med*. 2021;10(16). DOI: 10.3390/jcm10163457.
26. Adammek M, Greve B, Kassens N, *et al*. MicroRNA miR-145 inhibits proliferation, invasiveness, and stem cell phenotype of an in vitro endometriosis model by targeting multiple cytoskeletal elements and pluripotency factors. *Fertil Steril*. 2013;99(5):1346-55 e5. DOI: 10.1016/j.fertnstert.2012.11.055.
27. Chang L, Yuan Z, Shi H, Bian Y, Guo R. miR-145 targets the SOX11 3'UTR to suppress endometrial cancer growth. *Am J Cancer Res*. 2017;7(11):2305-17. PMID: 29218252.
28. Fabova Z, Loncova B, Bauer M, Sirotkin AV. Interrelationships between miR-34a and FSH in the control of porcine ovarian cell functions. *Reprod Sci*. 2023;30(6):1789-807. DOI: 10.1007/s43032-022-01127-2.
29. Wang Y, Wang J, Li Q, *et al*. Characterization of MicroRNA expression profiles in the ovarian tissue of goats during the sexual maturity period. *J Ovarian Res*. 2023;16(1):234. DOI: 10.1186/s13048-023-01318-8.
30. Zheng Y, Kong J, Li Q, Wang Y, Li J. Role of miRNAs in skeletal muscle aging. *Clin Interv Aging*. 2018;13:2407-19. DOI: 10.2147/CIA.S169202.
31. Chatterjee K, Jana S, DasMahapatra P, Swarnakar S. EGFR-mediated matrix metalloproteinase-7 up-regulation promotes epithelial-mesenchymal transition via ERK1-AP1 axis during ovarian endometriosis progression. *FASEB J*. 2018;32(8):4560-72. DOI: 10.1096/fj.201701382RR.
32. Pandit A, Das Mahapatra P, Saha P, Srivastava AK, Swarnakar S. Interleukin-1beta activated c-FOS transcription factor binds preferentially to a specific allele of the matrix metalloproteinase-13 promoter and increases susceptibility to endometriosis. *J Cell Physiol*. 2022;237(7):3095-108. DOI: 10.1002/jcp.30773.
33. Kvaskoff M, Horne AW, Missmer SA. Informing women with endometriosis about ovarian cancer risk. *Lancet*. 2017;390(10111):2433-4. DOI: 10.1016/S0140-6736(17)33049-0.
34. Bruner-Tran KL, Zhang Z, Eisenberg E, Winneker RC, Osteen KG. Down-regulation of endometrial matrix metalloproteinase-3 and -7 expression in vitro and therapeutic regression of experimental endometriosis in vivo by a novel nonsteroidal progesterone receptor agonist, tanaproget. *J Clin Endocrinol Metab*. 2006;91(4):1554-60. DOI: 10.1210/jc.2005-2024.
35. Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum Reprod*. 2012;27(3):712-21. DOI: 10.1093/humrep/der442.
36. Wang D, Luo Y, Wang G, Yang Q. CircATRNL1 promotes epithelial-mesenchymal transition in endometriosis by upregulating Yes-associated protein 1 in vitro. *Cell Death Dis*. 2020;11(7):594. DOI: 10.1038/s41419-020-02784-4.
37. Bartley J, Julicher A, Hotz B, Mechsner S, Hotz H. Epithelial to mesenchymal transition (EMT) seems to be regulated differently in endometriosis and the endometrium. *Arch Gynecol Obstet*. 2014;289(4):871-81. DOI: 10.1007/s00404-013-3040-4.
38. Bjorkman S, Taylor HS. MicroRNAs in endometriosis: biological function and emerging biomarker candidatesdagger. *Biol Reprod*. 2019;100(5):1135-46. DOI: 10.1093/biolre/ioz014.
39. Nasu K, Aoyagi Y, Zhu R, *et al*. Role of repressed microRNAs in endometriosis. *Med Mol Morphol*. 2022;55(1):1-7. DOI: 10.1007/s00795-021-00303-8.
40. Zhang J, He J, Zhang L. The down-regulation of microRNA-137 contributes to the up-regulation of retinoblastoma cell proliferation and invasion by regulating COX-2/PGE2 signaling. *Biomed Pharmacother*. 2018;106:35-42. DOI: 10.1016/j.biopha.2018.06.099.
41. Xiong Y, Wang Y, Wang L, *et al*. MicroRNA-30b targets Snail to impede epithelial-mesenchymal transition in pancreatic cancer stem cells. *J Cancer*. 2018;9(12):2147-59. DOI: 10.7150/jca.25006.
42. Otto T, Candido SV, Pilarz MS, *et al*. Cell cycle-targeting microRNAs promote differentiation by enforcing cell-cycle exit. *Proc Natl Acad Sci U S A*. 2017;114(40):10660-5. DOI: 10.1073/pnas.1702914114.
43. Yang T, Ge B. miRNAs in immune responses to Mycobacterium tuberculosis infection. *Cancer Lett*. 2018;431:22-30. DOI: 10.1016/j.canlet.2018.05.028.
44. Rosas-Hernandez H, Chigurupati S, Raymick J, *et al*. Identification of altered microRNAs in serum of a mouse model of Parkinson's disease. *Neurosci Lett*. 2018;687:1-9. DOI: 10.1016/j.neulet.2018.07.022.
45. Peng D, Wang H, Li L, *et al*. miR-34c-5p promotes eradication of acute myeloid leukemia stem cells by inducing senescence through selective RAB27B targeting to inhibit exosome shedding. *Leukemia*. 2018;32(5):1180-8. DOI: 10.1038/s41375-018-0015-2.
46. Hosseinahli N, Aghapour M, Duijf PHG, Baradaran B. Treating cancer with microRNA replacement therapy: A literature review. *J Cell Physiol*. 2018;233(8):5574-88. DOI: 10.1002/jcp.26514.
47. Agarwal S, Hanna J, Sherman ME, Figueroa J, Rimm DL. Quantitative assessment of miR34a as an independent prognostic marker in breast cancer. *Br J Cancer*. 2015;112(1):61-8. DOI: 10.1038/bjc.2014.573.
48. Daige CL, Wiggins JF, Priddy L, Nelligan-Davis T, Zhao J, Brown D. Systemic delivery of a miR34a mimic as a potential therapeutic for liver cancer. *Mol Cancer Ther*. 2014;13(10):2352-60. DOI: 10.1158/1535-7163.MCT-14-0209.
49. Xu D, Song Q, Liu Y, *et al*. LINC00665 promotes Ovarian Cancer progression through regulating the miRNA-34a-5p/E2F3 axis. *J Cancer*. 2021;12(6):1755-63. DOI: 10.7150/jca.51457.
50. Iqbal MJ, Javed Z, Sadia H, *et al*. Targeted therapy using nanocomposite delivery systems in cancer treatment: highlighting miR34a regulation for clinical applications. *Cancer Cell Int*. 2023;23(1):84. DOI: 10.1186/s12935-023-02929-3.
51. Rokavec M, Oner MG, Li H, *et al*. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest*. 2014;124(4):1853-67. DOI: 10.1172/JCI81340.
52. Li S, Wei X, He J, *et al*. The comprehensive landscape of miR-34a in cancer research. *Cancer Metastasis Rev*. 2021;40(3):925-48. DOI: 10.1007/s10555-021-09973-3.
53. Gumusoglu-Acar E, Gunel T, Hosseini MK, *et al*. Metabolic pathways of potential miRNA biomarkers derived from liquid biopsy in epithelial ovarian cancer. *Oncol Lett*. 2023;25(4):142. DOI: 10.3892/ol.2023.13728.

54. Cattaneo MG, Chini B, Vicentini LM. Oxytocin stimulates migration and invasion in human endothelial cells. *Br J Pharmacol*. 2008;153(4):728-36. DOI: 10.1038/sj.bjp.0707609.

55. Sun J, Xu Z, Mao Y, Zhang T, Qin Y, Hua D. Prognostic role of oxytocin receptor in colon adenocarcinoma. *Open Med (Wars)*. 2021;16(1):1762-76. DOI: 10.1515/med-2021-0387.

PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.