ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Elevated levels of adrenomedullin in eutopic endometrium and plasma from women with endometriosis

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Objective: To test adrenomedullin (*Adm*, ADM) as a downstream target of signal transducer and activator of transcription 3 (STAT3) in endometrial cells and to test midregional proadrenomedullin (MR-proADM) as a biomarker of endometriosis.
Design: Cross-sectional analysis of *Adm* expression in eutopic endometrium and of MR-proADM in plasma from women with and without endometriosis; and prospective study of MR-proADM levels in women with endometriosis undergoing surgical resection of ectopic lesions.
Setting: Academic medical centers.
Patient(s): Fifteen patients with endometriosis and 11 healthy control subjects who donated eutopic endometrial biopsies; and 28 patients with endometriosis and 19 healthy control subjects who donated plasma for MR-proADM analysis.
Intervention(s): None.
Main Outcome Measure(s): *Adm* mRNA levels according to quantitative real-time polymerase chain reaction after activation of STAT3 by interleukin-6 (IL-6) in Ishikawa cells; immunohistochemistry for ADM in eutopic endometrial biopsies from women with endometriosis compared with healthy donors; and MR-proADM levels measured by commercial immunoassay in plasma from healthy women and women with endometriosis who subsequently underwent surgical resection of ectopic lesions.
Result(s): Activation of STAT3 by IL-6 up-regulated *Adm* mRNA expression in Ishikawa cells. ADM protein levels were elevated in the eutopic endometrium of women with endometriosis. MR-proADM concentrations were higher in women with endometriosis but were not correlated with disease stage, corrected by surgery, or predictive of fertility outcome.
Conclusion(s): MR-proADM may be able to serve as a biomarker of endometriosis, but it is unknown whether elevated MR-proADM levels are secondary to the estrogenic and inflammatory properties of endometriosis or an inciting pathogenic factor. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Adrenomedullin, endometriosis, midregional proadrenomedullin

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Endometriosis is a common gynecologic disease characterized by the presence of endometrial tissue outside of the eutopic endometrium, commonly in the peritoneum of the pelvis and in pelvic organs, and often causing scarring and pain (1, 2). Although endometriosis is also strongly associated with subfertility and infertility, the mechanisms underlying fertility problems in women with stage 1–2 disease is a subject of debate (3, 4). The nonspecific nature of endometriosis symptoms makes the disease difficult to diagnose, and confident diagnosis usually requires visualization of ectopic lesions during surgical exploration. Endometriosis is equally difficult to treat; hormonal therapies and surgical excision of ectopic lesions are the mainstays of treatment but are not always effective (5, 6). These diagnostic and therapeutic challenges have fueled interest in identifying biomarkers and signaling pathways associated with endometriosis (7).

Recently, Kim et al. demonstrated aberrant activation of signal transducer and activator of transcription 3 (STAT3) in the eutopic endometrium of women with endometriosis (8). Other studies consistently point to

Received October 26, 2017; revised January 22, 2018; accepted February 2, 2018.
B.C.M. and K.M.C. have a pending patent application, Adrenomedullin Therapy to Improve Fetal Implantation. K.E.Q. has nothing to disclose. B.A.L. advises and consults for Abbvie and Exeltis and has received grant funding from Pfizer. B.A.L. and S.L.Y. have intellectual property licensed to CiceroDx.
Supported by U.S. National Institutes of Health grants HD060860 (to K.M.C.), HD067721 (to S.L.Y. and B.A.L.), HD085652 (to B.C.M.), and a Lalor Foundation Fellowship (to K.E.Q.).
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Fertility and Sterility® Vol. ■, No. ■, ■ 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.02.004

endometriosis-associated factors that affect STAT3 activation: the cytokine interleukin 6 (IL-6), which activates STAT3 via the IL-6 receptor, is elevated in the peritoneal fluid of women with endometriosis (9–11); miR120, which targets STAT3, is elevated in endometriotic cyst stromal cells (12); and protein inhibitor of activated STAT3 and dual-specificity phosphatase 2, negative regulators of STAT3, are down-regulated in endometriosis (13, 14). Kim et al. also found hypoxia inducible factor 1A (HIF1A), which is stabilized by STAT3, to be elevated in the eutopic endometrium of women with endometriosis. Taken together, these data strongly implicate the IL-6–STAT3–HIF1A pathway in the pathophysiology of endometriosis (8).

Both STAT3 and HIF1A have been previously identified as regulators of the versatile peptide hormone adrenomedullin (*Adm*, ADM) (15–18). ADM is expressed in the female reproductive system and has been associated with female reproductive physiology, including embryo implantation and placentation, and pathophysiology, including subfertility and complications of pregnancy, such as preeclampsia (19, 20). In endometriosis, ADM is higher in intrafollicular fluid and negatively associated with oocyte maturity and embryo quality in women with endometriosis, underscoring a potential link between ADM, endometriosis, and fertility (21). Collectively, these data imply that ADM may be able to serve as a biomarker of endometriosis.

Mid-regional pro-adrenomedullin (MR-proADM) is a by-product of post-translational processing of preproADM peptide and is a more stable analyte than the mature ADM peptide (22). In the past decade, many groups have found prognostic value for MR-proADM plasma concentrations as a biomarker of heart failure (23), community-acquired pneumonia (24), and sepsis (25), among other diseases. In reproduction, MR-proADM has been tested as a biomarker of gestational diabetes and preeclampsia (26, 27). Here, we test the hypothesis that MR-proADM, as a surrogate for ADM potentially downstream of the IL-6–STAT3 axis, can serve as a biomarker of endometriosis.

MATERIALS AND METHODS

Study Design and Human Subjects

The study was approved by the Institutional Review Boards of Greenville Health System and the University of North Carolina at Chapel Hill. Informed consent was obtained from all study participants, who were 18–45 years of age and had not used hormonal therapies or an intrauterine device in the 3 months preceding biopsy or plasma collection. Eutopic endometrial biopsies were collected from healthy donor women and women with endometriosis in both proliferative and secretory phases at the time of surgery at Greenville Health System and the University of North Carolina. Plasma samples for analysis of MR-proADM concentrations were collected from healthy women and from women with endometriosis in both proliferative and secretory phases at Greenville Health System and the University of North Carolina. Patients who wished to conceive were followed expectantly after surgery for up to 6 months and pregnancies recorded. Pregnancy was defined as a visible gestational sac with cardiac activity on ultrasound and referral for obstetrical care. The clinical characteristics of women from whom plasma was collected are presented in Table 1. MR-proADM concentrations were measured in undiluted plasma with the use of a commercial assay (Brahms MR-proADM Kryptor; Phadia Immunology Reference Laboratory).

Immunohistochemistry

Five-micrometer sections of paraffin-embedded endometrial biopsies were deparaffinized and hydrated. After antigen retrieval in 10 mmol/L citric acid/0.05% Tween-20 (pH 6.0), endogenous peroxidase activity was quenched by means of 3% hydrogen peroxide in phosphate-buffered saline solution (PBS). Tissues were permeabilized with the use of PBS/0.1% Triton X-100 (PBST) and then blocked in 10% normal goat serum/1% bovine serum albumin in PBST. Tissues were incubated in anti-ADM primary antibody (1:200; Abcam ab69117) in block overnight at room temperature. The following day, slides were washed and incubated in biotinylated goat antirabbit antibody (1:250, Jackson Immunoresearch) for 1 hour. Avidin-biotin complexes (Vectastain Elite ABC Kit; Vector Laboratories) were added to tissues for 30 minutes, and then diaminobenzidine (DAB Peroxidase [HRP] Substrate Kit; Vector Laboratories) was added for 2 minutes. Slides were rinsed with tap water, counterstained with hematoxylin (Vector Laboratories) for 20 seconds, and then rinsed with tap water again. Tissues were dehydrated and then coverslipped with the use of DPX mountant (VWR). Slides were imaged on a Zeiss AxioImager with ProgRes Capturepro software (Jenoptik). Staining intensity was determined by a blinded observer (K.E.Q.) and graded on a scale of 0 (no staining) to 4 (strong staining).

Cell Culture, Western Blot, and Quantitative Real-Time Polymerase Chain Reaction

Ishikawa cells were cultured in DMEM/F12 (Gibco) + 10% fetal bovine serum (FBS) + 1× penicillin/streptomycin (Gibco) in a 37°C incubator containing 5% CO_2. For Western blot analysis, Ishikawa cells were grown to confluency in 10-cm dishes and treated with a vehicle control or 1, 10, or 100 ng/mL human IL-6 (R&D Systems) for 15 minutes. Cells

TABLE 1

Clinical characteristics of study participants.

Characteristic	Control (n = 19)	Endometriosis (n = 28)
Age, y	26.2 ± 4.4 (20–33)	32.9 ± 4.9 (23–41)
BMI, kg/m^2	22.4 ± 2.9 (18.3–28.2)	23.7 ± 4.7 (18.7–42.5)
Gravidity at biopsy	0 (0–3)	0 (0–4)
Race		
White	9	26
Black	7	0
Asian	3	1
Multiple	0	1

Note: Age and body mass index (BMI) are presented as mean ± standard deviation (range). Gravidity is presented as median (range).

Matson. MR-proADM as biomarker of endometriosis. Fertil Steril 2018.