MicroRNA expression in adolescents and young women with endometriosis

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Disclosure

- I have no financial relationship with a commercial entity producing health-care related products and/or services
Endometriosis

- Implantation of endometrial glands/stroma outside of the uterus, in up to 10% of women.
- Associated with pelvic pain, dyspareunia, dysmenorrhea, and bladder and bowel dysfunction.
- Infertility in 30-50%: Altered pelvic anatomy, inflammatory and immune function, oocyte quality.

Endometriosis

- Symptoms often start at menarche, but diagnosis is often delayed (on average 6.7 years)
- Early identification and intervention can slow disease progression and may improve fertility and functional outcomes.
- Currently, definitive diagnosis requires surgical visualization

A Non-Invasive Test for Endometriosis

- Earlier diagnosis and referral to specialists
- Diagnosis for patients with geographic, insurance or other barriers to specialist referral
- Patients could potentially avoid unnecessary surgery
- Monitoring endometriosis activity and recurrence (avoid reoperation)
- Insights into the currently enigmatic pathogenesis of disease
- In the future: miRNA profile of endometriosis may allow for personalized precision medical treatment
MiRNA

- Small noncoding RNA involved in epigenetic gene regulation largely through messenger RNA silencing.
MiRNA Utility

- MiRNA are identified in a wide variety of biologic samples, including tissues, serum, plasma, saliva and urine
- RNAase-resistant, therefore biologically stable.
- MiRNA profiles of endometriosis and ectopic pregnancies, among other gynecologic conditions, are starting to be elucidated

MiRNA and Endometriosis

- Expression of over 20 specific miRNA shown to be altered in adults with endometriosis.
  - miRNA 16, 17, 18a, 20a, 21, 22, 29c, 34c, 100, 125a, 126, 141, 145, 195, 199a, 200a, 200b, 200c, 202, 222, 424, 449

- Inverse correlations are observed between certain miRNA dysregulated in endometriosis and
  - VEGF-A (upregulating angiogenesis)
  - COX-2 (an enzyme involved in inflammation)
  - TGF-beta (regulating cell growth, proliferation, differentiation and apoptosis).

Clinical application of miRNA

Though none are currently used in patient care, miRNA-based diagnostic tests for clinical use are in development.
Need for research

- Existing studies of miRNA profiles in endometriosis:
  - Small
  - Span endometriosis stages I-IV
  - Include only adults
  - Inconsistent in biologic sample analyzed (serum, plasma, peritoneal fluid, eutopic or ectopic endometrium, endometriomas)

- These issues limit comparisons among studies, and to adolescents
Specific Aims

• To test the hypothesis that certain miRNA are differentially expressed in adolescent patients with surgically-confirmed endometriosis, as compared to age-matched healthy controls.

• **Specific Aim 1**: “Discovery”—To perform quantitative real-time PCR using plasma from a representative subset of adolescent patients and age-matched controls.

• **Specific Aim 2**: To externally validate dysregulated miRNAs identified in Specific Aim 1, using the FirePlex™ Circulating microRNA Assay (Abcam®, Cambridge, MA), in a replication set of case samples and age-matched controls.
Study samples

- **Case samples:** Females 13 - 26 years with surgically-confirmed endometriosis, enrolled in The Women’s Health Study: From Adolescence to Adulthood, a longitudinal cohort study of endometriosis across the lifespan.
- **Controls:** Healthy volunteers, age 13-26, without signs or symptoms of endometriosis
Study samples

- Plasma collected and processed according to World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonization Project (EPHect) guidelines.

- Blood centrifuged 2,500 x g for 10 minutes at 4°C; the plasma was aspirated, aliquoted into cryovials, then frozen to -80°C.
An expanded WERF Endometriosis Phenome and Biobanking Harmonisation Project clinical questionnaire was completed upon enrollment.

- Includes extensive medical, surgical and family history
- Symptom onset, location, severity
- Endometriosis treatments past and present (NSAIDs, opiates, hormonal)
Discovery phase

- Matched 10 pairs of cases and controls
- 5 pairs using hormonal medications at the time of sample collection, 5 without hormone use for at least 30 days prior to collection
- Samples collected before any surgery performed, or ≥ 6 months postoperatively
- Cases and controls age-matched within 2 years
- For discovery phase, excluded patients with known inflammatory or autoimmune disease
MiRNA extraction and quantification
Quantitative real-time PCR

- Applied Biosystems TaqMan® Array MiRNA 384 well cards x 2
- Analyze 760+ miRNA in each sample
- Relative miRNA expression between cases and controls calculated using the $2^{-\Delta\Delta Ct}$ method.
Preliminary results

Upregulated:
- miR-1180
- miR-1183
- miR-122
- miR-1291
- miR-135b
- miR-144
- miR-15b
- miR-183
- miR-18a
- miR-202
- miR-204
- miR-205
- miR-208
- miR-218
- miR-25

Downregulated:
- miR-1276
- miR-1298
- miR-148a
- miR-181c
- miR-331-5p
- miR-487a
- miR-520D-3p
- miR-551b
- miR-571
- miR-597
- miR-654
- miR-661
- miR-770-5p
- miR-885-5p

Gene Symbols:
- TGF-BR1
- TNF-R
- MMP-24
- estrogen-related receptor-α
- GABA-B-R
- prostaglandin G/H synthase and cyclooxygenase
- Estrogen Receptor 1
- MMP16
- EGF
- FGF-9
- IGF2R
- SHOX
- TGF-B1

Molecular Functions:
- colony stimulating factor 1 (macrophage)
Next Step: Confirmation

- **FirePlex: Multiplex circulating miRNA assay**
- 68 miRNA per well, 96 wells
- Hydrogel technology—each well contains particles with code to identify individual miRNA
- Readout is performed on flow cytometer

**Per well:**
- 68 miRNAs
- 20 replicates
- 1,360 data points

True multiplexing: Multiple miRNAs are detected within a single well.
Thank you

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