Immunohistochemical analysis of GATA2 expression in endometrium and its relationship with hormone receptor expression in benign and premalignant endometrial disorders

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Abstract

The GATA gene family encodes highly conserved zinc-finger transcription factors that facilitate the development and function of multiple organ systems including the uterus. In the endometrium, GATA2 functions in a positive autoregulatory loop with the progesterone receptor (PGR) and colocalizes with PGR on chromatin to promote PGR transcriptional programs. GATA2 also has PGR-independent functions that maintain endometrial cell identity, and *GATA2* transcripts reportedly are down-regulated in endometrial disorders including endometriosis. This event is accompanied by a reciprocal increase in *GATA6*. Here, we applied custom anti-GATA2 monoclonal antibodies and performed GATA2 immunohistochemistry (IHC) on patient endometrial tissues corresponding to proliferative, secretory, inactive, and hormone-treated endometrium, as well as endometriosis and endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia (EAH/EIN). We also performed IHC for the estrogen receptor, PGR, and GATA6 in relevant groups. The results reveal a tight correlation between GATA2 and PGR expression in the glandular and stromal cells of benign endometrium. GATA2 expression is markedly reduced in stromal but not glandular cells in endometriosis and EAH/EIN. This reduction in GATA2 expression does not lead to a detectable increase in GATA6 expression in endometriosis. Although average glandular GATA2 expression was preserved in endometriosis and EAH/EIN cases, its expression was decoupled from PGR, implying that alternative pathways regulate GATA2 levels in these disorders. Our findings indicate that GATA2 dysregulation is a feature of endometriosis and EAH/EIN, and support a model whereby loss of stromal GATA2 in these disorders contributes to their progesterone insensitivity.

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Keywords

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INTRODUCTION

Uterine health and function are regulated by the ovarian steroid hormones estradiol and progesterone, which act on estrogen and progesterone receptors (ERs and PGRs) to mediate critical endometrial activities [1,2,3]. The endometrium constitutes the functional inner layer of the uterus and is composed of hormone-sensitive glandular and stromal elements (Supplementary Figure 1). At the start of the menstrual cycle, the endometrium proliferates under the influence of estrogen. Progesterone is secreted by the corpus luteum after ovulation and promotes a secretory transition and endometrial decidualization. These alternating proliferative and secretory phases constitute the endometrial component of the normal menstrual cycle, and result in stereotyped tissue reorganization that can be recognized on routine histologic examination. Following menopause, reduced production of estrogen and progesterone leads to inactive endometrium (absence of normal cycling) and endometrial atrophy [1]. Abnormalities of endometrial hormone receptor activity can compromise endometrial biology and function, and conversely, modulation of hormone receptor activity is leveraged therapeutically to treat endometrial conditions [4,5].

Unfortunately, disorders of the uterus and endometrium are common and may result in significant symptoms requiring clinical intervention. For example, endometriosis is characterized by abnormal growth of endometrium-like tissue outside of the uterus, frequently involving the ovaries, fallopian tubes, intestines, and other sites, causing pain, infertility, and tissue scarring [5]. Benign endometrial polyps are outgrowths of uterine tissue composed of endometrial glands and stroma. Their size can vary from millimeters up to several centimeters, growth appears to be hormone dependent, and larger pedunculated polyps may interfere with fertility [6]. Finally, endometrial hyperplasia is a disorder in which increased endometrial proliferation is driven by elevated and unopposed estrogen levels [7]. Although usually benign and self-limiting, hyperplastic glands may develop crowding and irregular architecture accompanied by concerning cytologic abnormalities leading to endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia (EAH/EIN), which portends a 22% risk of transformation to endometrioid carcinoma [4].

GATA binding protein 2 (GATA2) is expressed by the endometrium and is one member in a family of six conserved zinc finger transcription factors that bind to GATA nucleotide motifs throughout the genome to promote complex transcriptional programs [10]. GATA2 is a critical regulator of the development and function of the hematopoietic system, prostate, lymphatics, central nervous system, uterus, and other organ systems [11]. Homozygous deletion of *Gata2* in mice causes early embryonic lethality due to failure to form blood, and *Gata2* is critical for maintenance, proliferation, and differentiation of hematopoietic stem and progenitor cells [12]. Patients born with monoallelic mutations in *GATA2* or in enhancers that regulate *GATA2* expression suffer from the GATA2 Deficiency Syndrome, characterized by a constellation of symptoms that show incomplete penetrance and may

include bone marrow failure, immunodeficiency, lymphedema, deafness, and others [13,14]. However, the most devastating outcomes are myelodysplastic neoplasm and acute myeloid leukemia which occur with high penetrance. In addition, 3–5% of apparently sporadic acute myeloid leukemias harbor acquired mutations in *GATA2* [14,15]. The mechanism(s) by which loss-of-function mutations in *GATA2* and/or reduced GATA2 levels promote myeloid malignancies remains unclear, but the finding has generated interest in targeting GATA2 for the prognosis or treatment of hematologic disorders.

GATA2 also critically regulates endometrial function by driving expression of *PGR* and interacting with PGR on chromatin to reinforce PGR-driven transcriptional programs that are critical for endometrial decidualization and embryo implantation [16,17]. For this reason, conditional deletion of *Gata2* in the mouse uterus leads to infertility. Loss of *Gata2* is associated with PGR-independent squamous metaplasia of the endometrial lining coupled to a cancer-like gene expression profile, and reduced *GATA2* expression may correlate with aggressive behavior of endometrial carcinomas [18]. In human endometrium, expression of *GATA2* and *PGR* also show a strong positive correlation, while loss of *GATA2* expression may be a defining feature of both adenomyosis and endometriosis, possibly in conjunction with increased expression of *GATA6*, with both GATA family members postulated to play a role in endometriosis pathogenesis [19,20]. This finding suggests that a pathogenic "GATA switch", analogous to the GATA2 to GATA1 switch that occurs during normal erythropoiesis, could underlie select endometrial pathologies [21].

Whereas most studies of GATA2 function in the endometrium have focused on measuring transcript levels, it has been more challenging to evaluate the expression level of GATA2 protein in endometrial tissue biopsies from human patients due primarily to a lack of anti-GATA2 antibodies that can routinely detect physiologic levels of GATA2 protein. We recently developed anti-GATA2 monoclonal antibodies which are sensitive and specific for detection of human and mouse GATA2 across multiple assays, including routine immunohistochemistry (IHC) performed on standard formalin-fixed paraffin-embedded (FFPE) pathology specimens [22]. In the present study, we used one of these new antibodies to perform GATA2 IHC on FFPE endometrial biopsies and resections and scored GATA2 expression in endometrial glandular and stromal cells across a cohort of normal endometrium, hormone-treated endometrium, inactive endometrium, endometrial polyps, endometriosis, and EAH/EIN. We also performed IHC for ER, PGR, and GATA6 on relevant case subsets in which a relationship between their expression and GATA2 has been previously reported. In these cases, we scored their expression and analyzed their expression patterns in relation to GATA2. Our results show a strong coupling of GATA2 and PGR expression levels in the stromal and glandular elements of normal endometrium, which is lost in endometriosis and EAH/EIN. We find that while GATA2 expression is lost in the stromal cells of endometriosis and EAH/EIN, we detect no corresponding increase in GATA6. Although overall GATA2 levels are preserved in the glandular cells of endometriosis, GATA2 expression is decoupled from PGR levels, suggesting that PGR-independent pathways regulate GATA2 expression in these cells. Our findings support a model whereby GATA2 dysregulation is a feature of multiple endometrial disorders which can be routinely detected by IHC on patient tissue biopsies.

MATERIALS AND METHODS

IRB and Case Selection

This work was approved by the University of Wisconsin - Madison Institutional Review Board under protocol #2018–1510. All samples used in this study constituted residual material and per protocol patient consent was not required. Cases were identified by searching the electronic medical record at the University of Wisconsin Hospitals and Clinics (UWHC). Pediatric and Veterans Affairs cases were excluded as they were not covered by our IRB protocol. All cases had been previously examined and a diagnosis rendered by a board-certified surgical pathologist with special expertise in gynecologic pathology. The entire case was reviewed again by another board-certified pathologist and the block which best recapitulated the disease process selected for IHC and scoring. Cases with insufficient amounts of glandular and/or stromal elements for analysis were excluded. Except in the hormone treated group, cases in which patients received exogenous hormone treatment at time of endometrial sampling were excluded.

Immunohistochemistry

Generation and validation of GATA2 monoclonal antibodies and optimized IHC protocol have been described earlier [22]. IHC for GATA6 (Cell Signaling Technology, Clone D61E4), ER (Ventana, #790–4325), and PGR (Ventana, #790–2223) were performed on the Ventana Discovery Ultra BioMarker platform (Ventana Medical Systems) in a similar fashion. Deparaffinization was accomplished on the instrument followed by heat-induced epitope retrieval with cell conditioner 1 buffer (Ventana #950–224) for 32 min at 95°C. Primary antibodies against ER (Ventana # 790–4325) and PGR (Ventana #790–2223) were pre-diluted and applied to the slide for 60 min (ER) or 32 min (PGR) at 37°C. Primary antibody against GATA6 (Cell Signaling, Ref#5851) was diluted 1:750 with DaVince green diluent (Biocare Medical # PD900H) and applied for 32 min at 37°C. Slides were rinsed with reaction buffer (Ventana #950–300), incubated with Discovery OmniMap anti-rabbit horseradish peroxidase (Ventana #760–4311) for 16 minutes at 37°C and then rinsed with reaction buffer. Discovery ChromoMap DAB detection kit (Ventana #760–159) was used for visualization. The slide was then rinsed with warm water and dawn dish soap, and counterstained with Harris hematoxylin (1:5) for 45 seconds before rinsing, dehydration, dipping in xylene, and coverslipping with mounting media.

Microscopy and Immunohistochemistry Scoring

Brightfield images were acquired at room temperature on an Olympus BX43 model U-LHLEDC microscope using a 10X air objective with a 0.25 numerical aperture and a Lumenera Infinity 5 camera with Infinity Capture software. All images were white balanced in Adobe Photoshop. Scoring was performed separately by two board-certified pathologists (AK and DRM) and a graduate student (UP). The percent of positive glandular and stromal cells for each marker was evaluated by eye as an average across the entire section. The intensity of marker staining was evaluated on a four-point scale from 0 (negative) to 3 (dark/intense).

Tissue Culture and Western Blots

ECC1, Ishikawa, and Ark1 cells were cultured in RPMI-1640 + 10% FBS + 1% Penn/Strep. 12z cells were cultured in DMEM/F12 with 10% FBS and 1% Penn/Strep. Sequences for siRNAs are GATA2 Pool #1 (GCACAAUGUUAACAGGCCA, GCGCACAACUACAUGGAAC), GATA2 Pool #2 (GCUUCGAGGAGCUGUCAAA, CCAACAAGUCCAAGAAGAG), and Scramble pool (UAGCGACUAAACACAUCAA, UAAGGCUAUGAAGAGAUAC, AUGUAUUGGCCUGUAUUAG, AUGAACGUGAAUUGCUCAA). Depletions were performed using Lipofectamine RNAiMax (Life Technologies) and 20 nM siRNA according to the manufacturer's protocol. Depletion efficiency was measured after 48 hours by western blotting using the same custom mouse anti-GATA2 monoclonal antibody utilized for IHC at 1:1000 primary concentration. For shRNAs, stable doxycycline-inducible 12z lines were generated by transfecting cells with virions packaged in a pTRIPZ backbone (Horizon Discovery). After transfection, pools were selected for by treatment with 1 μg/mL puromycin and shRNA induced with 1 μg/mL doxycycline was confirmed by IRES-dependent RFP expression. GATA2 depletion was confirmed by western blot. shRNA sequences were shGATA2 #1 (TTCTCTACATAAAGTTGTC) and shGATA2 #2 (TCTTGCTCTTCTTGGACTT).

Invasion Assays

For Matrigel invasion assays, cells were transferred to Matrigel-coated membrane inserts (membrane 8.0 μm) (Fisher #8774122) 24 hours after shRNA induction and allowed to migrate for an additional 24 hours, after which Matrigel was removed and the bottom surface of inserts stained with Diff-Quick reagent and imaged by standard microscopy. Cell number was then quantified in a blinded fashion across 5 random fields.

Statistical Analyses

Student's T-test was used for between group analyses of two groups. Between group analyses with more than two groups were performed using two-way ANOVA followed by Šidák's multiple comparisons test. Correlations were calculated using the Pearson method. Curve fitting was accomplished using a variable slope with least squares fit. The threshold for significance was $p < 0.05$ for all analyses.

RESULTS

GATA2 IHC in Normal Proliferative and Secretory Endometrium

GATA2 reportedly promotes *PGR* expression under physiologic conditions and works in conjunction with activated PGR to drive PGR-directed transcriptional programs, while also inhibiting estrogen-mediated transcriptional signals [18,19]. In contrast, *GATA6* is reportedly not expressed at functional levels in normal endometrium [23]. To evaluate the expression of these markers in relation to ER and PGR in human patient samples, we assembled a cohort of 17 proliferative and 19 secretory endometrial biopsies or hysterectomy samples (Table 1) and performed IHC for GATA2 using a custom anti-GATA2 monoclonal antibody that we recently validated [22,24]. We also performed IHC for ER,

PGR, and GATA6. We chose to utilize IHC because it allowed us to directly measure factor protein expression in patient samples while maintaining native tissue architecture, supporting quantification across different cell types. In the case of ER and PGR this was performed using clinically validated antibodies that are routinely used for assessment of these markers in routine surgical pathology practice, while GATA6 IHC was performed using a commercial monoclonal antibody previously utilized for IHC on patient tissue [25,26]. For all markers, the percent positive cells was scored, as well as staining intensity on a scale from 0 (negative) to 3 (intense).

GATA2 IHC yielded crisp nuclear staining in a subset of glandular and stromal cells that correlated to menstrual phase (Figure 1A–C). The 83% of glandular cells and 57% of stromal cells that were GATA2-positive (GATA2+) in proliferative phase endometrium significantly exceeded the 57% of glandular and 34% of stromal cells that were GATA2+ in secretory phase endometrial specimens. The intensity of GATA2 staining also was significantly higher in glandular and stromal cells of proliferative endometrium compared to secretory phase endometrium (Supplemental Figure 2A–B). Like GATA2, levels of ER and PGR expression were lower in the glandular cells of secretory compared to proliferative phase endometrium (Figure 1A–B). However, only ER but not PGR expression was significantly reduced in stromal cells (Figure 1C). Despite this finding, the levels of GATA2, ER and PGR expression were highly correlated in glandular cells and stromal cells in normal endometrium (Figure 1D–E). As expected, no labeling for GATA6 was identified in the glands or stroma of normal endometrium (Supplemental Figure 1C). Fallopian tube epithelium showed appropriate nuclear GATA6 labeling and served as a positive control [27].

GATA2 IHC in Hormone Treated Endometrium, Inactive Endometrium, and Endometrial Polyps

GATA2 directly promotes *PGR* expression in the endometrium, and in conjunction with PGR on chromatin it may jointly regulate as many as 50% of endometrial PGR target genes [18]. However, *GATA2* also is a PGR target and endometrial PGR signaling upregulates *GATA2* [17]. This supports a model wherein a positive feedback loop between GATA2 and PGR reinforces cooperative activation of PGR target gene transcription. This model also infers that reduced PGR signaling will result in lower *GATA2* expression. We explored this possibility by performing IHC using anti-GATA2 antibodies on endometrial samples from 9 patients prescribed hormonal birth control comprising either progestin-only (n=5) or combination estrogen and progestin (n=4) regimens. Under both treatment regimens, the endometrium experiences relative progesterone excess, however absence of physiologically elevated estrogen levels also leads to reduced PGR expression, together leading an inactive and decidualized state. First, we confirmed that GATA2 levels in the glands and stroma of endometrium treated with progestin-only regimens was similar to that found in endometrium treated with combination regimens (p=0.88 for glandular cells and p=0.72 for stromal cells). Then, because among the normal endometrial phases, hormone-treated endometrium most closely approximates secretory phase endometrium, we compared GATA2 levels in hormone-treated endometrium against the GATA2 levels we already scored in secretory endometrium (Figure 1). We found no significant difference between the percent of GATA2-

positive cells or in the intensity of GATA2 staining in the glandular or stromal compartments of hormone-treated endometrium compared to normal secretory controls (Figure 2B–C and Supplementary Figure 3A–B).

Following menopause, reduced estrogen and progesterone expression by the ovaries compromises ER and PGR endometrial signaling and results in inactive endometrium. We hypothesized that GATA2 expression levels would be reduced in inactive endometrium due to reduced PGR signaling when compared to PGR levels in normal secretory endometrium. However, the percent of glandular and stromal cells positive for GATA2 as well as GATA2 staining intensity in inactive endometrium was similar to normal secretory controls (Figure 2A–C and Supplemental Figure 3A–B). Another setting in which endometrial PGR signaling is reduced is in endometrial polyps, which are overgrowths of endometrial glands and stroma that often extend into the uterine cavity [6]. However, we found no difference in GATA2 expression between glands or stroma of endometrial polyps compared to normal secretory endometrium (Figure 2A–C and Supplemental Figure 3A–B).

These results suggest that under benign conditions of relatively reduced progesterone receptor activity, GATA2 levels in the glands and stroma are low, and expressed a level similar to normal secretory endometrium.

GATA2 IHC in Endometrial Atypical Hyperplasia/Endometrioid Intraepithelial Neoplasia

EAH/EIN is a premalignant condition characterized by glandular crowding and cytologic atypia for which exposure to unopposed estrogen is a known risk factor [7,28]. PGR agonists are often given to patients with simple endometrial hyperplasia and PGR pathways also appear responsive in EAH/EIN considering that these patients often respond to progesterone therapy [29–32]. However, EAH/EIN carries a 22% risk of progression to endometrial carcinoma [33]. Whereas hysterectomy is the definitive treatment for EAH/EIN, continuous progestin-based therapy may be used for poor surgical candidates and young patients who desire fertility sparing therapy [34]. We scored GATA2 and PGR expression in 7 EAH/EIN cases. Because EAH/EIN is a proliferative process, we compared GATA2 expression levels to samples of normal proliferative endometrium that we scored earlier (Figure 1). We found that GATA2 expression in the glandular compartment of EAH/EIN was similar to normal proliferative endometrium (Figure 2A–B). However, GATA2 expression in the stromal compartment was markedly reduced, with virtually all EAH/EIN stromal cells showing absence of GATA2 expression (Figure 2C). A similar pattern of selective GATA2 loss in stroma but not glandular cells of EAH/EIN samples was identified when evaluating intensity of GATA2 and PGR (Supplemental Figure 3A–B). Moreover, there was no correlation between GATA2 and PGR expression in EAH/EIN, suggesting loss of autoregulation (Supplemental Figure 3C–D). This was not due to absence of PGR expression, as EAH/EIN cases showed PGR levels similar to normal proliferative endometrium controls (Supplemental Figure 3E–F).

GATA2 IHC in Endometriosis

Endometriosis is characterized by implantation of endometrium-like tissue outside of the uterine cavity, often on the ovaries and pelvic surfaces [34,35]. It arises through

still-unclear mechanisms but is associated with both genetic and environmental risk factors [36,37]. Work in pre-clinical models implicates epigenetic silencing of GATA2 in endometriosis pathogenesis, whereupon loss of *GATA2* expression blunts hormone-dependent transcriptional pathways that contribute to endometriosis pathology [23,38,39]. GATA2 loss is reportedly accompanied by upregulation of the related GATA family transcription factor GATA6, at least at the transcriptional level, with elevated *GATA6* blocking hormone responsiveness while simultaneously repressing *GATA2* [23]. However, mechanistic studies have been mostly limited to cultured endometriotic stromal cells, and data on GATA2 protein expression levels (especially in the glandular compartment) in primary endometriotic lesions is lacking.

We scored GATA2, GATA6, and PGR IHC in endometriosis biopsies from 18 patients. The number of GATA2 positive glandular cells in endometriosis lesions was similar to normal secretory endometrium and significantly less than normal proliferating endometrium (Figure 3A–B). In contrast, glandular PGR expression was elevated to levels measured in proliferative endometrium. However, the number of GATA2-positive stromal cells within endometriotic lesions was significantly reduced below levels measured in normal endometrium regardless of phase (Figure 3C). This was again in contrast to stromal PGR levels which were no different than normal endometrium. We further correlated the relationship between GATA2 and PGR on a case-by-case basis in endometriosis (Figure 4A–B). Interestingly, even though glandular GATA2 expression was similar to secretory endometrium controls, GATA2 expression correlated with PGR in benign endometrium whereas this relationship was lost in endometriosis (Figure 4A). Similarly, there was no significant correlation between GATA2 and PGR expression in the stromal compartment of endometriotic lesions (Figure 4B). When we stratified benign endometrium and endometriosis cases by menstrual phase, GATA2 expression in the glands and stroma was significantly different in proliferative but not secretory phase (Supplemental Figure 4). We found virtually no difference in the strength of GATA2 IHC across nuclei (Supplemental Figure 5A–B), indicating that the number of cells expressing GATA2 reflected the primary driver of differential GATA2 expression across samples. Although earlier studies reported that *GATA6* transcripts are upregulated in endometriotic lesions [23,40,41], we found no detectable GATA6 expression in either the glandular or stromal compartments of endometriotic lesions, while background fallopian tube epithelium stained appropriately positive and served as an internal positive control in multiple samples (Supplemental Figure 5C).

Finally, we sought to determine whether GATA2 depletion may promote extension of endometriosis lesions towards extra-uterine sites. We generated 12z cell lines [43,44] with doxycycline-inducible expression of anti-GATA2 shRNAs or shScramble controls (Supplemental Figure 6A). GATA2 depletion in 12z cells did not result in reproducible changes in GATA6 expression (Supplemental Figure 6B) and we were unable to detect PGR protein or transcripts. We then tested whether GATA2 depletion augmented the ability of 12z cells to invade through Matrigel-coated membranes (Supplemental Figure 6C). While there was a trend suggesting that GATA2 depletion may increase 12z cell invasion compared to shScramble control, this was not statistically significant (Supplemental Figure 6D).

DISCUSSION

Recent studies have established GATA2 as a critical transcription factor in the endometrium that is required for fundamental reproductive functions. An autoregulatory GATA2-PGR axis enables implantation of the fertilized embryo, and GATA2 also supports the fidelity of endometrial tissue identity in a PGR-independent manner. Aberrant *GATA2* expression has been linked to common endometrial disorders including endometriosis. In the current study, our recently validated [45] custom anti-GATA2 monoclonal antibody enabled us to directly evaluate GATA2 protein levels across a range of normal and pathologic patient endometrial samples (partially summarized in Supplemental Table 1).

Our findings in normal cycling endometrium confirm a very strong and positive correlation between GATA2 and PGR expression, which collectively supports the prevailing model in which GATA2 and PGR exist in an autoregulatory positive feedback loop [18]. Our tissue-level analyses extended this paradigm by demonstrating a positive correlation between GATA2 and PGR in both the glandular and stromal compartments of normal cycling human endometrium, and we found no such relationship between GATA2 and ER.

We subsequently evaluated endometrial GATA2 expression in the setting of common benign endometrial conditions associated with abnormal PGR activity including hormone-treated patients, peri/post-menopausal patients who have inactive endometrium, and in the setting of endometrial polyps. Interestingly, we found no significant difference in endometrial GATA2 expression between these conditions and normal secretory phase endometrium. In contrast, we quantified a striking loss of stromal GATA2 expression in EAH/EIN, which was lower than that found in proliferative or secretory phase endometrium. Interestingly, whereas average glandular expression of GATA2 in EAH/EIN appeared to be preserved, further analyses also revealed a decoupling of the GATA2-PGR autoregulatory axis in this cellular compartment as well. We identified the same findings in endometriotic lesions. Our findings support loss of GATA2 and PGR autoregulation in EAH/EIN and endometriosis, and suggest that loss of GATA2 in stromal cells may reliably separate these lesions from normal endometrial tissues (Figure 4C). It is interesting to speculate that the loss of GATA2 in these lesions may contribute to the progesterone insensitivity that is thought to contribute to disease pathogenesis in both disorders. Maintenance of average GATA2 expression in the glandular cells across these conditions suggests that PGR-independent pathways in this compartment maintain GATA2 at levels indistinguishable from normal endometrium.

Several studies have reported elevated levels of *GATA6* concomitant with loss of *GATA2* in endometriosis [23,40,41]. Current models suggest that *GATA2* hypermethylation leads to reduced GATA2 expression in association with *GATA6* hypomethylation. Increased GATA6 expression then both directly represses *GATA2* and independently drives disease pathology, potentially through dysregulation of hormone metabolism [42]. Similar findings have also been reported in adenomyosis, suggesting that a GATA2-GATA6 switch could be a generalizable mechanism across numerous endometrial disorders. This raises the possibility that GATA6 IHC could be worth pursuing as a diagnostic and/or prognostic marker. However, we failed to detect increases in GATA6 expression in endometriotic lesions, even in the presence of appropriately staining internal positive controls. Most prior

studies demonstrating elevated *GATA6* in endometriotic tissues have relied on transcript level analyses whereas information related to GATA6 protein expression in endometriosis has been comparatively sparse and sometimes contradictory. Dyson et al. detected elevated GATA6 by immunofluorescence in human cultured endometriotic stromal cells derived from ovarian endometriosis lesions [23]. However, Izawa et al. performed anti-GATA6 IHC on sections of ovarian endometriotic lesions and noted mostly epithelial staining [41]. They also observed that endometriotic lesions from the peritoneum show only marginal anti-GATA6 staining that is localized to glandular cells. Additional tissue-level studies will be required to confirm whether abnormal levels or patterns of GATA6 expression are features of endometriosis and other gynecologic disorders.

Some limitations of our study should be acknowledged. First, interpretation of GATA2, ER, PGR, and GATA6 levels is dependent on antibody sensitivity and specificity, in addition to counterstain properties. The ER and PGR antibodies utilized in our study are clinically validated and utilized in routine diagnostic surgical pathology practice across numerous institutions for the quantitative analysis of ER and PGR levels in human tissues, while our custom anti-GATA2 monoclonal has been validated in human tissues and shows identical staining to a previously-published rabbit polyclonal anti-GATA2 antibody [45,46]. The monoclonal anti-GATA6 antibody has also been previously utilized for IHC on human tissues [25,26] and in our hands labeled fallopian tube epithelium appropriately. However, as expression of GATA6 protein in normal and pathologic human endometrium has not yet been systematically interrogated, we cannot rule out that the absence of identifiable GATA6 protein by IHC could be due to an insufficiently sensitive antibody or a function of our IHC staining protocol. Second, all studies seeking to evaluate GATA factor expression must be cognizant of the fact that GATA factor expression levels are often finely regulated in context-specific manners, and levels of individual GATA factors alone may not predict the activity of a given GATA-responsive transcriptional program. Future studies may examine the levels of GATA2 targets in endometrial tissues to determine whether their expression is predicted by GATA2 expression levels measured by IHC. Future studies should also evaluate GATA factor expression in non-human primate models of endometriosis, which represent perhaps the most relevant pre-clinical model for this disorder.

Future studies also may focus on identifying PGR-independent pathways that regulate GATA2 expression in endometrium. In hematopoietic stem and progenitor cells, GATA2 binds to multiple *GATA2* enhancers generating a positive feedback loop and driving further *GATA2* expression. Whether a similar form of GATA2 regulation occurs in the endometrium is not known. It would also be interesting to define GATA2 patterns and expression levels in endometriosis and EAH/EIN following treatment with progestin therapy. Although we and others show that GATA2 levels are suppressed in these disorders, progestin therapy can be effective in treating these entities, suggesting an intact PGR signaling pathway. It may be informative to determine whether progestin therapy leads to upregulation of GATA2 expression, and whether this contributes to PGR transcriptional programs. Interventions which can increase GATA2 expression in these disorders would be expected to increase PGR levels and sensitivity, potentially reducing or ameliorating disease pathology. As EAH/EIN represents a pre-malignant endometrial disorder, and as GATA2 mechanisms are already implicated in malignancies of the prostate and bone marrow, it will also be critical to

examine GATA2 levels and activity in endometrial malignancies to determine if GATA2 is also lost in these cancers and whether this contributes to disease pathobiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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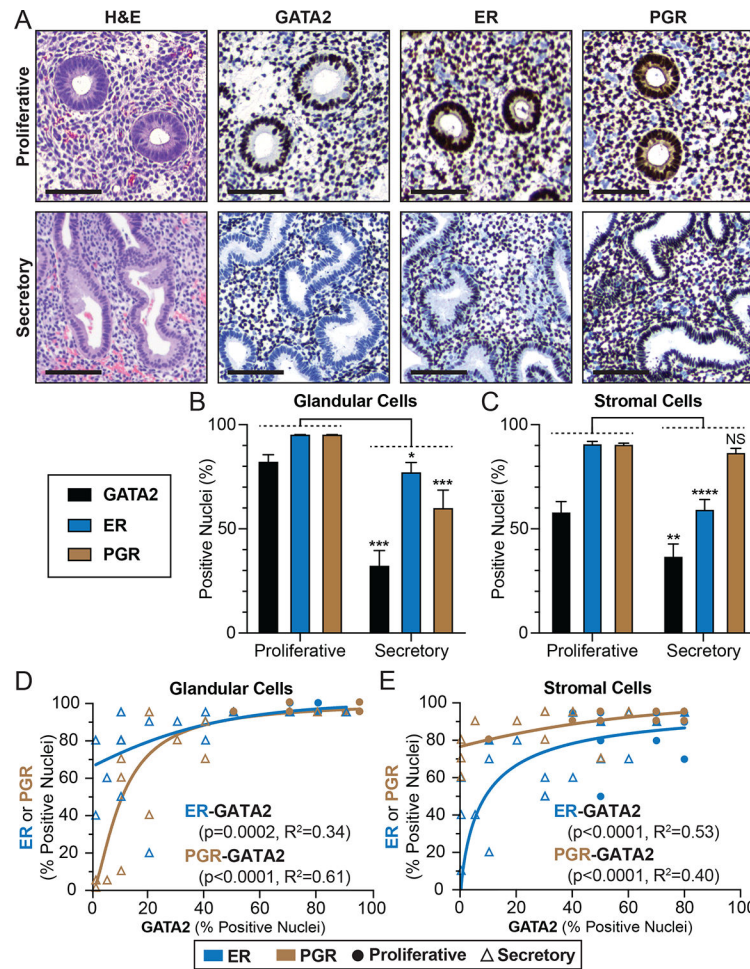
Figure 1: GATA2, ER, and PGR immunohistochemistry in normal cycling endometrium.
(A) Representative sections of proliferative and secretory phase endometrium stained for H&E and IHC for GATA2, ER, and PGR. (B-C) Percent GATA2, ER, and PGR positive nuclei in (B) glandular and (C) stromal cells from proliferative and secretory phase endometrium. Statistical comparisons made within individual markers. (D-E) Correlation between percent cells positive for GATA2 and either ER or PGR in (D) glandular and (E) stromal endometrial cells. All IHCs are DAB with hematoxylin counterstain. NS = not significant, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005. Scale bar = 50 microns.
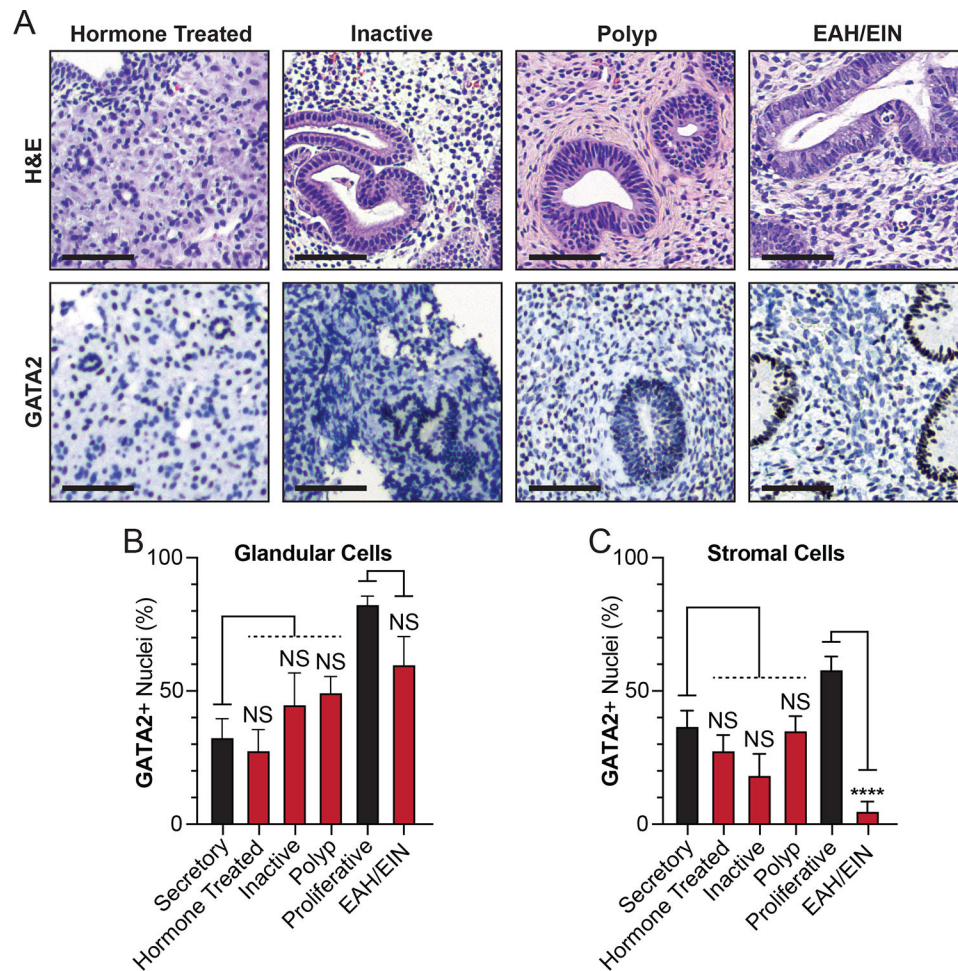
Figure 2: GATA2 immunohistochemistry in hormone treated endometrium, inactive endometrium, endometrial polyps, and endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia.

(A) Representative H&E and GATA2 IHC images. (B) Percent GATA2 positive nuclei in (B) glandular and (C) stromal cells. All IHCs are DAB with hematoxylin counterstain. NS = not significant, ****p<0.00005. Scale bar = 50 microns.
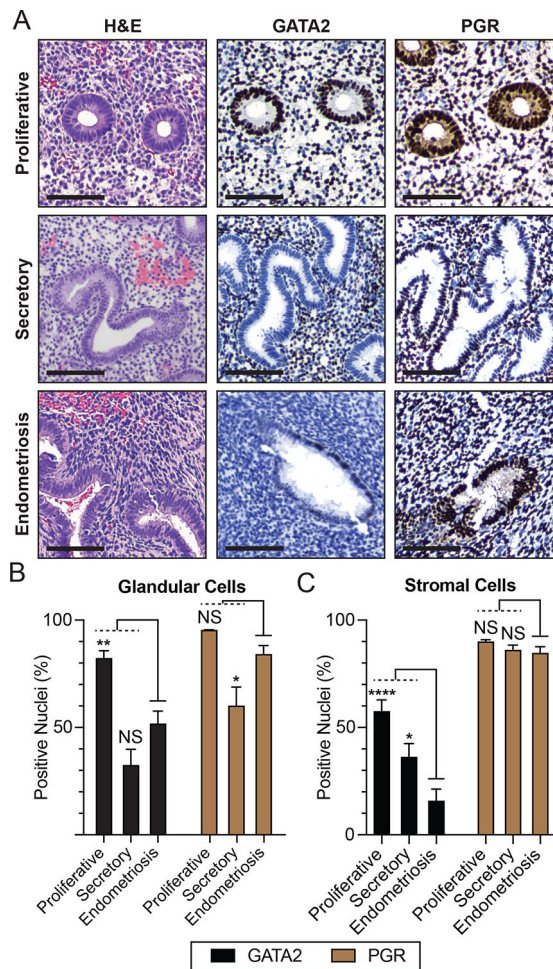
Figure 3: GATA2 and PGR immunohistochemistry in normal cycling endometrium and endometriosis.

(A) Representative H&E, and GATA2 and PGR IHC images from normal endometrium in proliferative or secretory phase, and in endometriosis. (B) Percent GATA2 positive nuclei in proliferative and secretory endometrium, and in endometriosis. All IHCs are DAB with hematoxylin counterstain. NS = not significant, *p<0.05, **p<0.005, ****p<0.00005. Scale bar = 50 microns.
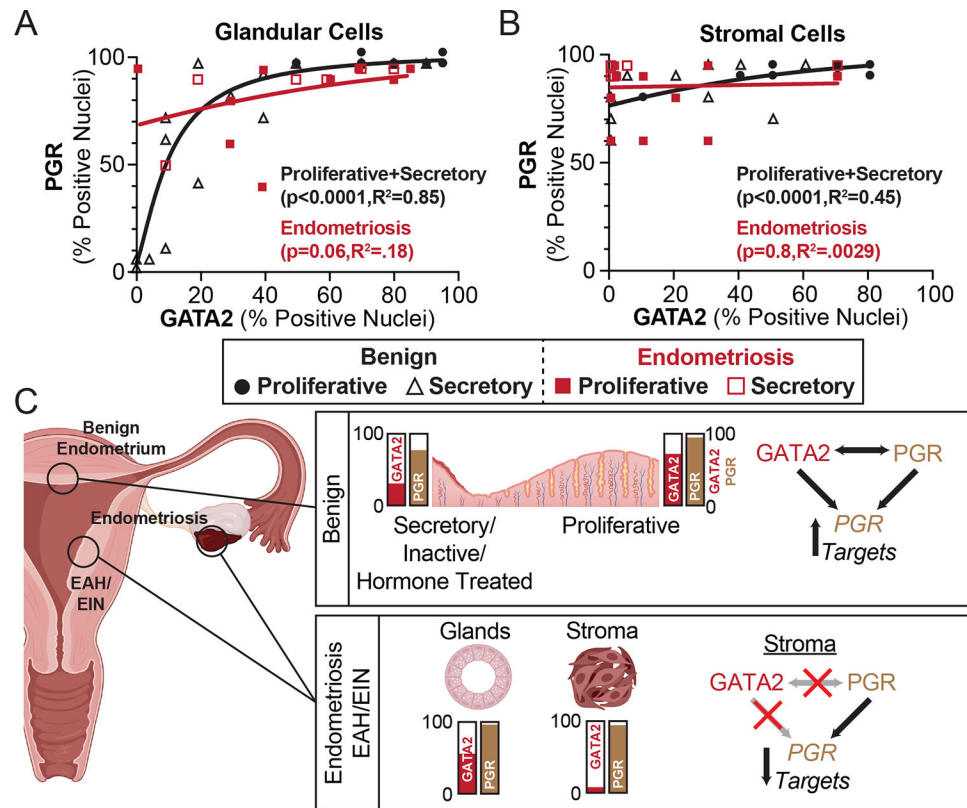
Figure 4: Correlation between GATA2 and PGR IHC expression in normal endometrium and endometriosis.

(A-B) Plot of percent nuclei positive for GATA2 and PGR in all analyzed cases of normal endometrium and endometriosis within (A) glandular and (B) stromal cells. (C) Diagram of GATA2 and PGR expression in normal cycling endometrium contrasted with endometriosis and EAH/EIN. In normal cycling endometrium GATA2 levels are low in secretory phase and high in proliferative phase, while PGR levels are high throughout but highest in proliferative phase. GATA2 levels are lower in inactive and hormone treated endometrium, similar to secretory phrase endometrium. In all conditions, GATA2 remains in a positive feedback loop with PGR while also contributing to PGR gene target transcription. In endometriosis and EAH/EIN, GATA2 levels in stromal cells are markedly reduced, while PGR levels remain high. In stromal cells, GATA2 no longer forms a positive regulatory loop with PGR, and we hypothesize that reduced GATA2 levels fail to contribute to PGR target gene transcription. EAH/EIN=endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia.

Table 1:

Characteristics of endometrial tissue cohort.

Immunohistochemistry columns indicates which targets were evaluated for each case cohort.

Endometrium Status	Number	Median Age (yrs) [Range]	Immunohistochemistry			
			GATA2	GATA6	ER	PGR
Benign Proliferative	17	38 [24–47]	X	X	X	X
Benign Secretory	19	37 [31–49]	X	X	X	X
Hormone Treated	10	44 [26–58]	X			
Inactive	7	48 [42–78]	X			
Endometrial Polyp	20	49 [31–72]	X			
EAH/EIN	7	60 [35–76]	X		X	X
Endometriosis	18	39 [33–76]	X	X		X