Northwell Health℠

Journal Articles

2020

Epithelial Cells in Endometriosis and Adenomyosis Upregulate STING Expression

H. Qu

L. Li

T. Wang

T. Seckin
Zucker School of Medicine at Hofstra/Northwell

J. Segars

See next page for additional authors

Follow this and additional works at: https://academicworks.medicine.hofstra.edu/publications

Part of the Obstetrics and Gynecology Commons

Recommended Citation

Qu H, Li L, Wang T, Seckin T, Segars J, Shih I. Epithelial Cells in Endometriosis and Adenomyosis Upregulate STING Expression. . 2020 Jan 01; 27(6):Article 7650 [p.]. Available from: https://academicworks.medicine.hofstra.edu/publications/7650. Free full text article.

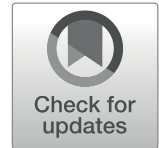
This Article is brought to you for free and open access by Donald and Barbara Zucker School of Medicine Academic Works. It has been accepted for inclusion in Journal Articles by an authorized administrator of Donald and Barbara Zucker School of Medicine Academic Works. For more information, please contact academicworks@hofstra.edu.

Authors
H. Qu, L. Li, T. Wang, T. Seckin, J. Segars, and I. Shih

This article is available at Donald and Barbara Zucker School of Medicine Academic Works:
https://academicworks.medicine.hofstra.edu/publications/7650

Reproductive Sciences (2020) 27:1276–1284
https://doi.org/10.1007/s43032-019-00127-z

ORIGINAL ARTICLE



Epithelial Cells in Endometriosis and Adenomyosis Upregulate STING Expression

Hong Qu[1,2] · Lihong Li[1,3] · Tian-Li Wang[1,3] · Tamer Seckin[4] · James Segars[1] · Ie-Ming Shih[1,3]

Received: 16 August 2019 / Accepted: 9 November 2019 / Published online: 6 January 2020
© Society for Reproductive Investigation 2019

Abstract

In response to cytosolic DNA, stimulator of interferon gene (STING) initiates and orchestrates host's innate immunity by inducing type I interferon. Since endometriosis is a chronic inflammatory disorder, we sought to determine whether STING pathway is activated in ectopic endometrium in comparison to eutopic endometrium. Immunohistochemistry was employed in evaluating the expression levels of STING in normal endometrium, endometriosis, and adenomyosis. The density of CD45+ intraepithelial lymphocytes was correlated with STING expression levels. A total of 39 cases of endometriosis and/or adenomyosis with normal endometrium were analyzed. Among them, 32 had adenomyosis, 26 had endometriosis, and 19 have both lesions. STING protein expression is mainly evident in the cytoplasm of epithelial cells but much less in stromal cells. Based on H-score, we found that the STING expression levels were significantly higher in the epithelial cells of adenomyosis and endometriosis than in eutopic endometrium (132.7 ± 12.20, 119.6 ± 12.57 vs. 19.74 ± 5.96, $p < 0.0001$). There was no significant difference in STING expression level between endometriosis and adenomyosis. More intraepithelial lymphocytes were detected in endometriosis and adenomyosis lesions than endometrium ($5.60 \pm 0.70\%$, $4.95 \pm 0.54\%$ vs. $1.25 \pm 0.12\%$, $p < 0.0001$). A positive correlation between STING expression and intraepithelial lymphocytic infiltrate was observed ($p < 0.0001$). In summary, STING was upregulated in the epithelium of ectopic endometrium as compared to eutopic endometrium. Its expression levels correlate with the degree of intraepithelial lymphocyte infiltration, suggesting a role in promoting chronic inflammation of ectopic endometrium.

Keywords Endometriosis · Adenomyosis · STING · Inflammation

Introduction

Endometriosis and adenomyosis are characterized by normal-appearing endometrial tissue located outside the endometrium. Both disorders significantly reduce the quality of life in many women because they cause menometrorrhagia, pelvic pain, and infertility. Endometriosis and adenomyosis are the sources of a significant economic burden worldwide

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s43032-019-00127-z) contains supplementary material, which is available to authorized users.

✉ Ie-Ming Shih
ishih@jhmi.edu

Hong Qu
quhong2014@163.com

Lihong Li
lli97@jhmi.edu

Tian-Li Wang
tlw@jhmi.edu

Tamer Seckin
drseckin@mac.com

James Segars
jsegars2@jhmi.edu

1 Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA

2 Department of Obstetrics and Gynecology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

3 Departments of Oncology and Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA

4 Lenox Hill Hospital and Zucker School of Medicine at Hofstra/Northwell , New York, USA

[1]. According to previous reports, local inflammation and immune dysregulation characterize both disorders, particularly in endometriosis[2–4], and the resultant chronic inflammation in both cases causes various symptoms in women. Therefore, understanding why ectopic but not eutopic endometrium tends to be inflammatory would be fundamental to developing new treatment options.

Protein levels in several key inflammatory mediators including COX-2, IL-1β, IL-8, TNF-α, PGE2, and estradiol (E2) are higher in ectopic endometrium than eutopic endometrium[5–10]. These mediators work synergistically to sustain and aggravate inflammation. Like cancer, endometriosis resembles a chronic wound that does not heal. In cancer, NF-kB, one of the most extensively studied inflammatory mediators, orchestrates many aspects of cancer-associated inflammatory phenotypes by coordinating cross-talk between NF-kB and other signaling pathways including STAT3, p53, IRF, NRF2, JNK, Notch, and WNT-beta-catenin pathways [11]. NF-kB apparently plays a critical role in the development of endometriosis[12–14]. The mechanisms behind the activation of the NF-kB pathway remain elusive; however, iron overload in endometrial stromal cells may activate IKKβ and results in the production of abundant reactive oxygen species that stimulate the NF-kB pathway.

In this study, we investigated whether stimulator of interferon gene (STING) is abnormally expressed in endometriosis and adenomyosis as compared to their corresponding eutopic endometrium. The reason why we focused on STING is because STING is not only the upstream regulator of NF-kB but also the molecular culprit thought to be involved in several auto-inflammatory diseases such as systemic lupus erythematosus (SLE) and silica-induced lung inflammation, which are related to STING-dependent sensing of self-DNA [15, 16]. STING plays a major role in detecting the presence of cyclic dinucleotides, either generated by intracellular bacteria or by cGAS in response to viral infection or self-DNA leak from the nuclei. The binding of cyclin dinucleotide activates STING, which then forms a complex with TANK-binding kinase 1 (TBK1). The complex is further relocated to the endolysosomal compartment where it phosphorylates NF-kB and the transcription factors interferon regulatory factor 3 (IRF3) [17, 18]. Both NF-kB and IRF3 signaling pathways work together to transcriptionally activate genes such as type I interferons (IFNs). IFNs and other inflammatory cytokines, in turn, recruit inflammatory cells to eliminate the infectious agents or transformed cells.

Since endometriosis is considered as an inflammatory disease, we reason that STING overexpression contributes to initiation and sustaining of the inflammatory environment surrounding the lesions. In this study, we performed immunohistochemistry using a STING-specific antibody in 39 different specimens containing normal uterine endometrium and matched either adenomyosis or endometriosis or both. We also quantify intraepithelial CD45+ inflammatory cells in all specimens. We found that epithelium in endometriosis and adenomyosis upregulated STING in comparison with eutopic endometrium and a positive correlation between STING protein upregulation and the density of intraepithelial inflammatory cells. Thus, endometriosis and adenomyosis are inflammation primed at least through the STING pathway.

Material and Methods

Tissue Samples and Patient Selection

We analyzed a total of 39 anonymous cases of the formalin-fixed and paraffin-embedded archival samples, which were obtained from the Department of Pathology at the Johns Hopkins Hospital. The paraffined tissue sample collection used in this study was approved by the Institutional Review Boards of the Johns Hopkins Hospital. The criteria for inclusion of cases were adenomyosis or endometriosis containing both epithelium and stroma confirmed by pathologists. All cases had normal-appearing uterine (eutopic) endometrium, without progesterone (progestin) therapy and no history of uterine neoplastic diseases. The hematoxylin-and-eosin stained slides of all cases were reviewed by two authors (IMS and HQ) before the preparation of unstained slides. All samples were divided into three groups: proliferative phase, secretary phase, and inactive phase. The inactive endometrium was atrophic either under physiological condition or under GnRH-antagonist treatment. There was one case who was a 75-years-old woman presenting with both deep infiltrating endometriosis in bladder and small bowel and adenomyosis lesions, but her endometrium was weakly proliferative. Eight patients showed inactive endometrium including one postmenopause and the other receiving GnRH-antagonist treatment.

Immunoblotting to Validate the Specificity of the Anti-STING Antibody

Cell lysates from HeLa (isogenic $STING^{-/-}$ and $STING^{+/+}$ clones), hEM, KLE, HHUA, HEC1A, SKOV3, and OVCAR8 cell lines were analyzed using Western blot, and the samples were prepared in the lysis buffer (Cell Signaling Technology) containing protease/phosphatase inhibitor cocktail (Cell Signaling Technology). Proteins were separated by 5–12% polyacrylamide gel with 120 V for 65 min and then transferred onto PVDF membrane using Trans-Blot Turbo Transfer System (Bio-Rad). The membrane was blocked with 5% nonfat milk in TBS-T at room temperature for 1 h, followed by primary antibodies incubated overnight at 4 °C (rabbit anti-STING/TMEM173: NBP2–24683, Novus Biologicals,

USA, 1/1000; rabbit anti-GAPDH: SAB2108668, Sigma, 1/2000). The membrane was incubated with donkey anti-rabbit IgG (H + L) (711,035,152, Jackson ImmunoResearch,1/5000) for 1 h at room temperature. Clarity Western ECL Substrate reagent (Bio-Rad) was used for detection.

Immunohistochemistry

After deparaffinization by xylene and ethanol, tissue sections were heated in Trilogy pretreatment solution buffer (Cell Marque) at 100 °C for 30 min, for antigen retrieval. Slides were then incubated with the primary antibody (rabbit anti-STING/TMEM173: NBP2–24683, Novus Biologicals, USA, polyclonal, 1/1200; rabbit anti-CD45, Abcam, 1/250) at 4 °C overnight. The following day after secondary antibody incubation, EnVision+System (Dako, Carpinteria, CA) was used to detect the immunoreactivity.

Scoring of Immunoreactivities

We used H-score to semiquantitatively analyze the STING expression levels in epithelial cells and stroma cells as previously described [19, 20]. We calculated the percentage of cytoplasm staining intensity, using the following scoring parameters: strong (3x), medium (2x), and weak (1x). The H-score was independently evaluated by two investigators, with scores ranging from 0 to 300. In addition to the continuous variables, we also use categorical scoring by arbitrarily designating cases to the STING high group if their H-scores exceeded 50 [20]. For measuring the percentage of intraepithelial lymphocytes (IELs), we counted the number of epithelial cells and IELs in three independently selected microscopic fields (200x) and then calculated the ratio of IEL/epithelial cells according to the count record. Similarly, we calculated the ratio of lymphocytes/stroma cells.

Statistical Analysis

One-way ANOVA was used to determine the significance of H-score (mean ± SD) among groups: normal endometrium, adenomyosis, and endometriosis. Unpaired Student's t-test was used to determine the significance between STING positive and STING negative groups. Chi-square test was used for ratio comparison. P value < 0.05 was considered significant. Linear regression was used to correlate the H-score of STING immunoreactivity and the abundance of IELs. All analyses were done using the software GraphPad Prism 6.

Results

Immunohistochemistry was performed on all specimens obtained from 39 cases (patients) including 13 cases with adenomyosis, 7 cases with endometriosis alone, and 19 cases containing both adenomyosis and endometriosis. A total of 74 slides including 23 slides with normal endometrium and adenomyosis, 16 with normal endometrium, 9 with adenomyosis, and 26 with endometriosis were reviewed. The age in this cohort ranged from 23 to 75 years (44.7 ± 8.5 years), and the median age was 44 years. All cases had normal-appearing endometrium including 19 cases at the proliferative phase, 12 at the secretary phase, and 8 at the inactive state (Table 1). The specificity of the anti-STING antibody was confirmed by immunoblotting, showing the presence of STING protein (~43 kD) in the cell lysate from the parental HeLa cells ($STING^{+/+}$) but not in the lysate from the isogenic cells with STING gene knockout ($STING^{-/-}$) (Supplementary Fig. 1A). We also found variable expression levels of STING in different cell lines analyzed (Supplementary Fig. 1A).

STING immunoreactivity, when present, was detected predominantly in the cytoplasm of epithelial cells but not in stromal cells. The H-scores of STING staining were significantly higher in epithelial cells from endometriosis and adenomyosis than those from the matched eutopic endometrium (p < 0.0001) (Fig. 1a). The H-scores in epithelial cells from endometriosis, adenomyosis, and eutopic endometrium were 119.6 ± 12.57, 132.7 ± 12.20, and 19.74 ± 5.96, respectively. Likewise, when the specimens were categorized into STING high and low groups using an arbitrary cutoff (H-score of 50), endometriosis (81.8%, $p < 0.001$) and adenomyosis (87.5%, $p < 0.001$) had more cases belonging to the high expression group than eutopic endometrium (12.8%) (Table 2). There was no difference in H-scores between endometriosis and adenomyosis ($p > 0.05$). In general, the H-scores of STING in the epithelial cells of eutopic endometrium were very low, irrespective of the proliferative or luteal phase (Fig. 1c). No significance of STING H-score was observed in stroma cells from different phases of endometrium (Fig. 1d). Representative photomicrographs of STING staining from the same cases were shown in Fig. 2. Besides, we did not observe any significance in STING immunoreactivity in stromal cells among endometriosis, adenomyosis, and eutopic endometrium (Fig. 1b and 1d).

CD45 immunohistochemistry was also performed in all cases. CD45+ immune cells were mainly present in the stroma of endometrium, adenomyosis, and endometriosis. In contrast, we found fewer CD45+ immune cells associated with epithelium in general. There was no significant difference in the density of CD45+ lymphocytes in the stroma among those groups (Supplementary Fig. B and C). Interestingly, the percentage of CD45+ IELs (associated with glands) was 1.25 ± 0.12% in normal endometrium, 5.60 ± 0.70% in adenomyosis

Table 1 Characteristics of the cohort

Normal endometrium phase	Cases (n = 39)	Average age	Endometriosis lesion location (n = 26)				Adenomyosis lesion (n = 32)
			Ovary (n)	Fallopian tube (n)	Peritoneum (n)	DIE (n)	
Proliferative	19	44.7	7	7	1	1	16
Secretary	12	42.9	5	2	0	1	9
Inactive	8	47.5	1	0	1	0	7

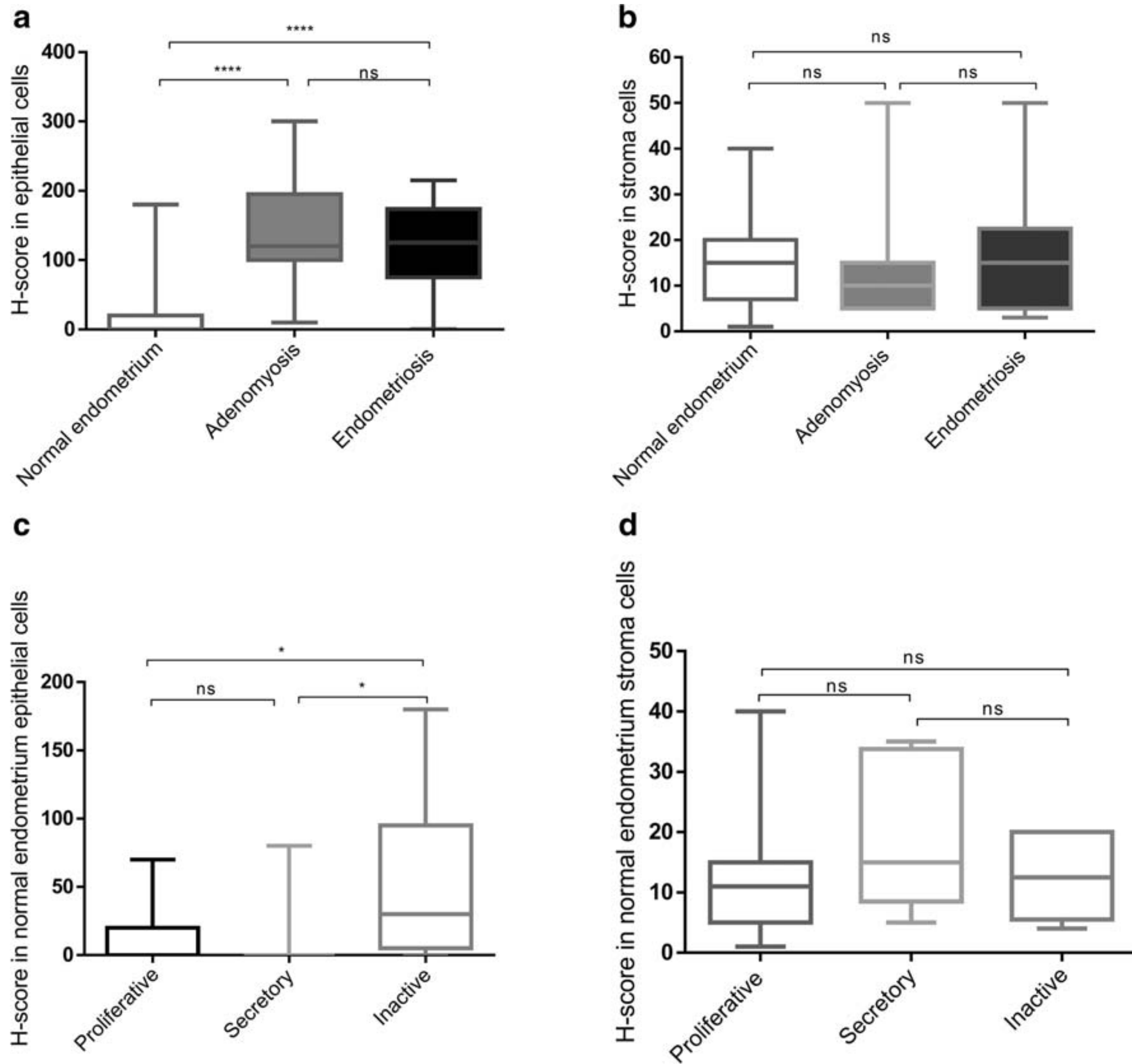


Fig. 1 STING upregulates in endometriosis and adenomyosis in comparison with normal eutopic endometrium. (A) The glandular epithelial cells in adenomyosis ($n = 32$) and endometriosis ($n = 26$) express higher levels of STING than those in eutopic endometrium ($p < 0.0001$), but no significance was observed between them ($p =$ 0.46). (B) The expression level of STING in stroma cells showed no significant difference between adenomyosis (n = 32) ($p = 0.53$), endometriosis (n = 26) ($p = 0.29$) and endometrium ($n = 39$), and no significance between adenomyosis and endometriosis ($p = 0.15$). (C) In epithelial cells from normal-appearing endometrium, no significant difference of STING expression levels was observed between the proliferative phase ($n = 19$) and secretory phase ($n = 12$) ($p = 0.63$). However, its expression level was higher in an inactive state ($n = 8$) when compared to the proliferative phase ($p = 0.011$) and secretory phase ($p = 0.028$). (D) In stroma cells from normal-appearing endometrium, there were no significance of STING expression levels between proliferative phase (n = 19) and secretory phase (n = 12) ($p = 0.087$), proliferative phase and inactive state (n = 8) ($p = 0.98$), secretory phase and inactive state ($p = 0.18$)

Table 2 Positive expression of STING in endometrium, adenomyosis and endometriosis

Group	Case (n)	H-score ≤50 (n, %)	H-score >50 (n, %)	P value
Normal endometrium	39	34(87.18%)	5(12.82%)	
Adenomyosis	32	4(12.5%)	28 (87.5%)	<0.001
Endometriosis	26	5(19.23%)	21 (81.77%)	<0.001

and 4.95 ± 0.54% in endometriosis (Fig. 3A). The percentage of IELs in adenomyosis and endometriosis lesions when combined or individually analyzed was significantly higher than in normal endometrium ($p < 0.0001$), but no significant difference was noted between adenomyosis and endometriosis groups. Next, we correlated the STING H-scores and the percentage of CD45+ IELs in all specimens and observed a positive correlation ($p < 0.0001$) (Fig. 3C and Fig. 4).

Discussion

In this study, we provided new evidence to support that an innate immunity increases in ectopic endometrium as reflected by STING upregulation in epithelial cells of endometriosis and adenomyosis as compared to eutopic endometrium. Importantly, STING expression levels correlate with more intraepithelial lymphocytes in ectopic endometrial lesions.

Endometriosis and adenomyosis are common gynecological benign disorders. Endometriosis is characterized by local chronic inflammation which conspires with aberrant hormonal homeostasis to promote its development, producing symptoms and resistance to hormonal therapy [21, 22]. Clinically, endometriosis exhibits a range of disease activity including increased angiogenesis without grossly detectable lesions, active and inflammatory endometriosis and fibrosis (Fig. 5). In this study, we found that STING is upregulated in the epithelial cells from endometriosis as compared to epithelial cells from their corresponding eutopic endometrium. Likewise, the epithelial cells from adenomyosis also upregulated STING as compared to the matched eutopic endometrium. The overexpressed STING may be likely associated with the STING pathway activation, rendering epithelial cells highly reactive to microenvironmental milieu and perhaps initiating an inflammation cascade.

STING is activated by cyclic dinucleotides in its canonical pathway, which activates the cyclic GMP-AMP synthase (cGAS) pathway. cGAS is an enzyme which senses cytosolic DNA fragments and catalyzes them to form a group of related cyclic dinucleotides, called cyclic GMP-AMP (known as cGAMP). There are several sources of cytosolic DNA including exogenous virus and bacterial infection, as well as endogenous self-DNA derived from tumor cells with genomic instability and necroptosis.

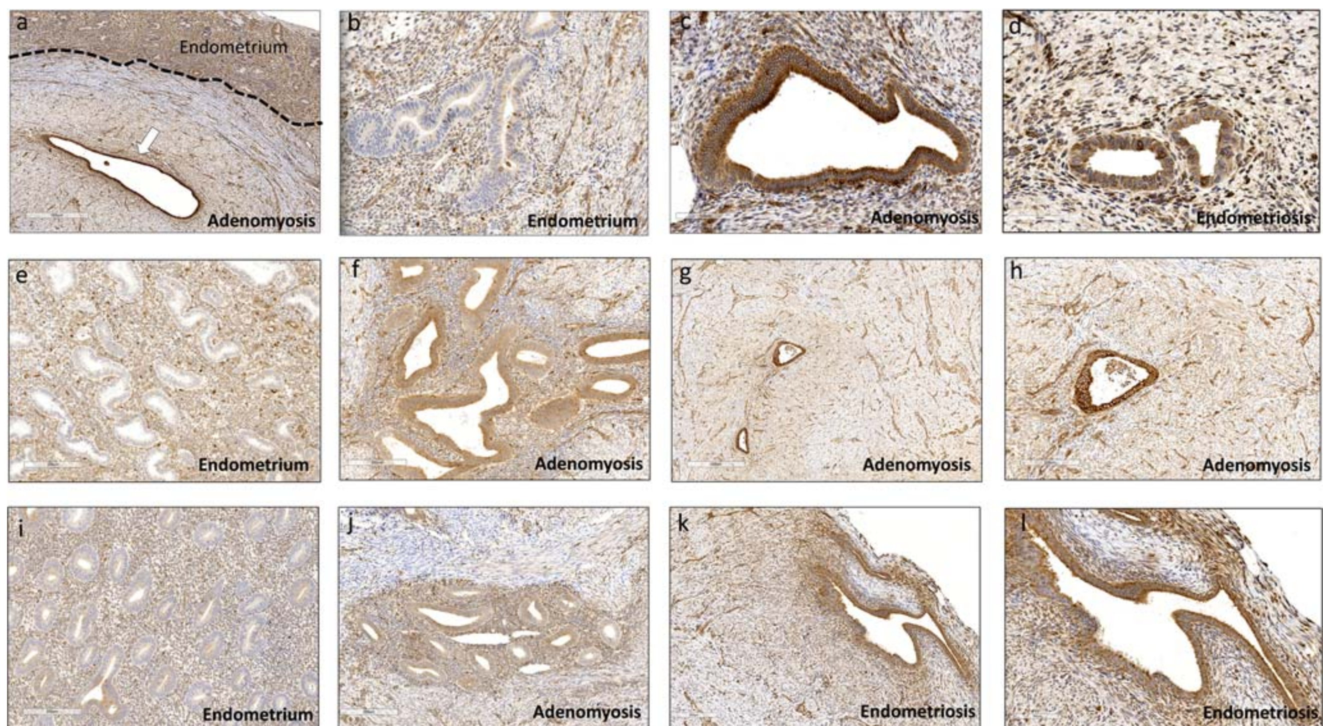


Fig. 2 STING expression patterns in adenomyosis and endometriosis. In epithelial cells from normal-appearing endometrium, STING immunoreactivity was undetectable. Case A, a(4x), b (20x), c (20x), d (40x); case B, e (10x), f (10x), g (4x) and h (10x); case C, i (10x), j (10x), k (10x) and and l (20x)

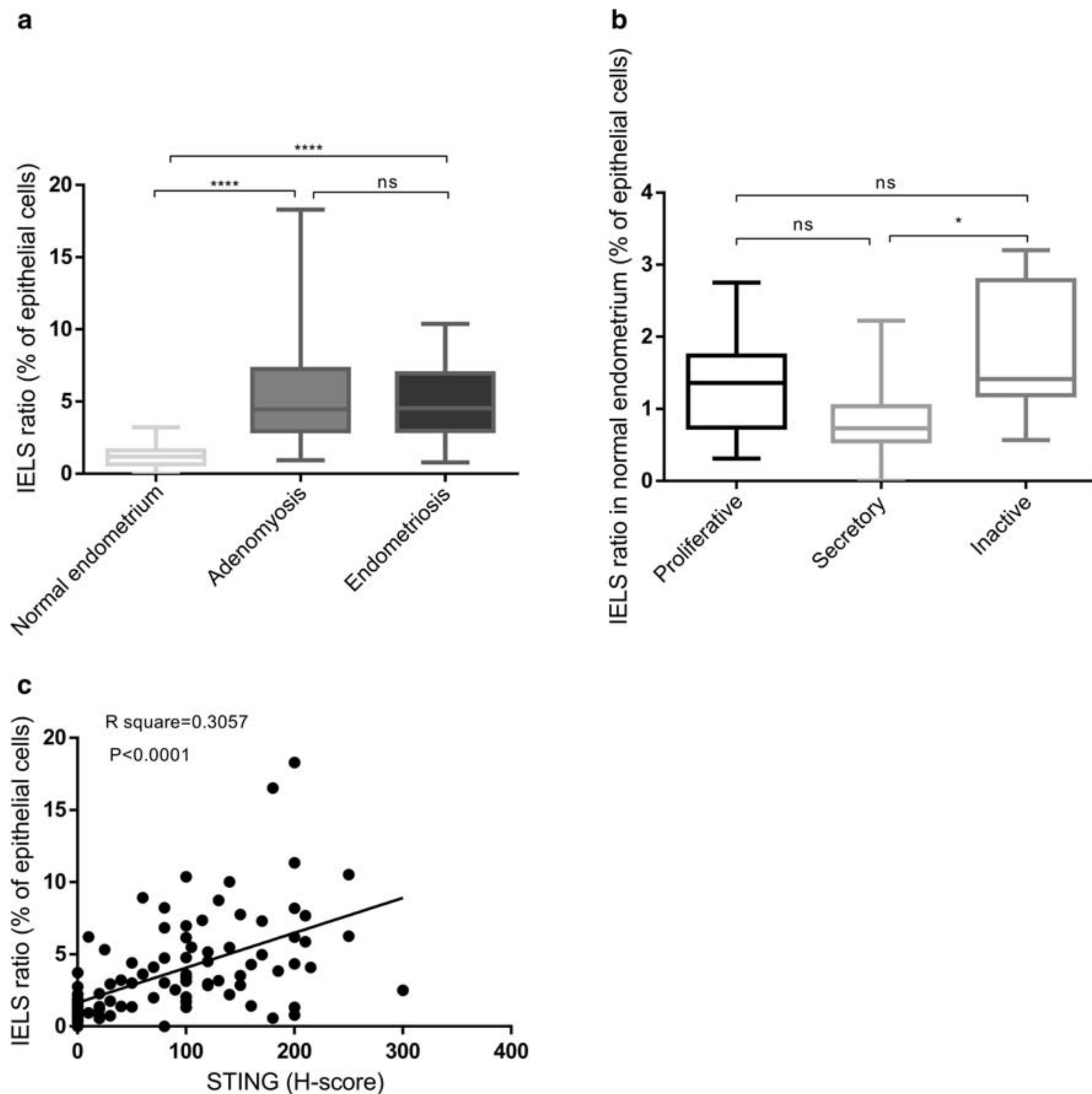


Fig. 3 Intraepithelial lymphocytes to epithelial cells ratio in normal endometrium, adenomyosis and endometriosis. (A) The ratio of CD45+ intraepithelial lymphocytes was significantly higher in adenomyosis ($p < 0.0001$) and endometriosis ($p < 0.0001$) than in normal endometrium, with no significant difference between adenomyosis and endometriosis ($p = 0.48$). (B) There was no significant difference in IELs ratio to epithelial cells among proliferative, secretory ($p = 0.15$) and proliferative and inactive endometrium ($p = 0.060$). But inactive endometrium had more IELs than secretory endometrium ($p = 0.015$). (C) The ratio of intraepithelial lymphocytes has a positive correlation with STING expression levels

The cGAS-STING is an evolutionarily conserved pathway, serving as the major mechanism to mount innate immunity to the host in eliminating the invading pathogens and nascent neoplastic cells. Activated STING forms a complex with TBK1, then trafficking from the endoplasmic reticulum to perinuclear Golgi where it is palmitoylated, resulting in phosphorylating interferon regulatory factor 3 (IRF3) and NF-kB. This leads to transcription upregulation of type I IFNs [23–25]. While type I IFN executes the killing of infected or neoplastic cells, it can also sustain chronic inflammation and is responsible for auto-inflammatory and autoimmune diseases [26, 27]. A study employing the STING-knockout mouse model demonstrates that STING pathway is required to develop acute pancreatitis [28]. The results as reported in this study that the expression level of STING was much higher in the epithelial cells of endometriosis and adenomyosis than normal endometrium suggest that STING signaling plays a role in initiating chronic inflammation as well as orchestrating the trafficking of different immune cells to the lesions. Our results are consistent with higher STING expression levels in cancer cells than in normal cells [29], providing another molecular evidence to support that endometriosis, especially the deep infiltrating ones, share several features with cancer in addition to

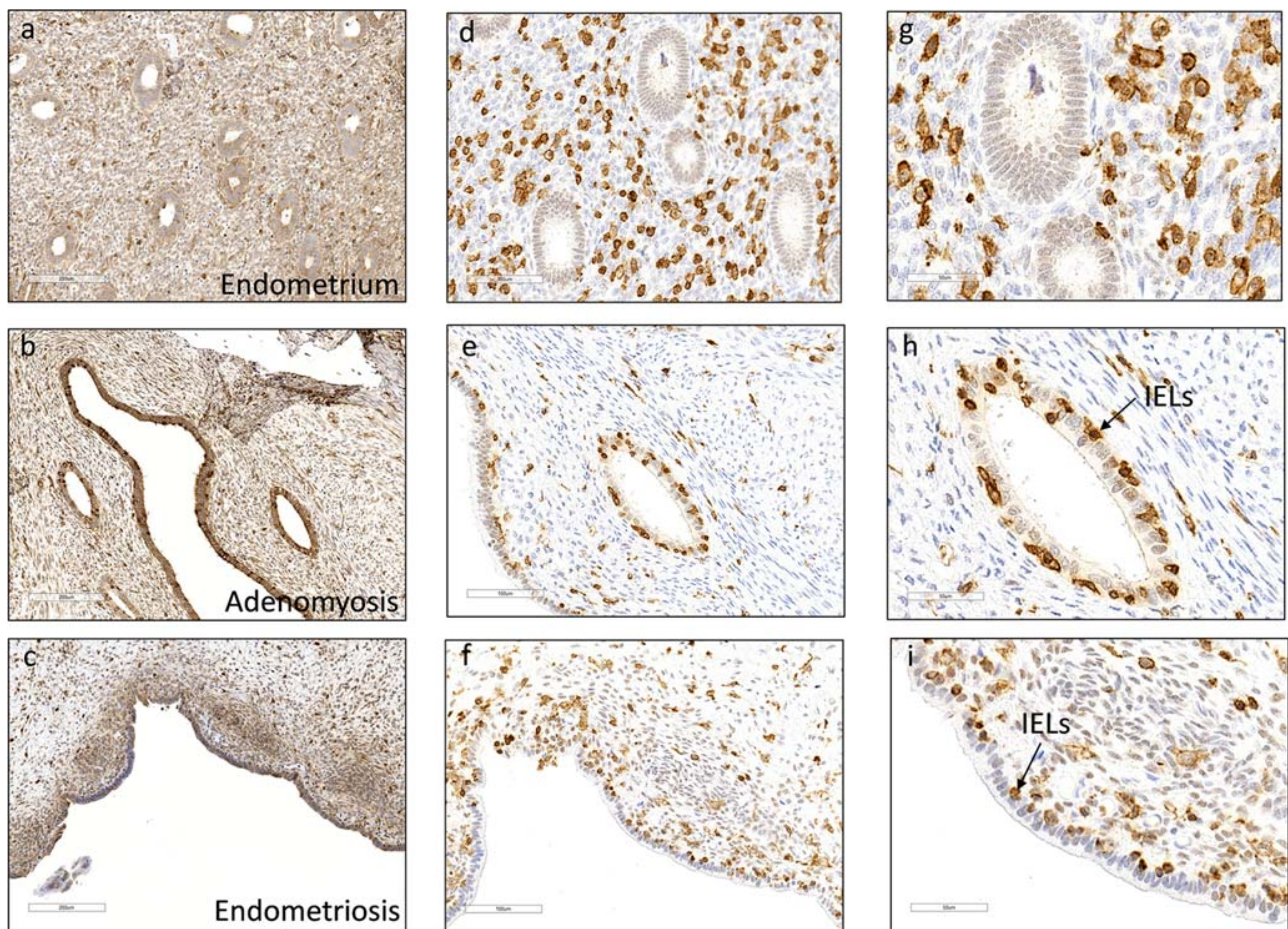


Fig. 4 There were more CD45+ intraepithelial lymphocytes in adenomyosis and endometriosis lesions than in normal endometrium from the same cases. a-c, STING immunoreactivity in endometrium, adenomyosis and endometriosis (10x). d-i, CD45 expression patterns in endometrium, adenomyosis and endometriosis (d-f 20x, g-i 40x)

somatic mutations in cancer-associated genes and their infiltrative/disseminated behavior [30].

For a fact, STING is rapidly degraded, if it does not bind to cyclic dinucleotides . Thus, it is likely that the upregulation of STING is a surrogate for STING activation in tissue sections [31]. Although the epithelium from endometriosis and adenomyosis is STING-primed, the source of cyclic dinucleotides that activate the signaling is yet to be identified. Occult viral or bacterial infection could be one of the sources, but direct evidence is lacking. Alternatively, endometriosis and adenomyosis could be cytosolic DNA-triggered autoinflammatory diseases. Epithelial cell injury may repeatedly occur as a result of the degeneration-regeneration cycles in response to estradiol and progesterone. Unlike eutopic endometrium, which is shed during menstruation, the damaged epithelial cells within endometriosis and adenomyosis remain in the lesions. Also, the nuclear or mitochondrial DNA may appear in the cytosol of epithelial cells which triggers activation of the cGAS-STING pathway. It is also possible that the presence of cytosolic DNA fragments can be generated by the underlying mitotic check point deficiency related to cancer-driver gene mutations including KRAS, PIK3CA, PPP2R1A and ARID1A [30]. Finally, the germ-line STING variants that constitutively induce type I interferon response may also be responsible at least in some patients [23].

In this study, we determined the expression of STING levels in endometriosis and adenomyosis. One of the approaches used is to correlate the STING expression levels and infiltration of inflammatory cells within the epithelium in both lesions and eutopic endometrium. In the previous report, no significant difference in intraepithelial lymphocytes existed between eutopic and ectopic endometrium in adenomyosis [32]. But in this study, we observed that the density of intraepithelial CD45+ cells was significantly higher both in endometriosis and adenomyosis than in normal endometrium in many of cases analyzed. More importantly, we found a positive correlation between the amount of intraepithelial CD45+ cells and the H-score of STING. This finding is consistent with the roles of INFs which were produced because of activation of the STING pathway in the

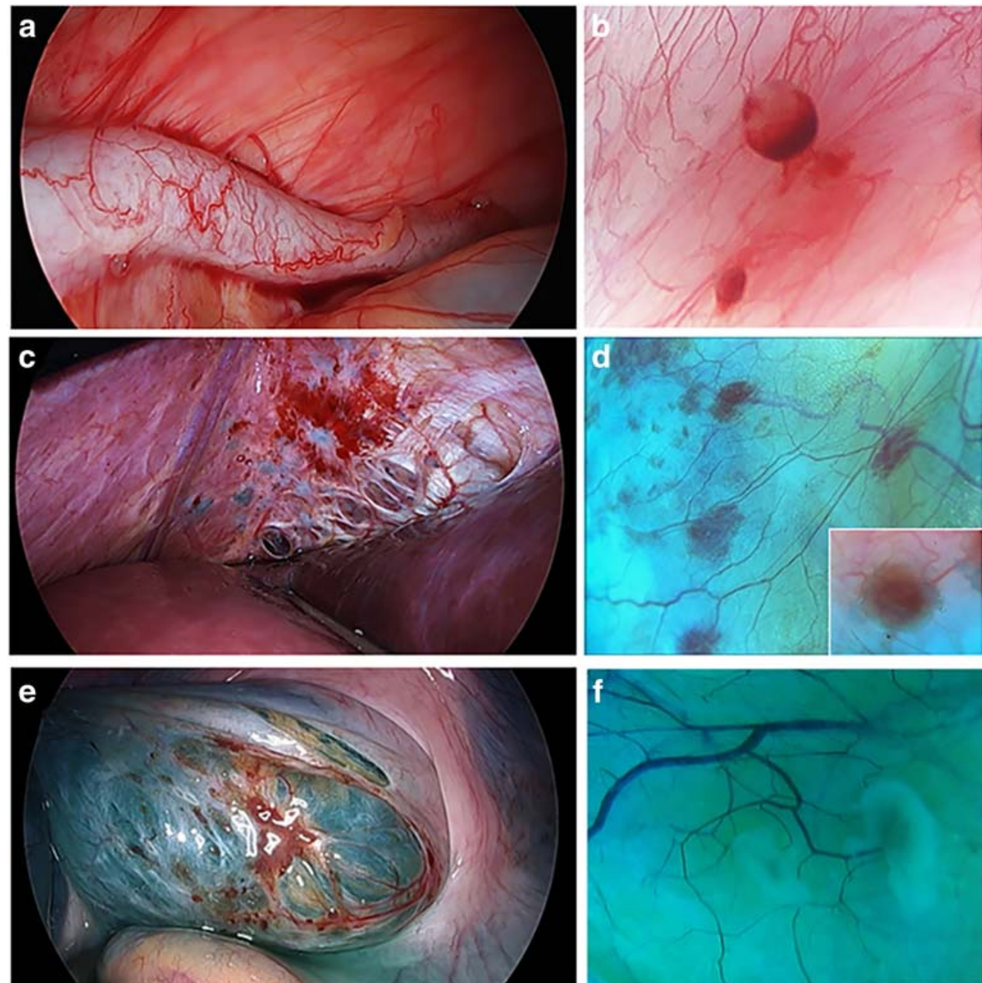


Fig. 5 Laparoscopic appearance of endometriosis at various stages of development. (A) Appendix showing prominent blood vessels on the serosal surface. No gross lesion is apparent. (B) An incipient endometriotic lesion on the peritoneal surface. (C) Endometriosis involves diaphragm. (D) Multiple "active" endometriotic lesions on peritoneum. (E) and (F) Endometriotic lesion showing fibrosis. The inset in D represents a close up view in a lesion. D-F, visualized with AquaBlue Contrast with diluted methylene blue dye

recruitment of lymphocyte. The majority of IELs are T cells and they represent a unique population of immune cells that would normally reside in the basal-lateral of the epithelial cells, serving as the first-line immune surveillance [33, 34]. These mucosa-associated IELs, whether naturally occurring or adaptively induced, are important for tissue defense to protect the mucosa from repeated damage of mucosal barrier integrity caused by infection and other physical assaults [33, 35], given that IELs increase in response to infection or inflammation [35]. In the current study, while IELs are expected to appear in eutopic endometrium, their higher density in endometriosis and adenomyosis may reflect an over-reaction to the underlying inflammatory microenvironment initiated and sustained by different mechanisms including the active cGAS-STING pathway.

In summary, STING, in general, is upregulated in the glandular epithelial cells from both endometriosis and adenomyosis as compared to eutopic endometrium. Its expression also correlates with the density of IELs. The current study is limited by the small sample size and a lack of profiles of different immune cells within lesions. Future studies are needed to determine the STING expression patterns and IELs in endometriotic cysts and hormone treated lesions. More efforts are also required to illuminate the biological functions of STING upregulation in the development of the endometriosis and adenomyosis and, to determine whether deactivating STING by compounds such as 2-bromopalmitate (suppressing STING palmitoylation) has a clinical benefit. Furthermore, it would be interesting to assess STING as a tissue biomarker for treatment response and recurrence in women with endometriosis and/or adenomyosis. Nevertheless, the current study may provide a rationale to study how STING signaling activation configures the immune cell landscape in the lesions.

Acknowledgments This study was supported by Richard W. TeLinde endowment for gynecologic pathology research from the Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine and Endometriosis Foundation of America.

Compliance with Ethical Standards

Conflict of Interest The authors declare no competing or financial interests.

References

1. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. Hum Reprod. 2012;27(5):1292–9.
2. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. Annu Rev Pathol Mech Dis. 2020;15:71.
3. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789–99.
4. Grund EM, Kagan D, Tran CA, Zeitvogel A, Starzinski-Powitz A, Nataraja S, et al. Tumor necrosis factor-alpha regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase kinase, p38, and nuclear factor kappaB in human endometriotic epithelial cells. Mol Pharmacol. 2008;73(5):1394–404.
5. Tseng JF, Ryan IP, Milam TD, Murai JT, Schriock ED, Landers DV. R. N. Taylor RN. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab. 1996;81(3):1118–22.
6. Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. J Clin Endocrinol Metab. 1997;82(2):600–6.
7. Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. J Clin Endocrinol Metab. 1996;81(1):174–9.
8. Juhasz-Boss I, Fischer C, Lattrich C, Skrzypczak M, Malik E. O. Ortmann O, Treeck O. endometrial expression of estrogen receptor beta and its splice variants in patients with and without endometriosis. Arch Gynecol Obstet. 2011;284(4):885–91.
9. Han SJ, Hawkins SM, Begum K, Jung SY, Kovanci E, Qin J, et al. A new isoform of steroid receptor coactivator-1 is crucial for pathogenic progression of endometriosis. Nat Med. 2012;18(7):1102–11.
10. Wu MH, Wang CA, Lin CC, Chen LC, Chang WC, Tsai SJ. Distinct regulation of cyclooxygenase-2 by interleukin-1beta in normal and endometriotic stromal cells. J Clin Endocrinol Metab. 2005;90(1):286–95.
11. Taniguchi K, Karin M. NF-kappaB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018;18(5):309–24.
12. Gonzalez-Ramos R, Defrere S, Devoto L. Nuclear factor-kappaB: a main regulator of inflammation and cell survival in endometriosis pathophysiology. Fertil Steril. 2012;98(3):520–8.
13. Gonzalez-Ramos R, Langendonckt AV, Defrere S, Lousse JC, Colette S, Devoto L, et al. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. Fertil Steril. 2010;94(6):1985–94.
14. Gonzalez-Ramos R, Langendonckt AV, Defrere S, Lousse JC, Colette S, Devoto L, et al. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. Fertil Steril. 2010;94(6):1985–94.
15. Ahn J, Gutman D, Saijo S, and Glen N. Barber GN. STING manifests self DNA-dependent inflammatory disease. PNAS. 2012. 109 (47): p. 19386–19391.
16. Benmerzoug S, Rose S, Bounab B, Gosset D, Dunear L, Chenuet P, et al. STING-dependent sensing of self-DNA drives silica-induced lung inflammation. Nat Commun. 2018;9(1):5226.
17. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science. 2013;339(6121):826–30.
18. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009;461(7265):788–92.
19. Goulding H, Pinder S, Cannon P, Pearson D, Nicholson R, Snead D, et al. A new immunohistochemical antibody for the assement of estrogen receptor status on routine formalin-fixed tissue samples. Hum Pathol. 1995;26:291–4.
20. Ishibashi H, Suzuki T, Suzuki S, Moriya T, Kaneko C, Takizawa T, et al. Sex steroid hormone receptors in human thymoma. J Clin Endocrinol Metab. 2003;88(5):2309–17.
21. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10(5):261–75.
22. Garcia-Solares J, Donnez J, Donnez O, Dolmans MM. Pathogenesis of uterine adenomyosis: invagination or metaplasia? Fertil Steril. 2018;109(3):371–9.
23. Mukai K, Konno H, Akiba T, Uemura T, Waguri S, Kobayashi T, et al. Activation of STING requires palmitoylation at the Golgi. Nat Commun. 2016;7:11932.
24. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014;41(5):830–42.
25. Corrales L, Gajewski TF. Endogenous and pharmacologic targeting of the STING pathway in cancer immunotherapy. Cytokine. 2016;77:245–7.
26. Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat Immunol. 2016;17(10):1142–9.
27. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, et al. Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 2014;371(6):507–18.
28. Zhao Q, Wei Y, Pandol SJ, Li L, Habtezion A. STING signaling promotes inflammation in experimental acute pancreatitis. Gastroenterology. 2018;154(6):1822–35 e2.
29. Ding L, Huang XF, Dong GJ, Hu EL, Chen S, Wang TT, et al. Activated STING enhances Tregs infiltration in the HPV-related carcinogenesis of tongue squamous cells via the c-Jun/CCL22 signal. Biochim Biophys Acta. 2015;1852(11):2494–503.
30. Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noë M, Horlings HM, et al. Cancer-associated mutations in endometriosis without Cancer. N Engl J Med. 2017;376(19):1835–48.
31. Konno H, Konno K, Barber GN. Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell. 2013;155(3):688–98.
32. Bulmer JN, Jones RK, Searle RF. Intraepithelial leukocytes in endometriosis and adenomyosis comparison of eutopic and ectopic endometrium with normal endometrium. Hum Reprod. 1998;13(10):2910–5.
33. Hoytema van Konijnenburg DP, Mucida D. Intraepithelial lymphocytes. Curr Biol. 2017;27(15):R737–9.
34. Lambolez F, Mayans S, Cheroutre H. Lymphocytes: Intraepithelial. In: eLS. 2013; John Wiley & Sons, Ltd: Chichester.
35. Mayassi T, Jabri B. Human intraepithelial lymphocytes. Mucosal Immunol. 2018;11:1281–9.