NLRC5 and autophagy combined as possible predictors in patients with endometriosis

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Objective: To investigate the levels of NLRC5 and autophagy in women with leiomyoma and endometriosis and the correlation between NLRC5 level and autophagy level.
Design: Case-control study.
Setting: Clinics.
Patient(s): Sixty-five patients were recruited: 30 women with endometriosis were compared with 35 women with leiomyoma.
Intervention(s): Endometriosis was definitively diagnosed during surgery by laparoscopy or laparotomy and was confirmed by histopathological evaluation (n=30). Secretory phase ectopic endometrium tissues and eutopic endometrium tissues were obtained from 30 women with endometriosis. Control endometrium tissues were collected at hysterectomy from 35 women with leiomyoma. Immunohistochemical staining of NLRC5, LC3, Beclin1 and P62 were performed.
Main Outcome Measure(s): A semiquantitative analysis was performed. Correlations between NLRC5 level and LC3, Beclin1, P62 levels were compared.
Result(s): The expressions of NLRC5 and P62 in the ectopic and eutopic endometrium of endometriosis groups were significantly higher than that in the endometrium of leiomyoma group. And their expressions in ectopic endometrium were significantly up-regulated compared to the eutopic endometrium. The expressions of LC3 and Beclin1 were down-regulated in the ectopic and eutopic endometrium of endometriosis groups compared to the leiomyoma group. LC3 and Beclin1 levels were lower in ectopic endometrium than in the eutopic endometrium. There is a negative correlation between NLRC5 level and LC3, Beclin1 levels. There is a positive correlation between NLRC5 level and P62 level.
Conclusion(s): There is a negative correlation between NLRC5 level and autophagy level. NLRC5 and autophagy combined may as promising predictors in patients with endometriosis. (Fertil Steril® 2018;110:949–56. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Endometriosis, NLRC5, autophagy, correlation, predictors

Endometriosis is a heterogeneous disorder and defined as the presence of functional uterine glands and stroma outside the uterine cavity including ovaries and pelvic peritoneum, rectovaginal septum. Women with endometriosis experience chronic pelvic pain, infertility, dysmenorrhea, dyspareunia, dysuria, and dyschezia. Prevalence has been estimated to reach 10% of the female population of reproductive age and 20% to 50% of infertile women (1, 2). In addition to the health-related diseases, endometriosis also has dramatic economic burden with healthcare expenses (3). Surgical approaches to extirpate the lesions and medical therapy to relieve the symptoms are present treatment options for endometriosis, but they are inadequate (4). Embarrassing, the pathophysiology of endometriosis remains unclear. It is now widely acceptable that the pathogenesis of endometriosis is multifactorial (5–7). Recent studies involved in the contribution of inflammation to the progression of endometriosis earned wide attentions. For instance, interleukin (IL)-17A was specifically elevated in the blood and endometrium of women with endometriosis, and its expression was reduced after treatment of endometriosis (8).

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J. L. and B. W. should be considered similar in author order.
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The Nod-like receptors (NLRs) are a recently discovered family of cytoplasmic pattern recognition receptors (PRRs) whose functions are mainly to regulate inflammation and play a critical role in innate and adaptive immunity (9). NLR family, a caspase activation and recruitment domain containing 5 (NLRC5) is a newly identified member of the NLR family which contains more than 20 members in mammalian genome (10). NLRC5 is well identified as a transcriptional regulator of major histocompatibility complex (MHC) class I genes in immune cells (11). Of special note is that recent studies proposed controversial roles of NLRC5 in innate and adaptive immunity. On the one hand, NLRC5 can act as a negative regulator of inflammatory responses, silencing of NLRC5 resulted in increased IL-6, tumor necrosis factor-alpha (TNF-α), C-X-C chemokine ligand 5 (CXCL5), and IL-1β secretion and decreased secretion of the anti-inflammatory cytokine IL-10 (12). On the other hand, NLRC5 also contributed to inflammasome activation and inflammasome-dependent cytokines secretion (13). Nevertheless, less is known about the expression and role of NLRC5 in the inflammation-mediated endometriosis.

Macroautophagy/autophagy is a pathway by which cytoplasmic components, including intracellular soluble macromolecules, organelles, and microorganisms, are delivered to lysosomes for degradation (14). The process of autophagy is controlled by the autophagy-related proteins groups (ATGs), of which, three vital proteins LC3, Beclin1, and P62 are essential to autophagy progression. Autophagy has been linked to various pathophysiological processes, including tumorigenesis, immunity, and inflammation and so on (15, 16). Notably, Mei and co-workers (17) investigated that estrogen promoted the survival of human secretory phase endometrial stromal cells (ESCs) via CXCL12/C-X-C chemokine receptor type 4 (CXCR4) up-regulation-mediated autophagy inhibition. Furthermore, CXCL12 repressed the autophagy of secretory phase ESCs partly depend on nuclear factor-κB (NF-κB) signaling pathway, indicating that autophagy seemed to be involved in inflammation in endometriosis (17). Of special note is that ATG16L1 was critical for Nod-dependent regulation of cytokine responses and that disruption of this nucleotide-binding oligomerization domain 1 (Nod1)- or Nod2-ATG16L1 signaling axis could contribute to the chronic inflammation associated with Crohn's disease, suggesting autophagy was implicated in NLRs-mediated inflammation (18). However, it is unknown whether autophagy plays a function in the progression of NLRC5-meidated inflammation.

Owing to the above mentioned pathophysiological correlations between NLRs, autophagy, and inflammation-mediated endometriosis. We hypothesized that there is a correlation between the levels of NLRC5 and autophagy related genes in endometriosis, the aim of the current study is to determine the levels of NLRC5, LC3, Beclin1, and P62 in ectopic and eutopic endometrium of women with endometriosis and endometrium of women with leiomyoma, with a particular emphasis on exploring the correlations between the levels of NLRC5 and LC3, Beclin1, and P62.

MATERIALS AND METHODS

Subjects

This was a case-control study jointly carried out in The Second Affiliated Hospital of Anhui Medical University. The subjects recruited into the study were women of reproductive age attending the Department of Gynecology and Obstetrics in The Second Affiliated Hospital of Anhui Medical University between January 2017 and December 2017. To be included in this study, patients had to fulfill the following criteria: be 25 to 45 years of age; have a regular menstrual cycle (25–35 days); have a complete medical record and pathology report available; have written informed consent on file sampling and endometrial biopsies that were taken solely for research purposes; and not have taken any medications or received hormonal therapy at least six months prior to surgery. The study indicated that the levels of autophagy in proliferative phase endometrium tissues did not significantly change (17), so we collected secretory phase endometrium tissues in our studies. The exclusion criteria for patients included: proliferation phase endometrium; and having complications including pelvic inflammatory disease, polycystic ovarian syndrome, endometrial polyps, hydrosalpinx and adenomyosis. Endometriosis was definitively diagnosed during surgery by laparoscopy or laparotomy and was confirmed by histopathological evaluation (n=30). Samples of secretory phase ectopic endometrium and eutopic endometrium were obtained from the 30 women. The staging and morphological distribution of peritoneal lesions of endometriosis were based on the revised classification of the American Society for Reproductive Medicine (rASRM) (19). The rASRM staging system is based solely on surgical visualized disease volume and location and does not incorporate symptomatology. Stage of disease (I-IV: minimal, mild, moderate, severe) was automatically calculated via the rASRM weighted point score. Among the ectopic endometrium, four were from stage I-II cases and were pelvic endometriosis and the remaining 26 were ovarian endometriosis from women with stage III-IV disease. Control secretory phase endometrium tissues were collected at hysterectomy from patients with leiomyoma (n=35). The phase of the menstrual cycle was determined by evaluating the endometrial histology and by comparing the date to the expected day of the menstrual cycle provided by the women. This study was approved by the institutional review board of the Anhui Medical University (20170076).

Sample Collection

Eutopic endometrium and ectopic endometrium were collected from four patients with pelvic endometriosis and 26 patients with ovarian endometriosis during laparoscopy or laparotomy and 35 patients with leiomyoma during hysterectomy. All endometrium samples were collected using using a Pipelle sampler (Prodimed) under sterile conditions. All collected endometrium samples were prepared for formalin-fixed paraffin-embedded tissue blocks for subsequent histopathological and immunohistochemical study.

Immunohistochemistry

Human endometrial tissues were fixed in 10% neutral buffered formalin solution, embedded in paraffin, and stained for routine histology. The sections were dewaxed in xylene and dehydrated in alcohol, antigen retrieval was achieved by microwaving in citric saline for 15 min. Thin sections were deparaffinized and treated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. The sections were further blocked by 2% bovine serum albumin followed by incubation with primary rabbit monoclonal antibody against NLRC5 (ab 105411, Abcam, diluted 1:100), LC3 (GB11124, Servicebio, diluted 1:500), Beclin1 (GB11228, Servicebio, diluted 1:2500), and P62 (18420-1-AP, Proteintech, diluted 1:200) for 16 h at 4°C. After rinsing, the sections were incubated with biotinylated secondary antibody (G1210-2-A, Servicebio) for 60 minutes at room temperature. NLRC5, LC3, Beclin1, and P62 expressions were visualized by 3, 3′-diaminobenzidine tetrahydrochloride (DAB) staining. Slides were counterstained with hematoxylin before dehydration and mounting.

Quantitative analysis was calculated from five random fields at ×200 magnification for each endometrial slice. All slices were observed under a fluorescence microscope and photographed. The background light of each photo was consistent. Dark brown staining indicated a positive reaction. The intensity of dark brown staining was analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc.).

Statistical Analysis

Statistical analysis was performed by using SPSS statistical software package, version 20.0 (SPSS). Continuous variables were expressed as mean ± standard deviation. Student's t-test was used to compare age, body mass index, and CA-125 level. Intergroup comparisons were performed using a Fisher's exact test for categorical variables. NLRC5, LC3, Beclin1 and P62 levels were analyzed by one-way analysis of variance (ANOVA) using Bonferroni's post hoc test in multiple groups. Correlation analysis was performed by Pearson correlation. $P < .05$ were considered statistically significant.

RESULTS

Demographic Characteristics of Patients with Leiomyoma and Endometriosis

The indications for surgery were shown in Supplemental Table 1. Table 1 summarized the demographic characteristics of patients from the leiomyoma group and the endometriosis group. There were no significant differences in age, body mass index, gravidity, and parity between the two groups. The level of CA-125 in endometriosis patients was significantly higher than in leiomyoma patients.

Comparison the Levels of NLRC5, LC3, Beclin1 and P62 Between Women with Leiomyoma and Women with Endometriosis

Figure 1 showed the immunostaining of NLRC5, LC3, Beclin1 and P62 in endometrium from women with leiomyoma and eutopic/ectopic endometrium from women with endometriosis. The expression of NLRC5 in the endometriosis endometrium groups was significantly higher than that in the leiomyoma endometrium group. Furthermore, the level of NLRC5 in ectopic endometrium was significantly up-regulated compared to the eutopic endometrium. In consistent with previous studies, the level of autophagy was significantly down-regulated in the endometriosis group which compared to the leiomyoma group. Furthermore, autophagy was also down-regulated in ectopic endometrium than in the eutopic endometrium, which was confirmed by the following results. The levels of LC3 and Beclin1 in endometriosis were significantly lower than in the leiomyoma group. And their expressions in ectopic endometrium were also lower than in the eutopic endometrium. Consistently, the expression of P62 was up-regulated in the endometriosis group compared to the leiomyoma group. Moreover, P62 level was higher in ectopic endometrium than in the eutopic endometrium. Table 2 showed the comparisons of levels of NLRC5, LC3, Beclin1, and P62 in leiomyoma endometrium, eutopic endometrium, and ectopic endometrium.

TABLE 1

Demographic characteristics of patients with leiomyoma and endometriosis.

Variable	Leiomyoma (N = 35)	Endometriosis (N = 30)	t	*P* value
Age, y	36.51 ± 7.37	33.97 ± 5.75	1.535	.130
BMI, kg/m^2	22.82 ± 2.57	22.65 ± 4.64	0.184	.854
Gravidity (%)[a]				.192
0	5 (7.7)	8 (12.3)		
1	3 (4.6)	6 (9.2)		
2	10 (15.4)	9 (13.8)		
≥3	17 (26.2)	7 (10.8)		
Parity (%)[a]				.056
0	6 (9.2)	9 (13.8)		
1	20 (30.8)	20 (30.8)		
≥2	9 (13.8)	1 (1.5)		
CA-125 (U/ml)	16.24 ± 9.06	92.51 ± 81.26	5.114	< .001

Note: Data are expressed as mean ± standard deviation, unless specified otherwise. BMI = body mass index.
[a] Fisher's exact test.

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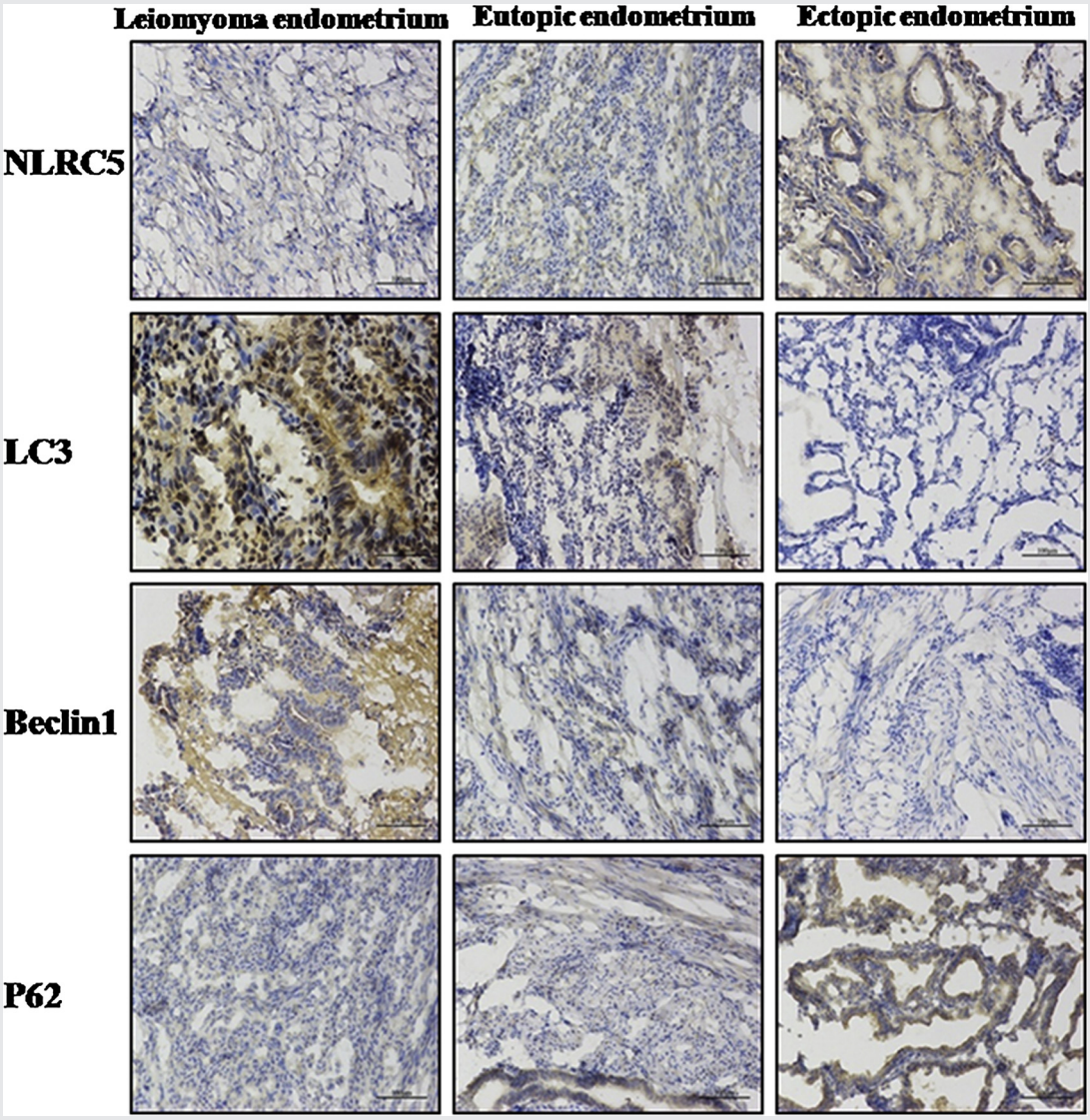
Correlation Between NLRC5 with LC3, Beclin1, and P62

Correlation analysis was performed by Pearson correlation. The correlation analysis between NLRC5 levels with the autophagy markers showed a significant negative correlation between NLRC5 level and LC3 (r -0.900, $P < .001$; Fig. 2A), Beclin1 levels (r -0.845, $P < .001$; Fig. 2B), and positive correlation between NLRC5 level and P62 level (r 0.920, $P < .001$; Fig. 2C), indicating that there is a negative correlation between NLRC5 level and autophagy level.

DISCUSSION

In this study, we compared the levels of NLRC5 and LC3, Beclin1, and P62 in endometrium tissues from women with leiomyoma and endometriosis and detected the correlations

FIGURE 1



Leiomyoma endometrium, eutopic endometrium and ectopic endometrium were stained by immunohistochemistry with NLRC5 and LC3, Beclin1, and P62 (original magnification ×200). The expression of NLRC5 in the endometriosis endometrium groups was significantly higher than that in the leiomyoma endometrium group. The level of NLRC5 in ectopic endometrium was significantly up-regulated compared to the eutopic endometrium. The levels of LC3 and Beclin1 in endometriosis were significantly lower than in the leiomyoma endometrium group. And their expressions in ectopic endometrium were also lower than in the eutopic endometrium. The expression of P62 was up-regulated in the endometriosis group compared to the leiomyoma endometrium group. P62 level was higher in ectopic endometrium than in the eutopic endometrium.

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between NLRC5 and LC3, Beclin1, and P62. Based on our findings, we found NLRC5 was up-regulated in endometriosis ectopic and eutopic endometrium of patients with endometriosis compared to the endometrium of patients with leiomyoma. Furthermore, the level of NLRC5 in ectopic endometrium was also significantly higher than in the eutopic endometrium. Autophagy was down-regulated in ectopic and eutopic endometrium of patients with endometriosis compared to the endometrium of patients with leiomyoma, and autophagy also was down-regulated in ectopic endometrium compared to the eutopic endometrium. Importantly, correlation analysis showed that there is a negative correlation between NLRC5 level and autophagy level.

Chronic inflammation is disruptive and a major cause of infertility and menstrual bleeding disorders (20). Endometriosis is one of the most common causes of infertility and pelvic pain and affects millions of women (21). More and more evidence indicated that increased and sustained inflammatory microenvironment was one of main factors which ultimately leading to endometriosis. It was indicated that endometriosis is a systemic and reversible inflammatory condition that alters endometrial function (22, 23). Mechanistic

TABLE 2

Comparison of levels of NLRC5, LC3, Beclin1, and p62 in leiomyoma endometrium, eutopic endometrium and ectopic endometrium.

Variable	Leiomyoma endometrium (N = 35)	Eutopic endometrium (N = 30)	Ectopic endometrium (N = 30)
NLRC5	0.23 ± 0.05	0.35 ± 0.04	0.56 ± 0.04
LC3	0.81 ± 0.07	0.50 ± 0.04	0.26 ± 0.03
Beclin1	0.59 ± 0.08	0.33 ± 0.07	0.18 ± 0.05
P62	0.23 ± 0.05	0.35 ± 0.03	0.61 ± 0.07

Note: Data are expressed as mean ± standard deviation. There are statistically significant differences between any two groups (using Bonferroni's method). $P < .001$ for all groups.

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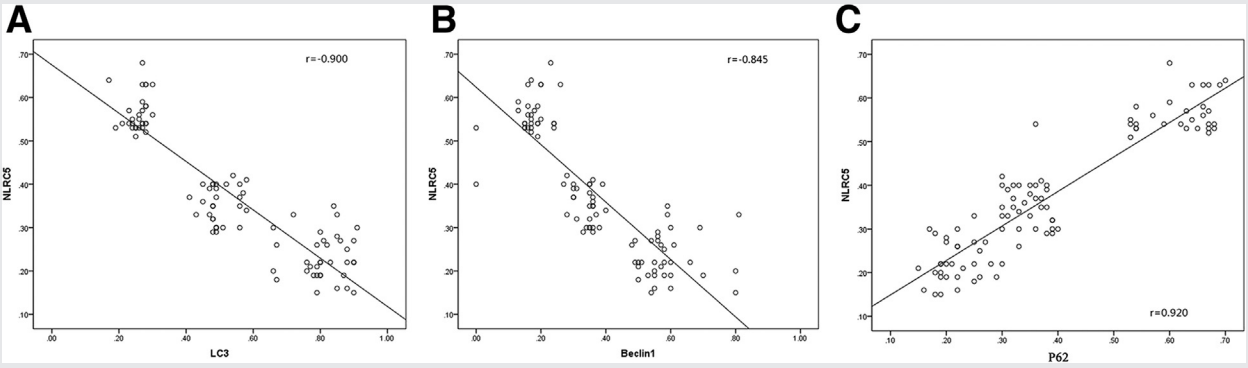
investigations suggested that the inflammatory response seen in endometriosis may be related to intrinsic programmatic endometrial responses to progesterone withdrawal and estrogen dominance, which further promote angiogenesis, cell proliferation, and immunosuppression (24, 25). Thus, an appealing therapeutic approach for blocking key elements of the inflammatory processes associated with abnormal ectopic growth would be utilized. Increasing evidence indicated that dysregulation of the function of multiple NLR family members has been linked to a propensity for inflammatory diseases (26–28). Notably, several recent studies identified that NLRC5 could be a potential therapeutic target for inflammatory diseases owing to its role in inhibiting inflammation (29–32). For example, Chen et al. illustrated that lack of NLRC5 resulted in severer hepatic ischemia/reperfusion injury (29). Furthermore, Ma and colleagues (30) indicated that NLRC5 knockout mice fed with high fat showed accelerated fibrosis and inflammation response by promoting α-smooth muscle actin, collagen I/III, toll-like receptor 4, myeloid differentiation primary response gene 88 and nuclear factor (NF)-kappaB expressions (30). Nevertheless, to date, the role of NLRC5 in endometriosis has not been well investigated yet. In our study, we first to compare the expression of NLRC5 between women with leiomyoma and endometriosis, we detected that NLRC5 was up-regulated in ectopic and eutopic endometrium of patients with endometriosis compared to the endometrium of patients with leiomyoma. Furthermore, the level of NLRC5 in ectopic endometrium was also significantly higher than in the eutopic endometrium. These results suggested that NLRC5 seems to be involved in the progression of endometriosis.

Autophagy is an evolutionarily conserved cellular self-digestion pathway for maintenance of homeostasis by recycling lysosome-dependent intracellular soluble macromolecules, organelles and microorganisms (33). It is evident that transcriptional inductions of autophagy-related genes LC3, Beclin1, and P62 are involved in different steps of the autophagic pathway. Recruitment of Beclin1 contributes to phosphatidylinositol-3-OH kinase-Beclin1-VPS34 complex formation in the initiation stage. LC3, which consists two forms the cytoplasmic form LC3-I and the processed form LC3-II, is the marker of autophagosome. LC3-II is present on both the outer and inner membrane of the autophagosome and is positively correlated with the autophagy level in cells. The adaptor molecule P62 enables autophagy to target designated cargo to nascent LC3-positive isolation membranes selectively, leading to rapid acidification and enhanced killing of the ingested organism (14). The most frequent words which described autophagy are "double-edged sword" because autophagy plays dual roles in cell survival and cell death (34). In these years, accumulating researches present potential evidence that the progression of endometriosis was crucially implicated in autophagy. However, seemingly opposing concepts concerning the role of autophagy in endometriosis have been proposed, with controversial evidence regarding the expression level of autophagy in endometriosis. For example, Allavena and colleagues (35) showed autophagy was up-regulated and contributed to ovarian endometriosis progression through interplaying with p53 and heme oxygenase-1. However, Zhang et al. (36) found the level of Beclin1 in endometriosis was significant lower than normal women. In our study, we found the levels of LC3 and Beclin1 in women with endometriosis were significantly lower than in women with leiomyoma. And their expressions in ectopic endometrium were also lower than in the eutopic endometrium. The expression of P62 was up-regulated in the endometriosis group compared to the leiomyoma group. Moreover, P62 level was higher in ectopic endometrium than in the eutopic endometrium. Nevertheless, these studies mainly focus on the role of autophagy in ESCs fate, such as cell proliferation and apoptosis, but very limit of the researches regard to the relationship between autophagy and inflammation are prompted (35,37–39). Specifically, there are two studies by Li and colleagues (17, 40) who present the potential role of autophagy in inflammation in endometriosis. They implied that the suppression of autophagy caused by estrogen was dependent on CXCL12-CXCR4 interaction, and CXCL12 inhibited the autophagy of secretory phase endometrial stromal cells was via activating the nuclear factor (NF)-kappaB signaling pathway (17). Furthermore, they also implicated that a decrease of the ESCs autophagic level promoted the expression of IL-15 receptors and increased the sensitivity of ESCs to IL-15 (40). From the above, it is reasonable to assume that low levels of autophagy in patients with endometriosis led to activation of inflammation, which further contributed to the development of endometriosis.

Noteworthily, NLRs have been recently implicated in the control of autophagy. For example, NLRX1 and its interacting partner mitochondrial Tu elongation factor (TUFM) promoted autophagy by association with ATG5-ATG12 complexes and ATG16L1 (41). NLRP4 interacted with Beclin1 and led to the activation of autophagy (42). NLRC5 is a newly identified member of the NLR family, human NLRC5 is in the 16q13 locus and consists of 1,866 amino acids while mouse NLRC5 is at chromosome 8 and contains 1,915 amino acids (11). NLRC5 possesses three structural domains including the N-terminal atypical caspase activation and recruitment domain, which is completely distinct from the other NLRs, the central NACHT domain, which contains the nucleotidebinding domain (NBD) and 27 leucine-rich repeats at the C-terminal. NLRC5 has the longest leucine-rich repeats domain of all human NLR

FIGURE 2



Correlation analysis between **(A)** NLRC5 level and LC3 level (r -0.900, *P*<.001); **(B)** NLRC5 level Beclin1 level (r -0.845, *P*<.001); and **(C)** NLRC5 level and P62 level (r 0.920, *P*<.001).

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proteins and is the largest member in the NLR family with a predicted size of more than 200 kDa (10, 11). However, the correlation between NLRC5 and autophagy is unknown yet. In our study, we found there is a negative correlation between NLRC5 level and autophagy level in endometriosis. Nevertheless, further study is needed to explore the exact molecular mechanisms between NLRC5 and autophagy.

In this study, the observed NLRC5 and autophagy levels changes in patients with endometriosis compared to patients with leiomyoma indicate that NLRC5 and autophagy are involved in the progression of endometriosis. Furthermore, there is a negative correlation between NLRC5 level and autophagy level. It is possible that high level of NLRC5 and low level of autophagy contribute to development of endometriosis partially through promoting inflammation. It remains possible that NLRC5 plays a role in autophagy in the inflammation-mediated endometriosis. But our findings support the proposed theory that NLRC5 and autophagy combined may as promising predictors in patients with endometriosis.

Although our results are promising, we are aware that the study has several limitations: the number of samples we assessed was not enough large to permit analysis of other confounding variables; our study only used immunohistochemistry and semiquantitative analysis to observe the expression of NLRC5 and autophagy markers, more quantification methods should be used, such as western blotting or reverse transcription-polymerase chain reaction; and confocal microscopic observations would be useful to validate the correlation between NLRC5 and autophagy.

CONCLUSION

In conclusion, our study showed NLRC5 was up-regulated in endometriosis endometrium compared to the leiomyoma endometrium and the level of NLRC5 in ectopic endometrium was also significantly higher than in the eutopic endometrium. Autophagy was down-regulated in endometriosis endometrium compared to the leiomyoma endometrium and autophagy also was down-regulated in ectopic endometrium compared to the eutopic endometrium. Furthermore, there is a negative correlation between NLRC5 level and autophagy level in endometriosis. We indicated that NLRC5 and autophagy combined seem as promising predictors in patients with endometriosis. However, the sample size in our study is limited, and the mechanism between NLRC5 and autophagy in endometriosis is still unknown, further study is needed to explore the exact molecular mechanisms between NLRC5 and autophagy in endometriosis.

REFERENCES

1. Giudice LC. Clinical practice. Endometriosis. N Engl J Med 2010;362:2389–98.
2. Hickey M, Ballard K, Farquhar C. Endometriosis. BMJ 2014;348:g1752.
3. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, et al. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. Hum Reprod 2012;27:1292–9.
4. Horne AW, Saunders PTK, Abokhrais IM, Hogg L. Top ten endometriosis research priorities in the UK and Ireland. Lancet 2017;389:2191–2.
5. Chwalisz K, Perez MC, Demanno D, Winkel C, Schubert G, Elger W. Selective progesterone receptor modulator development and use in the treatment of leiomyomata and endometriosis. Endocr Rev 2005;26:423–38.
6. Laux-Biehlmann A, d'Hooghe T, Zollner TM. Menstruation pulls the trigger for inflammation and pain in endometriosis. Trends Pharmacol Sci 2015;36:270–6.
7. Othman ER, Meligy FY, Sayed AA, El-Mokhtar MA, Refaiy AM. Stem cell markers describe a transition from somatic to pluripotent cell states in a Rat Model of endometriosis. Reprod Sci 2017:1933719117697124.
8. Ahn SH, Edwards AK, Singh SS, Young SL, Lessey BA, Tayade C. IL-17A Contributes to the Pathogenesis of Endometriosis by Triggering Proinflammatory Cytokines and Angiogenic Growth Factors. J Immunol 2015;195:2591–600.
9. Abrahams VM. The role of the Nod-like receptor family in trophoblast innate immune responses. J Reprod Immunol 2011;88:112–7.
10. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821–32.
11. Chelbi ST, Dang AT, Guarda G. Emerging Major Histocompatibility Complex Class I-Related Functions of NLRC5. Adv Immunol 2017;133:89–119.
12. Benko S, Magalhaes JG, Philpott DJ, Girardin SE. NLRC5 limits the activation of inflammatory pathways. J Immunol 2010;185:1681–91.
13. Davis BK, Roberts RA, Huang MT, Willingham SB, Conti BJ, Brickey WJ, et al. Cutting edge: NLRC5-dependent activation of the inflammasome. J Immunol 2011;186:1333–7.

14. Zhan L, Zhang Y, Wang W, Song E, Fan Y, Li J, et al. Autophagy as an emerging therapy target for ovarian carcinoma. Oncotarget 2016;7:83476–87.
15. Singh SS, Vats S, Chia AY, Tan TZ, Deng S, Ong MS, et al. Dual role of autophagy in hallmarks of cancer. Oncogene 2017.
16. Samie M, Lim J, Verschueren E, Baughman JM, Peng I, Wong A, et al. Selective autophagy of the adaptor TRIF regulates innate inflammatory signaling. Nat Immunol 2018.
17. Mei J, Zhu XY, Jin LP, Duan ZL, Li DJ, Li MQ. Estrogen promotes the survival of human secretory phase endometrial stromal cells via CXCL12/CXCR4 up-regulation-mediated autophagy inhibition. Hum Reprod 2015;30:1677–89.
18. Sorbara MT, Ellison LK, Ramjeet M, Travassos LH, Jones NL, Girardin SE, et al. The protein ATG16L1 suppresses inflammatory cytokines induced by the intracellular sensors Nod1 and Nod2 in an autophagy-independent manner. Immunity 2013;39:858–73.
19. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997;67:817–21.
20. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10:261–75.
21. Endometriosis and infertility: a committee opinion. Fertil Steril 2012;98:591–8.
22. Kim BG, Yoo JY, Kim TH, Shin JH, Langenheim JF, Ferguson SD, et al. Aberrant activation of signal transducer and activator of transcription-3 (STAT3) signaling in endometriosis. Hum Reprod 2015;30:1069–78.
23. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update 2016;22:104–15.
24. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 2012;13:277–88.
25. Li X, Large MJ, Creighton CJ, Lanz RB, Jeong JW, Young SL, et al. COUP-TFII regulates human endometrial stromal genes involved in inflammation. Mol Endocrinol 2013;27:2041–54.
26. Kumar SR, Paudel S, Ghimire L, Bergeron S, Cai S, Zemans RL, et al. Emerging Roles of Inflammasomes in Acute Pneumonia. Am J Respir Crit Care Med 2017;197:160–71.
27. Boro M, Balaji KN. CXCL1 and CXCL2 Regulate NLRP3 inflammasome activation via G-protein-coupled receptor CXCR2. J Immunol 2017;199:1660–71.
28. Ydens E, Demon D, Lornet G, De Winter V, Timmerman V, Lamkanfi M, et al. Nlrp6 promotes recovery after peripheral nerve injury independently of inflammasomes. J Neuroinflammation 2015;12:143.
29. Chen Z, Ding T, Ma CG. Dexmedetomidine (DEX) protects against hepatic ischemia/reperfusion (I/R) injury by suppressing inflammation and oxidative stress in NLRC5 deficient mice. Biochem Biophys Res Commun 2017;493:1143–50.
30. Ma SR, Xie XW. NLRC5 deficiency promotes myocardial damage induced by high fat diet in mice through activating TLR4/NF-kappaB. Biomed Pharmacother 2017;91:755–66.
31. Ludigs K, Jandus C, Utzschneider DT, Staehli F, Bessoles S, Dang AT, et al. NLRC5 shields T lymphocytes from NK-cell-mediated elimination under inflammatory conditions. Nat Commun 2016;7:10554.
32. Li L, Xu T, Huang C, Peng Y, Li J. NLRC5 mediates cytokine secretion in RAW264.7 macrophages and modulated by the JAK2/STAT3 pathway. Inflammation 2014;37:835–47.
33. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008;451:1069–75.
34. Liu J, Debnath J. the evolving, multifaceted roles of autophagy in cancer. Adv Cancer Res 2016;130:1–53.
35. Allavena G, Carrarelli P, Del Bello B, Luisi S, Petraglia F, Maellaro E. Autophagy is upregulated in ovarian endometriosis: a possible interplay with p53 and heme oxygenase-1. Fertil Steril 2015;103:1244–12451 e1.
36. Zhang L, Liu Y, Xu Y, Wu H, Wei Z, Cao Y. The expression of the autophagy gene beclin-1 mRNA and protein in ectopic and eutopic endometrium of patients with endometriosis. Int J Fertil Steril 2015;8:429–36.
37. Choi J, Jo M, Lee E, Lee DY, Choi D. Dienogest enhances autophagy induction in endometriotic cells by impairing activation of AKT, ERK1/2, and mTOR. Fertil Steril 2015;104:655–64.e1.
38. Liu H, Zhang Z, Xiong W, Zhang L, Xiong Y, Li N, et al. Hypoxia-inducible factor-1alpha promotes endometrial stromal cells migration and invasion by upregulating autophagy in endometriosis. Reproduction 2017;153:809–20.
39. Xu TX, Zhao SZ, Dong M, Yu XR. Hypoxia responsive miR-210 promotes cell survival and autophagy of endometriotic cells in hypoxia. Eur Rev Med Pharmacol Sci 2016;20:399–406.
40. Yu JJ, Sun HT, Zhang ZF, Shi RX, Liu LB, Shang WQ, et al. IL15 promotes growth and invasion of endometrial stromal cells and inhibits killing activity of NK cells in endometriosis. Reproduction 2016;152:151–60.
41. Lei Y, Wen H, Yu Y, Taxman DJ, Zhang L, Widman DG, et al. The mitochondrial proteins NLRX1 and TUFM form a complex that regulates type I interferon and autophagy. Immunity 2012;36:933–46.
42. Jounai N, Kobiyama K, Shiina M, Ogata K, Ishii KJ, Takeshita F. NLRP4 negatively regulates autophagic processes through an association with beclin1. J Immunol 2011;186:1646–55.

NLRC5 y autofagia combinados como posibles predictores en pacientes con endometriosis

Objetivo: investigar los niveles de NLRC5 y autofagia en mujeres con leiomiomas y endometriosis y la correlación entre los niveles de NLRC5 y el nivel de autofagia.

Diseño: Estudio de casos y controles.

Lugar: XXX.

Pacientes: sesenta y cinco pacientes fueron reclutadas: 30 mujeres con endometriosis fueron comparadas con 35 mujeres con leiomiomas.

Intervención(es): La endometriosis fue diagnosticada definitivamente durante la cirugía por laparoscopia o laparotomía y fue confirmada por evaluación histopatológica (n 30). Se obtuvo tejido endometrial ectópico en fase secretoria y tejido endometrial eutópico en 30 mujeres con endometriosis. Se colectó tejido endometrial en histerectomías de 35 mujeres con leiomiomas como control. Se realizó tinción con inmunohistoquímica de NLRC5, LC3, Beclin 1 y P62.

Principales medidas de resultados: se realizó un análisis semicuantitativo. Se compararon las correlaciones entre el nivel de NLRC5 y los niveles de LC3, Beclin1, P62.

Resultado(s): la expresión de NLRC5 y P62 en el endometrio ectópico y eutópico del grupo de pacientes con endometriosis fue significativamente mas alto que la expresión en pacientes del grupo de leiomiomas. Y su expresión en el endometrio ectópico estaba significativamente aumentada comparado con el endometrio eutópico. La expresión de LC3 y Beclin 1 estaba disminuida en el endometrio ectópico y eutópico de pacientes en el grupo de endometriosis comparado con el grupo de leiomiomas. Los niveles de LC3 y Beclin 1 fueron más bajos en el endometrio ectópico que en el endometrio eutópico. Existe una correlación negativa entre los niveles de NLRC5 y LC3 y Beclin 1. Una correlación positiva fue hallada entre los niveles de NLRC5y P62.

Conclusión(es): existe una correlación negativa entre los niveles de NLRC5 y el nivel de autofagia. El nivel de NLRC5 y de autofagia combinados pueden ser predictores prometedores en pacientes con endometriosis.