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ARTICLE

Are lower levels of apoptosis and autophagy behind adenomyotic lesion survival?



BIOGRAPHY

Marie d'Argent received a medical degree in 2021 from the Université catholique de Louvain, Belgium. She is currently an intern in gynaecology, with a strong interest in fertility preservation.

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KEY MESSAGE

Adenomyotic lesions are characterized by progesterone resistance, matrix metalloprotein 9-mediated invasiveness and aberrant cell survival. These findings provide new insights into the mechanisms underlying the development of adenomyosis and highlight the need to explore novel therapeutic options.

ABSTRACT

Research question: How are markers of cell death, invasiveness and progesterone signalling expressed in endometrium and ectopic lesions from adenomyosis patients?

Design: Formalin-fixed paraffin-embedded tissue was collected from 15 control and 15 adenomyosis participants . To assess cell survival capacity, caspase 3 and microtubule-associated proteins 1A/1B light chain 3B (LC3B) were immunolabelled as markers of apoptosis and autophagy respectively. Matrix metalloproteinase 9 (MMP9) expression served as a marker of extracellular matrix degradation and invasion activity. Progesterone receptors were immunostained to detect evidence of progesterone resistance.

Results: Caspase 3 expression was significantly lower in the stromal ($P = 0.0013$) and epithelial ($P = 0.0157$) compartments of adenomyotic lesions than in healthy endometrial tissue. In the stroma, caspase 3 expression was significantly weaker in lesions than in corresponding eutopic endometrium ($P = 0.0006$). LC3B immunostaining was significantly decreased in adenomyotic stroma compared with corresponding eutopic endometrium ($P = 0.0349$). A significantly higher expression of MMP9 was detected in eutopic stroma from adenomyosis patients than in healthy tissue ($P = 0.0295$). Progesterone receptor immunostaining was found to be significantly weaker in the stroma of endometrium and ectopic lesions from adenomyosis patients than disease-free women ($P = 0.0001$; $P = 0.0021$).

Conclusions: Adenomyotic lesions show lower levels of apoptosis and autophagy, suggesting that aberrant cell survival may be involved in disease pathogenesis. MMP9 appears to contribute to endometrial invasiveness in adenomyosis, as its expression is more pronounced in endometrium from these women than women without the disease. Evidence of progesterone resistance can be found in endometrium and ectopic lesions from adenomyosis patients, and may drive disease development and account for the failure of certain patients to respond to progestogens.

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KEY WORDS
Adenomyosis
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INTRODUCTION

Uterine adenomyosis is a commonly encountered, benign, oestrogen-driven chronic disorder affecting as many as 20% of women visiting the gynecology clinic (*Devlieger et al., 2003*; *Naftalin et al., 2012*). It causes heavy menstrual bleeding (menorrhagia), dysmenorrhoea and intense pelvic pain, and is linked to infertility (*Garcia-Solares et al., 2018a*; *Guo et al., 2013*; *Naftalin et al., 2014*; *Stratopoulou et al., 2021*b). Despite its prevalence and the severity of symptoms, the pathogenesis and aetiology of the disease have not yet been elucidated (*Donnez et al., 2018*; *Stratopoulou et al., 2021*b). Nevertheless, because of its impact on women's quality of life, the condition is becoming a worldwide health concern, as researchers work to shed light on its origins to be able to offer patients better therapeutic options (*Donnez et al., 2021*).

Adenomyosis is histologically characterized by the presence of endometrial tissue within the myometrium, resulting in an enlarged uterus with endometrial glands and stroma typically surrounded by hypertrophic and hyperplastic myometrium (*Donnez et al., 2018*). The most widely accepted hypothesis on the origin of the disease suggests invasion of the myometrium by basal endometrium upon traumatization of the endometrial-myometrial junctional zone (*Garcia-Solares et al., 2018a*; *Leyendecker and Wildt, 2011*).

In healthy women, endometrium undergoes apoptosis in order to maintain cellular homeostasis during normal menstrual cycles by eliminating senescent cells from the functional endometrial layer during the late secretory and menstrual phases (*Nasu et al., 2011*). It has been postulated that this mechanism is impaired in endometriosis, where decreased susceptibility to apoptosis allows endometrial cell survival and overproliferation, thereby promoting disease progression (*Kapoor et al., 2021*; *Nasu et al., 2009*; *Nasu et al., 2011*). Data on the involvement of these mechanisms in adenomyosis are more limited, but enhanced cell proliferation and motility have also been observed, potentially contributing to invagination of the endometrial basalis into the surrounding myometrium (*Zhai et al., 2022*).

Autophagy is another important biological cellular process that removes unnecessary or dysfunctional cell components in compromising conditions, such as hypoxia, starvation, lack of nutrition or extreme pH values (*Ryter et al., 2013*). Autophagy is known to occur in the endometrium, and growing evidence now points to the key involvement of dysregulation in the progression of endometriosis (*Yang et al., 2019*). However, opposing theories have been proposed on its function, with different studies claiming that autophagy is either up- or down-regulated in individuals with endometriosis (*Zhan et al., 2018*). Since endometriosis and adenomyosis appear to be associated (*Donnez et al., 2019*; *Stratopoulou et al., 2021a*), it would be of great interest to analyse the role of autophagy in adenomyosis.

Matrix metalloproteinases (MMP) are proteolytic enzymes expressed in the endometrium during the menstrual cycle to induce extracellular matrix breakdown, leading to menstruation and ultimately tissue regeneration (*Gaide Chevronnay et al., 2012*; *Marbaix et al., 1996*). MMP9 has been identified as one of the most crucial factors for endometrial function, since its expression markedly increases in stromal, epithelial and inflammatory cells throughout the cycle in response to ovarian steroids (*Cornet et al., 2005*; *Dong et al., 2002*; *Henriet et al., 2016*). The role of these enzymes has long been investigated in endometriosis, but their involvement in adenomyosis has yet to be clarified. Furthermore, the theory of progesterone resistance is now well established in patients suffering from endometriosis (*Donnez and Dolmans, 2021*; *Flores et al., 2018*; *Patel et al., 2017*; *Yilmaz and Bulun, 2019*), encouraging interest in exploring progesterone receptors in adenomyosis, a closely related disease.

With all this in mind, the goal of the present study was to evaluate apoptosis and autophagy patterns in healthy endometrium and compare them with adenomyotic eutopic and ectopic endometrium. An additional aim was to determine whether these phenomena are related to the invasion process and progesterone resistance involved in the pathophysiology of adenomyosis.

MATERIALS AND METHODS

Sample collection and patient selection

Tissue samples were recovered from the Biobank of the Cliniques Universitaires Saint-Luc (CUSL) in the form of formalin-fixed paraffin-embedded tissue blocks. Use of human tissue for this study was approved by the ethics committee of CUSL and the Université Catholique de Louvain on 31 August 2020 (reference 2020/14AOU/410). A retrospective cohort study was conducted based on immunohistochemical analyses.

Histology from 30 participants was investigated in this study after separating the samples into two study groups: adenomyosis ($n = 15$) and control ($n = 15$) groups. Both cohorts were further subdivided into three groups according to the menstrual cycle phase (proliferative, secretory or menstrual) after histological verification and confirmation. Tissue blocks were selected according to strict criteria. In all cases, the women had undergone hysterectomy, which allowed histological examination of the surgical specimens (in collaboration with the Anatomopathology Department under Professor E. Marbaix) to confirm the cycle phase and diagnosis in the case of adenomyotic tissues, and the absence of adenomyosis from all controls.

Individuals in the adenomyosis group were treated by hysterectomy for symptomatic adenomyosis resistant to medical therapy. Menorrhagia was the most prevalent symptom, affecting 73% of these women. They all experienced symptoms of the disease, including menometrorrhagia, dysmenorrhoea or pelvic pain. Imaging was used in these cases to corroborate the diagnosis of the pathology, using either transvaginal ultrasound or magnetic resonance imaging.

The control group was made up of women undergoing hysterectomy for reasons unrelated to endometrium. These included intramural (International Federation of Gynecology and Obstetrics type 4) leiomyomas (nine patients), uterine prolapse (two patients) and adenocarcinoma *in situ* of the cervix (one patient). The remaining three women resorted to surgery because of persistent menometrorrhagia with no sign of uterine pathology.

No participants in either study group were suffering from endometriosis or any other endometrial disease. All were premenopausal and none had received any hormonal therapy (contraceptive pill,

implant or hormonal intrauterine device) for at least three months prior to surgery.

The clinicopathological characteristics of the investigated patient groups are shown in TABLE 1. Patients were comparable in terms of age and parity. Women with adenomyosis had a mean uterine volume of 142.7 cm^3 (range 38.46–633.2 cm^3), significantly larger than the average uterine volume in healthy women, which is about 34–35 cm^3 (range 15–56 cm^3) ($P = 0.0183$) (*Chapron et al., 2017*). Uterine volume was calculated using the formula for the volume of an ellipse: volume (cm^3) = 0.5233 × A × B × C, where A, B and C are the longitudinal, anteroposterior and transverse diameters of the body of the uterus.

Immunohistochemistry was performed on consecutive slides from all 30 patient samples. Eutopic endometrium from adenomyosis patients was always paired with its corresponding ectopic endometrium.

Immunohistochemistry

Tissues were immunolabelled for cleaved caspase 3 as a marker of apoptosis, microtubule-associated proteins 1A/1B light chain 3B (LC3B) as a marker of autophagy, MMP9 as a marker of stromal remodelling for invasion capacity and progesterone receptors for progesterone responsiveness.

Paraffin blocks were cut with a microtome to obtain 5 μm tissue sections, which were mounted onto slides. After deparaffinization and rehydration, endogenous peroxidase was inhibited by incubating the slides in H_2O_2 solution. They were then heated in citrate buffer (pH 6) for 75 min at 98°C to allow antigen retrieval, and cooled at room temperature for 15 min. Next, they were incubated with the primary antibody overnight at 4°C (primary antibody against caspase 3 (1/200; Cell Signaling, Danvers, MA, USA 9661S), LC3B (1/1000; Cell Signaling 3868S), MMP9 (1/200; Sigma, St. Louis, MO, USA IM37) or progesterone receptors (1/500; Dako A0098). For negative controls, tissues were incubated with FLEX rabbit or mouse negative controls (IS 600–IS 750; Dako North America, USA) instead of the primary antibody (Supplementary Figure S1). The next day, the slides were rinsed and incubated with the secondary antibody for 60 min. Chromogen staining was achieved with diaminobenzidine prior to counterstaining with Mayer's haematoxylin. Finally, the sections were routinely dehydrated and mounted, before being digitized using the Pannoramic SCAN II (Sysmex,Terhulpen, Belgium) and magnified × 20. Details on immunohistochemical procedures can be found in Supplementary Table S1.

Visiopharm

Areas of interest stained with diaminobenzidine were analysed with Visiopharm software (Hamamatsu, MontSaint-Guiber, Belgium). The staining index was calculated as follows: % stained area × (255 − mean intensity in the stained area), where 255 represents the maximum intensity for 8-bit images. A total of 20–30 eutopic endometrial glands were manually encircled in each sample and eutopic stroma was delineated. In adenomyosis specimens, lesions were identified and glands and stroma were surrounded using the same technique as for eutopic tissue.

Statistical analysis

Statistical analyses were conducted with GraphPad Prism software (San Diego, CA, USA) v9.2.1. An unpaired t-test was used to compare observations from different groups. Where data did not follow a normal distribution, the Mann–Whitney *U*-test was applied. *P*-values below 0.05 were considered significant.

TABLE 1 CLINICOPATHOLOGICAL CHARACTERISTICS OF PATIENTS INVOLVED IN THE STUDY

Parameter	Control	Adenomyosis	*P*-value
Age (years)			
Mean	43.20	45.20	
SD	5.98	4.23	0.2992
Range	35–55	38–54	
Parity (*n*)			
Mean	2.6	2.7	
SD	0.99	1.5	0.7278
Range	1–4	0–6	
Menstrual phase			
Proliferative	5	5	
Secretory	5	5	
Menstrual	5	5	

$P < 0.05$ was considered statistically significant.

RESULTS

Caspase-induced apoptosis

To assess apoptosis in endometrial tissue, we analysed the expression of caspase 3 in the endometrial glands and stroma (FIGURE 1A). Positive signalling was detected inside the cell nuclei in both compartments (Supplementary Figure S2). Staining was found to be significantly less intense in ectopic adenomyotic stroma than in healthy endometrial stroma ($P = 0.0013$) (FIGURE 1B). Caspase 3 concentrations were also significantly lower in ectopic adenomyotic stroma compared with matched eutopic adenomyotic stroma ($P = 0.0006$) (FIGURE 1B). The caspase 3 staining intensity was significantly weaker in ectopic adenomyotic glands than in healthy endometrial glands ($P = 0.0157$) (FIGURE 1C). Expression levels of caspase 3 during the different phases of the menstrual cycle are shown in Supplementary Figure S3A, B.

LC3B-mediated autophagy

Autophagy was investigated by evaluating LC3B expression in endometrial glands and stroma (FIGURE 2A). This marker was observed to be positive inside the cell nuclei of both epithelial and stromal cells (see Supplementary Figure S2). LC3B immunolabelling was significantly weaker in ectopic adenomyotic stroma compared with the eutopic stroma of matched adenomyosis specimens ($P = 0.0349$) (FIGURE 2B). No significant difference was observed in the glandular expression of LC3B (FIGURE 2C). Expression levels of LC3B during the different phases of the menstrual cycle are shown in Supplementary Figure S3C, D.

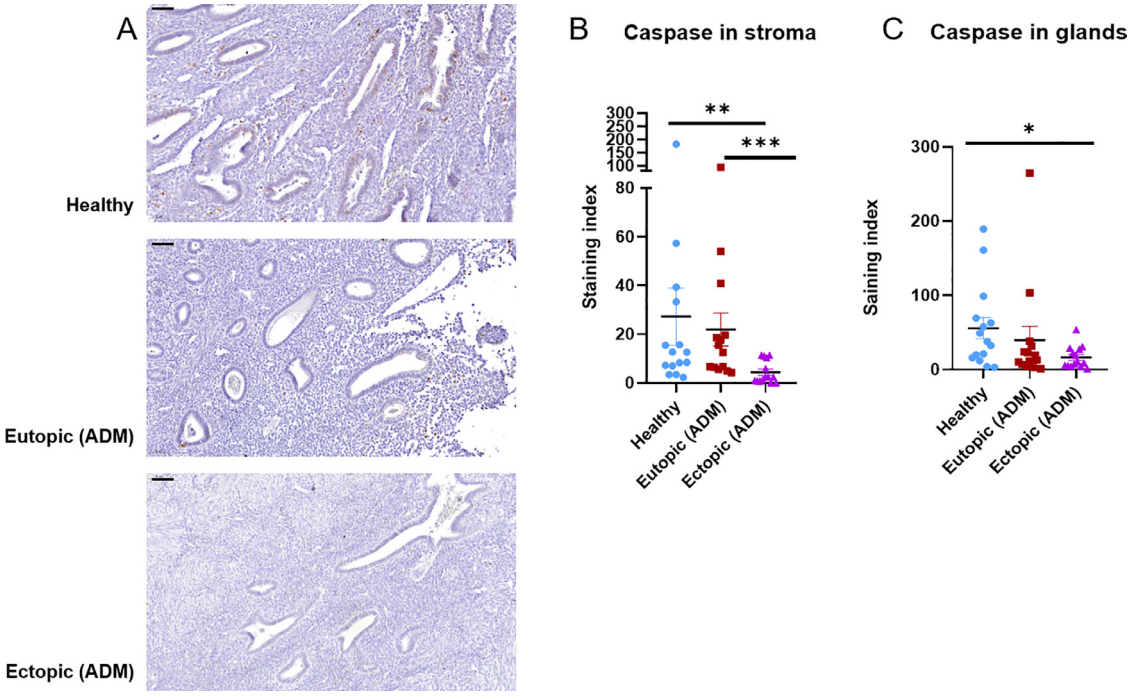


FIGURE 1 Caspase 3 results. (A) Immunoreactivity staining results for caspase 3 in healthy endometrium, eutopic adenomyotic endometrium and matched ectopic adenomyotic endometrium. Phase: menstrual. (B) Staining index of caspase 3 in endometrial stroma. Caspase 3 expression was found to be significantly stronger in healthy and eutopic adenomyotic stroma than in matched ectopic adenomyotic stroma (**P = 0.0013, ***P = 0.0006). Results are presented as mean ± SD. (C) Staining index of caspase 3 in endometrial glands. Caspase 3 expression was found to be significantly stronger in healthy endometrial glands than in ectopic adenomyotic glands (*P = 0.0157). Results are presented as mean ± SD. Scale bar = 50 μm. ADM, adenomyosis.

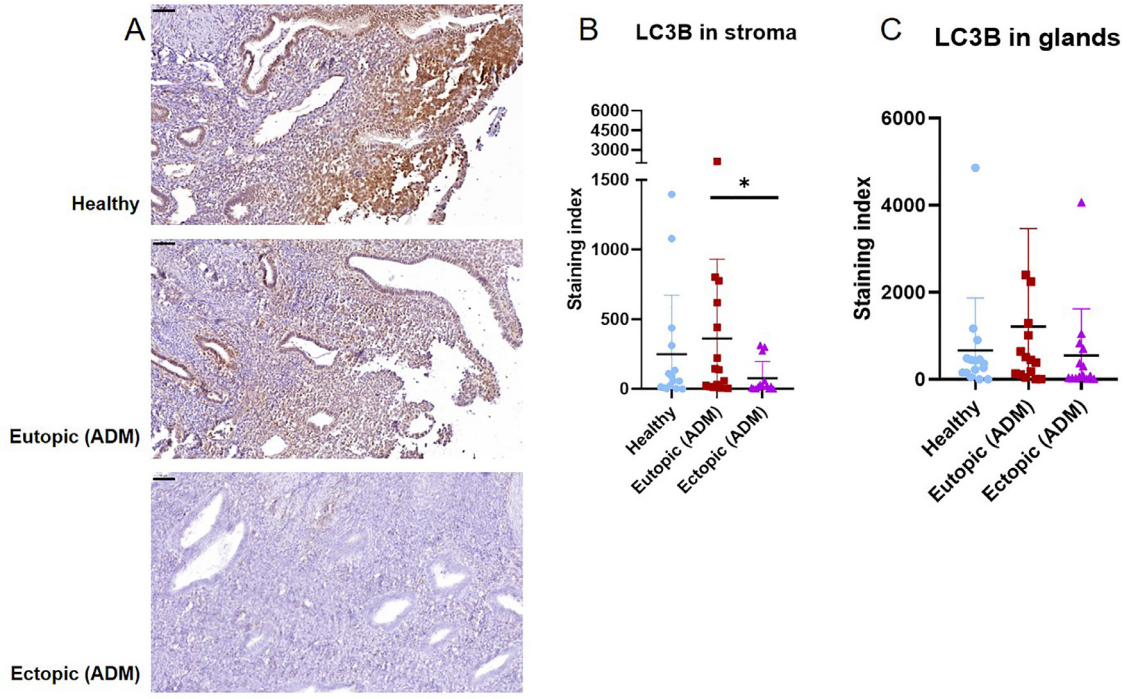


FIGURE 2 Microtubule-associated proteins 1A/1B light chain 3B (LC3B) results. (A) Immunoreactivity staining results for LC3B in healthy endometrium, eutopic adenomyotic endometrium and matched ectopic adenomyotic endometrium. Phase: menstrual. (B) Staining index of LC3B in endometrial stroma. LC3B expression was found to be significantly stronger in eutopic adenomyotic stroma than in matched ectopic stroma (*P = 0.0349). Results are presented as mean ± SD. (C) Staining index of LC3B in endometrial glands. No significant difference was noted. Results are presented as mean ± SD. Scale bar = 50 μm. ADM, adenomyosis.

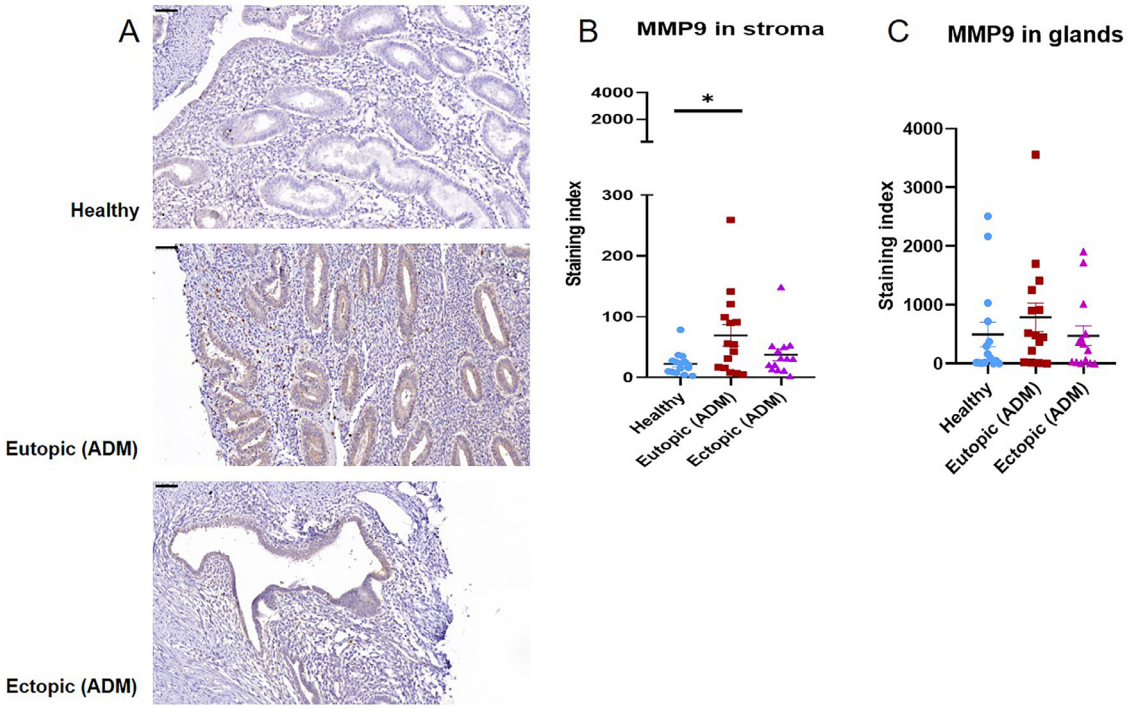


FIGURE 3 Matrix metalloproteinase 9 (MMP9) results. (A) Immunoreactivity staining results for MMP9 in healthy endometrium, eutopic adenomyotic endometrium and matched ectopic adenomyotic endometrium. Phase: secretory. (B) Staining index of MMP9 in endometrial stroma. MMP9 staining was found to be significantly stronger in eutopic adenomyotic stroma compared with healthy endometrial stroma (*P = 0.0295). Results are presented as mean ± SD. (C) Staining index of MMP9 in endometrial glands. No significant difference was noted. Results are presented as mean ± SD. Scale bar = 50 μm. ADM, adenomyosis.

MMP9

MMP9 expression was analysed as a marker of extracellular matrix remodelling and invasion (FIGURE 3A). Antibody positivity was detected primarily in cytoplasmic and sometimes extracellular locations (see Supplementary Figure S1). MMP9 staining was shown to be significantly more intense in eutopic stroma from adenomyosis patients than in healthy endometrium (P = 0.0295) (FIGURE 3B). No significant difference was noted in the glandular expression of MMP9 (FIGURE 3C). Expression levels of MMP9 during the different phases of the menstrual cycle are shown in Supplementary Figure S3E, F).

Progesterone receptors

Progesterone responsiveness was examined by assessing the expression of progesterone receptors (FIGURE 4A). The antibody showed nuclear positivity in both endometrial epithelial and stromal cells, consistent with previous findings on the subcellular localization of this receptor. Progesterone receptor staining intensity was significantly lower in the stroma of eutopic and matched ectopic adenomyotic endometrium than in healthy endometrium (P = 0.0001, P = 0.0021) (FIGURE 4B). No significant difference was encountered in the glandular expression of progesterone receptors (FIGURE 4C). Expression levels of progesterone receptors during the different phases of the menstrual cycle are shown in Supplementary Figure S3G, H.

DISCUSSION

Adenomyosis is an oestrogen-driven gynaecological condition characterized by invasive endometrium implanting within uterine muscle. It has been linked to endometriosis because of the similarity of the symptoms and the pathophysiology of both diseases (*Chapron et al., 2017*; *Donnez et al., 2019*; *Stratopoulou et al., 2021*).

Human endometrium is a dynamic tissue, periodically undergoing shedding and regeneration in response to the local hormone milieu (*Gargett et al., 2012*; *Marbaix et al., 1996*). Apoptosis occurs physiologically in the endometrium when circulating progesterone concentrations drop, triggering a transition from the secretory to the menstrual phase (*Vaskivuo et al., 2000*). This process is essential for endometrial regeneration and for maintaining its function (*Gargett et al., 2012*; *Vaskivuo et al., 2000*), while the dysregulation of apoptotic mechanisms has been found to contribute to the development of endometriosis by inhibiting physiological cell death and promoting aberrant cell survival and invasion (*Nasu et al., 2009*; *Nasu et al., 2011*).

By comparing caspase 3-induced apoptosis and the LC3B pathway in healthy and adenomyotic endometrium, this study investigated the possibility of abnormal cell survival in adenomyosis. Regarding caspase 3, signalling was detected inside the cell nuclei, where cleaved caspase 3 physiologically translocates to activate the apoptotic cascade, as previously reported (Supplementary Figure S2) (*Kamada et al., 2005*). The results demonstrated that ectopic adenomyotic lesions appear to resist apoptosis, expressing lower amounts of caspase 3 in both ectopic stromal and epithelial cells than in eutopic endometrium. This suggests that resistance to apoptosis allows these invasive cells to persist in ectopic locations.

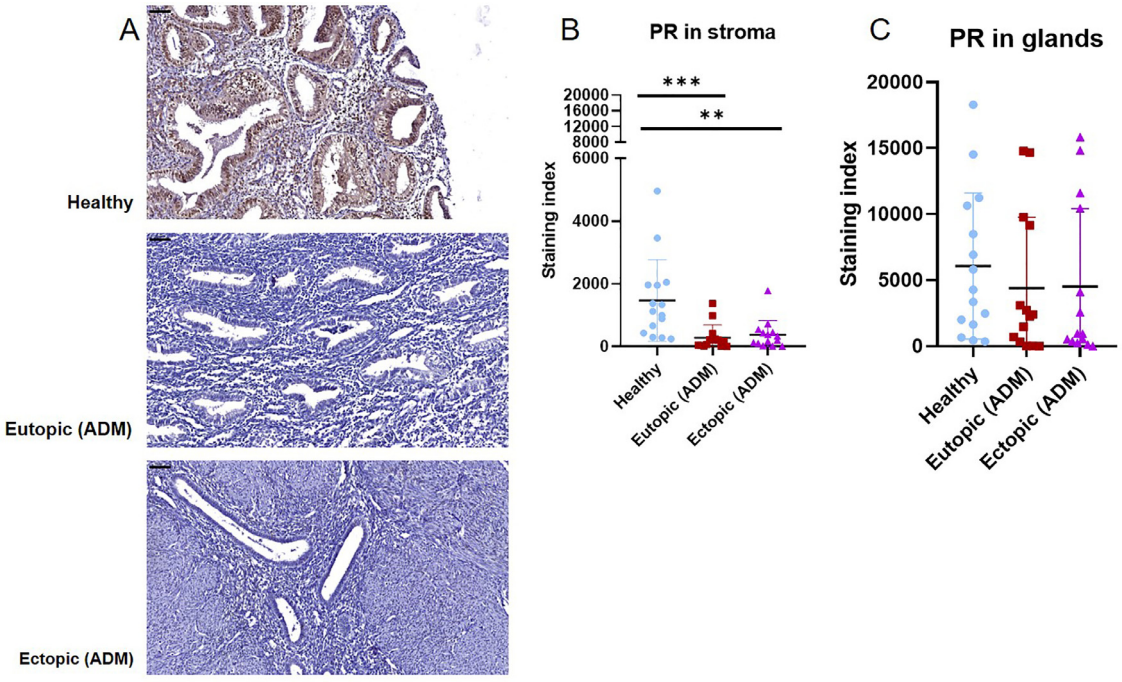


FIGURE 4 Progesterone receptor results. (A) Immunoreactivity staining results for progesterone receptors in healthy endometrium, eutopic adenomyotic endometrium and matched ectopic adenomyotic endometrium. Phase: secretory. (B) Staining index of progesterone receptors in endometrial stroma. Progesterone receptor expression was found to be significantly stronger in healthy endometrial stroma than in eutopic and matched ectopic adenomyotic stroma (***P = 0.0001, **P = 0.0021). Results are presented as mean ± SD. (C) Staining index of progesterone receptor in endometrial glands. No significant difference was noted. Results are presented as mean ± SD. Scale bar = 50 μm. ADM, adenomyosis; PR, progesterone receptor.

Studies have revealed that autophagy plays an essential role in physiological and pathophysiological processes related to the endometrium, such as cyclic menstruation, decidualization or reconstitution during normal pregnancy, and endometrium-associated diseases and disorders, such as endometriosis, endometrial carcinoma and infertility (*Yang et al., 2019*). In peritoneal endometriotic lesions, however, levels of autophagy are lower than in normal endometrium (*Choi et al., 2014*; *Honda et al., 2008*). Indeed, this study demonstrated that adenomyotic lesions undergo fewer autophagic processes than eutopic endometrium, as stromal LC3B expression was significantly diminished in ectopic endometrium, which may lead to ectopic survival and implantation of lesions. Ren and colleagues (*Ren et al., 2010*) also found a decreased expression of autophagy-related genes in eutopic endometrium from adenomyosis patients compared with control participants, but there were no investigations into ectopic tissue.

The current study reveals abnormal autophagy signalling in ectopic sites. Surprisingly, the majority of staining was observed inside the cell nuclei of both epithelial and stromal cells, inconsistent with previous reports of autophagosome localization inside the cytoplasm during autophagy induction (Supplementary Figure S2). Indeed, more recent research confirms the presence of LC3B in cell nuclei as well and suggests a key role for this phenomenon in activating autophagy (*Baeken et al., 2020*; *Huang et al., 2015*).

Collectively, the current results point to resistance to cellular autophagy and death in ectopic adenomyotic sites, allowing adenomyotic cells to survive and proliferate in a disorganized way, deviating from normal patterns of the menstrual cycle. As no significant difference was found between healthy and eutopic adenomyotic endometrium in terms of apoptosis and autophagy patterns, the authors believe that the ectopic implantation of endometrium into the myometrium cannot be ascribed to the aberrant cell survival of eutopic endometrium, but may require other factors such as local inflammation and macrophage infiltration. Indeed, this group previously found an accumulation of macrophages in adenomyosis, where their presence was accompanied by an enhanced capacity to rearrange the extracellular matrix and subsequent cell metastatic activity (*Stratopoulou et al., 2021*; *Stratopoulou et al., 2023*).

Similarly, this study detected higher MMP9 concentrations in eutopic endometrium from adenomyosis patients, which would allow adenomyotic tissue to remodel the stroma and invade surrounding uterine muscle, enabling lesions to establish themselves in ectopic zones. Consistent with earlier studies in endometrium, MMP9 was observed primarily in cytoplasmic and sometimes extracellular locations, and even inside vessels (Supplementary Figure S2) (*Jeziorska et al., 1996*). Other studies have previously reported elevated MMP9 activity in the endometrium of both adenomyotic and endometriotic tissues, where this enzyme is thought to contribute to the metastasis of lesions and ensuing angiogenesis (*Barbe et al., 2020*; *Li et al., 2006*; Zhai et al., 2020; *Zhai et al., 2022*). MMP9 has also been described in the context of deep nodular endometriosis, where it is believed to participate in collective cell migration during disease development and progression (*Garcia-Solares et al., 2018*b; *Zhai et al., 2022*).

Progesterone signalling is vital to physiological endometrial function, as its up-regulation during the secretory phase leads to proliferation arrest, differentiation of cells to a receptive phenotype, and eventually activation of the apoptotic mechanism with a view to menstruation (*Vaskivuo et al., 2000*). Progesterone resistance has long been demonstrated in deep endometriosis, a disease closely associated with adenomyosis (*Donnez and Dolmans, 2021*; *Nisolle and Donnez, 1997*). Both eutopic and ectopic adenomyotic sites showed decreased progesterone receptor expression, strongly suggesting that progesterone resistance is involved in this disease too. Given the multifaceted role of progesterone in the endometrium, it is likely that inadequate progesterone signalling in adenomyosis may not only impair endometrial receptivity, but also block apoptosis and promote overproliferation, thereby facilitating disease progression.

Interestingly, MMP9 expression has been shown to be down-regulated by progesterone (*Bulun et al., 2006*; *Marbaix et al., 1992*; *Singh et al., 2013*), so progesterone resistance itself may also be responsible for endometrial invasiveness in adenomyosis. Although the exact mechanisms underlying progesterone resistance in adenomyosis need to be further elucidated, it may well explain why a high proportion of patients do not respond to progestogen therapy, highlighting the need for alternative treatment options (*Donnez and Dolmans, 2021*; *Flores et al., 2018*; *Stratopoulou et al., 2021*b).

The present study has both strengths and limitations. Its strong points include the careful selection of patients in both groups, with women who were of similar age and gravidity status and free of hormone treatments affecting their menstrual cycle. Only patients who had undergone hysterectomy were included in the control group, allowing the presence of adenomyosis to be excluded at the histological level too. All phases of the menstrual cycle were represented equally (five samples per phase in both groups) to avoid potential discrepancies in the examined parameters due to menstrual status, thereby adding value to the findings.

In terms of drawbacks, the study is somewhat limited by its retrospective nature, preventing an evaluation of the impact of the investigated mechanisms during the course of the initiation and development of the disease. It should be pointed out, however, that adenomyosis is actually a very slow-moving condition and it is almost impossible to observe its progression in real time, let alone determine when the disease began. Unfortunately, typical mouse models of the disease remain insufficiently characterized. While a number of hormone-disrupting procedures have been reported to promote an adenomyosis-related phenotype, strain specificity and lack of consistency hamper an understanding of disease pathogenesis, calling for more studies to validate these models (*Marquardt et al., 2020*).

To overcome these limitations, the group's current research focuses on implementing primary endometrial organoids as an advanced in-vitro model that more accurately mimics the aetiopathogenesis of the disease. Using this approach, the group aims to dig deeper into the mechanisms of impaired apoptosis and autophagy in adenomyosis and elucidate their underlying causes.

CONCLUSION

The present study revealed that the development of adenomyosis depends on a number of dysregulated mechanisms, including resistance to physiological cell death, aberrant extracellular matrix-degrading ability and inadequate responsiveness to progesterone. The study showed that both apoptosis and autophagy are down-regulated in adenomyotic lesions, potentially contributing to disease progression by ensuring cell survival and invasion. MMP9 was found to be increased in eutopic endometrium from adenomyosis patients and is therefore likely to promote invasion of the myometrium during disease initiation. All in all, progesterone resistance appears to be a key component of the pathogenesis of adenomyosis, inhibiting cell death and promoting lesion invasion.

DATA AVAILABILITY

Data will be made available on request.

ACKNOWLEDGEMENTS

This research has been conducted using specimens from the CUSL Biobank collection (Belgian accreditation BB190044). The authors would also like to thank Mira Hryniuk, BA, for reviewing the English language of the manuscript, and Maria Dolores Gonzalez, Sarah Storder and Olivier Van Kerk for their technical assistance.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2023.06.003.

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