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Review

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Real world perspectives on endometriosis disease phenotyping through surgery, omics, health data, and artificial intelligence

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Endometriosis is an enigmatic disease whose diagnosis and management are being transformed through innovative surgical, molecular, and computational technologies. Integrating single-cell and other omic disease data with clinical and surgical metadata can identify multiple disease subtypes with translation to novel diagnostics and therapeutics. Herein, we present real-world perspectives on endometriosis and the importance of multidisciplinary collaboration in informing molecular, epidemiologic, and cell-specific data in the clinical and surgical contexts.

Endometriosis is an ancient disease with ancient treatments[1] and is poised to benefit from modern technologies and continued innovation for accurate diagnosis, disease classification, and novel medical and surgical therapies[2,3]. It is a chronic, systemic, estrogen-driven disorder wherein disease lesions comprising tissue similar to the uterine lining (endometrium) develop outside the uterus—mainly in the pelvis and less commonly at extra-pelvic sites as the umbilicus, vagina, thoracic cavity, and gastrointestinal (GI) and genitourinary (GU) systems[4,5]. These ectopic lesions invade resident structures and elicit inflammation, neo-neuroangiogenesis, fibrosis, pain, and organ dysfunction[6–8]. Endometriosis affects 10–15% of reproductive age persons with a uterus, 50% of patients with chronic pelvic pain, and 30–90% with subfertility/infertility and is associated with multi-system comorbidities including irritable bowel syndrome, migraine headaches, depression, and inflammatory disorders[2,9–12]. Central to its pathophysiology are enhanced estrogen and diminished progesterone signaling, inflammation in the eutopic endometrium, disease in the pelvis, and systemically, and fibrosis in ectopic sites[11–15].

Several theories exist to explain its pathogenesis[16–22], and it is likely that different mechanisms occur in different patients. For example, dissemination of endometrial fragments and cells shed retrograde into the pelvis during menses ("retrograde menstruation")[23–26] is supported by recent evidence wherein somatic mutations in eutopic (within the uterus) endometrial epithelium are shared with endometriosis lesions[19,27–29]. However, as 98% of persons with a uterus have retrograde menstruation[30] and only 10% have endometriosis, other mechanisms are likely operational. Other theories include benign metastasis of endometrial cells via uterine lymphatic drainage and vascular dissemination[24], coelomic metaplasia of abdominal viscera and peritoneum mesothelium[31], embryonic rests and endometrial and bone marrow-derived stem cell transformation[32–34], iatrogenic introduction of endometrium into tissues and/or surgical sites, dysfunctional immune clearance of ectopic tissue[11], and genetic, epigenetic, infectious, and environmental influences[35–41].

The gold standard to diagnose endometriosis is *surgical identification* of lesions and suspected lesions and histologic confirmation of endometrial-like cells in lesion types. While clinical symptoms overlap with some other gynecologic disorders, imaging techniques, and AI-designed applications are increasingly complementing diagnostic evaluation[2,42–45]. The long latency (on average 7–11 years) from symptom onset to surgical diagnosis is mainly due to the absence of non-invasive diagnostic biomarkers and can result in disease and symptom progression, not uncommonly leading to

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irreversible organ damage as loss of kidney function, undertreatment, social isolation, and depression among affected individuals[45–49].

Medical therapies for pain include reducing inflammation and estrogen action and/or synthesis, but these approaches have intolerable side effects for many patients, individual responses to specific medications are unpredictable, and 25–34% of patients exhibit no or poor response to these medical treatments[48–51]. Surgery is largely successful for pain, infertility, and subfertility management, although ~50% of patients have pain symptoms return within 2–5 years, requiring additional therapies[48–52], and surgical outcomes are highly surgeon- and patient-dependent[53]. For infertile patients with endometriosis, medical-assisted reproductive approaches and/or surgery benefit most, although treatment responses are unpredictable[48], and those with advanced versus early-stage disease[54] have higher risk of poor pregnancy outcomes[55]. Overall, apart from surgical management following the revolution and evolution of minimally invasive and robotic surgery in the past 3–4 decades, there have not been major paradigm shifts in endometriosis care, including diagnosis, medical therapies, prevention, and long-term management[2,3].

Thus, current unmet clinical needs comprise developing (1) non-invasive biomarkers to diagnose and stratify disease, (2) novel and personalized medical and surgical therapies targeted to individual lesion and lesion-niche interactions and pathophysiology, (3) prognostic outcome indicators for responses to therapies, risk of disease and symptom recurrence, and (4) ultimately, cure. Rapidly evolving single-cell and other advanced technologies are providing insights into endometriosis pathogenesis, pathophysiology, heterogeneity, and behavior, and can contribute significantly to fulfill these unmet needs. This manuscript focuses on endometriosis lesions and the centrality of the clinical context provided by surgeons, pathologists, gynecologists, and molecular and computational scientists to endometriosis lesion single-cell data generation, analysis, and interpretation. We focus on real-world perspectives of complexities of endometriosis diagnosis, lesion heterogeneity histologically, architecturally, and molecularly, and the importance of these to single-cell data analysis and integrative analyses of multi-omic datasets. With heterogeneity of lesion morphology and location, a more nuanced perspective of surgical assessment of lesions can provide insights into more detailed analysis of data derived from resected tissues. Other considerations for data interpretation, rigor, and reproducibility include disease symptoms (pain/infertility/both/neither), defining control groups, recruited subject clinical metadata including comorbidities, standard operating procedures for biospecimen processing, and designing computational pipelines for data analyses, subanalyses, and surgical/pathology findings. We conclude with an eye to the future tapping AI and digital technologies to further advance the field. Information conveyed herein is based on peer-reviewed scientific publications, systematic reviews, meta-analyses, commentaries, and professional society statements from 1991 to 2024.

Endometriosis staging

Classification systems of endometriosis are based on surgical observations and/or fertility outcomes. These include the revised American Society for Reproductive Medicine (rASRM) classification[54], the ENZIAN classification[56], the American Association of Gynecological Laparoscopists (AAGL) classification[57], and the endometriosis fertility index (EFI)[58]. The most well-known is the rASRM classification wherein numerical scores for extent of disease and adhesions stratify disease stages from minimum (stage I) to severe (stage IV). The ENZIAN classification was developed to classify deep infiltrating endometriosis and focuses on retroperitoneal structures. Neither system predicts treatment outcomes nor correlates with severity of pain symptoms. The AAGL classification reports scoring intraoperative surgical complexity and correlations with patient-reported pain symptoms or infertility[57]. The EFI is the only system to predict non-IVF fertility outcomes in patients who underwent surgery for endometriosis[58]. The World Endometriosis Society (WES) 2017 consensus statement[59] recommends using a "classification toolbox" that includes the rASRM system and the ENZIAN system for deep disease to improve disease classification. However, there is no classification system that is consistently used across the globe, and different systems are adopted according to clinical and surgical goals[60,61].

In contrast to the above, we developed a unique classification system[62] that is more descriptive of disease appearance, location, and adhesions, and includes concomitant adenomyosis which is similar in cell composition and somatic epithelial mutations with endometriosis but located in the uterine myometrium[63,64]. Our classification (Fig. 1) describes reproductive organ ("*genital*") (Fig. 1a) and non-reproductive organ ("*extragenital*") (Fig. 1b) endometriosis. Genital endometriosis affects various parts of the reproductive system, including the uterus, upper cervix, vaginal fornix, fallopian tubes, and ovaries, and is classified into four stages: minimal (stage I), mild (stage II), moderate (stage III), and severe (stage IV) (Fig. 1a). Minimal or stage I disease is characterized by <5 spots with <5 mm penetration, minimal to no signs of adenomyosis, and no significant adhesions. Mild or stage II is characterized by 5–11 spots, also with <5 mm penetration, and minimal to mild adenomyosis. Moderate or stage III disease is defined as >11 spots of endometriosis with <5 mm penetration or endometrioma(s) <2 cm on a single ovary. There may be filmy adhesions between the tubes, ovaries, pelvic sidewall, and the anterior and posterior cul-de-sacs. Adenomyosis in this stage ranges from minimal to moderate. Severe or stage IV disease is depicted with genital endometriosis involving any number of lesions with >5 mm penetration (deep infiltration), including unilateral or bilateral endometriomas of any size, with or without rupture. There may be thick adhesions between the tubes, ovaries, the pelvic sidewall, and the anterior and posterior cul-de-sacs. In this stage, adenomyosis can range from minimal to severe. Additionally, adenomyosis alone can be categorized into 4 stages mentioned above without endometriosis noted,

Extragenital endometriosis (Fig. 1b) affects areas outside the reproductive organs, and is classified into four stages: minimal (stage I), mild (stage II), moderate (stage III), and severe (stage IV) and can be further classified as pelvic and extra pelvic[62,65]. Pelvic extragenital lesions are most commonly found on uterosacral ligaments and less commonly on the rectum, rectovaginal septum, ureters, and/or bladder[66]. Extra-pelvic structures include the gastrointestinal system[67], lung and diaphragm[5], liver[68], genitourinary system[4], abdominal wall, thoracic cavity, and/or nervous system[5,62,65]. The most common site of extragenital disease is bowel endometriosis, occurring in 7–37% of individuals with endometriosis[67,69,70]. The wide range in prevalence can be explained by inconsistent definition of bowel endometriosis. The latter primarily impacts the rectosigmoid colon, followed by involvement of the appendix, cecum, ileocecal valve, small bowel, and rarely the transverse colon[62,67,69–71]. Lesions can range from superficial serosal disease to deeply infiltrative disease invading into the muscularis or mucosa. Lower urinary tract endometriosis is less frequently found than bowel disease, followed by ureteral involvement. These lesions can appear as superficial peritoneal implants and do not affect the muscularis or mucosa. Via theories mentioned above, extragenital endometriosis can exist widely throughout the body.

Fibrosis is an important component in the *progression* of endometriosis[12,15] and is further addressed below. Notably, different locations and niche environments of reproductive organ ("genital") and non-reproductive organ ("extragenital") lesions may contribute to altered pathophysiology of these distinct disease types, which is yet to be explored.

Endometriosis lesions

Endometriosis lesions are broadly categorized into 3 groups: superficial peritoneal endometriosis (SPE), ovarian endometriomas (OMA), and deep infiltrating endometriosis (DIE)[6,11,72–74] (Figs. 2 and 3). They reside in different anatomic locations, display diverse structural architectures, variable steroid hormone responsiveness and invasive properties, and are highly heterogeneous in extracellular matrix (ECM) composition, abundance of endometrial-like epithelial cells, stromal mesenchymal cells (fibroblasts, stem cells), immune cells, vascular endothelium/smooth muscle/other mural cells, and extent of fibrosis. Despite their heterogeneity, there is evidence that all three lesion types derive from eutopic endometrium by retrograde menstruation and oligoclonal expansion, as several studies have

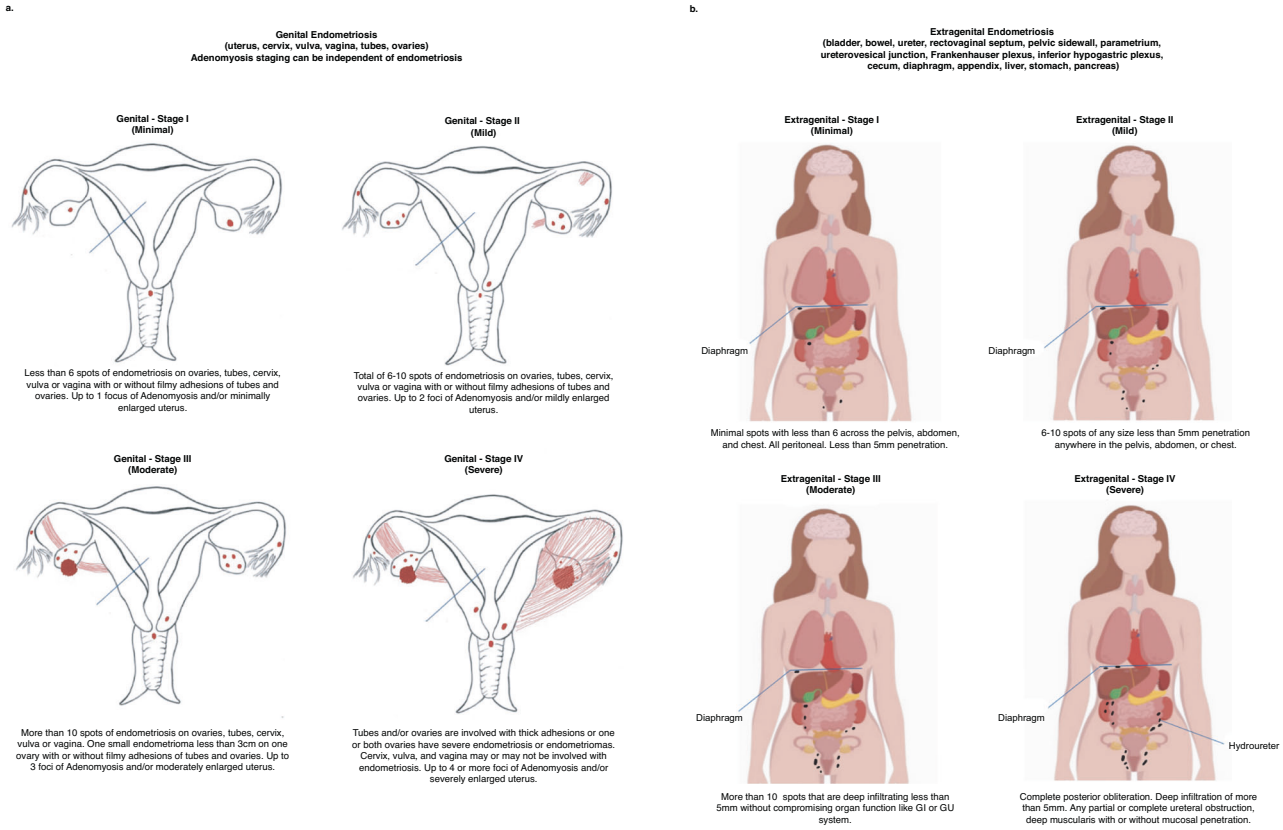

Fig. 1 | Graphic representation of "genital" and "extragenital" endometriosis. **a** Genital endometriosis and its subtypes (minimum, mild, moderate, and severe). Depiction of genital endometriosis and its stages used the image of the female reproductive tract from: Cream Cake Diva/getdrawings.com (https://getdrawings.com/get-drawing#external-drawing-21.jpg), adapted and used under the Creative Commons License CC BY-NC 4.0. **b** Extra genital endometriosis (pelvic and extrapelvic) and subtypes (minimum, mild, moderate, severe). Depiction of extra-genital endometriosis adapted the image used under the Shutterstock Standard License [Andrii Bezvershenko/Shutterstock.com (Stock Vector ID: 109207899).

shown that they share epithelial-specific somatic mutations with the intracavitary eutopic tissue[19,27–29]. Recently, abundance of *KRAS* mutations was found to correlate with lesion type: higher in patients with only DIE or only endometriomas (57.9%) and with mixed subtypes (60.6%) versus SPE (35.1% ($P = 0.04$))[75]. Moreover, greater mutational frequency was observed in rASRM stages II, III, and IV compared to stage I[75]. Although these data are highly supportive of eutopic endometrium as the source of pelvic disease, endometriosis outside the pelvis likely derives from hematogenous or lymphatic spread of endometrial tissue and cells and inside and outside the pelvis by stem and somatic cell trans-differentiation[24,31–34]. Overall, lesion types and subtypes and their cellular components and epithelial somatic mutations, coupled with surgical observations and clinical metadata are central to informing disease stratification and eventually identifying personalized and disease-specific therapeutic targets and prognostics for treatment responses and recurrence risk[10,73,76]. Single-cell technologies offer promise to contribute significantly to achieving these goals, which would be a huge step forward in the field. Herein, we focus on endometriosis lesions and lessons learned from recent single-cell studies, challenges in obtaining such data, and some real-world solutions.

Superficial peritoneal endometriosis (SPE)

Superficial disease is defined as lesions invading <5 mm into serosal structures[77] and is commonly characterized by morphology, pigmentation (red, black, brown, yellow, white, clear), histology, and other features[72,77–87] (Fig. 2a–c). Endometriotic lesions are considered histologically "active" when glandular epithelium is proliferative or unresponsive to hormones with typical endometrial stromal fibroblasts. Endometrial glands, stroma, estrogen receptor-α (ERα) and progesterone receptor (PR)-A/B expression, and collagen fibers increase from colorless through red to blue-black lesions[88]. Red lesions are most highly vascularized and have higher VEGF expression and highest proliferative activity versus white lesions[88], which appear as opacifications, sub-ovarian adhesions, yellow-brown patches, or circular peritoneal defects[89–91]. The black/brown or "gun-powder lesion" is a result of blood and hemosiderin deposits as well as glands, stroma, scar and other debris[91]. One report focused on a narrowly defined patient population and found a large overlap in proliferative activity and ER and PR expression across lesion color types, although differences were observed in lesion morphology, gland pattern, gland content, and adjacent stromal "reaction" of increased abundance of collagen and elastic fibers and smooth muscle bundles from red to brown to black to white lesions[87]. Based on these observations, red lesions are considered as fresh implants with progression to black/brown, yellow, and inactive white lesions[72,77–87]. Atypical lesions include peritoneal defects, flame-like lesions, peritoneal petechiae, glandular lesions, and highly vascularized lesions[89,91,92]. In some cases, the only lesions observed at laparoscopy are those that are more physiologically active yet less obvious and are best described by Nezhat et al. in 1991[81], Donnez et al.[90,93], and Nisolle et al.[88]. In a pilot study of patients with endometriosis and pelvic pain, Lessey et al.[94] showed that microscopic disease, not readily apparent during surgery, could be detected in areas of methylene blue dye staining of the peritoneum after excision and scanning electron microscopy (SEM)[94]. Such "occult" lesions have had limited characterization at the molecular level, and this technique has not been widely adopted likely due to use of SEM in the workflow. Nonetheless, the presence of occult disease may contribute to persistent pain after medical and surgical therapies and disease "recurrence" after surgery and preliminary analysis suggests they may be detected by scRNA seq of seemingly unaffected peritoneum (see below).

SPE demonstrates heterogeneity of cellular steroid hormone receptors expression and niche fibrosis biomarkers[92]. A recent immunofluorescent

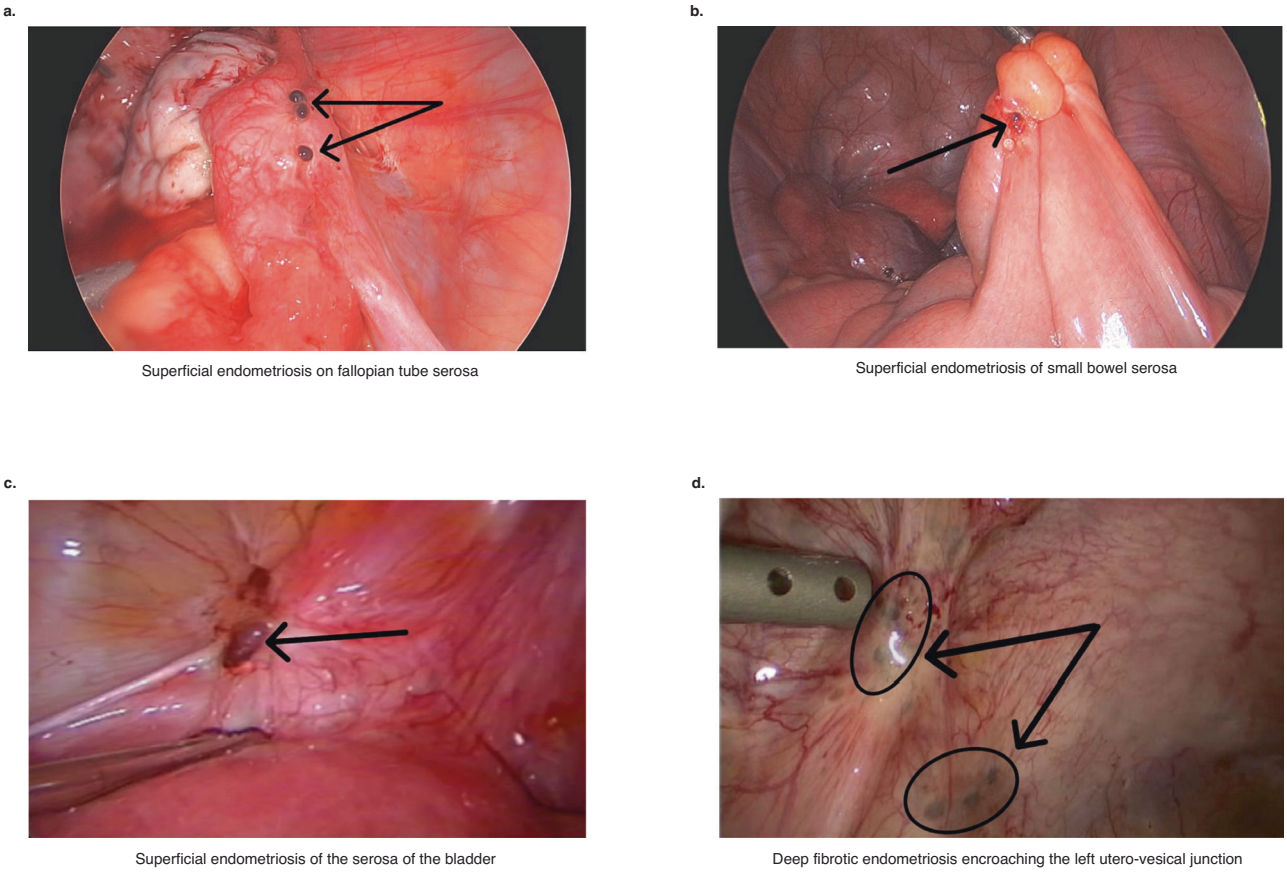

Fig. 2 | Examples of superficial endometriosis and deep infiltrating endometriosis identified intraoperatively. a Superficial endometriosis on fallopian tube serosa. **b** Superficial endometriosis of small bowel serosa. **c** Superficial endometriosis of the serosa of the bladder. **d** Deep fibrotic endometriosis encroaching the left utero-vesical junction. Arrows point to the lesions.

study of 271 superficial peritoneal lesions with ≧1 endometrial epithelial gland and contiguous CD10+ stromal fibroblasts from 64 patients across the menstrual cycle revealed extensive heterogeneity of ERα and PR-A/B expression in these cell types and variable degrees of co-localization, even in the same patient[95]. Highest ERα and PR-A/B expression was in epithelium and stroma, respectively, during the menstrual versus other cycle phases[95]. These data underscore the importance of integrating ER and PR analyses into single-cell data analyses—especially in multiple cellular subtypes identified (also see below). Moreover, they underscore the challenges of medical therapies with e.g., progestins, and the promise of cell-specific targets which may differ lesion to lesion, leading to combined therapies for pain and/or infertility.

Fibrosis biomarkers—alpha smooth muscle actin (αSMA)-positive myofibroblasts, smooth muscle bundles, and Type I collagen—have been consistently described over the past three decades as prominent features of superficial peritoneal lesions[12,96–102], and subsequently there has been a call to redefine endometriosis to include its pro-fibrotic nature[12]. A recent study found low numbers of αSMA, type I collagen fibers, and CD45+ leukocytes, and CD68+ macrophages in SPE, in contrast to the *adjacent* tissue microenvironment with significantly more SMA and Type I collagen, depending on lesion location and gland morphology[103].

Taken together, these data underscore the importance of lesion evolution, location, and niche environment, especially relevant to interpreting cell-specific signaling pathways, cell–cell communications and mediators thereof, classifying disease types and subtypes, and identifying and developing targets for therapeutics, diagnostics, and potentially for prognostic efficacy of responses to mono- or combined medical therapies. AI technology lends itself well here for pattern recognition, stage of disease, and standardization of care (see below).

Ovarian endometriomas

Ovarian endometriomas can be classified into Types I and II (Fig. 3). Type I endometriomas (Fig. 3a) develop from endometrial tissue that has implanted on the surface of the ovary. The ovarian cortex invaginates and blood accumulation results in growth of the cyst[104–109]. Type I endometriomas tend to be <5 cm and are difficult to remove due to their fibrous capsule that is densely adhered to the surrounding ovarian stroma[106,110]. On the other hand, Type II (Fig. 3b–d) endometriomas originate from endometrial implants entering existing functional cysts. They can be further categorized into Type IIA, IIB and IIC based on depth of invasion (<10%, 10–50%, and >50%, respectively)[104–108,110–112].

Ovarian endometrioma cyst walls are primarily composed of fibrotic tissue especially in almost all endometriosis Type I and Type IIC and to a lesser degree Type IIA and Type IIB, with αSMA immunostaining confirming the presence of myofibroblasts[12,104–108,111]. In Type IIA and Type IIB endometriomas, the level of fibrosis correlates with invasion of endometrial stroma into the functional cysts. While fibrosis is consistently observed in ovarian endometriomas, histological examination reveals higher fibrotic content in deep infiltrating endometriotic lesions[113]. Platelet activation in ovarian endometriomas may induce fibrosis via TGF-β1 release and smad signaling pathway activation in endometriosis cells, which can be reversed with TGF-β1 blockade[12,114]. Fibrosis can extend into the ovarian cortex surrounding endometriomas and is associated with more atretic early follicles and lower ovarian follicle density compared to nonaffected ovaries[115].

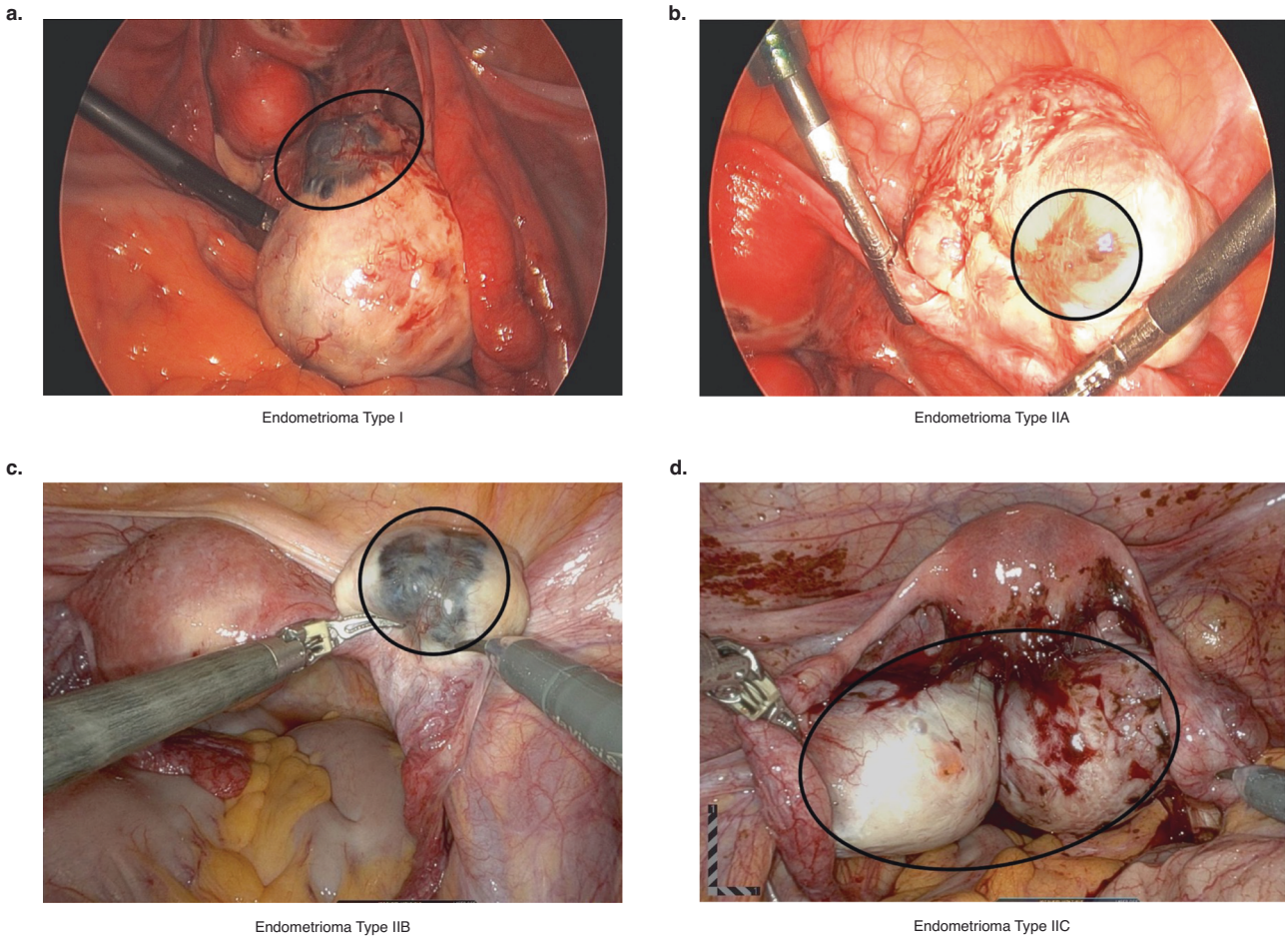

Fig. 3 | Photographs of ovarian endometriomas identified intraoperatively. a Type I endometrioma. **b** Type IIA endometrioma. **c** Type IIB endometrioma. **d** Type IIC endometrioma. Black circles around the endometriomas have been drawn on the photographs to highlight the extent of the endometriomas.

A recent study demonstrated that the extent of endometrioma fibrosis positively correlated with dysmenorrhea severity but did not impact levels of anti-Mullerian hormone (AMH), a marker of ovarian reserve; rather ovarian cortical fibrosis negatively correlated with serum AMH levels, supporting cortical fibrosis as a key driver of endometrioma-related impairment of the ovarian reserve[116]. Notably, intense inflammation in ovarian endometriomas[117] can result in fibrosis and affect ovarian follicle function[6,12]. In a recent study, it was further confirmed that the presence of ovarian endometrioma(s) indicates a higher stage of disease correlating mainly with stage IV[118].

Principal component analysis of microarray bulk gene expression data from endometriomas, SPE, and DIE[119], revealed non-cycle-dependent gene expression profiles, and that endometriomas were significantly differentiated from the other two lesion subtypes and uniquely displayed ERβ (*ESR2*)-altered gene expression profiles after estrogen suppression versus non-treated controls. Overall, these data importantly suggest the role of the niche in lesion features and possible disease modifying targets for further therapeutic development. Single-cell data can further refine these targets and may reveal cellular and pathway heterogeneity supporting expanded multi-drug targets for disease management.

Deep infiltrating endometriosis (DIE)

DIE is defined as endometriosis infiltrating >5 mm into the peritoneum and serosa covering pelvic/abdominal organs[120,121] (Fig. 2d). DIE can appear as nodularity of the rectosigmoid colon or rectovaginal septum, uterosacral ligaments, vaginal fornix and/or the bladder peritoneum[122–126]. It has been suggested that DIE can be classified into three types: (I) cone-shaped lesions that leave the pelvic anatomy intact, (II) adhesions covering lesions grossly disturbing pelvic anatomy, and (III) largely intact pelvic anatomy with the largest area of lesion beneath the surface[120]. Adenomyosis externa, which is characterized as endometrial tissue growing into the myometrium and extending outwards to surrounding pelvic tissue, can be included in type III DIE. Thus, despite similarities between DIE and adenomyosis externa, they are distinct entities[126]. Single-cell technologies are anticipated to inform the relationship between these two entities.

About 68% of deep infiltrating disease has been reported to be "active" versus 25–50% for lesions with <5 mm invasion, and 74% versus 38–57% were "in phase", respectively, with the eutopic endometrium[127]. These data suggest more steroid hormone responsiveness in deep versus superficial disease. Undifferentiated and mixed gland patterns were found mainly in DIE, often with scant stroma, similar to endometriomas[92]. These features contrast with SPE which contains mostly well-differentiated gland patterns that are highly responsive to medical suppressive therapies[92]. We and others have found examples of fibrosis in deep infiltrating disease, where endometrial epithelium is present deep in fibromuscular tissue without the classic stromal surroundings[81,93]. DIE involves glandular epithelium within fibromuscular tissue, with the latter potentially originating from trans-differentiation of endometrial stromal cells rather than pre-existing muscle tissue, suggesting that the local tissue environment may react to ectopic endometrium, inducing fibrosis[12,93,96,128–130]. Present evidence suggests fibrosis may be a self-propagating event, indicating that although not always the case for all lesions, fibrosis can represent end-stage endometriosis[12]. Notably, a recent immunohistochemical study revealed higher expression of markers of fibroblast-to-myofibroblast trans-differentiation, smooth muscle metaplasia, fibrosis, epithelial-to-mesenchymal transition (EMT), epigenetic modifications, and ERβ, and less vascularity in DIE versus

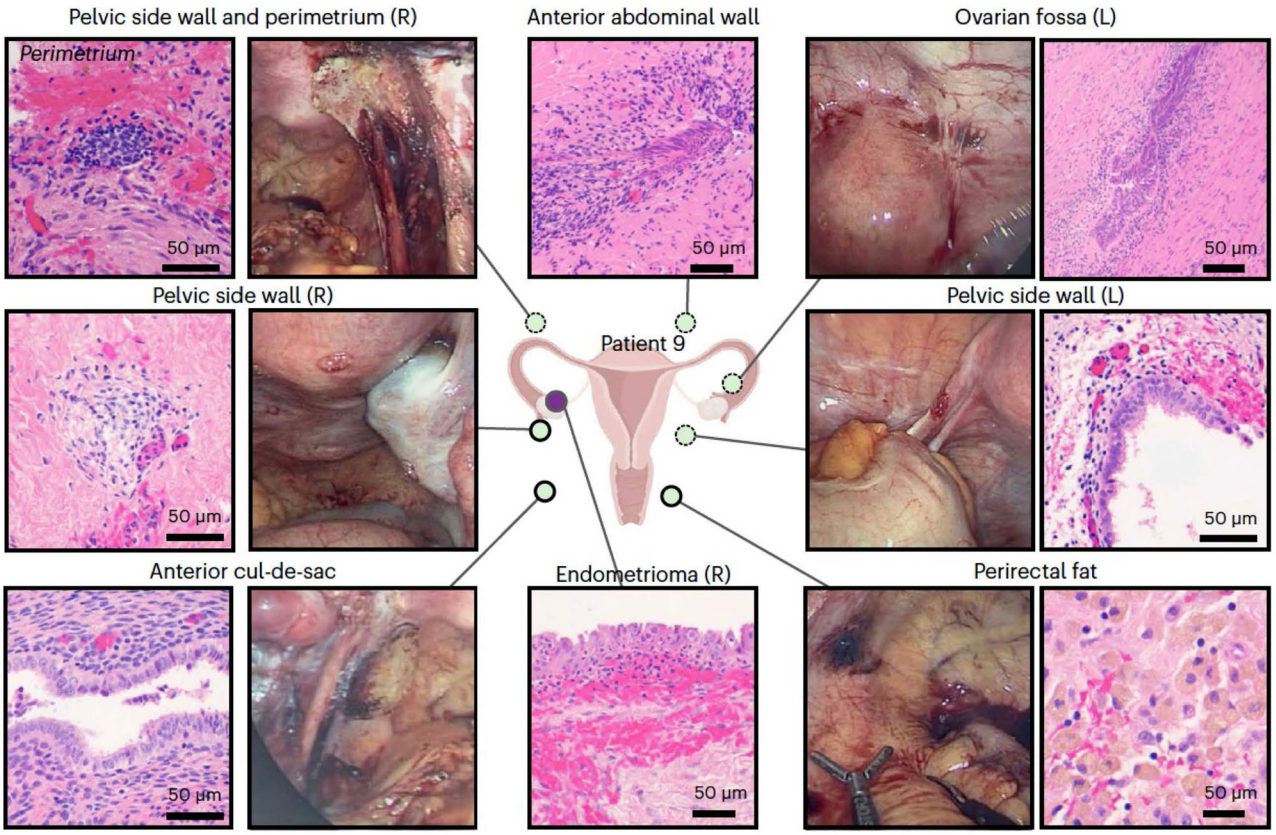

Fig. 4 | Examples of histologic and architectural heterogeneity of endometriosis lesions. The broad array of endometriosis lesions resected for research are shown here diagrammatically and histologically (hematoxylin & eosin (H&E) staining. From Fonseca et al.[139], with permission.

endometriomas. Single-cell analyses can further inform the diversity of cell and tissue features in DIE, important in disease classification and targeted therapies.

Recent single-cell analyses of endometriosis lesions

The transcriptome of intracavitary endometrium from volunteers and cadaveric subjects without endometriosis has been recently studied at single-cell resolution[131–134]. In addition, several studies have been published on scRNA seq of ectopic lesions and eutopic endometrium[135–141], laying the foundation for comparisons with features of the intracavitary tissue of origin and also among lesion types. Figure 4 shows architectural and histologic heterogeneity of lesions[131], underscoring significant lesion cellular components and unique niche environments identified at surgery and histologically. Major cell types identified in eutopic and ectopic endometrium using single-cell transcriptomics include epithelial, mesenchymal, vascular endothelial/smooth muscle/ mural, lymphovascular, immune (myeloid, lymphocytes), and stem/progenitor cells and subtypes, including ciliated, glandular, and luminal epithelium, numerous fibroblast subtypes, uterine natural killer (uNK), T, and B cells, and epithelial progenitors. These studies have identified a few novel cell types in lesions, as well as insights into cell–cell communications, lesion relatedness, and potential lesion evolution (see below). Table 1 provides a summary of recent endometriosis lesion single-cell transcriptomic studies wherein samples were obtained in different hormonal milieu (menstrual cycle phase, exogenous hormones), which is more extensively described in original data sources and in a recent review[142].

Ma et al.[135] sequenced 55,000 cells from 3 endometriomas and matched eutopic endometrium and 3 endometrium samples from controls without endometriosis in the proliferative phase. They found that immune cell subpopulations and fibroblasts were major contributors to a pro-inflammatory, angiogenic environment in endometriomas. T and uNK cell frequencies were lower, uNK cells more active, and macrophages (Mφ) were enriched in endometriomas versus the eutopic tissue, consistent with prior molecular analyses[117]. Thirteen fibroblast subtypes were identified with features of inflammation, fibroblast growth factor (FGF) stimulation, ECM reorganization, and EMT, further informing underlying processes observed histologically (see above). Moreover, *eutopic* endometrium from cases displayed a transitional state from normal to endometrioma, supporting the intracavitary tissue in the developmental trajectory of ovarian disease.

García-Alonso et al.[132] leveraged publicly available bulk microarray transcriptomic data from endometriosis peritoneal lesions (GSE141549) to characterize lesion cell types applied in the context of their scRNAseq data generated from endometrium (basalis and full thickness (basalis and functionalis)) from volunteers in different cycle phases and cadavers (Table 1). Key findings included upregulation of SOX9+ in pre-ciliated epithelial cells of peritoneal lesions and a SOX9+/LGR5+ subset as in proliferative endometrium, and similar expression of progesterone-associated endometrial protein (PAEP) and SCGB2A2 (Secretoglobin Family 2A Member 2) in secretory cells and ciliated cell PIFO and TP73 as in peritoneum. That SPE display of basalis epithelial stem cell markers further supports the intracavitary origins of ectopic disease.

An atlas of endometriosis lesion cell populations was recently published from an extensive dataset of scRNA seq >370,000 cells from endometriomas, SPE and DIE lesions, endometrium, unaffected ovaries and peritoneum[139]. Samples were derived from 17 cases (9 with endometriosis alone; 8 with endometriosis and adenomyosis, uterine fibroids, and/or uterine polyps); 14 were cycling (7 proliferative, 7 secretory phases), and 3 on hormonal contraception. There were 4 controls without endometriosis: 3 postmenopausal and 1 perimenopausal, all with uterine fibroids +/− adenomyosis +/− uterine polyps, and all on hormonal replacement regimens. Major cell types identified in all samples included epithelial, mesenchymal, smooth muscle, endothelial, mast, myeloid, B, and T/NKT cells, and

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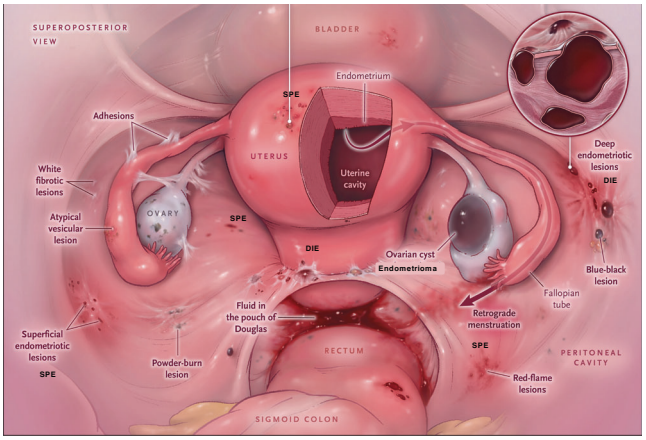
Table 1 | Recent scRNAseq studies word document is uploaded separately

Reference	Tissue analyzed	Cells sequenced	Technology/ platform	Total subjects	Endometriosis type and ASRM stage	Controls	Race/ ethnicity	Hormones/IUD	Cycle phase endometrial histology
Ma et al.[135]	Endometrium, ovarian endometrioma	55,000	scRNA seq (10X)	*n* = 6 subjects (*n* = 3 cases, *n* = 3 controls)	ASRM Stage III, IV ovarian endometriomas	Healthy controls without endometriosis	N/A	None	All in proliferative phase
Garcia-Alonso et al.[132]	Endometrium (functionalis, full thickness), endometriosis peritoneal lesions (red, white, black)	98,569	scRNA seq, snRNA seq, spatial profiling. Lesion microarray data (GSE141549)	*n* = 3 functionalis *n* = 6 full thickness; Microarray data controls: endom *n* = 42, peritoneum *n* = 12; *n* = 9 red, 9 white, 11 black	Peritoneal disease: red, white, black	Healthy controls (functionalis layer) *n* = 6 full thickness without reproductive disorders; normal peritoneum	N/A	None	Proliferative, secretory; Microarray samples: Control. Endo 17PE, 25SE; Peritoneal 4PE, 8SE; Lesions: Red 2PE, 7 SE; White 5PE, 4SE; Black 6PE, 5 SE
Tan et al.[137]	Endometrium, endometriosis lesions	122,000	scRNA seq (10X) IMC organoids	*n* = 27 subjects *n* = 19 cases, *n* = 8 controls; *n* = 14 sequenced	ASRM Stage II–IV, peritoneal lesions, ovarian "lesions", organoids	No endometriosis or inflammatory conditions	White: 9 cases, 3 controls Asian: 4 cases, 2 controls Hisp: 5 cases, 3 controls Black: 1 case, 0 controls	Progestin [NETA, LVN oral, IUD, drosperinone, norelgestromin] +/− ethinyl E2, medroxy-progesterone acetate, copper IUD	wkly PE: 3 cases, 2 control; inactive 3 cases, 0 control; menstrual: 1 case, 0 control, exog hormone effect: 7 cases, 7 control PE: 0 cases, 2 controls IE: 0 cases, 1 control ESE: 4 cases, 0 control N/A: 1 case, 1 control
Fonseca et al.[139]	Endometrium, endometriosis lesions, unaffected ovary and peritoneum	373,851	Digital scRNA seq	*n* = 21 subjects; *n* = 17 cases, *n* = 4 controls n-54 specimens collected	Cases: *n* = 17 Endometrium Endometrioma Superficial and deep disease. *n* = 9 w/o GYN disorders; *n* = 8 w/ adenomyosis, uterine fibroids +/− polyp	Controls *n* = 4 *n* = 3 PMP, 1 perimenopause All no evidence of endometriosis All w/ leiomyoma +/− adenomyosis +/− uterine polyp	White: 13 cases, 2 controls Black: 2 cases, 0 controls Asian: 1 case, 0 control Other: 1 case, 0 control	*Cases*: *n* = 14 on no hormones. *n* = 1 w/ vaginal ring E + P; *n* = 1 on E/T, P4; *n* = 1 on E2 + P4. *Controls*: 3 of 4 on E +/ − P4; NETA; *n* = 1 perimenopause in luteal phase	*Cases*: Proliferative phase: *n* = 7; secretory phase: *n* = 9; N/A (on hormones) *n* = 3. *Controls*: Proliferative *n* = 0, Secretory *n* = 1, N/A (on hormones) *n* = 3

Adapted from Giudice et al. (2023)[10], with permission.

ASRM American Society for Reproductive Medicine, *C* complement, *CD* cycle day, *c/w* consistent with, *Dx* diagnosis, *E2* estradiol, *E* estrogen, *eEC* endometrial epithelial, *exog* exogenous, *ESE* early secretory endometrium, *EuE* eutopic endometrium, *IE* interval endometrium, *IUD* intrauterine device, *LSE* late secretory phase endometrium, *LVN* levonorgestrel, *mens* menstrual, *PE* proliferative endometrium phase, *sc* single-cell, *sn* single nuclei, *RNA* ribonucleic acid, *SE* secretory endometrium phase, *seq* sequencing, *T* testosterone.



Key features:

- SPE lesions display basalis epithelial stem cell markers (Sox9+), supporting contribution to this lesion type.
- DIE has a unique perivascular cell type with features promoting neo-angiogenesis, immune cell trafficking, and an immunotolerant microenvironment conducive to escaping immune clearance and to lesion establishment.
- DIE epithelium uniquely differs from SPE epithelium in NGF and estrogen-signaling events, development and survival of nerves via NTRK1 signaling, and inhibition of apoptotic pathways.
- SPE and DIE epithelium and stromal fibroblasts share structural genes and processes of ECM organization and platelet degranulation, suggesting they are part of the same continuum and are distinct from endometrioma.
- Ovarian endometriomas have a pro-inflammatory, angiogenic environment mainly contributed by several of 13 fibroblast subtypes and by immune cells - especially T cells, uNK cells, and macrophages.
- Single cell deconvolution analysis reveals endometrial-type epithelium cell-type signatures in endometriosis-associated ovarian cancers (clear cell and endometrioid).

Fig. 5 | Key features of endometriosis lesion types revealed by single-cell RNA sequencing. The image on the left illustrates the three major lesion types and subtypes (e.g., red lesions, black lesions) revealing their heterogeneity and anatomic locations. Image is from Zondervan et al.[6] with permission. On the right are listed key features of endometriosis types (superficial peritoneal endometriosis (SPE), deep infiltrating endometriosis (DIE), and endometriomas) and how they differ or share features with each other, based on studies on single-cell RNA-seq of lesions and endometrium described in the text.

erythrocytes. All three lesion types and endometrium from cases versus control endometrium displayed different cell and molecular signatures, consistent with restructuring and transcriptional reprogramming in the lesions. Also, strikingly different transcriptomic signatures were noted in endometriomas versus SPE lesions, suggesting these are distinct disease entities, and SPE and DIE epithelium and fibroblasts displayed some distinct transcriptomic features and pathways, but structural genes were common, suggesting they are related and distinct from endometriomas. Interestingly, some histologically negative mesothelium had disease signatures, raising the possibility of occult abnormalities that may be precursors to lesion subtypes or reaction of normal peritoneum to different insults—physiological, biochemical, and/or perhaps genetic/epigenetic.

Tan et al.[137] studied peritoneal lesions, endometriomas, and endometrium from cases and control endometrium—all from patients taking progestins, a common class of hormones for contraception and also to treat endometriosis-related pain[48]. The peritoneal lesions had similar cell compositions as endometrium but dysregulated innate immune and vascular components, in contrast to endometriomas that displayed distinct cell compositions[137], supporting other studies of endometrioma cellular behavior as distinct from peritoneal disease. Overall, these data demonstrate that immune and vascular components of peritoneal endometriosis favor neoangiogenesis and an immune tolerant niche in the peritoneal cavity.

Key findings are summarized in Fig. 5 in the context of the 3 endometriosis lesion subtypes and their niche environments.

Lessons learned from proteomic and metabolomic profiling studies

In the ongoing quest to uncover the causes of endometriosis, investigators have also increasingly utilized varied proteomic and metabolomic approaches. These have been pivotal for exploring the molecular mechanisms underlying endometriosis and for identifying candidate disease biomarkers of clinical utility. Their integration with transcriptomic data awaits further analyses.

Proteomic studies

Early proteomic research focused on analyzing the abundance of target proteins in blood (serum, plasma), revealing molecules such as CA-125[143,144], CA-19-9[145], inflammatory and oxidative stress mediators, hormones, cell adhesion molecules and angiogenic regulators as potential biomarkers of endometriosis[146–149]. However, these studies have yielded inconsistent results in terms of accurately diagnosing endometriosis, highlighting the limitations of a blood-based approach[148–151]. Consequently, researchers have shifted their focus towards profiling proteins in endometrial tissues and lesions.

Initial proteomic studies of endometrial tissues using targeted approaches identified diverse candidate biomarkers linked to critical processes such as cell cycle control, cell adhesion, and angiogenesis[152]. Newer non-targeted methods revealed a wider range of differentially expressed (DE) proteins in endometrial tissues of cases versus controls. For example, an early study using 2-D gel electrophoresis/matrix assisted laser desorption ionization (MALDI)-time of flight (TOF) mass spectrometry (MS) identified 48 proteins, including those with roles in stromal cell identity and oxidative stress, as consistently DE between endometriosis cases and controls, independent of menstrual cycle phase and disease severity[153]. Another study using a similar MS method identified 119 DE proteins in eutopic endometrium collected during the secretory phase of the cycle between stage II endometriosis cases versus controls[154]. DE proteins included molecules with important roles in apoptosis, immune responses, glycolysis, cell structure, and transcriptional regulation. More recently, liquid chromatography (LC)-MS/MS identified 5301 proteins in samples of eutopic endometrium obtained during the mid secretory phase of the menstrual cycle[155]. Of these, 543 proteins were DE between endometriosis cases and controls. DE proteins were enriched for pathways governing focal adhesion and PI3K/AKT signaling, implicating their roles in disease. These findings, along with others[156,157], highlight the potential of proteomic analyses of endometrial tissues for uncovering disease mechanisms and identifying biomarkers.

Recognizing potential variations in the proteome across endometrial pathologies, investigations are conducting comparative studies. For instance, Zhu et al., using iTRAQ labeling combined with MS, identified 359 DE proteins upregulated in ovarian endometriotic cysts versus other ovarian tissues (i.e., ovarian cysts and normal ovarian tissues), with S100 calcium-binding protein A8 (S100A8) and A9 (S100A9) showing promise as predictors of postoperative recurrence[158]. Future investigations that delve deeper into the proteome of the endometrium and endometriotic lesions are a promising route to a better mechanistic understanding of this heterogenous disease and discovering biomarkers of its many forms, individually and as a group. In addition, the paired analysis of biopsies and blood (serum or plasma) has been proposed as an effective strategy to determine changes occurring on a tissue level that translate to serological biomarkers associated with the disease state. Early on, Zhang et al.[159] applied 2-dimensional gel electrophoresis/MALDI-TOF-MS to identify 11 proteins in both endometrial tissue and serum that were DE in endometriosis cases versus controls. More recently, Manousopoulou et al.[160] used a newer shotgun MS method to discover 1214 and 404 DE proteins (cases versus controls) in eutopic endometrium and serum, respectively. Among these, 21 proteins were DE in both matrices. They were enriched for carbohydrate/lipid

metabolism and organ development, suggesting potential mechanistic links between these pathways and endometriosis. Additional research is needed to determine the ultimate value of applying MS-based global proteomic profiling methods to matched tissues and serum samples. Nevertheless, the concept of discovering biomarkers proximally in endometrial tissues and/or lesions and determining if they can be detected at altered levels in body fluids such as urine and blood is appealing.

While limited in number, the existing proteomic studies demonstrate their utility in uncovering DE proteins and related pathways in the context of endometriosis. Recent advancements, such as data-independent acquisition (DIA) MS, promise even higher sensitivity and reliability in protein identification. While not yet applied to endometrial tissues, a recent study of serum in endometriosis cases versus controls highlights DIA's potential[161]. Furthermore, the application of *single-cell proteomics* will open new avenues for investigation of endometrial tissues and lesions[162]. Coupled with emerging high-dimensional proteomic imaging technologies, these single-cell approaches will enable incorporation of spatial information for a comprehensive phenotypic and functional proteomic mapping of the cellular architecture in solid tissue[163]. For example, in their recent study, Tan et al.[137] combined spatial proteomics analysis using imaging mass cytometry with single-cell transcriptomics to characterize the cellular landscape of the eutopic endometrium. The results provided additional spatial context to proteomic data and emphasizing relationships between single cells and tissue architecture underlying disease histopathology.

Moving forward, the application of high resolution, quantitative-based proteomic methods along with study designs that incorporate possible confounding factors—such as the stage of the menstrual cycle[164], severity[165], lesion type[166], and associated pain type[167]—is anticipated to enable robust identification of DE proteins, providing deeper insights into disease mechanisms and potentially clinically valuable biomarkers.

Metabolomic studies

The metabolome encompasses a wide array of metabolites, both endogenous—such as amino acids, organic acids, nucleic acids, fatty acids, amines, sugars, vitamins)—and exogenous, including environmental chemicals and pharmaceuticals[168]. Metabolomic approaches, including both targeted and non-targeted methods, have been used in endometriosis research to identify metabolic alterations linked with the disease's pathophysiology and progression[169–171]. The majority of these studies have concentrated on endogenous metabolites in blood[172]. In limited studies, MS-based metabolomics has been utilized to profile endometrial tissues in the context of endometriosis. For example, Dutta et al.[173] using proton NMR demonstrated differential levels of specific amino acids (e.g., lower alanine) and organic acids in endometrial tissues from women with endometriosis cases versus controls[173]. In another study, using ultra-high performance liquid chromatography coupled with electrospray ionization high-resolution mass spectrometry (UHPLC-ESI-HRMS), investigators found that levels lipid metabolites—phosphatidylcholine, phosphatidylserine, and phosphatidic acid—differed in the eutopic endometrium of cases compared to controls[174]. These initial studies suggest that distinct metabolic disruptions in endometrial tissues and lesions could be integral to understanding the pathophysiology of endometriosis, offering new insights into molecular mechanisms contributing to changes at the cellular level.

Exogenous factors contribute to endometriosis, including infections and environmental contaminants. Recent reviews have underscored possible roles of the gut microbiome[40] and the female reproductive tract microbiome and their metabolic products[40,41] in endometriosis pathophysiology by modulating immune function and other mechanisms. The time is optimal to evaluate these early observations with disease lesion phenotypes and clinical metadata with the promise of revealing new treatment strategies. Moreover, a recent systematic review of 50 epidemiological studies highlighted positive associations between endometriosis and several environmental contaminants, including polychlorinated biphenyls, dioxins, phthalates, organochlorines, and bisphenol A[175]. These substances may disrupt tissue homeostasis through various mechanisms, such as acting as endocrine disruptors, modulating immune function, inducing oxidative stress and perturbing epigenetic processes[176–178]. Consequently, such disruptions can perturb endometrial cell functions and pathways that regulate cell proliferation, differentiation, and decidualization[179,180], leading to perturbations on the cellular and/or organ level. While largely unexplored, the use of emerging MS-based non-targeted approaches, utilizing not only blood[181] but also endometrial tissues and lesions, provides great promise in identifying exogenous and endogenous factors that contribute to pathological changes in endometriosis and mechanisms of its pathogenesis.

Informative approaches in studying endometriosis lesions

The above studies, using single-cell and other advanced technologies, have provided huge insights into the heterogeneity of cell types/subtypes, unique clusters and signatures informing cell- and tissue-specific features, cell–cell communications, and pathways involved in cellular dysfunctions in endometriosis lesion subtypes and endometrium of patients with disease versus controls. It is anticipated that results across studies will further inform endometriosis lesion types and underlying pathophysiology. The future looks promising as advanced cell-specific transcriptomic and spatial localization provide insights into endometriosis and its comorbidities, and mass spectrometry-based methodologies enable quantification of thousands of proteins and metabolites in human blood and endometriotic tissues. However, significant hurdles remain, including sample/disease heterogeneity, diverse methodologies, differences in study designs, and complexities in data interpretation. These challenges contribute to inconsistent findings and hinder broader applicability, warranting careful consideration of their limitations for translating findings into clinical applications. Generating datasets from diverse populations, combined with the integration of -omic and clinical data, could ultimately provide a comprehensive understanding of disease pathways and manifestations, leading to the identification of reliable biomarkers for disease diagnosis and prognosis. However, these studies on a heterogeneous, hormone-sensitive disorder, also underscore real-world challenges and key informative approaches in conducting this type of research. Some of these include:

Lesion location and confirmation

Surgical phenotype data collection has been endorsed by the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking and Harmonization Project (EPHeCT)[182]. Documenting lesion locations, as described above, and proximity of lesions to each other are huge contributions of collaborating surgeons and warrant further study in how they inform data interpretation outcomes. Moreover, as lesions have multiple appearances, documentation of endometrial-like epithelial cells and stromal fibroblasts is important, although sometimes is challenging. The epithelium can be affected by hormonal changes, and some lesions ("stromal endometriosis") contain scant or no epithelium[183]. Moreover, the stroma can be obscured by hemosiderin-laden/ foamy histiocytes, fibrosis, elastosis, smooth muscle metaplasia, and decidual change[183], which can affect lesion identification, analysis, and data interpretation. About 60–80% of biopsied lesions have histologic confirmation of these two cell types[85]. If lesions are suspected but neither epithelium nor stromal cells are identified, pathologists often further section the lesions and may conduct immunohistochemical staining for the endometrial stromal fibroblast surface marker, CD10[184–186]. Recent computer-aided histopathological characterization of endometriosis lesions[187] may further inform diagnosis. While a typical eutopic endometrial biopsy comprises ~200–300 mg of tissue, biopsied lesion volumes in SPE and DIE often results in very low cell yields, and the fibrous nature of endometriomas can limit sufficient non-fibrotic tissue-derived cells for sequencing and other analyses.

Standard operating procedures (SOPs)

We have adopted SOPs for endometrial and endometriosis tissue and peripheral blood biospecimen collection, processing, and storage, as described by Sheldon et al.[178] and in the WERF-EPHeCT protocols[188–190].

Tissues are either flash frozen in liquid nitrogen, embedded in optimum cutting temperature (OCT) compound and/or formalin-fixed/paraffin embedded, depending on tissue volume and experimental design and goals. Frozen tissues and peripheral blood (plasma and serum) are stored at −80 °C until further use. As noted recently regarding unbiased approaches and advanced technologies for sequencing and the proteome and metabolome of endometrium and endometriosis lesions in the context of endometrial-based infertility, uniform protocols for sample collection and processing as described above and by WERF-EPHecT protocols[188–190] are key for rigor and reproducibility from sample to sample and study to study[191].

Determining hormonal status

As hormonal status at the time of sample acquisition is key to data interpretation of endometrial and endometriosis tissues, we assay estradiol (E_2) and progesterone (P_4) in serum collected on the day of surgery, complemented by histologic dating of the eutopic endometrium to determine menstrual phase cycle[192,193], and record the subject's last menstrual period and often menstrual cycle length for further context of cycle phase. Phase-specific transcriptomic signatures of select cell types are a huge advance in the field[131,194]. Samples from subjects on various hormonal treatments are a valuable cohort to study lesion responsiveness to select therapies. However, while ovarian-derived and synthetic hormones (e.g., progesterone and progestins, respectively) signal through common pathways, they also signal via unique pathways that could influence outcomes, as we have demonstrated in vivo and in vitro studies[195–199].

Defining cases, controls, and rigor of de-identified metadata

Well-defined cases and control populations, inclusion and exclusion criteria, and clinical metadata are essential for all clinical studies and especially in designing and conducting studies and interpreting results of endometriosis lesions. Storage of these metadata in the REDCap database for ready access and updating during the study is often necessary as new information may be forthcoming. Key items include whether the diagnosis of endometriosis is accompanied by pelvic pain, infertility, subfertility, organ dysfunction, all of the above, or is asymptomatic; what the hormonal status of the subjects is (cycling, menopause, exogenous hormone usage at the time of tissue sampling and time interval since stopping these therapies). Other covariate phenotypic data include age, BMI, gravidity (i.e., prior pregnancy), race/ethnicity, medical, surgical, and family history, non-hormonal medication use, and comorbidities. The latter are particularly germane, as there is a high prevalence of common gynecologic disorders (e.g., uterine fibroids, adenomyosis, endometrial polyps, abnormal uterine bleeding) in cases (and controls). Furthermore, for controls, whether they have documented absence of endometriosis or presumed absence should be noted, as well as comorbidities. A comprehensive description of clinical and covariate phenotype data collection in endometriosis research was developed in the WERF-EPHeCT protocol in 2014[200]. Notably, while numbers of cells sequenced enrich the phenotypic features of individual cell types (Table 1), the numbers of subjects are, by comparison, low, and diversity of recruited cohorts is limited or not always documented. To date, as most studies either did not describe ethnicity or had a preponderance of White subjects, the data across ethnicities are limited and offer opportunity to close this gap in future research[8].

Opportunities for the future

Integrating surgical, histologic, molecular, and genomic features

The cross-roads of decades of surgical, clinical, molecular, genetic, and epidemiologic research on endometriosis, and new data derived from advanced technologies at single-cell resolution offer an unprecedented opportunity to integrate these diverse data platforms and transform endometriosis disease phenotyping and elucidate further its pathogenesis and pathophysiology. For example, do cellular and sub-cellular features and processes differ in Type I vs Type II endometriomas, do they differ by location (e.g., "genital", "extra-genital", "extra-pelvic"), by nearest neighbor lesions, by ER and PR content and subtypes? Do they correlate with symptoms, treatment responses, are they impacted by comorbidities? As SPE, DIE, and endometriomas share common endometrial cellular origins, what is the role of the niche environments in which they are found, temporal effects of disease, and can this inform risk of developing disease and progression? How do cells within a niche and across niches communicate? What are the roles of genetics, dysbiosis of the gastrointestinal and female reproductive tracts, and environmental toxicants in these processes? It is hoped that more sophisticated disease stratification, including somatic genomic events[201], will lead to discovery of non-invasive disease biomarkers, personalized and novel mono- or multi-target therapies for pain and/or infertility or organ dysfunction and disease modification, prognostic indicators for responses to medical and surgical therapies, and risk of disease and symptom recurrence—to enrich the lives of patients with endometriosis and eventually result in cure.

Integrative omics computational approaches for diagnostics and therapeutics

Integrative computational approaches hold significant promise in advancing diagnostic and therapeutic strategies for endometriosis[202]. Single-cell transcriptomic datasets can be integrated with other molecular measures including proteomic, metabolomic, methylation as well as others, using approaches such as MOFA[203,204] or DIABLO[204] to identify common cell-type specific pathways and programs mis-regulated in disease. Importantly, while there is a high level of discordance between global transcriptomic and proteomic analyses at the level of individual mRNAs and their cognate proteins, there is a high degree of concordance at the pathway level, a principal recently demonstrated for human endometrium[205]. Also, unsupervised machine learning or clustering approaches can characterize lesions on the molecular level, and combined with precise phenotyping are anticipated to enable a better understanding of disease heterogeneity. Supervised machine learning approaches can be applied to these data to develop classifiers and predict which patients may have a particular outcome of interest. Furthermore, lesion-specific signals from transcriptomics and other types of molecular data can be used to advance drug discovery through both classical target-based approaches and more recent drug repurposing methods. In our own recent study[206], we leveraged gene expression data to identify new therapeutic candidates based on transcriptional reversal. While the aforementioned study relied on signals from bulk transcriptomic data of eutopic endometrium, extending this work to leverage lesion data, especially on a single-cell level, is an exciting prospect.

Analysis of electronic health records

Analysis of electronic health records (EHR) data has contributed to enhancing our understanding of endometriosis disease phenotypes. For example, a study that implemented a clustering approach on the data of approximately 4000 endometriosis patients represented in a primary care clinical database found that women with endometriosis could be classified into six clusters according to the presence of comorbidities, including a cluster associated with fewer comorbidities, multiple comorbidities, anxiety and musculoskeletal disorders, type 1 allergy or immediate-type hypersensitivity, anemia and infertility, or headache and migraine[207]. Another work that utilized individual-level genotype and EHR data from the UK Biobank and genome-wide association statistics from multiple international resources revealed genetic and phenotypic associations of endometriosis with depression, anxiety, and eating disorders[208]. Combined analysis of clinical and molecular data can provide greater insights into endometriosis, although there has yet to be a study to date leveraging EHR with single-cell data for this disease. Such analyses could enhance our understanding of processes and/or mechanisms involved in endometriosis pathophysiology or disease presentation.

The power and promise of artificial intelligence (AI) for endometriosis

AI technology lends itself well to pattern recognition and using historical data to make predictions—features that are increasingly being applied in

health care and translational research of complex diseases[209,210]. It holds great promise for screening and staging endometriosis with its complex classifications, varied clinical and molecular profiles, and unpredictable responses to therapies that are yet to be tailored for individual patients.

Recent technological advancements, such as the Nezhat Endometriosis Risk Advisor (EndoRA) application, highlight the growing role of AI in non-invasive screening[44]. EndoRA demonstrated a sensitivity of 93.1% in screening endometriosis in patients with chronic pelvic pain or unexplained infertility, although its specificity remains low (5.9%). This application's free and accessible platform empowers individuals to identify their endometriosis risk, potentially leading to earlier diagnosis and better outcomes[44].

With the recent advent and use of generative AI to democratize and standardize medicine, there may be opportunities for expedited progress in understanding, diagnosing, and managing endometriosis. For example, using large language models (LLMs) to analyze gene expression data could assist researchers with knowledge-driven candidate gene prioritization and selection which in turn could hasten the discovery of biomarkers and therapeutics[211]. In addition, analyzing molecular data combined with other types of data such as clinical records (e.g., electronic health records, patient registries) and environmental chemical and pollutant exposures data, could yield even greater insights into endometriosis including potential disease etiologies, mechanisms, and phenotypes[43,212,213]. While the current best LLMs may not be able to accurately diagnose patients across all diseases, future improved versions of LLMs could be used to analyze clinical records and assist clinicians in making potentially faster and more accurate diagnoses as well as recommending personalized treatment plans[214,215]. LLM-powered chatbots could provide support for patients affected by the endometriosis and education for individuals interested in learning more about this condition and even in pre-operative and patient education sessions[216]. Generative AI-empowered digital twins (DTs) could revolutionize drug discovery and development by enabling simulations across different biological models, from cells to clinical trials, although challenges remain including large data requirements, lack of straightforward interpretation, and regulatory oversight[217]. Addressing these obstacles as well as developing multimodal and foundation DT models could ultimately enhance drug development and personalized medicine[217].

Democratization and standardization of endometriosis care from diagnosis to treatment

As multi-omics research and data integration proceed, and as minimally invasive and robotic surgery evolves, the principles of individualization and democratization of care and standardization of procedures underscore the necessity of enhancing global access to such procedures. Video-assisted surgery, pioneered by Dr. Camran Nezhat and his team, revolutionized the field, enabling complex operations with fewer complications and faster recovery times[44]. Moreover, digital surgery integrates robotics, data analytics, AI, enhanced visualization, and instrumentation, and technologies as image-guided ultrasound and augmented reality are progressing towards incision-less surgeries. Due to rapid advances in technology, tasks that once took days can now be done in hours, with attendant benefits for patients. These innovations offer pathways to a more equitable and advanced surgical future[44].

Summary

In the "real world", we, as a research and clinical community, have come a long way, together, to mine the mysteries of endometriosis. It is anticipated that multidisciplinary and integrative approaches will reveal features of endometriosis and its various subtypes that will transform non-invasive biomarker development to diagnose disease, identify cell-specific targets for personalized single or combined medical therapies for endometriosis pain and sub/infertility, and develop risk prediction models for disease and symptom recurrence. Additionally, as technology advances, we predict that surgical management, as well as medical management of endometriosis, will become standardized and democratized within the next 2–3 decades. Furthermore, incorporating AI and computational approaches is viewed as vital in analyzing multiomics and clinical datasets and patterns and improving understanding of endometriosis. Training the next generation of surgeons, clinicians, computational, epidemiologic, and wet lab researchers is a major goal to assure a pipeline of collaborators whose focus and efforts will result in improved well-being of patients affected by endometriosis and the ultimate goals of prevention and cure of this enigmatic disease.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Author contributions

C.R.N., L.C.G., D.K.S., B.G., and M.S. conceived the theme of this review. C.R.N. and L.C.G. co-wrote the introduction and sections on endometriosis staging and lesions; L.C.G., J.C.I., and B.L. co-wrote the section on scRNA seq and the section on somatic mutations, and J.F.R., S.J.F., and B.G. co-wrote the section on proteomics and metabolomics. L.C.G. wrote the section on real-world needs; M.S., T.T.O., C.R.N., L.C.G., and A.T. co-wrote the section on AI and drug repurposing. All authors contributed to opportunities for the future section and mined the literature for their respective sections. L.C.G. prepared figures 4 and 5. C.R.N. prepared figures 1–3. All authors read, reviewed, and approved the final manuscript.

Competing interests

All authors declare no financial competing interests. Other competing interests: L.C.G. is on the Editorial Board of *npj Women's Health*, and L.C.G. and M.S. are Guest Editors of the npj Women's Health Collection, "Inflammatory Disorders and Women's Reproductive Health".

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