Reproductive endocrinology

A novel resource to study endometriosis at the single-cell level

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A transcriptomic analysis of endometriosis and comparison tissues has been conducted to reveal a rich and complex catalogue of single-cell based expression data. This resource is an invaluable building block toward single cell profiling at scale, to aid research into endometriosis pathogenesis and new ways of diagnosing and treating the disease.

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The endometrium, the lining of the uterus, undergoes a dynamic process of shedding, regeneration and differentiation throughout the menstrual cycle and serves as the site of embryo implantation [1]. Endometriosis is a condition where endometrial-like tissue (known as endometriotic lesions) grows outside the uterine cavity in response to ovarian hormones. It can have a substantial negative impact on quality of life, causing chronic pelvic pain and even leading to infertility. Endometriosis affects around 10% of cis-women (hereafter referred to as women) of reproductive age, which translates to around 190 million women across the world, yet its underlying causes are largely unknown [2]. Women can wait an average of 7 years for a formal diagnosis, and even when diagnosed, available treatments (such as exogenous hormones) do not provide a cure and have not advanced substantially in decades [2].

Developing new targeted diagnostic and treatment options for endometriosis requires a detailed molecular, cellular and tissue-level characterisation of the disease state and its potential subtypes. However, studying the endometrium and endometriotic lesions is challenging due to their heterogeneity and substantial dynamic changes. For instance, between individuals, lesions can be found in various locations in the body, and the

symptoms and severity of the disease can also vary widely. Furthermore, within an individual, endometriotic lesions undergo dynamic remodelling in response to ovarian hormones and changes over the menstrual cycle, and endometriosis is also influenced by age. To characterise such inter-individual and intra-individual variability and dynamics, new approaches to profile patients and control individuals at scale are needed. Fonseca and colleagues now provide a step towards this aim, with a study profiling gene expression patterns in endometriosis, endometrium, peritoneum and ovarian tissues at single cell resolution [3].

The authors profiled the transcriptomes of ~400,000 cells from endometrium, endometriotic lesions and unaffected ovarian and peritoneal tissue from 21 women aged 21–62 years. The majority (16 of 21) had previously been diagnosed with endometriosis (with or without adenomyosis or leiomyomas). The study sample included women of reproductive age and post-menopausal women, who had or had not undergone exogenous hormonal treatment. A caveat, as noted by the authors, is that the sample size did not enable detailed analyses or adjustment for potential confounding factors, such as menstrual cycle phase or age, which might influence the observed heterogeneity in the patient population. The resulting dataset is a complex but invaluable resource that complements previous efforts to atlas endometrial-like tissue in patients with endometriosis who were receiving exogenous hormonal treatment [4]. Additionally, the authors provide extensive histological and macroscopic features of the profiled samples, which gives additional depth to the dataset.

Overall, the study focused on the epithelial and stromal populations in the endometriotic lesions and quantified changes in cellular composition between the endometrium and the endometrial-like tissues. For example, the authors found that foci of endometrial-like epithelium within the lesions are composed of ciliated cells and cells that are positive for LGR5 and SOX9 expression, which were previously identified in unaffected individuals at the lumen of the proliferative endometrium [5]. In addition, peritoneal lesions and ovarian endometriomas also showed differences in cellular composition. Due to inter-individual variability, this result should be interpreted with caution but might highlight the ability of endometrial-like cells to adjust to different organ-specific microenvironments. This finding might also suggest that the pathogenesis of ovarian endometrioma is different from that of peritoneal disease, which would be consistent with studies of heritable genetic risk variants over the past few years [6]. By contrast, no differences in cell composition were observed between superficial peritoneal and deep infiltrating endometriosis, which suggests a shared aetiopathology.

The authors also studied the potential link between somatic mutations and cellular expression patterns as a possible basis for the association between endometriosis and ovarian cancer. Patients with endometriosis have a small increased risk of clear cell and endometrioid ovarian cancer, and the authors observed endometriosis epithelial cells carrying somatic mutations in the cancer driver gene *ARID1A*. These cells upregulated expression of paracrine factors that the authors speculate might promote local growth of lymphatic endothelial vessels. This observation suggests that non-cancerous endometrial-like epithelial cells carrying *ARID1A* somatic mutations could thus set the ground for pro-lymphangiogenesis and the conversion into malignancy. However, given that cancer driver mutations such as those in *ARID1A* naturally accumulate in endometrium with age [7], the link between these mutations and the pathogenesis of both endometriosis and endometriosis-associated ovarian cancer remains to be further explored.

Altogether, this study provides a rich source of information to mine, for example for new biomarkers for diagnosis of different endometriosis subtypes. If such markers are also enriched in the blood of patients with endometriosis, novel approaches for developing non-invasive methods of (earlier) diagnosis could be. Considering that the authors observed a potential inflammatory signature in endometriotic lesions, a deep profiling of immune cells might provide further insights into the mechanics of immune cell interactions and inflammation. Immune cells, such as macrophages, are known to modulate tissue remodelling and to have a major role in the inflammatory response in endometriosis [8]. Hence, immunoregulatory treatments might be a promising approach for the disease.

Looking forward, this study highlights the continued need for large-scale studies of endometriosis. So far, single cell expression studies have been underpowered, so have not been able to account for inter-individual and intra-individual variability (including menstrual stage, age, treatment with exogenous hormones, disease stage and/or ethnicity). To identify commonalities and differences between normal and eutopic endometrium or endometriotic lesions, disease atlases need to be contextualised with reference atlases from healthy control individuals [5,9]. Given the marked effect of endogenous hormones shaping the transcriptional profile of the endometrium [5,9,10] and endometrial-like cells [3,4] , it is imperative that reference and disease atlases fully account for the dynamic nature of the menstrual phase. Large-scale atlases of endometrial cell gene expression that are specific to the menstrual cycle stage and hormonal state and are integrated with other ‘omics’ profiling (such as genomics and proteomics), are crucial to understand the pathogenesis of diseases of the female reproductive tract and to develop new diagnostic methods and treatments. Fonseca et al. provide a valuable resource contributing to this aim.

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Figure 1. Schematic illustration of the samples collected by Fonseca and colleagues. The samples were profiled with single-cell RNA-sequencing and evaluated using immunohistochemistry.

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Competing interests

The authors declare no competing interests.