Chapter 1: Introduction

1.1 Overview

Endometriosis is a chronic inflammatory condition characterized by the growth, adhesion, and progression of endometrial-like tissues outside the uterus, mainly on the peritoneal cavity, ovaries, and recto-vaginal septum. It affects about one in ten women, or 5-10% of women in their reproductive age (which in average are between ages 15 and 49). However, the actual prevalence of this disease is unknown as the reliable diagnostic confirmation is accomplished by laparoscopy or surgical visualization with histological verification. In addition, there are no specific endometriosis-related symptoms; women with the disease may be asymptomatic or presented with a single or a combination of symptoms with various intensity that can be related to many factors. The common reported symptoms of women with endometriosis are dysmenorrhea or painful menstruation, cyclical or non-cyclical abdominal pain, debilitating pelvic pain (during and/or after sexual intercourse), and fertility issues (Zondervan et al., 2018).

The etiology and pathophysiology of endometriosis remain elusive besides a lot of hypothesis have been proposed to explain this issue. One of the most widely accepted hypothesis is retrograde menstruation proposed by Sampson in 1927 (D'Hooghe, 2002). This theory postulates the origin of endometriosis from a reflux of menstrual fluid through the fallopian tubes into the peritoneal cavity instead of being excreted out through cervix and vagina. The backward flow of menstrual debris containing viable endometrial glands and stroma provides the cell seeding into the surrounding pelvic cavity and/or ovaries (Zondervan et al., 2018). This theory was supported by endometriosis model in female baboons where the prevalence of retrograde menstruation was higher in animal with the disease than those without it (83% and 51%, respectively). However, the fact that not all cases of retrograde menstruation led to endometriosis indicates that other factors possibly contribute to the survival ability of endometriotic cells and their adhesion and invasion capacities in ectopic sites.

Endometriosis is a complex condition. It is also notable as a systemic gynecologic disorder which an impaired hormonal and immune homeostasis play as underlying factors to promote cell survival and lesion growth in ectopic sites post-retrograde seeding. Sex hormones, estrogen and progesterone are capable in regulating the growth of endometrial tissue by stimulating and inhibiting cell proliferation. The development of endometriotic tissue is estrogen-dependent and unusually high estrogen production has been observed consistently as the endocrine feature of endometriosis. Estradiol, a biologically active hormone of estrogen, is essential in the survival and growth of endometriotic tissue as well as neurogenesis and inflammation that are highly correlated with pain symptoms of the disease. Estradiol reaches endometriosis by both circulation and local production within the endometriosis niche. Its receptors (estrogen receptor – ERα and ERβ) are being upregulated in the disease state compared to normal endometrium, serving the estradiol hormone and its receptors as therapeutic targets for endometriosis (Dyson et al., 2012). Nevertheless, current hormonal therapies rarely provide a long-term relief with many adverse effects have been reported including infertility problems (Parasar et al., 2017).

As an inflammatory disorder, endometriosis pathophysiology is strongly associated with immunological dysfunction (Fig. 1). Recent studies show that endometriotic lesions harbor a unique microenvironment comprising of a diverse infiltrating immune cell types and their associated dysfunction featuring prominently (Symons et al., 2018). Accumulation of endometrial fragments in the peritoneal cavity naturally triggers innate and adaptive immune response to clear the menstrual debris and initiated tissue repair mechanism. However, the persistent or long-term exposure of endometriotic cells in ectopic sites may lead to overloaded immune response and subsequent immune dysfunction such as anergy, cell death, or establishment of pro-lesion immune system. These defective immunosurveillance against autologous tissue deposited in the ectopic sites facilitates successful lesion growth and development of new blood vessels. It is not clear whether this immune dysfunction is a cause or consequences of endometriosis.

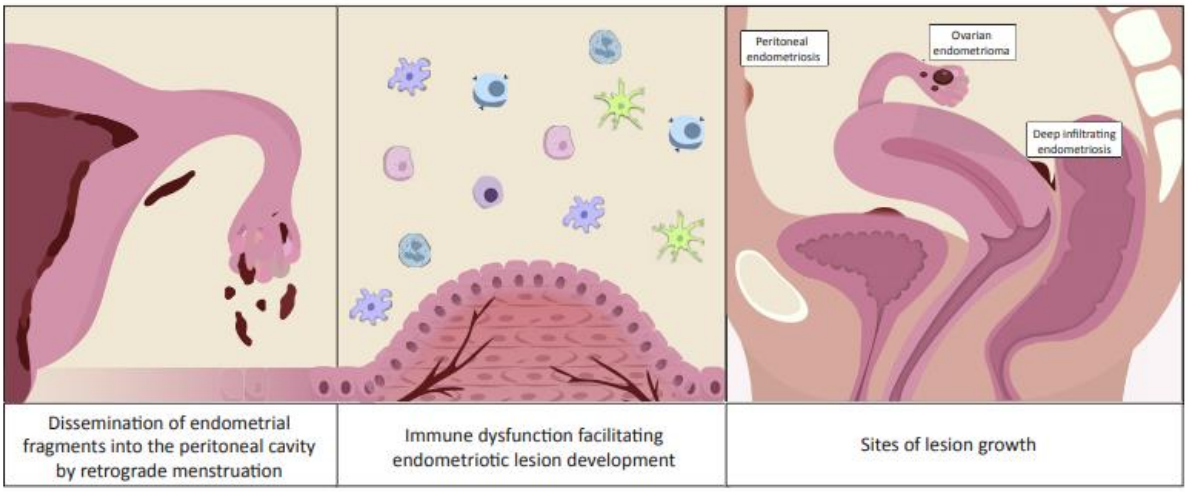

Figure 1. Retrograde menstruation and immune dysfunction facilitating endometriosis lesion growth in ectopic sites. (Image curtsey *Symons et al. Trends in Molecular Medicine. 2018*).

The nature of endometriosis is heterogeneous. It can be in a form of superficial peritoneal, serosal lesions, endometriosis cysts in the ovaries and nodules in depth. It sometimes be accompanied by fibrosis and adhesions as well (Zondervan et al., 2018). Along with infiltrating immune cell components and angiogenic factors, the microenvironment of endometriotic lesions is even more heterogenous. Therefore, the most ideal way to investigate this disease such as identifying differentially expressed genes and discovering possible rare cell types/subtypes presented in disease compared to normal should be achieved in a single-cell resolution.

Analysis in single-cell level is important to provide a deep understanding of cellular and functional differences in the identified cell population because each cell has its own unique phenotypes (Stuart & Satija, 2019). By understanding a disease in a single cell level, therapeutic target will be more specific and the outcome projections in each patient will be more precise. Recent advances in single-cell sequencing technologies such as single-cell RNA sequencing (scRNA-seq) allow a highly sensitive and multiplexed single cell transcriptomics approach in studying the nature of tissue-of-interest which in this case is endometriosis. The use of scRNA-seq has been reported previously to analyze the full transcriptome of CD13+ stromal and CD9+ epithelial cells of endometrial tissue biopsies versus cultured cells (Krjutškov et al., 2016). Whilst a study performed by Wang et al. in 2018 utilized scRNA-seq to observe the molecular and cellular

cartography of human endometrium across different time points of menstrual cycle and found signature profiles for each endometrial cell types in particular menstrual phase. Both of the studies have used single-cell approach in analyzing normal endometrium heterogeneity but not in the disease-state endometriosis. A meta-analysis of eutopic endometrium transcriptome microarrays using publicly deposited datasets has been done by Poli-Neto et al. to plot the immune profile differences between those samples coming from stage I-II and stage III-IV endometriosis patients (Poli-Neto et al., 2020). However, the lack of single-cell approach and the analysis of only eutopic without the ectopic endometrial tissue leaving a space for improvement.

Therefore, to achieve a complete understanding of endometriosis heterogeneity at a single-cell level and propose novel targets for improved therapeutics, we aimed to decipher the immune cell landscape in endometriosis biopsies using scRNA-seq technique. Additionally, to understand the significance of these immune cells under the light of endometrial niche, we also observed differentially expressed genes from each cluster. The immune cell types infiltrating endometriotic lesions and their phenotype differences with those in the normal endometrium are critical to the disease pathology and is a major component of this study.

1.2 Objectives

1. To analyze the single-cell RNA sequencing data from human endometriosis tissue biopsies and to determine their cell-type compositions
2. To identify the immune cell-types present in endometriosis and compare it with those in normal endometrial tissues.
3. To identify the differentially expressed genes from each immune cluster to project their phenotypes in endometriosis and compared it with those in normal endometrium.