Establishment of endometriotic models: the past and future

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Abstract

Endometriosis is a prevalent chronic disease that affects approximately 6% to 10% of reproductive-aged women. Although numerous researchers have endeavored to explore the etiology of endometriosis over a century, its etiology still remains an enigma. The exploration of pathophysiologic mechanism and novel therapy for endometriosis depends on ideal endometriotic models. In the previous decade, various endometriotic models have been established; therefore, we made a conclusion for available information on these models. This review summarized the common experimental models used in endometriotic studies, including their origins, characteristics, applications, and limitations. Endometriotic models played an important role in studying etiologies and novel treatments of endometriosis during the last decades. Among them, animal models and endometriotic cell lines were viewed as most common studying tools to explore the intrinsic entities of endometriosis. In addition, endometrial organoid also emerged and was regarded as an ideal studying tool for endometriosis research. Different research models collectively complement each other to advance the endometriosis research. The successful establishment of endometrial organoids means that organoids are expected to become an ideal model for studying endometriosis in the future.

Keywords: Endometriosis; Endometriotic studying models; Endometriotic cell lines; Animal models; Endometrial organoids

Introduction

As a prevalent disease, endometriosis affects approximately 6% to 10% of all women during their reproductive age.[1] The incidence of endometriosis ranges within 40% to 60% in women with dysmenorrhea and within 20% to 30% in women with subfertility.[2,3] Endometriosis is characterized by the presence of endometrioid epithelial and stromal cells outside the uterus. Endometriosis is mostly found in the pelvic cavity, and mainly consists of three categories: peritoneal, ovarian, and deep infiltrating endometriosis (DIE).[4,5] Patients often experience pelvic pain, dysmenorrhea, deep dyspareunia, ovary cyst and infertility, and even face a higher risk of epithelial ovarian cancer.[4,6,7] Present treatments contain pain medication, hormonal intervention, and surgery.[7]

Despite these numerous studies, the etiologies of endometriosis remain indistinct, which depends on ideal studying models. Among these, animal models and endometriotic cell lines are viewed as the most common tools to support the research of endometriosis. In addition, the basis on organoid cultures that concern the liver and gut, endometrial organoid (EO) has also emerged, and has been regarded as a reliable model for endometriosis research. Therefore, the present review summarizes the experimental models that have been used in endometriotic studies, and it was considered that organoid culture could be an ideal model complementary to others for endometriosis study in the future.

Published literatures were searched from the PubMed, Embase, and Web of Science databases with the following relative terms: "endometriosis cell line*," "(endometriosis epithelial cell line*[Title/Abstract]) OR endometriosis stromal cell line*[Title/Abstract])," "(endometriosis) AND (stem cell)," "organoid culture," "(endometrium) AND (organoid culture)," and "(endometriosis) AND (animal models)." The search was performed until December 2019. In addition, relevant reviews and the reference lists of all the included articles were analyzed to search for related articles.

In Vitro Cell Models

The characteristics and potential applications of endometriotic cell lines are respectively presented in Tables 1 and 2. In addition, the details of the *in vitro* cell models were introduced in each part.

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Table 1: The cell lines used in endometriosis studies.

Type	Origin	E or S	T-genes	Im	In	Steroid receptors by RT-PCR
EEC145T	PE	E	SV40T	N	Y	ERα(+) ERβ(+)PR(+)*
EEC10Z	PE	E	SV40T	Y	N	ERα(+) ERβ(+)PR(+)
EEC11Z	PE	E	SV40T	N	Y	ERα(+) ERβ(+)PR(+)
EEC12Z	PE	E	SV40T	Y	Y	ERα(+) ERβ(+)PR(±)
EEC49Z	PE	E	SV40T	N	Y	ERα(+)ERβ(+)PR(±)
ESC22B	PE	S	SV40T	N	Y	ERα(+) ERβ(+)PR(±)
EMosis-CC/TERT1	OMA	E	hTERT, cyclinD1, cdk4	Y	UK	ERα(+) PRB(+)
EMosis-CC/TERT2	OMA	E	hTERT, cyclinD1, cdk4	Y	UK	ERα(+) PRB(+)
EEC16-TERT	OSE	E	hTERT	Y	UK	ERα(−)PR(−)
St-T1b	EU	S	hTERT	Y	UK	ERα(+) ERβ(−)PR(+)

* EEC145T was also tested by immunocytochemical analyses. RT-PCR: Reverse transcription polymerase chain reaction; E or S: Epithelial or Stromal; Im: Immortal; In: Invasive; PE: Peritoneal lesion; UK: Unknown; ±: Barely detectable or not determined; OMA: Ovarian endometrioma; OSE: Ovarian surface endometriosis; EU: Endometrium; Y: Yes; N: No.

Table 2: Potential applications of endometriotic cell lines for preclinical research.

Research contents	References
Epigenetics	[8,9]
Inflammatory and immune reaction	[10,11]
LncRNA and microRNA	[12,13]
Therapy relevant	[14,15]
Neoplastic transformation	[16,17]

Characteristics of endometriotic primary cells

Gaetje et al reported that the E-cadherin negative epithelial cell of endometriotic primary cells is invasive, and could be distinguished from fibroblasts and stromal cells by its expression of cytokeratin.[18] Hence, this cell type, cytokeratin+/E-cadherin−, might play an important role in the development and invasion of endometriosis.[19] Primary cells can better characterize the disease, but have a limited life span. This lay a foundation for the subsequent establishment of endometriosis cell lines.

Endometriotic epithelial cell line 145T

Endometriotic epithelial cell line (EEC) 145T was established, and the detailed procedure was previously described.[18,19] Derived from peritoneal biopsies and transformed Simian virus 40 (SV40) T antigen,[20] EEC145T is invasive, cytokeratin+/E-cadherin−, and is expressed both in the estrogen receptor (ER) and progesterone receptor (PR) by immunocytochemical analyses, with a life span approximately 35 passages. At about passage 25, EEC145T begins to lose the expression of cytokeratin, and the invasive features and expression of fibroblast growth factor activating gene 1 (Frag-1) mRNA, but maintains its proliferative potential.[20] Thus, EEC145T can retain these initial characteristics for as long as 25 passages. Furthermore, the addition of 10% (v/v) peritoneal fluid considerably enhanced the invasive capacity of EEC145, while the removal of steroids and growth factors in peritoneal fluid or the treatment at 95°C did not change the invasion-promoting activity.[20] As a studying tool, EEC145T was once used to verify that ovarian cancer antigen CA125 influences the cell adhesion in vitro.[21]

EEC10Z, EEC11Z, EEC12Z, EEC49Z

Zeitvogel et al established a number of endometriotic cell lines derived from peritoneal endometriotic biopsies.[22] As immortalized cell lines using the SV40 T antigen, these endometriotic cell lines included two categories: one category exhibited stromal cell features, while the other category exhibited epithelial-like features. However, both of these were E-cadherin negative. Among these, cell lines 10Z, 12Z, 39Z, and 50Z escaped the crisis, and became immortal. Furthermore, cell lines 10Z, 11Z, 12Z, and 49Z, and the previous cell line EEC145T were selected for further investigation [Table 1]. Although E-cadherin was negative, N-cadherin was expressed in endometriotic cell lines, which is similar to metastatic EJ28 cells.[22] This coincided with previous studies, indicating that N-cadherin might be associated with invasion and migration.[23] Notably, Zeitvogel et al provided many useful study tools for endometriosis research, especially EEC12Z, which has been most frequently used. More than 40 studies have used this as a research tool. Although there was no more introduction about endometriotic stromal cell (ESC) 22B, ESC22B has been widely employed as a study tool, especially in cooperation with EEC12Z.[8,10,14] In addition, some cell lines, such as 11Z, 12Z, 49Z, 108Z, and 22B, have been further examined by Banu et al.[24] They focused on exploring gene expression profiles and the functional characterization of these cell lines, such as the mRNA expression of relevant cytokines and cell cycle regulation.[24]

EMosis-CC/TERT1 and EMosis-CC/TERT2

Due to the risk of carcinogenesis in endometrioma,[25] it is vital to study the ovarian EEC line, to explore carcinogenesis mechanism and treatment. However, ectopic ovarian epithelial cell lines are more difficult to culture, when compared to ectopic stromal cell lines,[26] and premature senescence and telomere-dependent senescence were the two paramount limitations.[27] To solve this problem, Bono et al attempted be different combinations of transfection genes,

and produced five independent cell populations from two patients by co-transfection of at least three genes.[28] These cells could grow for over 100 population doubling, maintaining the original morphology and negative senescence-associated β-gal staining.[28] The outcomes indicated that the co-expression of cyclinD1 and cdk4 could overcome the premature senescence of EECs, and that combining these with hTERT is sufficient for the immortal phenotypes, without the additional inactivation of p53. Then, they produced the EEC lines, parental cells, EMosis-CC/TERT1, and EMosis-CC/TERT2.[28] Unfortunately, the EMOsis-CC/TERT2 was contaminated with few interstitial cells. For the response to steroid hormone, the immortal epithelial cells exhibited a cell response to progestin, but there was no response to estrogen (E2). These managed to overexpress ERα in EMOsis-CC/TERT1 cells via the lentiviral introduction of ERα cDNA and obtained the ERα-over-expressing cell line EMOsis-CC/TERT1/ER. The sufficient expression of ERα in EMOsis-CC/TERT1/ER was confirmed by western blot, and the growth of EMOsis-CC/TERT1/ER cells was markedly activated by the addition of E2.[28] Finally, these successfully generated immortal EECs from ovarian endometrioma that still had the property of estrogen or progestin response and did not have tumorigenicity: EMOsis-CC/TERT1/ER cell line. In general, this cell line has been a useful tool for endometriosis research, especially for the carcinogenesis of ovarian endometrioma. In the introduction or knockdown of candidate genetic factors, this cell line enabled the identification of genetic factors required for transformation. For example, Mita et al used EMosis-CC/TERT1 and its deuterogenic cell lines to explore the pharmacologic mechanism of dienogest.[29]

EEC16 and EEC16-TERT

Brueggmann et al established an EEC16 from ovarian surface endometriosis lesions.[30] They cultured EEC16, EEC12Z, and normal ovarian surface epithelial cells (OSECs) in vitro three-dimensional (3D) culture models and examined morphologic and molecular characteristics. EEC16 displayed an epithelial morphology with mesenchymal factors: cytokeratin⁺/vimentin⁺, but E-cadherin⁻/N-cadherin⁻/P-cadherin⁻/ERα⁻.[30] After performing RNA-sequencing to compare the transcriptome between primary EEC16 and OSEC, Brueggmann et al found 1780 significantly differentially expressed genes. Intriguingly, EEC16 more closely resembled peritoneal lesions than cystic endometriomas within the ovarian cortex on the histologic examination. Compared with the two-dimensional model, the 3D culture model could better represent the characteristics of the endometriosis expression profile.[30]

Based on EEC16, Lawrenson et al established a novel TERT (human telomerase) immortalized cell line (EEC16-TERT).[31] The lifespan of EEC16 in vitro was increased following the transduction with either lentiviral-TERT (EEC16-TERT-L), or retroviral-TERT (EEC16-TERT-R), when compared to both control cells transduced with GFP (EEC16-GFP) and primary EEC16, from 60 to 70 days extended to over 200 days. In addition, EEC16-TERT

retained the normal karyotype, and did not appear with tumorigenicity in vivo.[31]

Primary and TERT-expressing EEC16 shared similar morphologies, but had differentially expressed epithelial and mesenchymal markers: the primary EEC16 cell was cytokeratin⁺/vimentin⁺/E-cadherin⁻,[30] while the EEC16-TERT cell was cytokeratin⁻/vimentin⁺, suggesting the occurrence of epithelial-mesenchymal-transition (EMT).[31] EMT is a key process in the pathogenesis of endometriosis[32] which does not occur in TERT-immortalized OSECs,[33] suggesting that this phenomenon is a feature of EEC16 and/or other endometriosis-derived cell lines. Furthermore, compared to primary EEC16, EEC16-TERT cultures exhibited a lower expression of tumor suppressor genes associated with clear cell ovarian cancer (ARID1A, MLH1, and MSH2).[31] Lawrenson et al hypothesized that Src activation could be a driver of endometriosis-associated ovarian cancer (EAOC). If the Src signature is differentially activated in the eutopic endometrium of women at highest risk of developing EAOC, endometrial biopsy might represent a non-invasive screening tool to detect early stage EAOC.[31] Therefore, EEC16-TERT is a potential model to deeply study the pathogenesis and identify the novel therapy for endometriosis, especially for EOAC.

In fact, as early as 2012, Boccellino et al reported the introduction of hTERT to construct EEC lines and stromal cell lines derived from deep endometriotic tissues. In addition to the absence of chromosomal abnormalities and long-term expansion, the cell line also maintains the natural characteristics of endometrial cells from the perspective of the phenotype and functional expression of estrogen and PRs.[34] In their study, for immortalized cell lines, epithelial cells expressed cytokeratin 7 and stromal cells expressed CD10. However, it was uncertain whether EMT would occur in later passages.

The ESC line Hs832cT (CRL-7566)

CRL-7566 was established by American Type Culture Collection (ATCC; Manassas, VA, USA). This cell line, which was derived from an ovarian cyst wall from a patient with endometriosis, has been partially characterized as stromal (vimentin⁺/cytokeratin⁻/E-cadherin⁻). This cell line had been broadly used as a research tool.[9,12,16]

Endometrial stromal cell line St-T1b

Samalecos et al established an ESC line St-T1b, which was immortalized by introducing hTERT into primary cells derived from the proliferative endometrium of patients with tubal disorder.[35] St-T1b displays the phenotype of vimentin⁺/CD90⁺/CK7⁻ by immunocytochemistry.[35] By reverse transcription polymerase chain reaction, PR, and ERα were readily detectable in St-T1b cells, and the ERβ transcripts were below the limit of detection.[35] However, neither PR-B nor PR-A was detectable by western blotting in St-T1b cells, and the combination of progestin with E2 failed to induce morphologic changes associated with decidualization in St-T1b cells.[35] Although cells did not respond to progestin alone, Samalecos et al demonstrated

that cyclic AMP (cAMP)-induced St-T1b cells respond to progestin, and the combination of cAMP with synthetic progestin MPA promoted the decidualization better, when compared to P4.[35] However, these phenomena would decline in later passages. In addition, when compared to that *in vivo*, E2 did not induce PR protein in cells, and no induction was obtained with steroids alone. Although St-T1b was originally established to study the decidualization, this could be used as a contrast to the endometriotic cell line while studying the etiology of endometriosis, such as the role of micro-200b in endometriosis.[13]

CRL-4003, which is a *hTERT*-immortalized human ESC line (T-HESC) established by the ATCC (CRL-4003), has also been frequently used as contrast in endometriosis research.[36,37] Furthermore, Kyo *et al*, Krikun *et al*, Barbier *et al*, and Chapdelaine *et al* also established some endometrial cell lines, which might be useful in endometriosis research.[38-41]

Epithelial progenitors and mesenchymal stem cells obtained from human endometrium

More than 90% reproductive women experienced retrograde menstruation, but merely 6% to 10% suffered from endometriosis.[42] Some experts hypothesized that in endometriosis patients, endometrial stem/progenitor cells were inappropriately shed during menstruation and reached the peritoneal to establish endometriotic implants.[43-45] Gargett *et al* also testified that adult human endometrium contained rare epithelial progenitors and mesenchymal stem cells (MSCs), which are likely responsible for its immense regenerative capacity and development of endometriosis.[46] In the study conducted by Chan *et al*, some cells from ovarian endometrioma exhibited somatic stem cell properties. Derived from ovarian endometriotic cysts, purified epithelial and stromal cells established colony-forming units, and presented with self-renewal and multi-potent capacities.[47] Meanwhile, the migration, proliferation, and angiogenic ability were more obvious in ectopic endometrial MSCs from patients with endometriosis, when compared to eutopic MSCs from the same patient or control MSCs from women without endometriosis.[48,49] Kao *et al* also observed ectopic MSCs with increased angiogenesis and invasion into the surrounding tissue in a scaffold transplantation mouse model.[48] Moggio *et al* demonstrated that sorafenib might decrease the higher migratory, proliferative, and angiogenic phenotype of ectopic MSCs, showing that MSCs are helpful for both illuminating the pathogenesis, and exploring novel non-hormonal therapy.[49]

Endometrial organoids

Organoids were stem-cell-derived structures generated *in vitro*, which display the 3D architecture and physiology of original organs.[50] These offer extraordinary opportunities for modeling and exploring the normal development and disease processes, and gave rise to novel approaches to drug screening and toxicology testing.[50] In recent years, organoid culture has again gained the attention of researchers and received further development. As a genetically stable and self-organizing 3D culture system, organoids contain both progenitor/stem and differentiated cells that resemble the original tissue. Human organoids have been derived from tissue-resident adult epithelial stem cells from the gut, liver, endometrium, as well as other organs.[51-53] Turco *et al* and Boretto *et al* were the first scholars to report the study of EOs, including mouse and human EOs.[53,54] Based on these, Fitzgerald *et al* and Boretto *et al* further investigated the characteristics of human EOs, including the different cell types of EOs and ectopic and eutopic EOs of endometriosis patients.[55,56] These studies explored the components of various EO culture mediums, which was usually referred to as the R-spondin-based culture method. This elucidated the characteristics of specific types of EOs at the phenotypic and genetic levels, and revealed that EOs are positive in cytokeratin, E-cadherin, steroid receptors, mucus, and intact epithelial polarity. Furthermore, this tested the responsiveness to hormones, which influenced the expression of ER and PR, gene profiles, and cell types.[53-56] Importantly, they verified that EOs could maintain the important phenotypic and genetic characteristics after long-term expansion. As an ideal model, EOs are expected to play an important role in pathophysiology research and the drug screening of endometriosis.

In Vivo Models

Compared with *in vitro* models, *in vivo* models have extraordinary advantages in exploring etiology, novel therapies, and the influence of endometriosis on patients, such as fertility or pain, which require behavior analysis. The most common animal models included non-human primates (NHPs) and rodent animals. Each category has its own merits and limitations [Table 3].

Table 3: The merits and weaknesses of animal models.

Animal model	Merits	Weaknesses
Non-human primates	Similar to human in phylogenetic, reproductive anatomy and physiology; presence of menstruation; and spontaneous endometriosis	Requires special infrastructure, logistics, and training for handling these animals; ethically sensitive and expensive
Rodent	Low cost, easy handling; the possibility of genetic manipulation, such as knockout mice and transgenic mice	Different to human in phylogenetic, reproductive anatomy and physiology; lack of menstruation and spontaneous endometriosis

Non-human primates

Spontaneous endometriosis only occurs in humans and NHPs, such as rhesus monkeys and baboons, which have nature menstrual cycles.[57] Endometriosis in NHPs resemble the human condition, in terms of phylogenetics, reproductive anatomy and physiology, laparoscopic and microscopic aspects.[58] Furthermore, NHPs are only species with spontaneous or induced endometriosis similar to the disease in women.[59] In the wild, endometriosis infrequently and slowly develops, which result in the establishment of an induced model *via* the injection of autologous menstrual effluent into the pelvic cavities of baboons.[60] In general, the presence or absence of endometriosis was checked by laparoscopy. After endometrium was injected into the abdominal cavity, laparoscopic procedures were performed at different time points to observe the endometriotic lesions and development of the disease.[61,62] The reasons why baboons were the most frequent models include noninvasive cycle monitoring based on perineal changes, continuous breeding, suitable size and strength, spontaneous peritoneal fluid, cross-reactivity between baboons and humans, vaginal transcervical uterine access, spontaneous retrograde menstruation, and human-like minimal to severe endometriosis.[59] In a classic design, D'Hooghe *et al* found that compared with retroperitoneal injection, intra-peritoneal implantation using menstrual endometrium, rather than luteal endometrium, could more successfully induce endometriosis.[62] Frequently, baboons have been used to explore the possible pathophysiology and potential therapies.[63,64] There is also a saying that new drugs that exhibit effective potential in the rodent model needs to be further tested, in terms of the general and reproductive side effect in NHPs.[59]

Rodent animal models

Although there are a lot of advantages in the NHP model, the limitation of price and ethical considerations make its frequent use difficult. On the contrary, rodent models are relatively more general with respect to economics, and easy to maneuver and perform genetic manipulation, such as the knock-out mice and transgenic mice.

The rat autologous model was developed by Vernon and Wilson in 1985,[65] and subsequently modified by Berkley *et al*,[66] who sutured small pieces of uterus not only to the mesenteric cascade, but also onto the abdomen and ovary, to further resemble the distribution of lesions in women. In addition, Prodromidou *et al* attempted to establish the DIE model in rats by suturing the resected uterine horn to the rectum of rats, and confirmed this macroscopically and microscopically.[67] Except for verifying the reduced fertility,[65] the rat model was also commonly used to explore the association between endometriosis and increase in pelvic nociception, such as the vaginal hyperalgesia and muscle hyperalgesia induced by a ureteral calculosis amid behavior analysis, and this might be partly explained *via* the "viscero-visceral referred hyperalgesia" and central sensitization.[66,68] At the same time, some studies explored relevant therapies for "viscero-visceral hyperalgesia" and central sensitization using rat models, such as ketoprofen.[68]

Uterine fragments have also once been grafted onto the sciatic nerve to imitate neuropathic pain in endometriosis.[69] In addition to exploring the pain mechanism, a rat model was used to study novel therapies and relevant pathophysiology. For instance, cisplatin and letrozole have been tested for the treatment of endometriosis on a rat model.[70] Furthermore, the rat model was used to analyze the gene expression profiles of ectopic tissues deposited in rats, and explore the association between lesions and inflammatory response, angiogenesis, extracellular environment, and so on.[71-73] However, the establishment of rat models were mainly surgically induced by suturing fragments of uterine tissue to the peritoneum and omentum from the same or syngeneic donor.[74] The injection method did not work in rats, because the fragments failed to attach and invade the peritoneal cavity.[65] In contrast, due to the involvement of knockout and transgenic mouse, the mouse model was more various. Apart from the autologous model, there was also the patient-derived xenograft (PDX), which indicated the humanized mouse model of endometriosis by grafting intact human tissue or human endometriosis cell lines.[75] In addition, even in the syngeneic mouse models, the experimental methods were different, ranging from the suturing tissue to the peritoneal lining, and the injection of whole uterine fragments to the injection of "menstrual" material, which was described by Greaves *et al* in detail.[75] Among these models, the use of steroid-induced menstruation as the source of syngeneic mouse menstrual endometrium and the introduction of this into the peritoneum of immunocompetent mice[76,77] further simulated the human disease process. These different mice models were complementary to each other. For example, although human tissues could be manipulated before xenografting in heterologous models, but these cannot be used to study the immune system due to immunodeficiency. In contrast, the immunocompetent mouse model could be used to study the effect of immune-modulating drugs and anti-inflammatory agents.[75] The mouse model has also been involved in genetic manipulation, which was applied to certain target genes to investigate alterations in ERβ activity during endometriosis progression.[78]

Conclusions and Perspective

As a debilitating, chronic and recurrent disease, endometriosis affects around 6% to 10% of women in their reproductive age, and this substantially affects the quality of life of women, and imposes costs on the society, which is similar to other chronic conditions, such as type-2 diabetes mellitus, rheumatoid arthritis and Crohn disease.[7] To better understand this enigmatic disease, the establishment of reliable endometriotic models for further research are indispensable. Traditional endometriotic models include cell lines and animal models. Primary cell lines could better represent the disease, but have a limited lifespan. For immortal cell lines, EEC12 was the most widely used, others like CRL-7566 and ESC22B have also been used in research. An ideal cell line should be able to passage and maintain the original phenotype and genotype in the long-term. However, SV40 T antigen transfected cell lines usually have karyotype abnormalities.[79] Although *hTERT* transfection can maintain the normal karyotype,

many formed cell lines do not express or express ER and/or PR only at the RNA level, when compared to the protein level, and these could not respond to hormonal stimulation.[28,35] Indeed, endometriosis is an estrogen-dependent disease. Therefore, although these cell lines are easy to culture for a long time, there are general limitations of using such cell lines, including their genetic background, potential changes occurring during transformation and culture.[80] In addition, ethical and economic reasons limit the use of NHPs. Although rodent models are valuable in research, the research outcomes are critical in view of the differences between animals and humans. For example, IFN-α-2b, which has been shown to be efficient in the treatment of rodent endometriosis, makes the endometriosis more severe in patients.[81,82]

Organoids partly solved above mentioned problems. In oncology research, researchers have found that tumor cell lines cannot retain certain important mutations, and the barcode complexity of cell lines was also progressively lost.[83] Organoids could maintain the genetic stability of the original tissue, even for tumor significant genetic heterogeneity, which is better than tumor cell lines.[84,85] Turco et al and Boretto et al concluded that EOs phenotypically and genetically resemble the original characteristics, even after long-term expansion, which are important for establishing an ideal disease model.[53,54] In addition, the transcriptomic and genetic analyses of EOs could also reveal disease-associated traits, such as the gene expression differences associated with the signaling pathway, hormonal response, and the adhesion/invasion factors exhibited among the normal EOs, eutopic EOs, and ectopic EOs of endometriosis patients.[56] Therefore, EOs can also be used as reliable disease models for pathogenesis research and drug screening, which are similar to other organ-derived organoids. Similar to drug screening for cystic fibrosis[86] and colorectal cancer,[87] endometrial cancer EOs present with patient-specific drug responses.[56] In addition to being superior to cell lines, Schutte et al discovered that the response to various drugs between parallel organoids culture and PDX was generally consistent,[88] while PDX demanded more time and resources. Therefore, before conducting clinical trials, screening out sensitive drugs by combining organoids and PDX greatly improve the efficiency of drug screening, and saves time and costs.[85]

Despite the numerous merits and potential application in clinical medicine, there are still some unresolved technical issues, such as the lack of blood vessels and immune cells in most of the present organoid protocols.[50,89] The communication between epithelial cells with stromal and immune cells play an essential role in the development of endometriosis, but present EOs models cannot solve this problem, which needs to be handled for endometriotic EOs in the future.

Endometriosis is a heterogeneous condition, and the subtypes may differ from pathogenesis and require different treatments, and even require different markers for diagnosis and stratification. Zondervan et al once put forward that future research must focus on understanding the pathogenesis, identifying disease subtypes, developing non-invasive diagnostic methods, and targeting non-

hormonal treatments appropriate for women who wished to conceive.[7] An exclusive endometriosis organoids biobank for pathophysiology research and drug screening are expected to solve these problems. Taken together, all kinds of models should corporate with others. As suggested by Bredenoord et al, organoids are complementary to, rather than in competition with, these classical research methodologies.[50]

Conflicts of interest

None.

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