The organoid: A research model for ovarian cancer

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INTRODUCTION

Epithelial ovarian cancer (EOC) is a heterogeneous disease with a wide range of distinct clinical and molecular features [1,2], and is divided into two types (type 1 and type 2 EOC) [1,2]. Type 1 EOC includes endometrial cancer, clear-cell carcinoma, low-grade serous ovarian carcinoma, and mucinous carcinoma, whereas type 2 EOC includes high-grade serous ovarian cancer (HGSOC), carcinosarcoma, and undifferentiated carcinoma [3]. Type 1 EOC shows genetic stability and contains a set of genes, including KRAS, PTEN, BRAF, and CTNNB1, that are frequently mutated [4]. The genetic changes in HGSOC include extensive structural genomic (copy number) variations, P53 pathway inactivation, homologous recombination-mediated DNA damage repair (HR DDR, BRCA1 mutation) deficiency, CCNE1 and NOTCH3 activation, and RB and NF1 inactivation [3]. A previous study has shown that HGSOC originates from the fallopian tube epithelium (FTE) [5].

The currently available, common research models for EOC include tumor cell lines and patient-derived xenografts (PDXs) [6,7]. However, these models have some shortcomings, including the time-consuming nature of cell line establishment processes, loss of genetic traits after long-term culturing, and investment of animal care facilities [8]. Therefore, newer research models are needed [9]. Organoid technology was originally developed from colorectal cancer [10]. The tumor organoid system, a three-dimensional culture system, can help accurately decipher the tumor phenotype from the original tumor [11] and overcome the previously mentioned shortcomings of the currently available research models. This review aims to provide a clear picture of the advantages and disadvantages of the currently available research models and the tumor organoid system used in ovarian cancer research.

ABSTRACT

Epithelial ovarian cancer (EOC) is a heterogeneous disease with a variety of distinct clinical and molecular characteristics. The currently available and common research models for EOC include tumor cell lines and patient-derived xenografts. However, these models have certain shortcomings: establishing a cell line is time-consuming, loss of genetic traits after long-term culture is a possibility, and investment is required in terms of animal care facilities. Therefore, better research models are required. Organoid technology was originally developed from colorectal cancer. Tumor organoid is a three-dimensional culture system and can help accurately recapture the tumor phenotype from the original tumor. Tumor organoid systems can overcome the above-mentioned shortcomings of the currently available research models. The organoid model can be used for culturing ovarian cancer subtypes, screening drugs, assessing genomes, and establishing biobanks. However, the currently available organoid models can only culture one type of cells, epithelial cells. Therefore, an organoid-on-a-chip device can be developed in the future to provide a microenvironment for cell–cell, cell–matrix, and cell–media interactions. Thus, organoid models can be used in ovarian cancer research and can generate a simulated in vivo system, enabling studies on the heterogeneity of ovarian cancer.

KEYWORDS: Epithelial cells, Epithelial ovarian cancer, Organoid-on-a-chip, Patient-derived xenografts, Xenograft
HIGH-GR ADE SER OUS OVARIAN CANCER DEVELOPMENT

HGSOC was thought to have originated from the ovarian epithelium; however, recently, it was found to have originated from the FTE. Ovarian cancer originating from the FTE was suspected from the BRCA1/2 germline mutations observed in fallopian tubes [12]. The immediate precursor lesion for HGSOC is serous tubal intraepithelial carcinoma (STIC), which is limited to the FTE and shows nuclear atypia, mitotic figures, abnormal p53 staining, and enhanced Ki-67 staining [13,14]. STIC cells can detach from the fallopian tube and spread to the pelvic cavity. The migrated STIC cells survive via environment selection and progressive nodule formation that leads to ascitic fluid production. Since the ovarian surface is located adjacent to the FTE, it is usually the first site to which STIC cells migrate [15]. p53 signatures are another fallopian tube epithelial lesion and present with intense p53 immunostaining, consistent with a missense TP53 mutation [16].

HGSOC harbors several characteristics, including a higher stage during diagnosis, TP53 mutations, homologous recombination DNA repair deficiency, and CCNE1 amplification, that distinguish it from type 1 EOC [17]. HGSOC development begins at the first ovulation and spans over >30 years [18]; 10 years from normal fallopian tube epithelial cells (FTECs) to p53 signatures, 15 years from p53 signatures to STIC, and 5 years from STIC to HGSOC.

CURRENT RESEARCH MODELS FOR OVARIAN CANCER

The currently available common research models for EOC include tumor cell lines and PDXs [6,7]. Both of these have been described below.

Cell lines

To recapture its biological and molecular significance, researchers need genetically and clinically evaluated cell lines to retake HGSOC [19,20]. Without reliable cell lines, it is challenging to translate in vitro study findings into in vivo studies on HGSOC [8,20,21]. The previous study surprisingly found a genomic dissimilarity between clinical HGSOC and the commonly used HGSOC cell lines [8]. They found that only 12 cell lines, which have been infrequently used, are compatible with HGSOC genotypes [8]. This number of cell lines is too small to efficiently research HGSOC heterogeneity [19,21-24]. Therefore, more cell lines are needed to address the necessity of HGSOC-related research and facilitate ovarian cancer research [1,8,20,25-27].

The current in vitro cell culture system precludes suppressed expression of hormone receptors, which may play a role in carcinogenesis:

After long-term in vitro culturing of either immortalized FTECs or HGSOC cells, the expression of most steroid hormones is typically silenced, likely via epigenetics [28]. Currently, FTECs (FE25) [29] and ovarian HGSOC cell lines, such as Kuramochi, OVSAHO, OVKATE, OVCAR3, and OVCAR4 [30], which are used in in vitro studies do not express estrogen receptor (ER) and progesterone receptor (PR). The cell line FE25 is derived from primary cultured FTECs and transfected by HPV E6 and E7, causing TP53 and Rb loss that mimics the characteristics of HGSOC [31]. In contrast, ER and PR are actually abundantly expressed in the FTE and in primary cultured FTECs, only at early passages [18,29,32]. Therefore, a suitable in vitro system is needed to study the role of hormones in the normal physiology and malignant transformation of the FTE.

Patient-derived xenograft model

The PDX model is a new kind of animal model used in cancer research. In the PDX model, a fresh tumor tissue is implanted into an immunodeficient animal, without in vitro cell culture. This model can preserve tumor cell heterogeneity and is used in studying various cancers, including breast, endometrial, and cervical [33-38]. It can also be used to explore tumor recurrence and chemosensitivity [39]. Nevertheless, there are many unresolved obstacles with regard to the PDX model. The success rate of PDX establishment is variable as per different studies (18%–100%) [27,40-42]; the factors influencing the success rate are unknown. Due to the lack of standard operating procedures, tumor subtypes, and the gene ontology of the molecular signatures, the role of the PDX model in ovarian cancer research remains to be elucidated [27,43]. Besides, PDX model establishment warrants investments in animal care facilities and a time-consuming animal breeding course. This model is not suitable for genetic manipulations or large-scale drug screening and would precede a mouse-specific tumor evolution process [44]. Therefore, an emerging model is warranted for ovarian cancer research.

The orthotopic (intrabursal) or intraperitoneal animal model

Another animal model for ovarian cancer is orthotopic and intraperitoneal injection model. The intraperitoneal model imitates peritoneal metastasis of ovarian cancer and is used for the evaluation of the efficacy of chemotherapy intraperitoneally [45]. Nevertheless, the limitation of this model is contributed from absence of primary tumor, the short life span of mice, and spontaneous metastasis [46]. The orthotopic model simulates the ovarian microenvironment for ovarian cancer survival. The gene expression pattern, histopathological characteristics, disease progression, and interaction between cancer cells and the microenvironment can be also imitated [47]. Both primary ovarian cancer and metastasis tumor exist in this model and well predicting the subsequent results for patients with metastatic cancer [48]. Nevertheless, this model harbors several disadvantages, including difficult techniques, time-consuming, expensive, and difficult to monitor the tumors [49,50].

Organoid introduction

Organoids are three-dimensional cell aggregates formed in vitro and can be derived only from primary tissue or embryonic stem cells. Organoids have self-renewal and self-organization capabilities and exhibit organ functionality [51]. The model has a composition and architecture similar to that of primary tissue. It could also...
serve as a relevant model for assessing in vivo conditions and as a stable system for extended cultivation [51]. The tumor organoid system was first used in colon cancer research in 2011 [10]. The tumor organoid can accurately recapture the characteristics of the original tumor [11,52-54]. Organoid culture techniques employ cocktail growth factors and matrigel for the long-term effects. An organoid can be derived from a single cell and demonstrates tumor heterogeneity [55]. Organoid models can be used for evaluating genotype–phenotype correlations, drug sensitivity, and for predicting patient treatment response [54,56-58].

Taken together, organoids can be used in cancer research and can help avoid the disadvantages of cell lines and PDX models.

Organoid in fallopian tube epithelial cell research

Because HGSOC may originate from FTECs, an organoid model established from FTECs was developed. FTEC organoids can be derived from the proximal or distal end of the fallopian tube [59]. Fresh fallopian tubes can be used to generate organoids. The organoids will aggregate within 24 h and reach 2.5 mm after 2 weeks of culture [60]. The organoids can be passaged every 2–3 weeks in a ratio of 1:3 and can be maintained in culture for 34–60 days [61]. A previous study used a medium containing 25% of conditioned medium from fibroblasts to provide WNT3A and RSPO1 for fallopian tube organoids [60]. They also achieved a 100% success rate from 52 donors [60]. There was a study previously that reported use of 100 ng/mL of WNT3A and 600 ng/mL of RSPO1 in the organoid culture [62]. Another study used a 10% homemade RSPO1-conditioned medium and 200 ng/mL of WNT3A [63]. Our group also developed the FTEC organoid model. We used low amounts of WNT3A and RSPO1 (50 ng/mL each) to generate FTEC organoids [64]. A previous study found that P53-mutant FTECs could lead to HGSOC [65]. Induced pluripotent stem cells can also be used to generate FTECs and then organoids [66]. Above all, organoid models can be used for studying the transition from FTECs to HGSOC.

Organoids in ovarian cancer research

Organoids derived from ovarian cancers are different from those derived from FTEC. The culture media components and signal transduction are different for both cell types. Neuregulin-1 is considered one of the key factors in the generation of organoids from ovarian cancer [67]. However, the success rate of organoid formation is only 44% [67]. Another study, which contained 56 organoids derived from 32 patients, used the organoid model as a research platform for various types of ovarian cancer [68]. They used the Wnt-conditioned and pure organoid culture media for culturing ovarian cancer organoids, the success rate of which was 85% [68]. The organoid model has been recently used in ovarian cancer DNA repair defect research [69]. Zhang et al. reported the use of a genetically defined syngeneic organoid system to develop combination therapy for ovarian cancer [70]. Low Wnt signaling is required for ovarian cancer organoid formation [71]. Taken together, the organoid model used for ovarian cancer research is extremely common.

Advantages of organoids

The success rate of the organoid model is higher than that of spheroid cells and PDXs [72]. The applications of organoids in cancer research include ovarian cancer subtype culturing, drug screening, genomic assessments, and living biobank establishment [54,56-58,73]. An organoid can be derived from a single tumor cell and can recapture the heterogeneity of a tumor [55]. In summary, the organoid model can overcome the disadvantages of cell lines and PDXs.

Disadvantages of organoids

The organoid model harbors several disadvantages. The success rate is not 100% [67], although a previous report demonstrated an 85% success rate using specific culture medium components [68]. Next, the required medium is expensive (3000 USD/month for growth factors and special ingredients). Further, maintaining the organoid culture system is labor-intensive and Western blots cannot be performed to confirm the expression of specific proteins. However, this shortcoming can be conquered using immunohistochemistry. Taken together, the expensive culture medium may be the main disadvantage of the organoid model.

Assembloids in cancer research

Assembloids have recently been introduced to cancer research [74]. The old organoid model can only be used to culture epithelial tumor cells. However, the tumor comprises three germ layers: the endoderm, mesoderm, and ectoderm. The assembloid model overcomes this problem; it can be used to culture epithelial cells, stromal cells, and myoepithelial cells. The assembloid model can recapture the composition of the normal bladder and bladder cancer. It needs a three-dimensional printing technique to mix the three types of cells. In the future, we hope to use this technique to establish an ovarian cancer model.

Future perspectives

The organoid model still needs improvements. The current organoid model can only be used to culture epithelial cells and lacks cell–cell interactions. The assembloid model, which mixes three types of cells with three-dimensional bioprinting, will be a huge step forward [74]. However, other types of cells, such as immune cells and endothelial cells, cannot be cultured yet with any of these models. An organoid-on-a-chip may be a possible solution [59]; this system can enable cell–cell, cell–matrix, and cell–media interactions, a feature that the current organoid system lacks. Currently, three-dimensional bioprinting is established in a bioprosthetic ovary [75] and cervical cancer model [76]. The organoid-on-a-chip can use the microfluidic technique to function as a self-assembling and self-organized organoid system. The microfluidic technique is applied on the endocrine loop involving the liver, ovary, fallopian tube, uterus, and cervix, which can communicate with each other within the system [77]. Another study used the microfluidic technique to coculture endometrial cells and endothelial cells and demonstrate steroid responsiveness [78]. Most importantly, the current organoid model needs to be improved. An organoid-on-a-chip system can provide a microenvironment conducive for cell–cell, cell–matrix, and cell–media interactions, and these can be taken up as future developments [Figure 1].

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Applications of the organoid model in ovarian cancer research.

**CONCLUSION**

The organoid model can serve as a sustainable model for ovarian cancer research. This model overcomes the shortcomings of cell lines and PDX models. Once organoid biobanking is established, this model can be used for ovarian cancer subtype culturing, drug screening, and genomic assessment [Figure 1]. In patients with cancer, this model can be used to test chemosensitivity and radiosensitivity and for improving the treatment response. However, the currently available organoid models possess some disadvantages, such as a moderate success rate, expensive culture media requirements, limited assay runs, and labor intensiveness. Moreover, they can only be used to culture epithelial cells. Therefore, in the future, an organoid-on-a-chip can be developed; this will provide a microenvironment for cell–cell, cell–matrix, and cell–media interactions. In conclusion, organoid models can be used in ovarian cancer research and can generate a simulated in vivo system, enabling studies on the heterogeneity of ovarian cancer.

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