

Review

Human-Derived Model Systems in Gynecological Cancer Research

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The human female reproductive tract (FRT) is a complex system that combines series of organs, including ovaries, fallopian tubes, uterus, cervix, vagina, and vulva; each of which possesses unique cellular characteristics and functions. This versatility, in turn, allows for the development of a wide range of epithelial gynecological cancers with distinct features. Thus, reliable model systems are required to better understand the diverse mechanisms involved in the regional pathogenesis of the reproductive tract and improve treatment strategies. Here, we review the current human-derived model systems available to study the multitude of gynecological cancers, including ovarian, endometrial, cervical, vaginal, and vulvar cancer, and the recent advances in the push towards personalized therapy.

Introduction

During human embryogenesis, the FRT arises from a common precursor, coelomic epithelium (also known as mesothelium) [1]. Despite the mutual origin, the adult FRT displays regional specification with distinct lineage-committed somatic **stem cells** (see Glossary) that self-renew the organ throughout life [2]. The proper functioning of each organ is required to ensure the overall reproductive capacity of the FRT; exemplified by the collective phase-dependent changes of the menstrual cycle [3,4]. This reflects the vast plasticity and proliferation capacity of the FRT. Consequential to such a continuous remodeling is the propagation of mutations in cells, which may lead to the development of cancers of the reproductive tract later in life. Indeed, the majority of gynecological cancers are commonly diagnosed in postmenopausal and elderly women [5]. In fact, imbalance in the levels of the primary female sex hormone, estrogen, and its receptors is associated with the etiology of many diseases, including but not limited to cancers of reproductive organs, wherein estrogen influences cancer initiation and progression [6,7]. Dependent on the exact location of origin, the FRT epithelium can give rise to a plethora of different cancer types with distinct genomic landscapes; most frequently to ovarian, endometrial, and cervical, but also, to a lesser extent, to vaginal and vulvar cancers (Figure 1). To study different epithelial gynecological cancers, transgenic mice have historically provided the opportunity to probe the effect of genetic hits on oncogenes and tumor suppressor genes towards cancer development in a near physiological environment [8]. However, in our pursuit of a representative model, we have to consider fundamental species-specific differences in the FRT, which raise questions about the extent of reliability that animal models can offer. For instance, female mice fail to spontaneously develop relevant gynecological tumors often observed in women with lack of intra-**tumor heterogeneity** and superior aggressiveness. To overcome such differences, alternative human-relevant models have been developed (Figure 2, Key Figure).

Immortalized Cell Lines

Primary cultures derived from gynecological cancers offer an *in vitro* model system for cancer research at a low maintenance cost, and have been invaluable tools for translational science, allowing genomic manipulation, cell biology studies, and high-throughput screenings beyond

Highlights

Tumors of the FRT represent a major gynecological burden, with high-grade serous ovarian cancer incurring the highest mortality rate.

Recent advances in sequencing technology and data mining have started to uncover the complex heterogeneity of gynecological cancers, reshaping our modeling approach.

Clinical management and overall 5-year survival rate have not substantially improved, due to decades of unsatisfactory models.

Tumor-derived organoids have emerged as an optimal compromise between *in vitro* and *in vivo* models, maintaining the flexibility of the former while capturing the complexity of the latter.

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what would be feasible in clinical trials or animal models. Such cell lines are generated from patient-derived tissues through immortalization; however, the success rate to establish a new line is often low and unpredictable [9]. When successful, these cell lines are regularly a product of long-term clonal selection and comprise a largely homogeneous cell population that no longer captures the cellular heterogeneity originally present. Importantly, key aspects of tumor metabolism such as nutrient and oxygen consumption are lost in a 2D environment and, even though they can generate tumors easily upon engraftment into immunocompromised hosts, the developed tumors still lack clinical relevance.

Many conventional 2D epithelial ovarian cancer (EOC) cell lines were established decades ago, when limitation in technology did not allow for their proper validation. Notoriously, an in-depth analysis of a panel of ovarian cancer cultures revealed a shocking truth that two of the most commonly used cell lines (SK-OV-3 and A2780) lack the main hallmarks of the high-grade serous ovarian cancer (HG-SOC) subtype they were originally believed to be derived from, including *TP53* mutations and extensive genomic instability, forcing a proper re-evaluation of many previous studies [10]. However, genome sequencing has made it possible to perform in-depth analysis of available EOC cell lines to better characterize their histological and molecular features, facilitating more intelligent selection of appropriate lines for subtype-focused studies [10–13]. Additionally, improved culture conditions have recently supported more successful derivation and maintenance of new EOC cell lines, which are thoroughly characterized and seem to better capture tumor heterogeneity than those established before [14,15]. A large proportion of EOC cell lines have been derived from malignant peritoneal ascites of neoadjuvant pretreated patients, and are resistant to platinum compounds among other drugs [16], providing a useful tool to study mechanisms by which cells acquire drug resistance. For example, the emerging role of miRNAs, such as miR-130 and miR-29 families, in regulating EOC chemoresistance has received increasing attention in recent years [17]. Additionally, intelligent attempts to integrate DNA, mRNA, and methylation alteration data gathered from EOC cell lines have started to reveal novel routes to achieve drug sensitivity [13].

For decades, cell lines established from endometrial adenocarcinomas have represented the cornerstone of endometrial cancer (EC) research. Molecular mechanisms fueling tumor growth, metastasis, and therapy response have been extensively studied using EC cell lines [18,19]. Genomic characterization of the most common commercially available EC cell lines detected **copy number alterations** (CNAs) and **single nucleotide variants** (SNVs) in top EC-mutated genes like *PTEN*, *PIK3CA*, *PIK3RI*, *CTNNB1*, and *KRAS* [20,21]. Such representative characteristics have made EC cell lines widely used to explore novel therapeutic approaches to target the PI3K/AKT pathway, uncovering a beneficial combination of PI3K and poly (ADP-ribose) polymerase (PARP) inhibitors towards *PTEN*-mutant lines [22]. Few EC cell lines retain hormone receptor expression; accordingly, its signaling has been investigated *in vitro*, highlighting the pivotal role of the transcriptional activator ETV4 in EC cell growth by controlling estrogen receptor genomic binding, which is particularly interesting for type I EC [23]. HEC1A and HEC1B are two EC cell lines derived from the same donor that differ in their microsatellite instability (MSI) status and have proved useful to outline the role of PMS2 in preserving EC genomic stability [24]. Chemoresistance remains a major hurdle in EC, and cell lines have been used to exploit its driving mechanisms, uncovering possible targets among noncoding RNAs and epigenetic regulators [25–27]. Nevertheless, EC cell lines harbor important shortcomings that limit their potential as valuable surrogates. Most of the commercially available lines are *TP53*-mutant and their phenotype is comparable with that of adenocarcinoma, covering only a minority of EC subtypes, leaving the majority of clinical cases under-represented.

Glossary

Clonal selection: biological process upon which mutational events confer a growth advantage to a single cell and its progeny which can therefore escape niche control.

Copy number alteration: aberration in chromosome structure that leads to a gain or a loss in DNA copies.

High-throughput: an experimental method that uses automated equipment designed for the simultaneous analysis of a large number of samples, impractical for conventional techniques.

Organoids: adult stem-cell-derived organotypic structures capable of long-term self-renewal *in vitro* in chemically defined media. Organoids recapitulate key biological features of the original tissue including morphology, function, gene expression, and differentiation dynamics.

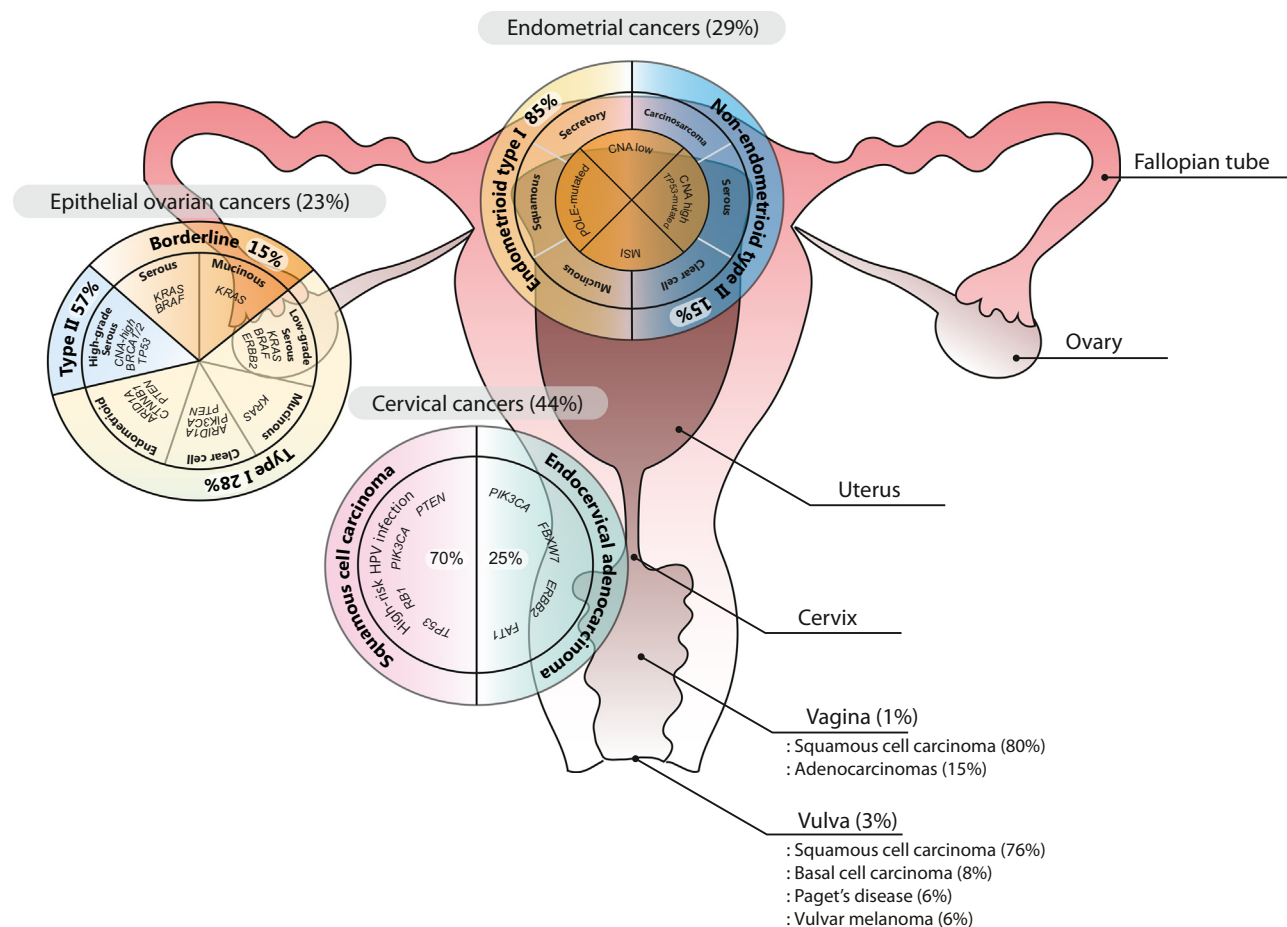
Single nucleotide variant: variation in a single nucleotide of the coding sequence of a gene that can either result in a silent, missense, or nonsense mutation.

Stem cell: undifferentiated cell with the unique capacity of unlimited proliferative potential and the ability to generate more specialized cells in each tissue.

Stem cell niche: specific area of a tissue with specialized cells directly interacting with stem cells by providing factors that induce either their self-renewal or their differentiation towards more functional cells. The presence of certain growth factors is geographically restricted within the niche creating a selective pressure towards mutations in tumor cells.

Tumor heterogeneity: refers to the presence of tumor cells with distinct profiles like cell morphology, gene expression, genetic mutations, and metabolism, as a consequence of clonal expansion of divergent clones.

Xenograft: transplantation of donor tissue into a host of a different species.



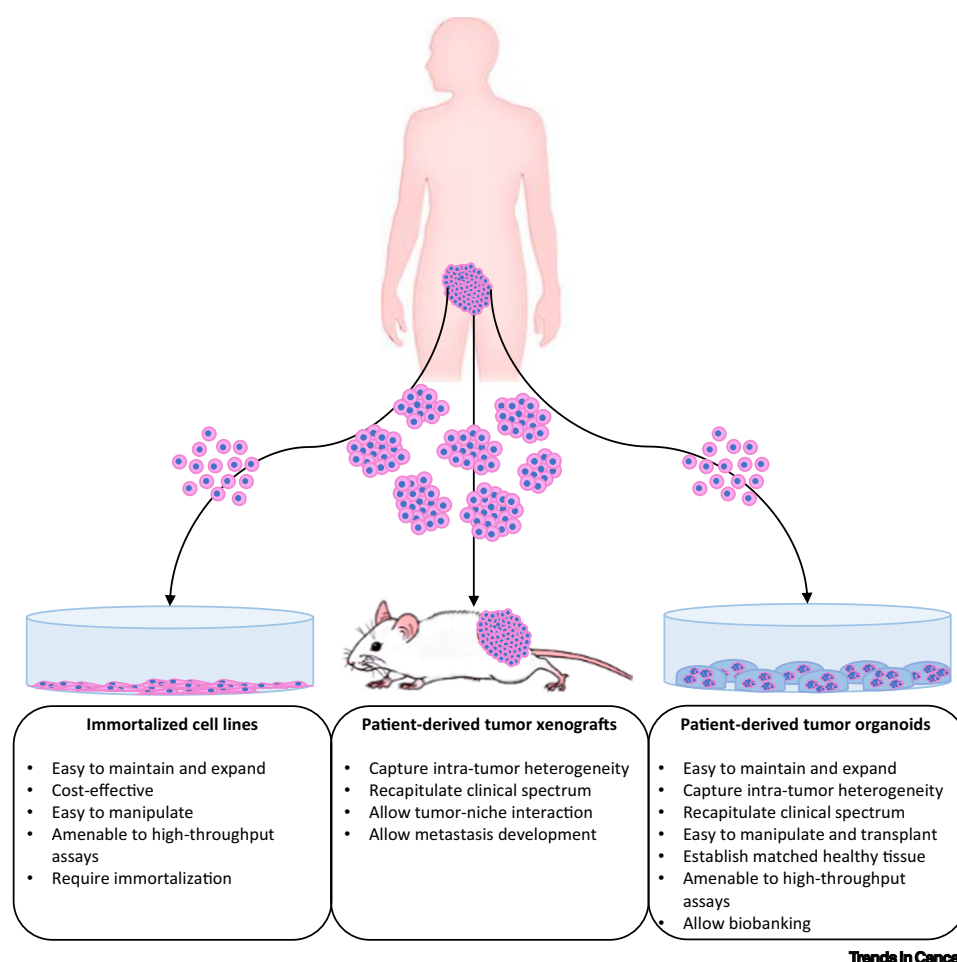
Trends in Cancer

Figure 1. Representation of the Main Tumor Types across the Female Reproductive Tract (FRT). Epithelial malignancies of the FRT affect mainly the ovaries, endometrium, and cervix. Each tumor type is represented with the appropriate clinical incidence and specific features such as histopathological spectrum and genetic alterations. Ovarian cancers are generally classified into borderline, type I (low grade) and type II (high grade) subgroups, with the latter covering most of the clinical cases. The more genomically stable low-grade and borderline tumors involve frequent alterations in *KRAS* and *PTEN*, while high-grade serous ovarian cancer is predominantly *TP53*-mutated and carry high CNA burdens. Mutations in *BRCA* genes predispose to ovarian cancer. Endometrial cancer is divided into two main subtypes. While the endometrioid type I is the more abundant, the nonendometrioid type II accounts for most deaths and recurrences. From a genetic standpoint, the serous type II endometrial cancer and most of the carcinosarcomas fall into the CNA high, *TP53*-mutated group, while the endometrioid subtype can be equally categorized into MSI and CNA low. Finally, a subcategory, marked by *POLE* mutations and displaying an ultramutator phenotype has better prognosis. Cervical cancers present as endocervical adenocarcinoma or squamous cell carcinoma. The adenocarcinomas are less common and are characterized by recurrent alterations in *PIK3CA*, *ERBB2*, *FBXW7*, and *FAT1* genes. The latter type accounts for 70% of diagnoses and is mainly caused by high-risk human papillomavirus infection. Mutations in *TP53*, *RB1*, *PIK3CA*, and *PTEN* are common in this type. Vulvar and vaginal cancers, typically diagnosed as squamous cell carcinomas, account for approximately 4% of malignancies in the FRT. Abbreviations: CNA, copy number alteration; MSI, microsatellite instability.

Immortalized cancer cell lines have also been instrumental in cervical cancer research and drug discovery. In fact, the oldest and most commonly used human cell line in cancer research, HeLa, was derived from a patient presenting with a particularly aggressive form of cervical adenocarcinoma [28]. Subsequently, additional cervical cancer cell lines emerged; however, new analyses have revealed several concerning findings that discourage the use of the conventional cancer cell lines due to evident divergences from the original tumor [29,30]. In regards to the tumor modeling, cervical cancer is a unique type, as the majority of them are caused by oncogenic human papillomavirus (HPV) infections [31] (Box 1). Both conventional immortalized and primary cervical cell cultures, however, fail to recapitulate the squamous differentiation of

Key Figure

Human-Derived Models for Gynecological Cancers



Trends in Cancer

Figure 2. Flowchart depicting the main models to study the tumors of the female reproductive tract. Tumor cells isolated from the resected specimen can be cultured indefinitely in 2D upon immortalization. Immortalized cell lines can be easily manipulated and subjected to high-throughput techniques such as drug screenings and genome editing. Despite being a cost-effective model, cell lines poorly recapitulate intratumor heterogeneity, the phenotypic diversity, and complex hierarchy of a tumor. Patient-derived xenografts (PDXs) are generated by heterotopic or orthotopic transplantation of tumor cells, isolated from biopsies, into immunocompromised mice. Despite the suboptimal establishment efficiency, PDXs preserve the heterogeneity of the primary tumor and allow tumor–niche interaction studies. PDXs can be amplified by sequential transplantation of tumor material over multiple generations. Finally, tumor cells can be embedded in extracellular-matrix-mimicking scaffolds (such as Matrigel, BME, or more chemically defined synthetic hydrogel systems, e.g. polyethylene glycol hydrogels) and, under appropriate medium conditions, cultured as 3D tumor organoids. Organoids capture features of the primary tumor and do not require immortalization for long-term propagation. These models are suitable for high-throughput applications, while their ease of manipulation is compatible with techniques across disciplines such as immunology and gene editing. The possibility of deriving matched healthy and tumor organoid pairs from the same donor is guiding the design of tumor-specific therapies. Living biobanks of patient-derived tumor organoids have been already generated for ovarian and endometrial cancer, recapitulating the subtype diversity of the diseases.

Box 1. HPVs in Cervical Cancer Oncogenesis

HPVs are a small group of nonenveloped icosahedral viruses with double-stranded circular DNA, coding for eight genes: E1–E7 (early genes) and L1 and L2 (late capsid-encoding genes) (Figure 1). HPVs belong to the *Papillomaviridae* family and can be further divided into three genera: alpha, beta, and gamma. While the alpha genus is mostly restricted to mucosal epithelial tissues such as anogenital tract and oral cavity, the cutaneous HPV types are represented mainly by the beta and gamma genera and are widely present in the skin of normal individuals. Alpha-HPVs are among the most commonly sexually transmitted infections and the majority of them is naturally cleared within 6–10 months. However, persistent infections with high-risk HPV types, such as HPV16 and HPV18, are known to be the etiological agents of mucosal cancers, including cervical cancer and its precursor lesions.

HPVs are extremely human species-specific and tissue-restricted viruses, showing high tropism towards stratified squamous epithelia, such as the ectocervix. HPVs can enter the targeted tissue via microabrasions in the genital epithelium and the viral infection is only productive when infecting the basal cells at the bottom of the stratified epithelium (Figure 1). HPV entry is mediated by initial binding to primary cell surface receptor, namely heparan sulfate proteoglycans [102], but it is also thought to involve secondary receptors, the identity of which is still debated. The subsequent conformational change in capsid proteins allows for virus internalization [103]; however, the exact mechanism is still poorly understood. Following endocytosis, the viral genome is transported to the nucleus for transcription and replication. Viral gene expression is strictly dependent on host cell differentiation, dynamically changing along the keratinocyte squamous differentiation trajectory. The new capsids are formed only at the most superficial layer of the epithelium, where cells die and shed off from the surface; at the same time releasing the progeny of new virions that enable to spread the infection (Figure 1). During the persistent infection, high-risk HPV DNA often integrates in the host genome, which causes changes in viral genome. Indeed, during integration viral E2 gene reading frame often gets disrupted, allowing for abrogation of E2-mediated transcriptional repression of the E6/E7 viral oncogene promoters [104]. Viral oncogenes (E6 and E7) directly interact with and inhibit the activities of two important tumor suppressor proteins, TP53 and RB1, respectively, targeting them for rapid proteasome-mediated degradation [105,106]. Taken together, such events promote HPV-associated carcinogenesis.

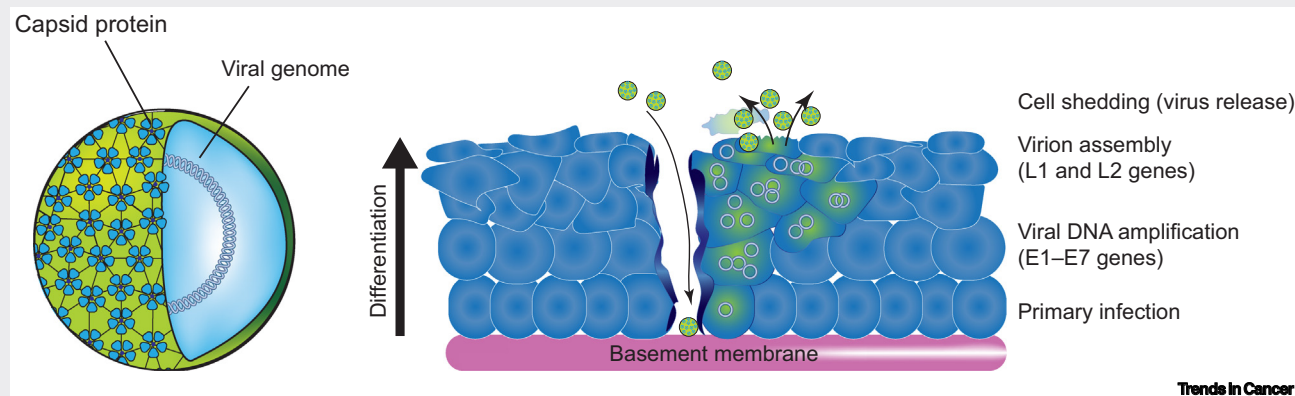


Figure 1. Human Papillomavirus Infection.

the ectocervix and thus do not support the stratification-dependent viral life cycle, rendering them largely unsuitable for cancer development studies. In order to generate models that permit squamous differentiation *in vitro*, more advanced organotypic raft cultures have been established. Raft cultures, where primary or immortalized human keratinocytes are seeded onto a gel and differentiated at the air–liquid interface, offered the first breakthrough in achieving keratinocyte differentiation *in vitro*, and supported the reproduction of the complete viral life cycle [32]. Still, these models do not support direct virus–host interaction and have short lifespans, hindering the studies on viral entry and the effects of long-term infection.

Vaginal and vulvar cancers are rare types of malignancies that together represent about 4% of all FRT cancers [33]. Due to their rarity, only a limited number of cytogenetically characterized cell lines for these cancer types have been reported [34–36], and most of the insight about relevant prognostic biomarkers for this cancer type has been simply gained by immunohistochemical or tissue microarray analysis of the resected specimens. Studies on vulvar cancer cell lines have demonstrated that these tumors are cytogenetically complex with multiple recurrent chromosome rearrangements [37]. Similar to cervical cancers, a proportion of vaginal and vulvar cancers is caused by infection with high-risk HPV, which is difficult to study in conventional culture systems.

In vitro human cell line models are commonly used for cancer pharmacogenomic and clinical response prediction studies. For example, NCI-60 is a traditional cell line panel that constitutes of a set of 60 cancer cell lines from nine different tumor types, and has been widely used for screening of anticancer compounds. However, among gynecological cancers, only ovarian cancer cell lines are represented in this panel. More recently, additional panels have emerged, such as GlaxoSmithKline (GSK) [38] and the Cancer Cell Line Encyclopedia (CCLE) [39], that combine larger set of cancer lines for chemical screening and omics data, also expanding the representation of gynecological tumors by inclusion of a number of endometrial and vaginal cancer cell lines. Cell lines with well-defined subtype-specific molecular alterations, such as BRCA1/2 or PI3K mutations, may be valuable tools for preclinical drug discovery, providing an opportunity to perform gene–drug association studies for specific patient populations. In this regard, the discovery of the efficiency of PARP inhibitors in the management of BRCA-deficient EOC was first realized in BRCA1/2-deficient cell lines [40,41]. Therefore, such models are useful for initial screening but the results always require proper validation.

Xenografts

Patient-derived **xenografts** (PDXs), in which cells or intact fragments from fresh human tumor tissue are transplanted into immunocompromised mice, have been instrumental in studies of *in vivo* chemotherapeutic responses and screening for novel compounds of clinical interest. Indeed, PDXs have been shown to recapitulate histopathological features of the primary tumor and maintain its molecular heterogeneity, even after propagation across multiple generations [42–45]. Furthermore, novel tools and methodologies seem to be emerging to facilitate the robustness of the PDX assays [46–48]. However, the genomic stability of the PDXs over serial passaging has been recently questioned on breast cancer models as **clonal selection** and rapid mouse-specific tumor evolution have been noted in two independent studies [49,50]. Such concerns have not been reported in the FRT PDXs to date. Although encouraging, this platform is not suitable for **high-throughput** screenings due to low rates of engraftment, slow tumor growth, and high costs. Additionally, PDX models cannot be genetically modified and rely entirely on the use of immunocompromised mice, precluding experimentation with novel immunomodulatory compounds. Pioneering work with establishing humanized mice that possess functional human immune systems might represent an alternative, despite challenging, solution.

PDX models have been developed from all the major EOC subtypes, with HG-SOC tumors showing the highest success rate [46,51]. Additionally, orthotopic PDXs have been shown to closely recapitulate EOC tumor progression, ascites formation, and metastasis as observed in human diseases [52]. Given their ability to maintain the original tumor heterogeneity, these models have been successfully exploited for platinum-based chemotherapy whose response seems to highly correlate with that of patients [53,54]. Targeted therapies have been applied as well, including PARP inhibitors on BRCA1/2-deficient xenografts [55] or HER2-targeted monoclonal antibodies on HER2-positive ovarian cancer PDXs [56]. Ovarian cancer PDXs have also been utilized to explore biomarkers and molecular mechanisms of chemoresistance. As an example, both CDK12 mRNA expression [57] and active Wnt signaling [58] in ovarian cancer PDXs have been linked to disease resistance to platinum therapy. Such features make PDXs a relevant model in EOC research.

Despite low engraftment rate, significantly more efficient for metastatic samples [42], EC PDX models have been developed and molecularly investigated to screen for a potential application of mammalian target of rapamycin (mTOR) and MEK inhibitors on tumors harboring *PTEN*, *KRAS*, and *PIK3CA* mutations [42,59]. Also, inhibition of AKT has been successfully exploited to restrain growth and invasion in a PDX model of EC [59,60]. An emerging strategy to address

MSI tumors, particularly relevant for EC, is through the modulation of the immune system with immune checkpoint inhibitors directed against the programmed death (PD)-1 and PD-ligand 1 proteins such as pembrolizumab, dostarlimab, avelumab, and durvalumab, which have shown success rates between 27 and 57% in clinical trials [61]. In this regard, PDXs in humanized mice might prove relevant preclinical models to address MMR-deficient and POLE-mutated ECs with novel combinatorial therapies, despite being a technical challenge.

PDX models have also been successfully developed from the two main subtypes of cervical cancer [62,63]. The tumor-take of these models has been reported between 48% and 70% [64,65]. Orthotopic cervical cancer PDXs show promise as they have demonstrated good correlation in transcriptomic landscapes as well as similar histological, metastatic, and stromal patterns when compared with the donor tissue [63]. These models also sustain causative high-risk HPV infection [64], which might be helpful in unravelling the viral gene expression dynamics in the already transformed cells, providing good opportunities for discovering novel therapeutic targets. In preclinical settings, HER2-amplified cervical cancer PDXs have shown promising results in terms of response to combined treatment with two clinically approved HER2 inhibitors, trastuzumab and lapatinib [66].

Compared with the aforementioned cancer types, no primary PDX models have been reported for neither vulvar and vaginal cancers to date.

Organoids

Throughout the last decade, human-derived cell culture models have rapidly evolved towards more advanced 3D organotypic cultures that hold promise in preclinical research. Stem cells-derived **organoids** are *in vitro* 3D cell cultures that closely recapitulate the biology and pathology of the primary tissue (Box 2). These minireplicas of organs or tumors do not require transformation for survival and allow for long-term expansion, while maintaining the genomic landscape of donor cells. As these systems support the growth of healthy epithelial cells, it gives the organoid models a considerable edge that no other human-derived model system has had before – suitability to study the early stages of tumor development in human-relevant settings [67]. Importantly, tumor organoids have been shown to capture inter- and intratumor heterogeneity and, as such, have potential in preclinical screenings.

Novel organoid cultures for EOC research have been steadily emerging throughout the last couple of years. Normal fallopian tube and ovarian-surface-epithelium-derived organoids that capture the transcriptomic and morphological features of the respective human tissues have been successfully established and offer a platform for studying cancer initiation from these potential origins [68,69]. To that end, human-derived fallopian-tube organoids have been shown to be susceptible to CRISPR/Cas9 genome editing by introducing knockouts of *TP53* and *RB1* genes [69], paving the way towards better characterization of ovarian cancer initiation and progression. In addition to the healthy organoid lines, both short- and long-term organoid cultures from a panel of solid ovarian cancer tissues, as well as from ascitic and pleural fluids, have been established, including all major subtypes of EOC [69–73]. Utilizing a novel single-cell DNA sequencing analysis, it has been confirmed that the organoids reliably maintain the tumor heterogeneity in culture, even after prolonged passaging [69]. A promising preprint on single-cell transcriptomic analysis of high-grade ovarian cancer organoids further supports the preservation of tumor heterogeneity in culture [74]. These recent studies have shown that tumor organoids recapitulate the histological and genomic features of original tumor subtypes and capture intra- and interpatient heterogeneity. Furthermore, the EOC organoids are suitable for medium-throughput drug screening assays [69,71–73,75] and have been shown to accurately predict clinical response of HG-SOC patients

to DNA repair inhibitors [70]. These advantages highlight the potential of tumor organoids to guide precision medicine, particularly in predicting patient-specific responses in preclinical drug screening.

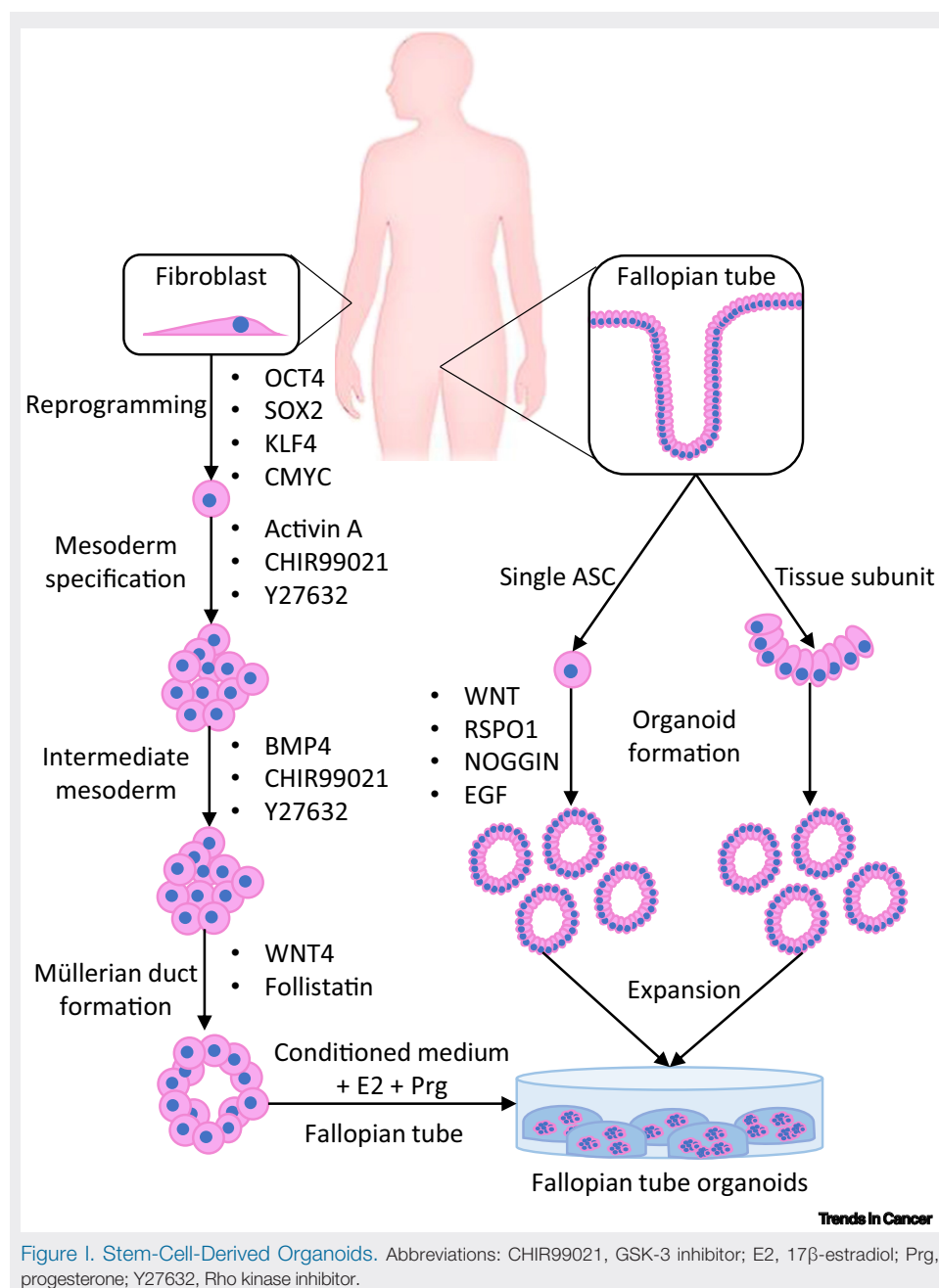
Despite the first organotypic model of human endometrium being reported in 1988 [76], progress in the field has been stalling for more than two decades due to inappropriate culture conditions. Recently, 3D adult stem-cell-derived endometrial organoids have been established and shown to recapitulate important aspects of endometrial biology, while maintaining genomic stability after extensive *in vitro* expansion [77,78]. Consequently, it was possible to establish patient-derived tumor organoids from both endometrioid and nonendometrioid subtypes that captured histopathological features of the primary tumor, such as hormone receptor status, preserved upon *in vivo* engraftment [79]. CNAs and SNVs were also conserved between primary tumor and organoids, covering the top mutated genes and genetic aberrations found in EC, such as *PTEN*, *PIK3CA*, *FBXW7*, *CTNNB1*, and *ARID1A* hotspot mutations [79,80]. Medium-based selection can be successfully used to confirm the association between genetic background of the organoids and niche factors independency. For instance, only *CTNNB1*-mutant lines expand in the absence of R-spondin (RSPO)-1 [79], as shown previously in other tumor organoids [81–83], demonstrating how niche composition shapes clonal selection in a tumor microenvironment. Encouragingly, the organoids have proved amenable to drug screening both as short- and long-term cultures showing line-dependent sensitivity to PI3K, mTOR, MEK, and histone deacetylase inhibitors, as well as common therapeutic drugs like paclitaxel and cisplatin [79,80,84]. Nevertheless, the preclinical predictive power of the organoids to guide individual therapies remains to be validated and no ongoing co-clinical trial has been reported so far.

Although reported for a single case of rare cervical clear-cell carcinoma [85], organoid cultures have not been published to date for the healthy cervix nor for the two most common

Box 2. Stem-Cell-Derived Organoids

Organoids, in their simplest definition, are 3D near-physiological structures that recapitulate key biological aspects of the original tissue. Two different stem cell types can support *in vitro* organoid formation: embryonic pluripotent stem cells (ESCs) or their *in vitro* artificial counterpart, the induced pluripotent stem cells (iPSCs), and tissue-committed adult stem cells (ASCs) (Figure I). Through the activation of developmental programs that lead to tissue specification, ESCs and iPSCs self-organize into organotypic structures that contain both epithelial and mesenchymal progenies. This is achieved by the sequential modulation of relevant pathways to induce lineage commitment towards anticipated fate. As for fallopian tubes, WNT, BMP, and TGF- β signaling activation is essential in the process of lineage specification towards Müllerian fate [107]. ESCs and iPSCs are routinely used to investigate the contribution of specific pathways to tissue development and how genetic defects interfere with it. At convenience, they serve as an alternative to more conventional patient-specific models in case of limitation in source tissue availability.

In adult tissues, there is a small population of ASCs with self-renewing and differentiation capacities located in a tightly regulated compartment termed the **stem cell niche**. This environment provides essential factors for stem cell maintenance, ensuring healthy tissue homeostasis throughout life. These cells can form organoids once embedded into an extracellular-matrix-mimicking scaffold and cultured in the presence of a cocktail of essential niche factors that promote proper mitosis, inhibit epithelial-to-mesenchymal transition, and prevent anoikis (a form of cell death of single cells). Commonly used niche factors in organoid cultures include WNT pathway activators (such as WNT3A and RSPO1) to retain stemness, NOGGIN to inhibit BMP-driven differentiation, and epidermal growth factor or fibroblast growth factors to induce proper mitosis. Among other tissues, these factors also enable the establishment of organoids from human fallopian tubes [68]. Under these serum-free culture conditions, single ASCs or tissue fragments, will undergo self-organization and morphogenesis to form miniature tissue replicas on the dish. Furthermore, upon tumor resection, or isolation of circulating tumor cells, organoids can also be established from malignant tissues. To avoid contamination with healthy epithelium, tumor cells can be positively selected by manipulation of culture media. As an example, this could be achieved by removing signaling factors such as WNT3A and RSPO1 or adding selection agents, such as Nutlin-3a, which negatively affects healthy cells and positively selects tumor clones due to mutations in core genes of the WNT or TP53 pathway, respectively.



cervical tumor subtypes, that is, squamous cell and adenocarcinomas. However, a promising preprint has been recently deposited to bioRxiv open repository that reports on establishment of human-derived normal ecto- and endocervical organoids that closely recapitulate the tissues of origin [86]. In our laboratory, we have made similar findings and, according to our unpublished data, long-term organoids could be also grown from cervical squamous cell and adenocarcinomas. These emerging hints will hopefully open up novel directions for the future of cervical cancer research, holding particular promise in advancing natural virus–host interaction studies.

There are currently no organoid cultures reported for vaginal nor vulvar cancers, or respective human normal epithelial counterparts.

Concluding Remarks

Over the past few decades, human-derived model systems for gynecological cancer research have made much progress. The systems have advanced from primary monolayer cultures to more advanced 3D models that better meet the demands of recapitulating the morphological features and the heterogeneity of donor tissues (see Outstanding Questions). In particular, 3D organoid cultures have emerged and quickly became a particular focus in cancer research, including in gynecological oncology. Indeed, tumor-derived organoids are rewiring our approach to address treatment strategies and improve therapy decision-making for individual patients. Although the amount of data collected on organoids derived from gynecological tumors so far is not as exhaustive as shown for other tumors [87–90], it supports their potential use in personalized cancer medicine, addressing type-specific features such as PTEN/PI3K pathway mutations for EC or use of PARP inhibitors for EOC. Moreover, as recently explored for other tumor types [87,90,91], organoids may serve as a companion diagnostic tool for clinical trials when screened in parallel with the same treatment option selected for the patient. Eventually, therapy response may be monitored in real-time in *in vitro* settings. In this particular scenario, combining multiomics analysis might help predict eventual drug resistance that can be targeted with a more-specific second-line intervention. Applying similar concepts for the most aggressive forms of gynecological cancers such as serous EC and carcinosarcomas, as well as HG-SOC, could help increase the therapeutic window and provide more specific options [75].

MSI and POLE mutations are recurrent features in EC [92]. The heavy mutational burden of these tumors confers high sensitivity to immune checkpoint inhibitors such as pembrolizumab and dostarlimab [61]. While the reconstruction of a human immune system in the host is challenging, the recombination of tumor-derived organoids with tumor-infiltrating T cells isolated from the same biopsy is easier [93]. Despite being still unproven for gynecological cancers, MMR-deficient patient-derived organoids and peripheral blood lymphocytes have already been cocultured for other tumor types and have shown promise to enrich tumor-reactive T cells, successfully probed for tumor-cell-killing activity *in vitro* [93,94]. In the field of cervical, vaginal, and vulvar cancer, studies about causative high-risk HPV infection have been hampered due to the difficulty of naturally infecting and completing the viral life cycle in conventional cell cultures, as well as due to the inability to track the infection-related events in the long term. The first hints about the emergence of cervical organoids that recapitulate the squamous differentiation and that can be expanded indefinitely will potentially offer a new tool to directly address questions about cervical oncogenesis that were not possible before. Similar models are demanded for rarer vaginal and vulvar cancers in the years to come.

The arrival of the sequencing era considerably increased the amount of data generated. In particular, integrated multiomics approaches improved our knowledge of the molecular basis of individual tumor types [92,95–99]. Such systematic analyses have already been applied to primary tumors of the reproductive system and enhanced our understanding of intratumor heterogeneity, which is now being harnessed at a single cell level [69,100,101]. In this regard, the organoids offer the potential to combine unlimited expansion with the requirements of a multiomics approach.

To conclude, with the rapid pace of technological advancements, cancer research has been offered a multitude of promising models whose full capacity should be thoroughly explored and benefited from to improve the treatment and prevention strategies for gynecological cancers in years to come.

Outstanding Questions

How can we improve gynecological cancer models to better understand mechanisms of tumor initiation, progression and metastasis?

How do we exploit the knowledge to start detecting the gynecological tumors at the early stage?

What is the predictive value of gynecological cancer models and can they feasibly assist in preclinical treatment decisions (are they compatible with clinical workflow)?

Could different tumor subtypes carrying identical aberrations benefit from the same treatment strategies?

Would it be feasible to build personalized cancer models for each patient or do we learn faster from the cohort studies to guide precision medicine?

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