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The latest animal models of ovarian cancer for novel drug discovery

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Abstract

Introduction—Epithelial ovarian cancer is a heterogeneous disease classified into five subtypes, each with a different molecular profile. Most cases of ovarian cancer are diagnosed after metastasis of the primary tumor and are resistant to traditional platinum-based chemotherapeutics. Mouse models of ovarian cancer have been utilized to discern ovarian cancer tumorigenesis and the tumor's response to therapeutics.

Areas covered—The authors provide a review of mouse models currently employed to understand ovarian cancer. This article focuses on advances in the development of orthotopic and patient-derived tumor xenograft (PDX) mouse models of ovarian cancer and discusses current humanized mouse models of ovarian cancer.

Expert opinion—The authors suggest that humanized mouse models of ovarian cancer will provide new insight into the role of the human immune system in combating and augmenting ovarian cancer and aid in the development of novel therapeutics. Development of humanized mouse models will take advantage of the NSG and NSG-SGM3 strains of mice as well as new strains that are actively being derived.

Keywords

Ovarian cancer; humanized mouse models; patient-derived xenografts; orthotopic

1. Introduction

Ovarian cancer is the seventh most common type of cancer in women worldwide [1]. At the time of diagnosis, only 15% of patients have localized disease and a 5-year survival rate of 92% [1]. In contrast, the majority of ovarian cancer patients are diagnosed with metastatic cancers with a 29% 5-year survival rate [1].

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Declaration of interest

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Ovarian cancer can metastasize via two distinct mechanisms. The first involves the original tumor expanding and directly infiltrating adjacent organs, including the bladder and colon. Alternatively, cancer cells can detach from the main tumor and seed the peritoneal cavity, an event typically associated with the formation of ascites [2]. Due to the ease by which ovarian cancer can metastasize, ovarian cancer patients often present with advanced pelvic disease, involving cancerous extensions into the uterus, fallopian tube, and ovaries [2].

The tumor microenvironment in ovarian cancer is highly immunosuppressive [3]. Myeloid leukocytes are one component of the microenvironment, supporting tumor growth through secretion of growth factors, inhibition of antitumor T cells through production of arginase, and maintenance and generation of the tumor vasculature [3]. Cancer-associated fibroblasts provide structural support for tumor growth and dissemination, while regulatory T cells (Tregs) promote immunosuppression and are associated with enhanced tumor growth. Even though their activity is often suppressed, T lymphocytes and NK-like T cytotoxic cells (CD3+ CD56+) are components of the microenvironment that exert pressure against the tumor [3,4].

Malignant epithelial tumors (carcinomas) account for 90% of all ovarian cancer cases and can be classified according to histopathology, immunohistochemistry, and molecular genetic analysis [5–7]. The five types of ovarian carcinomas include: high-grade serous carcinomas (HGSC), endometrioid carcinomas (EC), clear cell carcinomas (CCC), mucinous carcinomas (MC), and low-grade serous carcinoma (LGSC) [5]. HGSC accounts for over two-thirds of ovarian carcinoma cases. Until recently, these histotypes have not been differentiated in preclinical research or clinical trials [5]. However, these diseases are clinically distinct diseases that are dissimilar from each other. Integrated genomic analyses of ovarian carcinoma by The Cancer Genome Atlas (TCGA) Research Network showed that HGSC is genetically distinct from the other ovarian cancer histotypes [8]. HGSC is associated with mutations in the gene *TP53* and occasional germline mutations in the *BRCA1* and *BRCA2* genes [9]. Additional research has shown that two-thirds of LGSCs contain mutations in *KRAS*, *BRAF*, or *ERBB2* [7]. Most MCs are mutated in *KRAS* [10]. *ARID1A* mutations are found in half of CCs and one-third of ECs [7]. Histologically, these histotypes are classified according to the prominent pattern of differentiation of the tumor cells [6].

In order to enhance our understanding of the fundamental biological characteristics associated with ovarian cancer and to develop improved therapeutics, precise models must be established for EOC. Mice are often used as *in vivo* models for human disease, due to the similarity of the mouse and human genomes [11]. Many strains of mice have been used to interrogate the complexities of ovarian cancer and to discover and study the effect of putative treatments. However, only 5% of potential anticancer compounds that have been evaluated in mice demonstrated sufficient clinical activity in phase III clinical trials to eventually be licensed. These limitations highlight the necessity of improved preclinical models [12,13].

Successful mouse models for ovarian cancer drug discovery emulate the pattern of tumor growth and metastases that occur in the original patient and recapitulate the patient's response to therapeutics. Important factors to consider in mouse model design include tumor

cell source (i.e. cancer cell line or PDX), the location of transplanted tumor cells (i.e. orthotopic or heterotopic), and immune status (i.e. mouse immune system, human immune system, or immunocompromised) of the mouse. Attention to these considerations allows for the development of mouse models which are representative of both ovarian cancer histotype and the ovarian cancer tumor microenvironment.

This article focuses on the development of orthotopic, patient-derived xenograft (PDX), and humanized mouse models of ovarian cancer and the potential of these models to recapitulate human ovarian cancer and aid in drug discovery. Importantly, each of the aforementioned models plays an important role in the search for an accurate recreation of human ovarian cancer. For instance, orthotopic models allow for an examination of tissue site-specific pathology and studies of metastases. PDXs allow for a recreation of the molecular complexity and tumor heterogeneity associated with human ovarian cancer. Humanized mouse models provide insight into the dialog between the human immune system and tumor and are essential for the discovery and preclinical testing of immunotherapies. It is important to note that there are other models for ovarian cancer not described here, that is, syngeneic models and genetically engineered mouse models; however, these models warrant further discussion that is not within the scope of this article and is contained in several recent reviews [14–17]. Careful selection of preclinical models will enable the proper design and testing of novel therapeutics.

2. Orthotopic models for ovarian cancer using tumor cell lines

In orthotopic mouse models, tumor cells are transplanted in the anatomical location from which they were originally derived. The use of orthotopic transplantation has resulted in tumor models resembling human cancer with respect to tumor histology, vasculature, gene expression, response to chemotherapy, and metastatic biology [18]. In comparison to models based on subcutaneous tumor implantation, orthotopic models typically demonstrate the appropriate metastatic pattern associated with each disease [18]. Orthotopic models provide the necessary environmental regulators for cancer growth and an opportunity to study the microenvironment associated with the cancer of interest. The authors discussed below use varying surgical techniques to transplant tumor cells onto the mouse ovary, which is shielded from the peritoneal environment by a protective membrane called a bursa that provides a fluid chamber for oocyte development and ovarian function (Figure 1) [19–21]. These orthotopic mouse models have been generated using different ovarian tumor sources, that is, tumor cell suspensions, minced tumor fragments, tumor pieces, or cell sheet (CS) cultures.

Shaw et al. performed a comparative study of tumorigenesis in orthotopic and intraperitoneal models of ovarian cancer in CD-1 Nude mice using human ovarian cancer cell lines ES-2 and OVCA 429 [23]. The intraperitoneal ES-2 injected animals formed ascites with tumor cell accumulation on the omentum, which the authors classified as solid tumor. The intraperitoneally injected OVCA 429 injected animals formed solid tumors that adhered loosely to fat in the pelvic region, intestine, or omentum. Furthermore, the peritoneal cavities of these mice contained smaller tumors (less than 0.25 cm³) and bloody ascitic fluid. ES-2 and OVCA 429 were also injected intrabursally into CD-1 Nude mice, and both cell types resulted in tumor growth and enlargement of ovaries to 20 times their normal volumes.

However, the incidence of peritoneal disease was reduced compared to the animals injected intraperitoneally, suggesting that malignant cells are confined to the ovary by the bursal membrane. The bursal membrane is a unique feature in mice that separates the mouse ovary from the peritoneum. In contrast, the human ovary is located within the peritoneal cavity and not encased in a bursa, allowing for passive metastasis of tumor cells, which are carried by peritoneal fluid to the peritoneum and omentum [2,21]. This anatomical difference limits the ability of intrabursal ovarian cancer models to accurately mimic metastasis in human ovarian cancer [2].

One technical difficulty of orthotopic model establishment through ovarian bursa injection is potential cell leakage into the peritoneum. Cordero et al. highlight the importance of technical precision during establishment of an intrabursal orthotopic mouse model [24]. Removal of the needle from the bursa must be quick enough to seal the puncture site and prevent the leakage of injected cells but gentle enough not to tear the bursa. Zhang et al. developed an alternative method for orthotopic ovarian cancer mouse model generation that prevents bursal leakage [25]. In their method, a tumor fragment was surgically transplanted onto the ovary, and the ovary was subsequently wrapped with a hemostatic gauze that contained carboxymethyl cellulose and replaced into the body cavity to protect against displacement of the tumor graft and bursal leakage. Prior to harvest, the tumor fragment utilized to generate the orthotopic mouse model by Zhang et al. was harvested from a tumor that was established by subcutaneous injection of human ovarian cancer cells (HO-8910PM) in the scapular region of a female, Nude mouse. Sixty days after transplantation, abdominable masses could be palpated. Ovarian cancer occurred in 100% of mice. Intraperitoneal spread and ascites occurred in all mice, and metastatic foci were found in the omentum, intestine, liver, diaphragm, and lungs, events consistent with late-stage cancer.

Yi et al. examined additional methodologies for the establishment of orthotopic ovarian cancer models. In their studies, BALB/c mice were implanted with the mouse 4T1 breast cancer cell line, which was stably transduced with luciferase, to establish three different orthotopic models of ovarian cancer [26]. The models used either a suspension of cultured cells, a suspension of cells ground from subcutaneously transplanted tumors, or tumor pieces from subcutaneously transplanted tumors. The first model utilized a suspension of cultured cells and showed bioluminescence throughout the entire abdominal cavity, perhaps due to leakage after injection of the suspended cells into the ovary. The second model employed ground tumor cells from a previously generated subcutaneous tumor and showed limited bioluminescence after 1 week. The authors attributed this result due to decreased cell viability as a result of grinding the tumor cells. The third model that was established using tumor pieces obtained from the subcutaneous tumor resulted in bioluminescence localized to the ovary 1 week after implantation and expanded through the mouse's entire body after 50 days. The authors concluded that whole tumor pieces are advantageous compared to cell suspensions for orthotopic engraftment. Although this study has some technical challenges and used a breast cancer cell line, it highlights the advantage of using a whole tumor fragment for model development.

Arauchi et al. investigated the use of engineered CS cultures for the development of an alternative orthotopic ovarian cancer mouse model [27]. CS cultures allow for delivery of

tumor cells in the form of an intact cell monolayer. In contrast to conventional single-cell suspensions, CS cultures preserve all cell–cell junctions, receptors, and the relevant extracellular matrix. CS cultures are generated by growing cells on commercial plastic cell culture dishes, which are grafted with a thin layer of temperature responsive poly(N-isopropylacrylamide) (PIPAAm). Reducing temperature from 37°C to 25°C allows for detachment of the cell-sheet without enzymatic treatment. Noncancerous CS cultures have been utilized to improve graft quality and survival compared to tissue derived from injected cell suspensions [28]. Arauchi et al.'s syngeneic model utilized murine ID8 cancer cells, which is a murine cancer cell line derived from C57BL/6 mice, to form cancerous CS cultures. CS cultures were transplanted directly into the left ovarian bursa of C57BL/6 mice, allowed to adhere for a few minutes, and followed over time. A significant difference in tumor growth kinetics was found between tumors that were generated using CS cultures compared to single-cell suspensions. After 8 weeks, the weight of ovaries, which had CS culture implants increased more than twofold compared to tumors derived from single-cell suspensions. Further, at the same time point, twice as many metastases were observed for mice that received CS cultures compared to single-cell suspensions. Immunostaining was performed on both tumors derived from single-cell suspensions and those derived from CS cultures. Tumors derived from CS cultures stained positive for fibroblast activation protein (FAP) and matrix metalloproteinase-2 (MMP-2) 1 week after cell-sheet transplantation. For the single-cell suspension-derived tumors, FAP and MMP-2 staining were strongest 12 weeks after transplantation.

The CS culture approach may prove advantageous compared to use of single cell suspensions due to speed at which tumorigenesis occurs. Within 1 week, ID8 cell-derived tumors in the ovarian bursa were invading the ovary as detected by H&E staining with histological characteristics that emulated human spontaneous epithelial ovarian cancer. Enhanced speed of tumorigenesis may result due to inherent advantages in CS culture technology, which allows for preservation of the extracellular matrix [29].

3. PDX models

PDX models of ovarian cancer are derived from patient material, including primary tumor tissue or ascites fluid. PDXs are presumed to faithfully represent the genomic features of patient tumors, but a recent report suggests that copy number alterations occur during PDX passaging, rendering the PDX tumor genetically different from its matched primary tumor [30–34]. Similarly, most cancer cell lines have been passaged *in vitro* for years and due to the genetic modifications for growth on solid supports have lost their genomic similarity to the tumors from which they were derived [7]. Recent analysis by Domcke et al. revealed that many of the ovarian cancer tumor cell lines, which are most commonly used in *in vitro* and *in vivo* studies of high grade ovarian cancer, lack genetic similarity to patient tumors [7]. Furthermore, until recently, the histological subtypes of ovarian cancer (HGSC, EC, CCC, MC, and LGSC) were not differentiated in preclinical research or clinical trials, even though these ovarian cancer subtypes share few molecular similarities [35,36].

The first PDX model of cancer was established by Rygaard and Povlsen in 1969 by subcutaneously implanting fragments of human colon tumor into Nude mice [37]. All three

mice that received fragments of the human tumor developed tumor masses. This study provided proof-of-principle for feasibility of the development of PDX mice. In 1993, Fu et al. used this approach to develop ovarian cancer PDX mice [38]. This model was established orthotopically by transplantation of intact pieces of human ovarian tumors into the ovarian bursal membrane. Tumor engraftment was successful in two of five nude mice (40% tumor take) with a pattern of tumor growth and metastasis that was similar to that observed in the patient.

The research groups described below have developed PDX models for ovarian cancer to evaluate the antitumor responses in response to platinum-based chemotherapeutics using either Nude mice, severe combined immunodeficiency (SCID), or NOD.Cg-*Prkdc*^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice (Table 1). These three immunodeficient mouse strains have different genetic backgrounds that allow for engraftment of tumor cells. Briefly, Nude mice are T cell deficient; SCID mice are T cell and B cell deficient; and NSG mice lack B cells, T cells, NK cells, are hemolytic complement deficient, and have defective macrophages and dendritic cells [39,40]. A more detailed description of these immunodeficient mice is found in the Expert Opinion section of this chapter.

Ricci et al. developed PDX models of ovarian cancer in Nude mice implanted using tumor cells from clinical primary ovarian tumors and ascites fluid [41]. In total, 34 of 138 patient samples resulted in the formation of epithelial ovarian cancer xenografts. Of the mice that developed tumors, 22 xenografts were established subcutaneously by transplanting 2–4 mm pieces of intact tumor tissue into the flank; the remaining 12 xenografts were established through injection of tumor cells from ascites fluid into the peritoneal cavity of the mice. Subcutaneous and intraperitoneally transplanted xenografts displayed a significant difference in growth rate (1–15 months vs. 1–4 months, respectively), as defined by the time necessary for the xenograft to reach 1 g.

In order to recapitulate the dissemination pattern of ovarian cancer, a subset of the patient samples which formed subcutaneous or intraperitoneal xenografts were utilized to establish orthotopic PDX models by injecting tumor cells into the ovarian bursa [41]. Tumor dissemination was evaluated by scoring the size of metastases in the liver, diaphragm, omentum, pancreas, uterus, ovary, and lymph nodes. Diffuse tumor dissemination in the peritoneal cavity was observed in the orthotopic PDX mice, and the tumor dissemination scores for the orthotopic PDX models were similar to those established intraperitoneally. Furthermore, the ability of the orthotopic PDX mice to form ascites was evaluated and found to be correlated with the source of tumor cells. Xenografts that were established using tumor cells from patients' ascites resulted in ascites whereas xenografts generated from solid tumors did not.

The molecular features of the PDX xenografts were also compared to the original human ovarian cancer tumors. The mutational status of the xenografts was compared to DNA from the patient tumors. A total of 18 out of 23 (73%) xenografts displayed the same mutational status as the original patient tumor. Xenografts derived from HGSC patients harbored *TP53* mutations whereas xenografts derived from LGSC, CCC, EC, or MC exhibited wildtype *TP53*. The copy numbers of different genes, including *MET*, *MYC*, *PI3Ka*, *PTEN*, *FGFR1*,

ERBB2, *NF1*, and *RBI*, were determined for the xenografts and compared to the patient tumors. Although the distributions of these genes were similar, the xenografts contained higher copy numbers, suggesting genomic instability of the xenografts. Additionally, genes of the human immune system were downregulated in the xenografts compared to the patient tumors, suggesting a loss of human-infiltrating immune cells. The response of the PDX xenografts to cisplatin and paclitaxel was compared to the response of the corresponding patient. Nine out of 11 PDX xenografts had cisplatin responses, which correlated positively with the patient's antitumor response. This model highlights the ability of PDXs to serve as therapeutic testing platforms.

An additional group utilized a PDX model of epithelial ovarian cancer for preclinical drug evaluation of paclitaxel-carboplatin combination chemotherapy and the epidermal growth factor receptor (EGFR) inhibitor (erlotinib) [42]. Heo et al.'s PDX model was established by engraftment of a fresh fragment of epithelial ovarian cancer tissue obtained after primary or secondary debulking surgery into the subrenal capsule of the left kidney of a female BALB/C-nude mice. A total of 22 out of 45 patient samples successfully engrafted. Select PDX models were chosen and serially passaged *in vivo* to evaluate ovarian cancer therapies. A HGSC PDX was utilized to evaluate the effect of paclitaxel-carboplatin chemotherapy. The patient corresponding to the selected PDX had undertaken primary surgical debulking, which resulted in no residual cancer. The patient was treated with six cycles of adjuvant combination chemotherapy with paclitaxel and carboplatin. The PDX was responsive to the same chemotherapy regimen, suggesting that patient-specific response to therapy can be recapitulated by PDXs. Heo et al. also evaluated the promise of erlotinib in a PDX model of CCC. A strongly EGFR expressing EGFR responded to erlotinib therapy, suggesting this drug as a potential treatment for CCC.

Topp et al. utilized PDX models to understand cisplatin resistance in HGSC patients [31]. Fragments of chemotherapy-naïve, fresh ovarian cancer tumors were obtained from 12 HGSC patients and confirmed by immunohistochemistry to be HGSC, as evidenced by mutations in *TP53* and expression of *WT1*, *PAX8*, and *MKI67*. The PDX models were established by implanting unmanipulated tumor fragments either intrabursally or subcutaneously. PDX models were established in NSG mice by engrafting the unmanipulated tumor fragments either intrabursally or subcutaneously. Ten out of 12 (83%) tumor fragments xenografted successfully, regardless of transplantation method (i.e. intrabursal or subcutaneous). The intrabursal and subcutaneous xenografts both contained serous papillary structures, consistent with HGSC and retained baseline gene expression.

In this same study, the PDX mice were also utilized to determine the response to cisplatin [31]. Four PDX mice were sensitive to cisplatin or had a decrease in tumor volume with complete remission; three PDX mice were resistant to cisplatin or had an initial decrease in tumor volume followed by progressive ovarian cancer within 100 days; and the final three PDX mice were refractory to cisplatin treatment or failed to respond to treatment. Similar cisplatin responses were observed in mice that received serially passaged xenografts. PDX mice that had recurrent tumors were subjected to second-line cisplatin treatment. After the conclusion of second-line treatment, the time in days for the tumor to reach a volume of 0.5 cm³ was shorter than that of first-line treatment. The PDX mice that were resistant to

cisplatin treatment contained xenografts which were derived from tumor tissues that had high expression of oncogenes at baseline, including increased expression of *CCNE1*, *MYCN*, *BCL2*, or *LIN28B*. The response of the PDX mice to cisplatin was consistent with matched patient data. This study provided a mechanism to understand platinum-based therapeutic resistance.

Dobbin et al. investigated the ability of SCID PDX models to maintain the heterogeneous cell populations exhibited in patient tumors [43]. One subpopulation of cells within the tumor, cancer stem cells (CSC), is of interest due to its enhanced ability to initiate tumors. Immunohistochemistry confirmed that the PDX xenografts and patient tumors exhibited concordant expression of CSC markers ALDH1A1, CD133, and CD44. The effect of carboplatin/paclitaxel treatment was explored by comparing matched treated and untreated PDXs. IHC staining for markers ALDH1A1 and CD133 increased after treatment, suggesting that treated tumors are enriched in CSC populations. RNA-Seq revealed differential expression of five major pathways (protein kinase A signaling, GNRH signaling, Sphingosine-1 phosphate signaling, α -Adrenergic signaling, and cholecystokinin-/gastrin-mediated signaling) and key changes in molecular and cellular function between treated and untreated PDXs, suggesting that tumors respond to chemotherapy and modulate cellular function to ensure survival. This study revealed the ability of PDXs to model patient tumor heterogeneity, which could allow for the discovery of new treatment strategies.

Weroha et al. developed a tumor bank of PDX models for ovarian cancer [44]. PDX models were established by intraperitoneal injection of 241 ovarian, primary peritoneal, or fallopian tube tumors that were collected at the time of surgery from patients at the Mayo Clinic; an engraftment success rate of 74% was obtained. The mouse xenografts developed recapitulate ovarian cancer patient metastatic patterns and clinical response to carboplatin/paclitaxel chemotherapy. Significantly, this PDX library is heterogeneous and exhibits molecular diversity, which reflects the diversity seen in primary patient tumors. The presence of PDXs derived from common pathological subtypes and PDXs for less common subtypes enables these models to be utilized to develop subtype specific treatments.

Although the PDX models described above are advantageous for the testing of therapeutics, some limitations exist. For example, ovarian, breast, colon, pancreatic, bladder, and renal cancer PDXs are susceptible to the formation of human lymphocytic tumors and must be thoroughly evaluated for lymphocytic markers to ensure that the PDX model generated resembles the original patient's cancer [45,46]. A study by Butler et al. showed that B-cell lymphoma can be suppressed in ovarian cancer PDXs by inhibition with rituximab [46]. An additional limitation of PDXs is genomic instability, which manifests as copy number alterations after *in vivo* passaging of tumors [34]. This limitation may influence the response of the PDX to therapeutics. PDX copy number alterations influence the efficacy of therapeutics in PDX models of pancreatic cancer, colon cancer, and breast cancer [34]. These limitations should be taken into consideration by researchers developing ovarian cancer PDX models.

4. Humanized mouse models

An additional advance in the establishment of mouse models for ovarian cancer involves the use of ‘humanized mice.’ There are many types of humanized mouse models that contain human immune cells which may contribute to or prevent tumorigenesis. Different humanized mouse models are employed to evaluate the efficacy of immunotherapy for ovarian cancer treatment.

Bankert et al. utilized a humanized mouse model for ovarian cancer to evaluate exogenous IL-12 as a potential immunotherapy [47]. A humanized ovarian cancer mouse model was established by delivery of tumor-derived cell suspensions, which contained CD45+ leukocytes, cytokeratin positive cells, and trichrome positive collagen, which is produced by fibroblasts, into NSG mice by intraperitoneal injection. This mouse model is considered humanized, because tumor-associated CD3+ T cells, CD20+ B cells, and CD138+ plasma cells co-engrafted within tumor nodules and remained functional. This model was utilized to evaluate human IL-12 loaded liposomes as a potential immunotherapy for ovarian cancer. In comparison to mice receiving control liposomes, mice receiving human IL-12 loaded liposomes resulted in the production of IFN γ , suggesting that human tumor-associated T lymphocytes or possibly NK cells are functional within the tumor. Furthermore, human B-cells present in the tumor were also active and resulted in the presence of human Ig in the sera of the IL-12 liposome treated mice.

Koneru et al. also validated IL-12 based immunotherapy in an ovarian cancer mouse model humanized with chimeric antigen receptor T (CAR T) cells [48]. Armored CAR T cells are directed to MUC16^{ecto}, the retained extracellular portion of a glycosylated mucin protein found on the majority of epithelial ovarian cancer patient cells and secrete IL-12. In SCID-Beige mice, which contained intraperitoneally injected SKOV3 ovarian cancer cells, armored CAR T cell treatment resulted in the eradication of tumor within 2 weeks of treatment.

Chang et al. utilized a humanized mouse model to evaluate the effect of a monoclonal antibody (mAb) immunotherapy which depletes regulatory T cells and restores antitumor immunity in ovarian cancer [49]. The mouse model used human peripheral blood lymphocyte NSG (huPBL-NSG) mice, or mice which have received adult human peripheral blood lymphocytes. A mouse model for ovarian cancer was established using the ovarian cancer cell line Igrov-1, which secretes the chemokine CCL22 and attracts Tregs to the tumor site via their chemokine receptor CCR4. Igrov-1 cancer cells were injected subcutaneously into the dorsolateral flank of the huPBL-NSG mice to establish xenografts. Subsequently, luciferized human Tregs were administered to the mice intravenously in combination with anti-CCR4 humanized monoclonal antibodies that deplete Tregs (mAb2–3 IgG1) or that prevent Treg accumulation at the tumor site (mAb2–3 IgG4). In comparison to control antibody, bioluminescence imaging showed that Tregs were depleted by mAb2–3 IgG1 and were diffusely redistributed in mice treated with mAb2–3 IgG4 but were no longer accumulating at the tumor site. This experiment showed the promise of humanized mouse models for understanding the mechanisms of action of new immunotherapies. In a second experiment, Chang et al. showed that mAb2–3 IgG1 and mAb2–3 IgG4 treatment resulted in

a decrease in the size of Igrov-1 xenografts. Mice were humanized by intravenous injection of Igrov-1 specific T cells (TP-T cells) that were educated *in vitro* [49]. Characterization of the TP-T cells revealed that the population contained 31.6% CD25⁺CCR4⁺ T cells. In response to twice weekly treatment with mAb2–3 IgG1 and mAb2–3 IgG4 over the course of 5 weeks, the size of the tumors decreased, suggesting that the mAb treatment reversed immune suppression by Tregs. Collectively, these experiments show the power of humanized mouse models for testing novel ovarian cancer immunotherapies.

5. Conclusion

The aforementioned studies demonstrate that preclinical ovarian cancer mouse models provide lessons in ovarian cancer biology and aid in the discovery of novel ovarian cancer therapeutics. Careful design of orthotopic, PDX, humanized, or combination ovarian cancer mouse models allow investigators to understand metastases, drug resistance, tumor composition, and the immune component of ovarian cancer. These insights will assist in the unearthing and evaluation of new therapies.

6. Expert opinion

The previously discussed models provide insight into facets of ovarian cancer that will lead to drug discovery (Figure 2). Orthotopic and humanized mouse models promote an understanding of the role of the ovary and immune microenvironment in tumorigenesis, respectively, allowing for the discovery of therapies that modulate the tumor microenvironment. Equally important, PDXs reflect the tumor heterogeneity of ovarian cancer patients and are more reflective of human patient response to therapy. In combination, these models will lead to accurate preclinical evaluation of ovarian cancer therapeutics.

Future advances in ovarian cancer mouse models will take advantage of novel strains of immunodeficient mice that yield enhanced engraftment of tumor cells and human immune cells to further enhance understanding of the ovarian cancer tumor microenvironment and aid in the development of therapies. These humanized mice will be useful to understand platinum resistance and the influence of immune cells on platinum resistance. A recent report suggests that chemoresistance is mediated by stromal fibroblasts and that CD8⁺ T cells abolish fibroblast-mediated chemoresistance by secretion of IFN γ [50]. Furthermore, humanized mice are essential preclinical models for the development of human immunotherapies. This is evident by the inconsistency between anti-CTLA-4 efficacy in a preclinical syngeneic mouse model and in a Phase II study of ipilimumab (anti-CTLA-4) monotherapy (NCT01611558) [51,52]. In the syngeneic mouse model, anti-CTLA-4 treatment led to 50% tumor regression. In contrast, the overall response rate for patients in the Phase II clinical trial of ipilimumab was 10.3%.

The most common strains of mice that have been utilized for *in vivo* ovarian cancer mouse models are the Nude mouse and the SCID mouse. The Nude mouse contains the autosomal recessive *nu* mutation, which causes thymic dysgenesis [53]. Consequently, Nude mice lack T cells and suffer from a lack of cell-mediated immunity. While Nude mice offer the

advantage in that tumor growth can be easily observed due to their absence of hair, tumor xenografts in Nude mice are constrained by poor tumor growth, host-versus-graft reactivity, and an altered tumor microenvironment, due to the presence of functional humoral immunity and NK cells in these mice [54]. The SCID mouse contains a *scid* mutation which causes defective rearrangement of genes that code for antigen-specific receptors on B cells and T cells and consequently deficiencies in mature B and T cells [40]. Although the SCID mouse supports local and metastatic growth of tumor cells, SCID mice are prone to the development of thymic lymphoma and leakiness in which T cells and B cells develop in aged mice [39].

In contrast, the NSG mouse, which was utilized by Bankert et al., Topp et al., and Chang et al. is a severely immunodeficient strain of mouse, which can be readily humanized and engrafted with tumor cells [31,47,49]. The NSG mice have a *scid* mutation in the DNA repair complex protein *Prkdc* and a complete null allele of the IL2 receptor common gamma chain, characterized by an absence of T cells and B cells, a lack of NK cell function, defective complement, reduced levels of macrophage activation, and abnormal dendritic cell development. Furthermore, these mice contain a mutation in the IL2-receptor common gamma chain gene (*IL2rg^{null}*) of NOD-scid mice, which causes a lack of signaling through the IL2, IL4, IL7, IL9, IL15, and IL21 receptors.

NSG mice can be engrafted with a human immune system through introduction of human peripheral blood mononuclear cells or hematopoietic stem cells (HSCs). HSCs may be derived from several tissue sources, including umbilical cord blood, bone marrow, fetal liver, and G-CSF-mobilized peripheral blood [55]. Mice which are engrafted with HSC (hNSG) develop multilineage immune cells, including functional B cells and T cells, macrophages, monocytes, neutrophils, basophils, and mast cells [56]. These mice also support engraftment of tumor cell lines and PDXs. NSG mice which are engrafted with HSC and PDXs are available from a variety of vendors. The Jackson Laboratory offers these mice as part of their Onco-Hu mouse platform [56]. Tumor engraftment occurs despite HLA mismatch between the PDX tumors and the HSCs used for humanization, which may represent a limitation to these humanized mouse models [56,57]. However, a study by Wang et al. reveals the promise of these models for evaluating the efficacy and mechanisms of PD-1 targeted cancer immunotherapy [58]. A comparison between PDX mouse models of non-small cell lung cancer, sarcoma, bladder cancer, and triple negative breast cancer in nonhumanized NSG mice and in humanized NSG mice revealed that pembrolizumab (anti-PD-1 mAb) selectively inhibits tumor growth in humanized NSG mice. Inhibition of tumor growth was dependent on hCD8+ T cells, highlighting the power of the hNSG model for interrogation of immunotherapy mechanisms of action. Furthermore, hNSG mice from different HSC donors responded differently to pembrolizumab, recapitulating the clinical observation that not all patients respond to pembrolizumab. These models are applicable to ovarian cancer, because many HGSCs exhibit overexpression of PD-L1 which is correlated with decreased survival [52]. Moreover, the PD-1 and LAG-3 co-inhibitory molecules are frequently expressed by tumor-infiltrating lymphocytes in ovarian cancer [59]. Dual blockade of PD-1 and LAG-3 augments proliferation of CD8+ T cells, suggesting that these checkpoint inhibitors are putative therapeutic targets for ovarian cancer [59]. Future ovarian

cancer studies will utilize Onco-Hu mice to understand the efficacy of checkpoint blockade inhibitors.

A derivative of the NSG mouse called the NSG-SGM3 mouse may also be useful for future ovarian cancer studies. NSG-SGM3 mice express the hematopoietic human cytokines stem cell factor, granulocyte macrophage colony-stimulating factor, and IL-3. Similar to NSG mice, NSG-SGM3 mice also support robust engraftment with human immune and tumor cells. NSG-SGM3 mice that are humanized through introduction of HSC have increased cell counts of CD33+ myeloid cells, hematopoietic stem cells, myeloid progenitor cells, mast cells, myeloid dendritic cells, CD3+ T cells, CD4+ T helper cells, CD8+ T cytotoxic cells, and Tregs (CD4+ CD25 + FOXP3+) [56]. The increased number of myeloid cells and Tregs in comparison to humanized NSG mice will make these mice useful for future ovarian cancer mouse models. The immunosuppressive myeloid lineage tumor-associated macrophages (TAMs) are the most abundant infiltrating immune cell in tumors and ascites [60]. TAMs in ovarian cancer resemble M2-polarized macrophages and express the immunosuppressive chemokines CCL18 and CCL22 [60]. CCL22 causes recruitment of Tregs to the tumor site [60]. Higher intratumoral ratios of CD8T cell/Treg or CD4T cell/Treg are correlated with increased survival in epithelial ovarian cancer [61]. NSG-SGM3 mice may be utilized to understand how these immunosuppressive elements influence the course of ovarian cancer. Use of humanized mice coupled with advances researchers described in this review have utilized, such as orthotopic engraftment and PDX xenografts, will enable a better understanding of human ovarian cancer metastases and the ovarian cancer immune infiltrate.

Moreover, recent advances in the areas of transcriptomics, flow cytometry, and immunohistochemistry will enable researchers to unlock the breadth of information contained within humanized mouse models. For instance, single-cell RNA sequencing has been utilized to identify extensive heterogeneity within both tumor cell and tumor-infiltrating immune cell populations in breast cancer [62]. A multiparameter flow cytometry panel capable of measuring 51 immune cell populations showed that neutrophils dominate the immune environment of non-small cell lung cancer [63]. Furthermore, multiplex immunohistochemistry enables the simultaneous, geographic detection of 12 biomarkers and has been utilized to show that CD8+ T cell and PD-L1 status are correlated with outcomes of vaccinated pancreatic ductal adenocarcinoma patients [64]. Collectively, the information garnished from these studies will allow for the development of novel ovarian cancer treatments with increased therapeutic efficacy.

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Article highlights

- A wide variety of orthotopic, PDX, and humanized mouse models have been developed for ovarian cancer.
- Orthotopic mouse models enable recapitulation of the microenvironment of the ovary, allowing for metastasis studies.
- PDX models for ovarian cancer have been utilized to determine therapeutic efficacy of novel drugs but may not represent the precise genomic features of patient tumors.
- Humanized mouse models provide a platform to investigate the influence of the human immune system on tumorigenesis.
- These models may aid in the testing of novel immunotherapeutic anti-cancer drugs.

This box summarizes key points contained in the article.

Figure 1.
(a). Ventral view of a female NSG mouse showing reproductive organs (b). Diagram of mouse ovary and associated tissues; reproduced with permission from [22] with permission of Nature Publishing Group.

Figure 2. Advantages of orthotopic, PDX, and humanized models for showing metastasis, drug efficacy, and the tumor microenvironment.

Table 1

Important insights gained from PDX models of ovarian cancer.

Year	Investigators (Reference)	Strain of mice	Conclusions
1993	Fu et al. [38]	Nude	Orthotopic PDXs emulate tumor growth and metastasis of matched patients
2014	Ricci et al. [41]	Nude	PDXs had higher copy numbers than matched patients but emulated patient response to cisplatin/paclitaxel therapy
2014	Topp et al. [31]	NSG	PDXs are utilized to understand platinum-based therapeutic resistance
2014	Dobbin et al. [43]	SCID	PDX models maintain the heterogeneous cell populations of patient tumors
2014	Weroha et al. [44]	SCID	A tumor bank of 241 PDX models which represents common pathological subtypes and less common subtypes can be utilized for drug discovery
2017	Heo et al. [42]	BALB/C Nude	CCC PDXs respond to erlotinib therapy, which is a potential treatment for CCC
2017	Butler et al. [46]	SCID	Rituximab prevents the formation of lymphoproliferative tumors in ovarian cancer