Emerging and Evolving Ovarian Cancer Animal Models

Supplementary Issue: Animal Models of Cancer Biology

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ABSTRACT: Ovarian cancer (OC) is the leading cause of death from a gynecological malignancy in the United States. By the time a woman is diagnosed with OC, the tumor has usually metastasized. Mouse models that are used to recapitulate different aspects of human OC have been evolving for nearly 40 years. Xenograft studies in immunocompromised and immunocompetent mice have enhanced our knowledge of metastasis and immune cell involvement in cancer. Patient-derived xenografts (PDXs) can accurately reflect metastasis, response to therapy, and diverse genetics found in patients. Additionally, multiple genetically engineered mouse models have increased our understanding of possible tissues of origin for OC and what role individual mutations play in establishing ovarian tumors. Many of these models are used to test novel therapeutics. As no single model perfectly copies the human disease, we can use a variety of OC animal models in hypothesis testing that will lead to novel treatment options. The goal of this review is to provide an overview of the utility of different mouse models in the study of OC and their suitability for cancer research.

KEYWORDS: ovarian cancer, high-grade serous ovarian cancer, xenograft, patient-derived xenograft, genetically engineered mouse models, Adeno-Cre

Introduction

Every year in the United States, over 22,000 women are diagnosed with ovarian cancer (OC) and more than 15,000 die from the disease.[1] The poor prognosis for women diagnosed with OC is largely because of the presence of local and distant metastases in 81% of patients at diagnosis, leading to 5-year survival rates of 69% and 30%, respectively.[1] These numbers suggest that both early detection and treatment of metastatic disease are crucial in managing OC. These facts also highlight the need to better understand mechanisms driving progression from primary tumor to metastatic disease.

Ovarian tumors can arise in the ovary from germ cells, stromal cells, or epithelial cells. The vast majority (>90%) of OC arises from epithelial tissue. There are several distinct histotypes of epithelial OC. They are high-grade serous (HGS) and low-grade serous ovarian, mucinous, endometrioid, clear cell, and transitional or undifferentiated.[2] HGS is the most common form of OC. The high mortality associated with OC is a direct result of the process of tumor metastasis. OC invasion is a complex process that differs from metastatic programs of other solid tumors, since OC tumor cells shed from the primary site and spread throughout the peritoneal cavity without first entering the blood or lymphatic system.[3] OC was originally thought to be derived from the ovarian surface epithelium.[4] However, epidemiological, pathological, and molecular studies have made scientists question the origin of OC and hypothesize that different types of ovarian tumors may arise from different precursor cells, including those from the fallopian tube, endometrium, cervix, and gastrointestinal (GI) tract.[2,5–7] In addition to the OC subtypes being histologically different, they are also genetically distinct. Ovarian tumors have been divided into two classes. (1) Type 1 cancers progress from tumors with lower malignant activity, are usually low grade, and contain Ras pathway mutations; these include clear cell, mucinous, endometrioid, and low-grade serous tumors. (2) Type 2 tumors arise rapidly de novo and contain *TP53* mutations; these include HGS, undifferentiated, and carcinosarcomas.[8] Recent work suggests that some HGS tumors may be initiated in the fimbria of the fallopian tube.[8,9] Identifying the cell type of origin for OC will change how animal models are generated and evaluated.

The cause of mortality from most solid tumors is metastasis. Frequently, as OC progresses, tumor cells accumulate as single-cell and multi-cell aggregates in the ascites fluid.[10] The accumulation of ascites fluid in late stage cancer correlates with poor outcome.[11] These shed tumor cells metastasize locally. Preferred sites of metastasis include the omentum, peritoneal wall, the diaphragm, and small bowel mesentery.[3]

Therefore, design and utilization of mouse models of OC that recapitulate the steps of metastasis are critical. Here, we will review many of the common ways OC is studied in mice. We will first discuss xenografts derived from cell lines and patient samples, followed by genetic models of OC.

Xenograft Models of OC

Xenograft models are a common way of investigating OC growth, metastasis, and treatment response in a live animal. The process of injecting ovarian tumor cells into mice and monitoring tumor growth has been conducted since at least the early 1980s. In these early studies, the thymus of the mice was removed, mice were treated with cytosine arabinoside, given whole-body irradiation, and then engrafted subcutaneously (SC) with either ovarian ascites ($n = 5$) or a solid peritoneal metastasis from a papillary cystadenocarcinoma ovarian tumor biopsy ($n = 8$).[12] Four of the peritoneal metastases established tumor growth.[12] Xenograft studies have been used extensively since the 1980s and offer many advantages. Xenografts are effective at measuring tumor growth in response to mutations or exogenous gene expression. They allow for validation of therapies and studies of drug resistance. Xenografts also can faithfully recapitulate aspects of ovarian tumor progression and metastasis. However, xenografts do not recapitulate the initial transforming events leading to tumorigenesis. Furthermore, many xenograft mouse models are conducted using immunocompromised mice, which will lack the contribution of the full complement of immune cells to the tumor growth process.

The use of xenograft models enables researchers to test factors that influence tumor growth, spread, and drug response in a live animal. These elements cannot be entirely recapitulated in tissue culture. To study tumor growth in mice, murine or human OC cells are injected into mice. Cells from different genetic backgrounds are injected into immunocompromised mice such as Nude (Foxn1, Nu/Nu), SCID, NOD/SCID, or NOD-scid IL2Rcnull (NSG) to enable the cells to engraft without being eliminated by the immune system. Cells are injected SC, intraperitoneally (IP), or intrabursally [(IB) into the bursa that surrounds the mouse ovary]. The SC model is not well suited for ovarian metastasis studies as the tumors do not typically metastasize, and the tumor is not positioned in the right anatomic location or microenvironment. One advantage of the SC model is that it is well suited for investigation with imaging modalities such as two-photon microscopy (Fig. 1). In two-photon microscopy, tumor vasculature (with fluorescent dextrans), collagens (with second harmonic imaging), and fluorescently labeled tumor cells can be measured simultaneously in a live animal. This cannot be done with IP or IB injections because of the depth of the tumors. However, IP and IB tumors can be imaged in live animals with optical imaging approaches using fluorescence or luminescence. In contrast to SC tumors, IP and IB injections of tumor cells into mice can mimic aspects of tumor metastasis, particularly
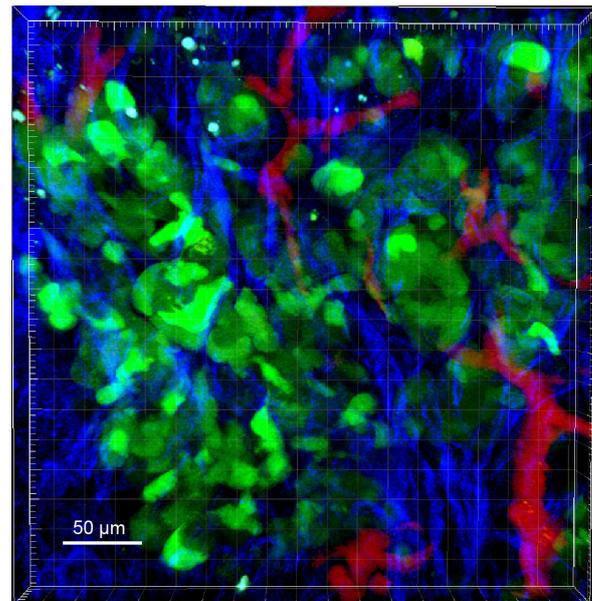

Figure 1. Two-photon microscopy image of SKOV3IP tumor expressing green fluorescent protein (GFP). SKOV3IP labeled with GFP were injected SC into nude mice. After one week, two-photon microscopy was conducted to image tumor cells (green), collagen (blue), and vasculature (red). Therefore, imaging subcutaneous tumor can provide information about how genetic variation affects tumor microenvironment.

metastatic dissemination. While IP injection cannot mimic the initial steps in metastasis, IP-injected tumor cells such as SKOV3 metastasize to the ovary, peritoneal wall, diaphragm, and form ascites fluid similar to human disease.[13] The SKOV3IP cell line is derived from ascites cells that developed in a mouse IP cavity injected with SKOV3 cells.[13] Compared to the parental SKOV3 cells, the SKOV3IP cells grow faster, disseminate more, and exhibit overexpressed ERBB2.[13] Many cells (including the SKOV3 lines) are frequently transfected or transduced with fluorescence or luminescence-expressing vectors to monitor tumor growth in vivo.[14] Similarly, OVCAR3 cells metastasize to the GI tract, omentum, pancreas, kidney, and liver (unpublished data) when injected IP. In some ways, the IB injection mimics the initial steps in metastasis, as the tumor cells exit the bursa to spread throughout the peritoneal cavity. Many cell lines metastasize following IB injection, including A2780, SKOV3, and HEY cells.[15,16] Sites of metastasis include diaphragm, mesentery, bowel, and liver.[16] In particular, the A2780 cells have been used to study not only metastasis but also chemoresistance. A2780 that are sensitive to cisplatin and a subline of A2780 cells that are resistant to cisplatin cells are also commonly used in xenograft studies of chemoresistance in vivo.[17] Many other cell lines not discussed also demonstrate growth in xenograft models.[18]

Compared to genetic models, xenograft models may require less time to form tumors, from a few weeks to a few months, depending on the cell line and the number of injected tumor cells. Tumor cells can be genetically manipulated in

culture, and then implanted in mice (Fig. 2). This allows for the study of how individual genes impact tumor development or metastasis. However, by using xenografts in immunocompromised mice to study OC, the response of the immune system is ignored.[19] A thematically related model of OC is patient-derived xenografts (PDXs). To derive PDXs, ovarian tumors are isolated from patients, and then surgically grafted or injected into mice. This system provides distinct advantages over using traditional xenografts with cell lines. Tumors from diverse genetic backgrounds can be grafted, and the tumor growth can be monitored. Furthermore, the PDXs recapitulate aspects of OC like metastasis and ascites formation. Typically, small fragments of a tumor are isolated and injected SC or orthotopically into immune compromised mice. Tumor engraftment percentages and time to develop tumors vary widely,[19] and some xenograft studies result in less than 50% of the mice developing tumors.[20] After the tumor reaches a critical size, it can be excised and reimplanted serially into subsequent mice. The original tumor and the serial PDX tumors are characterized for gene expression and mutation profile to assess if the PDXs retain the genetics and behaviors of the original tumor.

PDX models are used to examine tumor progression and metastasis when the PDXs are implanted into the peritoneal cavity. In one model, human OC biopsies were dissociated and injected in NSG mice.[21] The tumor aggregates that were injected contained tumor-associated cells, including lymphocytes and fibroblasts.[21] These tumors metastasized to the omentum, ovary, pancreas, uterus, spleen, and liver. Ascites also developed in these mice. Twenty-six percent of mice were found to have lung micrometastases as commonly found in stage IV disease.[21] Furthermore, a large study of 241 tumors demonstrated that the PDXs retain the genetics and metastatic spreading pattern of the original tumors.[22] Minced tumors were injected IP into female SCID mice, and 74% (168) of these tumors engrafted, mostly in the pelvis.[22] Almost 50% of the mice developed metastases in the intestines, mesentery, liver, spleen, diaphragm, and/or omentum, and 17 of these mice developed ascites.[22] One intriguing study found that PDXs with ascites or plural effusions were more likely to generate xenograft tumors than solid tumors.[23] This raises questions about the metastatic unit in OC. OC stem cells that have been isolated from serous adenocarcinomas based on surface expression of CD177 and CD44 were demonstrated to have increased tumorigenic capacity in xenografts.[24] A more recent study used a PDX model of OC to demonstrate that high expression type I receptor tyrosine kinase-like orphan receptor in OC stem cells results in higher tumorigenesis.[21,25] More studies on the link between cancer stem cells and metastasis should be conducted using models like the PDXs to discover novel strategies to limit metastatic spread and appropriately target cells with metastatic potential.

One of the major advantages of the PDX models is the ability to investigate the response of tumors to therapeutic agents. Treating PDX tumors with IL-12, FLT-3, and
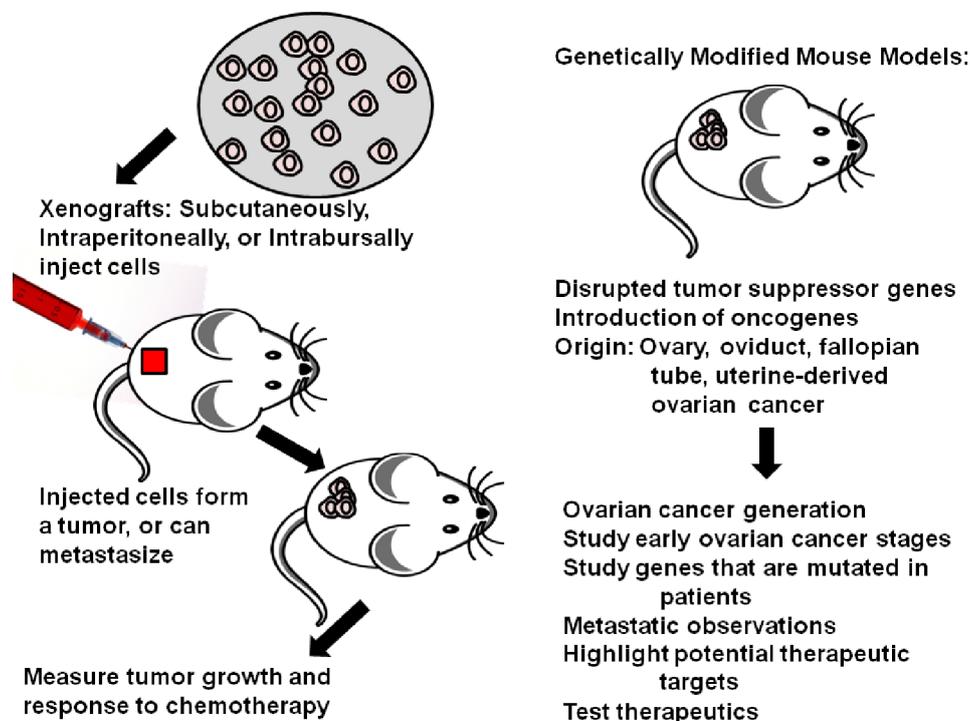
Figure 2. Tumor cells can be genetically manipulated in culture, and then implanted in mice to form a tumor. Growth of the tumor can be measured as well as response to chemotherapy. Genetic models of cancer have increased our understanding of how OC originates and what mutations or combinations of mutations may lead to tumor establishment by disruption of tumor suppressor genes, introduction of oncogenes, and tumor origin manipulations. These provide valuable information on metastasis and highlight potential therapeutic targets.

CD40 decreased tumor growth.[26–28] Similarly, PDX tumors have been studied for their ability to predict patient response to drug therapy (previously reviewed by Scott et al).[19] In one study, HGS tumors were engrafted into NGS mice with an 83% success rate.[29] After treatment with cisplatin, it was found that 40% of the mice were platinum sensitive, 30% were refractory, and 30% were platinum resistant.[29] These responses correlated with patient outcome,[29] which suggests that PDXs retain the genetic/epigenetic program of the original tumor and that the response to standard chemotherapy present in the original tumor is retained. Therefore, PDXs may someday be used to develop personalized patient treatment plans. One disadvantage of PDXs is the variable take rate of the tumors. Additionally, access to patient samples is an impediment to using PDXs as a model system.

Syngeneic mouse models of OC have also been developed. Murine ovarian surface epithelial cells from C57BL/6 mice were transformed (ID8 cells), and then injected back into C57BL/6 mice so that tumor metastasis or growth can be monitored using mice with intact immune systems.[30] More recent syngeneic mouse models use cells with spontaneously generated increased aggressiveness to study resistance to anoikis.[31] The tumors generated in this syngeneic model develop extensive metastasis in the peritoneal cavity reminiscent of advanced OC. McCloskey et al also described a strategy for creating spontaneously transformed ovarian surface epithelial cell lines by maintaining cells for many passages. These transformed cells exhibited aberrant Wnt and NFKB signaling and resulted in tumors resembling HGS cancer when injected into the IP cavity of FVB/N mice.[32] The syngeneic experimental design more accurately reflects the response of the immune system during tumor progression than immunocompromised models. However, the cells are of murine origin, and thus may not resemble human cancer as well as models using human cells.

Genetically Engineered Mouse Models of OC

Genetically engineered mouse models (GEMMs) have yielded many important advances in understanding cancer. Mouse models of leukemia, breast cancer, and colon cancer can faithfully recapitulate many aspects of human disease. The OC genetic models have been more complicated to generate, partially because of the lack of understanding of OC biology and the heterogeneity of OC. Infertility resulting from ovarian tumors has also hampered generation of transgenic models of OC. The controversy about the cell type of origin for OC and identification of transgenic promoters that reflect the proper cell type of origin are further complications. GEMMs are especially important for studying the beginning stages of OC that cannot be mimicked in xenografts. Some OCs may originate in the fallopian tube, while others may arise from ovarian epithelium.[5] Different GEMMs allow us to evaluate how tumors arising from mutations at different anatomic locations compare to human disease. Thus, GEMMs provide

an invaluable tool for studying the development of OC with mutations observed in patients. For example, women with familial *Brca1* mutations have increased the risk of developing OC and develop cancer at a younger age than those with sporadic disease.[33] Thus, mouse models with alterations to *Brca1* and other genes commonly mutated in OC, such as *Tp53*, *c–Myc*, *Kras*, *Akt*, and *Brca2*,[34–36] are important to increasing our understanding regarding how genetic predisposition leads to OC (summarized in Table 1).

The most common mutation in HGS OC occurs in *Tp53*,[37] which is mutated in about 48% of sporadic OCs and 96% of HGS cancers.[37,38] In addition, *Rb1* is dysregulated in about 67% of HGS OCs.[38] GEMMs can be used to study these tumor suppressors as well as oncogenes such as *c–Myc*, *Kras*, and *Akt1*. An early method for inhibiting both of *Tp53* and *Rb1* is to express *Simian virus 40* (SV40) large T antigen that binds to and inactivates both *p53* and *pRB*.[39] Transgenic mice expressing SV40 under control of the promoter for Mullerian inhibiting substance type II receptor (MISIIR) develop poorly differentiated ovarian carcinomas.[40] MISIIR is expressed in 92% of OC, but most nongynecological tissues do not express this receptor.[41] These carcinomas express cytokeratins 8 and 19 and lack alpha-inhibin, suggesting that they are derived from epithelium and not derived from granulosa cells.[40] These tumors result in ascites formation and metastatic spread to omentum.[40] One mouse demonstrated metastasis to the kidney and one had metastasis to the uterus, but metastasis to other organs was not observed.[40] This was the first published model of a transgenic model of epithelial OC. A major disadvantage to this approach is that the mice were infertile because of the ovarian tumors. Therefore, other approaches to inactivate tumor suppressors that may be more indicative of human cancer needed to be developed.

Conditional knockouts of *Tp53* and *Rb1* have also been used to generate mouse models of OC. As reported by Flesken-Nikitin, mice with *Tp53* and *Rb1* flanked by loxP sites (floxed) were injected into the bursa ovary with an adenovirus expressing Cre recombinase under control of a *CMV* promoter.[34] This double knockout resulted in a metastatic OC resembling human ovarian epithelial cancer, which spread to the lung, liver, and noninjected ovary.[34] A total of 97% of the mice with knockout of *Tp53* and *Rb1* in the ovary died within eight months.[34] Single knockouts of either *Tp53* or *Rb1* were much less efficient at inducing metastatic cancer: 13% of *Tp53* knockout mice and 3% of *Rb1* knockout mice developed tumors.[34] Importantly, these studies reveal that *Tp53* mutations alone are sufficient for ovarian tumor initiation, but mutation in both *Tp53* and *Rb1* lead to metastatic OC.[34]

In a more recent study, combinations of *Tp53* mutation or loss, loss of pocket protein function, and loss of *Brca1/2* were generated to thoroughly examine the contributions of these genes to OC. Expression of T_{121} (a domain of the SV40 large T antigen) in the surface epithelium is induced

Table 1. Summary of GEMMs.

REFERENCES	TRANSFORMING GENES	METHOD OF INDUCTION	TISSUE ORIGIN	HISTOLOGY
Flesken-Nikitin et al[34]	Tp53, Rb1	Injected Cre adenovirus-induced knockout	Ovary	Serous epithelial ovarian cancer
Szbova et al[35]	Tp53, Rb "pocket proteins", Brca1/2	SV40 T antigen expression, Injected Cre adenovirus-induced knockout	Ovary	Serous epithelial ovarian cancer
Orulic et al[36]	C-myc, K-ras, Akt1	Syngeneic cells transduced with RCAS viral vectors injected into p53-null mice	Ovary	Undifferentiated carcinoma
Connolly et al[40]	Tp53, Rb1	Chimeric expression of SV40A under MISIIR promoter	Ovary	Poorly-differentiated neoplasia
Xing et al[44]	C-myc, Tp53, Brca1	Syngeneic cells transduced with RCAS viral vectors, Cre-induced knockout	Ovary	Serous epithelial ovarian cancer
Perets et al[45]	Brca1, Brca2, Tp53, Pten	Cre-recombinase driven by Pax8 promoter	Fallopian tube	Serous tubal intraepithelial carcinomas
Sherman-Baust et al[46]	Tp53, Top2A	Expression of SV40A under Ovp-1 promoter	Fallopian tube	Serous tubal intraepithelial carcinomas
Tirodkar et al[47]	Pten, K-ras, Muc1	Injected Cre adenovirus-induced knockout	Ovary, oviduct, and uterus	Endometrioid adenocarcinoma
Wu et al[50]	Pten, Apc	Injected Cre adenovirus-induced knockout	Uterus	Endometrioid adenocarcinoma

by injecting Cre-expressing adenovirus (Adeno-Cre). T$_{121}$ is in a Cre-conditional loxP-GFP-stop-loxP (LSL) cassette driven by the cytokeratin 18 promoter. Injection of Adeno-Cre resulted in inhibition of *Rb1*, *p107*, and *p130*, also known as the "pocket proteins." In this study, antigen T$_{121}$ is used in concert with floxed alleles for *Tp53*, a point mutation in *Tp53* (*p53*^{R172H}), and inactivation of either *Brca1* or *Brca2*.[35] Inactivation of the pocket proteins was sufficient for tumorigenesis, resulting in stage I serous epithelial OC.[35] However, loss of *pRb*, *p53*, and *Brca1* or *Brca2* in mice more closely mimics serous OC.[35] When *Brca1* or *Brca2* were deleted in this model (in addition to inhibition of *Tp53* and the RB pathway), the tumors metastasized to the liver and demonstrated a gene expression profile resembling serous OC.[35] A later version of this GEMM was used to study drug response in *Brca1*-deficient mice, showing that tumors in these mice respond to a combination chemotherapy of cisplatin and olaparib.[42] This provides an example of how GEMMs can be used to test effective therapies. Through these varying combinations of genetic alterations, different stages of OC progression were modeled, from early stage I epithelial OC (Rb pathway disruption alone) to an advanced metastatic cancer (*RB loss/Tp53/Brca1/Brca2* alterations).

In addition to disrupting tumor suppressor genes, introduction of oncogenes has also been employed to generate mouse models of OC. Transgenic mice expressing the avian retroviral receptor TVA (with α-actin or keratin 5 promoter) were generated.[36] These mice were then crossed with *p53* null mice.[36] Ovarian surface epithelial cells were isolated from the mice and transduced in vitro with replication-competent avian-leukemia-virus splice-acceptor (RCAS)[43] viral vectors. These viral vectors expressed common OC oncogenes: *c-Myc*, *Kras*, or *Akt1*. Subcutaneous tumor formation resulted when any two of these oncogenes were expressed in ovarian cells that were injected SC into nude mice.[36] IB injection of the β-actin-TVA/p53$^{-/-}$ cells with the RCAS viruses containing *c-Myc* or *Kras* oncogenes resulted in metastatic OC.[36] Metastasis to the liver, spleen, intestine, kidney, and malignant ascites was observed.[36] This result mirrors that of later mouse models from the same laboratory, showing that TVA/RCAS-induced expression of *c-Myc* is sufficient to induce transformation in ovarian cells from mice with floxed *Tp53* and *Brca1*.[44] These transformed cells displayed chromosomal defects and an unusually high centromere count; injection of these modified cells into the ovary resulted in metastatic tumors that resemble serous ovarian carcinomas and spread to the peritoneum, pancreas, and intestines.[44] Collectively, these models demonstrate that *Tp53* mutation in concert with oncogene expression or loss of other tumor suppressors results in OC generation and metastasis.

As our understanding of the genetics and epigenetics of OC evolves, so must the ways of modeling the disease. It has been hypothesized that different cell types of origin and different genetic mutations are responsible for the distinct histological

subtypes of OC. Therefore, establishing models of fallopian tube and uterine-derived OC is essential. To determine if high-grade serous ovarian cancer (HGSOC) can originate from fallopian tube, Pax8-driven Cre recombinase was used to excise *Brca1*, *Brca2*, *Tp53*, and/or *Pten*.[45] Loss of *Brca1/2*, *Tp53* mutation, and *Pten* loss resulted in fallopian tube serous tubal intraepithelial carcinomas (STICs), ovarian metastases, and peritoneal metastases.[45] The histology, metastatic profile, and transcriptome of these tumors closely mimics HGS OC.[45]

Another method to transform the fallopian tube used SV40 large T antigen expressed under control of the Mullerian *Ovgp-1* promoter.[46] This model results in STICs and invasive adenocarcinomas that spread to the ovaries.[46] Therefore, this model is capable of recapitulating early development of OC[46] in the fallopian tube. One additional advantage of this model is that the *Ovgp-1* promoter ensures tissue-specific expression of SV40 in mice that have reached sexual maturity.

Since the ovary, uterus, and fallopian tube may be sites of origin for different types of OC, a comparison of transformation at each of these locations was conducted. To compare tumorigenesis in the ovary, oviduct, and endometrium using the same genetic backgrounds, a triple transgenic mouse model was created.[47] In these mice, Kras^{G12D} (using a LSL cassette) is induced, and floxed *Pten* is deleted using Adeno-Cre in different locations of the female reproductive tract.[48] Additionally, these transgenic mice also express the human glycoprotein MUC1 that is often overexpressed in OC.[48] Injection of Adeno-Cre into the ovary, oviduct, or uterus resulted in the formation of epithelial tumors capable of metastasis, spreading to the pancreas and diaphragm.[47] However, the nuclear grade of the tumors and survival of the mice varied depending on the anatomical site of injection.[47] Tumors arising from ovaries, the oviduct, and uterus generated endometrioid histology and metastasis to spleen, diaphragm, and liver were detected.[47] The oviduct tumors developed into poorly differentiated high-grade tumors, while ovarian and uterine tumors were more differentiated.[47] Mice with ovarian and oviduct tumors exhibited the shortest mean survival times compared to mice with uterine tumors (89, 82, and 132.5 days, respectively). A key feature of this model is that overexpression of *MUC1* results in a similar immune response as that seen in human patients.[47] This enables testing of immunotherapy treatments that target OC cells overexpressing *MUC1*.[47]

While many of the recent GEMMs are aimed at understanding the origin of OC or recapitulating HGSOC, mouse models of other OC histotypes have also emerged. Ovarian endometrioid adenocarcinoma is a subtype of OC originating from malignant uterine cells created during endometriosis.[6] Some models of endometrioid OC resulted from oncogenic *Kras* and a conditional *Pten* deletion.[47,49] However, *Kras* mutations are uncommon in human endometrioid cancer. To address this issue, a mouse model of endometrioid OC was

created in which *Apc* and *Pten* are deleted, since perturbations in Wnt and phosphoinositide 3-kinase (PI3K) signaling are linked in some endometrioid OC.[50] Mice with floxed *Apc* and *Pten* were IB injected with Adeno-Cre.[50] Resultant adenocarcinomas were similar to metastatic human ovarian endometrioid adenocarcinoma and[50] were generally low grade, unlike those resulting from *Tp53* mutations.[50] The tumors resulting from *Pten/Apc* knockout showed increased nuclear β-catenin and overactive AKT signaling.[50] These GEMMs have been used to test therapeutics against endometrioid cancer; a combination of cisplatin and paclitaxel together with inhibitors of mTOR and AKT signaling has proven effective in mouse models.[51] This work highlights the possibility of using treatments that target the Wnt and PI3K pathways to combat endometrioid cancer.

Summary

The survival rates and treatment for OC have remained relatively unchanged for the past two decades. Recent advances in understanding the genetic landscape of the histological subtypes of OC generate hope that targeted therapies can be devised that will increase patient survival. In order to see these targeted therapies come into practice, preclinical mouse models that reflect the genetics, metastatic potential, and different responses to therapy need to be used. In the last 30 years, mouse models have progressed from early xenografts to xenografts with immunocompetent mice, and PDXs that reflect individual patient's genetics and therapeutic response. Moreover, genetic models of cancer have increased our understanding of how OC originates and what mutations or combinations of mutations may lead to tumor establishment. A recent mouse model with mutations in *c-Kit* further allows the study of ovarian tumors in the context of a postmenopausal environment, potentially allowing for the above-discussed genetic mutations to be further improved by incorporating hormone responsiveness seen in the older population most likely to be affected by OC.[52] Now that we have a variety of mouse models (summarized in Table 2) (as well as other animal models, such as the laying hen)[53] at our disposal that reflect a wide range of steps of OC progression, we can develop diagnostic, prognostic, and therapeutic approaches that will increase OC survival.

Abbreviations

OC, ovarian cancer; GEMM, genetically engineered mouse model; PDX, patient-derived xenograft; HGS, high-grade serous; GI, gastrointestinal; HGSOC, high-grade serous ovarian cancer; IP, intraperitoneally; IB, intrabursally; SC, subcutaneously; STOSE, spontaneously transformed ovarian surface epithelial; LSL, loxP-GFP-stop-loxP; MISIIR, Mullerian inhibiting substance type II receptor; RCAS, replication competent avian-leukemia-virus splice-acceptor; STIC, serous tubal intraepithelial carcinoma; SV40, *Simian virus 40*.

Table 2. Ovarian mouse model systems.

MODEL TYPE	ADVANTAGES	DISADVANTAGES
Xenograft	Effective at measuring tumor growth in response to mutations or exogenous gene expression. They allow for validation of therapies and studies of drug resistance. Xenografts also can faithfully recapitulate aspects of ovarian tumor progression and metastasis.	Does not recapitulate the initial transforming events leading to tumorigenesis. Many xenograft mouse models are conducted using immunocompromised mice, which will lack the contribution of the full complement of immune cells to the tumor growth process.
Subcutaneous xenografts	It is well suited for investigating imaging modalities such as 2-photon microscopy.	It is not well suited for ovarian metastasis studies as the tumors do not typically metastasize and the tumor is not positioned in the right anatomic location or microenvironment.
Intraperitoneal xenografts	Can mimic aspects of tumor metastasis, particularly metastatic dissemination; IP-injected tumor cells, such as SKOV3IP cells, metastasize to the ovary, peritoneal wall, diaphragm, and form ascites fluid similarly to human disease. Tumor growth can be monitored using *in vivo* fluorescence or luminescence.	Tumor initiation and initial tranforming events cannot be studied. Cannot image *in vivo* using 2-photon microscopy due to the depth of the tumors.
Intrabursal xenografts	IB injection mimics the initial steps in metastasis, as the tumor cells exit the bursa to spread throughout the peritoneal cavity.	Tumor initiation and initial tranforming events cannot be studied. Cannot image *in vivo* using 2-photon microscopy due to the depth of the tumors.
Patient Derived Xenografts	Tumors from diverse genetic backgrounds can be grafted, and the tumor growth can be monitored. PDXs recapitulate aspects of OC like metastasis and ascites formation. PDX models have the ability to investigate the response of the tumors to therapeutic agents and could predict patient response to drug therapy.	PDXs have variable take rate and access to patient samples is a limitation for many investigators.
Syngeneic xenografts	The experimental design more accurately reflects the response of the immune system during tumor progression than immunocompromised models.	The cells are of murine origin, and thus may not resemble human cancer as well as models using human cells.
Genetically engineered mouse models	GEMMS are especially important for studying the beginning stages of ovarian cancer that cannot be mimicked in xenografts. Different genetically engineered mouse models allow us to compare evaluate how OC tumors arising from mutations at different anatomic locations compare to human disease.	OC genetic models have been more complicated to generate, partially due to the lack of understanding of OC biology and the heterogeneity of ovarian cancer. Infertility resulting from ovarian tumors has also hampered generation of transgenic models of ovarian cancer.

Note: This is an overview of the different mouse models used in the study of OC, summarizing advantages and disadvantages of each.

Author Contributions

Wrote the first draft of the manuscript: ASB and KDCD. Contributed to the writing of the manuscript: ASB, JMC, and KDCD. Jointly developed the structure and arguments for the paper: ASB, JMC, and KDCD. Made critical revisions and approved final version: ASB, JMC, and KDCD. All authors reviewed and approved of the final manuscript.

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