Animal Models of Gynecological Cancers

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Abstract

Gynecological cancers develop in the reproductive organs of a woman. The common gynecological cancers are cervical, ovarian, uterine, vaginal, and vulvar cancers. Most of the gynecological cancers including the ovarian cancers undergo metastasis to distant organs, acquire resistance to cancer therapy, and relapse. There are no specific diagnostic biomarkers available to detect the early stages of gynecological cancers. The challenges to diagnose and treat gynecological cancers stem from the fact that these cancers are heterogeneous diseases and, therefore, need physiologically relevant animal model systems in order to develop strategies to discover targeted therapeutics. In this book chapter, the authors elaborate on mouse models that serve as helpful tools to understand the biology of gynecological cancers as well as the identification of biomarkers and novel therapeutics.

Keywords

Ovarian cancer · Endometrial cancer · Animal model · Gynecological cancer · Cancer

Introduction

Cancer is a major global health problem with millions of people being diagnosed with cancer throughout the world, and nearly 50% of patients die from this disease (Ma and Yu 2006). Cancer is one of the primary causes of global mortality as it led to nearly ten million deaths in 2020. Gynecological cancer is a type of cancer that originates in one of the female reproductive organs, which include the ovaries, cervix, uterus, vagina, and vulva. In 2018, the American Cancer Society (ACS) reported over 110,000 newly diagnosed cases of gynecological cancers, resulting in over 32,000 deaths alone just in the United States. Each gynecological cancer is unique in its origin, symptoms, prognosis, and risk factors. Women with increased age are at a higher risk of developing gynecological cancers. In general, early detection of gynecological cancers can lead to an increased chance of survival. To improve the current treatment and increase the survival and quality of life of gynecological cancer patients, we need to understand the pathobiology of gynecological cancers. The extensive molecular characterization of gynecological cancers will facilitate the identification of new biomarkers of early growth, progression, and treatment outcome. Importantly, the gynecological cancers can be established in

animal models and could be used to determine the role of key genes/proteins by overexpression or knockdown studies, which can lead to the development of novel targeted therapies (Ramachandran et al. 2012; Ramadoss et al. 2017). In this book chapter, we discuss about the major types of gynecological cancers and the specific animal models that facilitate deeper understanding of the tumor biology of gynecological cancers.

Ovarian Cancer

Ovarian cancer (OC) ranks fifth among cancers in women, with more than 22,530 women diagnosed each year. In every 78 women, 1 will develop ovarian cancer in their lifetime, and about 1 in 108 women will die from ovarian cancer. The risk of developing ovarian cancer increases with age, and the lifetime risk is about 1.6%. Genetic predisposition is a major risk factor in the development of ovarian cancer (McLemore et al. 2009). Thus, women with a family history of ovarian cancer, especially first-degree relatives, have a 5% risk of developing ovarian cancer. Mutations in breast cancer gene 1 (BRCA1) or BRCA2 in women present a risk between 25% and 60% (McLemore et al. 2009; Pruthi et al. 2010).

Ovarian cancer subtypes are classified as endometrioid, mucinous, serous, and clear cell carcinoma (Bast et al. 2009). The most common form of ovarian cancer diagnosed in women aged 40 years or older is epithelial ovarian cancer (EOC). The incidences of other types of ovarian cancer include serous (about 50%), endometrioid (about 25%), mucinous (6–16%), and clear-cell (5–11%) ovarian carcinoma.

The high mortality rates associated with ovarian cancer are partially due to misdiagnosis or lack of diagnosis at an early stage, due to nonspecific or misinterpreted symptoms. About 70% of patients are diagnosed at an advanced stage with approximately 66% of late-stage patients eventually relapse and develop resistance to standard therapy. Importantly, the cancer stem cells (CSCs) are important contributors to tumor development and therapeutic resistance (Phi et al. 2018), and thus lead to tumor aggressiveness (Chengizkhan et al. 2020). Several pathways including Notch, Wnt/β-catenin, TGF-β, Sonic Hedgehog, PTEN, FGF, IGF1, and BMI1 are implicated in regulating the proliferation, survival, self-renewal, cell fate determination, as well as maintenance of CSCs in ovarian cancer (McAuliffe et al. 2012).

Animal Models to Study Different Types of Ovarian Cancer

Immunodeficient xenograft mouse models: The xenograft mouse model represents an immunocompromised mouse harboring a human tumor that is generated through orthotopic or heterotopic implantation of human tumor tissue, cell line, or primary cell culture. Strong immune rejection is the profound barrier for the engraftment of human cancer cells in immunocompetent mice (Yang and Sykes 2007). Therefore, immunodeficient mice were developed to overcome this rejection of human cancer cells facilitated by the mouse adaptive (B- and T-cells) as well as

innate system cells like macrophages and natural killer cells through perturbation of the genes critical for immune function (Tian et al. 2020). The discovery of congenitally athymic nude mice was a monumental breakthrough in the investigation of human cancers using immunodeficient mice in the 1960s. The nude mice are naturally homozygous for the *Foxn1*^{nu} or nude mutation. *Foxn1* gene encodes a transcription factor that is required for both hair follicle and thymic development. Hence, nude mice lack a thymus (where $CD4^+$ and $CD8^+$ T-cells differentiate and mature) and hair (nude). Due to T-cell deficiency, nude mice cannot support the majority of immune responses, including cell-mediated immune responses, antibody formation, hypersensitivity responses that are delayed, destruction of malignant T-cells, as well as graft rejection, thereby making the nude mouse immunodeficient. Essentially, nude mice can accept every type of human tumor. This attribute, however, is limited by the existence of both an intact humoral adaptive immune system and an innate immune system, which hinders the successful engraftment with some primary human tumor cells (Olson et al. 2018).

The next major development in the field was the identification of spontaneous mutations in C.B17 mice termed "severe combined immunodeficient (SCID)" (*Prkdc*^{scid}, protein kinase, DNA-activated, catalytic polypeptide) (Shultz et al. 2014). This mutation impedes the recombination of antigen receptor genes, thereby resulting in the arrest of B and T lineage-committed cells in their early development (Bosma and Carroll 1991). Though SCID mice are more receptive hosts to the engraftment of human cells and tissues as compared to athymic nude mice, they have an intact innate immune system consisting of NK cell action that limits the engrafting and growth of human tumors. Further research generated non-obese diabetic (NOD)/SCID strain mice that have intrinsic deficiencies in innate immunity, leading to lower activity of NK cells and decreased activation of macrophages, abnormal functioning of dendritic cells, and no hemolytic complement (Shultz et al. 2014). A major leap forward in developing higher-order immunodeficient mice was achieved when *NOD-Prkdc*^{scid} was combined with desired mutation in the interleukin-2 (IL-2)-receptor common gamma chain gene (*IL2rγ*^{null}), resulting in NOD-*Prkdc*^{scid} *IL2rg*^{null} (NSG) mice with the absence of adaptive immunity and deficiency in innate immunity and therefore being more susceptible to the engraftment of human tumors (Olson et al. 2018).

Cell line-derived xenograft (CDX) model involves the injection of established human cancer cells subcutaneously or orthotopically into immunodeficient mice. This model is used in cancer drug discovery and research (Day et al. 2015). Another widely used models include the intraperitoneal or intrabursal injection of tumor cells in immunodeficient mice (Shaw et al. 2004; Cordero et al. 2010; Magnotti and Marasco 2018). Intraperitoneal model closely represents the late-stage metastatic ovarian cancer, while the intrabursal model represents localized disease as tumors are mostly confined to the ovary. In contrast to mice in which the ovary is covered by the bursal membrane, humans do not have bursal membrane which allows the easy and rapid metastatic spread of tumor cells (Lengyel 2010). Because of this

anatomical difference, the intrabursal ovarian cancer models may not truly represent the metastasis in human ovarian cancer.

CDX models are useful to some extent, however, these models don't recapitulate the actual patient scenario as the biology and heterogeneity of human cancer cell lines differ greatly from the original tumor tissue. Furthermore, CDXs have failed to predict patient response to targeted therapies as observed by a very low approval rate of about 5% by United States Food and Drug Administration (FDA) (Day et al. 2015).

Syngeneic Mouse Models of Ovarian Cancer

Studies that require an intact immune system rely on immunocompetent animal models. Syngeneic mouse models can be used to examine the antitumor immune response in the tumor microenvironment including immune cell infiltration in ovarian cancers. The tumors can be initiated using established ovarian cancer cell lines that originate in the same mouse strain with similar genetic background, thereby, minimizing the immune rejection (Fig. 1). The syngeneic mouse models with orthotopic injection of tumor cells in the ovarian bursa/intraperitoneal cavity can model histopathological characteristics of ovarian cancer that can allow the examination of mechanisms underlying tumor immune evasion and ovarian cancer metastasis (Nunez-Cruz et al. 2010). The orthotopically implanted tumors into the organs from where the cancer originated can model early disease progression in the physiologically relevant tumor microenvironment (Quinn et al. 2010).

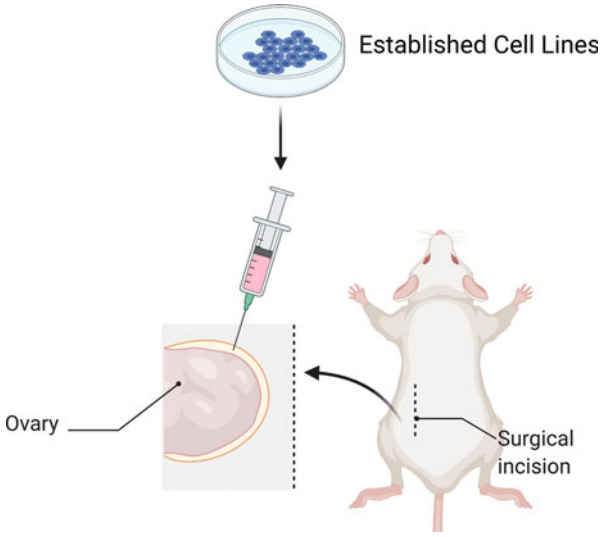

Fig. 1 Syngeneic orthotopic mouse model: Established ovarian cancer cell lines are prepared for injection. A dorsolateral incision is made, the ovary is located, and cells are injected

Applications of Syngeneic Mouse Models

Syngeneic mouse models are valuable preclinical tools used in identifying the response to immunotherapy. The ovarian syngeneic preclinical mouse models have led to several clinical trials investigating novel immunotherapy treatment strategies. Examples of such trials include testing of chemotherapy drug cisplatin in combination with rintatolimod, a toll-like receptor 3 (TLR3) agonist, and pembrolizumab, a programmed cell death protein 1 (PD-1) immune checkpoint inhibitor in patients with recurrent ovarian cancer (https://clinicaltrials.gov/ct2/show/NCT03734692).

Immune checkpoint inhibitors are used to treat various cancers including melanoma, where patients display durable response to immunotherapy. This new paradigm shift in cancer treatment led to numerous clinical trials using immune checkpoint inhibitors and ultimately approval of pembrolizumab (monoclonal antibody for PD-1) for patients who harbor the advanced stage of disease with a high mutational burden and high microsatellite instability (Matulonis et al. 2019). Although this is a huge milestone for treatment for patients with advanced ovarian cancer, many patients remain refractory to immunotherapy. Notably, current research efforts are geared toward the identification of novel factors that drive resistance to immunotherapy and investigation of the underlying mechanisms using these preclinical models.

Predictive biomarkers have also been investigated in syngeneic models to assess the efficacy of immune checkpoint inhibitors. Established syngeneic mouse models using validated mouse ovarian cancer cell lines are used to identify the biomarkers, such as CXCL9, as a driver of effective immune checkpoint blockade of PD-L1 in preclinical ovarian cancer (Seitz et al. 2022). The convenient and easy-to-use syngeneic models have facilitated a deeper understanding of the mechanism underlying the efficacy of immunotherapy and help to identify the proteins/factors that can be targeted to increase the response to immunotherapy in ovarian cancer patients.

Challenges and Limitations of Syngeneic Mouse Models

The main advantage of syngeneic mouse models is their feature of full murine immunity which allows to study the cross talk between the tumor, its microenvironment, and the surrounding immune cells. This model provides an opportunity to study various factors including secreted factors from immune cells, tumor intrinsic factors, and immune cell infiltration, among many other possible interactions, and explore the novel interventions that can overcome the challenges associated with the treatment of advanced ovarian cancer. However, this model also has major limitations that can often lead to the findings that are difficult to translate. This limitation is primarily due to the differences between mouse and human tumors. While they may share many similar features, human cancers are more complex and contribute significantly to the difficulty of interpreting the results derived from this model. Similarly, mouse cell lines do not recapitulate the heterogeneity present in human tumors, further limiting the physiological relevance of this model (House et al. 2014).

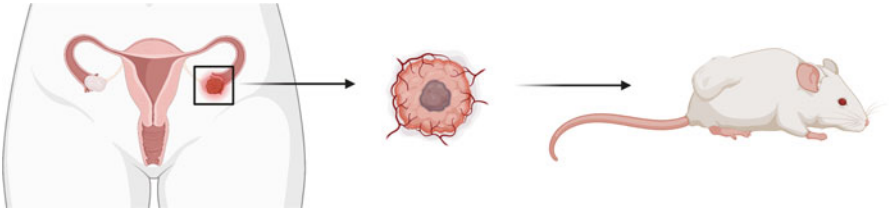

Fig. 2 Patient-derived xenograft (PDX) mouse model: Cancer tissue from patient surgery or biopsy is obtained and processed. Cancer tissue is engrafted in orthotopic or nonorthotopic sites in immunocompromised mice

Patient-Derived Xenograft Models of Ovarian Cancer

Patient-derived xenograft (PDX) models are widely used animal models that are developed by implanting human tumors derived from patients directly into an immunocompromised mouse (Fig. 2). PDX models are useful in representing the tumor of origin by retaining the complexity and heterogeneity that are deficient in other preclinical models. The development of PDX models overcomes some of these major limitations. The extensive handling of cell lines *in vitro* can result in drastic changes in their genome. PDX models, on the contrary, can be maintained *in vivo,* avoiding the *in vitro* culture steps and reducing the changes to the genome while retaining the histopathological features of the primary human tumors (Domcke et al. 2013). However, cultured cancer cells display altered genetic information including gain- and loss-of-function mutations that can lead to changes in the growth and invasion abilities (Gillet et al. 2011). PDX models have become one of the preferred methods for drug efficacy studies, for biomarker identification, and for other preclinical research on ovarian cancer to overcome the limitations of using conventional cell lines.

Applications of Patient-Derived Xenograft Models

There are several applications of PDX models that are employed to understand the different stages of ovarian cancer, and this depends on the study design including the location of the tumor implantation. This model provides the opportunity to study the ovarian cancer using subcutaneous or orthotopic engraftment of the tissue or a dissemination model to study the metastasis. The preclinical drug development for ovarian cancer relies on models that can recapitulate human disease and drug response by retaining the molecular and histological characteristics. PDX models can predict the clinical outcomes of new therapeutic approaches, identify the biomarkers, and shed lights on the biology of ovarian cancer to generate a strategy for personalized medicine (Hidalgo et al. 2014). Previous studies have shown a highly positive correlation between patient response and drug interventions and comparable experimental drug intervention in PDXs (Butler et al. 2017).

An earlier study using 20 distinct human ovarian tumors implanted into SCID mice demonstrated that >65% of the tumors reach a size large enough for passage into other SCID mice suggesting a moderate to high penetrance. Of those, several mice further developed metastasis and ascites representing the natural clinical progression of ovarian cancer (Xu et al. 1999). This study illustrates the flexibility of PDX model to conduct large-scale studies and to recapitulate human tumor progression. PDXs are powerful models for preclinical testing of new therapeutic strategies that can connect the findings from scientific studies to clinical translation. Several clinical trials have implemented the use of PDX models already, including combination therapy trials for patients with platinum-resistant ovarian cancer (https://clinicaltrials.gov/ct2/show/NCT02312245), thus highlighting the significance of utility of this animal model.

Challenges and Limitations of Patient-Derived Xenograft Models

PDX models are adaptable and can be used in parallel with other models to generate valuable preclinical data. While this model is promising, there are challenges and limitations to this model. Primarily, this model requires more time as tumor engraftment can be labor-intensive, and it takes several weeks for this model, to be prepared to passage into more animals. Additionally, obtaining patient samples can often be challenging for researchers and requires the generation of a tumor bank for this model to be more accessible. Cost can also become a challenge for researchers to maintain this model due to the requirement of expensive genetically engineered mice and the facility costs to continue studies over a long period of time. One major limitation of PDX models is the lack of immunity, which impedes the study of the immunotherapy and the immune response. For sufficient tumor engraftment, ovarian cancer PDX models require an immunodeficient host, and the absence of an immune system prevents the examination of the role of the immune system. Other limitations include the loss of human stroma and vessels, which is replaced by the mouse stroma and vessels 15–25 weeks after tumor engraftment. Therefore, this model may not be suitable to study the interventions targeting the human stromal components or vasculature (Hylander et al. 2013).

Specialized Mouse Models of Ovarian Cancer

Genetically engineered mouse models: Over the last decade, the development of genetic engineering techniques has resulted in an extraordinary growth in our understanding of the genetic basis of cancer (Frese and Tuveson 2007). Collectively, these pioneered the establishment of mouse models with the ability to incorporate specific genomic alterations to induce tumor development in a tissue-specific manner. Genetically engineered mouse models (GEMMs) are systems in which genetic changes are made in mice that can promote the development of a particular disease.

These models primarily employ tissue-specific promoters to enhance expression of an oncogene related to tumor formation or tissue-specific expression of recombinase enzymes to facilitate the deletion of tumor suppressor genes (Olson et al. 2018). The primary application and strength of GEMMs are the study of tumor growth and progression, as well as the identification of the role of specific genetic alterations in cellular transformation. In ovarian cancer studies, tumors are created by silencing appropriate tumor suppressor genes or activating the oncogenic genes using knock-out or knock-in approaches (Fig. 3). The use of this approach can examine driver mutations involved in tumorigenesis by the genetic manipulation of specific genes that are observed in human patients. The invaluable feature of GEMMs is the power to study the tumor initiation, progression, and metastasis of ovarian cancer while assessing the physiological relevance of specific genetic mutations found in human diseases (House et al. 2014).

GEMMs can be categorised as endogenous or transgenic. Endogenous GEMMs represent mutant mice with a loss of tumor suppressor genes (TSG) and over-expression of dominant-negative TSGs, as well as oncogenes. The transgenic mice models are developed through pronuclear injection of cDNA constructs consisting of promoter regions fabricated to inhibit tissue tropism. Transgenic GEMMs recapitulate identical genetic composition of amplified or translocated proto-oncogenes. To generate transgenic GEMMs, a direct injection of fertilized oocytes or through the gene targeting and lentiviral transduction in embryonic stem cells is often employed (Frese and Tuveson 2007). The advantages of transgenic GEMMs include the capability to control the target gene expression in a reversible manner.

Altered Genes	Cancer Histology	Reference
p53, c-Myc, Kras, Akt	Ovarian Carcinoma	Orsclic S. et al., 2002
p53, Rb1	Epithelial Ovarian Cancer	Flesken-Nikitin A. et al., 2003
p53, Rb, Brca1 or Brca2	Serous Epithelial Ovarian Cancer	Szabova L. et al., 2012
Pten, Apc	Ovarian Endometrioid Adenocarcinoma	Wu R. et al., 2007

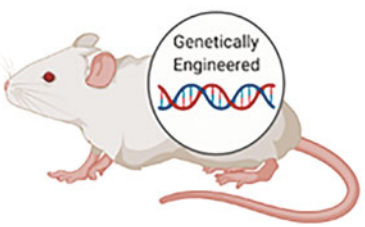

Fig. 3 Genetically engineered mouse model (GEMM): Specific genes are knocked out in the cell of interest to model the tumor development of ovarian cancer. The table summarizes the most utilized altered genes to create mouse models that develop into different ovarian cancer histologies

Applications of Genetically Engineered Mouse Models

A major advantage of GEMMs is the ability to examine the role of specific genes in the malignant cellular transformation in cancer. The study of prevention of disease can further be investigated using this model by observing the effects of interventions in models with particular gene alterations. The use of this model has led to a deeper understanding of cancer initiation, angiogenesis, invasion, metastasis, and the immune system overall in the context of cancer. In particular, GEMMs can provide the opportunity to study the spontaneous metastasis during tumor progression, which recapitulates human patient scenario (Hasan et al. 2015). GEMMs allow for specific gene alteration in a tissue-specific manner that can be regulated at critical times in the development or adulthood to mimic the human diseases. There are two methods of transforming cells in GEMMs: RCAS/TVA gene delivery system, which is performed *ex vivo*, and the Cre-LoxP system, which delivers the Cre recombinase to the orthotopic site. Both of these approaches allow for transformation specifically in the ovarian surface epithelia cells and are temporally controlled (Sale 2009). To study high-grade serous ovarian cancer (HGSOC), an investigation that utilized GEMMs found PAX8 as a driver of mouse HGSOC, and these PAX8-driven murine tumors were shown to have a strong correlation with human tumors. In this GEM model, the genetic alterations of *Brca, TP53,* and *Pten* resulted in intraepithelial precursor lesion. This study also outlined that HGSOC can initiate from the fallopian tube secretory epithelial cells (Perets et al. 2013).

GEMMs are suitable models to investigate the response of immunotherapy and can aid in the discovery of novel treatment strategies to treat patients with ovarian cancer. The study of immunotherapy in GEMMs requires a distinct approach compared to syngeneic models or other animal models; nonetheless, the use of GEMMs in immunological studies is increasing. GEMMs can model tumor intrinsic and extrinsic features that can initiate *de novo* tumor formation and the natural advancement of the cancer including metastasis, making these models essential for preclinical research and can be used in parallel with clinical trials, termed "co-clinical trial." The successful use of GEMMs has led to the validation of drug targets and cancer-causing genes, as well as for the assessment of the efficacy of therapeutics and evaluation of the mechanisms that contribute to treatment resistance.

Challenges and Limitations of Genetically Engineered Mouse Models

Several advantages of GEMMs have led to a more comprehensive understanding of the precise role of essential genes involved in tumor development and have enhanced our understanding in tumorigenesis. However, certain caveats exist with this model. The major limitation of using GEMMs is the time to develop this model. There is a latency period for *de novo* tumor formation to occur, and the mice can develop cancer at different stages with different levels of penetrance of mutations varying from 50% to 100%. This can result in complex and prolonged studies that can be

costly. The generation of GEMMs usually takes over a year to develop, and once developed, faster tumor growth and high tumor burden can force the termination of the study. This can impede the study of metastatic disease since animals are required to be sacrificed at an early tumor developmental stage. One more major limitation of this model is the absence of a complex genomic landscape that is prominent in human disease. Although GEMMs are labor- and resource-intensive and require time and careful consideration, they deliver valuable information that cannot be obtained from other animal models (Mullany and Richards 2012).

Humanized Mouse Models of Ovarian Cancer

PDX mouse models have been the most successful among the established models of gynecological cancers; however, they are primarily useful in the preclinical testing of chemotherapeutics drugs. Due to the site-specific differences, studies investigating the efficacy of immunotherapeutic drugs involving syngeneic animal models have largely been unsuccessful. Therein exists a void in the translatability of the immune system from mice to human, which has recently been addressed by the emergence of humanized mouse models. Humanized mouse models have been developed with the aim of incorporating an intact human immune system in mouse models to generate and study the immune responses following immunotherapeutic intervention (Choi et al. 2018). This model represents a paradigm shift in understanding the human immune response in the context of gynecological cancers and imparts high translational utility for novel immunotherapies for these cancers.

In order to develop this model, investigators engraft a functional human immune system into mouse strains that are murine immunodeficient. The three major humanized mice models are Hu-PBL-SCID, Hu-SRC-SCID, and BLT (bone marrow, liver, thymus) models. Hu-PBL-SCID mice models are established by engrafting human peripheral blood leukocytes (PBL) through intraperitoneal or intravenous injection into adult immunodeficient mice. Immunodeficiency can be achieved by irradiating the mice at sublethal doses. This method is relatively less time-consuming, and the development process is fairly simple. The Hu-SRC-SCID model is established by injecting the human $CD34^{+}$ hematopoietic stem cells (HSCs) into adult immunodeficient mice that are derived from various sources including HSCs from the peripheral blood mobilized with granulocyte colony-stimulating factor (G-CSF), blood from the umbilical cord, bone marrow, or fetal liver HSCs. The third major humanized mice model, the BLT model, is developed through the implantation of thymus and liver tissues derived from the same human fetus and then engrafted into immunodeficient mice. These mice are usually conditioned using renal capsule followed by the injection of autologous $CD34^{+}$ fetal liver HSCs intravenously. This model presents the most physiologically equivalent engraftment of a human immune system, which can be further strengthened when used in NSG mouse models (Yin et al. 2020; Tian et al. 2020).

A number of studies have employed advanced humanized mouse models in evaluating the efficacy of various immunotherapies. A combined dual-blockade

therapy of programmed cell death protein 1 (PD-1) and the immune checkpoint T-lymphocyte-associated protein 4 (CTLA-4) with autologous tumor-associated leukocytes has been shown to effectively reduce the ovarian cancer progression in PDXs of the humanized mice. Furthermore, it was also reported in this model that the combination of anti-CTLA-4 and anti-PD-1 increased the tumor-recognizing $CD8^+$ T cells that infiltrated the tumor microenvironment, showing an acquired memory phenotype in the T cells, and led to the protection of tumor growth upon tumor rechallenge in the animals (Odunsi et al. 2020).

Endometrial Cancer

Endometrial cancer (EC) is the sixth leading cause of cancer-related deaths among women in the United States, with an estimated ~76,000 deaths annually among women globally (Tang et al. 2021; Urick and Bell 2019). It usually originates in the cells that line the endometrium of the uterus and is also sometimes referred to as uterine cancer. Endometrial cancer accounts for about 5% of all diagnosed cancers and affects mainly postmenopausal women. An estimated 5% of EC occurs due to inherited cancer genetic predisposition syndromes, commonly Lynch syndrome, while the majority of EC diagnoses are considered sporadic. Notably, recent studies demonstrated that the number of deaths attributed to endometrial cancers is increasing. There are persistent racial disparities in the survival of patients with endometrial cancers, and this is illustrated by the variation between 60% and 80% 5-year survival rates depending on the different region of global population (Mukerji et al. 2018). Endometrial cancer consists of four distinct histological subtypes, which include endometrioid endometrial cancer (EEC, type I), serous endometrial cancer (SEC, type II), clear-cell endometrial cancer (CCEC, type III), and mixed endometrial cancer and uterine carcinosarcoma (type IV) (Urick and Bell 2019). Type I EC tumors represent around 70% of the diagnosed cases and are considered the most frequent subtype; they are considered low grade and are linked to estrogen stimulation. Type II EC tumors, in contrast, are less common, more aggressive, and commonly considered high grade, metastatic, and independent to estrogen stimulation and exhibit a higher risk of relapse after treatment. Around 10% of diagnosed endometrial cancers are type II tumors, accounting for 40% of deaths, and are associated with a poor prognosis (Sorosky 2012; Urick and Bell 2019).

Animal Models to Study Different Types of Endometrial Cancer

In accordance with other cancers, animal models of endometrial cancers provide a strong impetus for translational research, in vivo disease modeling, and therapeutic testing. Several animal models for endometrial cancers have been proposed and are presently utilized by research scientists and are highlighted in the sections below.

Rodent Models of Endometrial Cancer

Rodent models have been widely investigated in endometrial cancers, especially after the seminal discovery by Deerberg et al. (1981), where it was observed that there was an incidence rate of uterine tumors around 40% in female Wistar rats. Furthermore, Nagaoka et al. (1990) also demonstrated a 35.1% incidence rate of endometrial adenocarcinoma in Donryu rats, around 60% of which eventually develop tumor lesions in the endometrium. Moreover, findings by Tanoguchi et al. (1999) suggested similar signatures in tumors arising in Donryu rats to mutated KRAS as compared to endometrial cancers in humans. BDII/Han rats are also known for their high spontaneous tumor development of greater than 60% in their lifetime and are highly characterized at both genomic and molecular levels. Samuelson et al. (2009) showed how tumors in these rat models are comparable to type I human endometrial cancer and thus serve as excellent models to recapitulate the genomic and molecular features identified in the human endometrial cancers.

Chemical-induced rodent models of endometrial cancer are also of significant interest to researchers as tumors induced through chemical treatment serve as translational models that can be utilized to study the effects of chemoprevention. To study the effects of danazol on endometrial tumorigenesis, Niwa et al. (2000) utilized female ICR mice. The only major limitation of this type of model is the inimical effects on the metabolism and nonspecific toxicity upon chemical treatment.

Transgenic Mouse Models of Endometrial Cancer

Transgenic mouse models are the most widely used animal models to investigate the biological mechanisms related to endometrial cancer development. Phosphatase and tensin (PTEN) is one of the most altered genes in endometrial cancers, and knocking down its expression resulted in one of the first transgenic endometrial cancer models. To generate hyperplasia, knocking out of any one of the two alleles ($PTEN^{+/-}$) is adequate to form carcinoma in 20% of all cases. Knocking out both alleles ($PTEN^{-/-}$) is embryonically lethal for the mouse; however, to address this issue, conditional knockout systems including tamoxifen-inducible transgenic systems and AAV-mediated Cre-Lox are often utilized. The investigations with these systems have eluded that PTEN inactivation leads to rapid induction of endometrial carcinoma. This model also set the stage to investigate other genes associated with endometrial cancer development. A study conducted by Contreras et al. (2010) depicted that inactivating LKB1 further drives the progression of endometrial cancer development. Another study (Cheng et al. 2014) succeeded in establishing a transgenic mouse model with the combined deletion of LKB1 and PTEN that depicted reduced survival and modeled an advanced state of the disease.

TP53 mutations are often present in both type I and type II human endometrial cancer. The investigations by Daikoku and colleagues (2011) demonstrated that a combined deletion of $PTEN^{-/-}$ and $TP53^{-/-}$ led to an aggressive phenotype as well as reduced survival when compared to deletion of just $PTEN^{-/-}$ alone. While type I

endometrial cancer is the most commonly studied endometrial cancer, type II endometrial cancer is generally more aggressive and has a higher mortality in patients. Interestingly, studies (Akbay et al. 2013) demonstrated that the combined gene deletion of POT1A (along with TP53) resulted in an endometrial cancer resembling a type II phenotype at 9 months of age in these mice and further led to metastasis in all of the mice with the genetic deletions of POT1A and TP53 at 15 months of age.

Mitogen-inducible gene 6 (MIG-6) is a gene that is regulated by stress stimuli and mitogens, and it is known to negatively regulate EGFR signaling (Kim et al. 2017). Thus, another endometrial cancer model was developed using a transgenic mouse model with MIG-6 knockout in the uterus, and this model was utilized to uncover the estrogen-dependent tumor suppressive function. All these aforementioned transgenic mouse models have been widely investigated to assess the therapeutic response to various agents including Akt-mTOR inhibitors, PARP inhibitors, as well as palbociclib (CDK4/6 inhibitor). While these well-characterized models serve as excellent resources to further disseminate the tumor biology of endometrial cancers, they are insufficient to recapitulate the native heterogeneity of endometrial cancer and thus are not physiologically accurate (Van Nyen et al. 2018).

Recently, an orthotopic immunocompetent mouse tumor model of metastatic endometrial cancer was established from endometrial cancer that was developed in a GEMM. In this model, Fedorko et al. (2020) generated an immortal cell line MECPK (mouse endometrial cancer PTEN deleted K-ras activated) from a 4-week-old $Pgr^{cre/+}Pten^{f/f}K\text{-}ras^{G12D}$ GEMM that developed endometrial cancer. After tumor cell engraftment, the mice developed local and metastatic endometrial tumors particularly lung metastasis. This immunocompetent orthotopic tumor model may offer advantages of exposure of tumor cells to the physiologically appropriate microenvironment in the uterus and establishment of tumor in mice with an intact immune system.

Patient-Derived Xenografts (PDXs) of Endometrial Cancer

PDX models for endometrial cancer are generated by directly engrafting the tumor piece subcutaneously into immunocompromised mice (Jia et al. 2021). However, few studies have generated endometrial cancer orthotopic PDX models by intrauterine injection of tumor-derived cells (Fonnes et al. 2020). PDX models commonly used for uterine cancer research are generated by 2012 subcutaneous implantations (Shin et al. 2022). The generation of first PDX model where resected tumor tissue from an endometrial tumor was implanted orthotopically through transvaginal injection into nude mice (Cabrera et al. 2012). In another study, an orthotopic PDX model was developed by dissociating the primary tumor biopsy into cell suspension and then injecting them to uterine horn of NSG mice (Haldorsen et al. 2015). Over the last decade, seminal studies by Depreeuw and colleagues (2017) as well as Unno et al. (2014) have characterized over 24 endometrial cancer PDX models representing primary, metastatic, and recurrent endometrial cancer as

well as from patients undergoing surgery. Importantly, the development of PDX from patient-derived tumor organoids has gained a lot of attraction. Pauli et al. (2017) reported the generation of PDXs from 18 different endometrial cancer types. These PDXs had 86.4% engraftment rate and successfully formed endometrial cancer in multiple mice models. These PDXs offer an exciting avenue for personalized medicine and can lead to maximizing treatment efficacy in endometrial cancer patients.

Conclusion

Several gynecological cancer-related animal models are available. These animal models are useful tools to improve our knowledge on gynecological cancers. In particular, PDX-based models of gynecological cancers are expected to add significant value in animal model-based gynecological cancer research. The next few decades will likely witness further advancement in the modeling of various diseases with the ultimate goal of progressing patient care and increasing treatment outcomes for patients with gynecological cancers.

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