ZOOLOGICAL RESEARCH

Mouse models of epithelial ovarian cancer for preclinical studies

Sergey Karakashev[1], Ru-Gang Zhang[1,*]

[1] *Immunology*, *Microenvironment* & *Metastasis Program*, *The Wistar Institute*, *Philadelphia*, *PA* 19104, *USA*

ABSTRACT

Epithelial ovarian cancer (EOC) is the leading cause of gynecological cancer-related mortality in the developed world. EOC is a heterogeneous disease represented by several histological and molecular subtypes. Therefore, exploration of relevant preclinical animal models that consider the heterogenic nature of EOC is of great importance for the development of novel therapeutic strategies that can be translated clinically to combat this devastating disease. In this review, we discuss recent progress in the development of preclinical mouse models for EOC study as well as their advantages and limitations.

Keywords: Epithelial ovarian cancer; Patient-derived xenografts; Orthotopic mouse model; Subcutaneous mouse model; Intraperitoneal mouse model; Syngeneic mouse model; Genetic engineered mouse model

INTRODUCTION

More than 85% of ovarian cancers are of epithelial origin. Epithelial ovarian cancer (EOC) is broadly divided into two types. Type I EOC includes clear cell, endometrioid, mucinous, and low-grade serous carcinomas, while type II EOC consists primarily of high-grade serous carcinomas (Kurman & Shih Ie, 2011). Despite recent advances in our understanding of EOC pathology, it remains a devastating disease with poor outcome compared to many other types of cancer (Siegel et al., 2020). Indeed, EOC survival rates in patients have only slightly improved in the last several decades, and EOC remains a major cause of cancer-related deaths in women worldwide (Torre et al., 2018). The high mortality rate is, in part, a result of limited therapeutic options and therefore novel therapeutic strategies are greatly needed. Despite the significant progress that has been made in recent decades in our understanding of the molecular mechanisms underlying ovarian cancer initiation and progression, novel therapies to improve outcome in EOC patients remain elusive. EOC is a genetically heterogenous disease represented by several histological and molecular subtypes that are distinct in their response to different anticancer agents (The Cancer Genome Atlas Research Network, 2011). Given its heterogenic nature, EOC requires appropriate preclinical animal models to recapitulate the pathobiology of its various subtypes. Mouse models are crucial not only for improving our understanding of the pathobiology underlying EOC initiation and progression, but more importantly for developing clinically relevant therapeutic strategies (House et al., 2014). Recent advances in preclinical EOC *in vivo* models have led to the development of several FDA-approved therapies. Preclinical EOC *in vivo* models have been widely used to demonstrate

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*Corresponding author, E-mail: rzhang@wistar.org

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the efficacy of FDA-approved poly(ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, niraparib, and rucaparib, for EOC maintenance therapy (Alhilli et al., 2016; Kortmann et al., 2011). Bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor, is another effective anticancer therapy in EOC *in vivo* models and also recently approved by the FDA (Byrne et al., 2003; Hu et al., 2005). However, despite this success, novel preclinical EOC models are required due to the heterogeneity of EOC and the development of resistance to approved anticancer therapies.

XENOGRAFT EOC MODELS

Ovarian cancer cell lines derived from ascites or primary EOCs have been extensively used to study the molecular mechanisms involved in the regulation of tumor progression and chemoresistance and for the development of novel therapeutics. Historically, ovarian cancer cell lines engrafted in immunocompromised mice were the first EOC mouse models developed. Mice subjected to ionizing radiation were the first animal hosts used for ovarian cancer xenografts. Subsequently, several immunodeficient mouse strains were developed and gained wide popularity, including nude mice with low levels of T-lymphocytes due to spontaneous deletion of *Foxn1* (Cordier & Haumont, 1980), SCID mice with severe B-cell and T-cell immunodeficiency due to mutation of *Prkdc* (Pla & Mahouy, 1991), and NOD/SCID mice with B-cell and T-cell immunodeficiency and compromised cytokine signaling (Shultz et al., 2005). These mouse strains have allowed the use of the large panel of EOC human cell lines generated in the last few decades and represent various EOC subtypes. In general, EOC xenografts are fast growing tumors that permit *in vivo* testing of previously observed *in vitro* experiments. The low cost, high engraftment probability, and low tumor size variability mouse to mouse make these models popular in the EOC field. These xenografts are currently used to study various aspects of EOC progression such as tumor initiation, growth, invasion, metastasis, and sensitivity to anticancer agents. One of the greatest advantages of EOC mouse xenografts is the ability to use genetically modified cell lines. Xenograft EOC mouse models can be classified based on engraftment strategy or source of engrafted material, as follows:

Classification based on engraftment strategy

EOC cells can be engrafted subcutaneously, intraperitoneally, or orthotopically. Each method has its own advantages and limitations for meeting specific needs (Table 1). Some models are suitable for studies on the early stages of cancer, whereas others are useful for studies on invasion and metastasis. Subcutaneous xenograft models were the first animal models applied for the study of EOC and are still widely used today. Subcutaneous xenograft models gained popularity due to their simplicity, reliability, and low cost. Subcutaneous EOC cell injections do not require specialized expertise or equipment. Furthermore, tumors are formed within several weeks and their growth is limited to the injection site. Tumor size can be easily measured from the early stages of tumor formation, thus making subcutaneous xenograft models a good choice for drug response evaluation. However, the absence of a physiologically relevant tumor microenvironment, poor vascularization, and improper anatomical location are major

Table 1 Classification of EOC mouse models based on injection site

Advantages	Disadvantages
Subcutaneous model	
√ Easy to perform	√ Model is not physiologically relevant
√ Suitable for monitoring tumor growth	√ Absence of tumor microenvironment
√ Low variability in tumor size	√ Not suitable for studying angiogenesis
√ Good for routine evaluation of drug efficiency	√ Not suitable for studying tumor dissemination
√ Low cost	
Intraperitoneal model	
√ Easy to perform	√ Tumor growth measurements require advanced methods
√ Good model for studying late stages of EOC	√ Not suitable for studying early stages of EOC
√ Suitable for studying EOC dissemination	√ No primary tumor formation
√ Ascites formation	√ Not suitable for studying angiogenesis
√ Suitable for immunological studies	
√ Low cost	
Orthotopic model	
√ Tumor microenvironment recapitulates physiological conditions	√ Difficult to perform
√ Suitable for studying all stages of disease	√ Tumor growth measurement requires advanced methods
√ Suitable for studying angiogenesis and tumor microenvironment	√ High cost
√ Suitable for immunological studies	
√ Good model to study disease progression	
√ Ascites formation	

limitations, which present a barrier to the use of such models in the study of tumor development, invasion, metastasis, angiogenesis, and other processes. Although, subcutaneous xenografts are particularly useful at the early stages of *in vivo* drug-response evaluation, the results obtained need to be confirmed by more advanced physiologically and clinically relevant *in vivo* EOC mouse models.

Orthotopic xenograft models are free from the multiple constraints of subcutaneous xenograft models. Mouse ovaries are encapsulated in a membranous structure called ovarian bursa. Cancer cells can be injected intrabursally, thus allowing tumors to develop in a physiologically relevant microenvironment (Fu & Hoffman, 1993; Kiguchi et al., 1998). Orthotopic xenograft models can be employed to study cancer progression at various stages. Nutrients and signaling provided by the mouse ovary microenvironment allow for tumor development in conditions similar to those in EOC patients. Thus, orthotopic xenograft mouse models are an important tool for understanding the molecular mechanism underlying EOC progression. After the initial phases of tumor growth in the mouse ovarian bursa, cancer cells can invade and disseminate from the primary site to form ascites or metastasis, making it an appropriate model for studies on late-stage EOC progression. Orthotopic xenografts are also widely used in assessing the efficacy of novel therapeutic approaches. The tumor microenvironment plays a key role in determining responses to anticancer agents and contributes to drug resistance. As such, orthotopic xenograft models are an important step in preclinical studies (Hansen et al., 2016). However, despite their notable advantages, these models are technically challenging, which limits their application in EOC research. Intrabursal cell injections require specialized training and are also time consuming and labor intensive compared to other xenograft models. Furthermore, technical challenges result in low implantation success and tumor size variability among mice. These obstacles constrain the use of orthotopic xenograft EOC models, especially for studies on the efficacy of anticancer agents with marginal effects as they require large-sized cohorts to detect significant differences among treatment groups. The inability to monitor tumor growth by conventional methods also makes orthotopic xenograft models less desirable for routine *in vivo* experiments, although this disadvantage can be overcome by using non-invasive, advanced imaging techniques such as *in vivo* bioluminescence and computed tomography (Cordero et al., 2010). Another limitation of intrabursal xenograft models is the anatomical difference between mouse and human ovaries. Unlike the mouse ovary, human ovaries are not covered by bursa, which prevents certain cell lines from leaving the primary site of injection. Metastasis and formation of ascites are primary reasons of lethality among EOC patients, and therefore orthotopic models are not ideal for studies on the late stages of tumor progression.

Intraperitoneal xenograft EOC mouse models are widely used to study cancer cell dissemination. Cancer cells injected into the peritoneal cavity form tumor nodules on the surface of the liver and spleen, similar to that observed in patients with advanced stages of EOC (Ward et al., 1987). In addition, EOC cells can invade the peritoneum and diaphragm, which makes these models suitable for studying metastasis and dissemination. Ovarian cancer cells growing intraperitoneally can also lead to the development of ascites, one of the key clinical characteristics of EOC in patients (Ahmed & Stenvers, 2013). Ascites development is tightly linked to the immune response against cancer cells and therefore intraperitoneal EOC models are widely used to evaluate the efficacy of anticancer agents modulating ascites production (Shaw et al., 2004). Technical challenges associated with the assessment of tumor growth are a major disadvantage of intraperitoneal EOC xenograft models. Ascites development is highly variable animal to animal and therefore this model requires large groups to assess ascites production statistically.

Classification based on source of cancer cells

EOC xenograft mouse models can be classified by the EOC cell source (Table 2). Established EOC cell lines are the most common source of EOC cells used in *in vivo* mouse models. EOC is a heterogeneous disease represented by several subtypes, including high-grade serous ovarian carcinoma (HGSOC), ovarian clear cell carcinoma (OCCC), ovarian endometrioid carcinoma (OEC), and mucinous carcinoma. HGSOC is the most common type, representing ~70% of EOC cases (Bajrami et al., 2014). For many years, ovarian cancer surface epithelium was considered the primary origin site of EOC (Kurman & Shih Ie, 2010). In the last decade, however, extensive studies have demonstrated that HGSOC most likely originates from fallopian tube fimbria (Kurman & Shih Ie, 2011). This finding highlights the importance of choosing the right EOC cell line for relevant xenograft preclinical models. For example, the two most common cell lines used in earlier EOC xenograft mouse model studies were SKOV3 and A2870, which were later shown to have originated from ovarian surface epithelium and endometrium tissues, and therefore were not representative of HGSOC (Beaufort et al., 2014; Domcke et al., 2013). Inappropriate use of cell lines may contribute, at least partially, to the clinical failure of many previously developed therapeutic strategies. However, in the last decade, around 40 EOC cell lines representing different histo-subtypes of EOC have been characterized. Several studies have classified EOC cell lines based on their origin, histo-subtype, genetic alteration, and ability to grow *in vivo* (Beaufort et al., 2014; Domcke et al., 2013; Hernandez et al., 2016; Ince et al., 2015). These studies represent a valuable source of information and facilitate the choice of cell lines in experimental design. Appropriate choice of EOC cell lines and routine analysis of cell line authenticity have become requirements in the EOC field. Usage of established EOC cell lines *in vivo* has many advantages, including high engraftment rate, fast tumor growth, low tumor size variability, good reproducibility, and ability to use the same models in different research groups. However, despite these advantages, it is important to keep in mind that the majority of EOC cell lines

Table 2 Classification of EOC mouse models based on source of injected cells

Advantages	Disadvantages
Established human EOC cell lines	
√ Availability of cell lines	√ Cell alteration due to high number of passages
√ Large number of cell lines with various genetic backgrounds	√ Not suitable for immunological studies
√ Easy maintenance	
√ Cells can be genetically manipulated	
√ Low cost	
Established mouse EOC cell lines	
√ Availability of cell lines	√ Cell alteration due to high number of passages
√ Suitable for immunological studies	
√ Easy maintenance	
√ Cells can be genetically manipulated	
√ Low cost	
Patient-derived xenografts	
√ PDX derived from EOC patients, not altered by *in vitro* culture	√ Slow tumor growth
√ Recapitulates EOC tumor microenvironment	√ Difficult maintenance
	√ Specialized training required
	√ Limited access to tumor samples
	√ PDXs cannot be genetically manipulated

have originated from highly advanced tumors. These cells have been exposed to different levels of selection during chemotherapy and recurrence and have accumulated multiple genetic alterations required for survival *in vitro*.

Patient-derived xenografts (PDXs) are the most clinically relevant models currently used in the EOC field (Ricci et al., 2014). PDXs are developed by direct engraftment of EOC patient tissue into immunodeficient mice. Similar to EOC cell lines, EOC specimens can be engrafted subcutaneously, orthotopically, or intraperitoneally. After initial inoculation, PDXs are usually grown for several months before they are harvested for further analysis and transplantation to a new group of animals for *in vivo* experiments. PDXs clinically recapitulate EOC better than any other xenograft model and have gained popularity for preclinical development of novel therapeutic strategies (Heo et al., 2017). Correlations between drug response in patients and PDXs derived from the same patients before treatment have been observed in several studies (Izumchenko et al., 2017; Topp et al., 2014). PDXs are also suitable for studies on aspects of cancer biology that are difficult to study *in vitro* or in other animal models, e.g., vascularization, cancer cell stemness, and tumor microenvironment. However, one of the main disadvantages that PDXs share with other xenograft models is the lack of an immune response in immunocompromised mice. Xenograft mice cannot be used to study the effects of immunotherapies, one of the greatest achievements in cancer therapy in the past several years. This obstacle can be overcome by using humanized immune system mouse models, which utilize immunodeficient mice engrafted with human immune cells or immune cell producing tissues (Choi et al., 2018). Humanized immune system mice are effective at recapitulating an immune response and have gained traction in the development of immune therapies, such as immune checkpoint blockades (Gitto et al., 2020). Other disadvantages of PDX models are their high cost associated with long-term experiments and technical challenges associated with surgical procedures. In addition, the slow growth rate and lack of stability due to clonal selection during the first several passages limit the use of PDX models within small research groups.

SYNGENEIC EOC MODELS

Syngeneic EOC animal models have recently gained popularity in EOC research. They are particularly useful in studying antitumor immunity and are widely used in developing novel therapeutic strategies based on anticancer agents modulating antitumor immune response (Zhu et al., 2016). Syngeneic models are also suited for studying the tumor microenvironment as both the cancer cells and surrounding tissues belong to the same species (Said et al., 2007). The ID8 cell line is the most widely used in syngeneic EOC mouse models and is derived from C57BL/6 mouse ovarian surface epithelial cells transformed by subsequent serial passage *in vitro* (Roby et al., 2000). Similar to xenograft models, syngeneic models can be classified by the site of injection. Cancer cells, such as the ID8 mouse ovarian cancer cell line, can be injected subcutaneously, orthotopically, or intraperitonially into C57BL/6 mice depending on experimental needs. The ID8 syngeneic model has been further improved by the introduction of genetic alterations common in EOC such as inactivation of *Trp53*, *Brca1*, and *Brca2* (Walton et al., 2016). Recently, to address EOC heterogeneity, a new generation of syngeneic models have been developed with various genetic backgrounds. These models were developed by the introduction of serial genetic alterations such as loss of *Trp53*, *Brca1*, *Pten*, or *Nf1*, or overexpression of *Ccne1*, *Akt2*, *Kras*, *Brd4*, or *Smarca4* in fallopian tube cell lines, and are

able to better phenocopy EOC characteristics (Iyer et al., 2020; Zhang et al., 2021). These novel syngeneic models should improve our understanding of EOC progression and assist in the development of novel therapeutic strategies such as immunotherapies and PARP inhibition. Other advantages of syngeneic animal models are fast tumor growth, wide availability, and relatively low cost as there is no need to use immunodeficient mice. However, despite these advantages, the immune systems of different species may have substantial variances and therefore any finding obtained using such models will require further validation by other EOC mouse models.

GENETICALLY ENGINEERED MOUSE EOC MODELS

Genetically engineered mouse models (GEMMs) are another important tool for investigating different aspects of EOC pathobiology (Table 3). GEMMs have greatly improved our understanding of EOC initiation. For example, methods such as the Cre/*loxP* system have allowed tissue-specific gene knockin and knockout *in vivo* and have facilitated identification of several EOC driver genes (Hoess & Abremski, 1985). One of the early challenges in the development of EOC GEMMs was lack of knowledge about specific gene promotes associated with tissues involved in EOC development. This obstacle was initially overcome by intrabursal injection of the adenovirus encoding Cre-recombinase (Ad-Cre) under the control of the cytomegalovirus (CMV) promoter (Hardy et al., 1997). Ad-Cre injection into the ovarian bursa results in excision of conditional *LoxP* site-flanked alleles in surrounding tissues (Wu et al., 2007). Alterations in several oncogenes and tumor suppressor genes such as *Trp53*, *Rb1*, *Myc*, *Akt*, *Pik3ca*, *Pten*, and *Arid1a* are believed to be the major driver genes promoting cancer progression in different EOC subtypes (Marcotte et al., 2012). Indeed, early study demonstrated that simultaneous inactivation of *Trp53* and *Rb1*, but not inactivation of either of these genes alone, can lead to the development of EOC resembling HGSOC, with serous histology and metastases to multiple organs (Flesken-Nikitin et al., 2003). Several other studies have also demonstrated that inactivation of other combinations of genes such as *Myc*;*Trp53*;*Brca1* (Xing & Orsulic, 2006) or *Pten*;*Pik3ca* (Kinross et al., 2012) can promote HGSOC development. This approach has also been employed for the development GEMMs recapitulating other EOC subtypes. For example, the discovery of the *Arid1a* mutation as a major driver in the development of OCCC and OEC has been confirmed by several GEMMs (Wiegand et al., 2010). Genetically, loss-of-function *ARID1A* mutation typically co-exists with activation of the PI3K pathway through gain-of-function mutation in *PIK3CA* in OCCC or inactivation of *PTEN* in OEC (Wiegand et al., 2014). Indeed, simultaneous inactivation of Arid1a and induction of *Pik3ca* (*Pik3caH1047R*) mutation through intra-bursal Ad-Cre injection can drive the formation of mouse EOC that fully recapitulates OCCC pathobiology, including development of ascites and peritoneal dissemination (Chandler et al., 2015). In addition, simultaneous inactivation of *Pten* and *Arid1a* can lead to the development of mouse EOC resembling OEC (Guan et al., 2014). Interestingly, *Arid1a* inactivation promotes epithelial differentiation of cancer cells in OEC mouse models driven by concurrent inactivation of *Pten* and *Apc* (Zhai et al., 2016), highlighting the importance of genetic context in determining GEMM phenotypes.

Another approach for EOC GEMM development is selective expression of Cre-recombinase in tissues associated with EOC using tissue-specific gene promoters (Metzger & Chambon, 2001). Müllerian-derived epithelia, including fallopian tube epithelium, one of the tissues believed to be the origin of EOC, can be genetically modified using the *Amhr2* gene promoter (Connolly et al., 2003). For example, the use of the *Amhr2* promoter for Cre-mediated excision of *Dicer1* or *Pten* in combination with *Trp53* in Müllerian-derived epithelia can result in the development of tumors that morphologically resemble HGSOC (Kim et al., 2012). Recently, several other gene promoters specific to Müllerian-derived epithelia, such as *Pax8* and *Ovgp1*, have been used to introduce genetic alterations associated with EOC. Cre-recombinase expression controlled by the *Pax8* promoter has been applied to selectively inactivate *Trp53*, *Brca1*, and *B*rca2 in fallopian tubes and endometrium, resulting in the development of EOC histologically similar to HGSOC (Perets et al., 2013). Similarly, tamoxifen-induced expression of Cre-recombinase under the

Table 3 List of selected EOC GEMMs

Gene alteration strategy	Target genes	Phenotype	References
AdCre-intrabursal injection	*Trp53;Rb1*	HGSOC	Flesken-Nikitin et al., 2003
AdCre-intrabursal injection	*Myc;Trp53;Brca1*	HGSOC	Xing & Orsulic, 2006
AdCre-intrabursal injection	*Pten;Pik3ca*	HGSOC	Kinross et al., 2012
AdCre-intrabursal injection	*Arid1a;Pik3ca*	OCCC	Chandler et al., 2015
AdCre-intrabursal injection	*Pten;Arid1a*	OEC	Guan et al., 2014
AdCre-intrabursal injection	*Arid1a;Pten;Apc*	OEC	Zhai et al., 2016
Amhr2-mediated Cre expression	*Dicer1;Pten;Trp53*	HGSOC	Kim et al., 2012
Pax8-mediated Cre expression	*Trp53;Brca1;Brca2*	HGSOC	Perets et al., 2013
Ovgp1-mediated Cre expression	*Apc;Pten*	OEC	Wu et al., 2016
Ovgp1-mediated Cre expression	*Trp53;Pten;Brca1*	HGSOC	Zhai et al., 2017

Ovgp1 gene promoter has been used for inactivation of *Apc* and *Pten* in other animal models, resulting in tumors resembling OEC (Wu et al., 2016). Interestingly, tamoxifen-induced expression of Cre-recombinase under the *Ovgp1* gene promoter can inactivate *Trp53*, *Pten*, and *Brca1*, leading to the development of HGSOC (Zhai et al., 2017). Recently, CRISPR/Cas9 technology has allowed much more rapid development of GEMMs at a significantly lower cost compared with traditional breading protocols. CRISPR/Cas9-mediated knockout or knockin can be performed with mouse embryos of different genetic background, eliminating the need for crossing animals to achieve the desired combination of genetic alterations (Mou et al., 2015). CRISPR/Cas9-mediated gene editing can also be achieved *in vivo* by intrabursal injection of lentiviral particles encoding CRISPR/Cas9 machinery together with gRNA sequences targeting genes involved in EOC development. GEMMs have been widely used for studies on the basic mechanisms of EOC initiation and progression as well as the development of novel therapeutics. Notably, GEMMs allow the exploration of novel therapeutic strategies in an immunocompetent and genetically defined manner. GEMMs are also particularly useful in developing therapeutic approaches that aim to target EOC at the early stages. The major limitations of GEMMs are their inability to monitor tumor growth, high cost associated with breeding colony maintenance, technical difficulties associated with intrabursal injection of the adenovirus encoding Cre-recombinase, and the lack of tissue-specific promoters uniquely suited to drive Cre-expression for the various EOC histo-subtypes.

CONCLUSIONS AND FUTURE PERSPECTIVES

The past decade has seen unprecedented progress in our understanding of the genetic components of EOCs. However, multiple challenges for improving the survival of EOC patients remain. First, due to the lack of effective screening strategies and absence of specific symptoms, most EOC patients are diagnosed with advanced disease. Second, with the advance in knowledge on EOC biology, mechanistic understandings need to be urgently translated into new therapeutic approaches. To meet these challenges, animal models that faithfully recapitulate the initiation, progression, and response to therapeutics are of utmost importance. Although rat and hen spontaneous ovarian cancer models have been reported (Karnezis & Cho, 2017; Lengyel et al., 2014), mouse models are the main experimental platform in EOC research. It is also important to note that there is no superior EOC mouse model, and each model has its own advantages and disadvantages. Therefore, mouse model choice should be determined by the nature of the scientific questions or preclinical experiments on a case-by-case basis. The usage of several mouse models to answer the same scientific question may also increase confidence in *in vivo* observations and increase the potential of translating preclinical findings.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.K. and R.G.Z. conceived the review and wrote the manuscript. All authors read and approved the final version of the manuscript.

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