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Preclinical Models for Ovarian Cancer: A Review

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Abstract

Ovarian cancer is the most fatal gynecological disease and arises from epithelial cells, stromal cells, and germ cells. The incidence of ovarian cancer increases with age, with a peak incidence at the age of 50-60 years. Almost 60% of the women who develop ovarian cancer will lead to death. Some of the risk factors for ovarian cancer involve hysterectomy, pelvic inflammatory disease, and polycystic ovarian syndrome. In this review, we discussed various animal models which accurately represent the cellular and molecular changes associated with the initiation and progression of human ovarian cancer and have significant potential to facilitate the development of better methods for the early detection and treatment of ovarian cancer. Also, we reviewed the reliability and limitations of the existing tumor models.

Keywords: Genetically Engineered Models; Gynecological Disease; Ovarian Cancer; Risk Factors

Abbreviations: OSE: Ovarian Surface Epithelium; SCID: Severe Combined Immune Deficiency; PDX: Patient Derived Xenografts; GEMMs: Genetically Engineered Mouse Models; BC: Border Cells; EMT: Epithelial To Mesenchymal Transition; YAP: Yes Associated Protein; PDGFR: Platelet Derived Growth Factor Receptor; VEGFR: Vascular Endothelial Growth Factor Receptor

Introduction

Ovarian cancer is a gynecological disease commonly found in advanced stages, having spread to the peritoneal cavity, and it accounts for only 3 % of cancers in women. However, it is the fifth most common fatal cancer in women behind lung, breast, colorectal, and pancreatic cancers [1]. And the incidence of ovarian cancer increases with age, with a peak incidence at the age of 50-60 years. The pathophysiology of this ovarian cancer as well as its etiology is not completely known. Nearly all benign and malignant ovarian tumors originate from one of three cell types: epithelial cells, stromal cells, and germ cells. More than 90% of malignant ovarian tumors are epithelial in origin, 5%–6% of tumors constitute sex cord-stromal tumors, and 2%–3% are germ cell tumors [2]. Due to its unknown pathophysiology some of the proposed hypotheses for the development of ovarian cancer are such as "Incessant ovulation theory", Ovulation includes an inflammatory process with leukocyte infiltration and release of inflammatory mediators and reactive oxidants that can cause DNA damage. And this risk gets lowered by the prolonged use of anti-inflammatory drugs. According to the "Gonadotropin hypothesis," an Excessive level of gonadotropin plays a facilitating role in the development of ovarian cancer and it acts directly or via increased production of estrogen and stimulates malignant transformation. According to the Hormone stimulation hypothesis excessive estrogen

or androgen stimulation of the ovarian surface epithelium (OSE) promotes neoplastic transformation [3]. The risk factors for ovarian cancer such as hysterectomy, pelvic inflammatory disease, and polycystic ovarian syndrome, are less clear. Despite improved knowledge of the etiology of ovarian cancer, aggressive cytoreductive surgery, and modern combination chemotherapy, there has been little change in the mortality statistics over the last 30 years, and approximately 60% of the women who develop ovarian cancer will die from their disease [4]. Lack of an adequate screening test for early disease detection and the rapid progression to chemoresistance has prevented appreciable improvement in patients with ovarian cancer. Experimental models for human diseases are of crucial importance not only to understand the biological and genetic factors that influence the phenotypic characteristics of the disease but to utilize as a basis for developing rational intervention strategies [5]. In this review, we reviewed some of the most commonly used animal models for ovarian cancer and their pathological reliability to human ovarian cancer.

Animal Models for Ovarian Cancer

Mouse Models

Mice (Mus musculus) are the most widely used animal model for cancer studies. A tremendous amount of information about tumor biology can be obtained from mouse models. The benefit of using murine models relates to the fact that the physiology and molecular signalling pathways are similar to those of humans, as well as from the availability of mouse strains specifically created for an investigation into various diseases and molecular mechanisms. Syngeneic models, genetically modified animals, and mice with xenotransplanted human tumors are the most frequently used models [6].

Xenograft Models

Beginning in the early 1960s, experiments were conducted to xenotransplant human cancer cells into immunocompromised mice. There are many mouse strains available right now with low immune responses. Some of them originate from animals with naturally occurring gene mutations, such as NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice, SCID (severe combined immunodeficiency) mice, or nude mice (Foxn1Nu/Nu, with a spontaneous deletion in forkhead box N1 gene), which have low levels of T lymphocytes. Xenotransplants are appropriate for fast-growing tumors [7]. In Xenograft models, where ovarian cancer cells have been injected either subcutaneously, orthotopically, or into the peritoneal cavity have been used extensively for the testing of novel therapeutics or modified regimens for the administration of standard chemotherapeutic drugs [8-10] and also used for the analysis of Cancer cells tumorigenicity, tumor histology, and tumor responses [11]. In xenograft models, 2 promising techniques are involved i.e.,

- Xenograft using cell lines
- Patient-derived xenografts (PDX)

In Xenograft using the cell line model, established cell lines are particularly selected [12]. The various cell lines such as HEY, OVCA429, OVCA433, OCC1, OVCAR-3, SKOV-3, A2780-s, A2780-cp, OV2008, C13, and ES-2 are implanted through SC, IP, IB into mice. From these cell lines, SKOV-3 cells are more highly tumorigenic than OVCAR-3 cell lines [13,14].

In Patient-derived xenograft models, surgically resected, patient-derived cells or samples from ascites are used to induce ovarian cancer [15,16]. The tumor tissues were implanted through SC, IP, and IB into SICD or NOD-SCID-IL2 γ mice, rather than BALB/c nude mice [17,18]. Some Features of xenograft models depending on the site of cancer cells administration are mentioned in Table 1.

S. No.	Injection Site	Dose	Features
1	Subcutaneous	Cells (1–2 × 10^6) for 5-6 weeks	➢ The tumor is limited to the site of cells injection ➢ Easy observation of tumor growth ➢ A tumor develops in an unusual anatomical location and microenvironment
2	Intra peritoneal	Cells (1–2 × 10^6) for 5-6 weeks	➢ A good model of disseminated disease. ➢ Tumor growth can be monitored using in vivo fluorescence or luminescence techniques ➢ Not suitable for investigating the initiation of the neoplastic process and the early stages of the disease
3	Orthotopically / Intrabursal	Cells 2.5x10^5 cells/5µ for 5-6 weeks	➢ The tumor develops in a closed space limited by the ovarian bursa ➢ A good model for research on the early stages of the disease

Table 1: Features of xenograft models depending on the site of administration.

Syngeneic Mouse Model

In a syngeneic model, cancer cells are derived from the same mouse strain and are introduced into the immunocompetent host [19]. The tumors developed spontaneously when administering the MOSE cell line and ID8 cells are injected into the ovarian bursal cavity of C57B16 mice. The ID8 cells formed direct contact with the ovarian stroma, resulting in primary tumor formation, secondary peritoneal carcinomatosis, and extensive ascites fluid production between 80 to 90 days post-exposure [20]. Classical syngeneic model is based on ID8 cells (spontaneously transformed OSE from C57BL/6 mice), injected into mice of maternal strain. The cells can form intraperitoneal metastases after Intrabursal injection. This model allows investigating the tumor metastasis and immunological function. After Intrabursal inoculation to the syngeneic host, these cells eventually gave multiple tumors disseminated within the peritoneal cavity, accompanied by the formation of ascites. The benefit of this model is the capability to research tumor vascularization, epithelial-stromal interactions, and anticancer immune response. Moreover, compared to immunocompromised mice, the risk of infection is lower [19]. This model allows for specialized investigation into the function of the immune system in the formation of metastasis as well as the effectiveness of immunotherapy in preventing metastasis [21].

Genetically Engineered Mouse Model

Genetically engineered mouse models (GEMMs) have been widely used in studying cancer initiation and progression. Gene functions and pathways contributing to early tumorigenesis can be demonstrated in vivo using GEMMs. Numerous significant cancer research developments have emerged from genetically engineered mice. Immunocompetent mice called GEMMs with genetic flaws are introduced into animals by the use of RNA interference, inducible gene expression viruses, or DNA recombination techniques. It provides a means to investigate how gene mutations affect the development of cancer. Several genetic changes associated with malignancy have been discovered by extensive investigation of human ovarian cancer specimens, including TP53, C-MYC, K-RAS, AKT, and BRCA1 & BRAC2 [11]. The first model was constructed to examine which oncogenes are indispensable for the transformation of OSE cells having inactive p53. Different oncogenes could be introduced into p53 cells. These cells were further injected, either subcutaneously or intra bursally, into the syngeneic mice and their tumorigenicity was assessed. The similar experimental system was used to test tumor cells, sensitivity to Rapamycin, the mTOR inhibitor. It was shown that rapamycin was efficient in some tumors with Active Akt [22]. Triple transgenic mice were used with floxed P53 and Brca1 genes and with membrane expression of the TVA receptor [23]. These models helped to elucidate the role of several oncogenes and tumor suppressor genes and their engagement in different histological types of ovarian cancer (Figure 1).

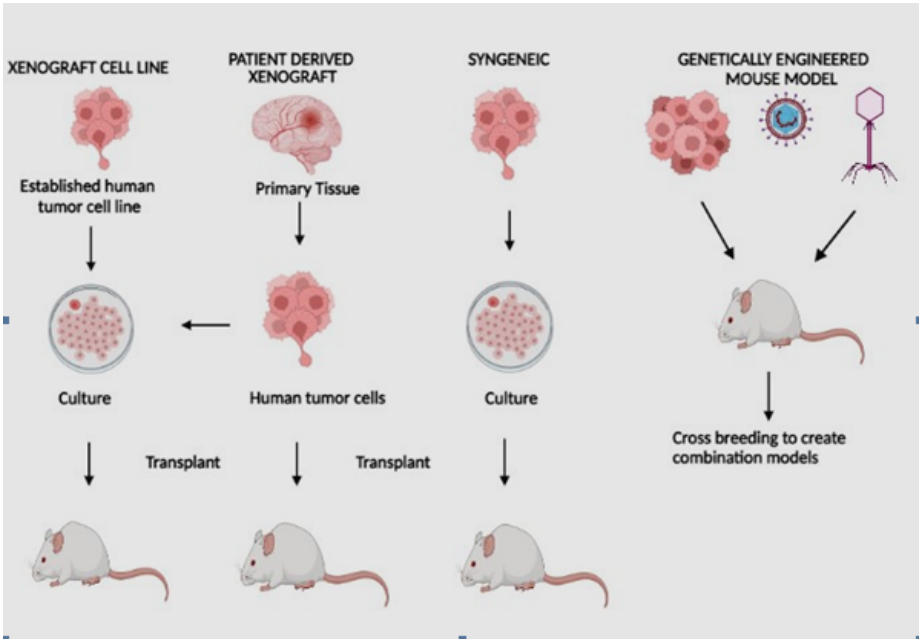


Figure 1: Different mouse models for induction of ovarian cancer.

Fruitfly Model

Fruit flies (Drosophila melanogaster) are practical and affordable model organisms due to their short life cycle and ease of propagation. Apoptosis, autophagic cell death, and necrosis occur in different cells in the ovary of D. melanogaster and have been associated with ovarian aging. Fruit fly border cells (BC) are recognized as a model suitable to study epithelial-to-mesenchymal transition (EMT) which occurs during ovarian cancer metastasis in higher animals [24,25]. Taiman is a protein involved in the control of BCs' migration and it initiates the migration of BCs in response to rising levels of ecdysone, a steroid hormone, by co-activating its receptor. This model was also used in the studies on Yap (Yes-associated protein) Oncogene [26,27]. In humans, YAP is engaged in promoting ovarian carcinogenesis by regulating the Hippo pathway which plays a crucial role in the control of cell proliferation and differentiation of both Drosophila and mammals [28-30]. Border cells express two receptor tyrosine kinases: epidermal growth factor receptor (Egfr) and Pvr, which shows similarity to the human platelet-derived growth factor receptor (PDGFR) and Vascular endothelial growth factor receptor (VEGFR). Overexpression of these receptor increases cellular motility, invasiveness, proliferation, and tumor progression resulting from enhanced angiogenesis and emerging vasculature of the tumor [31,32]. Thus, the Fruit fly model serves as a model to study the molecular mechanism of ovarian cancer.

Laying Hen Model

The laying hen (Gallus domesticus) is the only non-human animal with a significant rate of spontaneous ovarian tumor progression in women, ovarian cancer in the hen is age-related and it is also grossly and histologically similar to that in humans [33]. The incidence of tumors in this model is 10 times higher than that in humans, and they are highly similar to human diseases. Also, the similarities between then and human ovarian cancer are observed at the molecular level [34], as a result, this model provides the opportunity to research OC risk factors as well as the formation, progression, histological, and therapeutic response of tumors. Additionally, it serves as an important tool for preclinical cancer drug testing. In this model, some epithelial markers can be found that include CA-125, cytokeratin, EGFR, HER-2/neu, VEGF, COX-1, CYP1B1, E-cadherin, and PCNA and p53 mutations are observed in about 50% of hen ovarian cancer cases. Ras mutations in hens are less common than in women [35].

Frog Model (Xenopus)

Xenopus (clawed frog) is a genus of African frogs, of which two species, X. laevis and X. tropicalis, are widely used as model organisms for cancer studies. Naturally occurring tumors and spontaneously developed tumors in Xenopus are considered to be extremely rare; [36] however, ovarian germ cell tumors (dysgerminoma) have been observed in 5-7year-old frogs after long-term HCG treatment administered for breeding purposes. This species is used to study its function in cancer and embryogenesis because Xenopus embryos develop quickly and provide investigation without the interference of de novo mutations resulting from inherent genomic instability. Due to the presence of a variety of Anti-tumoral immune effectors like CD8 NKT-like cells and NK cells genetically modified Xenopus models are now under rapid development and they could provide a good alternative to the murine system for studying tumorigenesis and tumor immunity [37].

Zebrafish Model

In recent years, the zebrafish (Danio rerio) has emerged as an attractive alternative to the mouse in cancer research representing an efficient platform for investigating cancer and cancer therapeutics [38]. At present, it is one of the most promising models for cancer research. Both embryos and adult zebrafish can be used for drug screening, although for embryos, the drug administration in their water is easier. And also, facilitates the performance of reverse and forward genetic approaches, including mutagenesis and small molecule screens [39]. Using EOC cell line-bearing zebrafish embryos, zebrafish xenotransplantation models have been developed for assessing cancer progression or testing anticancer compounds [40]. This model provides a unique opportunity to monitor tumor-induced angiogenesis, invasiveness, and response to a range of treatments. Its embryo's high fecundity and quick generation times make it easier to create transgenic lines and carry out large-scale mutagenesis screenings that can find novel genetic pathways [41,42] (Table 2).

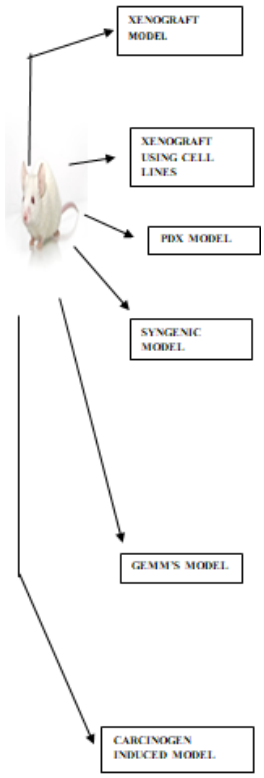
NO	Models	Pros	Cons
1		➢ A good model of advanced disease and study of the tumor microenvironment. ➢ Suitable for drug response testing and validation of new therapies. ➢ The rapidity of tumor formation, easy predictability, reproducibility synchronization. ➢ Retains the original characteristics of the tumor. ➢ A good model for basic research and preclinical studies. ➢ Immunocompetent host. ➢ Possibility to test the anti-cancer immune response study of the tumor microenvironment, its vascularization, and epithelial-stromal interactions. ➢ Reduced risk of infection in mice. ➢ Good model for basic research and for studies on ovarian cancer initiation and progression. ➢ Allows investigating the tumor metastasis and immunological function. ➢ Spontaneous disease induction occurs. ➢ Concomitant hormone treatment was shown to significantly affectthe rate of tumor formation	➢ Time-consuming. ➢ High cost of model construction and maintenance of immunodeficient mice. ➢ No host immune responses. ➢ Expensive. ➢ Technically Complicated. ➢ Time-consuming construction of the model. ➢ High cost of model constructionandmaintenane. ➢ Limited access to biological material. ➢ Not suitable for immunotherapy and host–cancer cells interactions. ➢ Only somewhat predictive. ➢ Mostly carcinogen derived. ➢ Time-consuming and costly construction ➢ Difficulty in noninvasive tumor burden in a small model.
2	 DROSOPHILA MELANOGASTER	➢ Suitable for basic research. ➢ Simple structure, short life cycle, easy propagation and maintenance. Conserved DNA repair mechanisms and signaling pathways	➢ Simple immune system. ➢ Tumors require induction and have a poor metastatic potential
3	 LAYING HEN	➢ Spontaneous development of cancer. ➢ Short time of tumor formation. Suitable for studies on genetic, biochemical, and environmental risk factors: formation, progression, histological, and therapeutic response of tumors	➢ Lower incidence of histological types that are predominant in humans
4	 CLAWED FROG (XENOPUS)	➢ Good model for studies of cell and Developmental biology. Lower cost and in a shorter period for tumor development.	➢ Spontaneous tumors are rare. ➢ Suitable models of epithelial ovarian cancer must be developed
5	 ZEBRAFISH	➢ High fecundity and quick generation times make it easier to create transgenic lines and carry out large-scale mutagenesis	➢ Moderate Flexibility and predictability

Table 2: Animal Models for Ovarian Cancer.

Carcinogen-Induced Tumor Model

Few chemical agents are used to induce ovarian cancer in such animal models as hens, some strains of mice, rats, and primate macaques [43]. Carcinogens that are used to trigger the formation of ovarian tumors include 7, 12- dimethylbenz(a) anthracene, 20-methylcholanthrene, 1, 3-butadiene, formic acid 2- [4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide, a nitrofuran antibiotic, and N-methyl-N'-nitrosourea, a direct-acting alkylating agent. Carcinogens were applied directly to the ovaries to cause tumors. In 1970, the 7, 12-Dimethylbenz (a) anthracene (DMBA) was exposed to the Guinea pig, the tumor developed spontaneously [44,45]. Numerous studies have revealed, the use of 7, 12-dimethylbenz[a]anthracene (DMBA) to induce ovarian tumors in rodents either by injecting DMBA directly into the ovary or injecting a DMBA-saturated suture/ gauze under the ovarian surface [46,47].

Conclusion

In conclusion, animal models are an indispensable tool for basic research and drug development. This model allows a better understanding of the pathology of the disease and identifies the potential therapeutic targets. And each of these models is used for a particular type of investigation. Various models like mice, fruit flies, laying hens, Xenopus, and zebrafish are used for the study of ovarian cancer. Comparing these models, the laying hen serves as a promising model for the incidence of tumors, the incidence of tumors in this model is 10 times higher than that in humans, and they are highly similar to human diseases.

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