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Current Models of Ovarian Cancer

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ABSTRACT

Ovarian cancer has proved to be one of the most difficult cancers to treat. It is often diagnosed in the late stages. When it is detected early, the 5-year survival rate is 93%. However, it is only detected early 15% of the time. For this reason, there is an emphasis on finding better tumor markers that can identify cancerous cells early. Ovarian cancers come from 3 different cell types. There are a variety of cancer subtypes from each type of cell. A one-size fits all treatment method isn't feasible with so much variation. Models of ovarian cancer help understand the pathway of cancer development, find tumor markers for early detection, improve imagining techniques, and test drug therapies. Current models include transgenic mice, xenograft mice, chick chorioallantoic membrane, the laying hen, and 3-D human tissue cultures. Unfortunately, there are plenty of flaws with these models that researchers are trying to overcome. Determining which model is the best representation of human ovarian cancer is crucial for making progress in treating ovarian cancer. In this review I will provide an overview of current models for ovarian cancer. I will be looking at current research done with these models to explore their benefits and disadvantages.

INTRODUCTION

In 2018, roughly 14, 070 women will die from ovarian cancer and 22,240 new cases will be diagnosed [1]. Women over 63 make up half of the cases. It is the 5th leading cause of cancer

related deaths in women [2]. The odds of a woman dying from ovarian cancer are 1 in 108 [2]. The symptoms of ovarian cancer include pelvic and abdominal pain, bloating, nausea, vomiting, indigestion, trouble eating, feeling full quickly, urinary urgency and frequency, fatigue, back pain, pain during sex, changes in periods, abdominal swelling with weight loss, and a change in bowel habits [3-4]. Unfortunately, early stages of ovarian cancer are usually asymptomatic and later stage symptoms are non-specific [5]. As a result, ovarian cancer is not typically detected in the early stages which leads to low survival rates. When it is detected early, before metastasis, patients have a 5-year survival rate of 93% [3]. Unfortunately, only 15% of ovarian cancer is detected in the early stages. The 5-year survival rate for stage 3 epithelial ovarian cancer ranges from 63%-28% depending on the sub classification. Stage IV epithelial ovarian cancer has a 19% 5-year survival rate [4].

Ovarian cancers are given a stage after diagnosis. Stages describe how far the cancer has spread with stage I indicating the least amount of spreading. Stage IV is the maximum. There are 3 factors used to stage the cancer in both the International Federation of Gynecology and Obstetrics and the American Joint Committee on Cancer TNM staging system. The factors include the extent of spreading (T), spreading to the lymph nodes (N), and metastasis to distant sites (M). Staging cancers help with talking about survival statistics as well as determining the severity and the best way to treat it. Most statistics are given in 5-year survival rate. These statistics look at how many people are still alive 5-years after treatment based on the stage the cancer was in when the patient was first diagnosed. They do account for new, improved treatment methods. They do not include cancers that come back or spread. They also do not adjust for many other factors such as age, overall health, and effectiveness of the treatment [4].

Cell-Type Specific Ovarian Tumors

There are 3 cells types in the ovaries and they develop into different types of tumors. The epithelial tumors are called carcinomas. They are the most common making up 85%-90% of malignant tumors. There are 4 main types [3]. These are differentiated by the same genes that are responsible for epithelial differentiation during embryonic development [6]. The serous carcinomas make up 52% of epithelial carcinomas. The serous membranes these carcinomas resemble are a type of epithelial tissue covers organs in closed cavities of the body. It is covered by a thin layer of serous fluid secreted by the epithelium [7]. The other main types are clear cell, mucinous, and endometroid carcinoma. Together they only make up 22% of epithelial carcinomas. They also are considered low-grade—grade 1—carcinomas because they look more like normal tissue. Grades are used to describe how close the cancerous tissue looks to non-cancerous tissue with 1 being the closest to non-cancerous tissue. Patients with low-grade carcinomas have better prognosis. While these tumors grow slowly, they cause fewer symptoms and typically do not respond well to chemotherapy. Serous carcinomas can be low-grade or high-grade—grade 3—carcinomas. High-grade carcinomas look less like normal tissue and usually have a worse prognosis for patents. While they grow and spread sooner, they seem to response better to chemotherapy [3].

The second cell type in the ovary are the germ cells. These come from the ova in females. They make up less than 2% of ovarian cancers and are typically benign. When they are cancerous, 5-year survival is at 90%. The most common subtypes are teratomas, dysgerminomas, endodermal sinus tumors, and choriocarcinomas. The most common cancer stemming from the germ cells are called dysgerminomas. They predominantly affect women in

their teens and twenties. However, they don't grow or spread rapidly. If they are removed before they spread beyond the ovary, 75% of patients do not need further treatment. In cases where the tumor has spread, 90% of patients can cure or manage the cancer with a surgery, radiation, and/or chemotherapy. Teratomas look like the 3 layers of the developing embryo when viewed under the microscope. There is a benign tumor called a mature teratoma that predominantly affects women of reproductive age. Immature teratomas are cancerous and predominantly affect women under 18. When they are still grade 1 and have not spread to other organs, they are treated by surgery. Grade 2 and 3 that have spread past the ovaries are commonly treated with a combination of surgery and chemotherapy. Endodermal sinus tumors and choriocarcinomas affect girls and young women. Although they tend to grow and spread rapidly, they are very sensitive to chemotherapy. Choriocarcinomas can start in the placenta or the ovaries. The ones that start in the placenta are more common and typically respond better to chemotherapy [3].

The third cell type of the ovary is the stroma. Tumors of these cells only make up 1% of ovarian cancers. Over half of them develop in women 50 years or older. However, 5% do develop in young girls. While they are less common, they do have better defined symptoms than the other ovarian cancers. They can cause abnormal vaginal bleeding due to the production of female hormones. They can start menstrual periods and breast development to happen before puberty in young girls. Conversely, these cells can also release male hormones that stop periods and cause facial and body hair to grow. These tumors can also bleed which can cause sudden, severe abdominal pain. The most common type are granulosa cell tumors which are malignant. The granulosa-theca tumors and Sertoli-Leydig cell tumors are also

cancerous stromal tumors. They are usually found in the early stages. Most than 75% of patients survive long-term [3].

Causes of Ovarian Cancers

For most ovarian cancers, the cause is still unknown. There are inherited genetic mutations like *BRCA1, BRCA2, PTEN, STK11*, and *MUTYH* that increase the risk of developing ovarian cancer. Risk factors for germ cell and stromal tumors of the ovaries are not well known. However, from the known risk factors for epithelial ovarian cancers, there are several theories about the causes. There seems to be reduced risk when the woman ovulates less. Irrespective whether that's due to pregnancy or birth control with hormonal contraceptives. The fact that tubal ligation and hysterectomy lowers the risk has led to a theory that cancer-causing substances travel to the ovaries from the vagina. There is another theory that androgens—male hormones—can cause ovarian cancer. Tests for acquired genetic mutations like the TP53 tumor suppressor gene or the HER2 oncogene may also predict the outcome for the patient indicating that these mutations are key in pathogenesis [8].

Ovarian Cancer Detection

During regular health exams, ovarian cancer is hard to catch. Pelvic exams check for the size, shape, and consistency of the ovaries and uterus but often miss early ovarian tumors. Advanced stages of ovarian cancer may show up on a pap test used to test for cervical cancer, but this is extremely rare. In combination with pelvic exams, transvaginal ultrasounds and cancer antigen protein (CA-125) blood tests may be given to screen for ovarian cancer. Unfortunately, both of these methods are flawed. Transvaginal ultrasounds help find masses in the ovaries, but most of those found are benign. The CA-125 blood test measures the amount

of CA-125 which is usually elevated in women with ovarian cancer. It is commonly used to test if treatment is working in women known to have ovarian cancer because successful treatment will lower it. Unfortunately, it is not always elevated in women with ovarian cancer. Levels can also rise from endometriosis and pelvic inflammatory disease. Screening is not recommended for women with average risk and no symptoms. Some germ cell cancers release human gonadotropin and alpha-fetoprotein which can be used to see if treatment is working or if the cancer has returned, but these are not recommended for screening. Other imaging tests may be used to determine if a pelvic mass is present and if it has metastasized. These imaging tests include: ultrasound, CT scans, barium enema x-ray, MRI, x-ray, and PET scans. Laparoscopy and Colonoscopy allow for biopsies to be done. Biopsies take a remove a piece of tissue for study in the lab. In a procedure called paracentesis, a syringe retrieves fluid from the abdominal cavity if the patient has ascites. The fluid can be tested for cancerous cells. Biopsies and paracentesis are the only way to know confirm a growth is cancerous. There are currently no other reliable screening tests although many are being heavily researched. Finding a reliable screening test to detect ovarian cancer early may be pivotal in preventing deaths [4].

Ovarian Cancer Treatment

There are a variety of treatments available. Local treatments that affect just the tumor and not the rest of the body include surgery and radiation therapy. Systemic treatments that affect the whole body include chemotherapy, hormone therapy, and targeted therapy. Surgery removing the uterus, both, fallopian tubes, the ovaries, and omentum is usually done when the patient has epithelial ovarian cancer. The tissue is then tested in order to accurately classify the cancer stage. If the patient is in early enough stages and is of childbearing age, they might not

have both ovaries and the uterus removed. It depends on the spreading of the cancer as well as the general health of the patient. When the cancer has already spread to the abdomen, the surgeon will try to remove as much of the tumor as possible. This is called debulking. Optimal debulking removes all visible cancerous tissue and leaves no tumors larger than a half-inch. Optimal debulking greatly improves prognosis for the patient. Germ cell and stromal cell cancers also lead to the removal of the uterus, fallopian tubes, and ovaries. However, keeping non-cancerous tissue until the patient has finished having children is more common with these types of cancer.

Chemotherapy can be useful after surgery to kill off any cancer cells left behind or metastasized. It can be used before surgery to shrink large tumors, making surgery easier. Chemotherapies are usually given through IV, intraperitoneal catheter, or orally. A combination of two different types of drugs are typically used. One is called a platinum compound and the other is called a taxane [9]. Platinum compounds covalently bind to DNA which ultimately results in apoptosis [10]. Taxanes bind microtubules so androgen receptors cannot move to the nucleus [11]. There are also several other chemotherapy drugs that are not platinum compounds or taxanes that may be helpful in treatment. Chemotherapy is typically given every 3-4 weeks for 3-6 cycles depending on the stage and type of the cancer. Cycles involve regular administration of the drug and then a period of rest. If the chemotherapy seems to work well and the cancer stays away for 6 to 12 months, the same chemotherapy regiment is recommended. If the patient is in stage III and the tumors have been optimally debunked, an intraperitoneal chemotherapy treatment may be used in combination with systematic chemotherapy. This is given through a catheter that has been placed in the abdominal cavity.

Although it may increase life-span, the chemotherapy side effects of nausea, vomiting, and abdominal pain can be more severe. For germ cell tumors, a combination of drugs called BEP are used. This includes bleomycin, etoposide, and cisplatin. Dysgerminoma may be treated with carboplatin and etoposide because these tumors are more sensitive to chemotherapy and this combination is less toxic. If the tumor doesn't response, the patient may be given a high dose chemotherapy treatment or an assortment of different drugs. Stromal tumors are not usually treated with chemotherapy [9].

Temporary side effects of chemotherapy include nausea, vomiting, loss of appetite, loss of hair, hand and foot rashes, mouth sores, increased risk of infection, easy bruising and bleeding, and fatigue. These usually go away after treatment is over. Unfortunately, certain drugs have long-term or permanent side effects. Cisplatin can cause kidney damage. Cisplatin and taxanes can cause nerve damage. Cisplatin specifically can damage nerves going to the ears which leads to hearing loss. Ifosfamide can cause irritation and bleeding of the bladder lining. Chemo can cause early menopause and infertility. Sometimes certain chemotherapies can permanently damage bone marrow. This may lead to second cancer of the blood [9].

Targeted therapies attack cancer cells while inflicting little damage to the normal tissue. Angiogenesis inhibitors such as bevacizumab attach to and inhibit a protein called VEGF. VEGF signals new blood vessels to form. Without new blood vessels to provide nutrients, the cancer's growth is slowed or stopped. This drug works better in combination with chemotherapy, but it doesn't seem to help increase the patient's life-span and has several side-effects. Common side effects include high blood pressure, tiredness, bleeding, low white blood cell counts, headaches, mouth sores, loss of appetite, and diarrhea. More serious side effects are rare but

include blood clots, severe bleeding, slow wound healing, holes in the colon, and abnormal connections between the bowel and skin or bladder. PARP inhibitors block the PARP enzyme that helps repair damaged DNA. Since the tumor cells cannot repair the damaged DNA, these cells usually die. Side effects include nausea, vomiting, diarrhea, fatigue, loss of appetite, taste changes, low red blood cell counts, belly pain, and muscle and joint pain. Like chemotherapy, these drugs may lead to blood cancers due to bone marrow damage [9]. People with BRCA 1 or BRCA 2 mutations may benefit from certain PARP inhibitors [4] like olaparib, rucaparib, and niraparib. Niraparib is also used to treat recurrent ovarian cancer if chemotherapy is not successful. A small portion of women with ovarian cancer have BRCA 1 and BRCA 2 mutations [9]. Genetic counseling is important to determine your risk as well as other family member's risk. There are over 1,000 known BRCA mutations [4].

Primary peritoneal carcinoma (PPC) is closely related to epithelial ovarian cancer. It looks the same as epithelial ovarian cancer that has metastasized in surgery. It also produces similar symptoms and elevates a tumor marker called CA-125 like ovarian cancer. PPC seems to start in cells lining the fallopian tubes and spreads along the surface of the pelvis and abdomen like ovarian cancer. For this reason, it can be difficult to determine exactly where the cancer started first [3].

The methods of studying cancer have evolved with new technologies. Ovarian cancer models have move way past the days of inducing tumors with carcinogens after it was shown that this is not a relevant model [12, 13]. More relevant models have been developed over time. This paper will explore the current animal models as well as human cell culture models.

Each model has benefits and drawbacks. Hopefully improvements to these models will result in better treatment and earlier detection of ovarian cancer.

GENETICALLY ENGINEERED MOUSE MODEL

High-grade serous ovarian cancers (HGSCs) are responsible for 70% of all deaths due to ovarian cancer. In the past few years, it has been proposed that the fallopian tubes may be the origin for this subset of ovarian cancer [14]. Development of a mouse model of HGSCs that would mimic the early alterations and disease progression by focusing on lesions in the fallopian tubes has been at the heart of many research studies trying to identify whether HGSCs can originate from this location [15]. Genetically engineered mouse models using Cre recombinase are popular methods for constructing human disease models including ovarian cancer.

Two examples include the Dicer-Pten and Pax8-Cre derived models. The Dicer-Pten was created by using an anti-Mullerian hormone receptor type 2-directed Cre recombinase to knock out *DICER* and *PTEN*. [14]. Anti- Mullerian hormone receptor type 2 is expressed in ovarian tissue [16] so this recombination event was not limited to the fallopian tubes. Dicer is an RNase III that is crucial in converting pre-miRNA to miRNA. Pten is a tumor suppressor that inhibits the PI3k pathway [14]. The Pax8-Cre model uses a *PAX8* promoter to control the expression of Cre recombinase. Pax8 is a transcription factor for fallopian tube secretory cells and appears to be absent from ovarian surface epithelium. This was confirmed by performing β-galactosidase stains on the organs from the first generation of Pax8-Cre mice crossed with Gt(ROSA) $26Sor^{tm1sor}$ that have a loxP-Stop-loxP LacZ transgene. The fallopian tubes had the highest LacZ

staining pattern. The uterus had some, but the ovaries had none [15]. Knowing which cells gene inactivation happens in allows for testing of the hypothesis that alteration in the fallopian epithelium can cause ovarian cancer.

While Dicer-Pten was used to create one double knock-out model [14], the Pax8-Cre created five distinct models. Murine cohorts were created by using Pax8-Cre mice crossed with mice containing different loxP flanked genes as well as mutations. Mutants or knock-outs of the four tumor suppressors genes *BRCA1*, *BRCA2*, *PTEN, and TP53* were used in different combinations to develop the five models [15]. *BRCA1* and *BRCA2* code for different proteins that help repair DNA damage [17, 18]. PTEN codes for an enzyme that is part of the stop replication signaling. This signaling induces apoptosis [19]. *TP53* codes for the tumor protein p53. This protein binds directly to damaged DNA. The protein can signal DNA repair mechanisms or signal the cell to undergo apoptosis [20]. Mutations in P53 are ubiquitous in HGSCs [21]. However, in the Pax8-Cre mice with wildtype *PTEN* and altered *BRCA2* and *TP53* did not develop tumors as quickly or as frequently as the other mutations. This suggests that *BRCA2* and *TP53* inactivation are not enough to initiate tumor development and progression. [15]

The highest incidence of serous tubal intraepithelial carcinomas (STIC) and fallopian tube transformation occurred in mice with an inactivated *BRCA1*, mutant *TP53*, and an inactivated *PTEN* as well as mice with *BRCA2,* mutant *TP53*, and inactivated *PTEN*. The fallopian tube transformations were characterized by secretory cell proliferation, loss of polarity, cellular atypia, and serous histology consistent with STICs. Their results are summarized in the following chart:

Genotype	Number of Mice	Survival (weeks)	STIC	FT Transformation	Ovarian Metastasis	Peritoneal Metastasis
Brca1$^{-/-}$;Tp53^{mut};Pten$^{-/-}$;Pax8-rtTA;TetO-Cre	4	5-17	4/4 (100%)	4/4 (100%)	1/4* (25%)	1/4* (25%)
Brca1$^{+/-}$;Tp53^{mut};Pten$^{-/-}$;Pax8-rtTA;TetO-Cre	12	9-20	10/12 (83%)	10/12 (83%)	6/12 (50%)	8/12 (67%)
Brca2$^{-/-}$;Tp53^{mut};Pten$^{-/-}$;Pax8-rtTA;TetO-Cre	12	7-15	9/12 (75%)	9/12 (75%)	9/12 (75%)	8/12 (67%)
Brca2$^{+/-}$;Tp53^{mut};Pten$^{-/-}$;Pax8-rtTA;TetO-Cre	3	17-18	3/3 (100%)	3/3 (100%)	3/3 (100%)	2/3 (67%)
Tp53$^{-/-}$;Pten$^{-/-}$;Pax8-rtTA;TetO-Cre	6	19-38	4/6 (67%)	4/6 (67%)	0/6 (0%)	0/6 (0%)

In both mice models, removal of the fallopian tubes prevented the development of HGSCs, but removal of the ovaries did not [14, 15]. Since the Dicer-Pten promoter was not cell type specific, it's not certain which type of fallopian tube cells the cancers originated in. Analysis of the fallopian tubes in earlier stages suggested fallopian stromal cells may be the origin. In the double-knock out of Dicer-Pten, 100% of the mice develop serous carcinomas in the fallopian tubes that spread to the ovaries. The tumors then metastasize to the abdominal cavity much like HGSCs in humans. The histology and upregulation of genes in the double knock-outs closely resembled human serous carcinomas [14].

In the Pax8-Cre models, the cohort's ability to develop STICs and fallopian tube transformations did not always correlate with the ability to metastasize. Although all the mice with inactive *BRCA1*, mutant *TP53*, and inactive *PTEN* develop STICs and fallopian tube transformations, only a fourth of the mice showed ovarian as well as peritoneal metastasis. When the carcinomas did metastasize, it was consistent with the pattern HGSC spread in humans. Immunohistochemical analyses showed that many of the tumor markers in mice mimicked human HGSCs and STICs. In addition, HGSC biomarkers and copy number alterations to human HGSC and found they correlated [15].

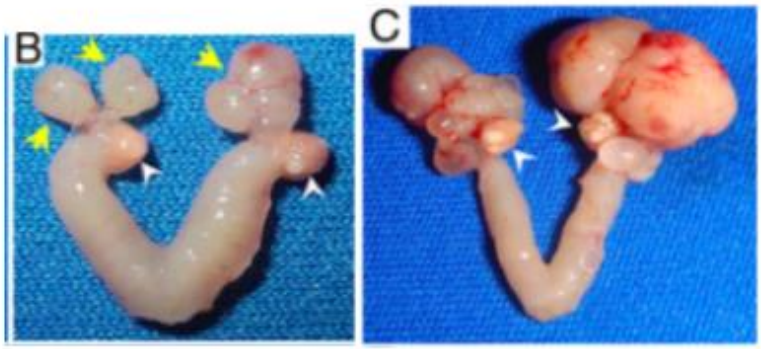

Figure 1. Side by side comparison of fallopian tubes (yellow arrows) and ovaries (white arrows) of 5 month old (B) and 8 month old (C) Dicer-Pten mice.

As shown in Figure 1, the Dicer-Pten mice develops tumors in the fallopian tubes [14]. However, In the Pax8-Cre mice, the fallopian tubes did not develop tumors that are visible from a gross inspection. This can be seen in Figure 2. It is often noted in human HGSCs cases that the fallopian tubes lack visible tumors [15]. Fallopian tube cancer itself if rare, but similar to epithelial ovarian cancer [3].

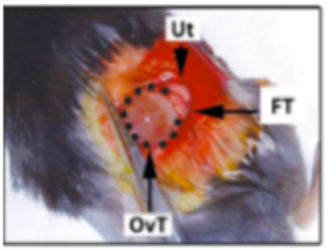

Figure 2. *Brca2+/-;Tp53R270H/-;Pten-/-* Pax8-Cre mouse with HGSC originating in the fallopian tube (FT) and metastasizing to the ovary (circled, OvT)

XENOGRAFTED MICE MODEL

Xenografts can be done with fresh cells or cell lines [22, 23]. Xenografts can be transplanted into the subcutaneous, intraperitoneal, subrenal capsule, and orthotopic tissue [24]. However, there is some debate about the orthotopic model. The orthotopic model transplants tumor cells directly into the ovaries. Bioluminescent images from an orthotopic model can be seen in Figure 3 [24]. In mice, bursa membrane surrounds the ovaries and creates a unique microenvironment. It provides a barrier to the peritoneal cavity that may impair tumor development [25]. On the other hand, this unique microenvironment may be more like the primary tumor's microenvironment than other transplant areas [24, 26]. Other injection sites may induce compensatory mechanisms. In intraperitoneal transplants of fresh patient tumors new murine stroma developed that may be imitating the primary tumor's microenvironment [22].

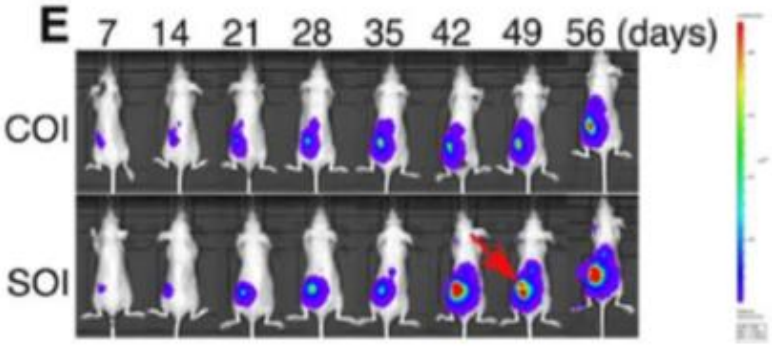

Figure 3. bioluminescent images of the tumor growth of a single mouse

By genomic characterization cell lines versus human ovarian cancer tissue, it was found that the most appropriate cell lines to model high-grade serous carcinomas were the least used in laboratories [23]. Although fresh tumor grafts allow for the best representation of the

primary cancer. The tumor tissue can be mixed with McCoy's media was injected intraperitoneally in female SCID mice [22]. SCID mice are mice that cannot produce T, B, and NK immune cells. Another popular immunocompromised mouse used in xenografts are the nude mice. These mice lack a thymus can cannot produce T cells [26]. SCID have the tendency to develop spontaneous lymphomas so the tissue type of the tumors must be tested. The mixture of tumor and McCoy's media leads to an engraftment rate of 74%. Serous, carcinosarcoma, and transitional tumor types can all engrafted. It is important to note that patients whose tumors did engraft successfully had lower overall survival than those that did not [22] indicating that there is a bias towards more aggressive cancer. The fresh tumor grafts are microscopically similar to the primary tumors. Glandular characteristics of adenocarcinoma and the proliferation index were intact in the tumor grafts. Testing for pan-cytokeratin expression which confirmed that the tumors are from epithelial tissue. Unfortunately, cells with leukocyte common antigen (CD45) from primary tumors do not coheterotransplant often [22].

The fresh tumor grafts show overlap between the genetic changes in the patient and the tumor graft. However, the levels of the tumor marker CA125 were only elevated high enough to detect in 3 models and they were not highly elevated unlike the patients [22]. Patient-derived xenografts are good at predicting how well anticancer therapies work (Pompili). Tumor grafts from platinum sensitive parent tumors are also sensitive to platinum. If treatment with carboplatin/paclitaxel that works on the patient, it also works on the tumor grafts [22]. Ideally, they could be used to test patient treatment methods before administering them to the patient [26]. There is also the possibility of using them in place of patient tissue for before and after treatment comparisons. This would eliminate the need for invasive procedures on patients to

collect more tissue. However, this method is not perfect and may prevent patients from getting all possible treatment methods if their tumor grafts do not respond well to the treatment [22]. Another drawback is that some patient derived xenografts are unable to metastasize. Until the method is perfected, the tumor grafts may serve as an ideal drug testing method or help with the discovery of new biomarkers [22]

CAM

The chick chorioallantoic membrane (CAM) model uses fertilized chicken eggs which is much less expensive than immune compromised mice. They also require less animal oversight. The methodology involves human cancer cells into the CAM through a small window in the egg shell. By using OV8GFP—which is a human ovarian cancer cell like that expresses green fluorescent protein—tumor formation can be monitored through the window in the egg shell. When compared to slides of the human tumor, the tumors grown in the CAM closely resembled the original tumor. Histopathological tests showed the growth of an extracellular matrix as well as stromal cells. This model can also grow tumors from human tissue transplants [27].

In order to show the CAM model could demonstrate cell invasion, *in vitro* assays were performed using the OVCAR-3, SKOV-3, and OV-90 ovarian cancer cell lines for comparison. The OV-90 proved to be the most invasive. These cells migrated to a chemo attractant the fastest while going through 12 μm pores in a Geltrex extracellular matrix. *Ex ovo* methods were not effective with a 10% rate of survival so this method was discarded. However, *in ovo* had much better results with a 70% survival rate. The *in ovo* test were done by creating a small window in the shell on the third day of embryo development which allows the CAM layer to detach from

the shell. 900,000 ovarian cancer cells were mixed with Matrigel and grafted on top of the CAM on day 11. On day 14, the ectoderm and the mesoderm were put through cytokeratin immunohistochemistry to assess the invasion of the cells from the ectoderm into the mesoderm. The results are shown in Figure 4. OVCAR-3 damaged the ectoderm more than the mesoderm. SKOV-3 damaged the mesoderm more than the ectoderm. The OV-90 cells invaded the mesoderm and destroyed the ectoderm. These results were consistent with *in vitro* assays. A neutralizing antibody was mixed with the Matrigel and OV-90 cells compared to a control anti-mouse IgG inhibited the OV-90 cells from invading the mesoderm [28]. This demonstrates how easy it is to test a new substance in the CAM model.

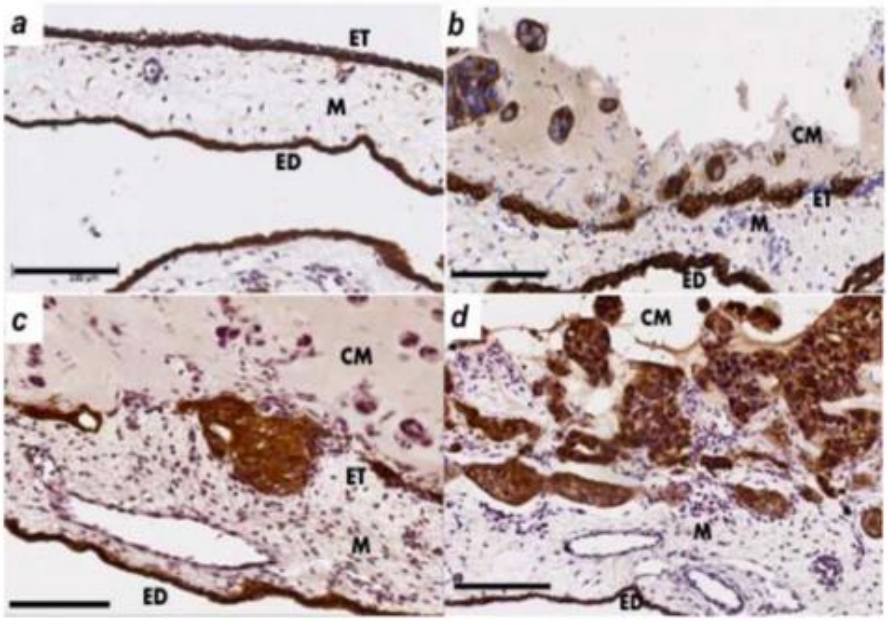

Figure 3. (a) normal structure of the CAM layers; ectoderm (ET), mesoderm (M) and endoderm (ED); (b) OVCAR-3; (c) SKOV-3; and (d) OV-90 cancer cell invasion after 14 days

Biodegradable PMO nanoparticles suspected to preferentially deliver drugs to the tumor were also tested in the CAM model. These nanoparticles were loaded with the chemotherapy drug doxorubicin. Doxorubicin damages DNA and induces apoptosis. These nanoparticles were injected intravenously once a tumor had been established. The cell death could be monitored from the window in the shell by bright field and green fluorescence using a fluorescent stereomicroscope. The organs of the fetal chicken were examined 3 days after injection. These were compared to eggs that had received no drug and eggs that had been given doxorubicin that was not bound to the nanoparticles. All of the eggs given nanoparticle bound doxorubicin survived the three days after injection and their organs appeared normal upon post-mortem examination. The eggs given 200 μg of unbound doxorubicin did not survive, and their organs showed severe damage [27]. This studied showed how easily the toxicity of a drug could be monitored by using the fetal chicken in the CAM model.

THE LAYING HEN MODEL

The laying hen has been used as a model of ovarian cancer due to the fact that they develop spontaneous ovarian cancers. In 4-year-old hens, 40% develop spontaneous ovarian cancers [30]. It could therefore be useful for testing new treatments [29]. Primary ovarian carcinomas from 2-year old hens were staged using the International Federation of Gynecology and Obstetrics Staging Systems for Ovarian Cancer in Humans as a reference. The tumor types were examined under light microscopy and classified by the WHO criteria for human ovarian cancers. Like humans, the hens developed serous, endometrioid, mucinous, clear cell, or mixed cell type carcinomas in their ovaries. All 4 stages of ovarian cancer were present. However, hens only have one functioning ovary so contra-lateral criteria used in human staging did not

apply. An illustration comparing the productive tracts of humans and laying hens is in Figure 5

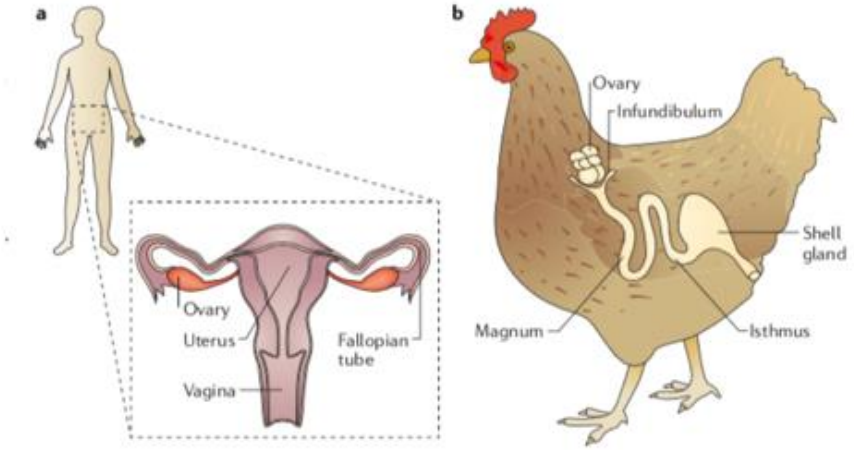

Figure 5. Anatomical comparison of human reproductive to laying hen reproductive organs [39].

Like humans, the hens also developed profuse ascites in some cases of advanced stage ovarian cancer. Precursor lesions were also present in the hens much like humans [29]. The hen carcinomas are similar to human ovarian cancers in their expression of tumor markers such as cytokeratin, epidermal growth factor receptor, Tag 72, proliferating cell nuclear antigen, TGF-a, E-cadherin, and CA125 [31-33]. Immune cells levels are increased in hens with ovarian cancer much like human cancers. The initiation events in models where ovarian cancer is induced are difficult to attribute to spontaneous cancers [34]. For this reason, hens serve as good model for improving imagining techniques. 3-year old hens with no abnormalities on the initial ultrasound were monitored daily for signs of abnormal ovarian function. They were given a gray scale ultrasound and a Doppler ultrasonography at 15, 30, and 45 weeks. Color Doppler imaging was used to identify vessels. The flow velocity waveform was measured to get an idea of blood flow. This was used to mark which hens seem to have tumor-associated neoangiogenesis. After 45 weeks, the hens were euthanized, and the ovaries examined. Out of 15 hens, 9 had ovarian

tumor-associated neoangiogenesis were detected by Doppler ultrasonography before they were visible on gray scale ultrasonography. 3 had microscopic tumor lesions that had not been detected by either ultrasonography method. The other 3 were normal on the ultrasonography and normal in post-mortem analysis [35]. Although the hen may be useful in many studies, it takes at least 2 years for hens to develop tumors [29]. Hens also require more space than mouse models [36]. These are two contributing factors to the laying hen model being less popular.

HUMAN TISSUE MODEL

The use of 3-D human tissue models would allow the use of human cells to be tested for drug interaction while maintaining their microenvironments. 3-D tissue models are closer to *in vivo* disease than monolayers cultures. Spheroids of epithelial ovarian cancer cell lines were created in hanging droplets [37]. Spheroids are sphere-shaped aggregates of cells [38]. An example of a spheroid is shown in Figure 6. To test cell viability, spheroid cultures were used to create xenografted mice. Highly vascularized and intraperitoneal tumors with hemorrhagic ascites formed in the same way monolayer xenografts do. The spheroids showed similar morphology to one another indicating they are highly reproducible. They can also be fixed and embedded in histogel for cross-sectioning.

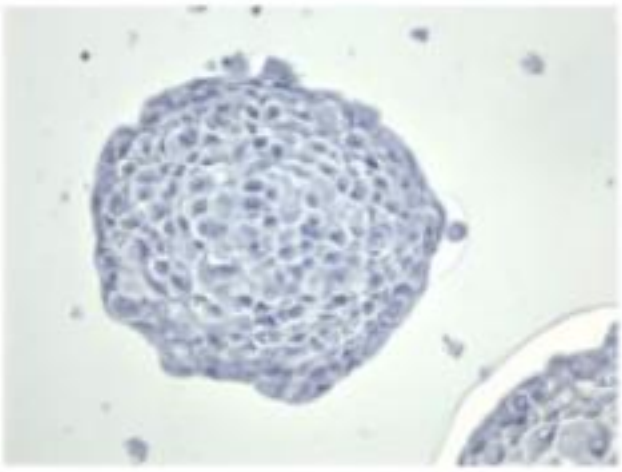

Figure 6. OV90 spheroid

While the spheroids did not develop hypoxic or necrotic cores like some do, they were admittedly cultured for only a short time and remained small enough during that time that there was no need for angiogenesis to maintain nutrient absorption throughout the cells [37]. Since hypoxic conditions have been such a problem in spheroid studies, specialized culture equipment and synthetic coats have been developed to prevent this [38]. This spheroid model showed tumor gene expression patterns to *in vivo* models that are not present in monolayers. With some improvements, it may replace *in vivo* modeling [37].

CONCLUSION

As technology improves, so do the models of ovarian cancer. Transgenic mice have so much potential to unlock the mysteries ovarian cancer. Pax8-Cre mice allowed for multiple gene interactions to be studied. This provided evidence that mutation of PTEN is essential for developing HGSCs [15]. Although getting 100% tumor formation is appealing, the Dicer-Pten mice may prove to be a poor representation of all HGSCs [14]. As mentioned earlier, the fallopian tubes in the Dicer-Pten developed tumors before spreading to the ovaries which is not

common in cases of human ovarian cancer [15]. The Pax8-Cre model has also received some criticism for not being able to develop ascites [21]. The fact that both studies were able to show their mice developed tumors resembling human HGSCs brings into question how to ensure that the model being used is the best one and which tests are the most crucial for making this determination.

While the laying hen model is able to form spontaneous tumors, it is often overlooked as an animal model. Even though hens produce tumors using the same cells types as humans, their reproductive anatomy is different [29]. While it may be useful for testing new drugs and risk factors, only 40% of laying hens develop tumors after 4 years [29]. The wait time necessary to use these animals and the cost of housing and care may not be worth it when there are on-going improvements to other cancer models.

The xenograft model has a lot of potential for testing drug-therapies for individual patients. However, it is quite costly and has about the same rate of successful transplantation as the CAM model [22, 28]. The CAM and 3-D tumor graft models are both cheaper than xenografted mice and have similar capabilities. The CAM model can grow fresh tissue from patients just as well as a xenograft [22, 28]. The 3-D cell culture model is still new and might need some time to catch on. The ability to use human cells *in vitro* with no difference to *in vivo* would completely change ovarian cancer research.

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