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Models of ovarian cancer metastasis: Murine models

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Abstract

Mice have mainly been used in ovarian cancer research as immunodeficient hosts for cell lines derived from the primary tumors and ascites of ovarian cancer patients. These xenograft models have provided a valuable system for pre-clinical trials, however, the genetic complexity of human tumors has precluded the understanding of key events that drive metastatic dissemination. Recently developed immunocompetent, genetically defined mouse models of epithelial ovarian cancer represent significant improvements in the modeling of metastatic disease.

Introduction

Epithelial ovarian cancer (EOC) can be effectively treated if detected early, but it is generally diagnosed after it has metastasized. The combination of cytoreductive surgery and chemotherapy has a modest impact on the long-term survival of patients with advanced disease. As a result, epithelial ovarian cancer has the highest mortality rate of all gynecological malignancies.

In addition to its unique biological behavior [1], EOC exhibits a distinctive pattern of metastatic dissemination in comparison with malignancies that arise at other organ sites. Malignant ovarian surface epithelial cells (OSE) primarily spread by local invasion to adjacent organs and exfoliation into intraperitoneal fluid, followed by implantation onto mesothelial surfaces. The obstruction of peritoneal lymphatic drainage combined with the secretion of vascular permeability factors by tumor cells results in the accumulation of ascites, which further facilitates mechanical dissemination of cells within the abdominal cavity. It is unclear whether most cells within the primary tumor have metastatic capacity or whether they acquire additional alterations to spread locally. This lack of understanding is partially because of the difficulties in establishing an appropriate model system for metastatic disease. This review will focus on recently developed mouse models of epithelial ovarian cancer and discuss their utility as metastatic models.

In vitro models

Much of what is known about molecular aberrations associated with ovarian cancer is based on studies conducted on human and rodent ovarian cancer cell lines derived from primary tumors or ascites and grown on artificial surfaces. Rodent (Fig. 1) and human OSE cell lines with defined genetic alterations have also been generated by *in vitro* transformation of normal OSE [2–4]. The relevance of cell lines as models of metastatic disease is limited because of the fact that *in vitro* conditions do not adequately mimic physiological processes involved in the induction and maintenance of a metastatic phenotype. Whereas some limitations (e.g. the lack of a host immune response, absence of neoangiogenesis, accumulation of numerous

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genetic aberrations because of extended propagation) are common to *in vitro* studies of all cancer types, others are unique to ovarian cancer.

In contrast to normal epithelial cells that undergo ANOIKIS [apoptosis triggered by loss of contact with the extracellular matrix (ECM)] when placed into suspension, exfoliated ovarian cancer cells maintain their viability while floating free in the peritoneal fluid. Their interaction and attachment to intraperitoneal mesothelial cells is a complex process mediated by several chemokines and cell–cell adhesion molecules such as CD44/hyaluronan, CXCR4/CXCL12, CA125/mesothelin, E-cadherin and β 1 integrins [5–8]. In addition to mediating cell attachment, these receptor–ligand interactions promote survival and growth of disseminated ovarian cancer cells by activating PI3K, MAPK and other signaling pathways. Once activated, many of these signaling cascades enhance the metastatic potential of the tumor and induce resistance to chemotherapy.

Although cell culture plates and membranes coated with components of extracellular matrix represent an improvement in culturing conditions (Fig. 1), it is still questionable how representative these conditions are of an *in vivo* environment. Standard *in vitro* cell migration and invasion assays are designed for analysis of single cells. However, a significant proportion of malignant cells in the ascites of ovarian cancer patients exist as multicellular aggregates. These can differ in size and morphology ranging from compact or loosely adherent groups of cells to spheroids with a central lumen surrounded by a cell monolayer [9]. Multicellular aggregates are particularly important from a clinical standpoint because they exhibit a high level of resistance to radiation and chemotherapy [10,11]. The contribution of spheroids to meta-static spread remained elusive for many years because of the lack of appropriate *in vitro* assays. Skubitz *et al.* recently developed a novel assay for quantifying migration of multicellular spheroids [12]. In addition, they modified the existing invasion assays and examined invasive properties of the spheroids by analyzing their ability to disaggregate and displace a live mesothelial cell monolayer. Surprisingly, multicellular aggregates were found to invade much more rapidly and to a much greater extent than single cells [12].

***in vivo* models**

Spontaneous malignant transformation of OSE cells in rodents is rare. Certain mouse and rat strains (CBA/J and Wistar, respectively) spontaneously develop ovarian cancers that are histologically very heterogeneous and include epithelial ovarian cancers, mesotheliomas, granulosa cell tumors, polycystic sex cord-stromal tumors and tubular adenomas [13,14]. The low incidence, long latency and heterogeneous nature of tumors in these models make them less attractive for experimental studies. Chemical carcinogens, such as 7,12-dimethylbenz(a)anthracene (DMBA), occasionally have been used for the induction of cancer in rodent models. The most extensive *in vivo* work has been done with xenograft models in which human cancer cell lines or tissues are transplanted into immunodeficient mice. Finally, several recently developed genetically engineered mouse models have the potential for being valuable models of metastatic ovarian cancer.

Xenografts

Xenografts of human ovarian cancer cells in immunodeficient mice have been used for decades in the analysis of cell tumorigenicity as well as for testing the efficacy of novel therapeutics. Xenografts are particularly useful for modeling the late stages of the disease. Because mouse ovaries are surrounded by a bursal membrane (Fig. 2), tumor cells can either be injected intraperitoneally or intrabursally (also referred to as orthotopically). It is unclear, however, whether orthotopic implantation reflects the physiological conditions in humans more accurately than intraperitoneal injection. In both methods of tumor inoculation, malignant cells spread throughout the abdominal cavity and attach to peritoneal surfaces. Gross pathology and

the pattern of dissemination of tumor nodules resemble human metastatic disease (Fig. 2). Such a diffuse pattern of spread makes it technically difficult to monitor the progression of the disease. Several techniques have been used to assess tumor burden including magnetic resonance, luciferase and green fluorescent protein (GFP) imaging and serum markers such as CA125. Tumor burden is much easier to assess in subcutaneous xenografts, however, these models are inappropriate for modeling intraperitoneal ovarian metastatic disease.

Although xenograft models recapitulate physiological conditions more accurately than *in vitro* models, there are still significant limitations of their relevance to human disease. The lack of immune response can significantly influence tumor formation as well as therapeutic efficacy. Interaction between cancer cells and intraperitoneal mesothelial cells can be altered because of the inter-species differences. Genetic polymorphisms in host animals also have been shown to influence the incidence of metastasis in both human and mouse xenograft models (reviewed by Hunter [15]).

The majority of ovarian cancer xenografts originate from OSE cell lines grown *in vitro* for extensive periods of time. As previously discussed, the adaptation of tumor cells to tissue culture conditions during *in vitro* propagation can cause an accumulation of genetic alterations resulting in changes in morphology, motility, invasiveness and proliferation rates. Because it has been well documented that patients with different histological subtypes of EOC have distinct genetic aberrations, which ultimately impact prognosis and responsiveness to therapies, it is clear that a detailed characterization of xenografts with respect to their genetic background and expression profile is a pre-requisite for successful evaluation of pathway-targeted therapies in pre-clinical trials.

Genetically engineered mice

One of the major challenges in designing genetically engineered mouse models of epithelial ovarian cancer has been the lack of tissue-specific promoters that regulate transgene expression exclusively in adult OSE cells. Malignant transformation of human OSE cells is thought to be a result of multiple genetic alterations that accumulate over many years, further complicating the generation of transgenic models that accurately recapitulate human disease. Several successes in the generation of transgenic mouse models have been recently reported, each addressing the aforementioned issues from different perspectives (summarized in Table 1).

Orsulic *et al.* [2] developed a model in which a defined set of genetic alterations can be introduced specifically into mouse OSE cells through an avian retroviral gene delivery system *ex vivo*. In this model, p53 deficient mice are engineered to express the avian tumor virus A (TVA) receptor exclusively in epithelial cells. Whole ovaries from adult TVA/p53^{-/-} transgenic mice are then isolated and cultured transiently *in vitro* in the presence of avian retroviral vectors carrying different oncogenes. Because only the cells expressing the TVA receptor can be infected, oncogene delivery is OSE specific. Upon *in vitro* infection, OSE cells are injected intraperitoneally or implanted orthotopically into nude or immunocompetent mice. Depending upon the combination of introduced oncogenes, the tumors in the recipient mice can be detected as early as 3–6 weeks after injection.

Connolly *et al.* [16] generated a transgenic mouse model in which expression of Simian virus 40 T antigen (SV40-Tag) is regulated by the Müllerian inhibitory substance type II receptor (MISIIR) gene promoter. The expression of endogenous MISIIR is restricted to the epithelium of the Müllerian tract origin (endocervix, endometrium, oviduct, ovary) and granulosa cells. Although MISIIR-TAG transgenic females develop both ovarian and nonovarian tumors, the appearance of epithelial ovarian cancer is much more frequent than cancers originating from other sites within the reproductive system. Unlike human ovarian tumors, which occur in adults,

the ovarian tumors in this system are initiated during embryonic development and are detectable in newborn females.

Two recently described mouse models have achieved a more accurate spatio-temporal regulation of transgene expression by combining the Cre-loxP recombination system with *in vivo* intrabursal delivery of the adenoviral vector expressing Cre-recombinase (Ad-Cre) [17, 18]. The ovarian bursa that encapsulates the mouse ovary can serve as a receptacle for injected viral supernatant. This results in prolonged exposure of OSE cells to high viral titers, thus increasing infection efficiency. Additionally, it limits the exposure of other intraperitoneal structures to the adenoviral vector, thus preventing malignant transformation of non-OSE cells. Flesken-Nikitin *et al.* used this adenoviral delivery system in p53loxP/loxP/Rb1loxP/loxP transgenic mice, whereas Dinulescu *et al.* used the same approach in LSL-K-RasG12D/+ / PTEN loxP/loxP mice. In both models, small areas adjacent to the bursa occasionally stained positive for transgene expression, presumably because of the leakage of Ad-Cre along the needle path.

Metastatic dissemination and ascites occur in all four models with variable incidence, latency and tumor burden (Table 1). Whereas variations in incidence between the models could be attributed to numerous factors including differences in genetic backgrounds and transgene delivery techniques, it is less clear why there is a considerable variation in the extent of metastatic disease within the same cohort of mice. Furthermore, there are significant differences between the four models in the incidence of distant metastasis. For example, none of the oncogene combinations in the TVA/p53^{-/-} transgenic mice resulted in extraperitoneal metastatic disease. It is possible, however, that extraperitoneal metastases could have been detected if the mice were sacrificed at a later time interval. The histology and gross appearance of primary tumors and metastatic nodules differ between the four models, which is probably because of different combinations of genetic lesions.

Model comparison

The choice of an ideal experimental model will depend on the nature of the question being asked (Table 2). All of the genetically engineered mouse models described above have the potential for being valuable models of metastatic ovarian cancer. The avian retroviral gene delivery technique described by Orsulic *et al.* is particularly useful for the analysis of different combinations of multiple genetic alterations and their collaboration in tumor initiation and progression [2] as well as for testing pathway-targeting therapies [19]. An important advantage of the MISIIR-TAg model is the short latency of metastatic disease because of germline expression of the transgene. Whereas this might be a disadvantage for the study of cancer initiation, it could accelerate pre-clinical evaluation of therapeutic efficacy in the pharmaceutical setting. The Cre-loxP models have the unique advantage of inducing malignant transformation of intact OSE cells in their natural environment in adult mice. It is probable that these models most accurately mimic *in vivo* carcinogenesis. The drawbacks of the Cre-loxP models are the relatively complex and time-consuming surgical procedures and the potential for the transformation of non-OSE cells because of leakage of the Cre-adenovirus.

Model translation to humans

A major concern in the interpretation of data obtained from mouse models is its relevance to human disease. It is encouraging that genetically engineered mice with ovarian tumors phenocopy human ovarian epithelial cancers and represent a spectrum of histologically distinct tumor types that include, depending upon the combination of oncogenes, serous, endometrioid and poorly differentiated cancers. Most of the individual genes altered in these mouse models have been previously implicated in human ovarian carcinogenesis. Genetically engineered mice are not only more useful for the identification of individual genes and signaling pathways

involved in the regulation of cancer dissemination, but can also serve as valuable models for the development of pathway-targeted therapies and investigation into tumor–host interactions and anti-tumor immune mechanisms. There are still some technical problems that need to be addressed, such as the complexity of surgical procedures [2,17,18] and the premature development of malignant disease [16]. The use of mouse models for drug testing will also require concurrent development of imaging techniques for monitoring tumor response to the drug.

Conclusion

The recent development of genetically defined ovarian cancer models will complement the existing data from xenograft models and provide further insight into the biology of meta-static ovarian cancer. It remains to be determined whether such models will have a superior predictive value over xenograft models in pre-clinical trials and drug discovery applications.

Links

- Mouse ovarian cancer models:
http://emice.nci.nih.gov/mouse_models/organ_models/ovarian_models
- Cancer models database (caMOD): <http://cancermodels.nci.nih.gov/camod/>
- Cancer image database (caIMAGE):
<http://cancerimages.nci.nih.gov/caIMAGE/index.jsp>
- National Cancer Institute ovarian cancer page:
http://www.cancer.gov/cancer_information/cancer_type/ovarian/
- The ovarian kaleidoscope database: <http://ovary.stanford.edu/>
- Daily ovarian cancer updates: <http://ovariancancer.blogspot.com/>

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Glossary

ANOIKIS, apoptosis triggered by loss of contact with the extracellular matrix..

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Figure 1.

Mouse ovarian surface epithelial cells. (a) H&E stained cross-section of the ovary. (b) OSE cells grown in a culture dish. (c) H&E stained section of a cell monolayer grown on a culture filter. (d) H&E stained section of cells grown in matrigel.

Figure 2.

Metastatic ovarian cancer dissemination in a mouse. (a) Normal mouse ovary *in situ*. (b) Metastatic spread of orthotopically implanted ovarian cancer cells (arrowheads) from the ovary (asterisk) to the adjacent mesentery (arrows). Tumor cells were engineered to express HA-tagged Akt protein and are detected by using an antibody against HA (brown staining). (c) Intraperitoneal ovarian carcinomatosis in a mouse model. Tumor nodules are attached to the intestinal mesentery. (d) A section of intestine and mesentery with tumor nodules (brown staining).

Table 1
Summary of the current genetically engineered mouse models of metastatic ovarian cancer

d	Additional oncogenes		Regulation of transgene expression		Primary ovarian tumor		Metastasis (% mice)		Ascites (% mice)
	Combination	Method of delivery	Induction	Start time	Latency	% mice	Histology	i.p.	e.p.
Drug Discov Today Dis Models	c-myc + k-ras	<i>Ex vivo</i>	Intraperitoneal injection of	Adult	Yes	100	S	100	No
	k-ras + Akt		OSE infected			100			
	Akt + c-myc		<i>in vitro</i> with RCAS			100			
	Akt + c-myc + k-ras					100			
	Akt + c-myc + Her2								
eP			Germline	Germline	No	50	P	100	n/a
			Intrabursal Ad-Cre	Adult	Possible	6	P	No	50
						97	S, P, U	27	18
			Intrabursal Ad-Cre	Adult	Possible	100	E	Yes (n/a)	43
N loxP/loxP									Some

intraperitoneal; e.p., extraperitoneal; E, endometroid; S, serous; P, poorly differentiated (CK8+); U, undifferentiated.

Table 2

Comparison summary table

	<i>In vitro</i> models	<i>In vivo</i> models	
		Xenografts	Genetically modified mice
Pros	Simple and cost-effective	Short latency of metastatic disease	Defined genetic background Immunocompetent
Cons	The lack of host—tumor interaction (immune response, neoangiogenesis etc.)	Inter-species differences	Complex surgical procedures Time-consuming
	Accumulation of genetic alterations because of the prolonged propagation <i>in vitro</i>	The lack of simple, accurate technique for monitoring of intraperitoneal disease progression	The lack of simple, accurate technique for monitoring of intraperitoneal disease progression
Best use of model	Characterization of cell—ECM and cell—cell specific interaction		Development of pathway-targeted therapy Identification and characterization of individual genes involved in the regulation of metastasis
		Pre-clinical evaluation of therapeutic efficacy	Pre-clinical evaluation of therapeutic efficacy
Access	Literature	Literature	Literature
			Contacting the authors [2,16-18]