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INVITED COMMENTARY

Precious GEMMs: emergence of faithful models for ovarian cancer research†

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†Invited commentary for Zhai *et al.* High-grade serous carcinomas arise in the mouse oviduct via defects linked to the human disease. *J Pathol* 2017; **243:** 16–25.

Abstract

The development of Genetically Engineered Mouse Models (GEMMs) has catalyzed tremendous progress in cancer research. However, it has been difficult to design adequate mouse models for high-grade serous carcinoma (HGSC), the most common and lethal form of ovarian cancer. The genetic complexity of the disease, as well as the recent appreciation that most HGSCs arise from the fallopian tube (FT) secretory epithelium rather than the ovarian surface epithelium, has stifled the development of robust GEMMs. In a recent issue of this journal, Zhai *et al* presented an elegant mouse model for ovarian cancer that uses *Ovgp1* as an FT-specific promoter to inactivate *Brca1*, *Trp53*, *Rb1*, *Nf1*, and *Pten*. The authors showed that loss of these genes in the mouse FT epithelium can mimic the different stages of human HGSC tumorigenesis. Their robust model emphasizes the importance of considering both the cell of origin and tumor genetics in developing accurate model systems. They provide a useful tool for studying mechanisms of disease *in vivo* and for research into novel methods of prevention, early detection, and treatment of HGSC.

Keywords: high-grade serous carcinoma; genetically engineered mouse models; ovarian cancer; fallopian tube

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Unraveling the pathways that lead to malignant transformation is a cornerstone of cancer research and a conduit to the discovery of new tools for diagnosis, management, and treatment. More than other animal models, mice have enabled the study of gene function *in vivo*, shedding light on genetic aberrations responsible for tumorigenesis. Increased sophistication in mouse cancer models has led to the development of Genetically Engineered Mouse Models (GEMMs). In a recent issue of this journal, Zhai *et al* [1] present an elegant GEMM of ovarian cancer that takes into account both the site of origin and the genetic complexity of the disease – prerequisites for GEMMs to accurately mimic the human malignancy.

Robust GEMMs have been difficult to develop for ovarian cancer. Epithelial ovarian cancer is a heterogeneous disease with many histological subtypes. High-grade serous carcinoma (HGSC) is the most common subtype and accounts for the vast majority of deaths from the disease. For decades it was thought that all epithelial ovarian cancers, including HGSC, emerge from the ovarian surface epithelium (OSE). However, the inability to reproducibly identify precursor lesions in the OSE ultimately led to a re-evaluation of the likely site of origin. The development of a protocol for Sectioning and Extensively Examining the FIMbria (SEE-FIM) [2] of the fallopian tube (FT) ultimately led to the realization that a majority of HGSCs emerge from secretory cells in the distal FT. Recent molecular studies have provided robust genomic evidence to support the FT as the site of origin for the majority of HGSCs [3,4]. This paradigm shift in our understanding of ovarian cancer pathogenesis has had a significant impact on the design and development of tractable GEMMs [5]. Realizing the importance of finding an FT-specific promoter to drive the inducible expression of the Cre recombinase, Cho and colleagues [1] honed in on the *Ovgp1* promoter. *Ovgp1* encodes for a glycoprotein that is expressed by the FT secretory cells. Previous work has demonstrated the restricted expression of *Ovgp1* and the utility of an *Ovgp1* tamoxifen-inducible system to target the FT with near pinpoint accuracy [6,7]. In addition to the importance of considering the cell of origin when selecting a promoter to drive the Cre recombinase, targeting genes that are relevant to the

Table 1. Phenotypes of FT-based GEMMs of ovarian cancer

Promoter	Targeted genes	Tumor features	Reference
Ovgp1	*Brca1*, *Trp53*, *Rb1*, *Nf1*, *Pten*	STIC, HGSC, ovarian carcinosarcoma	[1]
Ovgp1	*Apc*, *Pten*	Poorly differentiated tumors of the oviducts and ovaries	[7]
Ovgp1	SV40 large T antigen	Poorly differentiated tumors of the reproductive tract, except for the ovaries	[15]
Ovgp1	SV40 large T antigen	p53 signatures, STIC, HGSC	[6]
Pax8	*Brca1*, *Brca2*, *Trp53*, *Pten*	STIC, HGSC, endometrial hyperplasia and carcinoma	[10]
MISIIR	SV40 small and large T antigens	Poorly differentiated carcinoma Weak *MISIIR* mRNA signal in FT	[11]
Amhr2	*Dicer*, *Pten*	Serous tumor of stromal origin	[12]
Pgr	*Apc*	Endometrial hyperplasia, intraepithelial lesions and carcinomas of the oviducts, endometrioid ovarian tumors	[16]

human condition is vital. Zhai *et al* made a judicious choice of targeted genes in their GEMM, as loss of *Brca1*, *Trp53*, *Rb1*, and *Nf1* is frequent in HGSC. They used the *Ovgp1* tamoxifen-inducible system to target these genes in the oviduct – the murine equivalent of the human FT epithelium. Deletion of several different combinations of these tumor suppressor genes resulted in serous tubal intraepithelial carcinoma (STIC) formation that progressed to HGSC and, in some cases, carcinosarcoma, a much less common variant of HGSC. A portion of these mice also developed widespread metastatic disease in the peritoneal cavity with ascites formation. The cancer phenotype was highly penetrant and more rapid in mice carrying engineered alleles of all four tumor suppressor genes. Like STICs in humans, mouse STIC lesions had a predilection for arising in the distal portion of the oviduct [2].

Cho and colleagues also developed a GEMM where *Brca1*, *Trp53*, and *Pten* were inactivated in the oviduct. Although *PTEN* deletion only occurs in a minority of human STICs and HGSCs, the PI3K–AKT pathway is frequently activated [8,9]. These mice also developed STICs and HGSCs but tumors arose earlier in these mice than in those with *Brca1*, *Trp53*, *Rb1*, and *Nf1* inactivation. The longer latency for tumor development in the triple knockout mice (*Brca1;Trp53;Rb1* or *Brca1;Trp53;Nf1*) or the quadruple knockout mice (*Brca1;Trp53;Rb1;Nf1*) compared with the *Brca1;Trp53;Pten* mice suggested that the tumors derived in the absence of *Pten* loss required the acquisition of additional defects to achieve neoplastic transformation. This was supported by the finding that there was loss of the wild-type allele of *Rb1* in the tumors of mice carrying only one floxed *Rb1* allele. Importantly, these results are consistent with another study showing that *Pten* loss accelerates tumor development in a related HGSC GEMM [10].

A theme that emerges from these studies is that both the cell of origin and the genetics play a crucial role in determining tumor phenotype (Table 1). This is emphasized by Cho and colleagues' previous work, where they showed that deletion of *Apc* and *Pten* in the oviduct versus the ovarian surface epithelium led to tumors with markedly different morphologies and behaviors. The oviduct tumors resembled human endometrial carcinomas that were indolent and mimicked the classic type I tumors, while the ovarian tumors were strikingly more aggressive and poorly differentiated, with sparse glandular elements admixed with spindle cell areas [7]. In their current study, they provide further evidence that the 'right genes' in the 'right cell' matter. They also highlight the greater specificity of *Ovgp1* for the FT epithelium in comparison with other previously described promoters, such as the paired box 8 (*Pax8*) gene and the anti-Müllerian hormone receptor 2/Müllerian-inhibiting substance receptor 2 (*Amhr2/MISIIR*). Indeed, *Amhr2/MISIIR* is broadly expressed in the OSE and the stromal cells in the ovary, oviduct, and other portions of the female reproductive tract [11,12]. The *PAX8* gene is a marker of secretory cells in the human FT, and its expression is retained in the majority of STIC lesions and HGSCs. When used as a promoter, it targets the oviduct secretory epithelium [10], but lacks specificity because it is also expressed in other Müllerian epithelia, such as the endometrial glandular epithelium.

There is an issue with GEMMs that remains to be addressed. Since it is clear that a dialog between the immune system and the cancer cell can dictate responses to therapy, elicit resistance, and impact outcome; the ability to access the tumor immune microenvironment constitutes a major hurdle in modeling HGSC. Unfortunately, many of the published FT-based GEMMs are generated in a mixed genetic background [1,10,12], which precludes the development of tumor cell lines that can be used for syngeneic studies. Currently, faithful murine cell line models of ovarian cancer that would enable investigation of the tumor–host interface, especially the immune response, are lacking. The most commonly used murine line for this purpose is the ID8 cell line. This line was derived from the ovarian surface epithelium of a pure-bred C57BL/6 mouse and spontaneously transformed *in vitro* [13]. Although it is frequently used for syngeneic studies, the recent genomic characterization of ID8 revealed that this line does not harbor the typical mutations and copy number alterations that define human HGSC [14]. Hence, there is a need for additional models that can help orchestrate a more complete understanding of ovarian cancer biology. This may be accomplished by either developing GEMMs in a pure genetic background or developing

oviduct-derived cell lines from pure background animals. This issue notwithstanding, Cho and colleagues have provided the research community with a robust model for HGSC that will have an impact on research related to prevention, early detection, and therapeutic approaches.

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