Female Mice Chimeric for Expression of the Simian Virus 40 TAg under Control of the MISIIR Promoter Develop Epithelial Ovarian Cancer

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ABSTRACT

In women, >80% of malignant ovarian tumors are of epithelial origin. Early detection of these tumors is very challenging, and extensive i.p. dissemination is common by the time of diagnosis. The lack of adequate genetic mouse models of ovarian carcinomas significantly delays advances in early detection and treatment. We report that female transgenic mice expressing the transforming region of SV40 under control of the Mullerian inhibitory substance type II receptor gene promoter develop bilateral ovarian tumors in ~50% of cases. Histologically, these tumors are poorly differentiated carcinomas with occasional cysts and papillary structures present at the surface of the ovary. These tumors disseminate i.p., invade omentum, and form ascites as do human ovarian carcinomas. The epithelial origin of these tumors is supported by detection of cytokeratins 8 and 19, and the absence of α-inhibin, a protein characteristically expressed in normal granulosa cells and most granulosa cell tumors. Cell lines derived from the ascites exhibit the properties of epithelial ovarian cancer, such as anchorage-independent growth, tumorigenicity in immunocompromised mice, expression of epithelial cell markers, and organotropic implantation. The availability of a transgenic mouse model of disseminated ovarian carcinoma and respective cell lines should advance our understanding of this neoplasm, and serve as a useful tool for the evaluation of emerging detection and treatment strategies.

INTRODUCTION

Many different tumor types arise from the human ovary including germ cell tumors, sex cord stromal tumors, and EOCs. The EOCs are by far the most common tumor type and also the most deadly. Approximately 23,000 new cases of ovarian carcinoma are diagnosed, and 14,000 women die of the disease annually, making it the fifth most common cancer in women in the United States (1). When ovarian carcinoma is diagnosed at early stages, the survival rate can be quite high, ~90%. However, the vast majority of cases of ovarian carcinomas are not identified until late stage when the survival rate drops to only 30–40%. Controversy with regard to precursor lesions (2) and the lack of a well-defined disease spectrum consisting of benign, malignant, invasive and metastatic lesions have significantly hampered investigator efforts to understand the molecular changes that occur during the development and progression of ovarian carcinoma. Furthermore, EOC is a broad term that refers to tumors of at least four distinct histological subtypes including serous papillary, mucinous, endometrioid, and clear cell carcinomas. The distinct morphologies of ovarian carcinomas have led some investigators to speculate that these histological subtypes may represent different disease entities that arise via distinct molecular pathways. Another factor that has hindered investigations of EOC is the lack of a reproducible heritable model of the disease. Whereas epithelial cancers of many organ sites have been modeled successfully in transgenic mice including prostate, mammary gland, colon, lung, brain, and skin cancers (3–11), efforts to model human ovarian cancer have been delayed both by a general lack of understanding of the disease process in humans and of the cells widely believed to be the precursors of EOC, the OSE cells (12–16). The OSE cells are a single layer of relatively undifferentiated epithelial cells that cover the entire surface of the ovary. It is believed that these cells arise from the same celomic epithelial cells that give rise to the Mullerian duct, the progenitor of the epithelia of the structures of the female reproductive tract including Fallopian tube, uterus, and upper vagina (reviewed in Ref. 17). It is thought that OSE cells retain the potential to differentiate into the epithelia similar to each of those structures, thus explaining how the distinct histological subtypes observed in human ovarian carcinoma arise. During embryogenesis, after primary (genetic) sex determination occurs, secondary sex determination is dependent on the presence or absence of the MIS and its cognate receptors, MIS type I and type II receptors (reviewed in Refs. 18, 19). In the male animal, MIS is secreted from Sertoli cells of the developing testes and stimulates the regression of the Mullerian duct. Testosterone is also secreted from the developing testes and induces the differentiation of the Wolffian duct into the secondary structures of the male reproductive tract. In the absence of MIS in the developing female embryo, the Mullerian duct differentiates into the secondary structures of the female reproductive tract. This information suggested a possible strategy to develop a transgenic model of ovarian carcinoma. In this report, we describe the use of the 5’ upstream regulatory sequences of the mouse MISIIR gene to target expression of the SV40 TAg specifically to the epithelium of the female mouse reproductive tract including the OSE. Using this strategy, we have generated transgenic animals that develop ovarian carcinomas with metastatic spread to peritoneal organs.

MATERIALS AND METHODS

MISIIR Expression in Mouse Tissues. Expression of the MISIIR in mouse tissues was analyzed by RT-PCR. Total RNA from normal mouse tissues was isolated using a Mini-beadbeater tissue homogenizer (BioSpec Products, Inc., Bartlesville, OK) and Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Before cDNA synthesis, 1 µg of each RNA
The luciferase reporter construct, pMISIIR-LUC, was constructed by subcloning purified from the plasmid pRIP2TAg (Ref. 20, kindly provided by Dr. Doug Hind) into EcoRI/HindIII digestion of pMISIIR-TOPO TA and insertion of this restriction fragment into the promoterless pGL3 basic luciferase expression vector (Promega Corp., Madison, WI). Transfection efficiency was assessed and normalized by measuring β-galactosidase activity using the β-galactosidase Assay System (Promega Corp.), and luciferase activity stimulated by the pMISIIR-LUC reporter construct was measured using the Steady-Glo Luciferase Assay System (Promega Corp.).

Production and Screening of Transgenic Mice. The purified transgene DNA fragment (MISIIR-TAg) was injected into pronuclei of day-0.5 embryos of the first generation of a hybrid genetic background of C57BL/6 and C3H (B6C3F1) mice by microinjection. Injected embryos were implanted into the oviducts of day-0.5 pseudopregnant female Swiss Webster mice. Tails from the resulting pups were clipped 2 weeks after birth for genotype analysis. For the isolation of genomic DNA, mouse tails were digested in 300 μl of sodium chloride Tris EDTA buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.1% SDS) with the addition of 18 μl of 20 mg/ml proteinase K at 55°C for 2 h to overnight. Digested tails were centrifuged at 16,500 × g for 5 min to pellet insoluble material, 250 μl of the supernatant was transferred to a clean tube, and 2 volumes of 2-propanol were added. Mouse-tail DNA was precipitated by centrifugation at 16,500 × g for 5 min. Pellets were washed with 70% ethanol, centrifuged at 16,500 × g for 5 min, and supernatants were discarded. The DNA was air-dried briefly and resuspended in low concentration Tris EDTA [3 mM Tris-HCl (pH 8.0) and 0.2 mM EDTA]. Presence of the transgene was confirmed by PCR amplification of a 773-bp fragment of the large TAg using the Tag F4 forward primer (5′-TGCATGGTGTACAACAT-3′) and the Tag R1 reverse primer (5′-TTGGGACTGTGAATCAAGTGG-3′) using the same PCR conditions as described above.

Preparation and Analysis of Tissues. Tissues collected at necropsy were routinely fixed in 10% (v/v) NBF overnight, transferred to 70% ethanol, and paraffin-embedded. For histological analysis, 4-μm sections were cut and routinely stained with H&E. The global slide evaluation, digitizing, and photography were performed with the ScanScope (Aperio Technologies).

Immunohistochemical Analysis of Tissues. Sections for immunohistochemical analysis were cut on a Shandon Plus (Cherry Hill, NJ) chambered slides (Fisher). Antibens stained by immunohistochemistry included SV40 TAg, CK8, CK19, α-inhibin, and synaptophysin. The primary antibodies used were mouse monoclonal antibody pAb101 to SV40 TAg (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), rat monoclonal antibody TROMA1 to CK8 (Developmental Studies Hybridoma Bank, The University of Iowa, Iowa City, IA), rat monoclonal antibody TROMA3 to CK19 (kindly provided by Dr. Rolf Kemler, Max Planck Institute, Freiburg, Germany), mouse monoclonal antibody Clone R1 to α-inhibin (Oxford-Innovation Ltd.-DSL, Chewell Innovation Centre, Oxfordshire, United Kingdom), and polyclonal rabbit antibody to synaptophysin (DAKO). The unstained sections were deparaffinized in xylene and hydrated in a graded series of alcohols. Antigen retrieval was conducted by microwaving slides in citric acid buffer (0.01 M; pH 6.0) for 10 min. Endogenous peroxidase was blocked by preincubation with 0.3% hydrogen peroxide for 10 min. For CK8, CK19, α-inhibin, and synaptophysin, avidin and biotin were blocked by using the Avidin/Biotin Blocking kit (BioGenex, San Ramon, CA) according to the manufacturer’s protocol. The nonspecific antigens were blocked by 10% goat or rabbit serum in PBS. For α-inhibin immunostaining, a Mouse On Mouse kit (VECTOR M.O.M Immunodetection kit; Vector Laboratories, Inc., Burlington, CA) was used to reduce the nonspecific staining of mouse tissue by the mouse antibody. Dilutions of the primary antibodies for SV40 TAg, CK8, CK19, α-inhibin, and synaptophysin were 1:200, 1:25, 1:50, 1:50, and 1:50, respectively. Sections were incubated with the primary antibodies at 4°C overnight in a humidified chamber. The following day, detection was performed using the BioGenex kit according to

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6 D. C. Connolly, Tissue restricted transgene expression mediated by the Mullerian inhibitory substance type II receptor, manuscript in preparation.

7 D. C. Connolly, unpublished observations.
the manufacturer’s instructions. Briefly, sections were rinsed with PBS and exposed to appropriate species-specific secondary antibodies for 30 min. The sections were rinsed again and exposed to peroxidase-conjugated streptavidin for 30 min. Finally, each section was exposed to 3,3′-diaminobenzidine solution for 1–2 min after they were rinsed with PBS. Immunostained sections were lightly counterstained with Hematoxylin (Gill-3 hematoxylin; Fisher), dehydrated through a series of alcohols and xylene, and coverslipped. For immunohistochemistry on the cultured cells, the adherent cells were trypsinized and pelleted at 200 × g for 5 min in a tabletop centrifuge (Sorvall RT1000B; DuPont). The cells were washed once with PBS and pelleted again. After the PBS was removed from the tube, 1.5 ml of 10% NBF was added, and the cells were fixed for 10 min before the NBF was removed. Cell pellets were placed in paper, embedded, and processed as described above.

**RT-PCR Analysis of Tumor Tissues.** To verify expression of the transgene and the Msiir RNAs in ovarian tumors, total RNA from each tumor was isolated, DNase I-treated, and first-strand cDNA was synthesized as described. First-strand cDNAs were used as templates in PCR reactions using the oligonucleotide primers Tag F4 and Tag R1, and Msiir Forward (5′-CAGCCA-GAATGTCGCTTACCG-3′) and Msiir Reverse (5′-GCTCAATGTCCATCCAGT-3′) for amplification of the Tag transgene and the endogenous Msiir gene, respectively. PCR conditions were the same as described previously, and reaction products were routinely resolved on 2% agarose gels.

**Primary Cell Culture of MOSE Cells and MOVCAR Cell Lines from the Ascites Fluid.** Primary cultures of MOSE cells derived from ovaries of C57Bl/6 mice were obtained as described (21). For female mice found to have ascites present at the time of necropsy, the ascites fluid was collected aseptically, and aliquots of cells were made by washing several times with PBS to remove RBCs and pelleting at 200 × g for 5 min in a table top centrifuge. These cells were fixed in 10% NBF, paraffin-embedded, sectioned, and either H&E stained or subjected to immunohistochemical detection. Alternatively, to remove residual RBCs and other nonadherent material from these cultures, the ascitic fluid was washed in several changes of sterile PBS to remove the RBCs. The cellular material that had settled was seeded directly into T25 flasks containing DMEM supplemented with 4% FBS, 100 U/ml penicillin/100 μg/ml streptomycin, and 1% HEPES and incubated at 37°C in 5% CO2. The following day, most of the cell clumps from the ascites fluid had settled, cultures were incubated at 37°C in 5% CO2. The next day, a portion of the Msiir gene was removed from the T25 transfection. Cultures were incubated for 14 days and used in subsequent experiments.

**In Vitro MISIIR Promoter Activity.** A portion of the mouse **Msiir**’s upstream regulatory region was PCR amplified and cloned based on sequence homology to the rat **Msiir** promoter (23) to determine whether this promoter could be used to direct gene transcription in cells of OSE origin. Sequence analysis revealed amplification of a 1204-bp DNA fragment that shared >95% homology with the published rat promoter sequence with only minor nucleotide insertions, deletions, or substitutions. Transient transfection of an **Msiir-Luc** reporter construct was performed to test the functional activity and specificity of the cloned promoter element in vitro. Transfection efficiency was assessed and normalized subsequently by cotransfection of a constitutively expressed β-galactosidase reporter construct. Luciferase activity was measured in normalized cell line samples transfected with the p**Msiir-Luc** reporter plasmid and compared with the same cell line samples transfected with the promoterless pGL3 basic reporter. Results are depicted as fold increase in activity over the promoterless control (Fig. 1B), and demonstrate that the mouse **Msiir** promoter element can stimulate transcription of the luciferase reporter gene and that transcriptional activity is greater in cell lines of ovarian epithelial cell origin.

**TgMsiirTAg Transgenic Mice Develop Ovarian Tumors.** To determine whether the mouse **Msiir** promoter could be used to target expression of the SV40 TAg to mouse ovary as a means to generate a transgenic model of epithelial ovarian cancer, the cloned promoter element was fused to the early region of the SV40 virus including the coding regions of both the large and small TAg genes. The **Msiir-TAg** transgene construct was injected into fertilized eggs derived from B6C3F1 mice. Transgenic founders were successfully obtained from these injections, and both female and male mice developed tumors (results summarized in Table 1). Briefly, 18 of 36 (50%) female mice bearing the **Msiir-TAg** transgene developed ovarian tumors by 6–13 weeks of age. Necropsy consistently revealed the presence of bilateral ovarian masses (Fig. 2) ranging in size from 0.5 cm × 0.5 cm × 0.6 cm to 2.1 cm × 2.8 cm × 1.8 cm, and apparent tumor implants located frequently in the omentum and occasionally on other abdominal organs. In many cases, bloody ascites was present in the abdominal cavity of mice with tumors. Organs and tissues from all of the transgenic mice subjected to necropsy were collected, fixed, embedded, sectioned, and H&E stained for histological evaluation. Two individual female founder mice that were exhibiting signs of illness were sacrificed, and found to have a uterine mass and enlarged cystic ovaries.
kidneys, respectively. Tumors at other sites were not observed in female mice. Testicular tumors were observed in 7 of 25 male founders analyzed.

**Histopathological and Immunohistochemical Analysis of Tumors and Ascites.** Histological analysis revealed that ovaries were nearly completely substituted by poorly differentiated neoplastic cells with hyperchromatic nuclei and polygonal to oval eosinophilic cytoplasm (Fig. 3A). These cells were arranged in solid sheets and occasionally formed tubules (Fig. 3, B and C) and irregular slit-like spaces. On the ovarian surface, these cells formed papillary structures, with floating buds in the intrabursal space (Fig. 3B). The papillae were particularly well presented in the ascites (Fig. 3D). Evaluation of i.p. organs revealed extensive dissemination of the tumor with invasion of the omentum (Fig. 3E), the mesentery, and the parietal and visceral serosa. The majority of tumor cells contained nuclear TAg both in the ovary (Fig. 4A) and in i.p. masses (Fig. 4B). Many tumor cells also contained increased amounts of p53 (Fig. 4C), indicating functional activity of the large TAg gene. Similar to the normal ovarian surface epithelium (Fig. 4D), CK8 and CK19 were detected in the majority of tumor cells both in the ovary (Fig. 4, E and G) and in ascites (Fig. 4F). At the same time, in contrast to staining of granulosa cells in the remaining follicles, no α-inhibin was detected in the neoplastic cells (Fig. 4H). Synaptophysin, a marker of neuroendocrine differentiation, was not detected in any of the tumors (data not shown). Taken together, these observations allowed us to interpret these tumors as poorly differentiated carcinomas of the ovary with i.p. metastatic

<table>
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<tr>
<th>Organ Site</th>
<th>Incidence (%)</th>
<th>Tumor type</th>
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<tr>
<td>Females</td>
<td></td>
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<tr>
<td>Ovary</td>
<td>18/36 (50)</td>
<td>Poorly differentiated ovarian carcinoma</td>
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<tr>
<td>Uterus</td>
<td>1/36 (3)</td>
<td>Multiple leiomyomas</td>
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<td>Kidney</td>
<td>1/36 (3)</td>
<td>Polycystic kidney with neoplastic component</td>
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<td>Males</td>
<td></td>
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<tr>
<td>Testes</td>
<td>7/25 (28)</td>
<td>Poorly differentiated Sertoli-cell tumor</td>
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dissemination. The uterine mass found in one female founder was determined to consist of multiple leiomyomas that were found to express TAg (data not shown). In the case of the female with large polycystic kidneys, there was a neoplastic component of uncertain origin that also expressed TAg (data not shown). Histopathologic diagnosis of testicular tumors revealed poorly differentiated Sertoli cell tumors. Immunohistochemical analysis revealed strong expression of TAg in these tumors (data not shown).

Characterization of MOVCAR Cells. Ascites fluid contains a heterogeneous population of cells including tumor cells, blood cells, mesothelial cells, fibroblasts, and inflammatory cells. To confirm that the MOVCAR cell lines derived from the ascites of transgenic mice were epithelial and expressing the transgene, cells were subjected to immunohistochemical analysis for the expression of TAg, CK8, CK19, and α-inhibin as described. Consistent with the ovarian tumors and ascites cells from which they were derived, MOVCAR cells express TAg, CK8, and CK19, but do not express α-inhibin. These cells and several ovarian tumors were also tested for expression of the endogenous MISIIR and the TAg transgene by RT-PCR, and were found to express the mRNA for both genes (Fig. 1A; data not shown). The MOVCAR cells were additionally evaluated for a malignant phenotype by assaying the potential for anchorage-independent growth in soft agar and tumor formation in immunocompromised SCID mice. Anchorage-independent growth assays showed that many of the MOVCAR cells were beginning to grow as colonies by day 7, and by 21 days numerous colonies (>10,000 colonies/plate) were apparent, whereas no colony growth was observed in normal MOSE cells (data not shown). SCID mice injected i.p. with MOVCAR cells began to exhibit evidence of abdominal distention as early as 42 days after injection. All of the i.p. injected mice were found to have tumor nodules within the peritoneum and enlarged ovaries. Tumors were routinely observed within the intrabursal space surrounding the ovary, and in some cases, tumor nodules were observed within an otherwise normal ovary. Mice injected s.c. were found to have tumors under the skin occasionally invading the muscle wall. Histopathological evaluation of the tumor nodules collected from these mice revealed that these tumors displayed the histological features of papillary carcinoma and were nearly identical to tumor implants isolated from the original transgenic mice from which the ascites was initially collected. As in the transgenic mice, immunohistochemical staining of tumor nodules from the MOVCAR-injected SCID mice revealed strong uniform expression of TAg, CK8, and CK19 in tumors, and strong staining for α-inhibin in normal follicles in the ovary but not in tumor (data not shown).

DISCUSSION

Ovarian cancer is most typically diagnosed at advanced stage. Because of this, it has been difficult to establish the same type of model of disease progression from benign precursor lesions to frankly malignant lesions and metastasis as has been established for several other human carcinomas. In fact, the identification of the precursor cells that are the origin of ovarian carcinoma remains controversial. The widely held assumption is that the OSE cells are the precursor cells of ovarian carcinoma (2, 12–16). The OSE cells are a single layer of simple epithelial cells that cover the entire surface of the ovary. It is believed that the repetitive process of ovulation causing disruption, and subsequent wound healing and tissue repair in this cell layer can potentially result in mutations that lead to transformation and ultimately tumor formation. The issue that remains difficult to explain is how relatively simple and morphologically indistinct precursor cells such as the OSE cells can give rise to tumors that are histologically similar to the epithelia lining the Fallopian tube, uterus, and endocervix. In some cases, human ovarian carcinomas consist of more than one distinct histological subtype. To explain this, an additional assumption is made that the OSE cells have the capacity to differentiate into more complex epithelial cell types. However, some investigators question these assumptions and propose that components of the secondary Mullerian system may in fact be the precursors of ovarian carcinoma (24).

The Mullerian duct is the embryonic structure that gives rise to the uterus, Fallopian tubes, and vagina in female animals (reviewed in Refs. 18, 19, 25). MIS is a transforming growth factor β family member of peptide growth hormones that, in the male fetus, stimulates regression of the Mullerian duct. It stimulates this regression of the Mullerian duct via binding to a receptor complex consisting of the MISIIR and an MIS type I receptor, possibly ALK2 or ALK6 (18, 19). The MISIIR is a single transmembrane serine/threonine kinase that shares homology with the transforming growth factor β-receptor (26, 27). When MIS binds the receptor complex, MISIIR phosphorylates the type I receptor partner to initiate a downstream signaling cascade (18, 19). Expression of the MISIIR has been reported to be restricted to mesenchymal cells surrounding the Mullerian duct during embryogenesis, tubular and follicular structures of fetal gonads, Sertoli and Leydig cells of adult testes, and granulosa cells of adult ovary (26, 28, 29). More recently, expression of the MISIIR in established human ovarian cancer cell lines as well as cell lines derived from the ascites of patients with ovarian carcinomas has been demonstrated (30). These findings imply that the MISIIR is expressed in the cells that give rise to human EOCs. Indeed, one might anticipate this based on the embryonic relationship (celomic epithelium) of OSE to the tubal, endometrial, and endocervical epithelia, and the role of MIS in the embryonic regression of these structures to yield a phenotypically male fetus. This observation led us to evaluate purified populations of primary and transformed MOSE cells for expression of the MISIIR gene by RT-PCR. Although other investigators (27, 28, 31) have reported that the receptor is not expressed in rodent OSE cells, our results indicate the presence of the mRNA for the receptor in this cell type. Although it is possible that the protein is not expressed in these cells, other possible explanations are that the receptor binding or in
*in situ* hybridization methods of detection used in previous studies were not robust enough to detect expression of the *MISIIR* in OSE cells or that the OSE cell layer may have been disrupted in the handling and collection of specimens. On the basis of our expression data, the data of Masiakos et al. (30), and the very restricted expression of the *MISIIR*, we chose to pursue the use of the upstream regulatory elements of the mouse *MISIIR* as a candidate promoter element to direct expression of transgenic constructs to the female reproductive tract, including the OSE cells.

Our results indicate that the *MISIIR* promoter can indeed be used successfully to drive gynecological tissue-specific transgene expression in mice and that this often results in the formation of ovarian carcinoma. The ovarian tumors observed in our mice were poorly differentiated in parts of the tumor, but in some areas exhibited glandular structures and tubular differentiation consistent with serous carcinomas in humans. The peritoneal implants also exhibited papillary/cauliflower-like structures like those observed in humans. Tumors isolated from these mice were found to uniformly express TAg as measured by immunohistochemistry. Isolated areas of apparently normal surface epithelial cells remaining on the ovaries and the epithelial cells of both the Fallopian tube and uterus also exhibited focal positively staining cells indicating that the transgene can be expressed in tissues derived from the Mullerian duct. Although the ovarian tumors arising in TgMISIIRTAg mice display focal areas without any differentiation, two markers of epithelial cell differentiation (CK8 and CK19), and the absence of a typical marker of granulosa cell and Sertoli-Leydig tumors, α-inhibin, indicate their origin from surface epithelium, as opposed to a granulosa or sex cord-stromal cell origin. We cannot exclude the possibility that, under some circumstances, expression of the *MISIIR*-Tag transgene may result in this phenotype. Sequential characterization of the early stages of carcinogenesis in our model will allow for better understanding of both initial formation of early atypical cells and facilitation of our knowledge of gonad embryology.

![Fig. 3. Ovarian tumors in TgMISIIRTAg mice.](image-url)

*Fig. 3. Ovarian tumors in TgMISIIRTAg mice. A, ScanScope overview of the ovary substituted by tumor cells. Arrow and arrowhead indicate higher magnification of areas shown in B and C, respectively. Ov, ovary; B, intrabursal space. B, neoplastic cells form tubular and papillary structures and buds in the ovary (arrowheads) and in the intrabursal space (B, arrows), respectively. C, hyperchromatic, sometimes giant, neoplastic cells with apoptotic figures (arrow) forming occasional tubular structures (arrowheads). D, i.p. ascites. Papillary structures (arrow) consist of oval to polygonal relatively uniform cells with eosinophilic cytoplasm. E, neoplastic cells invading the omentum (Om) and forming cauliflower-like structures (arrow). H&E staining. Calibration bar, 1500 μm (A), 100 μm (B and C), 200 μm (D), and 360 μm (E).*
Because the MISHR is likely to be expressed in the epithelia of all of the organs of Mullerian origin (e.g., uterus, Fallopian tube, and cervix), we have carefully examined the reproductive tracts of all of the female transgenic mice for expression of the TAg transgene. In female mice that developed ovarian tumors, expression of TAg was observed frequently in the epithelia lining the uterus and Fallopian tube, but not in the cervix. We observed one animal that developed multiple leiomyomas of the uterus but have never observed endometrial carcinomas or tumors in the Fallopian tube. Whereas we have attempted to breed female TgMISHRTAg transgenic mice, mice that express the transgene have been found to be infertile, presumably because of the rapid onset of tumor initiation. Therefore, it seems unlikely that we will be successful in establishing stable transgenic lines via females. However, we are optimistic that we will be able to establish stable transgenic lines that are susceptible to ovarian carcinomas via the male transgenic animals that develop Sertoli cell tumors. Males with testicular tumors frequently breed successfully. In one case, a male MISHR-TAg transgenic animal that we were unaware had a testicular tumor, transmitted the transgene to both a female and a male pup. In the female, we observed bilateral ovarian tumors similar to those found in female transgenic founders and in the male we observed an early testicular tumor. These mice did not yield additional offspring; therefore, we were unable to establish a transgenic line. These animals demonstrate that it is technically possible to
transmit the transgene and the ovarian phenotype to female offspring via affected males. We continue to pursue this strategy to establish stable transgenic lines of mice that develop ovarian carcinomas. The SV40 TAg is a potent transforming gene that has been used successfully to model cancer in several organs in the mouse using the complete SV40 early region, or the intact or mutant large TAg driven by various tissue-specific promoters (20, 32–37). Moreover, a significant amount of information regarding the development and progression of these carcinomas continues to be gleaned from these transgenic models, suggesting the same will be true for our ovarian carcinoma model. Among prominent properties of our model is i.p. dissemination, which is a characteristic feature of human ovarian carcinomas. Furthermore, our experiments with i.p. transplantation of ascitic cells to SCID mice indicates that, similar to humans, the tumor cells tend to spread to ovaries and oviducts. These similarities in metastatic behavior make this model particularly attractive for developing and testing therapeutic approaches aimed at advanced stages of the ovarian epithelial cancer in humans. The ability to derive tumor cell lines from the ascites of transgenic mice also provides a useful tool to compare both gene expression profiles and chromosomal alterations between these cells and normal cultured MOSE cells or normal constitutional DNA to identify additional genetic changes other than the expression of the TAg that contributed to tumor formation.

We are unaware of other epithelial ovarian cancer-prone mice produced by transgenesis and are hopeful this model will complement existing alternative ovarian cancer models. Previous attempts to generate mouse models of ovarian cancer by transgenesis have resulted in mice that develop sex cord stromal cell tumors or germ cell tumors, but not EOCs (38–41). In addition to these efforts, we and others have developed experimentally induced in vitro models of ovarian malignancy by isolating OSE cells and subjecting them to a repetitious requirement for growth by repeated subculture (21, 42). In a simple way, this mimics the postovulatory wound repair at the ovarian surface. When exposed to growth-promoting conditions (i.e., cell culture) rodent OSE cells undergo spontaneous malignant transformation characterized by anchorage-independent growth and tumorigenicity in recipient immunodeficient (42) and syngeneic (21) animals.

More recently an alternative to these spontaneous in vitro transformation models has been produced (43). In this model, cultured mixed populations of ovarian cells isolated from transgenic mice bearing the avian retrovirus receptor TVA were infected with oncogene-bearing replication competent, avian leukosis virus LTR, and no splice acceptor (RCAS) retroviruses, and infected cells were reintroduced into the ovaries of recipient mice. Interestingly, these investigators found that regardless of whether the expression of the TVA receptor was restricted to the surface epithelial cells by use of the CK5 promoter, or restricted to the surface epithelial cells by use of the CK8 immunostaining and the ScanScope imaging, respectively.

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TRANSGENIC MOUSE MODEL OF OVARIAN CARCINOMA


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