Characterizing and evaluating preclinical suitability of a syngeneic mouse ovarian cancer model

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ABSTRACT SUMMARY
Epithelial ovarian cancer (EOC) accounts for over 90% of all ovarian malignancies. However, the lack of proper animal models that mimic the development and consequences of human EOC limits the ability of current preclinical ovarian cancer models to predict treatment response. The orthotopic development of syngeneic EOC within its relevant tumor microenvironment is an experimental ideal. Here, we demonstrate the development and characterization of a syngeneic EOC mouse model in immunocompetent C57BL/6 mice by orthotopic implantation of ID 8 mouse ovarian cancer cells.

INTRODUCTION
Ovarian cancer is the second most frequent invasive malignancy of the female genital tract and the most common cause of death among women with gynecologic malignancies with an estimated 22,280 cases diagnosed and 15,500 deaths annually in the U.S. alone. Epithelial ovarian cancer (EOC) accounts for over 90% of all ovarian malignancies. It has shown high mortality despite advances in treatment regimens, with overall 5-year survival rates of only 46% and even lower rates (28%) for patients with metastatic disease. Considering diagnosis of EOC at an advanced stage when the tumor is widely metastatic, development of appropriate disease models recapitulating tumorigenesis of EOC from early to advanced stage has immediate demand.

By orthotopic implantation of ID 8 mouse ovarian cancer cells, we demonstrated the development and characterization of a syngeneic EOC mouse model in immunocompetent C57BL/6 mice. Using conventional and ultrasound-based techniques to compare tumor weight, volume and vascularity we followed the development of primary tumors, metastases and ascites over 16 weeks.

EXPERIMENTAL METHODS
Six to 8-week old, female C57BL/6 mice were anesthetized with isofluorane and a single dorsal midline incision allowed access to both ovaries. $1.0 \times 10^6$ ID8 cells in 5 µl PBS were injected into the ovarian bursa. The contralateral ovary served as surgical control injected with equivalent amounts of PBS. Animals were monitored closely, weighed and assessed for health twice a week. At pre-defined time points, animals were euthanized and tumors were surgically harvested. Tumor size and weight were captured with digital calipers (Traceable Digital Carbon Fiber Calipers, Fisher Scientific, Pittsburgh, PA) and a microbalance (AG104, Mettler Toledo, Columbus, OH). Tumor volumes were calculated as $V=\frac{1}{2} (L \times W)^2$, L being length (longest dimension) and W width (shortest dimension).

Ultrasound measurements were conducted at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 16 weeks prior to sacrificing the animals. Mice were scanned with a 14 MHz transducer (Acuson Sequoia ultrasound, Siemens, Malvern, PA) images were obtained in transverse and sagittal planes. In addition to size measurements, spectral Doppler was employed to document blood flow within the tumor tissue.

Twelve weeks after implantation, ascites was collected from prior to surgical tumor harvest. Any remaining ascites after harvesting tumors was combined with the ascites collected prior to surgery to assess the total amount of ascites produced from each animal as accurately as possible. Metastatic lesions were visually confirmed in various organs at 12, 14, and 16 weeks post-exposure and quantified during surgery.

To evaluate microvessel density in tumor tissues, both CD 31 and Hematoxylin counterstaining were performed on a representative section of tumor from each time point by ARUP laboratory (ARUP, Salt Lake City, UT). Digital images were acquired through digital camera equipped with a microscope (Olympus BH-2 (Olympus America Inc., Center Valley, PA). The percentage of CD31 staining area was analyzed using ImageJ 1.44o software (N.I.H., Bethesda, MD) and minimum of 5 high power (400X) fields of view were evaluated for each tissue.

RESULTS AND DISCUSSION
Ovarian tumors grew consistently throughout the study period; ovarian weight and volume increased twelve and seven fold, respectively, compared to the contralateral, non-cancerous ovary. Ultrasound measurements of primary tumors and surrounding tumor tissue correlated with the actual size after surgical tumor harvest. Abdominal ascites were first observed at 12 weeks post orthotopic ID8 implantation with volume changes correlating with changes in abdominal circumference. Metastatic lesions were identified by ultrasound at 12 weeks after orthotopic ID8 implantation; sites included peritoneum, liver, and intestine. Histopathological analysis of tumors and metastases indicated similarities between orthotopic ID8 ovarian tumors and human ovarian tumors, except a significantly lower formation of angiogenic vasculature within the ID8 tumors (Figure 1 and 2). This study confirms the
successful development of a mouse model that closely replicates characteristics seen in human ovarian cancer patients. It shows the feasibility of using ultrasound to assess tumor formation, progression and vascularization.

![Figure 1. Immunohistochemical CD 31 stains highlighting tumor angiogenesis. A. CD 31 immunohistochemistry of MOSE-T at various time points in comparison with CD31 immunohistochemistry of high-grade serous human epithelial ovarian cancer (400 X; 100 µm scale bar).](image)

![Figure 2. Quantification (figure 1) (%) of areas positive for CD31 in mice and human epithelial ovarian cancer, respectively Mean ± S.D. (n=5).](image)

CONCLUSION

We demonstrate potential shortcomings, which could significantly limit the use of current animal models predicting therapeutic efficacy of novel agents, especially anti-angiogenic therapeutics, impacting the translation of preclinical information to actual clinical outcomes.

REFERENCES

3. ACS, American Cancer Society, 2011, 68

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