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A Syngeneic Mouse Model of Epithelial Ovarian Cancer Port Site Metastases

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Abstract

Epithelial ovarian cancer (EOC) is a deadly gynecologic malignancy, but animal models for the study of EOC pathophysiology and drug efficacy are limited. Based on the finding that women with EOC are at risk for metastasis at a trocar site after laparoscopy, we developed a syngeneic murine model of port-site metastasis of EOC. We leveraged the ID8 murine EOC cell line to induce intra-peritoneal tumors in mice. Once durable intraperitoneal tumor was confirmed with bioluminescence imaging, intra-abdominal wall tumors were induced by abdominal wall puncture with a hollow bore needle. This resulted in a robust system in which C57BL/6 mice developed metastatic deposits at a rate of 66.7% ± 10.77; no intra-abdominal wall metastases were seen in control samples ($P = .0003$, CI 41.16–90.84). Immunodeficient NOD SCID gamma mice developed puncture site metastases in 70% ± 10.0 of mice and also had no metastases documented in control sites ($P = .002$, CI 42.24–97.76). In addition we were able to demonstrate the presence of immune infiltrates within the metastatic deposits of C57BL/6 mice via IHC. Therefore, in this study we demonstrate the predictable development of invasive abdominal wall metastases in a syngeneic mouse model of EOC. This model enables studies of the metastatic process and provides a novel system in which to test the effect of therapies on a clinically-relevant model in an immune competent mouse.

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Introduction

Ovarian cancer is the deadliest gynecologic malignancy in North America killing an estimated 14,080 women in 2017 [1]. Epithelial ovarian cancers (EOC) are the most common type of ovarian carcinoma and include serous, endometrioid, mucinous, clear cell, and transitional cell histologies. Among epithelial ovarian cancer, high grade serous carcinoma is the most common histologic subtype [1]. At the time of diagnosis, the majority of women have metastases to peritoneal surfaces such as the omentum, bowel, and diaphragm. Although most women with metastatic cancer respond to initial therapy, approximately 70% of patients with metastatic disease experience a recurrence of their cancer with resultant mortality [1]. In order to study the pathophysiology and perform pre-clinical studies of potential therapies researchers have employed animal models of epithelial ovarian cancer. Current murine models of EOC involve transplatation of human or murine ovarian cancer cells into the peritoneal cavity, ovarian capsule, or subcutaneous spaces or genetic alterations that lead to the development of ovarian cancer in transgenic mice [2–9]. Researchers' ability to perform preclinical studies of ovarian cancer therapeutics and to study the pathophysiology of the disease is limited to the types of available animal models and thus we developed a novel and clinically-relevant metastatic model to add to the repertoire of available murine ovarian cancer research tools.

In humans, intraperitoneal metastasis of ovarian cancer occurs after the primary tumor is established and tumor cells exfoliate off of the primary tumor into the peritoneal cavity [2]. Women with ovarian

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carcinoma who undergo laparoscopic surgeries are at risk of developing metastatic tumor deposits within the tract of the laparoscopic trocar, also referred to as port site metastases [10–14]. This has also been observed in patients with other peritoneal cancers who undergo laparoscopic surgery and is thought to be the result of cells migrating from the peritoneal cavity into the trocar tract during or following surgery [15,16]. Thus, port site metastases are most frequently observed in humans with intraperitoneal tumor deposits and ascites [12,14]. Although the pathophysiology of this phenomenon has been investigated via animal models, the reproducibility of this process has not been established in murine models nor has this system been used as an outcome for the study of metastatic EOC [17].

Murine models of EOC typically rely on injection or surgical implantation of human or murine ovarian cancer cells into the peritoneal cavity, ovarian capsule, or subcutaneous spaces to establish tumor [2–7,13]. Grafting tumor into the ovarian capsule or subcutaneous tissue frequently fails to result in metastatic disease or ascites due to anatomic barriers to tumor spread [2–7,13]. An additional limitation of some murine models is the inability to establish clinically relevant tumors in an immune competent mouse for immunologic studies. Studies of xenographs of human ovarian cancer cells in immunosuppressed mice limit our ability to research the immune features of ovarian cancer and the effect of immune therapies [3,5]. A patient's immune response to their ovarian cancer and the immune cells within the tumor are known to play an important role in metastasis formation [18]. Syngeneic murine models enable focused studies of the immune system's role in metastasis formation and the effectiveness of immunotherapies at preventing metastasis. The development and application of a model of port site metastasis as a system for studying metastatic EOC allows for the use of a syngeneic mouse to model a clinically relevant and traceable metastatic process. Commonly used syngeneic models include the ID8 murine tumor cell line, which we apply in this study, and *MISIIR* transgenic mice and their derivatives [8,9,15]. There is a need for the development of a reliable and reproducible model of metastatic tumor deposit utilizing a syngeneic system.

In this study we describe a novel murine model of the pathophysiologic process that leads to port site metastasis in women with ovarian cancer. We were able to predictably induce a metastatic deposit within the abdominal wall in immune competent and immunocompromised mice using the syngeneic murine ID8 EOC cell line [15]. This metastatic model allows for study of a clinically-relevant metastatic implantation in an immunocompetent mouse and can be used as a secondary outcome for pre-clinical drug studies in mice.

Methods

Mice and Cells

C57BL/6 mice were purchased from Charles River (Wilmington, MA). NOD SCID gamma (NSG) mice were purchased from the Dartmouth Mouse Modeling Shared Resource (Lebanon, NH). All animal experiments were approved by the Institutional Animal Care and Use Committee. ID8 murine ovarian cancer cells transduced with pFB-neo-Luciferase (ID8-luc cells) were previously described and selected with 0.8 mg/ml G418 [15,16].

Establishment of the Port-Site Model

5×10^6 ID8-luc cells were injected into the peritoneal cavity via a left lower abdominal wall injection. Mice were imaged for in vivo luciferase activity 3–4 weeks following injection, and thereafter as indicated. Mice with radiographic evidence of intraperitoneal tumor were treated with puncture of the right inferior abdominal wall just medial to the nipple with an 18 gauge hollow bore needle. Control sites were identified in the midline of the upper abdomen remote from the ID8 injection site or the puncture site. Mice were sacrificed 3–4 weeks following abdominal wall puncture using CO_2 gas per institutional protocols.

Mouse Imaging

Imaging was performed as a modification of a previously described protocol [19,20]. Briefly, mice were injected with 200 μL of a suspension of 15 mg/mL D-Luciferin Potassium Salt (Gold Biotechnology, St. Louis, MI) in 9% sodium chloride (Baxter, Deerfield, IL) into the peritoneum via the left lower quadrant. Mice were then anesthetized with isoflurane gas. Images were obtained 10 min after Luciferin injection with the Xenogen VivoVision IVIS Bioluminescent and Fluorescent Imager (PerkinElmer, Waltham, MA).

Tissue Processing and Pathology

Biopsies of the abdominal wall were obtained immediately upon mouse sacrifice. Abdominal wall hair was removed with Nair™. If a palpable nodule or scar was identified in the right lower quadrant in the expected area of the needle puncture (just medial to the nipple), this was marked with a skin pen. If there was no scar or nodule, the area just medial to the nipple was marked. The anterior abdominal wall including the marked site was then excised using a 5 mm Keyes punch biopsy. Abdominal wall biopsies were taken in the same manner remote for the ID8 injection and contralateral to the puncture site and used as paired control sites. Specimens were placed in 4% paraformaldehyde within marked cassettes. Blocks were processed by the Dartmouth Pathology Core Resource. Specimens were embedded into a paraffin block and oriented such that a skin edge is visible on the slide. Slides were cut at 4 microns, air dried, and loaded onto Akura Tissue-Tek Prisma Autostrainer (Leica Biosystems, Buffalo Grove, IL). Slides were dried for 25 minutes, deparaffinized in Xylene, and hydrated through graduated alcohols to water. Cells were stained with Hematoxylin 2 for five minutes and washed in water. Cells were then washed in bluing agent for one minute then washed in water and then 95% alcohol for 30 seconds. Cells were then stained with Eosin-Y for 30 seconds. Slides were dehydrated in 100% alcohol and cleared with xylene. Slides were then mounted with Tissue Tek mounting medium. Staining and dehydrating materials were obtained from Richard Allen Scientific (Grand Island, NY). Pathology slides were interpreted by LJT, our collaborator within the pathology department, who was blind to specimen origin or treatment group.

Immunohistochemistry (IHC)

Slides were cut at 4 microns and air dried at room temperature. Staining was performed using the Leica Bond Rx Autostainer and Leica Biosystems reagents (Buffalo Grove, IL). The automated protocol includes baking slides for 30 minutes, dewaxing, antigen retrieval using Epitope Retrieval 2, pH 9.0 solution for 20 minutes at 100 °C. Primary anti- CD3 (Abcam, Cambridge, MA) and CD11b (Abcam, Cambridge, MA) antibodies were incubated for 15 minutes followed by washing. Primary antibody binding is visualized using Leica Bond Refine Detection kit with Dab chromogen and hematoxylin counterstain.