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New models of hematogenous ovarian cancer metastasis demonstrate preferential spread to the ovary and a requirement for the ovary for abdominal dissemination

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Abstract

Emerging evidence suggest that many high grade serous ‘ovarian’ cancers (HGSOC) start in the fallopian tube. Cancer cells are then recruited to the ovary and then spread diffusely through the abdomen. The mechanism of ovarian cancer spread was thought to be largely due to direct shedding of tumor cells into the peritoneal cavity with vascular spread being of limited importance. Recent work challenges this dogma, suggesting hematogenous spread of ovarian cancer may play a larger role in ovarian cancer cell metastasis than previously thought. One reason the role of vascular spread of ovarian cancer has not been fully elucidated is the lack of easily accessible models of vascular ovarian cancer metastasis. Here we present three metastatic models of ovarian cancer which confirm the ability of ovarian cancer to hematogenously spread. Strikingly, we observe a high rate of metastasis to the ovary with the development of ascites in these models. Interestingly, oophorectomy resulted in a complete loss of peritoneal metastases and ascites. Taken together our data indicates that hematogenously disseminated HGSOC cells have a unique tropism for the ovary and that hematogenous spread in ovarian cancer may be more common than appreciated. Furthermore our studies support a critical role for the ovary in promoting HGSOC cell metastasis to the abdomen. The models developed here represent important new tools to evaluate both the mechanism of cancer cell recruitment to the ovary and to understand and target key steps in ovarian cancer metastasis.

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Supplemental table 1. Summary of model systems used, tumor cell type, number of tumor cells used per experiment, sites of metastasis and rates of metastatic disease.

Introduction

Ovarian cancer is the most deadly gynecologic malignancy in the western world killing over 14,000 US women yearly (1). It is now believed that many ovarian cancers arise in the fallopian tube and then cancer cells are rapidly recruited to the ovary (2, 3). Once in the ovary, ovarian cancer is characterized by early metastatic spread into the abdomen often with diffuse peritoneal involvement, omental caking and ascites. Interestingly, in a murine ovarian cancer model arising in the fallopian tube, removal of the ovary significantly restricts metastases, suggesting a critical role for the ovary in promoting metastasis (3). The critical events necessary for cancer cell recruitment to the ovary and subsequent diffuse metastasis formation are not clearly defined.

It has long been assumed that direct shedding of malignant cells from the primary ovarian tumor into the intraperitoneal cavity is responsible for the development of metastatic disease. However, there is mounting evidence that ovarian cancer cells may also metastasize via the vasculature (4). Clearly, given the high rates of pelvic and para-aortic lymph node involvement, ovarian cancer cells can enter and spread via the lymphatic system. In stage III–IV epithelial ovarian cancer, pelvic and para-aortic nodes are involved in 70–75% of cases and nodal involvement significantly increases the risk of disease recurrence (5). Lymphatic disease is also independently associated with poor prognosis (6).

Hematogenous spread was thought to play a minimal role in ovarian cancer metastasis given the relatively low rate of extra-abdominal metastasis (7). However, lymphovascular space invasion (LVSI), while correlated with nodal involvement, is an independent predictor of progression and survival in early stage ovarian cancer (8, 9). Additionally, circulating tumor cells have been identified in ovarian cancer patients (10). More recently, an elegant study by Pradeep et al. used a parabiosis model (two mice, one with ovarian cancer and one cancer-free, who share a blood supply) to study hematogenous spread of ovarian cancer. In this model, the normal mouse develops ovarian cancer which requisitely had to have been the result of hematogenous spread (4).

As most did not consider hematogenous spread of ovarian cancer to be a common modality of metastases, there are few models to study mechanisms of hematogenous ovarian cancer metastasis. The parabiosis model, while powerful, is cumbersome and expensive. The development of appropriate models of ovarian recruitment of cancer cells and hematogenous spread is key to understanding the mechanisms of ovarian cancer metastasis and to developing therapies which target this lethal step in ovarian cancer progression. Traditional murine xenograft models utilizing ovarian cancer cells grown as subcutaneous tumors in immune-compromised mice rarely metastasize and direct intraperitoneal injection of human tumor cells does not assess the role of vascular dissemination.

We sought to investigate the pattern of ovarian cancer hematogenous spread. Here we report three *in vivo* models of ovarian cancer resulting in metastatic disease via hematogenous spread. Strikingly, all three models demonstrate the development of intra-ovarian metastatic disease indicating a unique tropism of ovarian cancer cells for the ovary and further supporting a role for vascular spread in ovarian cancer metastasis.

Materials and methods

Intravenous ovarian tumor cell injection

SKOV3, OVCAR3, KURAMOCHI, A2780 and COV362 ovarian cancer cell lines and SUM159, MDA-MB-231 breast cancer cell lines and the NIH-H522 lung cancer cell line were maintained in 10% FBS/1% Penicillin/Streptomycin supplemented RPMI. The SUM159 cells (labeled with Luc-mCherry via lentiviral transduction) were a kind gift from Dr. M. Burness. The KURAMOCHI cells were obtained from the Japanese Collection of Research Bioresources Cell Bank. SKOV3, OVCAR3, COV362, A2780, NIH-H522 and MDA-MB-231 cells were obtained through ATCC. Cells were trypsinized and re-suspended at a concentration of 5×10^6 cells/ml. For SKOV3 cells, 5 mice were injected with 1×10^6 cells and 8 mice were injected with 5×10^6 cells via tail vein injection. For OVCAR3 and COV362, 10 and 4 mice respectively were injected with 1×10^6 cells. For A2780, KURAMOCHI, SUM159, NIH-H522 and MDA-MB-231, 5 mice were each injected with 1×10^6 cells. Female nulliparous NSG (NOD/Shi-scid/IL-2R γ null) mice were used for all experiments. Mice were monitored for tumor size, ulceration, weight gain and end-stage illness scoring and mice were sacrificed at predefined thresholds for euthanasia in accordance with University of Michigan ULAM. Luc-mCherry-SUM159 injected mice underwent bioluminescence imaging prior to sacrifice to identify sites of metastatic disease. At time of sacrifice, mice underwent necropsy and macroscopic tumor sites were identified and either snap frozen or paraffin embedded for histologic analysis.

Eight mice per group underwent surgical oophorectomy or mock surgery as previously described (11, 12). Briefly, the mice were anesthetized (intraperitoneal injection of a mixture of ketamine chloride and xylazine chloride) under sterile conditions. A single 1-cm dorsal midline incision was made in the skin. Both the ovaries and oviducts were removed and the uterus was left intact. In the control group, the animal's abdominal wall was also opened to create similar surgical stress but the ovaries/oviducts were left intact. Animal usage and all procedures were in strict compliance with the guidelines of Institutional Animal Care and Use Committee (IACUC). Mice were injected with 1×10^6 OVCAR3 cells via tail vein 12 days after surgery. Mice were again monitored for tumor size, ulceration, weight gain and end-stage illness scoring. Mice were sacrificed by 6 ½ weeks following IV injection and mice were processed as above.

Murine ovarian metastasis model

The W2532T and W2476T murine ovarian cancer cell lines were derived from Apc^{-/-}/Pten^{-/-} murine ovarian endometrioid adenocarcinoma as previously described (11). Briefly, fresh ovarian tumor tissues were mechanically minced with sterile scalpels and further digested at 37°C with 0.05% Trypsin-EDTA for 20 minutes. Cells were cultured for 5 passages in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS/1% penicillin/streptomycin (P/S)/1% Insulin-transferrin-selenium (Invitrogen). Cells were maintained in 10% FBS 1% Penicillin/Streptomycin/1% Insulin-Transferrin-Selenium supplemented DMEM. 5×10^6 cells of each cell line were injected into the bilateral axilla of 10 NSG mice or injected into the intraperitoneal cavity of 3 NSG mice respectively. Mice were monitored, sacrificed, inspected for metastatic disease and processed as above.

Subcutaneous primary human ovarian tumor model

Primary patient derived ovarian tumor cells were isolated from human ovarian cancer surgical specimens in accordance with institutional IRB approved protocol. Tumor cells were isolated by manual mincing, filtering and centrifugation of tumor specimens. Cells were then grown on adherent cell culture dishes in supplemented RPMI. Cells were expanded in cell culture for 2–4 passages prior to injection. 1×10^6 primary patient derived ovarian tumor cells alone or mixed with human infantile hemangioma stem cells (HemSC) and patient derived carcinoma-associated mesenchymal stem cells (CA-MSCs) (isolated as previously described(13)) in a 1:1:1 ratio. Briefly, to isolate CA-MSCs primary patient derived tumor tissue was plated in supplemented MEBM media and MSCs were selected for plastic adherence. CA-MSCs were characterized by cell surface marker expression (positive for CD105, CD90, CD73, CD44; negative for CD34, CD24, CD45) and their ability to differentiate into adipocytes, osteocytes or fibroblasts (following guidelines presented by The International Society for Cellular Therapy on the minimal criteria for defining multipotent mesenchymal stem cells) (14). CA-MSCs used in xenograft models were derived from “matched” patient tumor samples in 1 of 4 xenografts, otherwise CA-MSCs were derived from a separate patient sample. HemSC were a kind gift from Dr. J Bischoff and were isolated from human infantile hemangiomas as described (15). HemSCs were maintained on fibronectin coated dishes ($1 \mu\text{g}/\text{cm}^2$) in supplemented Endothelial Basal Medium-2 + 20% FBS (Lonza). Eight NSG mice were injected with bilateral axillary tumors using primary patient tumor cells alone. Four additional NSG mice were injected with bilateral axillary tumors using primary patient tumor cells + HemSC + CA-MSCs. Mice were monitored, sacrificed and inspected for metastatic disease as above. Sites of tumor metastasis were likewise processed as above.

Immunohistochemistry

Snap frozen tumor blocks were cryo-sectioned through the University of Michigan microscopy and imaging lab. Sections were fixed in 4% formaldehyde and permeabilized with 0.1% triton. Sections were stained with either mouse anti-human nuclear antigen (Millipore), anti-TP53 (abcam), anti-PAX8 (abcam), anti-human mitochondrial antibody (Life Technologies), anti-human CD34 (abcam) or anti-smooth muscle actin-Cy3 antibody (abcam) in 1:100 dilution. Of note, anti-human CD34 antibody used is predicted to detect mouse in addition to human CD34. Goat anti-mouse-Alexa 488 secondary antibody was then applied (Life Technologies). Images were obtained using an Olympus BX41 microscope. Paraffin imbedded tumor tissue was prepared, sectioned and stained through the University of Michigan Microscopy & Image Analysis core.

Mice

Nulliparous NSG female mice between 8 and 12 weeks old were used for all the above experiments. Mice were maintained in accordance with institutional policies. All studies were performed with approval of the University Committee on Use and Care of Animals of the University of Michigan.

Statistics

Statistical significance was evaluated using a 2-sided student's T-test. For all experiments, $\alpha=0.05$. In all figures, error bars represent standard error of the mean.

Results

Human ovarian cancer cell lines preferentially metastasize to the ovary

Intravenous and intra-cardiac injection of cancer cells has been a useful model to study hematogenous cancer spread in multiple cancer models (16, 17). Given new evidence that ovarian cancer may also spread hematogenously, we first sought to identify the pattern of metastatic disease with intravascular dissemination of ovarian cancer cell lines. We used an IV model with SKOV3 ovarian cancer cells delivered via tail vein injection. Surprisingly, injection of 1×10^6 SKOV3 cells demonstrated gross metastatic disease within the ovary in 80% of mice (Fig 1a). The ovarian tumors generated were intra-ovarian and not surface deposits. Disease was also seen on the diaphragm and within the peritoneum with development of ascites (Fig 1a) as well as in the lung and liver. Only the lungs, which are anticipated to have a high degree of metastasis in a tail vein injection model given they may act as a filter, had a greater rate of metastases than the ovary and there was not a statistically significant difference in rate of metastasis to the lung or liver compared to the ovary. Injection of 5×10^6 SKOV3 cells lead to a high rate of death immediately after injection, likely due to embolic complications thus, while we observed ovarian tumors in 50% of the surviving mice, this data has been censored and not included in our final analysis. Histologic analysis of metastatic disease with H&E staining, human nuclear antigen staining and human PAX8 staining confirm tumors are of human origin with the anticipated malignant features (Fig 1b).

To determine if the pattern of SKOV3 hematogenous metastatic spread was likewise seen in other ovarian cancer cell lines, we subsequently performed tail vein injections with four additional ovarian cancer cell lines. We used three cell lines recently found to likely represent HGSO (OVCAR3, KURAMOCHI, COV362) and one cell line (A2780 cells) which likely does not represent HGSO. Analogous to SKOV3 cells, OVCAR3 cells (1×10^6 cells/mouse) resulted in an 83% rate of ovary metastasis (Table 1), 38% of these mice had bilateral ovarian involvement. Histologic analysis with H&E staining, human nuclear antigen staining and human PAX8 staining once again confirmed tumors are of human origin with appropriate malignant features (Fig 2D, E and data not shown). Similar to SKOV3 cells, OVCAR3 cells likewise metastasized to the peritoneal cavity in addition to the liver and lung. Interestingly, peritoneal disease and ascites were only detected in mice in which intra-ovarian metastatic disease was present, suggesting the possibility that ovarian disease may be important for the propagation of further intra-abdominal metastasis. Mice injected with KURAMOCHI cells appeared grossly healthy however upon sacrifice at week 12, 80% of mice had small (2–10 mm) ovarian tumors. Lung lesions were detected in 60% of mice and no other areas of gross disease were identified. Tail vein injection of COV362 cells failed to initiate any gross metastatic disease even 12 weeks after injection. The lack of any metastatic disease, even in the lung, suggests this cell line is not capable of hematogenous metastasis formation. A2780 cells were the only cell line which formed metastatic disease

that did not demonstrate gross ovarian cancer metastases. However, all animals developed ascites and some developed bowel obstructions (Fig 2C).

Of note, numerous cancer cell lines have been injected by others in the past and ovary is not commonly reported as a site of metastases (18–24). To confirm that the ovary is not just a general common site of hematogenous spread, we performed intravenous injections of NIH-H522 lung adenocarcinoma cells, MDA-MB-231 breast cancer cells and luciferase labeled SUM159 breast cancer cells (cell lines previously used in intravenous models of breast and lung cancer metastasis) (25–27), 5 mice per group. In all three groups, mice developed lung metastasis but no ovarian metastasis. In the NIH-H522 group, metastatic deposits were grossly detected in the lung (80%) and liver (20%) of mice. In the MDA-MB-231 group, metastasis were grossly detected in the lung (100%) and liver (40%) of mice. The luc-SUM159 mice demonstrated lung (80%) and bone metastasis (40%) as assessed by gross dissection and via bioluminescence imaging (Table 1). Composite analysis of ovarian cancer cell lines (OVCAR3, SKOV3, KURAMOCHI, A2780) vs non-ovarian cancer cell lines (SUM159, MDA-MB-231, H522) which formed metastasis demonstrates significant differences in the proportion of mice developing ovarian metastasis (66% vs 0%, $p=0.02$). There is no significant difference in the rate of lung metastasis (86% vs 93%, $p=0.6$) between ovarian cancer cell lines and non-ovarian cancer cell lines supporting the hypothesis that ovarian cancer cells have a unique tropism for hematogenous spread to the ovary.

Murine ovarian metastasis model

As direct intravenous injection is highly artificial bypassing all the necessary steps of cancer cell intravasation into the vasculature, we next used a subcutaneous murine ovarian tumor model to investigate patterns of metastasis. $Apc^{-}/Pten^{-}$ murine ovarian endometrioid adenocarcinoma-derived tumor cell lines W2532T and W2476T were used to create subcutaneous axillary tumors in NSG mice. Engraftment was achieved in 100% of bilaterally injected tumors in 6–8 weeks. Four out of 10 mice (40%) developed ovarian metastasis. Of the 4 mice with ovarian metastasis, one developed ascites and one developed liver and bowel metastatic disease. One mouse developed liver only metastasis and one mouse had ascites only without other sites of macroscopic tumor (Fig 3). Histologic analysis confirmed the murine ovarian cancer cell line origin of metastatic disease. Intraperitoneal injection of the murine ovarian cancer cell lines likewise yielded ovary metastatic disease at a similar rate of 33%. Liver and bowel disease developed in a higher percentage of mice with IP injection (66% and 33% respectively) indicating differences in pattern of spread with direct intraperitoneal cell exposure (similar to direct cancer cell shedding) vs hematogenous spread from axillary tumors.

Subcutaneous primary ovarian tumor cell model

Next, we sought to determine if human ovarian cancer cells demonstrate a similar pattern of metastasis from a subcutaneous site. Our previous work demonstrates that axillary xenografts with ovarian cancer cell lines SKOV3 and CAOV3 do not form metastatic disease (13, 28). We next utilized primary patient derived ovarian tumor cells in an axillary xenograft model. We isolated primary human ovarian tumor cells from surgical specimens from women with high-grade serous ovarian cancer. We found that 1 of 8 injected tumors

(n=4 for two patient samples) injected subcutaneously resulted in an ovarian metastases (Fig 4). No other gross metastatic disease was detected (either in the mouse with the ovarian metastasis or in the mice without ovarian metastasis).

We previously demonstrated that co-injection of cancer cells with carcinoma associated mesenchymal stem cells (CA-MSC) can promote cancer growth via the formation of multiple components of the human tumor microenvironment though these xenografts do not spontaneously develop metastasis (13, 28). We sought to determine if a human microenvironment can increase ovarian cancer metastasis. We co-injected patient derived primary human ovarian cancer cells and CA-MSCs. To enhance the human stroma with human tumor vessels, we also co-injected human infantile hemangioma stem cells (HemSCs). HemSCs have previously been demonstrated to create human blood vessels when injected into immune deficient mice (15). Subcutaneous tumors grew at all injected sites by 6–8 weeks thus yielding a 100% engraftment rate. Surprisingly, all mice with the humanized stroma developed overt macroscopic ovarian metastasis (unilaterally or bilaterally) by the time of sacrifice. Lung, liver and serosal bowel metastasis were also noted in 30–60% of mice. In contrast, only 1 of 8 mice without the humanized stroma developed metastases (as mentioned above). Histologic evaluation of the primary axillary humanized tumors and sites of metastasis confirm that the metastatic tumors are indeed derived from the primary axillary human ovarian tumor as evidenced by mutated p53 staining pattern of the primary tumor (Fig 4b). Additional human mitochondrial antibody staining of the metastatic ovarian lesion confirmed human origin (data not shown). Suggesting that spread was hematogenous in origin, clear vascular space invasion within the primary subcutaneous humanized tumor was observed, largely near the periphery of the tumor but also scattered throughout the body of the tumor (Fig 4c). A summary of cancer cells used per model system and sites of metastasis is provided in supplemental table 1.

Oophorectomy reduces the rates of intra-abdominal metastasis formation

In the models above, we noted the association of ovarian cancer metastases with ascites/peritoneal nodules. To assess the role of the ovary in propagating intra-abdominal metastasis formation, we next performed bilateral oophorectomies (or mock surgery controls) on female NSG mice (n=7 oophorectomy group; n=8 mock surgery group). 12 days postoperatively, mice were injected with 1×10^6 OVCAR3 cells via tail vein. 7 of 8 mice in the mock surgery control group developed overt ovarian metastasis. Of these 7 mice with ovary tumors, 5 demonstrated ascites and 3 demonstrated renal/adrenal metastasis (Fig 5a). All mice developed lung and liver metastasis (liver metastasis were largely intra-parenchymal). In the oophorectomy group, while all mice likewise developed lung and liver metastasis, no mice developed ascites, renal/adrenal or other abdominal metastasis (Fig 5a). Interestingly, the burden of liver disease was significantly different between the control group vs oophorectomy group with quantification of visible nodules demonstrating an average of 15 nodules in the control group vs 2 nodules in the oophorectomy group ($p=0.0002$) (Fig 5b, c). Surprisingly, 2 of 8 mice in the control group and 2 of 7 mice in the oophorectomy group developed tumor within the uterine horn (Fig 5d).

Discussion

Here we describe three models of ovarian cancer with hematogenous metastasis. We demonstrate that ovarian cancer cells can hematogenously spread with a striking predilection for spread to the ovary. This ovarian tropism is demonstrated in all three models 1) intravenous ovarian cell injection 2) murine subcutaneous tumors and 3) human subcutaneous tumors. Surprisingly, in the IV model, the rate of metastasis to the ovary was similar to rates of metastasis to the liver and lung which filter high volumes of intravenous cells during first pass circulation. In comparison, the ovary receives significantly less blood supply therefore likely was exposed to many fewer tumor cells yet resulted in the same rate of metastasis, indicating a tropism for the ovary and/or increased growth once within the ovary.

The subcutaneous models with spontaneous metastasis to the ovary also supports the idea of a unique predilection for the ovary as the burden of cells within the vasculature is significantly less in this model yet rates of ovarian metastasis remain high. The xenograft model is unique in utilizing both HemSCs and CA-MSCs to provide a humanized stroma which enhances the metastatic potential of this model. Of note, metastatic patterns of other cancer types using this model have not been reported and further studies will be necessary to define the role of the stroma in metastasis formation. The subcutaneous model resulted in fewer lung metastasis thereby suggesting the majority of lung metastasis in the IV model was likely due to cells trapped in the lung which serves as a filter in this model. The ovary tropism appears unique to ovarian cancer cells as IV injection of metastatic breast cancer cell lines and a lung adenocarcinoma line, while resulting in typical sites of disease in the lung, liver and bone, did not result in ovary involvement. Likewise, literature review of multiple non-ovarian cancer cell lines used in intravenous models rarely report ovarian metastasis (21–24).

The difference in metastatic pattern seen with the A2780 cell line (bowel predilection without ovarian metastasis) is interesting. The A2780 cell line is now recognized as not representative of high-grade serous ovarian cancer (29) and the difference in metastasis pattern may be due to differences in cancer cell origin and thus a different tropism for metastatic sites (though it is interesting to note that there remains a tropism for the intra-abdominal environment given the development of bowel obstructions and ascites). Of note, not all ovarian cancer cell lines produced metastatic disease when used in the tail vein injection model (COV362 failed to form any gross metastatic disease). This may be due to differences in cell lines and their ability to survive in non-adherent conditions within the blood stream or inherent differences in their capacity for hematogenous metastasis formation.

The importance of hematogenous spread in ovarian cancer is just starting to be realized. Our results support a role for hematogenous metastasis and indicate that ovarian cancer has a unique pattern of vascular spread. While the ovary is an area where tumors of multiple origin are known to spread (both via vascular dissemination and through intra-abdominal “drop metastasis” known as “krukenberg tumors”), these are rare events. It is estimated that “krukenberg tumors” make up 1–2% of all ovarian tumors and in one series of gastric

cancer patients, 0.4% developed ovarian metastasis (30, 31). The rate of ovarian cancer metastasis in our models ranging from 40–100% is much higher than would be expected based on the proportion of blood flow to the ovary alone. The recognition of hematogenous metastasis to the ovary may provide critical insight to the mechanism of ovarian cancer progression.

In support of this, there is growing evidence that high-grade serous ovarian cancer, the most common form of ovarian cancer, arises from precursor lesions within the fallopian tube and one of the earliest steps in cancer progression is metastasis to the ovary (2, 3, 32). It is important to note however that up to 40% of ovarian cancer cases present without an identified fallopian tube precursor lesion and there is murine data supportive of a possible ovarian epithelial cell of origin for HGSOC (33, 34).

While the definitive cell of origin remains controversial, the ovarian microenvironment appears to play a critical role in the dissemination of HGSOC to the abdominal cavity. Perets et al reported that in a genetically engineered murine cancer model of high grade serous cancer arising in the murine equivalent of the fallopian tube that oophorectomy, while not impacting cancer development, lead to a reduction of abdominal metastasis (3). Our studies further support a critical role for the ovary in promoting intra-abdominal metastasis. We noted a high correlation of ovarian metastases and ascites/peritoneal metastases. Further, removing the ovaries prior to intravenous injection of tumor cells significantly decreased the rate of intra-abdominal metastasis formation, including not only elimination of ascites and peritoneal metastases, but also decreased burden of liver metastasis. While intra-parenchymal liver metastasis were seen in both control and oophorectomy mice, significantly fewer nodules were seen in the oophorectomy group possibly suggesting the ovary is important in further seeding the liver (in addition to the initial liver seeding which occurs at the time of tail vein injection). However, given tumor nodules, in addition to being increased in number, also appeared larger (though not quantified), we feel it is more likely the ovary is also promoting growth of lesions within the liver. Given the increased burden of liver disease in the presence of ovarian disease, it is unclear if the development of ascites is secondary to or independent of the liver disease. However, given the ascites was clearly a malignant effusion (via grossly bloody appearance and the ability to isolate tumor cells from the ascites (data not shown)) it seems unlikely ascites was secondary to liver disease/portal hypertension.

The finding of uterine horn metastasis in both the control and oophorectomy group is also very interesting. This may be a result of inflammation and altered blood flow after surgery (mock surgery or oophorectomy) but may also implicate the microenvironment of the gynecologic tract in supporting HGSOC growth. It is important to note that while these lesions were present in 2 oophorectomy mice, none of these mice developed other non-liver intra-abdominal disease and burden of liver lesions remained low (1–2 liver lesions per mouse), further indicating a role for the ovary to propagate intra-abdominal dissemination. Consistent with a role of the ovary in metastatic disease in human disease, early bilateral intra-ovarian tumors (not merely on the surface of the ovary) are commonly seen (35) and in fact constitute their own stage of disease (stage 1b). These tumors appear clonally related therefore are not likely separate primaries (36, 37).

It is interesting to note that the parabiosis model of ovarian cancer hematogenous spread demonstrated omental disease rather than ovarian metastatic disease. This may be a reflection of differences in metastatic capacity/tropism between ovarian cancer cell lines used in this model. The parabiosis study used SKOV3.ip cells which are cells derived from SKOV3-produced ascites and SKOV3-OM3 cells which the authors describe as SKOV3.ip cells which metastasized to the omentum and were then re-harvested and passaged (4). Both of these cell lines already demonstrated the ability to metastasize to the peritoneal cavity and therefore may be able to bypass an initial ovary metastasis step. While clearly more work is needed to clarify the capacity and pattern of ovarian cancer hematogenous spread, understanding the importance of early homing of cancer cells to the ovary may be instrumental in furthering our knowledge and improving the treatment of ovarian cancer.

In conclusion, we describe three models of ovarian cancer which result in hematogenous metastasis and provide information on the pattern of vascular spread and importantly identify the ovary as a common site of hematogenous spread. These models can be enhanced with techniques such as luciferase labeling of tumor cells to detect *in vivo* metastasis patterns via bioluminescent imaging. Performing intracardiac rather than tail vein injection can also be used to bypass initial entrapment of cells in the pulmonary circulation. Further, stable cell labeling or “barcoding” would allow lineage tracing of metastatic cells in these models (38, 39). These models may be important tools in the future study of cancer cell recruitment to the ovary and metastatic spread from the ovary and may add significantly to the further investigation into the mechanism of metastasis and methods of blocking this deadly step in ovarian cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CA-MSC	carcinoma-associated mesenchymal stem cells
HemSC	human infantile hemangioma stem cells

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Background

New evidence suggests that most high-grade serous 'ovarian' cancers start in the fallopian tube and then rapidly spread to the ovary promoting widespread metastases. While metastasis from the ovary was thought to be from direct shedding, hematogenous spread has been postulated. Translational significance: We present three models demonstrating (1) ovarian cancer cells can metastasize hematogenously, (2) ovarian cancer cells are specifically recruited to the ovary and (3) the ovary is important for the propagation of intra-abdominal metastasis. These models will be important tools to define mechanisms of ovarian cancer metastases and identify therapeutic targets to restrict ovarian cancer metastasis.

Figure 1.

Murine intravenous injection of SKOV3 ovarian cancer cells result in a high rate of ovarian metastasis. A) percent of mice with organ specific metastasis after IV SKOV3 tumor cell injection B) representative macroscopic ovarian metastasis ii) H&E stain of metastatic ovary tumor iii) human nuclear antigen stain (green) of metastatic ovary tumor demonstrating human origin of tumor iv) human PAX8 stain of metastatic ovary tumor.

Figure 2.

Intravenous injection of multiple ovarian cancer cell lines result in ovary metastasis. A) percent of mice with organ specific metastasis after IV tumor cell injection with multiple ovarian cancer cell lines B) representative macroscopic ovarian metastasis C) dilated murine bowel/bowel obstruction after A2780 IV injection D) H&E stain of metastatic OVCAR3 ovary tumor E) human nuclear antigen stain (green) of metastatic OVCAR3 ovary tumor demonstrating human origin of tumor.

Figure 3.

Murine subcutaneous ovarian tumors metastasis to the ovary. A) Summary of metastatic sites after subcutaneous and intraperitoneal murine ovarian cancer cell injection B) representative picture of macroscopic ovarian metastasis from the subcutaneous model. C) H&E stain of ovarian tumor metastasis from the subcutaneous model.

Figure 4.

Subcutaneous primary human ovarian cancer cells in combination with HemSC and CA-MSCs form spontaneous ovarian cancer metastasis. A) tabular display of percent of mice which developed metastasis per axillary xenograft composition B) p53 stain of pt366+HemSC+CA-MSC xenograft C) CD34 stain demonstrating CD34+ vasculature surrounding tumor cells in flank pt366+CA-MSC+HemSC tumors indicating lymphovascular space invasion (arrows).

Figure 5.

Oophorectomy decreases the burden of abdominal metastasis in IV tail vein injection model. A) Prevalence of metastatic sites following IV tail vein injection of OVCAR3 cells demonstrates no ascites or kidney/adrenal metastasis in mice with oophorectomy vs mock surgery group which developed ovarian tumors, ascites and kidney metastasis. B) Quantification of the number of gross liver lesions identified in control vs oophorectomy mice demonstrating significant decrease in burden of liver lesions in oophorectomy mice. C) Representative liver of (i) control mouse (ii) oophorectomy mouse demonstrating fewer liver

lesions in the oophorectomy mice. D) (i) image of dissected uterine horn from oophorectomy mouse demonstrating nodularity corresponding with tumor deposits demonstrated in (ii, iii) H&E sections of the paraffin embedded uterine horn.

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Table 1

Summary of the percent of mice developing ovarian metastasis after IV injection of multiple cancer cells lines. Ovarian cancer cell lines: SKOV3, OVCAR3, KURAMOCHI, COV362, A2780. Breast cancer cell lines: SUM159, MDA-MB-231. Lung cancer cell line: NIH-H522.

Cell line	Ovarian metastasis
SKOV3, n =10	80%
OVCAR3, n=18	83%
KURAMOCHI, n=5	80%
COV362, n=4	0%
A2780, n=5	0%
SUM159, n=5	0%
NIH-H522, n=5	0%
MDA-MB-231, n=5	0%