Objective: The aim of this study was to review the latest research advances on the topics of the ovarian cancer tumor microenvironment and models of ovarian cancer.

Methods: In September 2016, a symposium of the leaders in the field of ovarian cancer research was convened to present and discuss current advances and future directions in ovarian cancer research.

Results: One session was dedicated to Tumor Microenvironment and Models of Ovarian Cancer, and included a keynote presentation from Anil Sood, MD, and an invited oral presentation from David Huntsman, MD. Eight additional oral presentations were selected from abstract submissions. Twenty-nine abstracts were presented in poster format and can be grouped into the categories of stromal cells in the microenvironment, immune cells in the microenvironment, epithelial-mesenchymal transition and metastasis, metabolomics, and model systems including spheroids, murine models, and other animal models.

Conclusions: Rapid advances continue in our understanding of the influence of the tumor microenvironment on ovarian cancer progression and metastasis. Vascular endothelial cells, stromal cells, and immune cells all modulate epithelial tumor cell biology and therefore serve as potential targets for improved treatment responses either in conjunction with or instead of current treatment modalities. Characterization of the underlying genetic alterations in both the tumor cells and surrounding microenvironment cells enhances our understanding of tumor biology. Model systems including both in vitro and in vivo approaches allow novel advances. Technological advances including sequencing strategies, use of mass spectrometry for metabolomics and other studies, and bioengineering approaches all complement conventional methodologies to push forward our understanding and ultimately the treatment of ovarian cancer.

Key Words: Ovarian cancer, Antivascular therapeutics, Mouse models, Spheroids, Metabolomics, Epithelial-mesenchymal transition, Mesenchymal stem cells, Cancer stem-like cells, Carcinoma-associated fibroblasts, Biomarker

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ANTIVASCULAR THERAPY AND ADAPTIVE CHANGES IN THE TUMOR MICROENVIRONMENT

The 11th Biennial Rivkin Center Ovarian Cancer Research Symposium was held in September 2016, and retreat proceedings have been published. The Tumor Microenvironment session opened with a keynote address by Anil Sood, MD, who discussed tumor microenvironment changes as patients develop resistance to ovarian cancer therapies and strategies to overcome this resistance. He first discussed microenvironment changes that result in the setting of antiangiogenesis therapy. In 2014, the Federal Drug Administration approved the monoclonal antibody bevacizumab, which targets vascular endothelial growth factor for treatment of ovarian cancer. Clinical trials in epithelial ovarian cancer consistently demonstrate an approximately 4-month improvement in progression free survival (PFS) with the addition of bevacizumab to standard cytotoxic chemotherapy, yet it is noted that this benefit “collapses” after cessation of therapy. In preclinical animal model systems, rebound growth is also observed when antiangiogenesis therapy is stopped. Thus, additional treatment strategies are needed to augment current clinical antivascular therapeutics (Table 1). Dr Sood presented data supporting an adaptive response in the tumor microenvironment after antiangiogenesis therapies characterized by hypoxia leading to increased neoangiogenesis and increased platelet extravasation. These new vessels are immature and demonstrate vascular leakage that is mediated by focal adhesion kinase (FAK). This posttreatment rebound growth can be blocked by FAK silencing or antiplatelet antibodies. At the time of antiangiogenesis resistance, increased tumor associated macrophages (TAMs) and myeloid-derived suppressor cells are also noted in the tumor microenvironment. The MD Anderson Cancer Center is beginning the REDIRECT clinical trial (ClinicalTrials.gov identifier NCT02923739) looking at the clinical effects of administering the monoclonal antibody emactuzumab against CSF-1 receptor in addition to paclitaxel and bevacizumab. The hypothesis is that emactuzumab downregulates the miRNA processing machinery including DICER. Dr Sood’s group has found that miR-192 is upregulated in epithelial ovarian cancer and be significantly associated with poor PFS and overall survival. Anirban Mitra, PhD, used an organotypic 3D culture model of early metastasis to investigate stromal-tumor cells signaling changes in metastasis. They found in this model system of the omental tumor microenvironment that tumor cells upregulate the ETS1 transcription factor to promote metastatic colonization.

Paul Campagnola, PhD, presented data on the role of collagen alterations in ovarian cancer. He showed data that targeting p130cas in tumor endothelial cells with siRNA approaches blocked tumor growth in xenografts and inhibited acquired resistance to bevacizumab antivascular therapy. Dr Sood next discussed the use of microRNAs (miRNAs) as an antiangiogenic therapy in the treatment of ovarian cancer. He reported that there are a number of barriers to the delivery of miRNAs to ovarian cancer tumors, including the finding that hypoxia downregulates the miRNA processing machinery including DICER. Dr Sood’s group has found that miR-192 is antiangiogenic and is now optimizing delivery strategies including nanoparticle and thioaptamer approaches. These efforts are now in phase I clinical trials at MD Anderson.

Role of Stromal Cells in the Tumor Microenvironment

Multiple presentations at the symposium addressed the role of stromal cells in the tumor microenvironment and interactions between stromal cells and epithelial tumor cells. LAN Coffman, MD, PhD, showed data on how tumor cells induce durable changes to mesenchymal stem cells (MSC) in the tumor microenvironment, and that this reprogramming of MSC to cancer-associated MSC results in enhanced protumorigenic effects. Ernst Lengyel’s group had a poster presentation investigating the effects of tumor cells on stroma through tumor development and metastasis. They reported that the serine-threonine kinase T-LAK cell-originated kinase (TOPK) is upregulated in high-grade serous ovarian cancer (HGSOC) and promotes a “mesenchymal” signature in the tumor cells as well as changes to the surrounding stroma including carcinoma-associated fibroblasts. The TOPK has been reported to be upregulated in epithelial ovarian cancer and be significantly associated with poor PFS and overall survival. Anirban Mitra, PhD, used an organotypic 3D culture model of early metastasis to investigate stromal-tumor cells signaling changes in metastasis. They found in this model system of the omental tumor microenvironment that tumor cells upregulate the ETS1 transcription factor to promote metastatic colonization.

Paul Campagnola, PhD, presented data on the role of collagen alterations in ovarian cancer. In his group’s work, stromal remodeling was investigated along the continuum of ovarian pathology from normal stroma, to benign tumors, to

<table>
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<tr>
<th>TABLE 1. Novel strategies to inhibit rebound growth after antivascular therapies</th>
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<td>Target</td>
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<tr>
<td>Platelet extravasation</td>
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<td>Immune cell infiltrates</td>
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<td>Vascular endothelial cell signaling</td>
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<td>Neoangiogenesis</td>
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CSF, colony stimulating factor.
low- and high-grade tumors using a biomedical engineering approach.\textsuperscript{11,12} Interestingly, stromal characteristics are unique among the pathology types, and characterization of the stromal architecture can predict the subtype of epithelial cell pathology.

In addition to tumor cells impacting stromal behavior, multiple investigators reported on the effects of stromal-derived signals on tumor cells. Karen McLean, MD, PhD, presented data that interleukin 6 and leukemia inhibitory factor cytokine signaling from cancer-associated MSC (CA-MSC) to tumor cells increases the percentage of cancer stem-like cells (CSCs) and promotes tumor growth, with JAK/STAT cascade inhibition blocking these protumorigenic effects of CA-MSC. Another specific stromal signal that may alter epithelial cell behavior is that of collagen type XI, α1 (COL11A1). Dong-Joo Cheon’s group has published that COL11A1 overexpression is associated with metastasis and poor survival in HGSOC.\textsuperscript{13} At this symposium, they presented new data that overexpression of COL11A1 in ovarian cancer stroma leads to NF-κB signaling and a proinflammatory gene expression signature, as well as an increase in CSCs, both of which are hypothesized to mediate chemoresistance.

**Immune Cells in the Ovarian Cancer Tumor Microenvironment**

Interactions between immune cells in the ovarian cancer tumor microenvironment and tumor cells are critical in tumor biology and represent an important potential therapeutic target in the treatment of malignancy. Dendritic cell vaccination to induce antitumor Th17 cells is one therapeutic approach now being investigated in clinical trials. However, preliminary data suggest that CD14+ TAMs have a suppressive effect blunting the efficacy of dendritic cell vaccines. Martin Cannon, PhD, presented data that the CD14+ cells in ascites suppress immune response through noncanonical STAT3 signaling as well as expression of interleukin 10 and indoleamine 2,3-dioxygenase.\textsuperscript{14} Consistent with Dr Cannon’s findings of immune cell changes in ascites of ovarian cancer patients, Sharon Robertson, MD, MPH, reported that sequencing comparison of primary HGSOC tumors and patient-matched ascites reveals an increase in T-cell markers and monocyte/macrophage markers in ascites compared with primary tumors. This again highlights the role of immune infiltrates in the metastatic process. Wei Wei, PhD, presented further data on the mechanisms by which TAMs mediate protumorigenic effects in ovarian cancer. He reports that fibroblast growth factor 18 promotes ovarian cancer tumor progression through NF-κB signaling.\textsuperscript{15} They find that in primary tumor samples, there is a significant correlation between fibroblast growth factor 18 expression and TAMs. They show data supporting a model in which there is crosstalk between tumor cells and TAMs, in which TAMs activate epithelial cell NF-κB signaling to increase proinflammatory cytokines.

Madhuri Koti’s group and others have reported that tumor cell STAT1 expression and CD8+ T-cells in the tumor microenvironment are significantly associated with improved PFS and enhanced response to chemotherapy.\textsuperscript{16,17} At this symposium, they present data that in chemosensitive tumors, there is upregulation of STAT1 and a type-I interferon pathway gene signature. Tumor cells with this gene signature may more effectively recruit CD8+ T-cells, resulting in a greater immunologic response and improved cancer cell treatment.

**The Origins and Early Development of Ovarian Cancer**

David Huntsman, MD, presented an invited talk on the origins of different histologic subtypes of ovarian cancer from a genetic perspective. He highlighted that although the different ovarian cancer types are highly distinct diseases, they share in common their growth in the ovary, suggesting that there may be unique features of ovarian tissue that support tumor initiation and progression. He hypothesized that specialized functions of the ovarian stroma that may impact tumorigenesis include the support of follicular development and the capacity to luteinize, both of which may result in unique or elevated growth promoting signals. He also suggested that the inherent immune privilege environment for dormant oocytes may favor cancer development.

Dr Huntsman then proceeded to highlight genetic changes that are characteristic of specific subtypes of ovarian cancer (Table 2). Granulosa cell tumors (GCT) are a true ovarian cancer, arising from ovarian stromal cells. The somatic mutation of the forkhead box L2 gene (FOXL2) has been identified in over 90% of GCT.\textsuperscript{18} Interestingly, no germline mutations in FOXL2 have been reported in patients with GCT. In addition, FOXL2 is expressed throughout the Mullerian tract stroma, yet it is unclear why the tumors arise specifically in the ovary, highlighting a need for further understanding of the ovarian microenvironment. Consistent with findings in testicular germ cell tumors, a subset of GCT cases demonstrate alterations in the telomerase reverse transcriptase gene. Another sex-cord stromal tumor, Sertoli-Leydig tumor, is characterized by germline or somatic mutation in the DICER1 gene.

Small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare ovarian tumor that comprises less than 1% of total ovarian cancer cases and is invariably unilateral. This tumor type is characterized by an activating mutation of the SMARCA4 gene that encodes the BRG1 protein.\textsuperscript{19} The finding of loss of BRG1 by immunohistochemical staining has been shown to be diagnostic of SCCOHT.\textsuperscript{20} Dr Huntsman proposes that this finding offers clues to the origin of SCCOHT, with 2 potential pathways for tumor development. One mechanism is that BRG1 silencing occurs leading to subsequent SMARCA4 loss. Alternatively, SCCOHT may arise when a BRG1-negative immature teratoma subsequently undergoes SMARCA4 allelic loss at the genomic level. The relative roles of these 2 pathways require further investigation.

Within epithelial histologic subtypes of ovarian cancer, both clear cell and endometrioid carcinomas are associated with the presence of endometriosis,\textsuperscript{21} and many believe endometriosis to be a precursor lesion of these cancers. Interestingly, clear cell and endometrioid ovarian carcinomas rarely arise outside the ovary, suggesting that there are heretofore unidentified factors within the ovarian microenvironment that promote tumor initiation and/or progression for these cancer subtypes. Genetically, clear cell and endometrioid ovarian cancers are characterized by mutations in ARID1A, the PTEN/ PI3K pathway and/or activating mutations in KRAS or BRAF. Whole-genome sequencing of ovarian clear cell carcinomas...
and endometriotic lesions from multiple patients reveals that there may be multiple classes of endometriosis with a specific high mutational subset serving as the precursor lesion for malignancy. Dr Huntsman also briefly touched on recent work demonstrating cystathionine γ-lyase as a novel clear cell carcinoma biomarker.

Finally, the mutational profile of HGSOC and the investigation into the site(s) of origin were discussed, and the implication on clinical strategies for risk reducing surgeries again highlighted. The cellular origin of HGSOC is discussed in greater detail by other speakers in the symposium.

**Epithelial-Mesenchymal Transition and Metastasis**

The role of epithelial-mesenchymal transition (EMT) in the initiation of metastasis in ovarian cancers, by inducing loss of epithelial cell polarity and cell-cell adhesion with a concomitant increase in migratory and invasive behavior, was well represented at the conference. Vermont Dia, PhD, presented the role of lysosphatidic acid in inducing EMT. Lysosphatidic acid treatment leads to an upregulation of EMT transcription factors including p65, SLUG and SNAIL in both macrophages and ovarian cancer cells. Hanming Wang, BSc, described the effect of TGFβ and EGF on EMT in ovarian cancers. The authors had previously shown that reprogramming of fibroblasts to induced pluripotent stem cells was reduced by SNAI1 knockdown and increased by its overexpression in human and mouse cells. They presented new evidence that levels of SNAI1 correlate directly with those of Nanog and inversely with Let-7. They also found that SNAI1 directly repressed transcription of Let-7. Furthermore, they found that on knocking down SNAI1, metastasis burden in the xenografts increased. They postulate that by repressing Let-7, SNAI1 induces CSC traits in the ovarian cancer cells. Maricela Gallardo, BS, showed the characterization of EMT-driven CSC traits in a 3D spheroid model of ovarian cancers. Her team labeled CSC with SNAI1, LIN28A, OCT4, SSEA4, ALDH, TRA1-60, CD133, E-CAD, and N-CAD. They found that cancer cell lines and patient-derived cells were positive for CSC markers to different degrees. They observed that higher expression of SNAI1 correlated with CSC traits, and they postulate that SNAI1 is a potential target for CSC in ovarian cancers.

Trillitye Paullin presented the characterization of EMT in 3D spheroid model of ovarian cancers in the context of heat shock factor 1 (HSF1). They found that TGFβ-induced EMT in 3D spheroids led to increased gene expression in stress response pathways and in heat shock response. They demonstrated that HSF1 knockdown had more significant impact on EMT markers in 3D spheroids compared with 2D cultures. They postulate that HSF1 could serve as a target chemotherapeutic for ovarian and other hormone responsive cancers.

Multiple presentations focused on the mechanistic pathways underlying metastasis of ovarian cancer. Akira Yokoi, MD, presented data demonstrating that extracellular vesicles including exosomes and microvesicles from highly metastatic ovarian cancer cell lines induce metastatic behavior. In addition, matrix metalloproteinase 1 mRNAs were selectively packaged into the extracellular vesicles and facilitate the metastatic phenotype. The authors highlight the potential of extracellular vesicles to be both a prognostic biomarker and a therapeutic target. The role of omental and peritoneal barriers in metastasis was also investigated by Tyvette Hilliard, PhD. A poster from the group investigated the role of the mesothelin-CA125 binding interaction on metastasis using mouse models comparing wild type to knockout mesothelin. They report that mesothelin functions in promoting metastasis, as fewer metastatic lesions were noted in the knockout mice. A final poster looking at the metastatic microenvironment of the omentum was presented by Venkatesh Krishnan, PhD. His group demonstrated that omental milky spots are the preferential sites for cancer
colonization of peritoneal adipose, and that omental macrophages are essential for the development of these milky spots and subsequent metastases.

Finally, Lilie Lin, MD, gave an oral presentation on the clinical use of a novel positron emission tomography tracer that can quantify poly ADP ribose polymerase (PARP) enzyme activity in vivo and may be used as a biomarker of response to PARP therapy.\textsuperscript{27,28} The 18F-fluourthanotrace was found to localize to known areas of tumor and level up date correlated with platinum sensitivity.

\section*{METABOLOMICS}

Multiple investigators reported on metabolomic changes that occur in ovarian cancer and their impact on tumorogenesis. Daniela Matei, MD, gave an oral presentation on the metabolic characterization of ovarian CSCs. She reported mass spectrometry data demonstrating that ALDH\textsuperscript{+}/CD133\textsuperscript{+} CSC have increased levels of unsaturated lipids. She presented a model in which there is a positive feedback loop in which increased unsaturated lipids promotes NF-\kappaB signaling, which increases the percentage of ALDH\textsuperscript{+} CSC. This leads to an increase in retinoic acid that in turn triggers metabolic changes further increasing unsaturated lipid levels. This feed-forward metabolic loop represents a novel pathway for targeting ovarian cancer and specifically CSC.

It is postulated that metabolomics signatures may predict responses to therapy. Nahid Razi, PhD, presented data on the identification and characterization of glycyl cell surface expression with response to therapy, and report 2 markers of response—Glycomarker 1 and Glycomarker 2. Paolo D’Amora, MD, PhD, reported on a phase-II clinical trial currently accruing patients that is correlating metabolic signatures with clinical outcomes including platinum sensitivity, PFS, and overall survival. They are using the Ex vivo Analysis of Programmed Cell Death chemoresponse assay on primary tumor tissue\textsuperscript{29} and mass spectrometric lipid component analysis of blood samples. To date, 21 of a target of 50 epithelial ovarian cancer patients have been enrolled.

Facundo Fernandez, PhD, reported on metabolomics profiling of Dicer-Pten double-knockout mice that develop HGSOC. Metabolic changes in both serum and tissue are noted.\textsuperscript{30} The presenters highlight that further characterization of such changes has the potential to be ultimately used as a needed approach for early detection of HGSOC in the clinical setting.\textsuperscript{31}

Justyna Kanska, PhD, presented data on the consequences of chronic glucose deprivation in ovarian cancer cell lines in vitro. Her team found that glucose deprivation results in upregulation of nicotinamide N-methyltransferase (NNMT) via genomic gain of NNMT and upregulation of the upstream transcription factor ZEB1. Furthermore, they demonstrated that ectopic expression of ZEB1 results in resistance to glucose deprivation in an NNMT-dependent manner. Finally, NNMT expression is correlated with poorer PFS and overall survival in patients. The ZEB1/NNMT pathway of acquired glucose-independent growth may be a therapeutic target in the treatment of ovarian cancer.

\section*{SPHEROID TUMOR MODELS}

To understand how the elements of the ovarian tumor microenvironment drive tumor progression, metastasis and chemoresistance, multiple models were presented at the symposium (Table 3). Geeta Mehta, PhD, described a 3D high-throughput hanging-drop spheroid model derived from as few as 10 CSCs isolated from patient ascites.\textsuperscript{32,33} The group demonstrated that the tumor heterogeneity and populations of CD133\textsuperscript{+} and ALDH\textsuperscript{+} were maintained between the CSCs freshly isolated from patient samples and cells harvested after growth as 3D spheroids. Patient CSC spheroids were capable of initiating tumors with short latency in immunodeficient mice, even when only 1 spheroid was injected. Most promisingly, there was a correlation in drug responses between the patient-derived CSC spheroids and the xenografts. Therefore, the patient tumor–based spheroid model shows great potential for improving predictive potency of sensitivity of conventional and novel chemotherapeutic agents. This model can also be used to study tumor re-emergence by creating spheroids from cells that escape chemotherapy.

Shu-Wing Ng, PhD, presented characterization of ovarian cancer spheroids using multiple approaches. Previously, their group characterized ALDH isoenzymes in ovarian cancer spheroids.\textsuperscript{34} Cells from spheroids were under constant oxidative stress with depleted endogenous glutathione, which was relieved by supplementation with N-acetylcysteine. These cells also demonstrated elevated expression of FOS/JUN gene, which activated glutathione-S transferase. The FOS knockdown abrogated chemoresistance in spheroids. Moreover, endosomal pathway genes for exosome secreted were upregulated in spheroids. They postulated that increased exosomes may promote cancer progression.

Yali Zai, MD, PhD, presented in vitro organoid models of HGSOC based on murine oviductal epithelium from Ovgpl-iCreERT2 animals where Ovgpl controls expression of tamoxifen inducible Cre. The oviductal organoids model the fallopian tube epithelium, and demonstrate both ciliated and secretory cells, and express oviductal epithelial markers (cytokeratin8, ER, PAX8, OVGP1). They also demonstrated organoid formation from Ovgpl-iCreERT2, Brea1\textsuperscript{+/−}, Pten\textsuperscript{−/−}, Rb1fl/fl(BPR) mice. Organoids can undergo Cre-mediated re-combination to inactivate tumor suppressor genes relevant to HGSOC in vitro, enabling rapid studies on oncogenic transformation in HGSOC.

Uday Veeramallu, MS, demonstrated the use of MetaCell separators to isolate, characterize, and grow invasive ovarian cancer circulating tumor cells (CTC) that have undergone EMT (http://metacell.cz/about/). They proposed using this device for selecting for subpopulations of CTC and using them for targeted therapies to arrest metastatic disease.

\section*{MOUSE MODELS OF OVARIAN CANCER}

Josephine Walton, PhD, gave an oral presentation on the establishment of ID8 murine models of HGSOCs with CRISPR/Cas9-mediated knockout of Trp53, Brcal, Brcax, and/or Pten. They characterized intraperitoneal growth, platinum and PARP sensitivity and immune cell infiltration in these ID8-based models with single (Trp53\textsuperscript{−/−}, Brcal\textsuperscript{−/−}, Trp53\textsuperscript{−/−}, Brcax\textsuperscript{−/−}, and Trp53\textsuperscript{−/−}, Pten\textsuperscript{−/−}) or double deletions (Trp53\textsuperscript{−/−}, Brcax\textsuperscript{−/−}, Trp53\textsuperscript{−/−}, Brcal\textsuperscript{−/−}, and Trp53\textsuperscript{−/−}, Pten\textsuperscript{−/−}). These models have great potential to probe relationships between tumor genotype, chemotherapy response, and the immune
To decode transcriptional heterogeneity and mechanisms of drug resistance, Benjamin Izar, MD, PhD, applied single-cell RNA-seq to ascites derived from patients with treatment-resistant cancers. They also used sc-RNA-seq to study microscopic residual disease in patient-derived xenograft models. They described gene sets related to the inflammatory pathways, including NF-κB and STAT3, as the drivers of transcriptional heterogeneity. They found significant intertumor heterogeneity via sc-RNA-seq and identified transcriptional programs related to cell cycle. A subset of noncycling cells demonstrated a CSC signature with CD133, ALDH1A, and AXL. Through hierarchical clustering, they identified 4 major clusters including an inflamed phenotype. With their comprehensive approach, they postulate that they will better be able to study treatment resistance in ovarian cancers.

Although abdominal shedding was long known to be the mechanism for ovarian cancer metastasis, new evidence suggests that ovarian cancers can also spread hematogenously. Lan Coffman, MD, PhD, revealed vascular spread of ovarian cancers with 3 different metastatic models. They used (1) mouse tail vein injection of ovarian cancer cells, (2) subcutaneous mouse ovarian tumor, and (3) human xenograft ovarian tumor with humanized microenvironment model to demonstrate formation of distant metastases by vascular spread. They observed high rate of metastasis to the ovary in all 3 models, with intra-abdominal metastatic disease and ascites. Their evidence suggests unique ovarian tropism for the hematogenously disseminated HGSOCs. These models will be valuable in studying recruitment of tumor cells to the ovary and progression of the ovarian metastases.

TABLE 3. In vitro and in vivo ovarian cancer models

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<tr>
<th>Model</th>
<th>Presenter</th>
<th>Strengths of the Model</th>
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<tr>
<td>In vitro</td>
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<tr>
<td>3D spheroids from patient-derived ascites cancer stem cells</td>
<td>Geeta Mehta, PhD</td>
<td>Spheroids generated from very few cells from patient samples. Prediction of in vivo drug responses with responses in spheroids matching those in parallel xenografts.</td>
</tr>
<tr>
<td>3D spheroids from cell lines and clinical specimens</td>
<td>Shu-Wing Ng, PhD</td>
<td>Characterization of gene and protein expression profiles as well as metabolomics signatures in spheroid growth conditions.</td>
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<tr>
<td>MetaCell separator isolation of cancer cells or CTC</td>
<td>Uday Veeramallu, MS</td>
<td>Isolation approach does not require cell labeling. Isolation of CTC from serum.</td>
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<tr>
<td>Organoids from oviductal epithelium of <em>Ovgp1</em>-iCreERT2;Bra1^del/fl;Trp53^mut/fl;Rb1^fl/fl* mice</td>
<td>Yali Zai, MD, PhD</td>
<td>HGSOC tumor suppressor gene inactivation in vitro in organoids via tamoxifen administration to allow rapid study of subsequent neoplastic transformation.</td>
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<td>In vivo, murine species</td>
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<td>ID8 mice with single (Trp53^-/-) or double deletions (Bra1^-/-, Trp53^-/-;Bra2^-/- and Trp53^-/-;Pten^-/-)</td>
<td>Josephine Walton, PhD</td>
<td>Development and characterization of ID8 immune competent mouse model system with genetic profiles mirroring human HGSOC tumor mutations.</td>
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<td>Tail vein injection of ovarian cancer cells</td>
<td>Lan Coffman, MD, PhD</td>
<td>Modeling of vascular metastases.</td>
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<td><em>Rgnef</em> knockout in MISIR-T-antigen induced C57BL/6 mouse model</td>
<td>Elizabeth Kleinschmidt</td>
<td>Characterization of Rgnef-FAK signaling in ovarian cancer, with the finding that this pathway modulates ovarian tumor initiation and progression, and the loss decreases tumor growth independent of stromal Rgnef status.</td>
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<td>In vivo, nonmurine species</td>
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<td>Jaguar with spontaneous ovarian adenocarcinoma with BRCA2 exon 11 c.3732C &gt; G variant</td>
<td>Sarah Corner, DVM, MS, DACVP</td>
<td>Spontaneous BRCA mutation resulting in hereditary cancer syndrome that is comparable to humans.</td>
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<td>Rhesus macaque</td>
<td>Manish Patankar, PhD</td>
<td>10%-30% of rhesus macaques of reproductive age develop endometriosis, thus serving as a model for spontaneous endometriosis-associated type I ovarian cancers. A murine model is not possible due to lack of menses and endometriosis in mice.</td>
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To investigate the effect of aging in ovarian cancer metastases, Elizabeth Loughan described a murine model based on C57BL/6 mice that were young (3–6 months) or old (20–23 months). These mice were injected intraperitoneally with syngeneic ID8 RFP-tagged cells. They observed more efficient ovarian cancer metastases in old mice compared with young mice. Aged mice showed heavier tumor burden in the gonadal fat compared with young mice, but no difference was observed in the omental metastases. Preference to metastasize in the gonadal fat in aged mice was attributed to upregulation of B cells. More studies are underway to understand how B cells contribute to metastases.

Expanding on their earlier work on the role of Rho guanine nucleotide exchange factor (Rgnef) activates FAK and RhoGTPase activity in fibroblasts, Elizabeth Kleinschmidt found that Rgnef is elevated in advanced stage HGSOC. The Rgnef knockout prevented ovarian tumor formation in MISIR-T-antigen–induced C57BL/6 mouse model. The Rgnef$^{−/−}$; TAg orthotopic and intraperitoneal tumors were much smaller than Rgnef$^{+/−}$; TAg tumors, but growth in 3D spheroids was not altered with loss of Rgnef.$^{36}$ Their work demonstrates that Rgnef-FAK signaling modulates ovarian tumor initiation and progression, and the loss decreases tumor growth independent of stromal Rgnef status.

In summary, multiple mouse models continue to be developed to aid in the study of ovarian cancers. New improvements to these models include background genotypes that more closely mirror the genetic alterations observed in epithelial ovarian cancer and consideration of modulators of response such as age. Tumor microenvironment interactions in the initiation, progression, and metastasis of ovarian cancer are an additional area of further mouse model refinement. Finally, as the role of the immune system in tumorigenesis becomes clearer, the need for immune competent models becomes more essential.

**NONMURINE MAMMALIAN ANIMAL MODELS OF OVARIAN CANCER**

Nonmurine animal models of ovarian cancer are less common but may provide unique and beneficial approaches for disease study. Sarah Corner, DVM, MS, DACVP, described ovarian adenocarcinoma in a jaguar model. They histologically confirmed ovarian adenocarcinoma in 40% of jaguars, and demonstrated autosomal dominant pattern of inheritance. Next-generation sequencing on paired ovarian adenocarcinoma and normal tissue samples revealed a BRC2 variant in the region essential for RAD51 binding in all jaguars. They postulate using the jaguar model to identify mutational carriers for ovarian adenocarcinoma, and make better breeding decisions for these endangered captive animals. Jaguars may be appropriate animal model for the study of hereditary breast and ovarian cancers due to shared genetic factors with the cancers in humans.

Manish Patankar, PhD, presented a rhesus macaque model for spontaneous endometriosis-associated type-I ovarian clear cell and endometrioid carcinomas. They highlight that nonhuman primates may be an ideal model system for studying the development of these malignancies, because 10% to 30% of female rhesus macaques of reproductive age develop endometriosis. Current immunohistochemical and genetic characterization of this model system is underway.

**SUMMARY**

Rapid advances continue in our understanding of the influence of the tumor microenvironment on ovarian cancer progression and metastasis. Vascular endothelial cells, stromal cells, and immune cells all modulate epithelial tumor cell biology and therefore may serve as targets for improved treatment responses either in conjunction with or instead of current treatment modalities. Rebound tumor growth after antivascular therapies is a clinical challenge that may require additional therapy either during or after antivascular therapy; multiple approaches were discussed by Dr Anil Sood in this symposium.

Characterization of the underlying genetic alterations in both the tumor cells and surrounding microenvironment cells enhances our understanding of tumor biology. Dr David Huntsman highlighted the dramatic genetic differences of cancer types that are considered to be ovarian cancers. Model systems including both in vitro and in vivo approaches allow novel advances. Technological advances including sequencing strategies, use of mass spectrometry for metabolomics and other studies, and bioengineering approaches all complement conventional methodologies to push forward our understanding and ultimately the treatment of ovarian cancer.

**REFERENCES**


