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Ovarian Cancer: Prevention, Detection and Treatment of the Disease and Its Recurrence. Molecular Mechanisms and Personalized Medicine Meeting Report

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Abstract

Objective—To review the current understanding of the underlying molecular, biologic and genetic mechanisms involved in ovarian cancer development and how these mechanisms can be targets for prevention, detection and treatment of the disease and its recurrence.

Methods—In May 2012, we convened a meeting of researchers, clinicians and consumer advocates to review the state of current knowledge on molecular mechanisms and identify fruitful areas for further investigations.

Results—The meeting consisted of seven scientific sessions, ranging from Epidemiology, Early Detection, and Biology to Therapeutics and Quality of Life. Sessions consisted of talks and panel discussions by international leaders in ovarian cancer research. A special career-development session by the CDMRP Department of Defense Ovarian Cancer Academy as well as an oral abstract and poster session showcased promising new research by junior scientists.

Conclusions—Technological advances in the last decade have increased our knowledge of the molecular mechanisms involved in a host of biological activities related to ovarian cancer. Understanding the role these mechanisms play in cancer initiation and progression will help lead to the development of prevention and treatment modalities that can be personalized to each patient, thereby helping to overcome this highly-fatal malignancy.

Keywords

ovarian neoplasms; epidemiology; etiology; screening; biomarkers; proteomics; genomics; metabalomics; BRCA1/2; cancer stem cells; micro RNA; nuclear receptors; individualized medicine; cancer vaccines; quality of life; patient reportable outcomes; therapeutics; clinical trials; survival

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Introduction

Ovarian cancer is the sixth most common cancer worldwide among women in developed countries and the most lethal of all gynecologic malignancies.(1) Currently, most women have advanced stage disease at the time of diagnosis. Despite aggressive surgery and chemotherapy, the prognosis for these women is poor, with a 5-year survival rate of less than 30%. This poor outcome is due in part to the lack of effective prevention and early detection strategies: when diagnosed at an early stage, the survival rate is approximately 85–90%. Thus, prevention and early detection are key to overcoming this disease. With the exception of oral contraceptives, there are no successful chemopreventive agents available. Bilateral oophorectomy has also been shown to reduce disease incidence, but the procedure has several drawbacks in terms of women's health.(2) Existing screening techniques (CA125, transvaginal ultrasound) have not been demonstrated to reduce morbidity or mortality. Thus, better prevention, detection and screening methods are urgently needed. As well, because of the virulent and usually fatal nature of the disease, most women with ovarian cancer live with fear of recurrence, which happens in about 85% of cases. Current treatments offer little hope and survival has remained virtually unchanged for almost three decades. New methods to prevent, detect and treat recurrence are urgently needed.

With advances in molecular biology and the emergence of new technologies, scientists are gathering remarkable knowledge about the genetic and biologic basis of ovarian cancer carcinogenesis. Such knowledge opens the door to new strategies for prevention, early detection and treatment of the disease. Importantly, it allows for the development of "personalized medicine," wherein prevention, detection and treatment modalities are aimed at the specific molecular mechanisms of an individual tumor and its microenvironment, as well as at the specific genetic and biologic profile of the host. Science stands on the precipice of a new era for making profound progress in ovarian cancer research.

To facilitate this progress, we convened a scientific symposium on ovarian cancer. The meeting was held May 10–11, 2012 in Pittsburgh, Pennsylvania. The meeting brought together over 300 researchers, scientists, clinicians, policy makers and advocates for an intensive two-day discussion of molecular mechanisms and personalized medicine in the prevention, detection and treatment of ovarian cancer and its recurrence.

This article summarizes the highlights of the main presentations. Also included are abstracts chosen by the program committee as among the top submissions, as well as abstracts of the presentations by members of the Department of Defense Ovarian Cancer Academy. Readers are referred to the conference website in order to view videos of the complete talks and interactive panel discussions that were part of each session (www.upci.upmc.edu/ovarian).

Julene Fabrizio Keynote Lecture: 40 Years of Ovarian Cancer Research

The meeting opened with a keynote address by **Nelly Auersperg, MD, PhD** who provided a look at the progress made in ovarian cancer biology during the last 40 years. The ovarian cancer cell of origin remains an ongoing debate. Early studies implicated the ovarian surface epithelium (OSE) as the origin of high-grade serous ovarian carcinomas (HGSOCs) based on the observation of early transformed cells within ovarian epithelial inclusion cysts (3) This view was held until quite recently when it was observed that lesions resembling HGSOC were found in the oviductal fimbriae of *BRCA1* carriers suggesting that some HGSOCs arise in the fallopian tubes.(4)

Despite evidence for both theories, none of the evidence supports either theory 100%. Thus it is possible that a subset of HGSOCs arises from the OSE while another subset has a tubal origin. This raises the question: how do two epithelia from different organs with very

different structure and function give rise to identical carcinomas? Emerging molecular evidence suggests that the OSE and distal fimbrial epithelium are a continuous, incompletely determined zone of epithelial transition from one epithelial type to another.(5) Such transitional epithelia in other parts of the body, such as the squamo-columnar junction of the cervix, are known to be prone to neoplastic transformation. This theory provides a partial explanation for why after prophylactic salpingo-oopohorectomies the remaining portion of the fallopian tube does not represent a cancer risk and why salpingectomy alone may not provide adequate protection against ovarian cancer development. Future work will provide greater insight into this theory and how it can help explain ovarian carcinogenesis.

Epidemiologic and Genetic Factors

C. Leigh Pearce, PhD presented work on endometriosis and ovarian cancer. Women with endometriosis are at an increased risk of ovarian cancer. Despite the prevalence of endometriosis (about 10% in the general population (6)), only a small percentage of women with the condition develop ovarian cancer. Identifying these high-risk women remains elusive. Moreover, the association between endometriosis-associated disease and well-known ovarian cancer protective factors, such as OC use and parity, remains unclear. The relationship between ovarian cancer risk and endometriosis-related factors, such as anatomical location, type and timing of treatments, and symptom and treatment response, are not also known. Clarifying these factors will help establish risk estimates based on an individual's profile and will help tailor prevention interventions. The Ovarian Cancer Association Consortium (OCAC) confirmed that the endometriosis-ovarian cancer link is limited to the invasive clear cell and endometrioid subtypes.(7) No association was found for borderline tumors, suggesting that contrary to current theories, borderline clear cell and endometrioid tumors may not be the pre-cursors of their invasive counterparts. Identifying the pre-cursor lesion for endometriosis-associated ovarian cancer as well as factors associated with progression from pre-malignant to malignant disease will support developing targeted prevention therapies.

Ellen L. Goode, PhD, MPH contended that ovarian cancer has a genetic component beyond the rare, high-risk, low prevalence (<1%) mutations in genes such as *BRCA1/2*. Combinations of common genetic variants with minor allele frequencies (MAFs) greater than 5% and which confer modest risk likely account for the remaining heritability. Candidate gene studies have identified many potential susceptibility variants. However, data have been inconsistent due to small sample sizes and heterogeneity across study populations. OCAC provides a large sample size, pooling of data and examining of between-study heterogeneity in order to address these limitations. OCAC has confirmed some variants associated with ovarian cancer while refuting others. Using GWAS, 6 novel susceptibility loci (in 2q31, 3q25, 8q24, 9p22,17q21, 19p13) were identified.(8, 9) Each of these variants is common (frequency >8%) and confers only a modest change in risk (< 20%); however, their functions are mostly unknown. Further work in understanding how they impact ovarian cancer and how they are affected by host factors is needed. Further research is also needed to understand the relationship between these loci, specific disease subtypes and other disease phenotypes such as survival. This will help evaluate the clinical utility of the markers as well as uncover novel prevention and treatment targets.

Kirsten Moysich, PhD examined the role of immunosuppression pathways in ovarian cancer and explored the hypothesis that a strong immunosuppressive genotypic and phenotypic profile is associated with ovarian carcinogenesis. Specifically, her work is investigating the roles of immunosuppressive regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) in ovarian cancer. Ovarian cancers appear to have much higher levels of Tregs and MDSCs compared to benign tumors. Work clarifying the meaning of

immunosuppressive cells in ovarian cancer development and outcome is needed. In addition to the phenotypic associations, functional SNPs in genes involved in the Treg and MDSC pathways were also associated with ovarian cancer risk and poorer survival in initial studies. When these same candidate genes were examined among 17,421 cases and 25,878 controls in OCAC, only a handful of associations were found and the effect sizes were of questionable relevance (less than 5–10%). However, when examining associations by histologic subtypes, several associations of modest size were found. The associations differed among the histologic types in both magnitude and direction. This raises interesting questions about the functionality of SNPs, the subsequent phenotype and the relationship to specific histology.

Prevention, Early Detection and Biomarkers

Robert C. Bast, Jr, MD discussed biomarkers for detecting and treating incident and recurrent ovarian cancer. Identifying sensitive (>75%) and specific (>99.6%) screening modalities are key to early detection. CA125 is currently the standard marker; however, it is only 99% specific. UKCTOCS showed improved detection when using ultrasound (US) after assessing CA125 levels relative to a woman's baseline value rather than a "standard" clinical value: early stage detection was doubled (48%) and the number of operations per cancer case detected was remarkably low (4 versus 36 for US alone). (10) Despite these promising findings, 20% of ovarian cancers will not be detected with this two-tier approach because they fail to express CA125. Moreover, cancers originating in the fallopian tube cannot be visualized on ultrasound prior to metastasis. Additional biomarkers are needed. Promising directions include panels of serum-based tumor markers and autoantibodies. To date neither has demonstrated the requisite sensitivity and specificity to be clinically useful. Better imaging techniques able to detect cancers at even smaller volumes are also needed. Finally, work is needed to understand how to combine both serum marker and visualization approaches in a way that will produce a cost-effective screening tool that can easily be adapted to the clinic.

Kristin Zorn, MD presented data on ovarian cancer development in "high-risk" women, defined as women who possess a germ-line mutation that confers an increased risk of ovarian cancer. In addition to garnering insight into how to best care for these women, studying the high-risk population helps understand the pathogenesis of sporadic disease. The discovery of incident fallopian tube cancers among women undergoing risk-reducing bilateral salpingo-oophorectomy (RRBSO) raises the intriguing notion that the fallopian tube and not the ovary is the origin for some HGSOCs. Further laboratory data support this hypothesis and even suggest that tubal intraepithelial carcinomas (TICs) may serve as the precursor lesion for HGSOC: both lesions exhibit cytological atypicia, high proliferative indices, and the presence of p53. (11) The presence of TICs in women diagnosed with HGSOC further supports this hypothesis. (12) However, the data are not consistent. For example, high rates of p53 foci are found in the tubal epithelium of normal-risk women. Thus, other molecular alterations must be necessary for the progression to malignant disease. Elucidating these alterations as well as developing techniques to more carefully profile specimens from high-risk women represent areas for future research. (13)

Marian Mourits, MD, PhD talked about the impact of the new fallopian tube hypothesis on preventive strategies in high-risk woman. Screening is ineffective for detecting ovarian cancer at an early stage and has not been shown useful in managing high-risk women. RRBSO is currently the only preventive option available. However, the procedure has many side effects that may negatively impact a woman's health and quality of life, including bone and cardiovascular health as well as sexual functioning.(2) Thus, care must be taken to manage postmenopausal symptoms arising from a RRBSO. In light of the tubal origin of the

disease, another option is for high-risk women to have a “risk-reducing” salpingectomy. However, no studies have been conducted to assess the effectiveness of this strategy. As well, while data support a tubal origin of the disease especially in murine models, (14) the evidence in humans is far from conclusive. Many questions remain, such as how cancerous or even pre-cancerous cells from the fallopian tubes migrate to the ovary and become HGSOC, what is the precursor to TICs, what is necessary for a TIC to convert to HGSOC, and through what mechanism does ovulation suppression affect the tubal epithelium?

Ovarian Cancer Biology – Mechanisms and Targets

Setsuko K. Chambers, MD presented data showing that micro RNAs (miRNAs) interact with messenger RNAs (mRNAs) to influence ovarian cancer etiology. RNA binding proteins control translational regulation of mRNA through a binding element typically located in the 3’ untranslated region (3’UTR). mRNA binding proteins can have both oncogenic and tumor suppressor roles. Emerging data also show that miRNAs are important mRNA regulatory components through both translational control as well as modulation of mRNA decay. miRNAs are frequently dysregulated in cancers, have both oncogenic and tumor suppressive roles, and are involved in ovarian proliferation, invasion and metastasis. Interaction between RNA binding proteins and miRNAs is another mechanism whereby each influences disease. For example, CSF-1, which plays a key role in ovarian cancer etiology,(15) is regulated by miR130a and miR301a. Both miRNAs are dependent on the RNA binding protein nucleolin for gene expression of CSF-1 and as well as for tumor cell motility. These data suggest that regulators of the 3’UTR can control gene expression and tumor behavior. Whether these mechanisms can be exploited for therapeutic intervention warrants further investigation.

There is limited understanding of the role of nuclear receptors (NRs) in HGSOC. **Steffi Oesterreich, PhD** presented *in silico* analyses of the Cancer Genome Atlas (TCGA) Project data sets, which identified members of the NR4A family of orphan receptors as potential drivers of a subset of HGSOC. The relevance of this finding is unclear and further studies of the mechanics and function of these receptors are needed. The association between hormonal exposures and HGSOC suggests that hormones play a role in the etiology of the diseases and, thus, endocrine therapies may be fruitful at least for some subset of cancers. However, little is known about the role of steroid hormone receptors such as the estrogen receptor (ER) in HGSOC. Preliminary studies in ovarian cancer cell lines indicate that ER expression is not a reliable biomarker of estrogen response and better predictive markers are needed.

Melanie Flint, PhD discussed the role of stress on cancer initiation and progression with a focus on the adaptive immune system. Stress triggers a complex response mechanism that affects various systems, including the immunes system. *In vitro*, release of stress hormones induces T cell activation and migration, while decreasing cell proliferation. (16) The mechanism underlying these changes may be through rearrangement of the actin cytoskeleton. In a transgenic mouse model of ovarian cancer, chronic stress decreases CD3+ T cell activation and results in earlier onset of tumors. However, the tumors appear to be more confined to the ovary compared cancers induced in unstressed mice. This suggests that stress hormones, in addition to affecting the immune system, may also directly interact with cancer cells thereby impacting proliferation. Elucidating the molecular mechanisms underlying these findings will be important in understanding the impact of chronic and acute stress on cancer initiation and progression.

Plenary Talk - Karen A. Johnson Memorial: Creating Effective Patient-Physician

Partnerships, Martha E. Gaines, JD. Dr. Gaines, an 18 year ovarian cancer survivor, founded the Center for Patient Partnerships to address the need to enhance the many partnerships that result from a diagnosis of ovarian cancer. Central to successful treatment for ovarian cancer is the physician-patient partnership. Choosing a healing path requires many elements, including understanding the roles and goals of both the physician and patient, clear and honest communication and a balance of power. Only through an effective patient-clinician partnership will a woman be truly empowered to face the fight of her life.

Novel Approaches to Symptom Assessment and Management

Dana Bovbjerg, PhD discussed biobehavioral models for symptom management. Managing symptoms of both the disease and treatment present significant challenges for women and clinicians. In addition to medical complications, behavioral comorbidities, such as depression, fatigue, disrupted sleep and cognitive dysfunction, often initiate with diagnosis and treatment, and may continue into the survivorship period. Post-operative pain is a significant symptom which often influences other comorbidities throughout treatment and beyond. However, there are great inter-individual differences in pain perception even in healthy adults and the determinants of pain remain poorly understood. Recent data suggest sleep disruption may be one such determinant. In women undergoing breast conserving surgery, lower sleep efficiency (i.e., frequently disrupted sleep the night before surgery) has been associated with greater pain severity and interference with daily activities in the week following surgery. (17) Similar results were found in women undergoing more invasive gynecologic surgeries. These data illustrate the intricate relationships among psychological, behavioral and physical factors. However, the biologic mechanisms underlying these relationships remain unclear. Recent work has focused on neuroendocrine-immune factors, which jointly influence central nervous system functions. Understanding these mechanisms will help identify at-risk women who can then be targeted for therapeutic interventions.

Sandra Mitchell, PhD, CRNP discussed using patient-reported outcomes (PROs) to measure therapeutic response and toxicities in clinical trials. PROs provide important insight into treatment efficacy, but to date have been underutilized. Many issues are involved in selecting an appropriate PRO instrument, including understanding the purpose of the measurement, assessing the correspondence between the instrument domain and scientific questions, and having clear scoring guidelines. Practical aspects must be considered, too, such as respondent burden, which can negatively impact results. The ability of current PRO systems to detect cancer treatment effects and their sensitivity to organ-specific issues remain unknown. Many challenges remain in incorporating PRO into gynecologic cancer trials, including measuring the value of the PRO instrument in the clinical trial setting, understanding when and how to incorporate a PRO system into trials, how to correlate PRO with diverse clinical and biomedical endpoints, how to handle missing data, developing methodologies to define responders and analyze results over time, understanding the clinical significance of changes in PRO over time, and how to report results in conjunction with other scientific findings. (18)

Heidi Donovan, PhD, RN presented data on multi-symptom management. Women with ovarian cancer report 10–14 concurrent symptoms during treatment. Identifying and prioritizing symptoms as well as providing clinicians and women with both medical and self-care strategies remain areas for further research. Effective symptom management poses many challenges because some symptoms, such as fatigue, have no efficacious medical treatment, while the treatment of some symptoms often causes or exacerbates others.

Symptom management is an ongoing part of care for women with ovarian cancer and may continue after active treatment ends. Thus, symptom management requires effective patient-clinician communication as well as substantial self-management on the part of women. Many trials exist to investigate symptom management, from those investigating a single method to address a single symptom to those investigating multiple methods to address multiple symptoms. Translating the findings on symptom management from the research setting to the clinic is an area for future investigation. The substantial self-management component for symptom management raises questions about its consequences on women with advanced disease. Identifying ways to support women in symptom management as well as to overcome barriers to self-management represent fruitful research directions.

Diane C. Bodurka, MD discussed cancer survivorship.(19) The number of cancer survivors, especially long term survivors, continues to grow. Many factors influence the health care and other needs of this growing population, including disease site and treatment, age at and time since diagnosis, comorbidities, lifestyle and behavioral factors and social support. Health issues affecting survivors, both pre-diagnosis issues and those resulting from treatment, are also not well understood. Such factors include fatigue, sexual dysfunction, sleep disturbance, neurological issues, urinary complaints and bowel complaints. Different treatments and combination of treatments impart different risk for these health effects and are experienced at all points on the survivorship spectrum. Interventions to mitigate these treatment-related effects throughout the entire survivorship period are needed. As well, educating both patients and primary care clinicians on managing short- and long-term survivor care is an important but currently unmet need.

New Directions in Therapeutics

Cancer stem cells are self-renewing cells capable of initiating tumorigenesis, recurrence and metastasis. **Patricia K. Donahoe, MD** proposed that at diagnosis, ovarian cancers have both stem and non-stem cell populations which must be differentially treated in order to ensure both cell populations are effectively targeted. An ovarian cancer stem cell-enriched population marked by three markers conserved across primary cancers and normal Fallopian tube fimbria (CD44, CD24 and Epcam) and by negative selection for Ecadherin has been identified. (20) These cells comprise less than 1% of cancer cells, have increased colony formation and shorter tumor-free intervals *in vivo*. Moreover, they are resistant to but stimulated by standard chemotherapeutic agents. They are inhibited by Mullerian inhibiting substance (MIS). These data support the use of combination of markers to develop targeted “tumor stem cell therapies” individualized to the specific tumor-initiating population identified in a lesion. Further work is needed to identify and understand mechanistic differences between different putative stem cell populations that could serve as therapeutic targets. Understanding the mechanisms underlying stem cell self-renewal or differentiation can also shed light on how these cells contribute to chemoresistence and whether modulation of these mechanisms can impact patient outcome.

Kunle Odunsi, MD, PhD discussed vaccine development. An effective immunotherapy will generate a robust, clonal expansion of T-cells that can differentiate into both effector cells with capacity to kill tumor targets and memory cells with capacity for recall response. Identifying targets for immune recognition is the first step in vaccine development. NY-ESO-1, a tumor-specific antigen, is one such target. Vaccination with NY-ESO-1 epitope induces integrated humoral CD4+ and CD8+ T cell response with the capacity to recognize tumor targets. (21) However, as the time from vaccination increases, functional immune response decreases. Thus, while the vaccine generates substantial effector T-cells, it does not generate a high frequency of memory T-cells. Understanding the mechanisms underlying this phenomenon as well as identifying agents that can influence the type of T-cells

generated are critical in designing vaccines with durable protection. mTOR blockade may be one possibility. In *In vitro* and animal studies, mTOR blockade influences T-cell differentiation towards memory cells, suggesting that including an agent that blunts mTOR may improve vaccine efficacy. However, even when an effective long-lived functional T-cell response is generated, most subjects will relapse. Improved understanding of how tumors escape immune attack is needed.

Mechanisms underlying immune system escape and exploiting those mechanisms to enhance vaccine efficacy was addressed by **Pawel Kalinksi, MD, PhD**. One way tumors escape the immune system is by creating a highly immunosuppressive environment through both the production of MDSCs and the suppression of type-1 immune effector cells. Local production of prostaglandin E_2 (PGE2) and COX2 appear to play a role. A COX2-PGE2 positive feedback loop controls CXCR4/CXCL12-guided accumulation of MDSCs as well as induction and stability of the immunosuppressive MDSC phenotype and function. PGE2 and COX2 also suppress induction and function of type-1 effector cells (Teff) and selectively inhibit production of Teff-attracting chemokines. Disrupting this feedback loop by suppressing COX2 restores local immunosurveillance *in vitro*. These findings have important implications for vaccine development. Cancer vaccine adjuvants can amplify PGE2-driven suppressive events when used alone. However, when combined with COX2 inhibitors, they induce Teff-attracting chemokines and also suppress MDSC and Treg attracting chemokines in tumor but not marginal tissue (22). This suggests that conditioning the tumor microenvironment by disrupting the PGE2-COX2 feedback loop prior to vaccination may enhance vaccine efficacy. Studies to test that hypothesis are needed.

William Zamboni, PhD presented data on the translational development of nanoparticles for drug delivery. Relative to non-encapsulated drugs, nanoparticle encapsulated drugs can have prolonged circulation and may selectively accumulate in different organs and blood components. They may also have different cellular distributions. Moreover, the size and surface properties of the encapsulating nanomaterial ("carrier") may lead to greater accumulation in tumors as a result of the enhanced permeability and retention (EPR) effect. Together these data suggest that nanoparticle encapsulation can alter the pharmacokinetics and distribution of a drug in ways that can improve drug efficacy while reducing toxicity. (23) However, recent studies suggest nanoparticle encapsulation results in greater pharmacokinetic variability – the individual rate of clearance of an encapsulated drug is highly variable between patients. Some agents can have 10 to 100 times the variability of their non-encapsulated counterparts. The mechanisms underlying this variability are poorly understood, although emerging data suggest that it may be due to the ability of cells to recognize the carrier and activate the drug, coupled with the effect of drug activation on those cells. A greater understanding of these mechanisms and their effects on toxicity and efficacy is needed.

David G. Huntsman, MD discussed how current genomic work can lead to new opportunities for cancer control. Advances in cellular and molecular biology confirm that "ovarian cancer" is not a single disease but rather a group of molecularly- and etiologically-distinct diseases that share an anatomical location. Prevention and treatment efforts must therefore exploit subtype-specific precursor lesions and tumor biology. Conducting subtype-specific clinical trials is challenging because of the sparse number of cases for subtypes of an already rare cancer. One possibility is to group cancers not by their site of origin and clinical presentation but by their molecular characteristics. Thus, clear cell and endometrioid ovarian cancers could be grouped with a subset of uterine cancers with which they share similar molecular profiles. However, successfully targeting a tumor-specific mutation in one cancer does not necessarily mean the same approach will be effective in another cancer expressing the same mutation: BRAF inhibition is highly effective in treating melanoma but

shows limited response in colon cancers harboring the same oncogenic lesion. (24) Understanding other factors that influence targeted therapeutics within a specific molecular context will be key to implementing this new paradigm in clinical trials.

Michael V. Seiden, MD, PhD presented data on the success of several therapies targeted at molecular alterations in other cancers. In almost all cases, these therapies targeted an activated oncogene (eg, EGFR, HER-2). Yet when these molecularly-targeted therapies have been tested in ovarian cancer, they have failed to show significant efficacy in progression-free survival or in clinical response. Data from TCGA provide insight: within HGSOC there is a great deal of genomic instability/variability with no single oncogene or group of oncogenes to target. (25) However, most HGSOC have lost at least three tumor suppressor genes (p53, OPCML, BRCA1/2) early in carcinogenesis. Mutated tumor suppressor genes cannot be directly targeted for reversal of function and the large number of pathways each affects makes targeting all subsequently activated genes prohibitive. Together, these observations raise the question of whether personalized, targeted, molecular therapies can really be developed in ovarian cancer and if so, what resources will be needed to accomplish that goal. Therapies targeting the tumor microenvironment and stem cells may prove more effective for ovarian cancer.

Conclusion

Technological advances in the last decade have increased our knowledge of the molecular mechanisms involved in a host of biological activities related to normal ovarian function as well as to ovarian cancer development. The advantages of applying molecular approaches and supporting technologies to ovarian cancer prevention and early detection are many. The most effective way to prevent any disease is to understand its underlying cause and change the conditions that permit it to occur. Identifying the precise molecular and biologic steps that characterize pre-malignant change will provide the foundation for the search to find agents that reverse these changes or block the steps critical to the full development of cancer. Similar steps can also be used to detect and prevent disease recurrence. Moreover, a precise characterization and understanding of the mechanisms involved in cancer initiation and progression will help lead to the development of prevention and treatment modalities that can be personalized to each patient, thereby helping to overcome this highly-fatal malignancy.

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Appendix 1: Ovarian Cancer Academy Abstracts

The Ovarian Cancer Academy, a virtual career development and research training platform, convened for its first inaugural meeting on May 9, 2012. These highly committed early-career investigators, their mentors, and the Academy Dean have been busy collaborating and networking as they work toward eliminating ovarian cancer and becoming the next generation of leaders in ovarian cancer research. Cumulatively, they have published more than 65 ovarian cancer research articles, presented at national conferences, filed patents, mentored trainees, and served on editorial boards as well as peer review panels. The DOD OCRP is proud of their accomplishments as they continue to make strides in their fields.

Martina Bazzaro, University of Minnesota, is targeting ubiquitin-mediated protein degradation pathways for ovarian cancer treatment. She is focused on diagnostic and prognostic markers, targeted therapy, and the inhibitors' potential mechanisms of anti-cancer activity. Dr. Bazzaro has found deubiquitinating enzymes (DUBs) involved in ovarian cancer cells, and she is testing probes to specifically target those DUBs and cells that overexpress them. Targeting the DUBs may lead to a new treatment for ovarian cancer in the future.

Jeremy Chien, University of Kansas, is investigating integrative functional genomics and proteomics to uncover mechanisms of chemotherapy resistance in ovarian cancer. Using the TCGA (The Cancer Genome Atlas) data, he has found a list of genes that could be used to predict prognosis of ovarian cancer. Dr. Chien observed a difference between tumors that express high and low levels of the gene FBN1, and while it may have no role in primary resistance, it may have a role in platinum-sensitive recurrence and acquired resistance. He

also proposed a new model of chemotherapy resistance that builds on other existing models. Results from Dr. Chien's work may be able to help improve patients' responses to chemotherapy.

Panagiotis Konstantinopoulos, Harvard Medical School, is working toward developing a biomarker for "BRCAness," a phenotype that is characterized by responsiveness to platinum and PARP (poly ADP ribose polymerase) inhibitors and improved survival in patients. He is investigating the association of the BRCAness gene expression profile with clinical outcome and molecular aberrations underlying defective homologous recombination in high-grade, advanced stage EOCs in the TCGA dataset. Dr. Konstantinopoulos found that the BRCAness profile is associated with certain defects (e.g., events involving BRCA 1/2 genes and deletions of homozygous PTEN) in homologous recombination DNA repair. Additionally, he found that patients with BRCA-like tumors had improved overall survival when compared to patients with non-BRCA-like tumors. Efforts are under way to improve the predictiveness of the BRCAness profile.

Charles Landen, University of Alabama at Birmingham, is looking at the emerging aspect of cancer stem cells (i.e., tumor-initiating cells). His research is supportive of the hypothesis that subpopulations within heterogeneous ovarian tumors contribute to chemoresistance. Dr. Landen has shown that markers in ovarian cancer cells, "side population," CD44, CD133, and aldehyde dehydrogenase (ALDH1), have cancer stem cell-like properties. Additionally, results from an analysis of primary and recurrent tumors showed chemoresistant tumors were enriched in CD133 and ALDH1-positive cells although they were not the only ones in the chemoresistant population. His findings could contribute important targets for chemotherapy and overcoming chemoresistance in addition to helping build better models to understand tumor heterogeneity.

Kathryn Terry, Harvard Medical School, is focused on understanding risk factors by etiologic pathway. Based on measurements from pathology reports, she and her colleagues have classified more than 1,700 cases from the New England Case Control Study and the Nurses' Health Study into dominant tumors (likely of ovarian origin) defined as those restricted to one side or with one side that is two times greater than the other and nondominant (likely tubal origin). They have found that dominant tumors are more strongly associated with multiparity, tubal ligation, and endometriosis, whereas nondominant tumors are more strongly associated with a family history of ovarian cancer and genetic variation in a telomere-associated protein, TERT. Results from Dr. Terry's work provide a better understanding of ovarian cancer risk factors, which is important for prevention.

Anda Vlad, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, is working on preclinical modeling of ovarian cancer for vaccine development—she is using genetically engineered mice with conditional mutations in the Kras and/or Pten pathways for the preclinical modeling of gynecologic malignancies. She is focusing on the well-defined tumor antigen, MUC1, as an oncoprotein/vaccine candidate because it is overexpressed in more than 80% of EOCs. Dr. Vlad has found that MUC1 expression in her transgenic mice mirrors human MUC1 tissue distribution. She is testing MUC1 as a vaccine candidate in the transgenic mice and demonstrated that the vaccine significantly prolonged survival in vaccinated mice with ovarian tumors as well as restored immune surveillance. Additional results from her research indicate that MUC1 influences ovarian cancer pathogenesis and will be a valuable target for immune therapy.

Rugang Zhang, Wistar Institute, is exploring how canonical Wnt signaling activated by loss of Wnt5a contributes to EOC development through overcoming senescence, a state of irreversible cell growth arrest. He has found that Wnt5a is expressed at lower levels in

primary EOCs, and the loss of Wnt5a correlates with a high cell proliferation index. It was a poor prognosis biomarker in EOC as well. Dr. Zhang observed that Wnt5a suppressed the human EOC cell growth in vitro and in an orthotopic mouse model. Reconstituting Wnt5a induced senescence in EOC cells too. Based on his results, he concluded that targeting Wnt signaling is a novel strategy for causing EOC cells to undergo senescence, which may be a possible mechanism for ovarian cancer therapeutics.

Appendix 2: Selected Abstracts

Molecular targeted ultrasound imaging of αvβ3-integrins expressing microvessels in association with anti-NMP antibodies detects ovarian cancer at early stage

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Background: Changes in nuclear morphology including nuclear matrix proteins (NMPs) followed by tumor associated neoangiogenesis (TAN) are the two earlier events in malignant transformation and progression of ovarian cancer (OVCA). Anti-NMP antibodies are produced in response to NMPs shed during malignant transformation. Expression of avb3-integrins by TAN vessels is one of the features of ovarian TAN. Anti-NMP antibodies and ovarian TAN may represent markers of early stage OVCA. Identification of patients at early stage OVCA is very difficult and laying hens have been shown to develop spontaneous OVCA with histopathology similar to humans. The goal of this study was to examine the feasibility of αvβ3-integrin targeted transvaginal ultrasound (TVUS) imaging and anti-NMP antibodies in detecting OVCA at early stage.

Methods: Hens with (n= 50) or without (n= 20) serum anti-NMP antibodies and without any TVUS detectable ovarian abnormality were selected for prospective monitoring by avb3-integrins targeted TVUS at 15 weeks interval up to 45 weeks. Hens were scanned before, during and after injection of targeted microbubbles at each interval. Pre- and post-injection images archived and analyzed off-line. Hens were euthanized at diagnosis for OVCA or at the end of the 45 weeks. Gross diagnosis was recorded at euthanasia. Serum and tissues were processed for histology, immunohistochemistry (SMA and αvβ3 integrins) and immunoblotting.

Results: Within 30–45 weeks of monitoring, 7 hens with serum anti-NMP antibodies developed OVCA spontaneously. αvβ3-integrins targeted microbubles enhanced the visualization of ovarian tumors remarkably. Targeted microbubbles bounded areas appeared as a ring on the ovarian surfaces of 5 hens on TVUS and suspected for OVCA. Tumors were confirmed in all hens predicted to have OVCA by targeted TVUS at euthanasia. In 4 hens, tumors were limited to the ovaries (early stage) and in one hen the tumor metastasized to abdominal cavity. Targeted TVUS imaging could not detect two hens with microscopic lesions without any solid tumor mass. Thus 7 of 50 hens had OVCA and targeted microbubbles detected approximately 72% (5 of 7 hens). Serum reacted against NMP antigens of various molecular wt from malignant ovaries in immunoblotting. The frequency of TAN vessels (SMA and αvβ3-integrins expressing) were significantly higher in OVCA hens than normal hens (P<0.05).

Conclusions: Targeted imaging enhanced TVUS detection of early stage OVCA. Anti-NMP antibodies together with targeted imaging may constitute an early detection test for OVCA. The results may form the foundation for a clinical study.

Support: Department of Defense (#09-3303), Sramek Foundation

Ovarian Cancer Survivors' Experiences of Self-Advocacy: A Focus Group Study

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Background: Women with ovarian cancer experience a multitude of co-occurring, severe, and distressing symptoms that directly impact their quality of life. Optimal symptom management depends on patients' ability to negotiate the health care system, communicate with health care providers, and manage complex medical and self-care strategies. The concept of self-advocacy is frequently promoted within cancer survivorship research and policy as a key factor ensuring patient participation and engagement in their care. However, a well-defined understanding of this phenomenon has yet to be established. The purpose of this qualitative, descriptive study is to explore the concept of self-advocacy from the lived experience of ovarian cancer survivors.

Methods: Participants were recruited through the WRITE Symptoms Study (GOG-0259) and Pittsburgh's National Ovarian Cancer Coalition (NOCC). Five focus group sessions comprised of 2 to 4 women with ovarian cancer were conducted (n=14). Verbatim transcriptions were systematically analyzed by 2 trained, independent researchers using a constant comparison method with axial coding. Each focus group was analyzed separately and then successively integrated together in order to uncover pervasive and meaningful themes and sub-themes. All participants were given the opportunity to validate the findings.

Results: Common themes, sub-themes, and exemplar quotations of self-advocacy and symptom management will be presented followed by a complete and succinct description of the phenomenon. Major themes include "learning how to live with my symptoms", "knowing how to manage my symptoms, and "overcoming obstacles". Complexities of self-advocating include "fighting against death while maintaining power", "leveraging personal strengths and working with others", and "trusting and being frustrated with your healthcare team". Self-advocacy was defined by these women as a strong will that activates survivors to stand up for themselves and address personally-meaningful obstacles to symptom management.

Conclusions: Exploring and describing the process of self-advocacy among cancer patients can provide needed insight into how patients engage in the symptom management process and participate in their healthcare in personally meaningful ways. A deepened understanding of self-advocacy can lead to improved clinical support for and research approaches to improving patient-centered care in addition to providing potential interventions for women with ovarian cancer.

Sex-steroid hormones and epithelial ovarian cancer: a nested case-control study.

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Background: The association of most established risk factors for ovarian cancer could be mediated by alterations in sex-steroid hormones concentrations. However, the results from the few epidemiological studies directly relating pre-diagnostic sex-steroids levels to risk of ovarian cancer so far have been unremarkable.

Methods: A case-control study was nested in the Finnish Maternity Cohort, which collects first trimester serum samples from all pregnant women in the country since 1983. For the current analyses, 257 invasive (114 serous, 79 mucinous, 42 endometrioid and clear cell) and 184 borderline (91 serous and 92 mucinous) epithelial ovarian cancers diagnosed by the end of 2007 among cohort members after recruitment were selected. Eligible samples were those from the last pregnancy preceding cancer diagnosis that led to the delivery of a singleton offspring. Three controls individually matching each case for age, parity and date at sample donation were selected. Progesterone, 17 hydroxyprogesterone (17-OHP), androgens (testosterone and androstenedione) and estradiol were measured by high-performance liquid chromatography tandem mass spectrometry. Odds ratios (OR) and 95% confidence intervals for doubling of hormone concentrations were estimated by conditional logistic regression.

Results: Doubling of androgen concentrations were associated with about 30% greater risk of ovarian cancer [OR 1.34 (1.13–1.58), p<0.0007 for testosterone and 1.36 (1.14–1.63), p=0.0006 for androstenedione], with similar risk estimates in analyses stratified by tumor invasiveness. There was no association of ovarian cancer (overall, invasive or borderline) with circulating concentrations of progesterone, 17-OHP and estradiol. However, these seemingly consistent overall associations differed substantially by tumor histology and invasiveness. For example, the associations of androgens were evident in all subgroups but serous invasive tumors (risk estimates of similar magnitude, but not all statistically significant), progesterone was significantly positively associated with invasive mucinous tumors only [OR 1.88 (1.03–3.44)] and the strongest association of estrogens was observed with endometrioid and clear cell tumors [OR 1.52 (0.91–2.54), p=0.11]. Switching perspective, the most consistent picture was observed for endometrioid and clear cell tumors with an indication for an inverse association with progesterone and 17-OHP and a direct one with androgens and estradiol. However the number of endometrioid and clear cell tumors was small and risk estimates reached borderline significance at most.

Conclusions: The study provides further evidence that sex-steroid hormones are involved in ovarian cancer pathogenesis and that the associations may differ by histological subtype and invasiveness of the tumors. An extension of the study is on-going.

Potential effect of the Risk of Ovarian Cancer Algorithm (ROCA) on the mortality outcome of the Prostate, Lung, Colorectal and Ovarian (PLCO) Trial.

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Background: Recently, the ovarian component of The Prostate, Lung, Colorectal and Ovarian (PLCO) Trial reported no mortality benefit of annual screening with CA-125 and trans-vaginal ultrasound (TVU) versus usual care. Currently ongoing is the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), a three-armed trial where one arm utilizes the Risk of Ovarian Cancer Algorithm (ROCA). In contrast to PLCO, which defined a positive CA-125 test based on the current CA-125 level only, ROCA considers serial CA-125 levels in assigning ovarian cancer risk probabilities of low, intermediate or high. The unfavorable stage distribution in PLCO of CA-125 detected cancers (85% stage III–IV) gives rise to the speculation that the CA-125 cutoff (35 IU/ml) is too high and catches cancers too late. A serial CA-125 algorithm may be able to catch cancers sooner, but without engendering too high a false positive rate, by considering CA-125 levels over time. A high false positive rate is problematic due to the frequent subsequent use of oophorectomy.

Methods: We investigate whether use of ROCA in PLCO could have potentially favorably affected the trial's outcome. Specifically, we utilized observed PLCO CA-125 values to calculate a ROCA score at each screen and analyzed how many women would have had their tumor detected earlier (or later) using ROCA than they did under the standard PLCO protocol (CA-125 ≥ 35 U/ml and/or positive TVU). Under a "best-case" scenario, any women dying of ovarian cancer who would have had her cancer detected earlier with ROCA is considered "saved" under ROCA.

Results: Updated PLCO data shows 132 ovarian cancer deaths in the screened versus 119 in the control arm (RR=1.11). Of the 132, 81 were "in play" to be detectable by ROCA, as defined by diagnosis within 3 years of a CA-125 screen. For ROCA cutoffs that classified 14% of all PLCO screens as intermediate and 3% as high risk, 25 of the above 81 women would have had their cancer detected earlier with ROCA screening. This gives, under a best-case" assumption, 107 screened arm ovarian cancer deaths and a RR of 0.90 (95% CI: 0.69–1.17).

Conclusion: Having utilized ROCA in PLCO likely would not have resulted in a significant mortality reduction for the screened arm, although it may have prevented some ovarian cancer deaths in that arm.

Body Mass Index and Risk of Epithelial Ovarian Cancer: The HOPE Study

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Objective: Studies examining the association between body mass index (BMI) and risk of epithelial ovarian, fallopian tube and peritoneal cancer (EOC) have been inconsistent. Notably, these studies use recent weight and height in examining the BMI-ovarian cancer

link. A potential explanation for the lack of association is that BMI in early adult life may play a role. As well, hormonal exposures throughout the life course, such as use of oral contraceptives (OCs), parity, breast feeding and use of hormone replacement therapy (HRT) may influence the BMI-EOC relationship. The purpose of this study is to examine the relationship between BMI and risk of EOC at multiple ages and in light of life course hormonal exposures.

Methods: Self-reported height and weight at ages 18 and at 9 months before interview were used to assess BMI in 902 incident EOC cases and 1802 community controls participating in the Hormones and Ovarian cancer PrEdiction (HOPE) Study, a population-based, case-control study of epithelial ovarian, peritoneal and fallopian tube cancers undertaken in the contiguous regions of western Pennsylvania, eastern Ohio and western New York between 2003 and 2008. Multivariable, unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) adjusting for age, race, education, oral contraceptive use, parity, breast feeding, tubal ligation, hysterectomy, talc use and family history of breast or ovarian cancer. Cross-product terms were included in the regression models to assess differences in any observed relationships based on ever use of OCs, ever use of HRT, ever breast fed and ever parous (independent analyses).

Results: BMI at age 18 was significantly associated with EOC (adjusted- OR=1.06; 95% CI=1.01–1.12). This relationship remained unchanged when adjusting for current BMI. In contrast, current BMI was not associated with EOC (adjusted-OR=1.00, 95%CI=0.98–1.01). Stratified analyses showed a significant interaction with HRT use. BMI at age 18 was significantly associated with EOC in never (OR=1.08; 95%CI=1.01–1.15) but not ever (OR=0.95; 95%CI=0.83–1.08) users of HRT (p for interaction < 0.008). No other hormonal-BMI interactions were found.

Conclusions: Early adult life BMI is significantly associated with ovarian cancer risk and this association is modified by HRT use. Given the relationship between increasing BMI and increasing levels of circulating endogenous estrogens, which is altered by HRT use, our findings suggest that endogenous estrogen exposure may play a role in EOC risk. Moreover, because early-life BMI is a modifiable factor and in light of the growing epidemic of childhood obesity, these data suggest that interventions to reduce obesity in young girls may prove fruitful in reducing their risk of EOC.

Monoclonal antibody-based immunotherapy of ovarian cancer: targeting of differentiated and cancer initiating cells with the B7-H3-specific mAb 376.96 and sunitinib

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Background: The high rate of relapse following surgical debulking and adjuvant chemotherapy in advanced ovarian cancer likely reflects the chemoresistance of cancer initiating cells (CICs) which play a crucial role in disease recurrence. This possibility has prompted us to develop therapy to target not only differentiated ovarian cancer cells, but also CICs. To this end, we combine the monoclonal antibody (mAb) 376.96, which recognizes a B7-H3 epitope with selective expression on malignant cells including ovarian

carcinoma cells, with the tyrosine kinase inhibitor sunitinib. We show that this combination targets not only differentiated ovarian cancer cells but also CICs. In addition we show that the mAb 376.96 is amenable to an intraperitoneal delivery method.

Methods: Eight ovarian cancer cell lines including 2 chemoresistant cell lines A2780.cp20 and SKOV3ip2.TR were stained with mAb376.96 and analyzed by flow cytometry to establish expression. In vitro studies to assess the effect of mAb376.96 ± chemotherapy ± Sunitinib were performed on chemosensitive (SKOV3.ip1) and chemoresistant (SKOV3ip2.TR and A2780.cp20) cell lines. The effect of mAb376.96 on CICs was evaluated via analysis of aldehyde dehydrogenase (ALDHbright) activity using an ALDEFLUOR kit. Cells isolated from an ovarian cancer patient were IP injected into immunodeficient e mice and radiolabeled mAb376.96 (99mTc-376.96) was injected IP and localization of antibody was assessed after 24 hours.

Results: The B7-H3 epitope recognized by mAb 376.96 is expressed by both chemosensitive and chemoresistant ovarian cancer cell lines. In vitro treatment of A2780.cp20 and SKOV3ip2.TR cells with single agent mAb376.96 - revealed cell growth inhibition of 30% and 45%, respectively. Combination treatment of SKOV3.ip1 cells with Sunitinib - and mAb376.96 - resulted in 28% cell growth inhibition vs. 10% inhibition with Sunitinib alone. Analysis of CICs revealed that treatment with Sunitinib - and mAb376.96 - reduced the proportion of CICs by one-third as compared with untreated cells. In vivo studies showed that 24 hours following IP dosing, 99mTc-376.96 showed higher uptake in tumors grown in mice (n=3) following IP injection of human ovarian cancer pleural fluid, as compared to 99mTc-labeled isotype control mAb (3 tumors had values of 15.1, 24.1 and 11.8% ID/g, vs. 4.3±1.4% ID/g in isotype control). Additionally, 99mTc-376.96 was retained in the tumors in peritoneal cavity.

Conclusion: In vitro studies using mAb376.96 showed an inhibitory effect against chemosensitive and chemoresistant ovarian cancer cells alone and in combination with Sunitinib. Importantly, the combination treatment inhibits CICs. Intraperitoneal 212Pb-376.96 is a feasible method for locoregional treatment delivery, which is of particular interest in the treatment of ovarian cancer.

Meso-TR3: A Novel TRAIL-Based Targeted Therapeutic in Ovarian Cancer

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Background: Chemoresistance limits the utility of cytotoxic agents in the treatment of ovarian cancer, as more than half of ovarian tumors eventually acquire inactivating p53 mutations. TNF-related apoptosis- inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) superfamily. It kills cancer cells via the extrinsinc death pathway independent of p53, thus offering a complementary approach to conventional cancer therapy. We have developed a novel TRAIL form, designated TR3, which represents a fusion protein of three consecutive TRAIL ectodomains that is generically extensible with stoichiometric control and improved stability. It has been shown that tethering TRAIL (TR3) to the surface of cancer cells enhances cell killing. Ovarian cancer cells have been described to express MUC16, which shows high-affinity interaction with mesothelin protein. Therefore, we reasoned that modifying the TR3 drug platform by attaching the mesothelin

protein sequence to the N-terminus of TR3 (generating Meso-TR3) would enhance target cell killing compared to its non-targeted TR3 counterpart.

Methods: Human embryonic kidney cells [HEK293T] were used for the production of soluble mesothelin, Meso-TR3, and TR3. Fluorescence activated cell sorter (FACS) analysis was used to determine the expression of MUC16 on different cancer cell lines (OVCAR-3, Jurkat-H, and HeLa) and the binding of mesothelin to MUC16-positive cells. The killing activity of Meso-TR3 and TR3 was compared using a luminescence-based cell viability assay (Cell-TiterGlo).

Results: While MUC16 was strongly expressed in OVCAR-3 cells (100% positive), it was completely absent in Jurkat cells (our reporter cells to determine antigen-independent killing activities of Meso-TR3 and TR3). Using soluble mesothelin alone, we were able to demonstrate strong binding to MUC16 on OVCAR-3 cells. Furthermore, at concentrations where Meso-TR3 and TR3 killed the same number of MUC16-deficient Jurkat reporter cells, significantly greater killing was seen with Meso-TR3 in the MUC16-positive OVCAR-3 cells. Finally, to obtain evidence for the targeted killing capacity of Meso-TR3, we challenged HeLa cells (a native mix of MUC16+ [80%] and MUC16- cells [20%]), where treatment with Meso-TR3 resulted in a selective reduction of the MUC16+ population to 54% (33% reduction), whereas TR3 alone did not change this ratio.

Conclusions: Meso-TR3, the novel tumor-targeted TRAIL, enhances TRAIL-mediated killing in MUC16 positive ovarian cancer via selective drug delivery to the tumor marker. The ability to target TRAIL to a cell surface protein via a native ligand/receptor interaction presents a unique opportunity to create a cancer selective drug with fewer off-site toxicities and enhanced killing capacities.

Enhancement of the COX2-PGE2 axis by activated lymphocytes promotes the activity of myeloid-derived suppressor cells in ovarian cancer patients

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Background: Myeloid-derived suppressor cells (MDSCs) are crucial contributors to tumor environment-associated immune suppression, representing a key mechanism for tumor progression and a significant barrier to effective immunotherapy.

Methods: We demonstrate that the development, accumulation, and persistent suppressive functions of CD11b+CD14+CD33+CD34+CXCR4+ MDSCs in the tumor ascites of ovarian cancer patients critically depend on the positive feedback between the tumor-associated inflammatory mediator prostaglandin E2 (PGE2) and cyclooxygenase 2 (COX2), the key regulator of PGE2 synthesis. We further demonstrate that activated T cells and NK cells enhance MDSC activity within the ovarian cancer environment through IFNγ-dependent activation of the COX2-PGE2 axis. Inhibition of the COX2-PGE2 axis was capable of reversing MDSC-associated immune suppression and the hyper-activation of MDSCs by activated T cells and NK cells.

Results: These data reveal the central importance of COX2-PGE2 feedback in supporting MDSCs within the human ovarian cancer environment, and provide strong rationale for the therapeutic targeting of PGE2 signaling in promoting spontaneous and therapy-induced immune responses in ovarian cancer patients.

A genetically engineered mouse model for high grade serous “ovarian” carcinoma arising in the fallopian tube.

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Background: Ovarian cancer is the most lethal gynecologic malignancy because the vast majority of cases are detected in late stage, a finding that has thwarted attempts to understand the pathogenesis and cell-of-origin of this disease. The traditional view of epithelial ovarian pathogenesis asserts that all tumor subtypes share a common origin in the ovarian surface epithelium (OSE). There is robust data to support the OSE as the site of origin for many ovarian tumors, including low-grade carcinomas and borderline tumors.

However, the pathogenesis of high-grade serous ovarian carcinoma, the most common type of ovarian cancer, continued to defy explanation by the OSE model. More recent studies suggested that the fallopian tube epithelium, rather than the OSE, may be the site-of-origin for a majority of pelvic serous carcinomas (PSC, defined as ovarian, peritoneal and tubal high grade serous carcinomas).

Methods: We show here that the fallopian tube epithelium can be site of origin for PSC by genetically engineering a mouse model that specifically targets the fallopian tube secretory cell with defined genetic alterations that are characteristic of human PSC. These mice developed tubal intraepithelial serous carcinomas, a precursor to PSC, that are morphologically and immunophenotypically similar to the lesions described in human patients.

Results: Furthermore, these intraepithelial lesions progress to widespread peritoneal disease that recapitulates the presentation of high-grade PSC in women. The tumors express common serous markers such as P53, PAX8 and WT1. Tumor bearing mice show high levels of the best characterized serum marker of ovarian cancer, CA-125.

Taken together our model is the first genetically engineered mouse model that truly recapitulates human serous carcinoma by means of pathogenesis, clinical characteristics, immunophenotype and serum biomarkers. Our model serves as proof-of-concept that the fallopian tube epithelium can be site-of-origin to PSC.

An optimized primary ovarian cancer xenograft model mimics patient tumor biology and heterogeneity.

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Background: Current xenograft and transgenic models of ovarian cancer are predominantly homogeneous and inadequately predict response to therapy in clinical trials. Use of patient tumors may represent a better model for tumor biology and offer potential to test personalized medicine approaches, but poor take rates and questions of recapitulation of patient tumors have limited this approach. We have developed a protocol for improved feasibility of such a model and examined its similarity to the patient tumor.

Methods: Under IRB and IACUC approval, 23 metastatic (omental) ovarian cancer samples were collected at the time of tumor reductive surgery. Samples were implanted either subcutaneously (SQ), intraperitoneally (IP), in the mammary fat pad (MFP), or in the subrenal capsule (SRC) and monitored for tumor development. Cohorts from eight xenolines were treated with combined carboplatin and paclitaxel chemotherapy or vehicle, and response to therapy was compared between xenografts and patients. Expression of tumor-initiating cell (TIC) markers ALDH1, CD133, and CD44 was assessed by immunohistochemistry in tumors from patients and treated and untreated xenografts.

Results: At least one of the SQ implanted tumors developed in 91.3% of xenografts, significantly higher than in the MFP (63.6%), IP (23.5%), or SRC (8%). Xenografts were similar in expression of putative TIC's compared to patient tumors (ALDH: 17% vs 19%, CD44: 2.4% vs 5%, CD133 10% vs 3%, p>0.05). The patients and the xenografts also have similar responses to chemotherapy in that xenografts from patients with a partial response to therapy responded more slowly than those xenografts from patients achieving a complete response (45 vs 21 days, p=.004). Interestingly, xenografts that were treated with chemotherapy were more densely composed of TICs, with ALDH1 increasing to 36.1% from 16.2% (p=0.002) and CD133 increasing to 33.8% from 16.2% (p=0.026).

Conclusions: Xenoline development can be achieved at a high rate when tumors collected from metastatic sites are implanted SQ. These xenografts are similar to patient tumors with regard to chemotherapy response and TIC expression profiles. Our model may represent a more accurate model for in vivo pre-clinical studies as compared to current models. As this xenograft model is developed directly from patient samples and the treated xenografts become enriched in chemoresistant cells, this model represents a novel mechanism to test patient-specific therapies.

Wild type TRP53 (53) promotes ovarian cancer cell survival

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Introduction: Ovarian cancers have been divided into two categories – low-grade type I and high-grade type II. One distinguishing and relevant feature of type I and type II ovarian cancer is the expression of the tumor repressor protein 53 (Trp53; or p53): almost all high-grade serous adenocarcinomas (96%) have Trp53 mutations whereas low-grade tumors express elevated levels of wild type Trp53. We have recently shown that Pten/Kras (Ptenfl/fl;KrasG12D fl/fl;Amhr2-Cre) mice exhibit many features similar to human low-grade invasive serous ovarian carcinomas, including elevated levels of wild type Trp53. To investigate the functions of TRP53 in the mutant mouse OSE cells, Trp53 was conditionally deleted in Pten/Kras mice.

Methods: Purified OSE cells were isolated from mutant and control mouse ovaries and RNA was prepared for gene profiling and Q-PCR analyses. Purified mutant and control cells were also grown in culture and matrigel to characterize the transformed phenotype of the TRP53+ (Pten/Kras (Trp53+)) and TRP53- (Pten/Kras(Trp53-)) OSE cells.

Results: In the TRP35 +cells, wild type TRP53 controls or enhances the expression of genes regulating proliferation, DNA repair and mitotic activity and markedly decreases genes with tumor suppressor functions. Rather than activating cell cycle arrest or apoptosis, wild type TRP53 in the Pten/Kras(Trp53+) OSE cells promotes the formation of papillary-like structures, cell migration, adhesion and invasion. By contrast, cells lacking Trp53 exhibit a less aggressive phenotype and gene expression profiles more like control OSE cells. Thus, we have unveiled: a novel role for wild-type TRP53 as a major promoter of ovarian cancer cell survival, differentiation and migration. These results provide a new paradigm: wild type TRP53 at low levels of activity does not preferentially induce apoptotic or senescent related genes in the Pten/Kras(Trp53+) cells. To test this paradigm, purified TPR53+ positive and TRP53- negative OSE cells were exposed to the p53 activator nutlin-3a. In TRP53+ OSE cells, nutlin-3a stimulated TRP53 activity as indicated by the rapid induction of cell cycle arrest and apoptotic genes.

Summary/Conclusion: In the Pten/Kras mutant mouse OSE cells and likely in human low grade ovarian cancer cells,TRP53 controls global, molecular changes that are dependent not only on the level of wild type TRP53 expression but also on its activation status. Low levels of TRP53 activity promote tumor survival and growth, whereas higher TRP53 activity induces cell cycle arrest and apoptosis. Thus, nutlin-3a provides a promising therapeutic for managing type I ovarian cancer and other cancers where wild-type TRP53 is expressed.