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TITLE: Early Precursor Escape and High-Grade Serous Carcinogenesis

PRINCIPAL INVESTIGATOR: Christopher P. Crum, MD

CONTRACTING ORGANIZATION: The Brigham and Women's Hospital, Inc

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project is intended to establish a novel carcinogenic pathway to extra-uterine high grade serous carcinoma (HGSC) that originates in early serous precursor lesions (ESPs) with TP53 mutations that escape the fallopian tubes and undergo malignant transformation in the peritoneal cavity to form "primary peritoneal" HGSCs. To date we have assembled paired ESPs and concurrent peritoneal HGSCs to further establish a lineage relationship between the two by sequence analysis, have collected and are currently analyzing paired ESPs and peritoneal washings to document escape of the altered cells into the peritoneal fluid, and have collected multi-site HGSCs to establish the direction of tumor spread with the goal of identifying not only the site of origin for the precursor but the site of initial malignant transfomation. The analysis is in progress to be completed in year two of the grant. If successfully characterized and confirmed, this concept will identify a new pathway to malignancy relevant to early prevention.

15. SUBJECT TERMS
Extra-uterine high grade serous carcinoma, BRCA, Serous tubal intraepithelial carcinoma, p53 signature, W

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1. **INTRODUCTION:** The subject of this grant is the origin of high-grade serous carcinoma, The purpose of this grant is to develop the hy0 that precursor cells with TP53 mutations escape from the fallopian tube into the peritoneal cavity, where they eventually undergo malignant transformation to form high-grade serous carcinomas. The research focuses on linking the precursor and malignant phases of serous carcinogenesis across space and time.

2. **KEYWORDS:** Extra-uterine high grade serous carcinoma, BRCA, Serous tubal intraepithelial carcinoma, p53 signature, Whole exome sequencing, Serous tubal intraepithelial lesion, Early serous proliferation.

3. **ACCOMPLISHMENTS:**

   - **What were the major goals of the project?** The study has three aims to address the hypothesis that early serous proliferations (ESPs) are directly responsible for subsequent intra-peritoneal high grade serous carcinomas (HGSCs). In AIM 1 both ESPs and HGSCs are analyzed by whole exome sequencing (WES) to determine if they share lineage specific biomarkers. In AIM 2, the possibility that ESPs release cells into the peritoneal fluid will be addressed. DNA from cells isolated in the peritoneal fluid will be examined for TP53 mutations and compared to ESPs from the same case. In AIM 3, WES will be used to determine the temporal relationships between ESPs, and tumor deposits on the peritoneal surface, associated STICs and circulating tumor cells.

   - **What was accomplished under these goals?** AIM 1 involved identifying cases of HGSC with tubal ESPs, isolating nucleic acids from the ESPs and the tumors, confirming that the two sites shared the same TP53 mutation, and then performing WES to strengthen the lineage relationship between the two. Ten cases with both ESP and tumor were identified. Isolated DNA was analyzed by ion torrent sequencing, but sensitivity was not sufficient to identify mutations in the ESPs, due to the small amount of material. To address this limitation, DNA was isolated in a second round and sequenced by PlexSeq diagnostics for TP53 by deep sequencing. The sequencing has been completed and is being analyzed. AIM2 involved isolating DNA from cells in peritoneal fluid and searching for TP53 mutations that could be traced to ESPs in the fallopian tube. The focus has been on fluids obtained from women with germ-line BRCA mutations undergoing risk-reduction surgery, and also including a small number of positive controls from women with HGSC. To date, samples from 40 women have been obtained and DNA isolated. In 18 the fallopian tubes were analyzed for the presence of ESPs containing TP53 mutations. In seven cases TP53 positive (putative mutation) ESPs were identified. DNAs isolated from these ESPs and corresponding fluid have been interrogated for TP53 mutations by deep sequencing. The sequencing has been completed and the data is under analysis. AIM 3 was scheduled to be addressed in the second year of the project, but cases are being identified for analysis. One in particular displays a unique tumor distribution combining a peritoneal serous carcinoma with a neuro-endocrine carcinoma of the ovaries. We hypothesize that the former is the primary site and the ovary the metastasis. Currently we are awaiting the initial DNA analysis to confirm that both tumors are directly related after which we will attempt to prove that the tumor initiated

not in the ovary but the peritoneal cavity following assessment of 447 discrete markers (OncoPanel).

We are pleased with the progress of the study in terms of specimen acquisition, characterization, nucleic acid isolation and scheduling the analyses. The only frustration has been with timing in obtaining the final results, owing to delays imposed by the COVID19 pandemic.

- **What opportunities for training and professional development has the project provided?** Dr. David Chapel, a clinical and research fellow funded partially by this project has been able to obtain additional funding to extend a component of the project to address the hypothesis that endometrial precursors could also contribute to the pathogenesis of intraperitoneal HGSC. He has obtained support from the OCRA to pursue this in the following year in addition to his activities on this grant. He has presented preliminary work at the United States Canadian Academy of Pathology.

- **How were the results disseminated to communities of interest?** The PI (Dr. Crum) presented the concepts in this work in a keynote presentation (the Maude Abbott Lecture) at the United States Canadian Academy of Pathology in Los Angeles in March 2020.

- **What do you plan to do during the next reporting period to accomplish the goals?** *In* the second and last year of the grant we will complete the current data analyses described above and continue to address the AIMs as follows: For AIM1, we will employ laser capture microdissection to isolate RNA from a select group of cases in which ESPs and HGSCs share identical TP53 mutations and analyze by WES to establish lineage identity. In AIM2, we will confirm the existence of common TP53 mutations between ESPs and peritoneal fluid samples. We will then refine this link by selectively capturing epithelial cells from the fluids and proving that the mutations are epithelial cell specific, an important step in proving that the mutations are not occurring in inflammatory or mesothelial cells. In AIM3, we will complete the genetic analysis of the cases described above and select an additional 4 cases to compare peritoneal and ovarian tumor cells by genetic panel analysis to prove the directionality of tumor spread.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?** The data is under analysis but the questions is critical, which is whether high grade serous carcinogenesis always goes to completion in the tube prior to spread, or initiates in the tube and completes in the peritoneal cavity. This has important implications for expectations from prophylactic salpingectomy as well as strategies to intercept the process of "unsuspected" carcinogenesis in the peritoneal cavity of currently healthy women.

- **What was the impact on other disciplines?** Nothing to report.

- **What was the impact on technology transfer?** Nothing to Report

- **What was the impact on society beyond science and technology?** The major impact would be on expectations of women who wish to lower their risk of HGSC by opportunistic or prophylactic surgery.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**. No changes are anticipated at this time.
- **Actual or anticipated problems or delays and actions or plans to resolve them**. The major delays have been related to the COVID19 impact on turn-round time for analyses. We anticipate that this will be less of a problem going forward.
- **Changes that had a significant impact on expenditures**. Nothing to report.
- **Significant changes in use or care of human subjects.** Nothing to report.
- **Significant changes in use or care of vertebrate animals.** Nothing to report.
- **Significant changes in use of biohazards and/or select agents.** Nothing to report.
- **PRODUCTS:**
- **Publications, conference papers, and presentations**
  - **Journal publications.** Nothing to report
  - **Books or other non-periodical, one-time publications.** Nothing to report
  - **Other publications, conference papers, and presentations.** Four abstracts were presented at the United States Canadian Academy of Pathology in Los Angeles, March 2020.

    1. Chapel DB, Robinson C, Goebel EA, Soong TR, Kolin D, Crum CP. Landscape of Putative Precursors to High Grade Serous Carcinoma (HGSC) in the Female Genital Tract (Poster).

    2. DaSilva AF, Crum CP, Kolin D. Primary Peritoneal High Grade Serous Carcinoma Revisited: Precursor Frequency and Implications (Poster)

    3. Goebel EA, Zian X, Xie J, Chapel DB, Hill S, Garber J, Xian W, Crum CP. Detection of TP53 Mutations in the Peritoneal Washings of Women with Germline Mutations in Ovarian Cancer Susceptibility Genes (Poster)

    4. Brouwer J, Yoon J-Y, Xie J, Hill S, Xian W, Crum CP. Tubal p53 Signatures in Li-Fraumeni Syndrome (LFS) are Geographically Unique, Multi-Clonal and Temporally Dynamic (Poster).
- **Website(s) or other Internet site(s)**
  https://www.brighamhealthonamission.org/2018/11/28/could-precursor-escape-explain-advanced-high-grade-serous-carcinoma/
- **Technologies or techniques.** Nothing to report.

- **Inventions, patent applications, and/or licenses.** Nothing to report
- **Other Products.** Nothing to report

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
- **What individuals have worked on the project?**

Name:	*Christopher P Crum MD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0001-7746-1436
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Crum supervised and mentored Dr. Chapel. He also isolated cases, performed DNA isolation, interfaced with the sequencing facilities and analyzed data.*
Funding Support:	Current grant and Brigham and Women's Hospital
Name:	*Wa Xian, PhD*
Project Role:	*Consortium PI*
Researcher Identifier (e.g. ORCID ID):	0000-0001-6315-8858
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Xian performed an analytic method of whole genome sequencing data on cancer stem cells and interpreted data.*
Funding Support:	The current grant, The University of Houston, NIH, Rivken Center for Ovarian Cancer
Name:	*David B. Chapel, MD*
Project Role:	*Clinical and Research Fellow*

Researcher Identifier (e.g. ORCID ID):	0000-0002-9733-5442
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Chapel isolated cases, performed DNA isolation, interfaced with the sequencing facilities and analyzed data.*
Funding Support:	The current grant (0.5 FTE) and Brigham and Women's Hospital (0.5FTE)

o **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** There has been no change in support for the PI. Dr. Xian has two new active grants and two grants that have closed. For Dr. Chapel an OCRA grant has been awarded to fund the other 50% of his salary to enable full time commitment to the research laboratory in 2020-2021. His salary from the DOD grant for 0.5 FTE is unchanged. Dr. Xian's annotated Other Support follows on page 9.

o **What other organizations were involved as partners?** Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *N/A*
   - **QUAD CHARTS:**
9. **APPENDICES:** Four abstracts attached.

Award chart attached separately.

Previous/Current/Pending Support
Xian, Wa

Previous

***(now closed) Title:** Inflammatory Transformation of Intestinal Stem Cells Underlie Crohn's Disease
Agency: University of Texas Health Science Center in Houston
Agency contracting/Grant Officer: Michael Musters- Michael.V.Musters@uth.tmc.edu
Performance Period: 02/01/2017 - 01/31/2019
Level of Funding: $200,000
Time Commitment: 0.5 CM
Description: The goal of this proposal is to address mechanistic questions of Crohn's disease from a standpoint of these patient-derived stem cells and their corresponding differentiated epithelia.
List of Specific Aims: 1. Develop platform of stem cell "pedigrees" of ileum and colon from 20 adult-onset Crohn's cases and 10 normal cases.
2. Define key "nodes" in the inflammatory gene signature across cohorts and testing these nodal genes via gene editing and overexpression analyses.
Overlap: No

Title: Development of an Efficient Method for Cloning Colonic Stem Cells from Ulcerative Colitis via Endoscopic Biopsies
Agency: American Gastroenterology Association
Agency contracting/Grant Officer: Wykenna S.C. Vailor - Wvailor@gastro.org
Performance Period: 07/01/16-06/30/17
Level of Funding: $31,000
Time Commitment: 0.6 CM
Description: The goal of this grant is to develop an efficient method for cloning colonic stem cells from ulcerative colitis patients via endoscopic biopsies.
List of Specific Aims: 1. To employ advanced stem cell cloning technologies to establish defined pedigrees of patient-matched, regiospecific colon stem cells of control and ulcerative colitis (UC) patients including those ascending, transverse, descending, and sigmoid colon.
2. To compare the in vitro differentiated colon epithelium from stem cells of 10 control and 10 UC patients using histology and RNA-sequencing for phenotypic insights into epithelial defects driving this disease.

Overlap: No

Title: The Oviduct and Serous Cancer Risk Assessment
Agency: Department of Defense
Agency contracting/Grant Officer: Susan M. Dellinger, Grants Officer, USAMRAA, 820 Chandler Street, Ft. Detrick, MD 21702-5014, Ph: 301-682-5507
Performance Period: 09/01/14-09/01/16
Level of Funding: $225,000
Time Commitment: 0.5 CM
Description: The goal of this project is to address the hypothesis that HGSC is a disease of fallopian tube stem cells, is reflected in altered stem cell biology that accumulates with time, and this altered biology can be quantified to detect women at risk for HGSC
List of Specific Aims: 1. Clone fallopian tube stem cells of BRCA carriers and general population.
2. Compare the fallopian tube stem cells derived from at-risk, cancerous and normal population to uncover predictive markers.

Overlap: No

***(now closed) Title:** Iron addiction and the biology of ovarian cancer
Agency: National Institutes of Health
Agency contracting/Grant Officer: Suzy Torti – mail to: storti@uchc.edu
Performance Period: 09/16/2014 - 08/31/2019
Level of Funding: $110,472
Time Commitment: 0.72 CM
Description: The goal of this proposal is to understand mechanisms underlying iron addiction in ovarian cancer stem cells, the impact of alterations in iron metabolism on the biology of ovarian cancer, and the potential of these alterations to impact treatment resistan
List of Specific Aims: 1. Generating cancer stem cell lines derived from patients with high grade serous cancer.
2. Analyzing these cell lines through in vitro assays and marker staining.
3. Performing RNAseq analysis on selected clones to identify the molecular basis of iron addition of ovarian cancer stem cells.

Overlap: No

*** (now closed) Title:** Patient-Specific Strategies for Targeting Therapy-Resistant Cells
Agency: Department of Defense
Agency contracting/Grant Officer: Karen Wylie – karen.m.wylie.civ@mail.mil
Performance Period: 05/01/2017 – 04/30/2020
Level of Funding: $984,505
Time Commitment: 3 CM
Description: The goal of this proposal is to clone cancer stem cells from high-grade ovarian tumors and to use these patient-matched resistant and sensitive cancer stem cell clones to dissect the molecular basis of chemotherapy resistance.
List of Specific Aims: 1. Do resistant clones pre-date therapy and promote recurrent disease?
2. Dissecting the "resistance expression signature" in patient-matched CSCs for therapeutic insights.

Overlap: No

Title: Identifying and Targeting Pre-Therapy Resistant Clones in High-Grade Ovarian Cancer
Agency: Cancer Prevention Research Institute of Texas
Agency contracting/Grant Officer: Patty Moore - pmoore@cprit.texas.gov
Performance Period: 09/15/2015-9/15/2019
Level of Funding: $3,699,997
Time Commitment: 3 CM
Description: The goals of the present work are to exploit new cloning technologies to preempt recurrent disease in high-grade ovarian cancer and to develop a platform for "pre-clinical" trials based on 100 cases of ovarian cancer.
List of Specific Aims: 1. Generate libraries of CSCs from high-grade ovarian cancer derived from three patients who have never been exposed to chemotherapy and validate the CSC properties and genomic stability of these clones.
2. Define the overall tumor heterogeneity of each library via CNV and sequence analysis.
3. Select for and characterize rare resistant CSCs in these libraries via CNV and gene expression to understand the origins, mutational profiles, and physiology of resistant cells in these tumors.

Overlap: No

Current

Title: Pathogenic Heterogeneity in Mucosal Stem Cells in Pediatric Crohn's Disease
Agency: National Institutes of Health
Agency contracting/Grant Officer: Frank Hamilton – hamiltonf@extra.niddk.nih.gov
Performance Period: 8/10/18 – 6/30/22
Level of Funding: $2,394,516
Time Commitment: 3 CM
Description: The goal of this proposal is to address mechanistic questions of Crohn's disease from the standpoint of these patient-derived stem cells and their corresponding differentiated epithelia.
List of Specific Aims:
1. To Generate and intestinal stem cell platform for the analysis of Crohn's disease.
2. To identify and functionally test putative "nodal" genes in inflammatory signature.
3. To compare barrier properties in differentiated, 3-D cultures of Crohn's stem cells.
4. Does stem cell heterogeneity underlie the "skip lesion" patterning in Crohn's.
5. Patient-matched stem cells from active and remitting disease.

Overlap: No

*** (new) Title:** Resistant Cancer Stem Cell Profile in Multi-Site Metastasis of HGSC
Agency: Rivkin Center for Ovarian Cancer
Agency contracting/Grant Officer: Kiran Dhillon – Kiran.Dhillon@swedish.org
Performance Period: 04/01/2019 – 09/30/2020
Level of Funding: $74,715
Time Commitment: 0.5 CM
Description: The overarching goal of the present proposal is to understand whether metastatic lesions typically present in HGSC patients harbor resistant CSCs and if so are they similar to those found in the primary tumor or alternatively evolve to distinct resistant CSCs.
List of Specific Aims:
1. What is the spectrum of genetic diversity in primary tumor and multiple metastases in one high-grade serous cancer patient?
2. Can we identify cancer stem cell clones resistant to standard carboplatin/paclitaxel therapy?
3. Can we identify therapeutics that specifically target resistant clones?

Overlap: No

*** (new-this grant) Title:** Early Precursor Escape and High Grade Serous Carcinogenesis
Agency: Department of Defense
Agency contracting/Grant Officer: Chris Baker, Grant Officer, email: Christopher.1.baker132.civ@mail.mil.
Performance Period: 07/01/2019 - 06/30/2021
Level of Funding: $45,000
Time Commitment: 0.72 CM
Description: The goal of this proposal is to test the hypothesis that early serous precursors are directly responsible for subsequent disseminated HGSCs. If so it would provide evidence that malignant transformation occurred in the peritoneal fluid prior to solid tumor development.
List of Specific Aims:
1. If widespread serous cancers are directly related to these seemingly benign precancers in the Fallopian tube.
2. If the mechanism of cancer development begins with detachment of

these cells into peritoneal fluids.

Overlap: (This Grant)

*** (new) Title:** Clonal Reconstruction and Targeting of the Correa Sequence
Agency: National Institutes of Health
Agency contracting/Grant Officer: Wong, Alice Chi (240-276-6299; chia@mail.nih.gov)
Performance Period: 07/01/2019 – 06/30/2023
Level of Funding: $466,237
Time Commitment: 1 CM
Description: The overarching goal of this proposal is to identify new vulnerabilities in the lesions that lead to esophageal adenocarcinoma and to exploit these for the discovery of lead compounds to improve therapeutic options.
List of Specific Aims: 1. Reconstruct the Correa sequence at a stem cell level in ten patients with early esophageal adenocarcinoma.
2. Develop high-throughput screens of Correa sequence stem cells for lead preemptive therapeutics.
3. Develop stem cell-based xenograft models of the Correa sequence for lead validation.

Overlap: No

*** (new) Title:** Subset of Pre-Existing, Poly-Resistant Cancer Stem Cells in High-Grade Serous Ovarian Cancer
Agency: Department of Defense
Agency contracting/Grant Officer: Karen Wylie (karen.m.wylie.civ@mail.mil)
Performance Period: 06/01/2020-08/31/2023
Level of Funding: $700,614
Time Commitment: 2CM
Description: The goal of this proposal is to test the hypothesis that recurrent HGSOC arises from a discrete subset of pre-existing CSCs marked by a uniform gene expression profiles and profound polyresistance to an array of common chemotherapeutics.
List of Specific Aims: 1. Identifying Pre-existing, Poly-Resistant Clones Across Cohort of HGSOC cases.
2.Establish the Epigenetic Basis of Polyresistance within Each Case.
3. Mechanisms Underlying and Therapeutic Targeting of Poly-Resistance.

Overlap: No

<u>Pending</u>

Title: Sensitizing Resistant Cancer Stem Cells to Paclitaxel in Treatment-Naïve HGSCO
Agency: MDACC Ovarian Cancer SPORE
Agency contracting/Grant Officer: Erusulan Hampton (ehampton@mdanderson.org)
Performance Period: 09/01/2020-08/31/2021
Level of Funding: $50,000
Time Commitment: 0CM
Description: In this proposal, we will leverage exciting preliminary studies based on novel technologies that clonally dissect the intratumor heterogeneity of HGSOC to identify highly resistant clones in therapy-naive tumors, test the uniformity of these clones within a given patient, and identify drug candidates to specifically eliminate them.
List of Specific Aims: 1. Identify resistant clones in 15 additional, therapy-naïve HGSCO cases
2.Identify common and targetable signaling pathways that regulate paclitaxel sensitivity

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683

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Jingzhong Xie, University of Houston
Sarah Hill, Brigham and Women's Hospital
Wa Xian, University of Texas Health Science Center
Christopher P. Crum, Brigham and Women's Hospital

Title:
Tubal p53 Signatures in Li-Fraumeni Syndrome (LFS) are Geographically Unique, Multi-Clonal and Temporally Dynamic

Background:

Li-Fraumeni syndrome, with a germ-line TP53 mutation, is susceptible to clonal TP53 inactivating mutations in the FT resulting in p53 signatures. This study addressed three questions relevant to the biology of p53 signatures including 1) their clonality, 2) distribution in the FT and 3) change in frequency over time.

Design:

FTs from four cases of LFS were studied. To determine geographic distribution of clonal TP53 mutations events , DNA from p53 signatures in three separate regions from one FT was subjected to ion torrent NGS and the mutational burden analyzed for regional differences. Regional variations in distribution of p53 signatures per se were evaluated by mapping frequency of p53 immuno-positive foci into inner (luminal), middle and outer third of a cross section and fimbria (Fig 1). p53 signatures were recorded and the number(s) of p53+ nuclei counted. An analysis of temporal changes in frequency of p53 signatures was determined in a single case in which right and left tubes were removed at different times over a span of 10 years.

Results:

Sequencing of TP53 mutations in one case confirmed the germ-line mutation (c.A643G). Different somatic mutations were identified in sites 1 (c.1083delG & c.254delC), 2 (c.304delA), and 3 (c.1083delG) confirming multiple unique genotoxic events. In the second analysis, the number of p53 signatures per section was markedly higher in the outer 1/3 of the cross sections and in the fimbria. The mean number of cells per p53 signature varied across all regions. However, the number of p53 signatures with high numbers of p53 positive cells was significantly higher in the outer 1/3 relative to all other regions ($p = .0001$) and to the inner and middle 1/3 of the tubes ($p = .0097$)(Fig 2). Analysis of two fallopian tubes from the same patient disclosed a dramatically higher number of p53 signatures in the second tube removed 10 years after the first.

Conclusion:
P53 signatures in fallopian tubes of women with LFS are genetically independent events. The outer one third of the fallopian tube as well as the fimbria harbor a significantly higher proportion of p53 signatures. This could reflect a higher proportion of susceptible cells (non-ciliated) as well as the possibility that the peripheral 1/3 of the endosalpinx contains cells with greater capacity for cell division. The higher number of p53 signatures in the second tube years later in one case suggests that genotoxic injury accumulates in the tube over time during the reproductive years.

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1862

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David L Kolin, Brigham and Women's Hospital
Christopher P. Crum, Brigham and Women's Hospital

Title:
Landscape of Putative Precursors to High Grade Serous Carcinoma (HGSC) in the Female Genital Tract

Background:

In the now-widely accepted model of high-grade serous carcinoma (HGSC) development, a serous tubal intraepithelial carcinoma (STIC) develops in the distal tube, and tumor cells spread from the STIC to the peritoneal cavity. A second related model proposes that inconspicuous early serous proliferations (ESPs) exfoliate ("precursor escape"), spread to the peritoneal cavity, and progress to HGSC in a subset of cases. Both models may account for a sizable proportion of extrauterine HGSCs but fail to explain every case. We examined the frequency and histomorphologic features of endometrial ESPs in women with extrauterine HGSC.

Design:

The study cohort included two groups. Group 1 comprised consecutive extrauterine HGSCs in which the endometrium and fallopian tubes were entirely submitted for evaluation (11 cases, 129 endometrial and 72 tubal blocks). Group 2 consisted of cases in which exhaustive sectioning of entirely submitted fallopian tubes disclosed no STIC or ESP (from prior work, Soong et al 2018). All endometrial sections from Groups 1 and 2 were immunostained for p53. The frequency of ESPs was recorded and compared to a prior study of benign polyps (Jarboe et al, 2009).

Results:

In Group 1, 3 of 11 (27%) endometria contained an ESP, and 2 of 6 (33%) endometrial polyps harbored an ESP, in contrast to 6 ESPs seen in 137 benign polyps by Jarboe et al (P=0.037). A tubal ESP was identified in 4 of 11 (36%) cases. In Group 2, 2 of 11 (18%) cases harbored an endometrial ESP. Across both groups, ESPs included two morphologic types: 1) lesions limited to the surface epithelium (Fig 1) and 2) sub-surface lesions showing endometrioid differentiation (Fig 2).

Conclusion:

This study indicates that ESPs may be more frequent in the endometria of women with extrauterine HGSC than in healthy women, and that ESPs may be particularly prone to develop in endometrial polyps. Endometrial ESPs show two different morphologic patterns, correlated to surface versus sub-surface location. Molecular studies are in progress to compare the p53 mutations between these ESPs and concurrent HGSCs to elucidate the possible role of the endometrial lining and endometrial polyps in extra-uterine HSGC pathogenesis.

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2482

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Christopher P. Crum, Brigham and Women's Hospital
David L Kolin, Brigham and Women's Hospital

Title:
Primary Peritoneal High Grade Serous Carcinoma Revisited: Precursor Frequency and Implications

Background:

In the current model for high grade serous carcinogenesis (HGSC) a serous tubal intraepithelial carcinoma (STIC) develops in the distal tube and tumor cells spread to the peritoneal cavity. A complementary model proposes that early serous proliferations (ESPs) lead to HGSC via “precursor escape” with HGSC emerging later in he peritoneal cavity. The latter explains "primary peritoneal" HGSC but is not established. Moreover the validity of every STIC as an invariable "launching point" for HGSC has been questioned.

Design:

Consecultive cases classified as primary peritoneal high-grade serous carcinoma (HGSC) in which tubes were evaluated by the SEE-FIM protocol were identified from the pathology archives. Diagnosis of PPHGSC based upon the presence of a tumor distribution confined primarily to the extra tubo-ovarian extraovarian tissues, with involvement of the tubes or ovaries limited to the serosal surfaces. where available, all histologic material was reviewed with attention to the presence of STIC or ESPs. Tissue blocks resectioned and immunostained for p53 to maximized identification of any potential precursors. Fallopian tubes from a small group of tumors classified as "ovarian" HGSC were included.

Results:

43 cases classified by pathology report as PPHGSC were identified over a 10-year interval. STIC was reported in 9 cases (21%). In 23 of 43 cases with no STIC reported, blocks were available and sections immunostained for p53. Fourteen associated tumors were strongly p53 positive, 6 displayed a p53 null immunophenotype, one wild type and in two tumors was not available for analysis. 228 tissue sections, including HE and p53 stains were evaluated from the fallopian tubes of the 23 cases. In 2 (9%) a previously unappreciated STIC was identified following sectioning and immunostaining; 6 (26%) contained an ESP, one with a p53 null immunophenotype; 15 (65%) did not display a detectible lesion. Of fallopian tubes from 8 cases with extensive ovarian involvement ("ovarian" distribution) on gross exam 3(38%) contained a STIC and 4 (50%) an ESP.

Conclusion:

Although estimates of the frequency of STIC in PPHGSC to approach 50%, this followup study has shown the frequency to be significantly lower (20%). Additional putative precursors (ESPs) were identified in 26% of the remainder but in nearly one-half of PPHGSCs an origin in the fallopian tube is still not evident. This underscores the importance of evaluating other potential origins (?endometrial lining) in the gynecologic tract.

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Title:
Detection of TP53 Mutations in the Peritoneal Washings of Women with Germline Mutations in Ovarian Cancer Susceptibility Genes

Background:

Women with germline mutations in the BRCA genes are at increased risk for developing high-grade serous carcinoma (HGSC). The presumed origin for these tumors in many cases is the fallopian tube and the source of the tumor cells is presumed to be exfoliated cells with TP53 mutations from either intra-mucosal serous carcinomas (STIC) or early serous proliferations (via precursor escape). Biologic progression of these cells with TP53 mutations thus can result in “primary peritoneal” HGSC. Recent studies of HGSC cases and controls have confirmed the presence of cells with TP53 mutations in the peritoneal fluid.

Design:

The purpose of this pilot study was to determine if healthy women at genetic risk for HGSC harbored cells with TP53 mutations in the peritoneal cavity. Peritoneal washings from women undergoing risk-reduction salpingo-oophorectomy were selected. Discarded samples were centrifuged and cell pellets were processed for DNA extraction. DNA was then subjected to next-generation Ion torrent sequencing and the data were analyzed manually and using the analysis program gatk4 mutect2 (gatkforums.broadinstitute.org). Results were interpreted with the following conclusions: 1) there is a low possibility that the same mutation would be shared by multiple patients, 2) Ion torrent sequencing platform is prone to homopolymer-sequencing-errors (indels), 3) there is a very low possibility that two independent mutation events happened in a single cell.

Results:

After excluding mutations that were presumed to be germline variants, 5 samples were identified with unique frameshift mutations in TP53 (Table). One sample contained multiple TP53 mutations, interpreted as likely signifying more than one cell population with a unique mutation.

Results Table:

TP53 mutations in peritoneal fluid of women at risk for HGSC

Sample	Genetics	TP53 indel
3	BRCA1	chr17: 7579420
4	BRCA1	chr17:7578280

5	BRCA1	chr17:7577031;7036;8280;8464,9547,9861
6	BRIP1	chr17: 7578474
7	BRCA2	chr17: 7579373

Conclusion:

Women at increased genetic risk for HGSC frequently harbor cells with TP53 mutations in their peritoneal fluid. The origin of the cell populations with these mutations remains unclear. The possibility that these cells share lineage with endometrial or salpingeal cells with similar mutations is currently under investigation.