Genetic Discoveries and Pathologic Variations in Ovarian Cancer

Moderators: Charles N. Landen, MD, and Barbara S. Norquist, MD

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Histopathological characterization of the tubal fimbria reveals a subgroup of *BRCA1* mutation carriers with tumor-promoting gene-set enrichment

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Objective: Fallopian tube secretory epithelial cells have been implicated as the origin of high-grade serous ovarian cancer (HGSOC). Most ovarian cancers are diagnosed in postmenopausal women, although it is unclear how postmenopausal conditions contribute to cancer formation. Germline *BRCA1* mutations are associated with a 40% lifetime risk of ovarian cancer. This high-risk population is characterized by early onset of disease and predominant serous histology. The events that lead to secretory cell transformation are unknown. We aimed to identify potential precancerous niches in normal human fallopian tubes.

Method: Normal fallopian tube sections of 38 *BRCA* mutation carriers and 36 noncarriers were stained for PAX8, a marker for secretory cells and FOXJ1, a marker for ciliated cells. Digital image analysis was applied to determine secretory-to-ciliated cell ratios in the tubal fimbria. The same areas of interest were subjected to transcriptomic analysis.

Results: The PAX8/FOXJ1 cell ratio distinguishes two subgroups of *BRCA1* mutation carriers: ~70% resemble noncarriers and ~30% with a high PAX8/FOXJ1 cell ratio (**Figure 1A**). In postmenopausal patients, this segregation is maintained at the transcriptomic level (**Figure 1B** and **Figure 1C**). Strikingly, fimbriae of postmenopausal *BRCA1* mutation carriers with a high PAX8/FOXJ1 cell ratio are significantly enriched for ovarian cancer related pathways, mainly Ras, a known oncogene, its effectors PI3K and AKT and Rap1, a Ras associated protein shown to promote ovarian cancer and metastasis. JAK/STAT3, a major signaling pathway associated with ovarian tumor progression and poor prognosis is also enriched (**Figure 1D**).

Conclusion: The PAX8/FOXJ1 cell ratio demarcates a unique population of postmenopausal *BRCA1* mutation carriers in which cancer-related gene sets emerge in healthy tubal fimbria. Further investigation is needed to determine whether these represent the earliest stages of cancerous transformation in hereditary ovarian cancer patients.

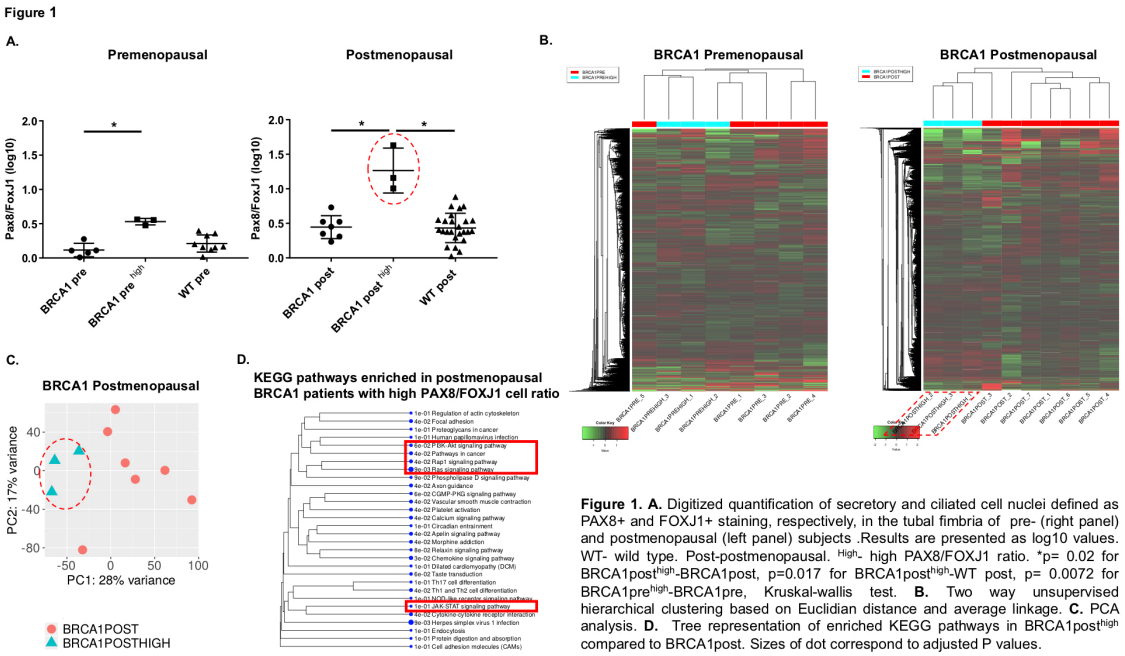

Figure 1. A. Digitized quantification of secretory and ciliated cell nuclei defined as PAX8+ and FOXJ1+ staining, respectively, in the tubal fimbria of pre- (right panel) and postmenopausal (left panel) subjects .Results are presented as log10 values. WT- wild type. Post-postmenopausal. ^{High}- high PAX8/FOXJ1 ratio. *p= 0.02 for BRCA1post^{high}-BRCA1post, p=0.017 for BRCA1post^{high}-WT post, p= 0.0072 for BRCA1pre^{high}-BRCA1pre, Kruskal-wallis test. **B.** Two way unsupervised hierarchical clustering based on Euclidian distance and average linkage. **C.** PCA analysis. **D.** Tree representation of enriched KEGG pathways in BRCA1post^{high} compared to BRCA1post. Sizes of dot correspond to adjusted P values.

Fig. 1.

Metabolomic and transcriptomic response to neoadjuvant chemotherapy in HGSOC

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Objective: Variability exists between genetic expression and metabolic profiles of primary tumor and ascites in patients with high-grade serous ovarian cancer (HGSOC). The objective of this study was to utilize primary patient samples to better characterize these differences and to analyze the effect of chemotherapy on gene expression and tumor metabolism.

Method: Tissue and ascites were collected from patients with suspected HGSOC. A subset of patients received neoadjuvant chemotherapy (NACT) and thus tumor was also collected at interval debulking. Full RNA sequencing was performed on 166 samples. Forty of these samples included tumor from patients (*n* = 20) pre- and post-NACT. Mass spectrometry-based metabolomics was performed on a subset (*n* = 26). RNA expression and metabolic profile differences between primary tumor and ascites were compared in 29 patients.

Results: Differences in gene expression pre- and post-NACT suggested changes in cell proliferation, cell cycle, and DNA damage response. The TCGA-based gene expression subtype changed in over half of patients following treatment. There was no statistically significant difference in immune signature post-NACT. In ascites, high lactose dehydrogenase was associated with malignant ascites. When comparing 29 matched tumor and ascites samples, the Cancer Genome Atlas (TCGA)-based subtype was the same in about 50% of patients. Differences between ascites and tumor from the same patient were significant for higher expression of genes associated with proliferation, cell migration, MAPK, and TGFb in the ascites. In a comparison of pre-NACT tumors from platinum-resistant and -sensitive patients, expression was significantly different in 84 genes. Platinum-resistant tumors were highly enriched for genes involved in nucleotide metabolism, redox, and oxidative phosphorylation. In a comparison of gene expression to metabolomic data, TCGA cycle metabolism was correlated with increased cellular proliferation in tumor tissue.

Conclusion: In this study, we demonstrate the importance of understanding the heterogeneity that exists in the transcriptomic and metabolomic profiles of the primary tumor, metastatic ascites, and changes induced by standard chemotherapy. Our data highlight the importance of understanding patients' initial response to treatment based not only on DNA-based next generation sequencing, but also on changes in RNA expression and metabolic profiles, which may help direct subsequent therapy.

Compositional and architectural characterization of high-grade serous ovarian carcinomas using single cell technologies and multiplex microscopy

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Objective: Therapy of high-grade serous ovarian carcinoma (HGSOC) remains challenging partly due to tumor heterogeneity and complex interactions with the tumor microenvironment (TME). The objective of the study was to determine whether immune cell states are related to their spatial localization within ovarian tumors.

Method: Patients with advanced ovarian cancer undergoing primary debulking surgery or diagnostic laparoscopy were prospectively identified. In order to analyze the interpatient ovarian cancer TME heterogeneity using orthogonal approaches, CD45− and CD45+ populations sorted from tumors were subjected to single cell RNA-sequencing (scRNAseq) analysis. Multiplex immunofluorescence (IF) analysis of the TME was performed on site-matched tumor samples. DNA from the primary site-matched tumors is being subjected to massively parallel sequencing.

Results: Ten patients undergoing primary debulking or diagnostic laparoscopy were included. Median age at diagnosis was 64 years (range 48–79 years). Nine of the 10 (90%) patients had HGSOC, and 1 (10%) had endometrioid ovarian cancer. scRNAseq data demonstrated quantitative and qualitative heterogeneity in tumor-infiltrating immune cell types among patients. The endometrioid ovarian cancer displayed a predominance of B cells compared to the HGSOCs. Multiplex IF analysis of the samples revealed an additional layer of information, demonstrating predominant localization of the CD8+ T cells and macrophages to the stromal rather than tumor compartment: CD8 tumor (median 0.15%, range 0.06–0.78%), CD8 stroma (median 0.56%, range 0.26–1.65%, $P = 0.009$), CD68 tumor (median 1.92%, range 1.18–5.55%), and CD68 stroma (median 11.96%, range 8.40–22.33%, $P < 0.001$). Confirming scRNAseq data, there was significant heterogeneity in the tumor-infiltrating CD8 cells, macrophages, and regulatory T cells among patients. See **Figure 1**.

Conclusion: Our findings highlight that broad characterization of the immune cell types and functional states in the ovarian cancer TME using scRNAseq fails to capture their geographic distribution (i.e., tumor vs stroma). Orthogonal datasets highlighting the TME composition as well as the functional state and distribution of T cells will be essential to understanding the mechanisms promoting tumor immune infiltration or exclusion and development of therapeutic approaches targeted to the specific TME phenotypes.

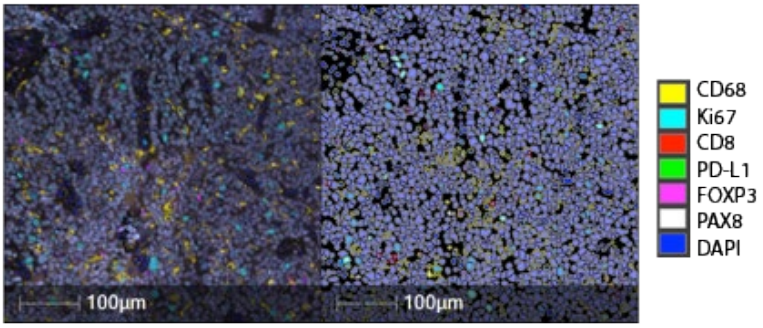

Fig. 1. Multiplex immunofluorescence imaging of omental sample acquired from a patient with HGSOC stained with select immune and tumor-specific markers (left). Segmentation masks demonstrating accurate recognition of individual cells, markers and colocalization (right).

***BRCA* tumor-testing in a tertiary referral center: Are we missing something or not?**

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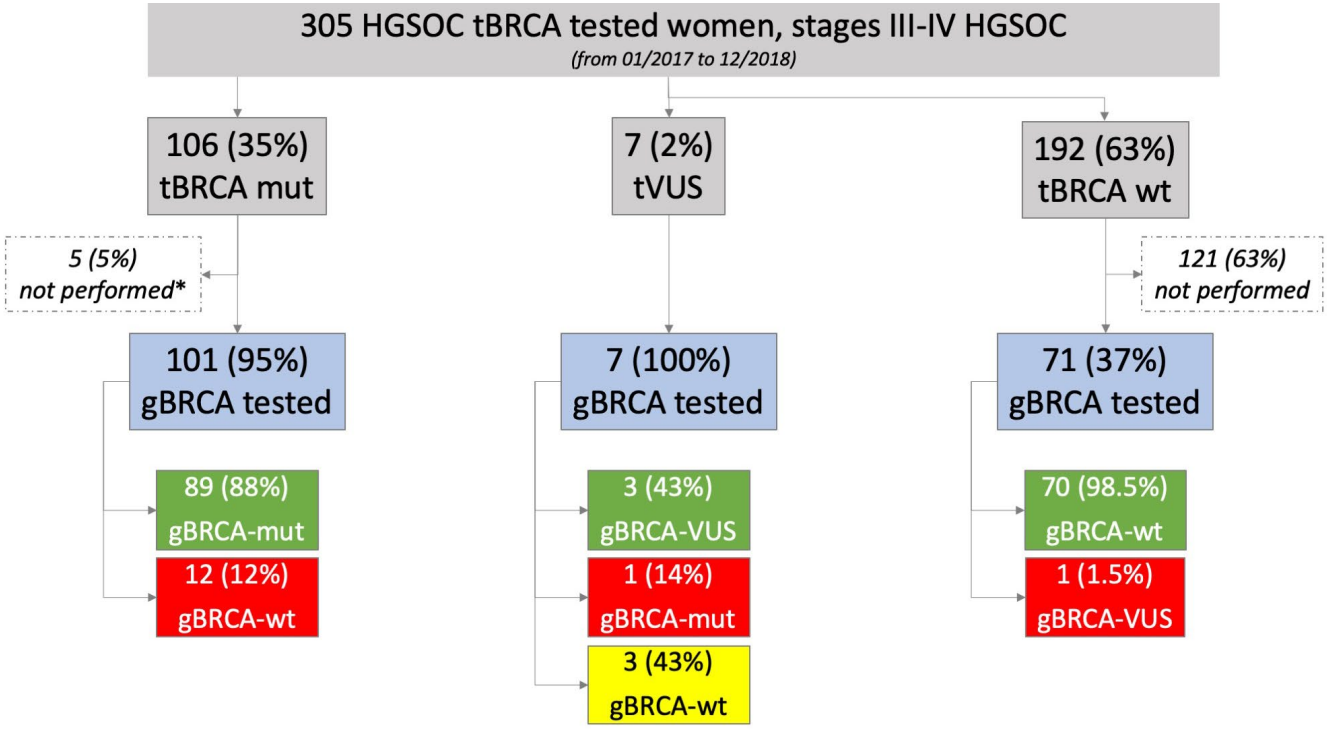
Objective: Investigating *BRCA* mutational status in ovarian cancer patients has a key role, both to identify hereditary cancer predisposition and to address therapeutic choices. Approximately 20–25% of patients with high-grade serous ovarian cancers (HGSOC) present with a germline *BRCA1/2* mutation, but a further 5–7% of patients will have a somatic *BRCA1/2* mutation, which might be missed if tumor genomic profile is not performed. The objective of this prospective study is to investigate the feasibility and reliability of a *BRCA* screening workflow based on tumor-tissue *BRCA* analysis and secondary germline screening, in a tertiary referral center.

Method: All newly diagnosed HGSOC patients with FIGO stage IIIC–IV treated from January 2017 to December 2018 underwent tumor *BRCA* testing and were recommended for blood *BRCA* analysis when a pathogenic variant was identified. Data concerning patients' clinical characteristics, treatments, outcomes, and genetic assessment were collected and analyzed.

Results: Overall, 305 patients with stage III–IV HGSOC were primarily treated at our institution and underwent tumor genomic profiling for assessing *BRCA* mutational status. In the whole population, 106 (35%) patients had a somatic pathogenic variant, specifically 66 patients with *sBRCA1*-PVs (62%) and 40 with *sBRCA2*-PVs (38%). Seven patients (2%) showed a variant of uncertain significance (s-VUS), and 192 (63%) a wildtype variant (s-WT). Among patients with s-PVs, 101 (95%) received the germline test, which confirmed the mutation in 89 (88%) patients and showed a wildtype variant in 12 patients (12%) (**Figure 1**). Also, a germline mutation was searched for in 71 (37%) of the s-WT patients; in 70 (98.5%) of them results were confirmed, while in 1 (1.5%) a germline-*BRCA2* VUS was identified.

Conclusion: In our experience, tumor-tissue sample has allowed the identification of 12% of patients potentially eligible for treatment with PARP-inhibitors (PARPi) that would have been missed if only germline testing had been performed. Waiting for results of PARPi trials in the first-line setting, tumor-tissue testing represents a reliable, feasible and cost-saving screening procedure for *BRCA* mutational status assessment.



HGSOC: high grade serous ovarian cancer.; tBRCA : tumor-tissue BRCA testing; tBRCA-mut: tumor-tissue BRCA 1 or 2 mutated; tVUS: variant of unknown significance on tumor-tissue testing; tBRCA-wt: tumor-tissue BRCA wild-type; gBRCA: germline BRCA testing; gBRCA-mut: germline BRCA 1 or 2 mutated; gVUS: variant of unknown significance on germline testing; gBRCA-wt: germline BRCA wild-type; *PATIENT'S REFUSAL OR DECISION

Fig. 1.

Comparing mutation frequencies for homologous recombination genes in uterine serous and high-grade serous ovarian carcinomas: A case for homologous recombination deficiency testing in uterine serous carcinoma

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Objective: Our goal was to compare the frequencies of somatic homologous recombination (HR) gene mutations identified in next-generation sequencing (NGS) genomic profiling of uterine serous carcinomas (USCs) and high-grade serous ovarian carcinomas (HGSOCs).

Method: Data for this analysis were obtained from AACR Project GENIE (*Cancer Discov.* 2017 Aug;7[8]:818-831), a multiinstitutional dataset of clinical-grade NGS genomic profiling results for many cancer sites and histologic subtypes, through cBioPortal (http://genie.cbioportal.org). Patient/specimen groups used for analysis were USC and HGSOC. Eighteen HR genes were queried for each group with respect to mutation frequency. For each HR gene, the difference in mutation frequency between the 2 groups was evaluated using Fisher exact test. The threshold for statistical significance was $P < 0.05$.

Results: In the USC group, there were 340 samples from 336 patients. In the HGSOC group, there were 1,208 samples from 1,193 patients. There was no overlap of patients between the 2 groups. Median patient age was 67 years for USC versus 63 years for HGSOC ($P < 0.001$). See **Table 1** for HR gene mutation frequencies for USCs and HGSOCs. The most frequently mutated HR gene for USC was *BRCA2* (4.12%) and for HGSOC, *BRCA1* (7.20%). Mutation frequency was significantly different between USC and HGSOC for *BRCA1* ($P < 0.001$) and *BRCA2* ($P = 0.034$). For the 16 non-*BRCA* HR genes, mutation frequency was not significantly different between USCs and HGSOCs.

Conclusion*: BRCA2* was the most frequently mutated HR gene identified in NGS genomic profiling of USC. Mutation frequency for non-*BRCA* HR genes was not significantly different between USCs and HGSOCs. These data add to the growing rationale for HR deficiency tumor testing and targeting (e.g., with PARP inhibitors) in future clinical trial development for women with USC.

Table 1. Mutation frequencies for homologous recombination (HR) genes analyzed by clinical-grade NGS genomic profiling of uterine serous carcinomas (USCs) and high-grade serous ovarian carcinomas (HGSOCs). *P* values listed if significant (< 0.05).

HR gene	**USC** n = 340 (%)	**HGSOC** n= 1208 (%)	**p-value**
ATM	11 (3.24)	47 (3.89)	-
BARD1	1 (0.29)	6 (0.50)	-
BRCA1	6 (1.76)	87 (**7.20**)	< 0.001
BRCA2	14 (**4.12**)	84 (6.95)	0.034
BRIP1	2 (0.59)	19 (1.57)	-
CDK12	9 (2.65)	31 (2.57)	-
CHEK2	2 (0.59)	9 (0.75)	-
FAAP20	1 (0.29)	1 (0.08)	-
FAN1	1 (0.29)	2 (0.17)	-
FANCE	1 (0.29)	9 (0.75)	-
FANCM	2 (0.59)	3 (0.25)	-
MRE11	0	5 (0.41)	-
NBN	3 (0.88)	14 (1.16)	-
PALB2	6 (1.76)	12 (0.99)	-
POLQ	0	9 (0.75)	-
RAD51B	0	1 (0.08)	-
RAD51C	1 (0.29)	2 (0.17)	-
RAD51D	1 (0.29)	3 (0.25)	-

Inherited mutations in fallopian tube, ovarian, and primary peritoneal carcinoma: Changes in diagnoses and mutational frequency over 20 years

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Objective: Fallopian tube carcinoma (FTC) had a higher reported frequency of inherited *BRCA* mutations than ovarian carcinoma (OC) or primary peritoneal carcinoma (PPC). We hypothesized that the adoption of serial sectioning of the fallopian tube would lead to an increased proportion of cases designated as FTC and could change mutation fraction. We investigated the prevalence of inherited mutations in women with FTC compared with OC and PPC, and how the fraction of FTC and inherited mutations changed over time.

Method: Women diagnosed from 1998 to 2018 were enrolled at diagnosis into an institutional tissue bank. Germline DNA from 700 women with primary FTC (n = 124), OC (n = 511), and PPC (n = 65) was assessed using targeted capture and massively parallel sequencing for mutations in ovarian cancer susceptibility genes. To assess the fraction of FTC over time, cases were split between those prior to the adoption of serial sectioning (1998–2008) and those after (2009–2019).

Results: Inherited mutation frequency was similar among women with FTC (24/124, 19%), OC (106/511, 21%, P = 0.42), and PPC (16/65, 25%, P = 0.25). In FTC, 16 mutations were identified in *BRCA1* (13%), 2 in *BRCA2* (1.6%), and 6 in non-*BRCA* genes (4.8%). The proportion of carcinomas attributed as FTC after 2009 was 31.3% (107/342), significantly higher than prior to 2009 (4.7% (17/358, P < 0.0001, OR = 9.1, 95% CI 5.4–15.7). Germline mutation rates in FTC were lower after 2009, with 16/107 cases (15.0%) compared to 8/17 cases (47.1%) prior to 2009 (P = 0.005, OR = 0.20, 95% CI 0.06–0.64).

Conclusion: The prevalence of inherited mutations is similar in FTC compared to OC or PPC when using modern pathological assignment. The adoption of complete serial sectioning of fallopian tubes has significantly increased the diagnosis of FTC and subsequently decreased the frequency of inherited mutations. All women with FTC, OC, and PPC should be offered genetic testing for inherited mutations in cancer predisposition genes.