OVARIAN CANCER

The ability to detect small amounts of tumor cells in blood and vaginal specimens offers big advances in diagnosis and treatment for this deadly malignancy

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The authors report no financial relationships relevant to this article.

Ovarian cancer remains the deadliest gynecologic malignancy in the United States, with more than 22,000 women newly diagnosed and more than 14,000 deaths each year. We have made slow progress in terms of survival with new drugs and applications, such as intraperitoneal chemotherapy combined with more aggressive cytoreductive efforts. Five-year survival rates have increased—from 36% to 44%—since the late 1970s.[1] To make the leap from molecular genetics to successful screening, early diagnosis, and targeted treatment, we must first:

- **Enhance our understanding of the changes that lead to ovarian cancer.** Currently, malignant transformation of the fallopian tube epithelium is thought to result in high-grade papillary serous cancer.[2] If this is indeed the pathologic origin of ovarian cancers, then early detection or even detection in the premalignant phase may be possible using tests of vaginal fluid. Are early detection, and even screening, possible and how would it effect treatment and survival?
- **Develop new and powerful tools to detect molecular changes that might impact treatment and survival.** Just a few years ago, initial sequencing of the human genome cost more than $100 million, but DNA sequencing technologies have evolved rapidly, with current estimates at less than a few thousand dollars per genome.[3] Knowing the mutations responsible for an individual's cancer would allow for targeted, individualized treatment plans. Would one patient benefit from neoadjuvant therapy while another needs primary surgical debulking?

In this article, we highlight the historical basis and recent developments in the field of ovarian cancer, focusing on:

- etiologic heterogeneity and molecular biology
- detection of small numbers of cancer cells in vaginal secretions and the blood stream.

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What mutations are we looking for?

Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609–615.

In last year's *Update*, we discussed the role of The Cancer Genome Atlas (TCGA) project in endometrial cancer.[4] For ovarian cancer, TCGA analyzed messenger RNA expression, microRNA expression, promoter methylation, and DNA copy number in 489 high-grade serous ovarian adenocarcinomas and the DNA sequences of exons from coding genes in 316 of these tumors.

Almost all tumors (96%) were characterized by mutations of the gene encoding TP53 in addition to statistically recurrent mutations in nine other loci, including NF1, BRCA1, BRCA2, RB1, and CDK12, although these were of low prevalence. Analyses also brought new insight regarding the survival impact of tumors containing BRCA1 or BRCA2 and CCNE1 mutations. Findings included NOTCH and FOXM1 signaling involvement in serous ovarian cancer pathophysiology as well as defective homologous recombination in approximately half of the tumors studied.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

With these mutations as our targets, we can screen vaginal secretions as well as blood for markers of ovarian cancer.

FAST TRACK

"Shed" cancer cells were identified in 100% and 41% of cases of endometrial and ovarian cancer, respectively

Ovarian and endometrial cancer cells detected in the vagina

Kinde I, Bettegowda C, Wang Y, et al. Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Sci Transl Med. 2013;5(167):167ra4.

Erickson BK, Kinde I, Dobbin AC, et al. Detection of somatic TP53 mutations in tampons of patients with high-grade serous ovarian cancer [published online ahead of print October 2014]. Obstet Gynecol. 2014;124(5).

Ruth Graham, Papanicolaou's cytology technician in the 1940s, first described ovarian cancer cells detected in vaginal/cervical cytology obtained from vaginal secretions.[5] Current studies now demonstrate that we have technology capable of more than simple cytologic detection. We can isolate and evaluate these cancer cells in very small numbers.

Ovarian and endometrial cancer DNA identified in Pap specimen

Kinde and colleagues assembled a catalog of common mutations previously found in ovarian cancer as well as new data on 22 endometrial tumors. They tested 24 endometrial and 22 ovarian samples from patients with endometrial or ovarian cancers and confirmed that all 46 harbored at least some component of the common genetic changes in their catalog. Hypothesizing that the cancers likely shed cells from their surfaces, they sought to determine whether they could detect these cells among the cervical cells in a Pap smear.

These investigators used massively parallel sequencing to test DNA collected in modern liquid-based cytologic specimens for the same mutations found in the cancer cells. They found that 100% of the endometrial

cancers and 41% of ovarian cancers were detectable by this method.

TP53 mutations in ovarian cancer cells detected in vaginally placed tampon

With similar technology, but a different collection method, Erickson and colleagues sought to detect tumor cells in the vagina of women with serous ovarian cancer by TP53 analysis of DNA samples collected via vaginal tampon.

Thirty-three women with pelvic masses suspicious for malignancy and scheduled to undergo diagnostic or therapeutic surgery were enrolled. Of the 25 patients who placed the tampon 8 to 12 hours prior to surgery; 13 had benign disease; three had nonovarian malignancies; and nine had serous adenocarcinoma of ovarian, tubal, or primary peritoneal origin. DNA from tumor specimens of eight patients with serous carcinoma and adequate DNA samples were analyzed for TP53 mutations. The corresponding DNA extracted from the tampon was then probed for the mutation identified in the tumor.

Mutational analysis of the tampon specimen DNA revealed no mutations in the tampon DNA of the three patients who had previously undergone tubal ligation, while mutations were observed in three of the five patients with intact tubes—producing a sensitivity of 60%. The fraction of mutant alleles in the tampon DNA was extremely low at 0.01% to 0.07%, requiring ultra-deep sequencing and increasing the importance of paired primary tumor specimens.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

While sensitivity in a population of high-risk patients with intact tubes was found to be 60%, it is unclear what it would be in patients with less advanced disease. The ability of the test to detect mutations at exceptionally low limits is impressive; however, it increases the risk that a variant represents a sequencing error or a sample-to-sample contamination. This study is novel in its approach to diagnosis of ovarian cancer and is a stride toward screening, providing an opportunity to further validate the technology prior to widespread use and clinical application.

FAST TRACK

Circulating tumor cells were found in 24.5% of the 216 patients with ovarian cancer

Circulating tumor cells—the future of cancer management?

Obermayr E, Castillo-Tong DC, Pils D, et al. Molecular characterization of circulating tumor cells in patients with ovarian cancer improves their prognostic significance: a study of the OVCAD consortium. Gynecol Oncol. 2013;128(1):15–21.

Similar in concept to noninvasive prenatal testing for fetal aneuploidy, high circulating tumor cell (CTC) numbers have been correlated with aggressive disease, increased metastasis, and decreased time to relapse. As with cancer cells in vaginal secretions, CTCs also may provide an opportunity for early detection and targeted treatment.[6]

While many CTC studies have used epithelial cell adhesion molecule (EpCAM)–based CTC detection, results have been found to be highly variable between tumor subtypes and phase of disease.[7] Therefore, Obermayer and colleagues sought to identify novel markers for CTCs in patients with epithelial ovarian cancer and elucidate their impact on outcome.

Details of the study

Matched ovarian cancer tissues and peripheral blood leukocytes of 35 patients underwent microarray analysis to identify novel CTC markers. Gene expression of the novel markers as well as EpCAM were analyzed using blood samples taken from 39 healthy females and from 216 patients with ovarian cancer before primary treatment and

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6 months after adjuvant chemotherapy. Overexpression of at least one gene, compared with the healthy control group, was considered CTC positivity.

CTCs were detected in 24.5% of the baseline and 20.4% of the follow-up samples, of which two-thirds showed overexpression of the cyclophilin C gene (PPIC), and just a few by EpCAM overexpression. PPIC-positive CTCs during follow-up were detected significantly more often in the platinum resistant group, and indicated poor outcome even when controlling for classical prognostic parameters.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

The study authors found that molecular characterization of CTC is superior to CTC enumeration. Ultimately, CTC diagnostics may lead to earlier detection and more personalized treatment of ovarian cancer.

Therefore, this technology could have great impact on screening for and the survival of a large subset of patients with ovarian cancer. In addition, the cells obtained preoperatively could help assess the risk of malignancy in an ovarian mass prior to surgery, or even help in treatment planning, as we enter an era in which we have the ability to assess cancers for prognosis and features of treatment response.

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