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Original Research

Detection of Somatic *TP53* Mutations in Tampons of Patients With High-Grade Serous Ovarian Cancer

Britt K. Erickson, MD, Isaac Kinde, BS, Zachary C. Dobbin, BS, Yuxuan Wang, BS, Jovana Y. Martin, MD, Ronald D. Alvarez, MD, Michael G. Conner, MD, Warner K. Huh, MD, Richard B. S. Roden, PhD, Kenneth W. Kinzler, PhD, Nickolas Papadopoulos, PhD, Bert Vogelstein, MD, Luis A. Diaz Jr, MD, and Charles N. Landen Jr, MD

OBJECTIVE: To investigate whether tumor cells could be detected in the vagina of women with serous ovarian cancer through *TP53* analysis of DNA samples collected by placement of a vaginal tampon.

METHODS: Women undergoing surgery for a pelvic mass were identified in the gynecologic oncology clinic. They placed a vaginal tampon before surgery, which was removed in the operating room. Cells were isolated and DNA was extracted from both the cells trapped within the tampon and the primary tumor. In patients with serous carcinoma, the DNA was interrogated for the presence of *TP53* mutations using a method capable of detecting rare mutant alleles in a mixture of mutant and wild-type DNA.

RESULTS: Thirty-three patients were enrolled. Eight patients with advanced serous ovarian cancer were included for analysis. Three had a prior tubal ligation. *TP53* mutations were identified in all eight tumor samples. Analysis of the DNA from the tampons revealed mutations in three of the five patients with intact tubes (sensitivity 60%) and in none of the three patients with tubal ligation. In all three participants with mutation detected in the tampon specimen, the tumor and the vaginal DNA harbored the exact same *TP53* mutation. The fraction of DNA derived from exfoliated tumor cells ranged from 0.01% to 0.07%.

CONCLUSION: In this pilot study, DNA derived from tumor was detected in the vaginas of 60% of patients with ovarian cancer with intact fallopian tubes. With further development, this approach may hold promise for the early detection of this deadly disease.

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From the Departments of Obstetrics and Gynecology and Pathology, University of Alabama at Birmingham, Birmingham, Alabama; the Ludwig Center of Cancer Genetics and Therapeutics and the Swim Across America Laboratory at Johns Hopkins University, Baltimore, Maryland; and the Departments of Obstetrics and Gynecology and Pathology, University of Virginia, Charlottesville, Virginia.

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Corresponding author: Charles N. Landen Jr, MD, University of Virginia, Department of Obstetrics and Gynecology, PO Box 800712, Charlottesville, VA 22908; e-mail: clanden@virginia.edu.

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Unlike other gynecologic malignancies, epithelial ovarian cancer typically presents at an advanced stage. This is in part a result of the fact that no effective screening methods exist to detect early-stage disease and patients with advanced-stage ovarian cancer usually have nonspecific symptoms at the time of diagnosis. Thus, despite modest improvements in treatment of advanced ovarian cancer, most patients eventually die of their disease.

To date, no effective serum biomarker or imaging-based strategy has proven to reduce mortality related to ovarian cancer. As an alternative to these screening approaches, identifying tumor cells through detection of somatic mutations may provide a different method of early cancer detection. The vast majority of epithelial ovarian tumors of the serous histologic subtype harbor *TP53* mutations.[1,2] Given that the intraabdominal cavity communicates with the vagina through the upper genital tract, we speculated that we could detect ovarian cancer cells that exfoliate and descend through the cervical os and into the vagina. We considered the possibility that malignant cells that have exfoliated from the tumor might be detected by deep sequencing of *TP53* exoms, which would allow for the detection of even a small fraction of mutant DNA (as little as 0.001%) within the context of a majority of wild-type alleles present in the DNA sample.[3] In this study, we hypothesized that, if these tumor cells and fragments containing tumor DNA are present in the vagina of women with known ovarian cancer, they could be collected using a tampon.

MATERIALS AND METHODS

The study was approved by the institutional human subjects protection review board at the University of Alabama at Birmingham and Johns Hopkins Hospital and carried out in accordance with their standards. Patients were approached for enrollment at the gynecologic oncology clinic from August 2012 through January 2013. Eligible participants included patients with a pelvic mass suspicious for malignancy and planned diagnostic or therapeutic surgery. Exclusion criteria included previous hysterectomy or bilateral salpingo-oophorectomy, age younger than 19 years, heavy vaginal bleeding, and inability or unwillingness to place a vaginal tampon. Patients with serous carcinoma of the ovary comprised the study group for this report.

After obtaining informed consent, patients were given a commercially available, plastic applicator, vaginal tampon (Tampax Pearl). Patients were instructed to place the tampon in their vagina 8–12 hours before their scheduled surgery. The tampon was removed in the operating room after induction of anesthesia and placed in a sterile phosphate-buffered saline buffer solution. Tumor specimens were collected at the time of surgery from either the primary or metastatic site and immediately snap-frozen in liquid nitrogen and stored at –80 C. Because we hypothesized that patients with tubal intraepithelial carcinomas may be more likely to have detectable malignant cells in the vagina, all fallopian tubes were subjected to assessment in accordance with the Sectioning and Extensively Examining of the Fimbriated end (SEE-FIM) protocol.[4]

The tampons were manually compressed into sterile phosphate-buffered saline solution and then discarded. The remaining suspension then was centrifuged at 8,000 rpm for 2 minutes and DNA extracted from the cell pellet using a modified protocol and reagents from the QIAamp DNA mini kit. The cell pellet was resuspended in 500 microliters of Buffer AE and then pelleted for 1 minute at 8,000 rpm (6,941 g). The supernatant was discarded and the pellet suspended in 300 microliters of Buffer ATL and 30 microliters of Proteinase K, pulse-vortexed for 10 seconds, then incubated at 56 C for 60 minutes with a 10-second vortex every 15 minutes. Postincubation, 300 microliters of Buffer AL plus 560 microliters of molecular-grade 100% ethanol was added and mixed by vortex. The solution was then transferred to a QIAamp Mini spin column, spun at 8,000 rpm for 75 seconds, and the follow-through discarded. The column was washed with 500 microliters of Buffer AW1, centrifuged at 8,000 rpm (6,941 g) for 2 minutes, then washed with 500 microliters of Buffer AW2 and centrifuged at 10,000 rpm (10,845 g) for 2 minutes. The column was dried by centrifugation at 15,000 rpm (24,400 g) for 3 minutes. DNA was eluted using 50 microliters of Buffer AE by centrifugation after 5-minute room temperature incubation. DNA concentration and 260/280 ratios were quantified using a microvolume spectrophotometer.

For tumor specimens, a small portion of snap-frozen tumor immediately adjacent to hematoxylin and eosin-confirmed malignant tissue was thawed and 10–20 mg of tumor was processed for DNA extraction using the DNEasy kit as instructed by the manufacturer.

A sequencing error-reduction technology described previously[3] was used to sequence *TP53* in DNA from the tumor and tampon samples. Amplification primers were designed to amplify segments containing all exons of the *TP53* gene. The polymerase chain reaction products were purified with AMPure and sequenced on a MiSeq instrument. Data were collected and analyzed as previously described.[3]

Descriptive statistics were used to describe mutation rates and frequencies. Fisher's exact test was used to measure association between variables. A P value of $<.05$ was considered significant.

RESULTS

During the study period, 33 patients were enrolled into the trial. Tampons were successfully placed and processed in 25 patients (76%, 95% confidence interval [CI] 58–89%). Reasons for failed collection

included patients forgetting or electing not to place the tampon, the tampon falling out prematurely, and incorrect application. Adequate quantity and quality of DNA were obtained from all patients who successfully placed the tampon and had it removed preoperatively. Of the 25 patients, 13 had benign disease, 3 had nonovarian malignancies (uterine carcinosarcoma and appendiceal carcinoma), and nine patients had serous (ovarian, tubal, or primary peritoneal) adenocarcinoma. One patient with serous adenocarcinoma had inadequate DNA obtained from her primary tumor. Thus, for this initial analysis, DNA from the tumor specimens from the eight patients with serous carcinoma and adequate DNA samples were analyzed for the presence of *TP53* mutations in any coding exon. The mutation identified in the tumor was then queried in the corresponding tampon DNA sample using the same technique. The clinical characteristics of the eight patients examined are listed in Table 1. One patient had identifiable tubal intraepithelial neoplasia. Of note, three patients had bilateral tubal ligations.

Information regarding *TP53* mutational analysis is demonstrated in Table 2. The average amount of DNA recovered from the vaginal tampon was 133.2 ng/microliter (range 14–609 ng/microliter; Table 2). No clinical factors correlated with the amount of DNA recovered from the tampons, including length of time the tampon was in the vagina. Tumor specimens from all eight patients showed at least one mutation in *TP53*.

The sequencing method not only allows identification of mutations, but also quantification of the number of DNA fragments containing the mutation. Interestingly, although all tumor specimens contained at least 50% of malignant cells in the tumor, generally mutated DNA in the specimens made up a smaller percentage of the total DNA, suggesting that histologic assessment may overestimate the overall makeup of malignant cells in a heterogeneous tumor.

Mutational analysis of the tampon specimen DNA revealed mutations in three of the eight patients (38%, 95% CI 9–76%). No mutations were observed in the tampon DNA of the three patients who had undergone tubal ligation, whereas mutations in the tampon DNA were observed three of the five patients (sensitivity 60%) without tubal ligation (0% compared with 60%, $P=.20$). The mutation identified in the tampon DNA was the same mutation identified in the tumor in all three patients (Table 2). The fraction of mutant alleles in the tampon DNA, which approximates the percentage of vaginal DNA that was tumor-derived, ranged from 0.01% to 0.07%. The fraction of mutant alleles in the corresponding tumors varied from 32% to 59%.

There was insufficient statistical power to evaluate the relationship between any clinical parameters (including age, race, tumor characteristics, CA 125, presence of ascites, length of time of tampon placement) and ability to detect of *TP53* mutations (data not shown).

DISCUSSION

In this pilot study, vaginal tampons were used to collect DNA from women newly diagnosed with advanced papillary serous ovarian cancer. Deep sequencing of this vaginal DNA yielded detectable *TP53* mutations in 60% of patients with serous carcinoma without a history of tubal ligation. *TP53* mutations were not detectable in the vaginal DNA obtained from all three patients with serous carcinoma with a prior tubal ligation. The *TP53* mutations detected in the tampon DNA samples were identical to the mutations found in the primary metastatic tumor. This supports the hypothesis that ovarian cancer cells survive transit through the fallopian tube and uterus and reach the cervix and vagina intact. We postulate that the exfoliated cells from the primary ovarian

Table 1. Patient Characteristics

Patient No.	Age (y)	Race	BMI (kg/m^2)	Pathology	Preoperative CA 125	Tubal Ligation
1	60	White	55	Stage 3C ovarian adenocarcinoma	739	No
2	54	White	55	Stage 4 papillary serous ovarian adenocarcinoma	929	Yes
3	71	White	24	Stage 3C papillary serous ovarian adenocarcinoma	696	No
4	29	White	34	Stage 3C mixed histology ovarian adenocarcinoma	233	No
5	56	White	33	Stage 3C mixed histology ovarian adenocarcinoma	2,570	No
6	61	Black	37	Stage 3C papillary serous fallopian tube adenocarcinoma	455	Yes
7	47	White	21	Stage 3C papillary serous primary peritoneal adenocarcinoma	2,365	No
8	67	White	28	Stage 3C papillary serous ovarian adenocarcinoma	2,770	Yes

BMI, body mass index.

Table 2. Mutational Analysis of Tumor and Vaginal Tampon DNA

Patient No.	Histologic Percentage of Malignant Cells	Tissue Mutation(s) (Percentage of Template Molecules With Mutation)	Mutation Detected in Tampon DNA	Percentage of Mutant Tumor DNA in Tampon
1	70	TP53 g.chr17:7577538C>T, c.743 G>A, p.R248Q (32%)	TP53 g.chr17:7577538C>T, c.743 G>A, p.R248Q	0.01
2	90	TP53 g.chr17:7579707delT, c.89delA, p.N30fs (69%)	Not detected	
3	80	TP53 g.chr17:7577559 G>T, c.722C>A, p.S241Y (21%)	Not detected	
4	80	TP53 g.chr17:7578190T>C, c.659A>G, p.Y220C (39%)	TP53 g.chr17:7578190T>C, c.659A>G, p.Y220C	0.02
5	70	TP53 g.chr17:7578234_7578235delAT, c.614_615delAT, p.Y205fs (36%)	Not detected	
6	70	TP53 g.chr17:7578394T>C, c.536A>G, p.H179R (86%)	Not detected	
7	90	TP53 g. chr17:7577115A>G, c.823T>C, p.C275R (59%)	TP53 g. chr17:7577115A>G, c.823T>C, p.C275R	0.07
8	50	TP53 g.chr17:7577120C>T, c.818 G>A, p.R273H (66%)	Not detected	

malignancy (ovarian, tubal, or primary peritoneal) migrate through the fallopian tubes, the uterus, and then through the endocervix and into the vagina.

Recent research suggests that a majority of serous ovarian cancers appear to originate from dysplastic lesions in the distal fallopian tube.[5,6] Vaginal tumor DNA was not detected in the one patient with tubal intraepithelial neoplasia. The rate of identifiable tubal intraepithelial neoplasia was lower in this cohort than in previously published studies; however, the diagnosis of tubal intraepithelial neoplasia is still variable among pathologists in different institutions. With only one patient with intraepithelial neoplasia, we cannot make any specific conclusions regarding the feasibility of this method in patients with early-stage or preinvasive disease.

This study gives further support to the potential utility of ultrasensitive genomic analysis for the detection of low-frequency mutations in clinical samples. In a recent study using the same sequencing technology, Pap test solvents (rather than tampons) in 22 patients with ovarian cancer were analyzed.[3] Nine of these 22 patients (41%) had detectable *TP53* mutations in the DNA from the Pap test fluids. It is possible that the sensitivity of this approach might be improved by the use of a method that potentially collects both descending tumor cells and tumor-derived fragments (eg, exosomes) reaching the vaginal cavity.

The fraction of mutant alleles in the liquid-based Pap test solvent was higher (median 3%, range 0.01–80%) than that observed in the current study with tampon extracts (range 0.01–0.07%). The advantage of tampons, however, is that they do not require a medical professional for sample procurement and can be more easily used for serial sampling. Frequent, serial sampling, especially in selected high-risk populations, can significantly improve the predictive value of diagnostic tests such as CA 125 in women with germline *BRCA* mutations. Our approach takes advantage of relatively noninvasive sampling and uses materials that would routinely be collected and discarded but could potentially be shipped to a central laboratory for testing. For this method to ultimately be clinically useful, several factors should be considered. The cost of the sequencing analysis of *TP53* is currently not amenable to a screening setting; however, it is anticipated that costs of next-generation sequencing methods will continue to drop exponentially. Most importantly, this approach will have to be shown to be able to adequately detect early stages of disease to provide sufficient lead time for an effective intervention.

One limitation of the current study was that all samples were obtained from patients with late-stage cancer. Because many serous cancers may originate in the fallopian tube in precancerous tubal intraepithelial carcinomas, it may be feasible to detect precancerous disease or cancer before metastatic spread outside of the genital tract with this next-generation sequencing method.[5] Detection of preinvasive disease has been modeled in other tumor types, most notably the detection of preinvasive colorectal cancer through fecal DNA testing.[7] Another limitation is that we did not sequence the DNA from tampons from patients with benign disease. Therefore, specificity could not be calculated.

In summary, the current study suggests that tumor DNA reaches the vagina in patients with advanced ovarian cancer and can feasibly be collected using tampons and detected therein using high-throughput *TP53* mutational analysis. The majority of patients with ovarian cancer present at an advanced stage because screening and early-stage diagnostic methods are imprecise, and none can currently be recommended for screening. This method may represent an innovative way to detect an intraabdominal tumor through a noninvasive process. Larger studies are needed to further validate this method and identify a more precise detection rate. As methods of DNA extraction and sequencing improve, we are hopeful that this may lay the groundwork for an opportunity to detect ovarian cancer at early or even premalignant stages.

REFERENCES

1. Ahmed AA, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, et al. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol 2010; 221:49–56.
2. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609–15.
3. Kinde I, Bettegowda C, Wang Y, Wu J, Agrawal N, Shih Ie M, et al. Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Sci Transl Med 2013;5: 167ra4.
4. Mingels MJ, van Ham MA, de Kievit IM, Snijders MP, van Tilborg AA, Bulten J, et al. Müllerian precursor lesions in serous ovarian cancer patients: using the SEE-Fim and SEE-End protocol. Mod Pathol 2014;27:1002–13.
5. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am J Surg Pathol 2007;31:161–9.
6. Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, et al. Dysplastic changes in prophylactically removed fallopian tubes of women predisposed to developing ovarian cancer. J Pathol 2001;195:451–6.
7. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget stool DNA testing for colorectal-cancer screening. N Engl J Med 2014;370:1287–97.