Retooling the Pap Smear for Ovarian and Endometrial Cancer Detection

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Although the Papanicolaou (Pap) smear was intended to be used to screen for cervical cancer and its precursors, ovarian and endometrial cancers are infrequently detected via abnormal cervical cytology. However, the diagnostic sensitivity of cytology alone is too low to be clinically useful. A recent publication in Science Translational Medicine (1) demonstrated that DNA mutational analyses of samples collected during the common Pap smear may be capable of detecting ovarian and endometrial cancer. Such a test would be highly advantageous considering that ovarian cancer is usually diagnosed in advanced stages and is highly lethal and that endometrial cancer is the fourth most common cancer in women. Furthermore, no effective screening test exists for either disease. This recent study combined with a prior report (2) provides proof of principle that tumor DNA from the proximal female reproductive tract may be detected in a minimally invasive fashion from the lower tract. Although promising, challenges remain before this concept can be applied as a global screening test for gynecologic cancer.

Analyzing disease-specific DNA from Pap smears to enhance cervical pathology screening has been performed clinically for a decade. The recent study (1) exploits DNA stability and newer technological advances to identify abnormalities in the upper genital tract. Before Pap DNA analyses, the primary tumors underwent whole-exome sequencing to identify both anticipated and novel tumor-specific mutated genes. Investigators successfully identified the same mutations within the cervical sample as were in the respective primary tumor for 100% of the endometrial cancers and 41% of the ovarian cancers (1). Several technologic advances are leveraged in this study: (a) detecting scarce ovarian cancer DNA from cervical scrapings underscores the sensitivity of the sequencing method used; (b) the heterogeneity of each cancer type requires analysis of more than one gene, which may be accomplished in a multiplex assay; and (c) mutational analysis is quantifiable and objective. Additionally, the costs of sequencing are dramatically decreasing (1), which will make complex mutational analyses financially feasible in the clinic.

However, the identification of a mutation does not always equate to malignancy or even a precursor lesion, because DNA mutations are known to occur in benign settings or have no clinical relevance. For example, TP53 (tumor protein p53, also known as p53) signatures are present in 25% of tubal epithelium samples in healthy controls (3). Additionally, the potential of finding DNA mutations in normal cycling endometrium has not been well investigated. Another limitation of early studies is that the patients investigated had biopsy-proven disease (1, 2). Biospecimen purity could be confounded by endometrial sampling techniques that disrupt tumor architecture. Whether iatrogenic transfer of tumor and its DNA to the cervix occurs will not be known until investigations are conducted in women before biopsy. Thus, the utility of this detection approach for endometrial cancer will require validation of both the sampling technique and the gene panel through studies on women with and without cancer who have not yet undergone endometrial sampling.

Fortunately, the sojourn from benign endometrium to malignancy is thought to take years, with precursor lesions such as hyperplasias or intraepithelial carcinoma developing during the process. As such, there is a potential window of opportunity to identify women who are destined to develop endometrial cancer. DNA mutation is one step in the process of carcinogenesis and can be identified in precursor lesions. Hormonal manipulation will in some instances reverse premalignant changes, and not all untreated precursor lesions progress to invasive cancer. At the present time no clinical test exists to identify which precursor lesions will progress to malignancy. If unique DNA sig-

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natures that correlate with high risk of precursor progression could be detected in biospecimens from the lower genital tract, the findings of the recent study (1) could guide the development of a stratifying, predictive test. This is an important public health issue because the incidences of both endometrial cancer and obesity, a well-known risk factor for endometrial cancer, are rising in the US.

Additionally, women with Lynch syndrome (LS) have a genetic predisposition that places them at increased risk of ovarian cancer and endometrial cancer. Current surveillance for endometrial cancer in LS patients who have not undergone risk-reducing surgery includes an annual endometrial biopsy. While endometrial biopsies may be performed in the outpatient clinic in most patients, in some it is not technically feasible; biopsies often cause unpleasant uterine cramping, and the cost of the test is several-fold higher than the cost of a Pap smear. Kinde et al. reported that 12 of the endometrial cancers in their cohort were highly mutated with >100 somatic mutations. Among the 12, 8 were classified as MSI-H (microsatellite instability-high) and 6 had MSH2 [mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)] or MSH6 [mutS homolog 6 (E. coli)] mutations (1). This suggests that exploitation of DNA from the lower genital tract could be an attractive, more patient-acceptable alternative for screening in the LS population.

An all-inclusive screening test for gynecologic malignancies is attractive in that it would be incorporated into a collection technique that is already accepted by women as part of their well woman care. However, one question that arises is what the ideal screening interval would be for such a test. The current recommendations for cervical cancer screening are for Pap smears every 3 years or cotesting [Pap and high-risk HPV (human papillomavirus)] every 5 years. However, guidelines for cervical cancer screening are continually under review, and further investigation of cotesting may allow for lengthier intervals between Pap smears. As noted above, most endometrial cancers arise following a process of precursor lesion development, and although current recommendations are for annual surveillance in high-risk patients, a lengthier surveillance interval may be possible. Additionally, mathematical modeling suggests ovarian cancer may be present as a subcentimeter-sized mass for up to 4 years (4). Thus, while performance of surveillance for patients at highest risk of ovarian cancer is currently recommended every 6 months, a longer screening interval may be possible for ovarian cancer as well.

One crucial limitation of utilizing cervical smear DNA for endometrial and ovarian cancer screening is the potential for the female reproductive tract to be interrupted in its continuity from ovary to cervix. The endometrium is essentially contiguous with the vagina via the cervix and, while cervical stenosis may block this connection, medical or procedural approaches can ameliorate this blockage. However, for ovarian epithelium to reach the cervix, it must traverse the fallopian tube and uterus. Common procedures to prevent pregnancy (tubal sterilization, intrauterine device placement), treat menorrhagia (endometrial ablation), or definitively manage benign uterine pathology (hysterectomy) will severely limit this screening method. Also, the role of the fallopian tube in serous carcinogenesis is becoming clearer; future approaches to ovarian cancer risk reduction may include interval salpingectomy with ovarian preservation. In high-risk patients who have undergone bilateral salpingectomy, biospecimens from the lower genital tract will not reflect ovarian abnormalities. Thus, while 41% of ovarian cancers in the recent study were detected via Pap DNA analyses (1), such a screening tool will have little or no utility in women who undergo several common gynecologic procedures. Regardless, finding DNA mutations in cervical smears that are identical to the primary tumor mutations (1) is promising because this is proof that genetic material from the ovary successfully navigates through the reproductive tract. Ovarian cancer DNA is also shed into the bloodstream (5), and an alternative approach for ovarian cancer would utilize free DNA from serum.

In conclusion, leveraging the stability of DNA, contemporary molecular technology, and a collection technique that is already acceptable to women provides an exciting preliminary version of a detection test for endometrial cancer and ovarian cancer. Limitations to the clinical applicability of this test include the need to have it collected by a healthcare provider and its limited utility for ovarian cancer screening among women who have undergone certain gynecologic procedures. Additionally, validation of the biospecimen and gene panel before biopsy and investigation of a larger number of women with benign conditions are needed to further develop this detection strategy.

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