CORRESPONDENCE

Re: Detection of Hypermethylated Genes in Women With and Without Cervical Neoplasia

Feng et al. *(1)* examined whether changes in DNA methylation of 20 genes, selected on the basis of their role in cervical cancer, could be used as markers of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC). The authors found varying frequencies of promoter hypermethylation in these 20 genes in 319 exfoliated cell samples and matched tissue biopsy specimens. For four of these genes (DAPK1, RARB, TWIST1, and CDH13), increasing frequency of hypermethylation was statistically significantly associated with increasing severity of disease. The estimated specificity of the three-gene panel (DAPK, RARB, and TWIST1) was 95%, which is higher than specificities reported for cytology and human papillomavirus (HPV) testing *(1)*. Although the study was well designed and a molecular genetic test with high specificity such as this one is needed, some of the findings are inconsistent with previously reported results and the conclusions may therefore not be valid.

We believe that the following points are important to consider when interpreting the results of Feng et al. First, the high frequency of hypermethylation in the SFN, HIC1, and APC genes observed in the samples of histologically normal cells and atypical squamous cells of undetermined significance (ASCUS) is unusual. Although low frequency of methylation in a group consisting of ASCUS compared with CIN and ICC is not unexpected because of the diagnostic dilemma involved in classifying these cytologic changes into specific diagnostic categories, none of the previous studies reported such a high frequency of promoter hypermethylation for HIC1 and APC in normal cervical epithelium or other normal tissues *(2–5)*. In fact, in our series of normal and ASCUS (N = 59) and ICC (N = 82) specimens, we observed a different pattern of promoter hypermethylation in these two genes. That is, we saw promoter hypermethylation in 4% and 0% of the normal/ASCUS group and 18.3% and 11% in ICC for HIC1 and APC genes, respectively. Similarly, the authors reported a higher frequency of promoter hypermethylation of SFN than has been seen in previous studies *(6,7)*. Misclassification of methylation could occur due to amplification of nonspecific targets in the genome, which ultimately makes the results on these three genes difficult to interpret. Whether these genes have utility in cervical cancer screening needs to be confirmed in other studies.

A number of previous studies have also shown a high frequency of promoter hypermethylation of DAPK, CDH1, RARB, and p16 in ICC *(2–4)*. However, the frequency of promoter hypermethylation of these genes in CIN lesions was not well studied. Thus, well-designed studies of CIN are required to determine whether inclusion of additional genes, such as HIC1, to the three-gene panel proposed by Feng et al. might improve its sensitivity in detecting high-risk lesions that progress.

In the context of previously published studies and our series, it is premature to propose a panel of genes that would be the best for screening CIN3/CIS and ICC or for identifying high-risk CIN. Before such a test based on promoter hypermethylation can be proposed for screening cervical cancer, further studies are required on a larger series of specimens from different geographical populations, and these studies should carefully address the issue of false-positive rates.

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Notes

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RESPONSE

We agree with Murty and Narayan's comment that it is premature to conclude that the specific panel of genes that we reported to be associated with increasing severity of cervical cancer among Senegalese women to be generalizable to other populations. Studies in lung, liver, and gallbladder cancer from different geographic sites suggest that the methylation profile seen in cancers may vary by population and environmental exposures *(1–3)*. Clearly, the sensitivity and specificity of our panel of genes need to be assessed in cancer and normal tissues from various populations.

Although Murty and Narayan suggest that the high frequency of methylation of HIC1, APC, and SFN we observed in normal cervical samples is unusual and may have obscured an association with cervical cancer, others have also reported observing methylation of these genes in other benign tissues. For example, HIC1 was methylated in 30% of benign breast tissues *(5)*. APC methylation was also observed in 30% of normal liver tissues

(6), although the level of methylation was lower than what was seen in liver cancer tissues. Moreover, SFN is known to be methylated in normal lymphoid cells *(4)*. Because many of our normal samples contained inflammatory infiltrates as well as blood, the high frequency of promoter methylation observed in this gene may be attributable to these noncervical cells.

Another explanation for the difference in our respective results may be due to the different methods used to detect methylation in the two studies. Because the methylation-specific polymerase chain reaction assay we used is not a quantitative assay, there may have been a difference in methylation levels of APC and HIC methylation between normal and cancer tissues that could not be distinguished. However, unmethylated (U)-DNA (human sperm DNA) and methylated (M)-DNA (in vitro methylated human sperm DNA) were included as positive and negative controls in all experiments. Furthermore, methylation of a specific gene was considered to be present only if both the specimen and the M-DNA but not the U-DNA were amplified by methylation-specific primers after sodium bisulfate modification. By taking such measures, which have not been incorporated in many other studies, we are confident of the specificity of our findings. Finally, the differences observed between our study and that of Narayan et al. *(7)* may be related to the use of different primers detecting different CpGs within the same CpG island.

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