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DNA Methylation Profiling of Anal Intraepithelial Lesions and Anal Squamous Cell Carcinoma

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ABSTRACT

Background:

Anal intraepithelial neoplasia (AIN) is HPV-associated and may progress to invasive squamous cell carcinoma (SCC), which has recently been detected with increasing frequency in immunocompromised patients. Unfortunately, the biology of AIN is poorly understood and screening programs are not optimal. We hypothesize that AIN is associated with abnormal DNA methylation and that detection of these events may be utilized to improve screening programs.

Design:

We identified 144 patients who underwent anal cytology screening and subsequent high-resolution anoscopy and biopsy at our institution between 1999 and 2004. The cytologic and histologic diagnoses on these patients were correlated. Next, a subset of these patient specimens was selected for DNA methylation analysis. The specimens included 184 anal biopsies (normal, n=57; AIN I (LSIL), n=74; AIN II-III (HSIL), n=41; and SCC, n=12) and 37 residual liquid-based anal cytology specimens (normal, n=11; LSIL, n=12; HSIL, n=14). DNA was extracted from each specimen and then bisulfite treated in preparation for real-time methylation-specific PCR (MSP). The methylation status of the following genes was determined for each biopsy and cytology sample using real-time MSP: *HIC1*, *RASSF1*, *RARb*, *p16*, *p14*, *p73*, *APC*, *hMLH1*, *MGMT*, *DAPK1*, and *TSLC1*.

Results:

The histologic diagnoses on the biopsies included 19% normal mucosa (n=28), 47% AIN I (n=69), and 34% AIN II-III (n=48). Cytologic diagnoses on these cases included 5% negative (n=7), 30% ASC-US (n=30), 56% LSIL (n=81), and 9% HSIL (n=14). Using a cut point for referral of >ASC-US (ASC-US, LSIL or HSIL), sensitivity for detection of biopsy-confirmed HSIL was 100%, but specificity was only 35%. Increasing the threshold of referral to HSIL increased the specificity to 85%, but reduced sensitivity to 25%. Real-time MSP analysis of biopsy samples revealed that aberrant DNA methylation was more common in SCC and HSIL than LSIL and normal mucosa. Specifically, methylation of *TSLC1* and *DAPK1* occurred at a high frequency in SCC (75% and 75% of cases, respectively) and HSIL (59% and 71%) but was absent in LSIL and normal biopsy samples. Aberrant promoter methylation of *RARb*, *p14* and *MGMT* also occurred at a higher frequency in SCC/HSIL than LSIL biopsy samples and was absent in normal biopsy samples. Methylation profiles of cytologic samples were similar to those found in the biopsy samples.

Conclusions:

1) Anal cytology is highly sensitive for AIN II-III, but lacks specificity. 2) Aberrant DNA methylation is a frequent event in AIN II-III and anal SCC. 3) Methylation of *TSLC1* and *DAPK1* is unique to AIN II-III and SCC, and may serve as a useful molecular biomarker. 4) Aberrant DNA methylation can be detected in anal cytology specimens and the methylation profiles resemble those found in biopsies.

