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Chromosomal gains measured in cytology samples from women with abnormal cervical cancer screening results

Patricia Luhn [a,b,*], Jane Houldsworth [c], Lynnette Cahill [c], Mark Schiffman [a], Philip E. Castle [d], Rosemary E. Zuna [e], S. Terence Dunn [e], Michael A. Gold [f], Joan Walker [g], Nicolas Wentzensen [a]

[a] Division of Epidemiology and Genetics, National Cancer Institute, Rockville, MD, USA
[b] Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, Rockville, MD, USA
[c] Cancer Genetics, Inc., Rutherford, NJ, USA
[d] Albert Einstein College of Medicine, New York, NY, USA
[e] Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[f] Department of Obstetrics and Gynecology, Tulsa Cancer Institute, University of Oklahoma School of Community Medicine, Tulsa, OK, USA
[g] Department of Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

HIGHLIGHTS

- Chromosomal gains at multiple loci are associated with cervical disease severity.
- FISH was used to evaluate gains of four genomic loci (3q26, 5p15, 20q13 and cen7) simultaneously.
- FISH signal enumeration was achieved through an automated scanning process.

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ABSTRACT

Objective. Chromosomal gains at 3q26, 5p15 and 20q13 have been described in cervical precancer and cancer. We evaluated a novel fluorescence in situ hybridization (FISH) assay that detects gains at these three loci simultaneously as a possible biomarker for detecting cervical precancer.

Methods. Chromosomal copy numbers at 3q26, 5p15, 20q13 and the centromere of chromosome7 (cen7) in liquid-based cytology specimens from 168 women enrolled in the Biopsy Study were determined by FISH. The number of cells with ≥3 or ≥4 signals for a genomic locus was enumerated and diagnostic test performance measures were calculated using receiver operating characteristic (ROC) analyses. Sensitivity and specificity values were determined for the detection of CIN2+ and/or HSIL.

Results. The median number of cells with ≥3 signals increased with the severity of cervical lesion for each genomic locus (p-trend < 0.02 for each locus). ROC analysis for the number of cells with ≥3 signals resulted in area under the curve values of 0.70 (95% CI: 0.54–0.86), 0.67 (0.52–0.83), 0.67 (0.51–0.83) and 0.78 (0.64–0.92) for 3q26, 5p15, 20q13 and cen7, respectively, for the detection of CIN2+ and/or HSIL. Positivity for gains at multiple loci resulted in only slightly better test performance measures than those for the individual probes for four distinct combinations of probes.

Conclusions. Chromosomal gains at 3q26, 5p15, 20q13 and cen7 are associated with severity of cervical lesions. Further studies are required to quantify risk stratification of FISH assays for cervical cancer screening.

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Introduction

The implementation of cytology-based screening programs, or Pap smears, has greatly reduced the incidence and mortality of cervical cancer in the United States and other developed countries [1]. However, population-based screening programs have changed since the identification of carcinogenic types of human papillomavirus (HPV) as the necessary cause of cervical cancer. Several HPV-based tests have been developed that are more sensitive than Pap smears for identifying women with high-grade cervical disease [2,3] and therefore are superior to Pap tests in ruling out disease over extended follow-up periods [4]. However, because many HPV-positive women will clear their infection without developing cancer, HPV-based tests have limited specificity and a secondary test is needed to determine women who are HPV positive and at increased risk of progressing to high-grade disease.

* Corresponding author at: National Cancer Institute, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, 9609 Medical Center Drive, Bethesda, MD 20892-9774, USA. Fax: +1 240 276 7838.
E-mail address: luhnpa@mail.nih.gov (P. Luhn).

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Chromosomal abnormalities such as gains, losses and rearrangements of whole or parts of chromosomes are a hallmark of cancer and have been described in many tumor types, including cervical cancer [5–10]. The most commonly described abnormality in cervical cancer is gain of the q26 region of chromosome 3 (3q26) where two genes important for carcinogenesis are located, the RNA component of human telomerase (*TERC*), and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*). Genomic gain of the 3q26 locus has been associated with the progression from high-grade cervical disease to cancer [11–13]. In addition, copy number gains of several genomic regions have been detected in earlier stages of cervical carcinogenesis and have been shown to increase with increasing severity of cervical lesion, making them potentially useful biomarkers [10,14,15]. Specifically, gains at 3q26 have been suggested to have clinical utility in identifying women who are likely to progress to high-grade cervical disease [16–19].

In addition to 3q26, gains at 5p15 have also been frequently described in cervical cancer ([5–8,20] and https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). Within this region, *TERC*, the other functional component of the telomerase enzyme is thought to be one potential oncogene. Another commonly reported chromosomal gain in cervical cancer is 20q13, where *EYA2* has been suggested to be the target oncogene ([5,20–22] and https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). Furthermore, gains of 20q13 are detectable in precancerous lesions, suggesting potential utility in screening for cervical cancer [23]. Including multiple markers may improve the diagnostic measures of an assay, however, data on these and other chromosomal markers and marker combinations are very limited.

In the present analysis, we examined the feasibility of using an automated scanning method to enumerate signals from a fluorescence in situ hybridization (FISH) assay that simultaneously detects gains at 3q26, 5p15 and 20q13 in cytology specimens from women referred to colposcopy following an abnormal screening result. We evaluated the frequency of chromosomal abnormalities in disease categories and explored the diagnostic accuracy of genomic gains for the detection of high-grade cervical disease.

Materials and methods

Study population

Samples from 168 women enrolled in the Biopsy Study between 2009 and 2010 were used in the current analysis. Women in the Biopsy Study were referred to colposcopy at the University of Oklahoma Health Sciences Center (OUHSC) following an abnormal cervical cancer screening result. Details regarding the study design and inclusion/exclusion criteria have been reported elsewhere [24]. Briefly, women were excluded if they were less than 18 years of age, pregnant at the time of their visit, or previously treated with chemotherapy or radiation for any cancer or for cervical intraepithelial neoplasia (CIN). Written informed consent was obtained from all women enrolled in the study. Institutional Review Board approval was provided by OUHSC and the US National Cancer Institute.

Colposcopy and specimen collection

Details regarding sample collection have been previously described [24]. Prior to colposcopy, a cervical cytology sample was obtained using a Papette broom device and transferred directly into PreservCyt solution (Cytyc Corp). The cytology specimen was used for ThinPrep liquid-based cytology and HPV DNA testing using the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics). Up to four biopsies were taken from distinct acetowhite areas or large heterogeneous lesions extending over two quadrants. As per standard practice, all CIN3 and most CIN2 were treated by loop electrosurgical excision procedure (LEEP) of the transformation zone. Analyses presented here were based on the worst histologic diagnosis including biopsy diagnoses and LEEP outcomes.

Fluorescence in situ hybridization (FISH)

Upon receipt of specimens (0.5–1.0 ml per specimen) at Cancer Genetics Inc., cell pellets were resuspended in Carnoy's fixative (methanol: acetic acid, 3:1), and aliquots dropped onto 12 mm diameter circles on slides. Slides were aged (2 h at 37 °C) and treated with pepsin (Sigma-Aldrich, 0.5 mg/ml, Cat# P7012) for 10 min at 37 °C. After a 1× phosphate-buffered saline (PBS) wash, cells were fixed in 1% formaldehyde and washed again in 1× PBS, all at room temperature (RT). Slides were dehydrated (70%, 85%, and 100% alcohol) for 1 min each at RT and air dried. A four-color DNA-FISH probe [3q26 (904 kbp, red), 5p15 (601 kbp, green), 20q13 (493 kbp, gold), and cen7 (70 kbp, aqua), Cancer Genetics, Inc.] was applied (3 μl) to each circle, followed by co-denaturation for 3 min at 80 °C and hybridization for 48 h at 37 °C. Slides were then washed briefly in 2× saline sodium citrate (SSC) (pH 7.0), two times in 2× SSC/0.1% Tween-20 at 45 °C for 5 min each, and finally rinsed in distilled water. After air drying, DAPI/antifade (1:10, Vector) was applied to the hybridized area.

Signal enumeration

Calibration of the instrument software (Metafer4, Metasystems) for automated scanning was done using an independent set of 10 specimens with a variety of cellular and hybridization characteristics to ensure that each nuclei was counted and proper signal enumeration for each FISH probe was achieved. Initially, we evaluated the feasibility of using the multi-colored FISH assay on cervical cytology specimens, enumerating signal counts in each cell manually as previously described [13,16,17]. We then used an automated scanning method to enumerate signals, as manual scoring of FISH signals would not be feasible on a large scale. Due to the previous manual scoring of samples, volumes were limited for a number of samples for the automated scanning process, leading to sample dropout. All signal counts for nuclei with greater than 2 signals were manually confirmed. All nuclei within the 12 mm diameter circle were scanned, imaged and signals enumerated.

Final analytic population

Samples were excluded (n = 34) from the final analysis based on the following criteria: low cellularity (<100 cells in the sample; n = 21), poor sample quality (i.e., excessive cellular debris; n = 2) and weak or uneven signals for all probe colors (n = 11), leaving a final analytic population of 134 samples. Of these, 7 had uneven signals for one FISH color which was excluded from analyses (aqua/cen7, n = 6; red/3q26, n = 1). Three samples had two consecutive enumerated slides which were combined for the final analysis.

Disease categories were based on a composite endpoint using histology and cytology results, as previously described [25]: a) <CIN2 histology and normal cytology (NILM) (n = 32); b) <CIN2 histology and LSIL or ASC-US cytology (n = 45); c) CIN2 histology or <CIN2 histology with HSIL or ASC-H cytology (n = 34); and d) CIN3+ (CIN3, adenocarcinoma in situ (AIS) or cancer, regardless of cytology; n = 23). Two women were missing cytology results and were categorized based on available histology diagnoses. For analyses with dichotomized outcomes, we combined groups a) and b) from above into <CIN2 and <HSIL and c) and d) into CIN2+ and/or HSIL. Similar results were observed for endpoints based on histology or cytology alone.