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Clinical Utility of Molecular Biomarkers in Cervical Squamous Intraepithelial Lesions in a Young Adult Population

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Abstract

Objective—Although human papillomavirus (HPV) infection is necessary for cervical squamous intraepithelial lesion (SIL/CIN) and cancer to develop, exposure to HPV is not predictive of which women will develop cervical squamous intraepithelial lesion and cancer. This study examines mRNA expression of several potential biomarkers in exfoliated cervical cells collected from college age women.

Methods—Freshman female students were recruited into the Carolina Women's Care Study (CWCS) which was designed to prospectively evaluate factors that contribute to persistent HPV infections. One component of this study was to extract mRNA from exfoliated cervical cells. In this study, mRNA expression of FZD, GDF15, IL1 β and N-cadherin was assessed through real-time PCR. Statistical analysis was performed with a Student's t-test; all results were standardized with GAPDH.

Results—Fifty samples were selected that reflected the demographics of the CWCS participants. IL1 β mRNA expression was 9.4-fold higher in cervical cells from women with abnormal Pap tests ($p=0.0018$); LSIL had 12.7-fold higher expression than negatives ($p=0.0011$). FZD mRNA expression was 5.7-fold higher in CIN2 as compared to CIN1 ($p=0.0041$) and 8.5-fold higher compared to cytology/pathology-negative ($p=0.0014$). Other differences in mRNA expression showed trends but not reaching statistical significance for each condition.

Conclusions—It appears that several biomarkers involved in the cytokine/inflammatory pathway (IL1 β), cell adhesion pathway (N-cadherin), growth factors (GDF-15), WNT signaling

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pathway (FZD) may be potential biomarkers in conjunction with the Pap test and HPV that help predict which women are at highest risk for developing CIN3 and cervical cancer.

Keywords

Human papillomavirus; cervical cancer screening; cervical squamous intraepithelial lesion (SIL/CIN); molecular biomarkers

Introduction

Since the advent of the Papanicolaou (Pap) test fifty years ago, cervical cancer has become one of the most screened for and therefore preventable cancers. Human papillomavirus (HPV) is the cause of genital warts, cervical squamous intraepithelial lesion (SIL/CIN), and cervical cancer. Although, cervical cancer rates have dropped over 70%, overall rates have plateaued, and it has been at the cost of performing more colposcopies and excisional procedures [1–5]. Current screening guidelines developed by the American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologist, U.S. Preventive Services Task Force and American Cancer Society has incorporated screening for high risk HPV types to occur concurrently or reflexively depending on the age of the patient [6–11]. Primary HPV screening will also likely be used in the future, given FDA approval this past year [12].

While HPV infection is necessary for cervical SIL/CIN and cervical cancer to develop, exposure and infection with HPV is not predictive of which women will develop cervical SIL/CIN or cancer [4, 5].

Clinically, it would be useful to have an adjunct test or “biomarker” to accompany cytological evaluation to help predict which women actually have cervical intraepithelial neoplasia (CIN) 2/3 without undergoing colposcopy, biopsies, and possible surgical excision with the potential long-term complications [13 – 15]. It has been estimated that in about 35% of the women undergoing surgical excision of lesions ranging from moderate (CIN2) to severe SIL/CIN (CIN3), the lesions would have regressed spontaneously and would not have progressed to invasive cancer [1, 4, 5, 16–32]. In adolescents and young women, it has been shown that as high as 68% of CIN 2 will regress by two years, with only 15% progressing to CIN3. Current methodologies cannot predict which women with high grade lesions will progress and those that would regress [1, 5, 6, 16–32].

We propose to determine a molecular profile of HPV infections involving the intracellular pathways involved with cellular adhesion and communication (junctional complexes, growth factors) and immunologic response (cytokines) which are integrated through WNT signaling and their relationship with epithelial mesenchymal transition (EMT), SIL/CIN, and ultimately cancer. These pathways were initially identified through gene expression profiling studies using RNA isolated from HPV16 immortalized human keratinocytes during different stages of *in vitro* progression in the laboratory of Drs. Pirisi and Creek [33, 34].

In this study, we investigated mRNA expression of Interleukin 1 Beta (IL1- β ; immune and inflammatory modulator), Frizzled (FZD; WNT signaling pathway), N-cadherin (NCAD;

cell adhesion) and Growth Differentiating Factor – 15 (GDF-15; transforming growth factor β superfamily) to determine their role in cervical SIL/CIN following HPV infection in a college-aged population.

Methods

Study Population

These studies were performed in a random sample of participants from Carolina Women's Care Study (CWCS) described in detail elsewhere [35, 36]. IRB approval was obtained from the University of South Carolina Institutional Review Board. Briefly, freshman female students (18 – 22 years old) were enrolled at the Women's Care Clinic at the Thomson Student Health Center at the University of South Carolina and followed until graduation with biannual visits. At each visit, blood samples, two samples of exfoliated cervical cells and cervical mucus samples were obtained and lifestyle questionnaires were completed [35, 36]. The first Papanicolaou test sample was collected in 20 mL of PreservCyt solution (Cytoc Corporation, 2005, Marlborough, MA, USA) and sent for routine cytology; referrals for colposcopy were made in accordance with guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) and American College of Obstetricians and Gynecologists (ACOG) established at the time of the initiation of the study [35, 36]. During the time of recruitment, Pap test screening guidelines by ASCCP and ACOG were that cervical cancer screening should commence at 18 years of age or after three years of sexual activity (which is different than the 2015 guidelines).

We also selected the samples for these experiments to reflect HPV persistence and clearance and Pap test distribution of the parent population; there were multiple time points for each study participant and only one was selected to experimental analysis. The sample of study participants selected for these studies were randomly chosen among all recruited women to reflect the population's race/ethnicity, age, HPV persistence and clearance, and Pap test distribution as published elsewhere [35, 36].

Five mL of PreservCyt[®] Solution (Cytoc Corporation, 2005, Marlborough, MA USA) containing exfoliated cervical cells were removed and placed into a barcoded 15 mL conical tube for HPV detection and typing, and the remaining sample was sent for cytological evaluation. The second cervical sample was collected in 2 mL of RNeasy (Ambion, Life Technologies, Carlsbad, CA, USA), placed in a 15 mL conical tube labeled with the participant's barcode, and stored at -20°C . Additionally, all relevant demographics and medical information was abstracted from the patient's medical record. All data was placed into a password-protected Microsoft Access database (Microsoft Office, 2010, Redmond, WA) [35, 36]

HPV DNA Testing

All samples were screened for HPV as described previously [36]. Briefly, DNA was extracted from the exfoliated cells in PreservCyt[®] Solution (Cytoc Corporation, 2005, Marlborough, MA USA) using sodium dodecyl sulfate/proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation. Samples were then screened for

presence of any HPV type through a PCR amplification protocol developed by Gravitt *et al.*, utilizing the My09/My11/HMB01 primers (PGMY primer set) which also contains primers for Beta-globin which served as a positive control for human DNA [35 – 37]. Definitions of clearance and persistence of HPV infections for the samples analyzed in these studies were detailed in a previous publication [35, 36].

Extraction and quantification of mRNA

Total mRNA was extracted from exfoliated cervical cells stored in RNAlater® Stabilization Solution (Ambion, Life Technologies, Carlsbad, CA, USA) with the RNeasy Mini Kit (QIAGEN Sciences, Germantown, MD USA), which has reproducibly provided the greatest yield (1,000 to 1,500 ng per sample) and quality (RNA integrity numbers (RINs), 5.9 – 7.8) of total RNA. Total RNA was reverse transcribed using IScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc, Hercules, CA USA) to generate cDNA. Real-time PCR analysis was used to quantify mRNA expression in our samples. Upstream and downstream primers were generated against FZD, NCAD, IL1-β, and GDF-15 as well as Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; our control gene). Primer sequences: GAPDH Forward- 5'-CAA TGA CCC CTT CAT TGA CC-3', Reverse -5'-GAC AAG CTT CCC GTT CTC AG-3'; GDF-15 Forward-TGT CTC AGG AAC CTT GAG CCC ATT-3', Reverse-5'TGT CGC TCC AGA CCT ATG ATG ACT-3' (purchased from Integrated DNA Technologies, Inc., Coralville, Iowa 52241). Frizzled Forward-5'CAT GCT CAT GAA AGC AAC TG-3', Reverse-TGG AAT CCT TAC CCC CTA AT-3'; Interleukin-1 beta Forward-5'-TTC GAC ACA TGG GAT AAC GA-3', Reverse-5'TCT TTC AAC ACG CAG GAC AG-3'; Neural cadherin - Forward-5'- TGG ATG GAC CTT ATG TTG CT -3', Reverse 5'- AAC ACC TGT CTT GGG ATC AA -3' (purchased from Real Time Primers, LLC Elkins Park, PA 19027). Real-time PCR was then performed using the ICycler IQ™ PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA USA) with a 1:10 dilution of the cDNA synthesis reaction with specific upstream and downstream primers for each specific gene studied.

Statistical Analysis

Analysis of Real-time PCR data is based upon the cycle number where product is first expressed (Ct value). All data was normalized to the house-keeping gene GAPDH and standardized by a method based on the Livak et al ($2^{-\Delta\Delta Ct}$ method; 38). Initial statistical analysis will be performed with two sample two-tailed Student's t-tests on the normalized data, with statistical significance at $p < 0.05$ using JMP statistical software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina). Data is presented as fold-difference either positive/increase (+) or negative/decrease (–) gene expression.

Results

Cervical cells from 50 patients from the CWCS were analyzed. The average age and ethnic distribution reflected that of the parent CWCS study population (Table 1). Mean age was 19.02 (standard deviation 0.68), with ethnic distribution of 64% White and 22% Black.

There were multiple time points for each participant chosen; 46 study participants were HPV-16 positive during at least one time point during their participation of CWCS and only

four were never exposed to HPV. 50% had persistent HPV-16 infections, and 14% were able to clear HPV-16 infections after three visits; 14 patients were considered uninformative for HPV clearance or persistence; there were inadequate patient visits to determine persistence or clearer status [previously published 37]. Of the specific samples/time points used for these experiments, 37 (74%) analyzed in these experiments were HPV-16 positive and 13 (16%) were HPV-16 negative.

Pap test distribution reflected what would be expected for a young adult population at any specific time point, with the majority with normal cytology (82%) and very few patients with a high grade squamous intraepithelial lesion (HSIL) (2%) (Table 2). Referrals to colposcopy were made according to the 2003 ASCCP guidelines (ASCUS or greater) and were based solely on Pap test cytology analysis by Professional Pathology Services, Inc. (PPS, Inc., Columbia, SC) and not on any experimental analysis or HPV typing performed in our laboratories. At this specific time point only 9 women would have been referred for colposcopy. Only four presented for colposcopy and had cervical biopsies; three women with LSIL who had Cervical Intraepithelial Neoplasia (CIN) 1 and the woman with HSIL had CIN2; 5 women did not show for colposcopy. Four patients were too small of a number to make a meaningful analysis. Additionally, one of the clinical usefulness of a molecular marker for SIL/CIN would be if its expression is different even when SIL/CIN is not present so these women could be tracked closer. When analyzing all years of study participation at some point during their multi- year study participation, another 21 referrals for colposcopy were made secondary to abnormal Pap test cytology.

Colposcopy data for worst pathology for each was reported for each patient (Table 3). 54% of patients had negative cytology during entire enrollment of the study. Cervical intraepithelial neoplasia (CIN) 1 was found in 12 patients (31%) of patients, and 5 (13%) with CIN2 and 1 (3%) with CIN3 during their participation (Table 3).

Real-time PCR was used to assess mRNA expression of IL1-Beta, FZD, N-cadherin, and GDF-15 with standardization to GAPDH expression. Fold change was determined for the various conditions, including HPV status, Pap test cytology, and worst cervical pathology (Tables 4 and 5). Biomarker expression did not vary according to race or age (data not shown).

IL1- β mRNA expression was increased 9.3-fold in women with abnormal Pap tests compared to normal Pap tests and 12.3-fold when comparing LSIL to normal Pap tests ($p < 0.01$; Table 4). There were no statistically significant differences when comparing fold-change of mRNA expression of IL1- β when stratified by cervical pathology (CIN1: normal; CIN1:CIN2; CIN2: normal), HPV positivity or HPV persistence/clearance status (Tables 4 and 5)

FZD mRNA showed an 8.5- ($p < 0.01$) and 5.7-fold ($p < 0.01$) increase in mRNA expression when comparing CIN2 to normal or CIN2 to CIN1 cervical (Table 5). Although it was not statistically significant ($p = 0.25$), there was a 6.7-fold increase in mRNA expression when comparing HPV positive to HPV negative patients. There was no difference when

comparing women who had a persistent HPV infection versus those who cleared, abnormal versus normal Pap test cytology and LSIL compared to normal cytology (Tables 4 and 5).

Although there was a 5-fold difference in mRNA expression of N-cadherin when comparing HPV-positive patients to those who are negative, this was not statistically significant ($p=0.13$). There were no statistically significant differences in mRNA expression for N-cadherin or GDF-15 under any of the conditions we analyzed (Tables 4 and 5). (Table 4, 5).

Discussion and Conclusions

Our sample population reflects the study population in age, ethnicity, and cytology results from the parent CWCS population. IL1- β mRNA expression was increased in abnormal and LSIL Pap tests ($p<0.01$) versus normal Pap test, and FZD mRNA expression was increased in CIN 2 ($p<0.01$). GDF 15 mRNA expression was increased in ASCUS Pap tests over normal and LSIL (data not shown; $p<0.01$). Other results were not statistically significant. Post-hoc sample size analysis performed for each arm in the analyses indicated that to obtain a power of 80% and confidence interval 95% would require enrollment of 50 – 100 in each group, depending on the gene being analyzed.

These results do suggest that expression of IL1- β and FZD may show promise as molecular biomarkers that may have a role in predicting which women will develop cervical SIL/CIN. It is unlikely that one marker from one specific molecular pathway will ultimately be utilized clinically. It is more likely that a panel of molecular markers in conjunction with cytology, HPV status and type, and p16 will be used in the future to predict which women are at risk of developing cervical SIL/CIN. It would be most clinically useful if a “cut-off” value for these biomarkers could be identified prior to the women developing the cervical SIL/CIN, so the clinician could target these women for closer surveillance.

There were several constraints to our study. Specifically, it focused on a young adult population. It is well known that in this young population (<24 years old), HPV infections in general are transient, and cervical cancer rarely develops in the age group targeted by the CWCS [16 – 32]; most HPV infections and SIL/CIN will resolve by 24 – 36 months; this time frame was longer than many of our study participants were enrolled. Consensus guidelines have since also evolved since the inception of the CWCS, and Pap tests are not recommended until 21 years old (unless immunocompromised; [6–11]). One of the major limitations of the young study population of the CWCS is that there were very few women with abnormal Pap tests and even fewer with colposcopy-directed cervical biopsies with CIN 2/3. Only one woman had an HSIL Pap and only one patient had CIN3 pathology. Studies are already commenced in an older population that are more at risk for persistent HPV infections and severe cervical SIL will be more indicative of clinical scenarios.

In conclusion, in the young adult population, increased mRNA expression of members of the immune pathway and WNT signaling may predict which patients have moderate and severe cervical SIL/CIN. Thus far in this small population, we were unable to determine any molecular marker that could predict HPV persistence or clearance. It will be useful if a clinical test could identify which women, prior to exposure to HPV or developing an

abnormal Pap test, are at increased risk for developing cervical cancer in the future prior to the development of SIL/CIN.

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ABBREVIATIONS

SIL	Squamous intraepithelial lesion
IL1-β	Interleukin 1 Beta
HPV	Human Papillomavirus
mRNA	message Ribonucleic Acid
CWCS	Carolina Women's Care Study
ASCCP	American Society for Colposcopy and Cervical Pathology
ACOG	American College of Obstetrics and Gynecology
FZD	Frizzled
GDF	Growth Differentiating Factor
N-cad	Neural Cadherin
PCR	Polymerase Chain Reaction
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase
LSIL	Low Grade Squamous Intraepithelial Lesion
CIN	Cervical Intraepithelial Neoplasia
Pap	Papanicolaou
FDA	Food and Drug Administration
EMT	Epithelial Mesenchymal Transition
IRB	Institutional Review Board
mL	Milliliters
DNA	Deoxyribonucleic Acid
cDNA	complementary Deoxyribonucleic Acid

SAS	Statistical Analysis Systems
HSIL	High Grade Squamous Intraepithelial Lesion
ASCUS	Atypical Squamous Cells of Undetermined Significance
NIH	National Institutes of Health
NCI	National Cancer Institute
CRCHD	Center to Reduce Cancer Health Disparities
CCE-SPHERE	Coordinating Center of Excellence in the Social Promotion of Health Equity through Research, Education, and Community Engagement
PI	Principal Investigator
LBS	Lisa Beth Spiryda
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References

1. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol.* 1993; 12:186–192. [PubMed: 8463044]
2. zur Hausen H. Papillomavirus infections--a major cause of human cancers. *Biochim Biophys Acta.* 1996; 1288:F55–F78. [PubMed: 8876633]
3. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer.* 2002; 2:342–350. [PubMed: 12044010]
4. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. *Obstet Gynecol.* 1998; 92:727–735. [PubMed: 9764690]
5. Schlecht NF, Platt RW, Duarte-Franco E, et al. Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. *J Natl Cancer Inst.* 2003; 95:1336–1343. [PubMed: 12953088]
6. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, Solomon D, Wentzensen N, Lawson HW. 2012 Updated Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors. 2012 ASCCP Consensus Guidelines Conference. *Obstet Gynecol.* 2013; 121:829–846. PMID:23635684. [PubMed: 23635684]
7. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC. Members of LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *J Low Genit Tract Dis.* 2012; 16:205–242. PMID:22820980. [PubMed: 22820980]
8. American College of Obstetricians and Gynecologists. Practice Bulletin No. 140: management of abnormal cervical cancer screening test results and cervical cancer precursors. *Obstet Gynecol.* 2013; 122:1338–1367. PMID:24264713. [PubMed: 24264713]
9. Moscicki AB, Cox JT. Practice Improvement in Cervical Screening and Management (Picsm): Symposium on Management of Cervical Abnormalities in Adolescents and Young Women. *J Low Genit Tract Dis.* 2010; 14:73–80. [PubMed: 20043357]
10. Moyer VA. Screening for Cervical Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2012; 156:880–891. W312. [PubMed: 22711081]
11. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening

- Guidelines for the Prevention and Early Detection of Cervical Cancer. *Am J Clin Pathol.* 2012; 137:516–542. [PubMed: 22431528]
12. Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. *Gynecol Oncol.* 2015; 136:189–197. [PubMed: 25579108]
 13. Khan AM, Singer A. Biomarkers in cervical precancer management: the new frontiers. *Future Oncol.* 2008; 4:515–524. PMID: 18684062. [PubMed: 18684062]
 14. Martin CM, Kehoe L, Spillane CO, O'Leary JJ. Gene discovery in cervical cancer: towards diagnostic and therapeutic biomarkers. *Mol Diagn Ther.* 2007; 11:277–290. PMID: 17963416. [PubMed: 17963416]
 15. Dehn D, Torkko KC, Shroyer KR. Human papillomavirus testing and molecular markers of cervical dysplasia and carcinoma. *Cancer.* 2007; 111:1–14. PMID: 17219448. [PubMed: 17219448]
 16. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. *Obstet Gynecol.* 2009; 113:18–25. PMID: 19104355. [PubMed: 19104355]
 17. Moscicki AB, Ma Y, Wibbelsman C, Darragh TM, Powers A, Farhat S, Shiboski S. Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women. *Obstet Gynecol.* 2010; 116:1373–1380. PMID: 21099605. [PubMed: 21099605]
 18. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. *Am J Clin Pathol.* 2012; 137:516–542. PMID:22431528. [PubMed: 22431528]
 19. Kiviat NB, Hawes SE, Feng Q. Screening for cervical cancer in the era of the HPV vaccine-the urgent need for both new screening guidelines and new biomarkers. *J Natl Cancer Inst.* 2008; 100:290–291. PMID: 18314471. [PubMed: 18314471]
 20. Evander M, Edlund K, Gustafsson A, et al. Human Papillomavirus Infection Is Transient in Young Women: a Population-Based Cohort Study. *J Infect Dis.* 1995; 171:1026–1030. [PubMed: 7706782]
 21. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med.* 1998; 338:423–428. [PubMed: 9459645]
 22. Syculo LK, Xi LF, Hughes JP, Feng Q, Winer RL, Lee SK, O'Reilly S, Kiviat NB, Koutsky LA. Persistence of genital human papillomavirus infection in a long-term follow-up study of female university students. *J Infect Dis.* 2008; 198:971–978. [PubMed: 18694334]
 23. Winer RL, Hughes JP, Feng Q, Xi LF, Chernes S, O'Reilly S, Kiviat NB, Koutsky LA. Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women. *Cancer Epidemiol Biomarkers Prev.* 2011; 20:699–707. [PubMed: 21173170]
 24. Winer RL, Kiviat NB, Hughes JP, Adam DE, Lee SK, Kuypers JM, Koutsky LA. Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis.* 2005; 191:731–738. [PubMed: 15688287]
 25. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol.* 2003; 157:218–226. [PubMed: 12543621]
 26. Insinga RP, Perez G, Wheeler CM, et al. Incident cervical hpv infections in young women: transition probabilities for cin and infection clearance. *Cancer Epidemiol Biomarkers Prev.* 2011; 20:287–296. [PubMed: 21300618]
 27. Moscicki AB, Shiboski S, Hills NK, et al. Regression of low-grade squamous intra-epithelial lesions in young women. *Lancet.* 2004; 364:1678–1683. [PubMed: 15530628]
 28. Haidopoulos D, Voulgaris Z, Protopapas A, et al. Cervical intraepithelial neoplasia in young women. *J Obstet Gynaecol.* 2007; 27:709–712. [PubMed: 17999299]
 29. Monteiro DL, Trajano AJ, Russomano FB, Silva KS. Prognosis of intraepithelial cervical lesion during adolescence in up to two years of follow-up. *J Pediatr Adolesc Gynecol.* 2010; 23:230–236. [PubMed: 20471873]

30. Fuchs K, Weitzen S, Wu L, Phipps MG, Boardman LA. Management of cervical intraepithelial neoplasia 2 in adolescent and young women. *J Pediatr Adolesc Gynecol*. 2007; 20:269–274. [PubMed: 17868892]
31. Moscicki AB, Ma Y, Wibbelsman C, Powers A, Darragh TM, Farhat S, Shaber R, Shiboski S. Risks for cervical intraepithelial neoplasia 3 among adolescents and young women with abnormal cytology. *Obstet Gynecol*. 2008; 112:1335–1342. [PubMed: 19037044]
32. Moore K, Cofer A, Elliot L, Lanneau G, Walker J, Gold MA. Adolescent cervical dysplasia: histologic evaluation, treatment, and outcomes. *Am J Obstet Gynecol*. 2007; 197:141 e1–141 e6. [PubMed: 17689626]
33. Spiryda LB, Brown J, Zhang HM, Burgis JT. Delaying Pap test screening in the adolescent population: An evidenced based approach. *J Pediatr Adolesc Gynecol*. 2014; 27:3–5. [PubMed: 23726136]
34. Pirisi L, Yasumoto S, Feller M, Doniger J, DiPaolo JA. Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. *J Virol*. 1987; 61:1061–1066. PubMed PMID: 2434663; PubMed Central PMCID: PMC254063. [PubMed: 2434663]
35. Wan F, Miao X, Quraishi I, Kennedy V, Creek KE, Pirisi L. Gene expression changes during HPV-mediated carcinogenesis: a comparison between an in vitro cell model and cervical cancer. *Int J Cancer*. 2008; 123:32–40. PMID: 18398830. [PubMed: 18398830]
36. Banister CE, Messersmith AR, Chakroborty H, Wang Y, Spiryda LB, Glover SH, Pirisi L, Creek KE. HPV prevalence at enrollment and baseline results from the Carolina Women's Care Study, a longitudinal study of HPV persistence in college-age women. *Int J Women's Health*. 2013; 5:379–388. [PubMed: 23861602]
37. Banister C, Messersmith AR, Spiryda LB, Glover S, Pirisi L, Creek KE. Increased risk of persistent human papillomavirus infection and abnormal Pap tests in African American compared to European American women in a college-age cohort. *J Infect Dis*. 2015; 211:100–108. [PubMed: 25028692]
38. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. Improved amplification of genital human papillomaviruses. *J Clin Microbiol*. 2000; 38:357–361. [PubMed: 10618116]
39. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods*. 2001; 25(4):402–408. [PubMed: 11846609]

Table 1

Demographics

	Study Group	CWCS [reference 36]
Age	19.02	18.8
Race (%; n)		
White	64% (32)	69.8%
Black	22% (11)	24.2%
Hispanic	6% (3)	2.8%
Asian/Other	8% (4)	3.2%

Table 2

Pap Test Cytology

Cytology % (n)	Study Sample	CWCS [36]
Negative	82% (41)	88.0%
Atypical Cells of Undetermined Significance (ASCUS)	6% (3)	4.9%
Low Grade Squamous Intraepithelial Lesion (LSIL)	10% (5)	5.8%
High Grade Squamous Intraepithelial Lesion (HSIL)	2% (1)	1.1%
No Result	0	0.2%

Table 3

Cervical Pathology Results from Colposcopy Directed Biopsies (Worst Pathology at any time during enrollment)

Cervical Pathology	% (n)
Negative	53.8% (21)
CIN1	30.8% (12)
CIN2	12.8% (5)
CIN3	2.6% (1)

Table 4

Differences in mRNA expression HPV status and Pap test result: Presented as Fold-Change

Condition	IL1- β	FZD	N-Cadherin	GDF 15
HPV-16 status				
Positive vs. Negative	(+) 1.8 p=0.63	(+) 6.7 p=0.25	(+) 5.0 p=0.13	(+) 1.4 p=0.63
Cleared vs. Persistent HPV	(+) 1.2 p=0.88	(-) 1.9 p=0.55	(+) 3.1 p=0.34	(+) 2.1 p=0.20
Pap test				
Abnormal vs. Normal	(+) 9.3 p<0.01	(+) 1.7 p=0.50	1.0 p=0.99	(+) 2.5 p=0.11
LSIL vs. Normal	(+) 12.3 p<0.01	(+) 2.3 p=0.43	(-) 1.5 p=0.72	(-) 1.1 p=0.94

Data is presented as fold-change or ratio between normalized mRNA data in each condition. (+) value indicated increased difference and (–) value indicates decreased amount as discussed in the Methods section.

Table 5

Difference mRNA Expression and Worst Cervical Pathology: Presented as fold change

Cervical Pathology	IL1-β	FZD	N-Cadherin	GDF 15
CIN 1 vs. Normal	(-) 2.4 p=0.43	(+) 1.4 p=0.78	(-) 1.4 p=0.77	(+) 1.4 p=0.57
CIN 2 vs. Normal	(-) 1.6 p=0.74	(+) 8.5 p<0.01	(-) 4.2 p=0.68	(-) 1.3 p=0.80
CIN 2 vs. CIN 1	(+) 1.5 p=0.85	(+) 5.7 p<0.01	(-) 3.0 p=0.82	(-) 1.9 p=0.79

Data is presented as fold-change or ratio between normalized mRNA data in each condition. (+) value indicated increased difference and (-) value indicates decreased amount as discussed in the Methods section.