Use of DNA Methylation Markers to Predict Cervical Dysplasia Outcomes

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Objective

This study aims to identify women with high-grade cervical dysplasia among women who test positive for HPV using established DNA methylation markers that have been shown to be involved in cervical cancer.

Background

Cervical Dysplasia and HPV

Each year, approximately 3 million women in the United States are diagnosed with Human Papillomavirus (HPV) -associated cervical dysplasia. Most individuals with a low-grade dysplasia diagnosis will clear it naturally, however, a few women will progress to high-grade dysplasia which increases their risk of developing cervical cancer.[1] Similarly, not every woman who has HPV needs treatment, but there are no clinical tests currently that can distinguish benign infection from clinically significant infection. Ultimately, we hope to apply DNA methylation testing in conjunction with at home HPV testing to identify women who need treatment.

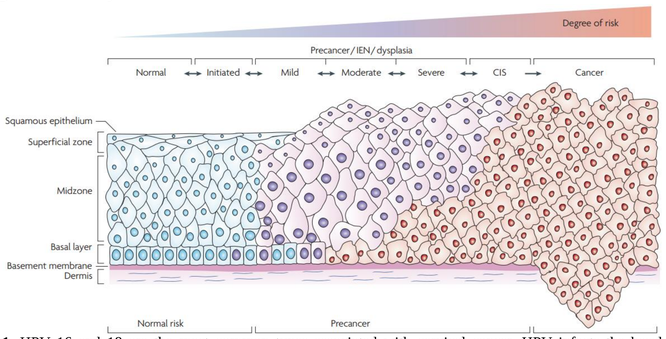

Figure 1. HPV 16 and 18 are the most common types associated with cervical cancer. HPV infects the basal layer of epithelial tissue and induces abnormal cellular proliferation (purple nucleated cells). During the infection, cells become more dysregulated, and the number of abnormal cells correlates with the severity of the dysplasia diagnosis. Without treatment, some women develop cancer.[2]

DNA Methylation

DNA methylation is the chemical modification of DNA.
- Plays an important role in normal development and cellular biology
- Alters gene expression and protein production
- Maintenance of genome integrity

DNA Methylation in Cancers
Hypermethylation:
- DNA repair genes are silenced
- Silencing of tumor suppressors
- Aids in the development and progression of cancer

Hypomethylation:
- Dysregulation of tumorigenesis
- Upregulation of oncogenes and proto-oncogenes [3]

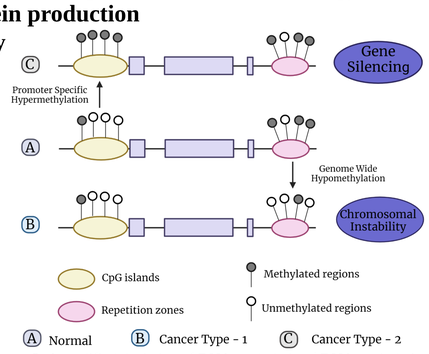

Figure 2. A modified depiction of DNA methylation.[4] DNA methylation of the promotor and the gene is dysregulated in oncogenesis.

Cervical Cancer Markers

DNA methylation markers ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671 have been found to be hypermethylated in cervical cancer cases.
- Regulate cell differentiation
- Proliferation
- Apoptosis
- Tumor suppression

DLX1 is another cervical cancer marker found to be hypomethylated in aggressive cancers.

Figure 3. Methylation marker panel was identified by Oncgnostics, GmbH.

We hypothesize that this methylation panel will be able to distinguish patients who require early intervention treatment and predict patient's prognosis among women who test positive for HPV.

Results

Demographics

Characteristics	HPV Positive	HPV Negative
Sample Size (n, %)	13 (100)	13 (100)
Age (avg, range)	40 (20-64)	50 (26-62)
<35	5 (38.46)	7 (53.85)
≥35	8 (61.54)	6 (46.15)
HPV Positive (n, %)		
High-risk HPV	9 (69.23)	-
Low-risk HPV	4 (30.77)	-
Race (n, %)		
White/Caucasian	3 (23.08)	1 (7.69)
Black/African American	8 (61.54)	8 (61.54)
Other/Unknown	2 (15.38)	4 (30.77)
Education Level (n,%)		
High school diploma or less	9 (66.23)	5 (38.46)
Some college and beyond	3 (23.08)	7 (53.85)
Unknown	2 (15.38)	1 (7.69)
Income (n, %)		
≤$20,000	7 (53.85)	9 (69.23)
>$20,000	4 (30.77)	3 (23.08)
Unknown	2 (15.38)	1 (7.69)
Baseline Cytology Result (n, %)		
LSIL	3 (23.08)	3 (23.08)
HSIL	3 (23.08)	2 (15.38)
Negative	5 (38.46)	7 (53.85)
Unknown	2 (15.38)	1 (7.69)

Figure 4. Demographics table of the cohort used for this study. Statistical significance was not observed between HPV positive and negative individuals. Calculations were done using Fisher's Exact Test.

- Total sample size is 26
- Similar number of HPV positive and negative individuals were identified in the cohort
- No significant difference was observed between the two groups
- Majority of population is:
  - Black/African American
  - Has a high school diploma or less
  - Has an income of less than or equal to $20,000

HPV Results

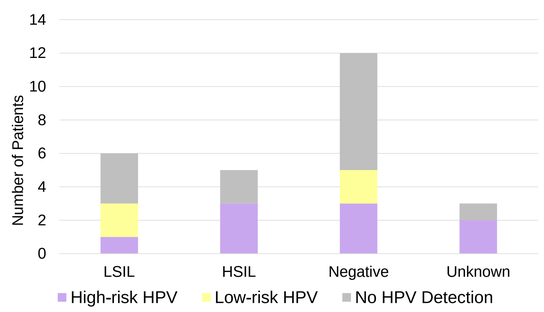

Figure 5. High-risk and low-risk HPV detection stratified by the corresponding Pap test with confirmed biopsy results. No significant difference was observed among HPV detection and diagnosis. Significance was determined by Fisher's Exact Test.

- No significance seen between high-risk and low-risk HPV types in LSIL
- HSIL is associated with high-risk HPV types
- Quality of samples may have diminished over time leading to less HPV detection

Methods

- DNA extractions were performed on 26 archived cytology specimens (pap tests) and HPV was detected and genotyped with Roche linear array (LA)
- Gyntect® for detection of methylation markers-Methylation was scored using GynTect's protocol
  - Bisulfite treatment and purification of DNA
  - Real Time PCR

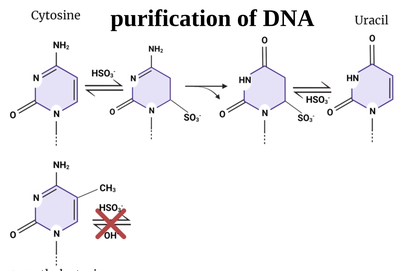

Figure 6. A modified depiction of bisulfite conversion of unmethylated cytosine. Methylated cytosine is protected.[5]

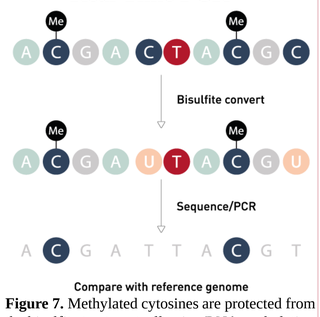

Figure 7. Methylated cytosines are protected from the bisulfite treatment, allowing DNA methylation to be determined at a nucleotide level.[6]

Methylation Analysis and Cytology

	HSIL	LSIL	Negative	Unknown	Total
Tested samples (n,%)	5 (100)	6 (100)	12 (100)	3 (100)	26 (100)
Valid	4 (80)	6 (100)	6 (100)	3 (100)	19 (73.08)
Invalid	1 (20)	-	6 (50)	-	7 (26.92)
Analysis of valid samples (n,%)	4 (100)	6 (100)	6 (100)	3 (100)	19 (100)
Positive	4 (100)	1 (16.67)	2 (33.34)	2 (66.67)	9 (47.37)
hr-HPV+	2 (50)	1 (100)	-	1 (33.34)	4 (44.45)
Negative	-	5 (83.34)	4 (66.67)	1 (33.34)	10 (52.63)
hr-HPV+	-	-	1 (25)	1 (100)	2 (20)
Detection rate (%)	100	16.67	33.34	66.67	-

Figure 8. Data table depicting patients whose methylation status was scored as GynTect positive, negative, or invalid. These data are stratified by cytology and hr-HPV results. Table does not include specific methylation markers each patient had detected.

- HSIL
  - All samples with valid results scored GynTect positive
  - 50% hr-HPV positive by LA
- LSIL
  - 83.34% scored GynTect negative
  - 1 patient scored GynTect positive and had hr-HPV; this patient is predicted to progress to HSIL
- Negative
  - Majority of samples were either invalid or GynTect negative
  - Negative cytology results are not expected to receive methylation analysis as this may lead to over treatment
- 9 (47.37) patients scored as GynTect positive
  - It is predicted that these patients are at risk of developing cervical cancer

Conclusions

- The GynTect DNA methylation panel is a promising diagnostic tool that can detect HSIL
- GynTect DNA methylation status, along with HPV genotyping and cytology results may be able to predict progression in patients with LSIL
- *It can be postulated that patients who were not scored as GynTect positive but still had some DNA methylation markers present will regress*

Future Work

- Examine patients who are overall GynTect negative but positive for DNA methylation markers
- Retrospective study looking at methylation status of patients with LSIL and known outcomes (HSIL or resolution) to determine the predictive quality of the assay
- Ultimate goal: create an at home test that can test for HPV as well as progressive cervical dysplasia, allowing for women to have easier access to screening and appropriate treatments

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References

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