HPV integration and methylation patterns in cervical intraepithelial neoplasia and invasive cervical cancer via HPV capture and high-throughput sequencing

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Abstract

Persistent infection with high-risk human papillomaviruses (HPV) types is essential for the development of cervical cancer and its immediate precursor, cervical intraepithelial neoplasia 3 (CIN3). However, HPV infection alone is not sufficient to promote cervical carcinogenesis, thus it is hypothesized that the progression to invasive disease is dependent on viral and host factors. For example, scattered CpG sites are located throughout HPV genomes, with CpG islands becoming increasingly methylated by the host cell’s DNA methyltransferases, potentially causing alterations in the expression patterns of viral genes relevant to transformation. In addition to epigenetic changes, HPV integration into the host genome is strongly associated with cancer progression and considered a signature of invasive cervical cancer (ICC). HPV integration contributes to the malignant phenotype in cervical cancer by modifying host cell genes and HPV gene expression. The goal of the current study was to investigate the landscape of HPV integration into the cervix and its relationship with HPV gene expression. The goal of the current study was to investigate the landscape of HPV integration into the cervix and its relationship with HPV gene expression.

Methods and Sample Information

HPV typing for each sample

Conclusions and Future Directions

- We have successfully utilized bait designed to target 63 unique HPV types to examine methylation and integration profiles in CIN and ICC samples.
- Bisulfite sequencing demonstrates increased methylation in and around the HPV L2 region in ICC versus CIN samples.
- Multiple HPV subtypes can integrate into the host genome in ICC. Validated integration sites never had breakpoints in the viral E6 or E7 oncoproteins. Different patterns of HPV integration occurred within the same host genomics.
- HPV variant analysis of samples run on the same lane in an Illumina flow cell shows that cross-contamination may occur during sequencing. Additionally, we have determined that in order for a sample to be considered positive for any HPV type, the number of reads for that type must account for at least 0.1% of total viral reads.
- Future work will include: (1) determination of HPV methylation and integration in 48 remaining samples, (2) pinpoint methylation differences in CIN versus ICC that could potentially aid as future biomarkers for screening and diagnosis of cervical cancer (3) detailed investigation of HPV methylation patterns adjacent to integration sites, and (4) further examination of the functional consequences of integration sites and how they may contribute to cervical carcinogenesis. 

Using HPV Variant Analysis to Identify Sample Cross-Contamination During Sequencing

Bisulfite sequencing analysis of 32 ICC and 8 CIN specimens. The table contains a percentage of reads that are in the same lane during sequencing. Multiple HPV variants were identified in each sample. Sample Prep 11 2 2 2 3 3 1 1 1 1 1 1 1 Sample Prep 2 1 1 1 1 1 1 1 1 1 1 1 1

HPV typing for each sample

Sample Prep 1 1 1 1 1 1 1 1 1 1 1 1 1