

(229399_1) - Combined approach using the Illumina Infinium Methylation27 BeadArray and Affymetrix Tiling Chip Set: Preliminary results illustrate methylation differences in HPV(+) and HPV(-) head and neck cancers

Maureen A Sartor, Dana C. Dolinoy, Tamara Jones, Thomas E. Carey, Laura S. Rozek

University of Michigan, Ann Arbor, MI

Human papillomavirus (HPV)-associated head and neck cancers (HNSCC) have a distinct risk profile and appreciate a prognostic advantage compared to HPV-negative HNSCC. Promoter hypermethylation has been widely recognized as an important mechanism in the progression of HNSCC, but the extent to which this mechanism is consistent between HPV(+) and HPV(-) tumors is unknown. The purpose of this study is to assess the performance and benefits of the Illumina Infinium HumanMethylation27 BeadArray versus the whole-genome Affymetrix Tiling Chip Set using 5-Me-C antibody to enrich for methylated regions (performed by Genpathway, San Diego, CA) for genome-wide identification of regions of altered methylation (RAMs) in HPV(+) vs HPV(-) tumors.

DNA were isolated from two HPV(+) and two HPV(-) cell lines. Subjects were matched by age and one female and one male each of HPV(+) and HPV(-) was used. The HumanMethylation27 BeadArray provides quantitative measurements of DNA methylation for 27,578 CpG sites spanning 14,495 genes. It is inherently different from virtually all other cy5/cy3 platforms in that the dye for unmethylated and methylated probes at any single chromosomal location is the same, and dye is determined based on the nucleotide following the CpG. Using bioinformatics approaches, we show that even though a single dye is used for unmethylated and methylated probes at any one location, a dye bias still exists that affects results when not accounted for. Since local regression is, therefore, not applicable in this situation, we developed a novel normalization strategy based on quantiles to correct for dye biases that does not assume equal global promoter methylation among samples. In testing for RAMs, we demonstrate an empirical Bayes spline-based method that accounts for the changes in probe variance across the % methylation range (for Illumina) and across the intensity range (for Affymetrix).

Results from both the BeadArray and Tiling analyses indicate that HPV(+) tumors have overall higher gene promoter methylation than HPV(-) tumors. Although this result was not significant treating subjects as random ($p=0.089$) with this small sample size, it is consistent with reported genome-wide hypomethylation and promoter hypermethylation in HPV(+) HNSCC tumors. Top target genes, including the estrogen receptor alpha, *ESR1*, are being validated in primary HPV(+) and HPV(-) HNSCC tumors using Pyrosequencing (Qiagen) technology. Data from the Illumina BeadArray indicates *ESR1* methylation of 4% in HPV(-) tumors compared to 82% in HPV(+) tumors, while the Affymetrix Chip Set estimates a 25-fold increase in methylation in the HPV(+) tumors. Together, a combined approach using tiling and bead arrays is a powerful unbiased method of detection for genome-wide differentially methylated regions. We conclude that the tiling array platform provides much higher coverage and an unbiased view across the genome, while the HumanMethylation27 BeadArray offers a direct and clear test of many important gene promoter sites. This approach identifies novel epigenetically labile genes associated with HPV status in HNSCC and will aid in the development of diagnostic and therapeutic strategies.