DIFFERENTIAL HYPERMETHYLATION IN ORAL SQUAMOUS CELL CARCINOMA USING MICROARRAY-BASED DNA METHYLATION ANALYSIS


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Introduction: DNA methylation is an essential epigenetic modification that indirectly regulates the mammalian genes expression, both in health and disease. Aberrant promoter hypermethylation will silence the gene expression. Thus, methylation-induced gene silencing is as important as mutation in cancer progression. The identification of differential hypermethylation using methylation array analysis is expected to be useful in diagnosing and predicting oral squamous cell carcinoma. If this study found to be reliable, the hypermethylated genes could serve as biomarkers for early cancer diagnosis.

Objective: To investigate the differential hypermethylation in oral squamous cell carcinoma (OSCC) using microarray-based DNA methylation analysis

Methodology: Extracted and bisulfite-converted DNA of 3 normal subjects and 20 patients OSCC from frozen section tissues were used for the DNA methylation array, from Illumina, Infinium Methylation 450K assay. Raw data obtained were normalized and filtered using GenomeStudio software. The significant methylated gene list was generated using one-way ANOVA with p-value of less than 0.05 and fold change more than 2.0 by Partek Genomics Suite 6.5. These genes were further classified for differentially methylated pathway using Panther Classification System. Few highly significant hypermethylated genes were selected to further validate the differential hypermethylation by using methylated-specific PCR (MSPCR) techniques.

Results From Partek Genomics Suite 6.5, Principal Component Analysis (PCA) clustered the normal subject samples differently from OSCC cases. In addition, heat map showed distinct methylation profile. 91 hypermethylated and 85 hypomethylated genes were identified in supervised cluster analysis. The selected hypermethylated genes that found in the pathway deregulation have showed high positivity of evidence using MSPCR technique.

Discussion and Conclusion: Methylation array analysis of highly differential promoter hypermethylation in OSCC cases was evidenced and validated using MS-PCR. Our hypermethylation biomarker approach shows that methylation array analysis can produce specific hypermethylated genes that used as biomarkers for early oral squamous cell carcinoma detection.

Keyword: promoter hypermethylation, microarray-based DNA methylation, oral squamous cell carcinoma, methylated-specific PCR (MS-PCR)