

Promoter Hypermethylation Quantification in Oral Dysplasia and Cancer

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Chi Tonglien Viet

University of California, San Francisco, CA

Background

Promoter hypermethylation, a major mechanism in silencing cancer-associated genes, is potentially an early diagnostic marker for oral cancer. Saliva is an ideal diagnostic fluid because of ease of collection. However, to date there has been no study quantifying promoter hypermethylation in the saliva of oral dysplasia (pre-cancer) and cancer patients.

Objectives

1. To quantitatively analyze promoter hypermethylation for five genes (p15, p16, MGMT, E-cadherin, and APC) using tissue and saliva from three groups of patients: normal, dysplasia, and cancer.
2. To determine the correlation between saliva and tissue promoter hypermethylation.

Methods

Saliva was collected from 11 oral cancer, 3 oral dysplasia and 5 normal subjects. MethyLight, a fluorescence based real-time PCR technique, was used to quantify DNA methylation at the locus of interest in the samples. The percentage of fully methylated molecules at the locus of

interest, indicated as PMR (percentage of methylated reference), was calculated for each sample and compared to normal. Cancer and dysplasia saliva samples with a PMR higher than the normal threshold were classified as hypermethylated.

Results

Hypermethylation of >1 genes was demonstrated in the saliva of 71% of samples; >2 genes in 29%; >3 genes in 7% and >4 genes in 7%. Hypermethylation status between saliva and tissue samples matched at 87.5%, 87.5%, 62.5%, 62.5%, 12.5% for p16, E-cadherin, p15, MGMT, and APC, respectively, with a mean correlation of 62.5%.

Conclusions

We report for the first time quantitative analysis of promoter hypermethylation in the saliva and tissue of oral dysplasia and cancer patients. Promoter hypermethylation in at least one gene was detected in 79% of saliva samples with a high correlation between tissue and saliva, which makes detection of promoter hypermethylation in saliva a promising early marker for oral dysplasia and cancer.