CHAPTER 8

EPI Early Detection Tool for Oral Cancer: Promoter Hypermethylation

Khor Goot Heah[*a], Gabriele Ruth Anisah Froemming[b], Rosnah Binti Zain[c]

*[a]*Centre of Studies for Preclinical Science, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buluh campus, Selangor, Malaysia.*
[b]Faculty of Medicine, Universiti Teknologi MARA, Sungai Buluh campus, Selangor, Malaysia.
[c]Oral Cancer Research and Coordinating Centre, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

Abstract

The present invention provides an early detection for of oral cancer using biomarker of promoter hypermethylation. Oral cancer become one of the major public health problems and is the sixth most common human malignancy with approximately 50% of five year mortality rate. High morbidity rate can be attributed to majority patients are diagnosed at disease late stage. Thus, appropriate biomarker for early oral cancer identification is very crucial to reduce the cancer morbidity. Promoter hypermethylation has been observed in different types of cancer. Thus, identification of appropriate biomarkers, TP73 promoter hypermethylation can be useful for early diagnosis and prognosis of oral cancer. To evaluate TP73 hypermethylation as early and sensitive detection tool for identifying oral cancer. TP73 was chosen as a signature candidate after screened using methylation microarray, followed by test validation of methylation-specific polymerase chain reaction analysis and immunohistochemical staining in tumour tissues. In tumour cases, 93% in promoter hypermethylation and 15% protein expression were observed in methylation-specific polymerase chain reaction and immunohistochemistry respectively. TP73 hypermethylation acts as early and sensitive diagnostic tool for oral cancer. This invention can be applied as diagnostic tool for early cancer detection. In addition, it also aids as cancer

screening test for healthy population. In short, this invention improves social sustainability by creating a healthy community.

Introduction

Oral cancer is the sixth most common cancer with more than 300,000 cases reported in worldwide yearly. Its incidence has been increasing over the last decade according to GLOBOCAN. Its 5-year survival rate is less that 50%. The 5-year survival rate is related to stage at the time of cancer diagnosis, thus early diagnosis and treatment still remain as main factors in improving the overall survival rates of oral cancer patients.

Promoter hypermethylation leads to suppression of housekeeping and cell cycle control genes, tumor suppresser genes and DNA repair genes, leading to tumor growth and progression. Silencing of the genes by promoter hypermethylation or induction of oncogenes by promoter hypomethylation is frequent mechanisms in different types of cancer and potentially may be of increasing diagnostic and therapeutic importance since the DNA methylation alterations are reversible.

Identification of appropriate gene's promoter hypermethylation in the recent years could be used as biomarker panels for better cancer detection, or prognosis, an individual's cancer risk assessment of recurrence and/or progression after diagnosis, and prediction of the therapeutic response. Therefore, TP73 promoter hypermethyklation for oral cancer identification was investigated in the present study.

Research Methodology

Archived specimens of healthy and tumour tissues were used for this study. Extracted materials of human genomic DNA was applied in microarray platform. TP73 gene was selected as signature candidate based on appropriate selection criteria. MSPCR and IHC assays were applied to validate the TP73 significant hypermethylation and protein expression respectively. Methylation specific PCR (MSPCR) was applied to rule out differential methylation level in tumour tissues. The bisulfite-converted DNA was subjected to PCR machine following cycling conditions: Initial activation step: 95oC for 10 mins; 35 cycles of PCR in the denaturation step: 94oC for 15 sec; annealing step: 54 oC for 20 sec; extension step: 72oC for 30 sec and final extension: 72oC for 10 mins.

Immunohistochemical staining was applied for protein expression validation. performed with DAKO REAL EnVision Detection System (Dako, USA, Capinteria, CA). Protocol was performed according to the manufacturer's instructions (Dako, USA, Capinteria, CA).

The data was further analysed by Statistical Package for Social Sciences (SPSS software, version 17, Chicago, USA), with Chi-square or Fisher's Exact for categorical variables and independent sample T-tests for continuous normally distributed variables. When p value was less than 0.05, the statistical difference was regarded as significant.

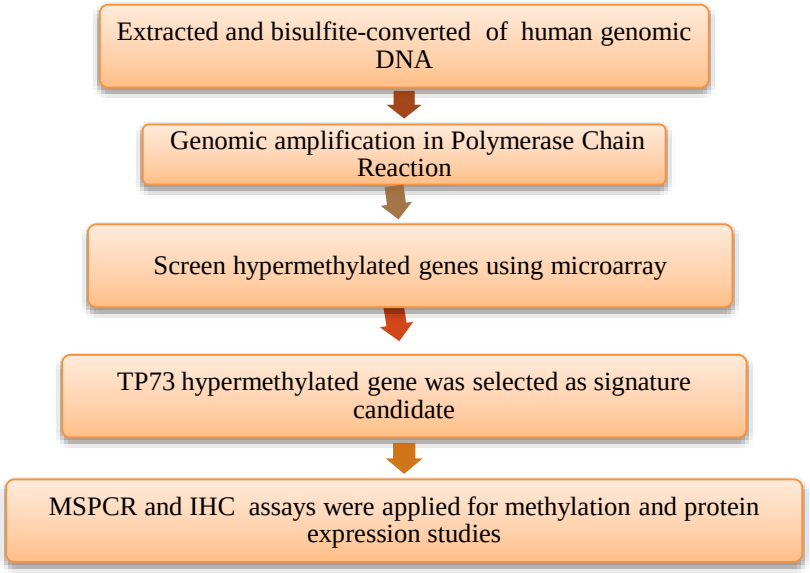

Fig. 1: Workflow of the study

Results

Analysis of the methylation profile resulted in selection of significate candidate of TP73 promoter-associated hypermethylationwith with an average β value of 0.4 in methylation ($p < 0.001$).

In MSPCR analysis, TP73 was found positivie in 93% and negative in 7% of tumour cases respectively. TP73 hypermethylation status was shown in agarose gel electrophoretic images as in Fig. 2. In Immunohistochemical analysis, 15% of positive and 85% negative in immunostaining were demonstrated in tumour cases for TP73 protein expression as shown in Fig. 3.

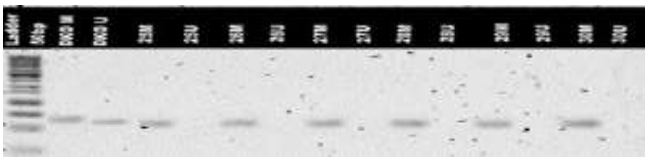

Fig. 2: Agarose gel electrophoretic image shows bands of TP73 methylation status.

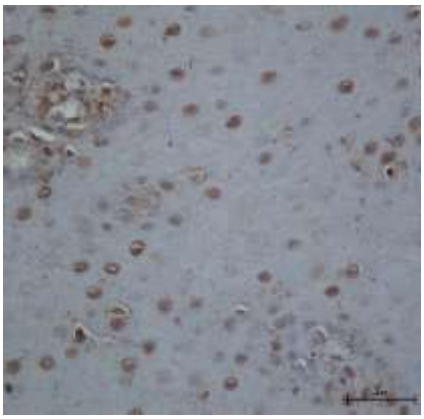

Fig. 3: Oral Squamous Cell Carcinoma tissue section shows positive TP73 immunostaining (20X magnification).

Conclusion

The present invention can be used as an early and sensitive tool to identify oral cancer. In addition, it also aids as cancer screening tool for healthy population as an early preventive measurement. In short, It promotes social sustatibility by maintaining the healthy community.

Acknowlegment

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