ABSTRCT

DNA Methylation Analysis of
E-cadherin, β-catenin, $p16^{INK4\alpha}$ and MGMT
in Oral Lichen Planus.

2016
Health Sciences University of Hokkaido
Graduate School of Dentistry
Takatoshi Chujo

Introduction

Oral lichen planus (OLP) is known as a chronic inflammatory disease with dyskeratosis that affects oral mucosa. The etiology of OLP is unknown, and environmental factors such as allergy caused by dental metals and drug or viral infection may be involved in the OLPs.

Epigenetics modifications are cased by the environmental factors. Epigenetics are patter of gene expression not involved in the DNA sequences. DNA methylation is a phenomenon of the epigenetic changes. Although the DNA hypermethylations of E-cadherin, ß-catenin, $p16^{ink4a}$ and O^6-methylguanine-DNA methyltransferase (MGMT) were shown followed by their regulated expressions in oral cancers and precancerous lesions, these phenomena have not been observed in OLP thus far.

The purpose of the present study is to investigate DNA methylation in promoter regions of E-cadherin, ß-catenin, $p16^{ink4a}$ and MGMT in OLP, compared with non-inflammation gingiva (Control), radicular cyst (RC) and oral squamous cell carcinoma (SCC).

Materials and Methods

1. Materials

The paraffin-embedded tissue samples obtained from biopsy specimen and operation material of OLP, RC, SCC and Control were used in this study. This study was approved by the ethics committee of Institute of Personalized Medical Science, Health Sciences University of Hokkaido (No. 2012-005). Paraffin slices were made using Microtome.

2. DNA purification

1)DNA extraction

Genomic DNA was extracted from paraffin slices using Epitect Plus FFPE Lysis kit®.

2)Bisulfite conversion

Genomic DNA was treated with sodium bisulfite using EpiTect Plus DNA

Bisulfite Kit®.

3. Primer design

For Methylation Specific PCR (MSP), The methylated and unmethylated primers were designed between the promoters and retro-element of the promoters in E-cadherin, ß-catenin, $p16^{ink4a}$ and MGMT.

4. Semi-quantitative MSP (semi-qMSP)

The expression levels of methylated and unmethylated DNA were analized using
agarose gel electrophoresis.

5. Quantitative MSP (qMSP)

The expression levels of methylation were analized using SYBR quantitative MSP.

6. Statistical analysis

Results were compared using the Kruskal-Wallis test with $p < 0.05$ accepted as statistically significant.

7. Immunohistochemistry

Monoclonal Mouse Anti-Human E-Cadherin , Polyclonal Rabbit Anti beta Catenin, Monoclonal Rabbit Anti clone EPR1473 p16 and Monoclonal Mouse Anti MT3.1 MGMT were used as primary antibody. EnVision+System-HRP Labelled Polymer Anti-mouse and EnVision+System-HRP Labelled Polymer Anti-Rabbit were used as secondary antibody.

The evaluation of immunostaining was graded as − (under 10% positively stained cells), ± (10–25% positively stained cells: Weak expression), + (25–50% positively stained cells: Mild to moderate expression) and ++ (50–100% positive cells: Moderate to strong expression).

Results

1. DNA methylation analysis by MSP

E-cadherin

The band intensity of OLP and SCC was high in methylated primer, and that of Control and RC was high in unmethylated primer by semi-qMSP.

The DNA methylation level in OLP was significantly higher than in Control and RC, but no significant in SCC by qMSP.

ß-catenin

The band intensity of OLP and SCC was high in methylated primer, and that of Control and RC was high in unmethylated primer by semi-qMSP.

The DNA methylation level in OLP was significantly higher than in Control, RC and SCC by qMSP.

$p16^{ink4a}$

The band intensity of OLP and SCC was high in methylated primer, and that of Control and RC was high in unmethylated primer by semi-qMSP.

The DNA methylation level in OLP was significantly higher than in RC, and significantly lower than in SCC, but no significant in Control by qMSP.

MGMT

The band intensity of OLP and SCC was high in methylated primer, and that of Control and RC was high in unmethylated primer by semi-qMSP.

The DNA methylation level in OLP was significantly higher than in Control and RC, but no significant in SCC by qMSP.

2. Immunohistochemistry

E-cadherin

The positively stained cells were ++ in Control and RC, and + in OLP and SCC.

ß-catenin

The positively stained cells were ++ in Control and RC, and + in OLP and SCC.

$p16^{ink4a}$

The positively stained cells were ++ in RC, and + in Control, OLP and SCC.

MGMT

The positively stained cells were + in Control and RC, and − in OLP and

SCC.

Conclusion

The results indicate that the DNA hypermethylations of E-cadherin, ß-catenin and MGMT may be involved in the pathogenesis of OLP. These hypermethylations may be usd for a predictive diagnosis and a therapeutic target for OLP.