A B S T R A C T O F T H E S I S

Involvement of *sirtuin 1* (*SIRT1*) in betel quid chewing related oral cancer

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INTRODUCTION

Sirtuins (SIRTs) are class III histone deacetylase family protein, comprising seven members, SIRT1-7. SIRT1 was the first family member to be discovered and is the most studied molecule. Dysregulation of *SIRT1* expression has previously described in many human malignancies including oral cancer. However, the physiological relevance of *SIRT1* in betel quid (BQ)-related oral cancer remains unexplored. BQ quid-related oral cancer is often preceded by the development of precancerous lesions, characterized by the disruption of epithelial integrity and, consequently, the transformation to invasive cancer. *SIRT1* has been identified as playing a role in the maintenance of epithelial integrity and contributing to the prevention of both the invasion and metastasis. These observations suggest that decreased *SIRT1* expression may occur in oral cancer induced by BQ chewing habit. Since the downregulated expression of *SIRT1* has been attributed to DNA hypermethylation, we hypothesize that DNA hypermethylation of *SIRT1* may be observed followed by its transcriptional downregulated expression in BQ chewing oral cancer patients.

In the present study, we analyzed whether the hypermethylation of *SIRT1* followed by its transcriptional downregulation in the human gingival epithelial cells could be caused by arecoline, a major component of BQ. In addition, we investigated the methylation status of *SIRT1* in smear samples of macroscopically healthy buccal mucosa from subjects with a habit of BQ chewing. Furthermore, we examined the methylation status of *SIRT1* in paraffin-embedded tissue samples of oral squamous cell carcinoma (OSCC) obtained from BQ chewing and non-chewing patients and in tissues samples from healthy control subjects for clarifying the role of *SIRT1* in carcinogenesis.

MATERIALS AND METHODS

1: Cell culture and arecoline exposure

Human gingival epithelial progenitors (HGEPs) cells were cultured and treated with

arecoline at a concentration of 50 μg/mL. The culture media, alternating with and without arecoline, was replaced every 3 days for 30 days. Untreated samples were used as controls. The DNA methylation, mRNA expression, and protein production were assessed.

2: Buccal smear samples and clinicopathological data collection

A convenience sample of 70 study subjects were recruited and classified into two groups: controls (45 healthy, BQ non-chewers) and BQ chewers (25 healthy, chewers). Genomic DNA was extracted from buccal smear samples and DNA methylation status of the *SIRT1* gene was analyzed.

3: Tumor specimen and tissue collection

Twelve oral squamous cell carcinoma (OSCC) tissue samples were obtained from patients with BQ chewing habit. Twenty-two OSCC tissue samples were obtained from patients without BQ chewing habit, and 13 normal oral mucosae were obtained from individuals who underwent oral surgical intervention. Genomic DNA was extracted from tissue samples and DNA methylation status of the *SIRT1* gene was analyzed.

4: Statistical analysis

Comparisons between two groups were performed using the Mann-Whitney U test. Multiple variables comparisons were performed by using multivariable regression analysis. Results with p-values of <0.05 considered to be statistically significant.

RESULTS

1. The effects of arecoline on *SIRT1* DNA methylation, mRNA and protein expression

The methylation level of *SIRT1* in cells treated with arecoline was significantly increased compared to that of control cells. The expression levels of *SIRT1* mRNA in the cells

treated with arecoline were significantly decreased compared to the control group. The protein levels of SIRT1 (bands of 120 kDa) was reduced by arecoline treatment in HGEPs cells compared to that in controls. On the other hand, the protein levels of GAPDH (bands of 37 kDa) was the same in all cells ($p<0.05$).

2. The effects of betel quid chewing on DNA methylation status of *SIRT1*

The habits of BQ chewing was the only significant predictor of *SIRT1* DNA methylation level. The duration of chewing habit was positively correlated to the levels of *SIRT1* DNA methylation ($p<0.05$).

3. DNA methylation status of *SIRT1* in OSCC obtained from BQ chewing and non-chewing patients

SIRT1 was significantly hypermethylated in tissue samples of OSCC from BQ chewers and BQ non-chewers than in oral mucosa from healthy control subjects. Results also showed that hypermethylation of *SIRT1* was significantly higher in OSCC from BQ chewing patients than in that from BQ non-chewing patients ($p<0.05$).

DISCUSSION

Our *in vitro* model showed that the hypermethylation is followed by downregulation of the transcriptional level of *SIRT1*. A higher level of methylation of *SIRT1* was observed in smear samples obtained from macroscopically healthy buccal mucosa in BQ chewers than in non-chewers. Results also demonstrated DNA hypermethylation of *SIRT1* in OSCC, and the methylation levels were significantly higher in the OSCC of BQ chewers than in that of non-chewers. These results suggest that *SIRT1* is involved in the oral cancer caused by BQ chewing, and that hypermethylation of *SIRT1* in the oral mucosa of BQ chewers could be a predictive marker for detecting early events of multistage carcinogenesis.

These results support previous findings suggesting *SIRT1* as a tumor suppressor. *SIRT1*

has been reported to play a role in maintaining epithelial integrity by inducing the expression of epithelial-cadherin. Downregulation of *SIRT1* expression may weaken epithelial-epithelial interaction leading to malignant transformation of the epithelia. The hypermethylation of *SIRT1* caused by arecoline in BQ chewers epithelium may be related to the instability of epithelial-epithelial interactions causing malignant transformation. Cigarette smoking and alcohol consumption are other risk factors for oral cancer. Those habits also cause alteration of DNA methylation. The hypermethylation of *SIRT1* may be a target for the prediction of oral carcinogenesis caused by those habits, as well as BQ chewing. Therefore, examination of *SIRT1* hypermethylation, as well as, other tumor suppressor genes (TSGs) in smears of buccal mucosa could be useful for the detection of early changes caused by BQ chewing habits.

CONCLUSION

DNA hypermethylation of *SIRT1* occurs in OSCC and normal oral mucosa obtained from BQ chewers and that the methylation status in buccal smear samples can be an applicable routine oral screening procedure in high-risk populations. Further investigations will be needed to validate the clinical utility of this method and to improve the accuracy of risk assessment for oral cancer induced by various environmental exposures.