ROLE OF TGF BETA-1, MMP-1, TIMP-1 AND PCNA IN PATHOGENESIS AND MALIGNANT TRANSFORMATION OF ORAL SUBMUCOUS FIBROSIS

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Introduction

Oral submucous Fibrosis (OSF) is a potentially malignant fibrotic oral disease. There is strong epidemiological evidence to suggest the association of OSF with the habit of chewing areca quid (AQ) which consists mainly of areca nut, betel leaves, slaked lime and tobacco. A primary ingredient of AQ, areca nut was classified as a group I carcinogen by the World Health Organization in 2004. Recent studies showed that malignancy develops in about 13% of OSF lesions (Tilakaratne et al., 2006). The carcinogenesis of OSF into OSCC is still unclear although a variety of approaches have been applied to analyze the carcinogenic process. It is likely that in OSF, the normal regulatory mechanism of the extracellular matrix (ECM) is either down- or up- regulated, causing imbalance in the normal equilibrium of collagen deposition and breakdown (Tilakaratne et al., 2006). Therefore, the present study aims to elucidate the ECM remodeling process and fibrosis in OSF and their influence on the malignant transformation of the OSF epithelium into oral squamous cell carcinoma.

Materials and Methods

Forty- two archival specimens in the form of paraffin blocks which had been histologically diagnosed as OSF were selected from the Department of Oral Pathology of Faculty of Dental Sciences, University of Peradeniya together with eight normal oral mucosa (NOM) specimens from patients who have neither pathological mucosal lesions nor habits of smoking and/or betel chewing. The clinically diagnosed patients of OSF for this study were 36 men (86%) and 6 women (14%), ranging in ages from 21 to 65 years, with an average age of 40 years.

We compared the expressions of TGF Beta-1, TIMP-1, MMP-1, PCNA in OSF and NOM by immunohistochemistry. For each slide, 10 non-overlapping fields were randomly selected (5 fields for epithelium and 5 fields for connective tissue) and photographed using light microscopy with a digital camera (Olympus, BX51T, Tokyo, Japan x 200). Sections were considered either negative or positive according to the absence or presence of brown staining in epithelial or stromal cells. The positive cases were graded into three

easily reproducible sub categories depending on the frequency of positively stained cells: (a) Positive expression in less than 30% of cells, (b) Positive expressions in 30%- 70 % of cells, (c) Positive expressions in more than 70 % of cells. To evaluate proliferative index (PCNA index), the total number of epithelial cells and the number of nuclei with positive PCNA expression were counted using Image-pro plus version 3.0 software (Media Cybernetics Inc., MD, USA) and the proliferative index was calculated using the following formula:

PI = Total number of positive cells/Total number of epithelial cells x100

Expression of TGF β-1, MMP-1, and TIMP -1 in OSF and NOM statistically analyzed using the Chi-square test and mean values of PCNA index among different groups were analyzed using the student's t- test.

Results

The fibrotic portion of most of the cases of OSF clearly showed positive expression of TGF β-1 over 30% compared to submucosal portion of NOM. This increase was statistically significant ($p<0.05$). MMP-1 expression in OSF is attenuated compared to NOM. There was a detectable difference in the prevalence and staining intensity of positive fibroblasts between NOM and OSF. This difference of MMP-1 expression pattern in OSF was found to be statistically significant ($p<0.05$).
Statistically, there was no significant difference in TIMP-1 expression between OSF and NOM in the connective tissue portion ($p> 0.05$). But when considering the staining intensity, it was observed stronger in fibroblasts of OSF than those of NOM. The OSF had a PCNA index of 12.77 ± 3.13 compared to the PCNA index of 5.96 ± 0.86 in the NOM which was statistically significant ($P<0.05$). The OSF with dysplasia had a PCNA index of 14.43 ± 2.24 compared to the PCNA index of 10.3 ± 2.68 in the OSF without dysplasia. These results were also statistically significant ($P<0.05$). Statistical analysis revealed that there is no significant correlation between epithelial dysplasia and expression of TGF β-1, MMP-1, and TIMP-1, respectively. Similarly, no correlation was found between the degree of tissue fibrosis and expression of TGF β-1, MMP-1, and TIMP -1, respectively. P values were found to be beyond the significant level ($p>0.05$).

Discussion

Taken together the results of the present study indicate that the increased expression of TGF β-1 followed by fibroblast proliferation and collagen synthesis are contributing factors to cause fibrosis in OSF. This proves the hypothesis that external stimuli such as areca nut may induce the development of OSF by increased levels of cytokines in the lamina propria (Tilakaratne et al., 2006). Also the imbalance between TIMP-1 and MMP-1 enhances the ECM accumulation which further affects the tissue architecture irreversibly in OSF. Its malignant transformation potential is also evident by demonstrating an increased PCNA index in dysplastic OSF compared with OSF without dysplasia. However, there is no correlation between deregulation of the collagen remodeling process and

epithelial dysplasia, suggesting that the deregulated collagen remodeling may not directly affect the cancerous transformation of OSF. Therefore, it supports our previous hypothesis that the stiffness of the submucosa due to fibrotic bands affected by fibrogenic cytokines such as TGF β-1 may provide carcinogen-rich soil derived from areca nut exposure, predisposing the epithelium to undergo malignant transformation (Tilakaratne et al., 2006, Tilakaratne et al., 2008).
Further study based on the relationship between fibrosis and dysplasia will provide insight in elucidating mechanism of cancer transformation in OSF.

Conclusion

This study provides further evidence that the fibrotic process in OSF is not only due to excessive collagen deposition but also due to disequilibrium in the ECM remodeling process. Neither ECM remodeling process nor tissue fibrosis of OSF directly shows any significant effect on their malignant transformation potential.

References

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