行政院國家科學委員會專題研究計畫　成果報告

第一型胞漿素原活化抑制劑基因在口腔黏膜下纖維化症的表現

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-040-016-
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計畫主持人：張育超
共同主持人：周明勇，謝易修

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第一型胞漿素原活化抑制劑基因在口腔黏膜下纖維化症的表現

計畫編號：NSC 91-2324-B-040-016
執行期限：92 年 08 月 01 日至 93 年 07 月 31 日
子計劃主持人：張育超　中山醫學大學牙醫系
計畫參與人員：

一、Abstract

Objective: Oral submucous fibrosis (OSF) is a pre-malignant fibrotic lesion of the mouth in areca quid chewers. It is probably a consequence of disturbances in the hemeostatic equilibrium between synthesis and degradation of extracellular matrix molecules. Recently, we found that plasminogen activator inhibitor-1 (PAI-1) mRNA and protein were be upregulation in OSF (*Oral Oncology* 2003:367–72). However, the detailed molecular mechanism is stilled remained to be elucidated. **Methods:** In this study, we investigated the genetic analysis of PAI-1 in the upstream -675bp of the promoter region between OSF and normal buccal mucosa. PAI-1 genotyping with allele-specific restriction enzyme site analysis was performed in the specimens from 52 OSF and 32 normal buccal mucosa. The Chi-square test was used as statistical analysis in this study. **Results:** There is a single guanosine deletion/insertion 4G/5G polymorphism -675 bp upstream from the start of transcription within the promoter. Our findings suggested that the distribution pattern of PAI-1 promoter were different between OSF and normal buccal mucosa ($P< 0.05$). It had a high frequency of PAI-1 (4G/4G) genotypes in the OSF group (42.3%) than those in normal buccal mucosa group (21.9%). The ratio of 4G/5G from OSF and normal buccal mucosa were 44.2% and 46.9%. In addition, the ratio of 5G/5G was 13.5%, 31.2% respectively. **Conclusions:** We concluded the results that PAI-1 4G allele, with a higher transcription activity, was more prevalent in OSF. This may be the molecular mechanism associated with an increased risk for OSF. This study was supported by NSC 92-2314-B-040-016.

二 Introduction

Oral submucous fibrosis (OSF) is a pre-malignant fibrotic lesion of the mouth in areca quid chewers, and is a chronic insidious disease of oral subepithelial connective tissue resulting in stiffness of the oral mucosa and inability to open the mouth. OSF is histologically characterized by epithelial atrophy and progressive accumulation of collagen fibers in the lamina propria and submucosa of the oral mucosa. Although the etiology of OSF is not completely understood, there is a close epidemiological association with the habit of chewing areca quid. Fibroblasts obtained from OSF subjects revealed a higher elevation for collagen synthesis than normal buccal mucosa roblasts (BMFs). It is probably a consequence of disturbances in the hemeostatic equilibrium between synthesis and degradation of extracellular matrix molecules (ECM).

Plasminogen activators and their inhibitors are thought to be key participates in the balance of proteolytic and antiproteolytic activities that regulates ECM turnover. There are 2 types of PAs: the tissue-type activator (t-PA) and the urokinase-type activator.PA activity is balanced by 2 distinct, specifically acting PA inhibitors (PAIs): (1) PAI-1, produced by endothelial cells and various normal and malignant cells, and (2) PAI-2, produced in the placenta and in macrophage, epithelial cells, and other cells. PAs and PAIs are thought to be key participants in the balance of proteolytic and antiproteolytic

activates that regulate extracellular matrix turnover.

In the previously studies, we found that plasminogen activator inhibitor-1 (PAI-1) mRNA and protein were found to be upregulation in OSF (*Oral Oncology* 2003:367–72). However, the detailed molecular mechanism is stilled remained to be elucidated. Furthermore, Genetic variation in PAI-1 gene is associated with different levels of PAI-1 activity. PAI-1 4G/4G is associated with the highest PAI-1 activity and PAI-1 5G/5G with the lowest level. In this study, we have therefore investigated the genetic analysis of PAI-1in the upstream -675bp of the promoter region between OSF and normal buccal mucosa.

三、Materials and methods

Subjects

32 healthy individuals without areca quid chewing habits were selected from the Department of Oral Surgery (Chung Shan Medical University Hospital, Taichung, Taiwan) with the informed consent for this study. The OSF specimens were obtained from 52 patients with areca quid chewing habits during surgical biopsy.

specimen collection

DNA of tissue was isolated with the QIAamp Blood Kit (Qiagen). The DNA was resuspended at a concentration of 100 mg/L in distilled water or 10 mmol/L Tris, pH 7.4, containing 0.1 mmol/L EDTA. DNA that was not used immediately was stored at 2–8 °C for not longer than 7 days or frozen at -20 °C.

Polymerase chain reaction (PCR)

For confirmation of genotypes, allele-specific restriction enzyme site analysis (ASRA) was performed. This protocol uses a modified upstream primer producing a *Bsl*I cutting site with the *5G* allele but not with the *4G* allele. In brief, a 99- or 98-bp fragment, depending on the promoter allele present, was amplified from genomic DNA using the primers. Amplification was carried out using 1 µg of genomic DNA, 100 ng of forward and reverse primers, 200 µM dNTP, and 1 U Tag polymerase in 30 µl of reaction mixture containing 1× PCR buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 0.01% gelatin). Samples were subjected to initial denaturation at 95°C for 3 min, followed by 35 cycles of 30 s at 94°C for denaturing, 30 s at 60°C for annealing and 1 min at 72°C for extension, and a final extension at 72°C for 7 min in a DNA Thermal Cycler (Perkin-Elmer Corporation, Foster City, CA, USA).

Restriction fragment length polymorphism (RFLP) analysis

Aliquots (10 µL) of the PCR mixture were then digested for 4 h at 55 °C with 5 U of *Bsl*I, using the restriction buffer recommended by the manufacturer (New England Biolabs). The resulting fragments (77 and 22 bp for the *5G* allele, and 98 bp for the *4G* allele) were electrophoresed on 8% polyacryalamide gel, visualized with 0.5mg/L ethidium bromide, and examined under ultraviolet illumination.

Statistical analysis

Genotype was treated with Chi-squared test for comparison. Statistical significance was defined as $P < 0.05$.

四、Result and conculsion

Our study showed that the distribution pattern of PAI-1 promoter were different between OSF and normal buccal mucosa. It had a high frequency of PAI-1 (4G/4G) genotypes in the OSF group. However, genetic variation in PAI-1 gene is associated with different levels of PAI-1 activity. PAI-1 4G/4G is associated with the highest PAI-1 activity and PAI-1 5G/5G with the lowest level. Thus, we concluded the results that PAI-1 4G allele, with a higher transcription activity, was more prevalent in OSF. This may be the molecular mechanism associated with an increased risk for OSF.

六 Figure

Table I Comparison of PAI-1 polymorphism between OSF and BMF

	BMF		OSF		
	N=32	%	N=52	%	*P*-value
PAI-1 Genotype					
4G/4G	7	21.9%	22	42.3%	<0.005
4G/5G	15	46.9%	23	44.2%	
5G/5G	10	31.2%	7	13.5%	
Allele frequencies					
4G	29	45.3%	67	64.4%	<0.05
5G	35	54.7%	37	35.6%	

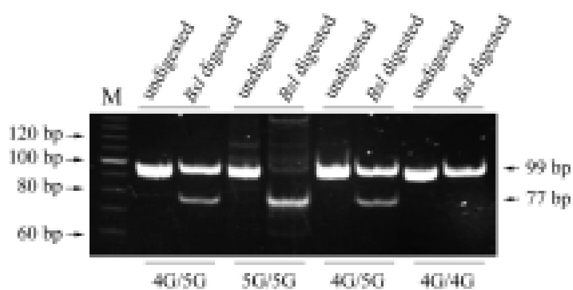

Fig.1 Genotyping of the 4G/5G polymorphism by primer-mediated PCR.

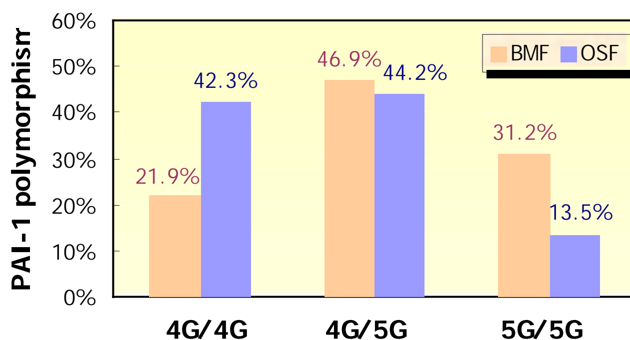

Fig.2 Different Genotyping of the PAI-1 polymorphism in OSF and MBF.