行政院國家科學委員會補助專題研究計畫成果報告

口腔黏膜下纖維化中膠原蛋白胺基因之調控

計畫類別：▒個別型計畫　　□整合型計畫
計畫編號：NSC　89－2314－B－039－015
執行期間：　88年　8月　1日至　89　年　7月　31日

計畫主持人：顏華馨
共同主持人：

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中　華　民　國　　89　年　　10　月　　30　日

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一 中文摘要

檳榔所致之口腔黏下纖維化之纖維母細胞因口腔黏膜下纖維化被認為是癌前期病變，已被研究有十年之久。然而，目前已有的研究仍無法對檳榔所致之及口腔鱗狀細胞癌有最終的結論。一研究口腔黏膜下纖維化的嶄新方法是找出它的訊息傳遞路徑，本實驗室正朝此方向努力並有些初步結 果 (由 NSC 87-2314-B-039-014， NSC 88-2314-B-039-031支持)，結果顯示分裂素活化蛋白動力胺(MAP kinase)的活化和受檳榔素刺激之纖維母細胞有關,在此實驗中我們證明了黃樟素(來自台灣檳榔的附加物),會促進轉錄因子ATF2的表現 並和轉錄調控區上有AP-1之結合序列的基因有關。

關鍵詞 ： **口腔黏膜下纖維化，檳榔，** MAP**動力媒**

Abstract

Fibroblasts from oral submucous fibrosis (OSF) has been studied for more than ten years [1] [2], due to betel nuts related OSF was recognized as a premalignant condition [3]. Unfortunately, the previous studies still haven't reached the conclusion as how betel nuts induce OSF and oral squamous cell carcinoma (SCC). A novel way of searching for the mechanism of OSF is to find its signal transduction pathway. Our lab had been searching toward this direction and had preliminary results (supported by NSC 87-2314-B-039-014, NSC 88-2314-B-039-031). The results show MAP kinases activation are involved in arecoline stimulated fibroblasts. From our experiment results, we are able to demonstrate safrole, a major component in Taiwan's betel quids complex preparation, can induce transcription factors ATF2 expression and has implicated the AP-1 sequence binding in the transcriptional regulation of genes.

Keywords: OSF, betel nuts, MAP kinase,

二 計畫緣由與目的

Betel nuts chewing is a popular habit in certain areas among the world, Taiwan is one of the prevalent area. According to epidemiology study, betel nutchewing is related to submucous fibrosis which is considered as a premalignant lesion, and oral squamous cell carcinoma.

The arguments of the nature of OSF fibroblasts existed for a long time. The earlier study showed arecoline and arecidine can stimulate collagen synthesis and fibroblasts proliferation [2], no significant difference between OSF and normal fibroblasts in the rates of proliferation in cell culture, nor in the rate at which they hydrolysed arecoline to arecaidine [4]. Recently, Jeng et. al. showed genotoxic and non-genotoxic effects of betel quit ingredients on human oral mucosal fibroblasts [5]. It was known that exposure of cells to genotoxic agents evokes a series of phosphorylation events leading to the modification of transcription factors and gene expression [6]. Experiments suggest tumor promoter mimic biological moleculars

important in signal transduction pathways that mediate growth, and the persistent stimulation of these pathway can lead to cancer. Other non-genotoxic chemical carcinogens may also act by mimicking the effect of biological mitogens. The most well known example is phorbol ester, a tumor promoter, can mimic the action of diacylglycerol and activate protein kinase C. Ultimately, this signal is transmitted to the nucleus via induction and phosphorylation of the proto-oncogene fos and jun that encode a transcription, AP-1, which is able to regulate gene transcription. Although the mechanism of carcinogenesis is not well known, a common feature of the carcinogen is its ability to activate intracellular receptors, leading to changes in gene expression. The mechanism of betel-quid-induced carcinogenesis might also follow this rule. The purpose of this study is to test whether AP-1(Activating Protein-1) binding can be regulated by components from betel quids. We choose arecoline and safrole as two major stimultants because arecoline is the main component in betel seeds, safrole is the main component in betel piper. Our previous study demonstrate arecoline can induce p42/44 MAP kinase activation, and safrole induce p38 MAP kinase activation. These two groups of MAK kinase were known to responsible for the phosphorylation and activation of various transcription factors [7], therefore it will be interesting to look at what transcription factors are involved in arecoline and safrole's signaling.

三 結果與討論

1. Transcription factor ATF2 is phosphorylated by safrole in a time(fig.1) and dose-dependented manner.

Western blot analysis show ATF2 phosphorylation can be induced by safrole in fibroblasts. Since ATF2 is one of the transcription factors known to be induced by p38 MAK kinase pathway, this result correlates with our previous study shown p38MAP kinase activation by safrole. We further demonstrate SB203580, a p38 MAK kinase pathway inhibitor can block safrole induced ATF2 phosphorylation.

2. AP1 and CRE(cAMP response element) are responsible for safrole stimulation.

Several cis-acting enhancer element luciferase vectors constructs includes AP1, CRE, GRE(glucocorticoid response element), HSE(heat shock response element), NFkB (nuclear factor kB cells), SRE (serum response element) are tested under safrole stimulation. AP1 and CRE show luciferase activity, ranged from 5 to 1.5 times relative increase. It is known AP1 is a key element for JNK pathway activation and CRE for JNK/p38 pathway. These results not only coin the role of AP1 and CRE in safrole stimulation, but also rule out pathways by stimulating transcription factors bind GRE, HSE, NFkB and SRE elements.

四計畫成果自評

This project aimed at finding out the signaling pathway for the components of betel quids. We show one of the component, safrole, can induce stress reaction by way of the know p38 stress pathway. For the continue experiments, we would like to know how this stress pathway refer to physiological or pathological condition in oral submucous fibrosis and oral cancer. Collagenase family genes contain AP-1 binding site, is one of the gene family we are interested in, we would like to know how the genes are regulated by this stress pathway.

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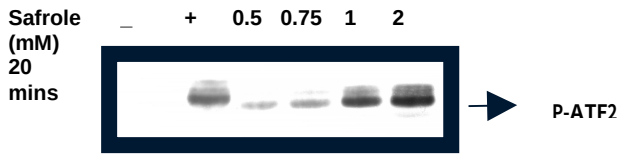
Figures

1. Transcription factor ATF2 is activated by safrole in a time-dependented manner and inhibited by SB203580.



+ : 20ug/ml anisomycin as a positive control