行政院國家科學委員會補助專題研究計畫成果報告

檳榔鹼訊息傳導路徑與泛激素結合系統之相關

計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC 89－2314－B－039－039

執行期間：89年 8月 1日至 90 年 7月 31日

計畫主持人：顏華馨

共同主持人：顏宏真

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執行單位：中國醫藥學院牙醫學系

中 華 民 國 90 年 10 月 30 日

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一 中文摘要

台灣80%口腔癌患者咀嚼檳榔, 因此檳榔成份和癌症的關係正廣泛的被研究, 也成了目前主要研究方向.對於檳榔成份的研究有各種方向, 然而目前對於口腔癌機轉仍無一致共識. 我們對於口腔癌機轉以找尋檳榔成份之訊息傳導方式來著手. 我們發現檳榔中主要成份檳榔鹼可以造成 p42/44分裂素動力媒活化及初現基因c-jun, c-fos的表現, 但其下游因子仍未知. 我們繼續研究此路徑, 尤其是c-jun, c-fos的調控.

因為降解短暫調節蛋白質多經由泛激素-蛋白小體路徑, 而p42/44分裂素動力媒及c-jun, c-fos的表現也許亦可經由此路徑, 因此我們的假設是檳榔鹼可造成 p42/44分裂素動力媒磷酸化, 接著是c-jun, c-fos的表現 , 而c-jun, c-fos的降解是和泛激素-蛋白小體路徑有關. 此假設經由我們實驗受支持,此實驗以一泛激素結合媒的cDNA為引子, 在受檳榔刺激的NIH3T3 細胞中發現有表現. 且蛋白小體受抑制後c-jun, c-fos的表現證明受影響

關鍵詞：檳榔鹼, p42/44分裂素動力媒, 泛激素-蛋白小體路徑

二英文摘要

Betel quids chewers contribute more than 80% Taiwan's oral cancer patient; therefore, the ingredients of betel quids are under studied and the correlation with cancer had became one of the major topic for researchers in Taiwan.

There were several lines of studies for ingredients from betel quids, yet no consistency for the mechanism of betel quids-related oral cancer. We started to approach this issue by examining the signal pathway by betel quids extracts and their major components. We found arecoline, one of the major components of betel quids can induced p42/44 MAP kinase activation and immediate early gene c-jun and c-fos expressions but downstream events and regulation still is an enigma. We would like to continue following this pathway and aim particularly at regulation of c-jun and c-fos.

Since degradation of most short-lived regulatory cellular proteins is mediated by the ubiquitin-proteasome pathway, the expression pattern of p42/44 MAP kinases, c-jun and c-fos might also fit into this notion, therefore, we made an hypothesis as following: Arecoline can induce p42/44 MAP kinase phosphorylation, subsequent c-jun and c-fos activation, and the degradation of c-jun and c-fos is ubiquitin-proteasome pathway related. The hypothesis was supported by our experiment using a cDNA clone encoding an ubiquitin-conjugating (UBC) enzyme, showing UBC is expressed in betel quids stimulated NIH 3T3 cells. It was demonstrated that c-jun, c-fos expression were affected by proteosome inhibition.

三計畫緣由與目的

It was known that exposure of cells to genotoxic agents will evoke a series of phosphorylation events leading to the

modification of transcription factors and gene expression [1]. Experiments suggest tumor promoter mimic biological moleculars important in signal transduction pathways that mediate growth, and the persistent stimulation of the pathway can lead to cancer. Tyrosine kinases provide a universal mode of signal transmission in response to extracellular cues that regulate cell proliferation and differentiation[2]. Uncontrolled activation of tyrosine kinases is implicated in proliferation of cancerous cells, and their deficiencies result in pathological conditions such as developmental abnormalities and immunodeficiencies. Tight regulation of tyrosine kinase cascades is therefore critical to elicit an appropriate type and level of response to external stimuli. Negative regulation of tyrosine kinase-mediated signaling is achieved through a number of distinct biochemical mechanisms. 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. The mechanism of betel-quid-induced signal was shown to induce c-jun and c-fos expression on our previous study. Although we aware the time dependent expression of c-jun and c-fos is a fact but what cause it is still untouched. One known pathway for protein degradation is the ubiquitin pathway [3]. Proteins ligated to polyubiquitin chains are usually degraded by the 26S proteasome complex that requires ATP hydrolysis for its action. A hypothesis dervied from our previous studies and the preliminary result is: Arecoline can induce p42/44 MAP kinase phosphorylation and subsequent c-jun and c-fos activation. An idea came from our preliminary study using a cDNA clone from Dr. H. E. Yen [a gene encoding ubiquitin-conjugating (UBC) enzyme (Accession No. 165422)], showing UBC is expressed in betel quids stimulated NIH 3T3 cells. Therefore, a hypothesis dervied from our previous studies and the preliminary result is: Arecoline can induce p42/44 MAP kinase phosphorylation and subsequent c-jun and c-fos activation. The degradation of c-jun and c-fos is ubiquitin-proteasome pathway related.

四結果與討論

1. Northern blot analysis showed that MG132 (Carbobenzoxy-leucinyl-leucinal- leucinal) rapidly induced the expression of c-jun, but not c-fos.
2. Immunoblot analysis showed that MG132 prevented degradation of c-Jun protein.
3. Kinase assay revealed that c-Jun N-terminal kinase (JNK) was rapidly activated by MG132.

The overlapping induction of c-jun by proteasome inhibitor (eg MG132) and arecoline hinders the study of c-jun degradation, whereas the sustained expression of c-fos after proteasome inhibition suggest proteasome-ubiquitin pathway is involved in arecoline induced c-fos expression.

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