行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC　89-2314-B-002-573

執行期間：89 年 8 月 1 日至 91 年 1 月 31 日

計畫主持人：湯月碧

計畫參與人員：林季玫

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

☑出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：台灣大學醫學院

中　華　民　國　91　年　9　月　15　日

行政院國家科學委員會專題研究計畫成果報告

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一、中文摘要

口腔癌在台灣的盛行率與日俱增,此與台灣流行的嚼檳榔有絕對的相關,然而,有關口腔癌的致病機轉仍未明瞭。目前由於整形外科介入口腔癌的重建,可選用各種不同種類組織或使用顯微手術進行組織轉移,使病人於第一次口腔癌術後獲得根治的機會大幅增加。這些病人活得夠久,因而出現第二個首次癌或第三個首次癌的機會大增。使吾人不得不對 field cancerization 的致病機轉,做進一步的瞭解。

另一方面,部分口腔癌的病人在術後接受深部的放射線治療後,於治療之附近(與原癌之位置不同)產生癌細胞變化的機會明顯增加。是否口腔黏膜原本已有化學物質刺激的病變,對於放射線的照射更容易造成基因的突變。而使細胞的轉化為癌細胞更早發生,而且腫瘤之形成更為迅速,更不易控制。如果對這一系列的基因變化有更深刻的瞭解,可使我們對於過去所沿用的腫瘤切除後放射線深部治療的必要性重新探討。

本計劃共搜集33例口腔癌病患之資料與檢體,做三年預期性的(prospective)追蹤,研究病變或正常細胞在染色體的 Simple Repeat Sequence Instability 與口腔癌術後放射線治療與第二個首次癌的關係。期由此研究能了解台灣口腔癌之致病機轉,並能進一步對早期癌症之偵測及術後放射線治療之使用多所助益。

關鍵詞：口腔癌、上皮細胞癌、遺傳改變、癌化機制

Abstract

The incidence of oral cancers in Taiwan is rapidly increasing and the cancers are always found in patients with habitual betel nut chewing. However, the molecular mechanism of oral tumorigenesis is still not clear. As the reconstructive modality invites various kinds of vascularized viable tissues or even the microsurgical free tissue transfers, many patients can be saved from the primary cancers. However, as the patients survived for sufficient period of time, the emergence of a second primary cancer or even a third primary cancer attracts the attention of the reconstructive surgeons. These observations lead us to further understand the concept of "field cancerization", in which there are carcinogen-induced changes throughout the mucosa of the oral cavity.

On the other hand, part of the oral cancer patients who received radiotherapy after surgery developed a second cancer close to the site of the previous cancer. It is possible that the oral mucosa have already damaged by chemical substances. In addition, radiation produces even more damage to the cells (e.g. gene activation or inactivation by mutations) and induces tumorigenesis. By understanding the molecular mechanism of radiation damage to the oral mucosa cells, we can design a better screening and treatment protocol for oral cancers.

In this project, we had included 33 oral cancer patients for a three-year prospective study. The cancer, lesion, and normal tissues will be subjected to simple repeat sequence instability. These results will be analyzed together with the clinical data and the outcome of radiation treatment. This could prompt us further understand the molecular

mechanism of oral tumorigenesis and help us designing better strategy for early detection and treatment.

Keywords: oral cancer, genetic alteration; squamous cell carcinoma; tumorigenesis

二、緣由與目的

In Taiwan, oral cancer has become one of the deadly killers. It is almost always found in patients with habitual betel nut chewing. It affects people who are in their productive age. Although epidemiological studies have long associated betel nut chewing, tobacco and alcohol use with the development of squamous cell carcinoma of the head and neck, the molecular targets of these carcinogens have yet to be identified.

Betel nut can produce serial pathologic changes of the oral mucosa, i. e. leucoplakia, submucous fibrosis, verrucous hyperplasia, squamous cell hyperplasia, verrucous carcinoma, and squamous cell carcinoma. They may occur at different locations of the oral cavity simultaneously or at different timing. As the reconstructive modality invites various kinds of vascularized viable tissues or even the microsurgical free tissue transfers, many patients can be saved from the primary cancers. However, as the patients survived for sufficient period of time, the emergence of a second primary cancer or even a third primary cancer attracts the attention of the reconstructive surgeons. These observations lead us to further understand the concept of "field cancerization" (Slaughter et al., 1953), in which an entire field of tissue developed malignant or premalignant change in response to a carcinogen. In the case of oral cancers, there are carcinogen-induced changes throughout the mucosa of the oral cavity by repeated exposure. Radiotherapy after surgery may induce changes in the radiation-sensitive cells, especially the adjacent, carcinogen-exposed regions. Thus it is urgent to understand the effect of radiation on premalignant tissues.

Oral cancers have become one of the deadly killers in Taiwan because of the obvious popularity of betel nut chewing in this country. Knowledge of the mechanism of oral tumorigenesis could help us designing better protocol for treatment and early diagnosis. The specific aims of this project are to:

(1) investigate patients' genetic alterations by LOH and gene mutation screening and link with the clinical data and treatment outcome;
(2) build a genetic progression model for oral squamous cell carcinoma;
(3) further understand the "field cancerization" hypothesis;
(4) find an early diagnosis marker; and
(5) use genetic alterations as markers to modify the treatment protocol.

三、實驗結果

本計劃共搜集 33 位口腔癌患者之檢體，檢體包括了正常、周邊、腫瘤部位的細胞組織，並登錄該患者的病歷資料。登錄項目有: name、chart no.、age、sex、first visit date、location of tumor、history、pathology、betal guid chewing、drinking、smoking、leucoplakia、submucous fibrosis、operation time、pre-operation chemotherapy、post-operation chemotherapy、pre-operation radiotherapy、post-operation radiotherapy、mode of operation、result、recurrence。病患之檢體收集儲存於-154℃之冰箱中，並將 DNA 萃取後儲存於-80℃的冰箱。

Table.1 為 DNA primers 的反應組態，其中 BRCA1, WTAP 這兩對 primers 的 PCR 結果在 Anneal Temp 中有所變更，由本來的 60℃改為 45℃，因降低 Annealing 的溫度，使得 DNA 的 primer 和 template 更容易結合在一起。

Primer	Anneal Temp	Base pair
Bax	68℃	94
hMSH3	60℃	154
hMSH6	68℃	94
WTAP	60℃ DMSO 3%	141
BRCA1	60℃	120
TGFRII	60℃	73
DNAPK	Stop	121
PTEN	55℃	132

我們將 33 位病人的正常、周邊、腫瘤部位的 DNA 進行 isotope 35^s labeling PCR 以比較兩者的 gene 有沒有差異。

各 Primer 之 isotope 35^s labeling PCR 的反應結果良好。所有反應進行實驗步驟均為，先進行 denature 95℃ 3 分鐘，再 denature 95℃ 30 秒、annealing 30 秒(各個 primer 依表上溫度進行)、elongating 72℃ 30 秒，並且重覆此步驟三十個循環，再 elongating 5 分鐘，最後維持在 4℃。待所有 PCR 反應結束後跑電泳，電泳結果如下列圖片所示。

Figure 1. The electrophoresis of primers included hMSH6 and BRCA1.

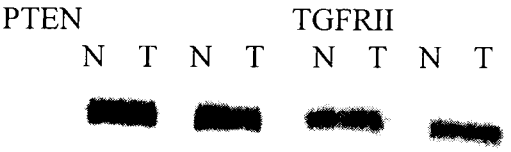

Figure 2. The electrophoresis of primers included PTEN and TGFRII.

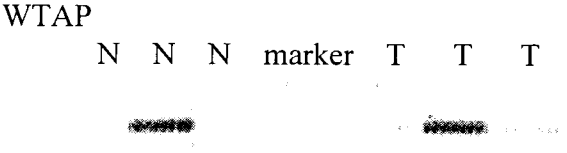

Figure 3. The electrophoresis of primers included WTAP.

Figure 4. The electrophoresis of primers included hMSH3

四、討論

我們設計 7 個 primers 的組態設定完成後，接下來是進行病人 DNA 的比對，但是並沒有發現特殊差異性存在，推論此表現結果可能是因為 PCR Target 在 normal cell 和 tumor cell，並沒有差別；也可能是這些基因在 Normal 變成 Tumor 的結果並沒有影響力。另外，僅僅三年觀察 DNA 的時間過於短暫，亦很可能因此難於發現 Normal 和 Tumor DNA 之間的細微變化，而導致本實驗中無發現基因差異性存在，對整體結果構成影響，本計劃研究人員亦會在接下來數年，持續地收集這 33 位口腔癌病人的檢體，並追蹤比對並紀錄其 DNA 的可能變化。

五、參考文獻

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行政院國家科學委員會補助國內專家學者出席國際學術會議報告

年　月　日

附件三

報告人姓名	湯月碧	服務機構及職稱	台灣大學醫學院醫學系外科 教授
會議時間 會議地點	October 3-7, 2000 Ann Arbor, Michigan, USA Seattle, WA, USA	本會核定補助文號	
會議名稱	(A)University of Michigan Visiting Professor Invited Speech (B)American Society for Surgery of the Hand 55th annual meeting		
發表論文題目	(中文)下顎骨重建 (英文)Mandible Reconstruction		

報告內容應包括下列各項：

一、參加會議經過

此開會應 University of Michigan 整形外科 Dr. Kevin Chung 之邀做專題演講，並順道參加第 55 屆之美國手外科醫學會。

二、與會心得

本次出國開會順道應邀至名聞國際之密西根大學整形外科作專題演講，著名之 Dr. Gilles 與 Grabb Smith ，Textbook of Plastic Surgery 之作者都是該中心之教授。

接著至 Seattle 參加美國手外科醫學會。此次會議之主題為 Overcoming adversity，由一家醫科醫師攀登喜馬拉雅山，發生山難，導致手指壞死及鼻部潰爛變形之整形重建經過，證明人定勝天。

三、考察參觀活動(無是項活動者省略)

參觀 University of Michigan 附設醫院之整形外科、新成立之兒童醫院。

四、建議

本次 ASSH meeting，整個 presentation 皆由電腦經單槍投影機放映，會場次序控制得非常好，完全沒有 down 機的問題，是值得學習之處。

五、攜回資料名稱及內容

American Society for Surgery of the Hand Meeting and Exhibits Program.
Overcoming Adversity ~ Hand Surgeons Shaping the 21th Century

六、其他