

行政院國家科學委員會專題研究計畫 成果報告

人類乳突瘤病毒嵌入對肺癌形成之影響及其與 E6/E7 蛋白表現之關係

計畫類別：個別型計畫

計畫編號：NSC92-2314-B-040-031-

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計畫主持人：邱慧玲

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中文摘要

肺癌是當前我國重要的醫藥衛生研究課題。從歐美國家的肺癌研究中已知抽煙是肺癌發生的最重要病因，但在台灣地區則有一半的肺癌發生無法以抽菸來說明，尤其台灣女性有90%以上是不抽菸者。因此不抽者罹患肺癌之病因學研究，是我們目前獨特且急迫的研究目標。由與李輝教授實驗室的合作研究中，我們發現高危險性人類乳突瘤病毒16和18型(human papillomavirus 16/18)與不抽煙之女性的肺癌生成有極高的關連性。另外，也發現病人周邊血液中是否有HPV DNA的存在可作為肺癌的危險指標。因此更進一步證明人類乳突瘤病毒參與肺癌的形成為本研究之主題。從病毒的觀點來看，病毒基因嵌入宿主染色體是病毒導致癌化機制中的重要關鍵。由E2-PCR方法我們證實大約70%的肺癌組織中有嵌入現象，並經由南方墨點法、RS-PCR等等不同方式證明人類乳突瘤病毒基因在肺癌組織中的嵌入現象。另一方面，我們的初步結果顯示有80%以上有E6/E7 mRNA的腫瘤切片中的p53/Rb蛋白無法被染出，反之則會被染到。這結果暗示HPV 16/18可能透過E6/E7致癌蛋白去活化p53/Rb蛋白之致癌路徑參與肺癌形成。而從子宮頸癌的研究中已知HPV基因嵌入絕大部分由E2斷裂，造成E2無法抑制E6/E7的表現。我們從E2、E6及E7的mRNA是否與嵌入現象同時存在來著手

分析，結果顯示血液中或組織中的E2 mRNA與E6 mRNA表現均為負相關性。另外E6 mRNA的表現和腫瘤類型(腺癌為86.7%，鱗狀上皮癌為47.6%)、以及腫瘤分期有關(Stage I及II為8.3%，Stage III及IV 87.9%)。這結果不僅能證明HPV參與肺癌之形成，同時也提供許多線索作為將來探討HPV引起之人類其他肺癌的研究基礎。

關鍵詞：肺癌、人類乳突瘤病毒、嵌入、E2、E6、E7

Abstract

Lung cancer is the leading cause of cancer death in Taiwan and the incidence rate increases at a steady rate every year. By looking at the prevalence rate of human papillomavirus (HPV), a powerful inactivator of p53 and close alliance to cervical cancer, in lung cancer patients, we have found that infection of HPV 16/18 could be a very possible determinant of lung cancer risk in Taiwan, especially for non-smoking patients and. HPV has been long and well known to be related with cervical cancer and integration of HPV genome into the host chromosome has been observed in most of the advance cervical cancer tissue. Since the integration of HPV will result in the loss of E2 open reading frame and subsequently the overexpression of E6 and E7 proteins, cells will continually grow without the control. All these indicate the involvement of integration of HPV genome in the tumorigenesis. Since our previous study has revealed that the presence of HPV DNA in peripheral blood may serve a risk biomarker, in this study, RT-nested PCR was employed to investigate the expression status of E2 and E6 and their

relationships to clinical parameters. As the results show, expression of E2 mRNA showed a significant mutual reverse relationship with E6- mRNA, in blood or cancer tissue samples, as well as for HPV 16 or 18. For HPV 16, E6 mRNA was profoundly detected in blood cells of female lung cancer patients than in that of male patients (88.2% vs. 61.1%). The presence of E6 mRNA was also well associated with tumor type (86.7% for adenocarcinoma vs.47.6% for squamous carcinoma) and tumor stage (8.3% for Stage I & II vs. 87.9% for Stage III & IV). These results may be of importance for clinical intervention.

Introduction

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Results and discussion

A total of 70 blood samples and 38 tumor tissues were obtained from HPV-positive patients with primary lung cancer and subjected to RT-nested PCR analysis for E2- and E6-mRNA. Results have showed that HPV 16 E6 mRNA was detected in 74.3% of blood samples and 71.4% of tumor tissues while HPV 18 E6 mRNA was present in 63% of blood samples and 74.4% of tumor tissues. It was therefore clear that HPV viral

integration was a common event during lung tumorigenesis. Statistical analysis showed that there was an inverse relationship between the expression of E2 mRNA and E6 mRNA, for both HPV 16 and HPV18. Such relationship was also found in both blood and tissue samples.

Table The inverse relationship between the presence of E2- and E6 mRNA

HPV 16					
E6/E2	+/+	+/-	-/+	-/-	
In blood	11	41	13	5	P<0.001
In tissue	2	21	8	7	P=0.002
HPV18					
E6/E2	+/+	+/-	-/+	-/-	
In blood	2	27	12	5	P<0.001
In tissue	1	16	8	2	P<0.001

Furthermore, HPV 16 E6 mRNA was more frequently detected in blood cells of female lung cancer patients, patients with adenocarcinoma, or with advanced tumor stage. These results may be of importance for clinical intervention.

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