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（II）

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-040-026-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：中山醫學大學醫學檢驗暨生物技術學系

計畫主持人：邱慧玲

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 10 月 27 日

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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執行期間：93 年 8 月 1 日至 94 年 7 月 31 日

計畫主持人：邱慧玲
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執行單位：中山醫學大學醫技系

中　華　民　國　94 年　10 月　28 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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中文摘要

從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。我們之前的研究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。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。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 IV87.9%）。另一方面，我們的結果顯示 HPV 16/18 E6 mRNA 及 p53 蛋白表現之間有負相關性存在，同樣的負相關性也存在於 HPV 16/18 E7 mRNA 及 Rb 蛋白表現之間。經過性別及抽煙狀況的校正後，肺癌組織中出現 HPV16 及 18 E6 mRNA 的 45 位不抽煙女性患者中分別有 17 及 19 位的 p53 免疫染色結果為陰性。不抽煙女性肺癌病人同時出現 HPV E6 mRNA 表現及 p53 免疫染色陰性的比例明顯高於不抽煙或抽煙男性。同時出現 HPV E7 mRNA 表現及 Rb 免疫染色陰性的不抽煙女性肺癌的比例也明顯高於不抽煙或抽煙男性。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。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. 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). In addition, a reciprocal relationships existing between HPV 16/18 E6 mRNA and p53 immunostaining, as well as between HPV 16/18 E7 mRNA and Rb immunostaining were observed in the same location of lung tumor sections. When lung tumors were stratified by gender and smoking status, 17 and 19 of 45 nonsmoking female lung tumors with HPV 16 and 18 E6 mRNA expressions, respectively, were negative in p53 immunostaining. The frequency of concurrent HPV E6 mRNA expressions and p53 negative immunostaining in nonsmoking female lung cancer patients was significantly higher than that in nonsmoking or smoking male patients. The frequency of nonsmoking female lung tumors with HPV 16 E7 mRNA expression having Rb negative immunostaining was also higher comparing to those of nonsmoking maleand smoking male. These results may be of importance for clinical intervention.

Introduction

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. 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 as a risk biomarker, the presence of E2- and E6- mRNA and their relationships to clinical parameters were investigated in this study

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.

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. A reciprocal relationships existing between HPV 16/18 E6 mRNA and p53 immunostaining, as well as between HPV 16/18 E7 mRNA and Rb immunostaining were observed in the same location of lung tumor sections. When lung tumors were stratified by gender and smoking status, 17 and 19 of 45 nonsmoking female lung tumors with HPV 16 and 18 E6 mRNA expressions, respectively, were negative in p53 immunostaining. The frequency of concurrent HPV E6 mRNA expressions and p53 negative immunostaining in nonsmoking female lung cancer patients was significantly higher than that in nonsmoking ~~and~~ or smoking male patients. The frequency of nonsmoking female lung tumors with HPV 16 E7 mRNA expression having Rb negative

immunostaining (11 of 45, 24.4% for HPV 16; 17 of 45, 37.8% for HPV 18) was also higher comparing to those of nonsmoking male (2 of 30, 6.7% for HPV 16; 2 of 30, 6.7% for HPV 18) and smoking male (3 of 62, 4.8% for HPV 16; 2 of 62, 3.3% for HPV 18).

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