行政院國家科學委員會專題研究計畫成果報告

人類乳突病毒與血管新生-從臨床到基礎

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一、 中文摘要

人類乳突病毒 HPV 是一群變異性極大的病毒，近來它們在人類惡性腫瘤的生成所扮演的角色引起許多研究者的興趣。部分的 HPV 感染特別是 HPV16，18，31，39，45，51，52，56，58，目前被認為與 90%以上的子宮頸癌生成是有相關的。病毒致癌蛋白 E6, E7 的基因表現可在腫瘤組織發現, 而且此蛋白會使 keratinocytes 變性. 研究顯示, HPV16 的 E7 蛋白會與 retinoblastoma antioncogene product 結合; 而 E6 會與腫瘤抑制基因 p53 結合, 使得病毒有能力改變細胞的生長和分化. 最近的研究則顯示子宮頸血管新生與人類乳突病毒感染有相關. 基於人類乳突病毒與血管新生在子宮頸癌的高度相關性, 我們設計這個研究計劃, 期以探求人類乳突病毒是否會調控子宮頸癌腫瘤血管新生及其可能的機轉. 血管新生(Angiogenesis)在原發性及轉移性腫瘤的生成上扮演著重要的角色。許多的細胞激素能夠促進血管新生，其中血管內皮細胞生長因子(vascular endothelial growth factor，VEGF), 纖維母細胞生長因子(basic fibroblastic growth facto, bFGF), 和 platelet derived growth factor 等細胞激素, 可能與人類惡性腫瘤的新生血管有相關聯。在子宮頸癌的研究裡，Guidi et al 證實在侵襲性子宮頸癌的腫瘤細胞有大量的 VEGF m-RNA, 相反地只有局部的正常上皮細胞表達 VEGF。我們最近的研究則顯示子宮頸癌組織中 VEGF 的含量與疾病的進行有正相關。腫瘤組織中 VEGF 的含量愈高者, 有較高比例的子宮間質侵犯、子宮旁組織的侵襲及淋巴血管的轉移這部分的臨床研究結果已發表於 Gynecologic Oncology 82,49-56(2001)。在細胞實驗上我們利用轉植入未被感染之子宮頸癌細胞發現其產生的能力明顯的上升此與 Robert S Kerbel 發表於 Oncogene(2000, 19, 4611)相同, 顯示人類乳突病毒會調控子宮頸癌腫瘤血管新生。

關鍵詞：血管新生, 血管內皮細胞生長因子, 纖維母細胞生長因子, 人類乳突病毒

二、 英文摘要

HPV, an important virus, has been implicated in cervical cancer progression, though its role remains elusive. This study was an attempt to elucidate the role of HPV in the pathogenesis of cervical cancer , with particular emphasis on tumor angiogenesis. METHODS: Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) levels were determined via enzyme immunoassay in 60 FIGO stage IB-IIA cervical cancer patients. Immunohistochemical staining in tissue sections was performed to analyze the distributions of VEGF. Meanwhile, human papillomavirus (HPV) DNA was

detected by polymerase chain reaction-based survey. In vitro studies of seven cervical cancer cell lines, C33A and SiHa, for the interaction between IL-6 and VEGF were also performed. RESULTS: Consistently higher expression of IL-6 and VEGF was evident in cancerous tissues than in adjacent noncancer tissues in early-stage cervical cancer patients ($P < 0.01$). After recombinant human IL-6 was added, VEGF was induced in a time- and dose-dependent manner in cervical cancer cell line C33A. Correspondingly, interrupting the IL-6 autocrine machinery with either anti-IL-6 or anti-IL-6 receptor antibody markedly reduced the expression of VEGF at the transcriptional level in SiHa cells. Significantly higher levels of IL-6 in cancer tissues were observed in patients older than 45 ($P < 0.01$), patients with tumors >2 cm ($P < 0.01$), patients with oncogenic HPV-16 or -18 infections ($P < 0.01$), and patients with squamous cell carcinoma ($P = 0.02$). Patients with a deeper stromal invasion, vaginal invasion, lymphovascular emboli, or lymph node metastasis appeared to have higher intratumoral IL-6 levels, although the differences were statistically insignificant. CONCLUSIONS: Substantially high microenvironmental IL-6 levels promote tumor angiogenesis and the development of cervical cancer. Thus, inhibition of the biological activity of IL-6 may be potentially beneficial. Copyright 2001 Academic Press.

Keywords：angiogenesis, vascular endothelial growth factor (VEGF), human papilloma virus

三、緣由與目的

原發性與轉移性腫瘤的生成與血管的新生有密切的關係。此一過程我們稱之為"tumor angiogenesis"。(1,2) 腫瘤的生長依賴此一新生血管網路，此點我們從 Angiostatin 能促使原發性及轉移性腫瘤停止生長得到佐證。(3,4) 新生血管是由許多細胞激素所促使，這些細胞激素包括 basic fibroblastic growth，placental growth factor，intenleukin-8，vascular endothelial growth factor(血管內皮細包生長因子)等。(2,5,6) 血管內皮細胞生長因子是一種多功能的細胞激素，它藉由增加血管的通透性與處進血管內皮細胞的有絲分裂來達成血管的新生。血管內皮細胞生長因子可能是與人類惡性腫瘤新生血管最有關連性的細胞激素。這些腫瘤包括 adenocarcinomas of the GI tract，breast carcinomas，melanomas，glial tumours 及 angiosarcoma。

關於子宮頸癌的研究，Dobbs et al 發現在 CIN 的病灶有 VEGF 的分泌;(7) Guidi et al 也證實在侵襲性子宮頸癌的腫瘤細胞有大量 VEGF 的 m-RNA。(8) 最近，我們也發現與正常子宮頸組織比較，子宮頸癌的組織其 VEGF 的含量有顯著的增加，而且我們的研究顯示 VEGF 的含量與深部組

織的侵襲、子宮旁組織的侵襲及淋巴管的轉移有著定量的關係。(9) 根據目前已有的報告，血管的新生早在子宮頸癌的癌前期經由不正常上皮細包分泌 VEGF 就已發生。疾病的演進隨著 VEGF 含量的增加而進行。

人類乳突病毒 HPV 是一群變異性極大的病毒。目前已有 77 種不同的 genotypes 被確認，近來它們在人類惡性腫瘤的生成所扮演的角色引起許多研究者的興趣。部分的 HPV 感染特別是 HPV16，18，31，39，45，51，52，56，58，目前被認為與 90%以上的子宮頸癌生成是有相關的。(10) 病毒致癌蛋白 E6, E7 的基因表現可在腫瘤組織發現, 而且此蛋白會使 keratinocytes 變性. (11) 研究顯示, HPV16 的 E7 蛋白會與 retinoblastoma antioncogene product 結合;(12)而 E6 會與腫瘤抑制基因 p53 結合,(13) 使得病毒有能力改變細胞的生長和分化. 最近的研究則顯示子宮頸血管新生與人類乳突病毒感染有相關.(14)

基於人類乳突病毒與血管新生在子宮頸癌的高度相關性,我們設計這個研究計劃,期以探求人類乳突病毒是否會調控子宮頸癌腫瘤血管新生及其可能的機轉.

血管新生 (Angiogenesis)在原發性及轉移性腫瘤的生成上扮演著重要的角色。許多的細胞激素能夠促進血管新生，其中血管內皮細胞生長因子(vascular endothelial growth factor，VEGF), 纖維母細胞生長因子(basic fibroblastic growth facto, bFGF), 和 platelet derived growth factor 等細胞激素, 可能與人類惡性腫瘤的新生血管有相關聯。在子宮頸癌的研究裡,Guidi et al 證實在侵襲性子宮頸癌的腫瘤細胞有大量的 VEGF m-RNA, 相反地只有局部的正常上皮細胞表達 VEGF。我們最近的研究則顯示子宮頸癌組織中 VEGF 的含量與疾病的進行有正相關。腫瘤組織中 VEGF 的含量愈高者, 有較高比例的子宮間質侵犯、子宮旁組織的侵襲及淋巴血管的轉移。

根據報告，至少 90%以上的子宮頸癌與人類乳突病毒(Human papilloma virus, HPV)的感染是有相關的，其中又以 HPV16，18 等感染關連性最強。以往我們藉由彩色都卜勒超音波檢查顯示，子宮頸癌的血管新生與 HPV 的感染有關 (Hsieh et al . Cancer 1995)。結果我們發現子宮頸癌的血管新生與 HPV 16, 18 的感染有關。因此本年度的主要工作是以體外細胞株實驗定量分析血管生長因子與 HPV 感染的關係。並探求其分子機制經由這個研究計劃, 將可使我們對人類乳突病毒在子宮頸癌血管新生所扮演的角色有更進一步的了解,進而提供臨床治療的思考方向。

六、結果與討論

In this section,we also disscuss the expression of IL-6,and the result were publish on Gynecologic Oncology 82,49-56(2001)Levels of VEGF, but not of PDGF, were

markedly raised in cancer tissues as compared with non-cancer tissues in early stage cervical cancer patients (Table 1). Using a log transformed paired t-test, significantly higher expressions of VEGF were observed in cancer tissues (11.6-70.6 vs 9.4-27.8 *pg*/mg, $p<0.01$), while expressions of PDGF were unchanged (23.4-41.9 vs 23.2-37.4 *pg*/mg, $p=0.43$). These findings suggested the essential role of VEGF in tumor angiogenesis during the early stages of cervical cancer. Since levels of IL-6 and VEGF were coincidentally increased in cancer tissues, the effect of IL-6 on the expression of VEGF was investigated herein.

Recombinant human IL-6 was added to an HPV-negative cervical cancer cell line C33A, revealing that VEGF proteins were induced by IL-6 in a time- and dose-dependent manner (Fig. 2A, 2B). Moreover, as Figure 2C shows, 32% and 38% reduction of VEGF expression were detected in SiHa cells treated with anti-IL-6 and anti-IL-6 receptor α chain antibodies, respectively. Consistent with the VEGF mRNA expressions, VEGF proteins were markedly reduced in the culture medium from the anti-IL-6 or anti-IL-6 receptor α chain antibody-treated SiHa cells (data not shown). Based on this clinical and laboratory evidence, we suggest that IL-6 is involved in tumor angiogenesis through regulation of VEGF during the early stage of cervical cancer.

Subsequently, the association between clinicopathological variables and concentrations of IL-6 was considered (Table 2). In the young age group (age < 45 y/o), IL-6 expression did not differ significantly in cancer tissues as compared with adjacent non-cancer tissues (5.0-35.2 vs 3.3-54.7 *pg*/mg, $p=0.66$; Log transformed paired t-test). However, markedly higher expression of IL-6 in cancer tissues was observed in patients older than 45 y/o (5.8-113.0 vs 2.8-79.9 *pg*/mg, $p<0.01$; Log transformed paired t-test). Though not statistically significant, the expressions of IL-6 in cancer tissues were slightly higher in the older age group (median 20.4 vs 10.1 *pg*/mg, $p=0.07$; Log transformed t-test).

Patients with HPV-16 or -18 infections had higher intra-tumor IL-6 productions (16.1 vs 8.8 *pg*/mg, $p=0.04$; Log transformed t-test) (Table 2). Using the log transformed paired t-test, higher IL-6 expressions were observed in cancer tissues as compared with adjacent non-cancer tissues in patients with HPV-16 or -18 infections (5.8-105.9 vs 2.8-23.7 *pg*/mg, $p<0.01$). Meanwhile, the distributions of IL-6 did not differ significantly between cancer and adjacent non-cancer tissues in patients without HPV-16 or –18 infections (4.6-20.5 vs 3.7-99.8 *pg*/mg, $p=0.15$; Log transformed paired t-test). Expression of IL-6 differed according to histologic type (Table 2). In patients

七、計畫成果自評

本研究承蒙國科會支助得以對血管新生與 HPV 16, 18 的感染進行研究，結果我們發現子宮頸癌的血管新生與HPV 16, 18 的感染有關。因此本年度的主要工作是以體外細胞株實驗定量分析血管生長因子與HPV 感染的關係。並探求其分子機制經由這個研究計劃, 將可使我們對人類乳突病毒在子宮頸癌血管新生所扮演的角色有更進一步的了解, 進而提供臨床治療的思考方向。由於此研究之人力配置為臨時工讀生對研究之進度有時間上的限制期望往後關於細胞實驗之人力配置以專職研究助理為主。

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