行政院國家科學委員會專題研究計畫　成果報告

E-cadherin 基因之 promoter 區域(-160 C→A)多型性與大腸直腸癌致癌危險性的關係

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（一）摘要

中文摘要

曾有報告指出 E-cadherin 的 promoter region 之-160℃→A 的基因多型性會增減 E-cadherin 基因的表現性。因此，評估 E-cadherin 基因表現的多型性或許可以鑑定出那些人具有罹患大腸直腸癌的危險性。然而，至今此類的報告卻只有零星數篇而已。本研究是依個以台大醫院病例為基礎之病例控制性分析研究，我們共收集了 200 位大腸直腸癌病患與 200 正常人。DNA 標本取自病患周邊血液白血球，經聚合酶鏈鎖反應放大欲檢驗之 DNA 片段，然後以限制酶切割分析基因多型性。我們以回歸分析統計學計算此基因多型性的危險係數及 95%信賴區間。並經過統計調整其飲食生活習慣等因素（如嗜食紅肉、抽菸、喝酒、肥胖、糖尿病）的影響。我們發現在 200 位健康台灣人中，E-cadherin 基因型的分布是 CC 佔 42%（n=84），C/A 占 48.0%（n=96），而 AA 占 10%（n=20）。而在大腸直腸癌病患中 AA 基因型的頻率（2%，n= 4 of 200）統計學上明顯比控制組的健康成人低（p<0.005），其罹患大腸直腸癌的危險性約只有 CC 基因型者的五分之一（OR=0.20；95% CI=0.06-0.56）。將這些大腸直腸癌患者依照病灶位置（左側 vs. 右側大腸），組織學型態（分化良好、中度、以及不良），腫瘤期別（stage 0、Ⅰ對 stageⅡ、Ⅲ、Ⅳ期），以及淋巴腺轉移（陽性對陰性）等因子與 E-cadherin 的關聯分析中，並未發現任何相關。我們的結論 E-cadherin-160A/A 基因型會降低大腸直腸癌罹病的危險性。接下來必須探討 A 型態的基因型到底在腫瘤組織中對 E-cadherin 功能所產生的影響，以及收集更多的病例，以進一步確定相關性。

英文摘要

A 160℃→A polymorphism in the promoter region of E-cadherin has been reported to decrease gene transcription. This allelic variation might be a potential genetic marker for identifying individuals at risk for colorectal cancer. Up to date, only very few reports were published the regarding clarification of the association between the polymorphism of E-cadherim and colorectal cancer. A hospital-based case-control study, including 200 colorectal cancer patients and 200 unaffected controls, was performed. DNA from peripheral blood samples was examined by polymerase chain reaction-restriction fragment length polymorphism. Logistic regression analyses were used to computed odds ratio (OR) and 95% confidence interval (CI) after adjusted for the influence from diet and life-style factors (e.g. red meat, smoking, drinking, obesity, and diabetes mellitus). In 200 healthy Taiwanese, the distribution of genotype C/C was 42% (n=84), C/A was 48.0% (n=96), and AA was 10% (n=20). The frequency of variant A/A genotype in colorectal cancer cases (4 of 200, 2%) was significantly lower than that of controls (20 of 200, 10%) (p<0.005), conferring a 5-hold decrease in the risk of colorectal cancer (OR, 0.20 : 95% CI, 0.06-0.56) compared with the C/C genotype. Stratification of the colorectal cancer cases according to their location (left-sided vs. right-sided colon), histology (well, moderate, and poor differentiation), tumor stage (stage 0, Ⅰ vs. stageⅡ,Ⅲ,Ⅳ), and lymph node metastasis(positive and negative) fail to reveal any heterogeneity with

resped to E-cadherin genotype. The present data suggest that individuals with E-cadherin-160 A/A genotype have a decreased risk of colorectal cancer. Further study is mandatory to clarify the functional relevance of the A allele *in vivo* and to confirm the inverse association of the A/A genotype with colorectal cancer in large epidemiologic studies.

關鍵字（Keywords）：E-cadherin，基因多型性，黏著分子，大腸直腸癌，轉移，正位種植

（二）報告內容

前言

大腸直腸癌在國內的發生率日漸提高，因此，熟悉致癌危險因子十分重要。再者，大部份的病例在診斷時已屬晚期。大部份早期侷限性的腫瘤可以用傳統的手術方法完全清除而得到成功地治療。因此，若欲提高目前大腸直腸癌的治療成績，應首重腫瘤轉移的預防。然而，大腸直腸癌轉移是一複雜的生物現象。先前的研究已闡明大腸直腸癌在發生過程牽涉到致癌基因與抑癌基因的變化與累積，吾人以為進一步研究這些腫瘤之進展 (progression) 與轉移 (metastasis)，尤其是 E-cadherin 遺傳多型性，將可以闡明大腸直腸癌之生物特性的多樣化，進而提供臨床實際應用在大腸直腸癌的防治上。

研究目的

本研究係基於我們初步實驗顯示 E-cadherin 基因之 promoter 確實會出現上述 (-160C→A) 之基因型多型性。我們擬收集至少 200 例以上大腸直腸癌病患，以及 200 例以上控制組病患，分別分析基因型 CC, CA, AA 之致癌危險性是否有明顯差異，其次再比較此三種基因型是否與病患之飲食生活習慣及腫瘤的臨床病理因子諸如腫瘤的期別，分化，位置等具相關性。

文獻探討

細胞彼此之間或細胞與周圍環境之間的黏附作用主導許多重要的生物現象，例如胚胎發生，細胞的成長及分化，以及傷口的修補等。此外，細胞之間的黏附作用在腫瘤的成長及轉移方面亦扮演相當重要的角色。近年來許多報告也相繼指出黏膜上皮分化作用的消失，以及伴隨而發生之腫瘤細胞的高移動力和侵襲性，均是細胞間黏附作用喪失的結果。由於癌細胞的侵犯和轉移決定癌症病患的臨床預後，因此，闡明腫瘤細胞間之各種不同型式之細胞黏附作用，便成為癌症生物學之重要一環。在各種型態的黏著分子中，cadherin 是一種穿透細胞膜的醣蛋白，而其作用是同型鈣離子依賴性的細胞間黏附作用。E-cadherin 是上皮細胞中最主要的 cadherin 成份，其主要的功能是維持細胞分化成功之後的表現型。再者，E-cadherin 在質和量方面起了變化也在許多惡性腫瘤，如頭頸部、乳腺、前列腺，以及胃腸道上皮癌中發現。目前已有一些活體內及體外的研究指出 E-cadherin 表現的低下 (down-regulation) 會增加腫瘤細胞的侵犯性 (invasiveness)。目前有關 E-cadherin 在大腸直腸癌轉移機轉的研究國外只有一些零星的報告，其中大抵提到 E-cadherin 的表現低下與腫瘤細胞分化，腫瘤期別，或甚至肝轉移有關。而國內的研究則仍闕如。再者，近年來一些零星的報告，如本院吳明賢醫師在 Cancer

雜誌上發表 E-cadherin promoter (-160 C→A)基因多型性與胃癌的致癌危險性有關，Li LC 等人在 Cancer Research 發表此種基因型的多型性與攝護腺癌的致癌有關，至於有關大腸直腸癌方面，唯一可查到的一篇是 Porter TR 等人在 Oncogene 發表此種基因型多型性與腫瘤位置有關。鑑於此，有關 E-cadherin 基因型多型性在大腸直腸癌致癌危險性的比重，與實際 E-cadherin 蛋白在大腸直腸黏膜的表現量，以及各種臨床病理乃至轉移能力的相關性，實在需要全面性的釐清。

研究方法

The experiment was sequentially conducted as follows :

1. Patients and Samples

We will recruit more than 200 cases of colorectal cancer and more than 200 cases of control group for this study. The patients' life style and clinicopathological data will be well recorded. The specimens were surgically obtained from the colorectal surgical department in the National Taiwan University Hospital. Clinical information was obtained from medical records. Samples were taken from the representative cancerous lesions including adjacent non-cancerous mucosa and were handled according to the guidelines of the Japanese Research Society for colorectal Cancer. Tissue for microscopic examination was formalin fixed and paraffin embedded following the routine procedures in the laboratory. Histological examination was done on hematoxylin-eosin-stained sections.

2. Genotyping analysis of E-cadherin Promoter

Laboratory personnel were blinded to the case-control status of the subjects. DNA was purified from peripheral blood lymphocytes by SDS/ proteinase K treatment and phenol/chloroform extraction. PCR-RFLP analysis of the E-caderin originally described by Li er al (41). Was modified to identify its genotype. The two primers were 5'-TCCCAGGTCTTAGTGAGCCA-3' and 5'-GGCCACAGCCAATCAGCA-3'. Each PCR reaction mixture (50 μ1) contained 8 pmol of each primer, 200 nM each dNTP, 2 unit of Taq polymerase and 150ng genomic DNA. Reaction mixtures were preincubated for 5 min at 96℃.PCR conditions were 10 cycles of 96℃ for 30s, 60℃ for 30s, and 72℃ for 30s. A final extension of 72℃ for 5 min was performed. After confirmation of the amplified fragment of the expected size on an agarose gel, the PCR products were digested with 5 units of restriction enzyme HphⅠ or AflⅢ site. DNA fragments were then eletrophoresed on a 7% polyacrylamide gel. Those subjects with inadequate quantity of DNA sample or unsatisfactory genetic analysis were also excluded form analysis. Finally, a total of 200 cases and 200 controls were included in further statistical analysis.

3. Statistical Analysis

The χ^2 test for association was used to test differences in the distribution of the genotypes or demographic variables. Exact 95% confidence intervals (CIs) for odds ratios (ORs) were computed by Cornifield's method. Unconditional logistic regression was used to compute ORs and 95% CIs, with adjustment for several covariates found associated with risk. All these tests were performed by SPSS software. A p value less than 0.05 was considered statistically significant.

結果與討論

結果

大腸直腸癌患者與對照組的人口學分析資料整理於表一。此二組在性別、種族、年齡方面並沒有統計學上的差別。而大腸直腸癌的病患卻明顯教育程度較高、嗜食紅肉、糖尿病、肥胖、以及抽菸（$p<0.001$）。在表二中顯示出大腸直腸癌患者與對照組患者之 E-cadherin 基因型的分布頻率。在健康人的控制組中，基

因型頻率分別為 C/C（42%，84/200），C/A（48%，96/200），和 A/A（10%，20/200）。在大腸直腸癌患者中，A/A 基因型的頻率統計學上比大腸直腸癌患者明顯更低（2%，4/200 vs 10%，20/200，p<0.005）。因此，罹患危險性（Odd ratio, OR），在調整嗜食紅肉、肥胖、糖尿病、教育程度、抽菸等因素後，計算起來在大腸直腸癌患者中，A/A 對 CC genotype 的比例是 0.20（95%CI，0.06-0.56）。C/A 異基因型並未發現有降低大腸直腸癌危險性的影響。當大腸直腸癌患者再以腫瘤位置、組織學、期別、淋巴腺轉移等因子次群作分析時，我們發現 E-cadherin 基因型差異以這些次群並未有統計相關。

討論

在本研究中，何以 A/A 基因型會比 C/C 基因型罹患大腸直腸癌的機會更低？其轉機是頗令人費解的。就我們過去的認知而言，E-cadherin 是屬於抑癌基因（tumor suppressor gene），E-cadherin promoter 的 C allele 理論上是強力的 transcription activator，A allele 則可以預期的應該會降低 E-cadherin 蛋白的表現。因此欲釐清 A/A 基因型具罹患大腸直腸癌保護能力的機轉，下列之工作仍須進行：第一，有文獻指出 mRNA 的 level 不一定與蛋白表現有正相關。因此，將這些具 A/A 基因型病患之腫瘤組織及正常組織的 mRNA 乃至 E-cadherin 的蛋白進一步定量分析誠屬必要；第二，在 *In vivo* 模型（如轉殖 E-cadherin 高與 E-cadherin 低表現之腫瘤組織至裸鼠身上，以觀察腫瘤進展情況）可能有助於釐清問題；第三，也許與研究 promoter polymorphism 有關的 linkage study 在生物統計學有其限制，也許 candidate genes 與疾病的關聯只是反映 linkage disequilibrium 的現象而已。

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圖表說明

Table1:Demographic Characteristics of Colorectal Cancer Patients and Controls

Characteristics	**Patients** (n=200)	**Control** (n=200)	**P-value**
Gender			
Male	124	120	NS
Female	76	80	NS
Age, yrs(mean±SD)	62.4±14.0	61.4±12.6	NS
Ethic group			
Fukien Taiwanese	141	132	NS
Hakka Taiwanese	24	22	NS
Mainland Chinese	35	46	NS
Education Level			
Primary school or lower	72	84	P<0.05
Junior high school	47	67	
Seniot higt school and above	82	49	
Cigarette Smoking			
No	56	138	P<0.05
Yes	144	62	
Diabetes Mellitus			
No	174	189	P<0.05
Yes	26	11	
Red Meat Consumer			
Very frequency	44	18	P<0.05
Frequency	84	94	
Rare	72	88	
Body-Mass Index(kg/m^2)			
Mean±SD	28.6±1.4	21.4±2.2	P<0.05

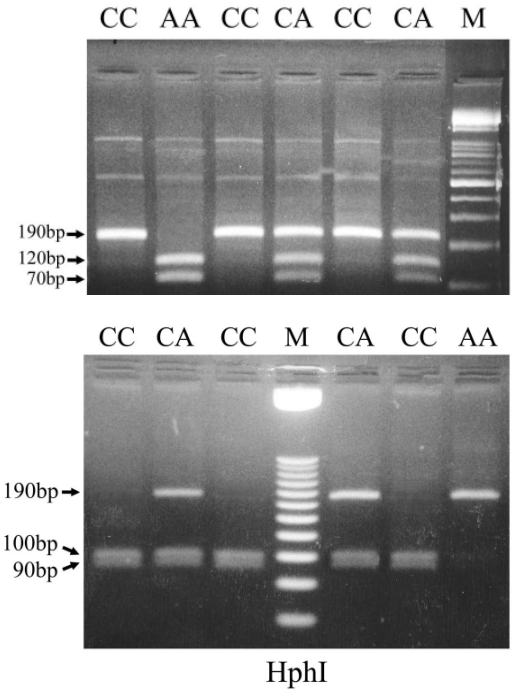
*NS： Not significant

Table2: Distribution of E-cadherin -160℃→A Polymorphism in Controls and colorectal Cancer Patients

Characteristic	E-cadherin Polymorphoism			
	CC	CA	AA	P-value
Control	84	96	20	P<0.05
Cases	94	102	4	
Sites				
Right Colon	34	36	6	NS
Left Colon	144	162	18	
Histology				
Well	20	19	2	
moderate	150	167	20	NS
Poor	8	12	2	
Stage				
Early(0, Ⅰ)	18	22	5	NS
Advance(Ⅱ,Ⅲ,Ⅳ)	160	176	19	
Lymph node metastasis				
Positive	90	104	14	NS
Negative	88	94	10	

*NS： Not significant

Fig.1:以限制酶（Hph Ⅰ及 Afl Ⅲ）分析的 200 位大腸直腸癌病患及控制組時，發現大腸直腸癌病患在 E-cadherin 基因的-160bp 位置，具有 C 及 A 的 polymorphisms。



（三）研究成果自評

1. 本計劃與原計畫相符程度達 90%以上。
2. 本計劃尚未達成者包括 E-cadherin 表現之免疫螢光染色，腫瘤組織之 mRNA 與各 genotype 之比較。
3. 本計劃研究成果對世界上尚稱新穎，應可以發表在一流的學術期刊。
4. 本計劃使相關研究人員在大腸直腸癌分子流行病學討論及研究方式，獲取相當多的經驗。將有助於此領域進一步研究。