行政院國家科學委員會專題研究計畫 期中進度報告

以多基因表現組合探索賁門部與非賁門部胃癌致病機轉之生物標記(2/3)

期中進度報告(精簡版)

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行政院國家科學委員會補助專題研究計畫 □ 成 果 報 告
■期中進度報告

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計畫主持人：林肇堂教授
共同主持人：郭明良教授
計畫參與人員：

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中　　華　　民　　國　九　十　六　年　五　月　三　十　日

中文摘要

雖然胃癌仍是常見的惡性腫瘤，但是最近幾年腫瘤生長的位置有明顯的改變。流行病學研究指出從胃竇部和體部的胃癌，尤其是組織型態屬腸道型者，顯著減少；而另一方面，在西方國家，特別是白種人男性，起源自賁門或胃食道交接處的胃癌有增加的趨向。造成這種流行病學變遷的決定因素以及這兩種不同型式胃癌的致病機轉仍不清楚。

胃賁門癌和胃食道交接處胃癌具有一些共同特色，例如診斷癌症之年紀較輕，男性為主，使用煙酒的危險性增加，較少合併慢性胃炎和較多的瀰漫型組織型態，這些特徵與非賁門部胃癌明顯不同，而且賁門癌與非賁門癌的癌症前驅病灶也不同。此外，有關幽門桿菌和 Epstein-Barr 病毒的病因角色也有所差異。幽門桿菌為非賁門癌之重要危險因子，而 Epstein-Barr 病毒則和近端處胃癌相關。總而言之，這些不同的流行病學和臨床病理差異顯示源自胃不同處的胃癌實為二種不同的疾病。由於癌症的發生起因於基因之改變，因此釐清基因變化並將其與臨床和流行病學特徵比較，將可促成對癌症致病機轉的深入了解。因此本三年期研究計劃，目的在於探討胃賁門癌和非賁門癌與致病機轉相關之基因變化標記。

吾人首先回溯性分析台大醫院從 1997 年至 2001 年，共 1112 例胃癌(ICD code 151)之臨床病理特徵差異。根據內視鏡和手術發現有 155 例(13.9%)屬賁門癌，957 例(86.1%)為非賁門癌。賁門癌明顯男性較多(男/女比：3.31 vs. 1.66, P=0.0006)、年紀較大(67.6±13.3 vs. 62.2±14.5 歲，p<0.0001)且存活較短(平均存活月數 24.7 vs. 30.4 月，p=0.0041)，但在喝酒和抽煙等個人生活習慣則未見統計差異。這些初步結果顯示賁門癌與非賁門癌確實有一些表現上的差異，而且這 155 例賁門癌組織也可做為進一步基因分析的素材。除了這些回溯性分析外，吾人也以雷射顯微分離術合併微陣列分析胃癌之全基因表現差異，發現有一些基因(表一)在賁門癌與非賁門癌的表現明顯不同。我們選擇以免疫組織染色法分析 annexin A1 在 81 位胃癌病人組織表現情形，結果在 81 位病人中，賁門部胃癌有較高比例的 annexin A1 是過度表現(表二)，進一步分析 11 例高表現 annexin A1 與 70 例低或未表現 annexin A1 胃癌，結果發現，高表現者除了賁門癌比例較高外(5/13，38.5% vs. 6/68，8.9% p=0.004)，也較易有肝臟轉移(2/3，66.7% vs. 9/78，11.5%，p=0.021)。上述結果顯示 annexin A1 在不同部位的胃癌和胃癌進展扮有一定角色，目前吾人正以 in vitro system 探討 annexin A1 表現與血管新生和腫瘤侵襲性間之關係。

Abstract

While gastric cancers (GCs) are still among the most common malignancies affecting human beings, the distribution of cancer within the stomach has changed dramatically. Epidemiologic studies have pointed out cancers arising from the antrum and body of the stomach, especially intestinal type tumors, have declined. On the other hand, the increase in incidence of cancers of the cardia and gastroesophageal junction (GEJ) is most pronounced in Western countries, especially among Caucasian males. The factors determining this epidemiologic trend and the pathogenesis of these two subsets of GC remains unclear.

The cardia cancers share important features, found in adenocarcinomas arising from the esophagus and the GEJ, which distinguish them from those so-called non-cardia GC. These features include a younger age at the time of diagnosis, male predominance, an increased risk associated with tobacco and alcohol use, a greater frequency of concomitant hiatal hernia, and a less common association with chronic gastritis, as well as an increased in diffuse type cancer. Precursor lesions of cardia or GEJ adenocarcinoma are pathologically less well-defined than those of non-cardia GC. The etiologic role of H. pylori and Epstein-Barr virus (EBV) is also different. H. pylori infection is a significant risk factor for non-cardia GC, while EBV infection is more closely associated with cancer of the proximal stomach. Collectively, these clinicopathological and epidemiologic differences suggest that cancers arising from different locations within the stomach may in fact represent diffent disease entities. Since cancer is a disease of misbehaved genes, delinating these genes involved and correlating these events with clinicopathologic and epidemiologic characteristics may lead to important new insights into carcinogenesis. However, the molecular events regarding cardia and distal stomach cancers remain ill-defined. Hence, this 3-year granted study will determine genetic biomarkers relevant to pathogenesis of cardia and non-cardia GCs.

We have first retrospectively analyzed the clinicopathologic differences between cardia and non-cardia GCs. From year 1997 to 2001, a total of 1,112 patients with primary GCs were identified from the cancer registry unit (ICD code 151) of National Taiwan University Hospital. Of them, 155(13.9%) patients were classified as cardia GCs according to endoscopic or surgical findings, while 957 (86.1%) patients were non-cardia GCs. Male predominance was noted for cardia GCs (M/F ratio: 3.31 in cardia vs. 1.66 in noncardia, $p=0.0006$). Cardia cancers tended to be older than non-cardia GCs (67.6 ± 13.3 vs. 62.2 ± 14.5 years, $p<0.0001$). Significant difference of mean survival between the two groups of GCs was also observed, with 24.7 months in cardia cancer and 30.4 months in non-cardia GC ($p=0.0041$). However, no differences in personal habits including alcohol and tobacco consumption were noticed. These preliminary findings demonstrated there indeed exist differences in these two groups of GCs and the selected 155 cardia cancers will be submitted for future genetic analyses. In addition to retrospective enrollment and

comparison of cardia and non-cardia GCs, we have performed gene expression profiling of GC by microarray combined with laser capture microdissection. There were several genes which have significantly different expression between cardia and non-cardia GCs (Table 1). We then analyzed annexin A1 expression in 81 patients with gastric cancer by immunohistochemistry. The results revealed cardia cancer tended to have high expression of annexin A1 (Table 2). Further stratification analyses between 11 cases with high annexin A1 expression and those without demonstrated that in addition to cardia cancer (5/13, 38.5% vs. 6/68, 8.9%, p=0.04), gastric cancer with liver metastasis also had high expression of annexin A1 (2/3, 61.7%, vs. 9/78, 11.5%, p=0.021)(Table 3). Taken together, these results suggested that expression of annexin A1 may play a role in gastric carcinogenesis in relation to different location and capability of metastasis. To further clarify the role of annexin A1 expression, in vitro experiments focusing on angiogenesis & tumor invasion are ongoing.

Table 1 Different expression profiles between cardia and non-cardia gastric cancer – delineated by microarray study

Ranking	ID	Name of genes	cardia	non-cardia
1		Human TAXREB67 pseudogene, complete sequence	↑	↓
2		Cytokeratin 20	↑	↓
3		Human pS2 protein gene ↑	↓	
4		Trefoil factor 2 (spasmolytic protein 1)	↑	↓
5		Cytochrome P450 isoform 4F12	↑	↓
6		Human, clone MGC:4172 IMAGE:3631177, mRNA, complete cds	↑	↓
7		Human CA11 mRNA, complete cds	↑	↓
8		Fructose-1, 6-bisphosphatase 1	↑	↓
9		Alcohol dehydrogenase 1C (class 1), gamma polypeptide	↑	↓
10		Cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 18	↑	↓
11		Cytochrome P450 isoform 4F12	↑	↓
12		Human gene for cytoderatin 20	↑	↓
13		Human L-glycerol-3-phosphate:NAD oxidorductase mRNA,	↑	↓
14		RecQ protein-like 4	↓	↑
15		Glycerol-3-phosphate dehydrogenase 1 (soluble)	↑	↓
16		Human mRNA for skin-antimicrobial-peptide 1	↑	↓
17		Heat shock 60kD protein 1 (chaperonin)	↓	↑
18		DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21	↓	↑
19		Human mRNA for putative carboxylesterase	↑	↓

(Reference: Wu MS, et al. World J Gastroenterol 2005 ;11 :7401-8)

Table 2 Clinicopathological Features According to Annexin Expression

	Annexin expression (Cytoplasm)			
	No (n=47)	Low (n=23)	High (n=11)	p-value
Gender				
Female	14	9	5	0.532
Male	33	14	6	
Age				
Mean (SD)	62.9 (14.2)	59.0 (14.2)	62.6 (17.2)	0.626
MVD				
Mean (SD)	29.7 (19.8)	33.3 (19.3)	32.7 (20.7)	0.743
Location of GC				
Non-cardia	40	22	6	0.009
Cardia	7	1	5	
Stage				
Early	5	1	2	0.443
Advanced	42	22	9	
Type (Lauren's)				
Intestinal	24	13	4	0.598
Diffuse	20	9	5	
Mixed	3	1	2	
LN meta				
No	13	3	5	0.120
Yes	34	20	6	
Lymphatic invasion				
No	22	10	7	0.674
Yes	23	10	4	
Vessel invasion				
No	24	10	7	0.760
Yes	21	10	4	
Liver Meta				
No	45	23	9	NS
Yes	1	0	2	

Table 3 Clinicopathological Features According to Annexin Expression

	Annexin expression (Cytoplasm)		
	No+Low (n=23)	High (n=11)	p-value
Gender			
Female	23	5	0.414
Male	47	6	
Age			
Mean (SD)	61.6 (14.2)	62.6 (17.2)	0.845
MVD			
Mean (SD)	30.89 (19.6)	32.73 (20.7)	0.774
Location of GC			
Non-cardia	62	6	0.006
Cardia	8	5	
Stage			
Early	6	2	0.330
Advanced	64	9	
Type (Lauren's)			
Intestinal	37	4	0.280
Diffuse	29	5	
Mixed	4	2	
LN meta			
No	16	5	0.112
Yes	54	6	
Lymphatic invasion			
No	32	7	0.377
Yes	33	4	
Vessel invasion			
No	34	7	0.486
Yes	31	4	
Liver Meta			
No	68	9	NS (McNemar)
Yes	1	2	

參考文獻

1. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000: the global picture. Eur J Cancer 2001;37:s4-s66.
2. Fuch CS, Mayer RJ. Gastric carcinoma. N Engl J Med 1995; 333;32-41.
3. Thompson GB, Ran Geerden JA, Sarr MG. Adenocarcinoma of the stomach: are we making progress? Lancet 1993;342;713-718.
4. Devesa SS, Fraumeni JF Jr. The rising incidence of gastric cardia cancer. J Natl Cancer Inst 1999;91:747-749.
5. Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factor. J Clin Epidemiol 2003;56:1-9.
6. Blot WJ, Devesa SS, Kneller RW, et al. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287-1289.
7. Siewert JR, Stein HJ. Classification of carcinoma of the esophago gastric junction. Br J Surg 1998;85:1457-1459.
8. Rasch VW. Are cancers of the esophagus, gastroesophageal junction and cardia one disease, two or several ? Semin Onclol 2004;31;444-449.
9. Correa P. Helicobacter pylori and gastric cancer: state of the art. Cancer Epidemiol Biomarkers Prev 1996;5:477-481.
10. Wu MS, Chen SY, shun CT, et al. Increased prevalence of Helicobacter pylori infection among patients affected with intestinal-type gastric cancer at non-cardiac location. J Gastroenterol Hepatol 1997;12;425-428.
11. Anagnostopoulos I, Hummel M. Epstein-Barr virus in tumors. Histopathology 1996;29;297-315.
12. Wu MS, Shun CT, Wu CC, et al. Epstein-Barr-virus associated gastric carcinoma: relation to Helicobacter pylori infection and genetic alterations. Gastroenterology 2000;118:1031-1038.
13. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;;61:759-767.
14. Stemmermann GN, Heffelfinger SC, Noffsinger AMY, et al. The molecular biology of esophageal and gastric cancer and their precurors: oncogenes, tumor suppressor genes, and growth factors. Hum Pathol 1994;25:968-981.
15. Wright PA, Quirke P, Attanoos R. Molecular pathology of gastric carcinoma: progress and prospects. Hum Pathol 1992;23:848-859.
16. Tahara E, Semba S, Tahara H. Molecular biological abservations in gastric cancer. Semin Oncol 1996;23:357-365.
17. Beuzen F, Dubois S, Flejou JF. Chromosomal numerical aberrations are frequent in esophageal and gastric adenocarcinoma: a study using in situ hybridization. Histopathology 2000;37:241-249.
18. El-Rifai W, Frierson HF Jr, Hoskaluk CA, et al. Genetic differences between

adenocarcinoma arising in Barrett's esophagus and gastric mucosa. Gastroenterology 2001;121;592-598.
19. Taniee P, Martel-Planche G, Maurici D, et al. Molecular and clinical differences between adenocarcinoma of the esophagus and of the gastric cardia. Am J Pathol 2001;158:33-40.
20. Wu MS, Chang MC, Huang SP, et al. Correlation of histologic subtypes and replication error phenotype with comparative genomic hybridization in gastric cancer. Genes Chromosomes Cancer 2001;30:80-86.
21. Huang SP, Wu MS, Shun CT, et al. Interleukin-6 increases vascular endothelial growth factor and angiogenesis in gastric carcinoma. J Biomed Sci 2004;11;517-527.
22. Huang SP, Wu MS, Shun CT, et al. Cyclooxygenase-2 increase hypoxia-inducible factor-1 and vascular endothelial growth factor to promote angiogenesis in gastric carcinoma. J Biomed Sci 2005;12:229-241.
23. Chang CC, Shih JY, Jeng Ym, et al. Connective tissue growth factor and its role in lung adenocarcinoma invasion and metastasis. J Natl Cancer Inst 2004;96:364-375.

已發表文章

1. Wu MS, Lin YS, Chang YT, Shun CT, Lin MT, Lin JT. Gene expression profiling of gastric cancer by microarray with laser capture microdissection. World J Gastroenterol 2005;11:7401-7408.
2. Chang YT, Wu MS, Chang YT, Chen CC, Lin YS, Hsieh T, Yang PC, Lin JT. Distinct gene expression profiles in gastric epithelial cells induced by different clinical isolates of Helicobacter pylori: Implications of bacteria and host interaction in gastric carcinogenesis. Hepatogastroenterology 2006;53:484-490.

出席國際學術會議心得報告

計畫編號	95-2314-B-002-054-
計畫名稱	以多基因表現組合探索貴門部與非貴門部胃癌致病機轉之生物標記(2/3)
出國人員姓名 服務機關及職稱	林肇堂 台大醫學院內科
會議時間地點	2007 年 5 月 19 日至 24 日在美國華盛頓
會議名稱	美國消化醫學週
發表論文題目	1. Role of interleukin-6 gene promoter polymorphism -634G/C and serum interleukin-6 level in gastric adenocarcinoma 2. MMP-9-1562 C/T promoter polymorphism associated with gastric cancer development in female

一、參加會議經過

此次美國消化醫學週(Digestive Disease Week，簡稱 DDW)自 2007 年 5 月 19 日至 24 日在美國首府華盛頓之會議中心舉行，共有來自世界各地的醫師及專家學者約 2000 人參加。其主題涵蓋消化系統(包括食道、胃、小腸、大腸、肝、膽、胰)疾病之病態生理學及其治療與預防。DDW 大會除了有 12 個臨床討論會 Clinical Symposia，約 60 場之”與教授或專家座談”，另外在肝病方面也有 10 場研究討論會。在內視鏡方面，一如往例，現場有許多小型的 DVD 播放場地，讓學員可以自由學習，稱為”學習中心”。在當代肝臟學講座方面，以 C 型肝炎、肝移植、酒精性肝病為主流。在胃腸學方面，以胃食道逆流(GERD)、NSAID 及抗血小板藥物對胃粘膜之傷害、炎性腸症(IBD)、大腸癌篩檢、胰腫瘤、腸道營養術、益生菌(Probiotics)…等主題最為熱門。在內視鏡方面，則以小腸鏡及膠囊內視鏡之突破最多。在內視鏡診斷技術上，以窄頻成像(Narrow band imaging, NBI)之技術進展最大，特別是在食道疾病及大腸息肉經內視鏡檢查加上 NBI 處理後，其影像可以大幅改善，並提高癌症及其他病變之診斷能力。治療內視鏡方面則是以內視鏡粘膜切除術(EMR)及內視鏡粘膜下剝離術(ESD)兩項技術突破最多，都有許多熱烈的討論。本人今年共有二篇論文在 DDW 發表，其一為”Role of interleukin-6 gene promoter polymorphism -634G/C and serum interleukin-6 level in gastric adenocarcinoma”主要是探討決定胃癌手術後之存活因素 IL-6 之基因多形性及血清 IL-6 濃度的關係。其二為”MMP-9-1562 C/T promoter polymorphism associated with gastric cancer development in female”，主要是探討女性胃癌病人 MMP-9 之基因多形性的特徵，在 5 月 20 日以壁報方式發表，當天也有一些學者在壁報展示時，詢問相關的問題並討論到一些未來可能合作的主題。

二、與會心得

此次大會有幾個具有潛力的主題都有熱烈的討論，其中包括

(1) 自然管孔經體腔內視鏡手術(Natural orifice transluminal endoscopic surgery，簡稱 NOTES)－這是突破傳統思維的手術方式，包括經口穿胃之膽囊手術或經陰道膽囊切除術等，都是很有創意的手術，未來是否可能成為膽囊手術之主流，仍有待觀察。

(2) 代謝症候群(Metabolic syndrome)、脂肪肝、胃腸腫瘤的關係愈來愈被重視，大會甚至邀請曾獲諾貝爾醫學生理學得獎的 Silverstein 來演講代謝症候群，可見其重要性。

(3) 肥胖之併發症及治療肥胖之胃部手術之利弊也有許多熱烈的討論

(4) 食道之巴雷氏食道與腺癌的關係仍有許多爭議存在，特別是在治療方面是否採取積極的手術或內視鏡療法也仍有一番辯論。

(5) 大腸癌之篩檢一直是西方人最熱門的話題，今年除了加入 NBI 這項新技術以改善醫療品質之外，傳統的糞便潛血反應，糞便 DNA 檢查及虛擬大腸鏡都是重要的診斷工具，孰優孰劣，自然引起一番論戰。

(6) 腸道菌株的研究是今年最新的熱門話題。過去對大腸內的菌株往往無法深入研究，因為大腸內已有許多細菌，有些細菌在普通的培養狀態常常長不出來，因此一直無法了解大腸內真正有那些細菌群落。直到最近，在分子醫學上有所突破，可以利用分生的方法找出糞便中的細菌 DNA 片斷，用它可以鑑認出細菌之群落，再比較這些細菌群落彼此之消長，可以用來判斷它與肥胖、或使用抗生素之關係。甚至在其他疾病狀況下都可利用此項技術提供很有價值的參考。這種研究，甚至已推向美國國家衛生院(NIH)，試圖研究所有存在於人類體內之微生物體(Microbiotome)，進而成為 Human microbiotome program。

雖然 DDW 之會議時間只有五天，但其內容多而不亂，參加人數也日益增加，表示這項會議仍是國際第一流的消化醫學活動。國人近年來參加國際學會之人數逐年遞減，發表論文更形減少，因為 DDW 的學術水準很高，發表論文的原創性也高，應大力鼓勵國內學者參與此會，以加速提昇我國消化醫學之水準。