行政院國家科學委員會專題研究計畫成果報告

以比較基因雜交和微衛星體分析研究與胰臟癌有關的基因變化特徵(1/3)

Characterization of genomic alterations associated with pancreatic cancer by comparative genomic hybridization and microsatellite analysis

計畫類別：■個別型計畫　□整合型計畫

計畫編號： NSC89-2314-B-002-241

執行期間： 88**年** 8**月** 1**日至** 89**年** 7**月** 31**日**

個別型計畫：計畫主持人：林肇堂醫師

整合型計畫：總計畫主持人：
子計畫主持人：

註：整合型計畫總報告與子計畫成果報告請分開編印各成一冊，彙整一起繳送國科會

處理方式： □**可立即對外提供參考**
■**一年後可對外提供參考**
□**兩年後可對外提供參考**
(**必要時，本會得展延發表時限**)

執行單位：台大醫學院內科

中華民國八十九年五月十八日

中 文 摘 要

胰臟癌約 90%是從胰管長出的腺癌，它們和從膽管或十二指腸附近所發生的腺癌，及至黏液性囊腺癌在臨床表現及治療結果上有明顯差異。目前有愈來愈多証據指出胰臟癌的多步驟致癌過程中累積許多基因變化，而且認為胰臟癌在腫瘤生物學上的差異可能與其基因變化之種類不同有關，因此釐清參與胰臟癌發生及進展的基因，將可反映其原因及臨床病理的差異性。雖然如此，過去有關胰臟癌的基因變化研究，由於受限於傳統顯微分離技術及細胞遺傳方法上的限制，欠缺系統性的了解。本研究的目的在於進一步(1)探討胰臟癌基因體的整體變化(2)尋找胰臟致癌相關之基因表現(3)釐清胰臟癌發生與進展時的基因變化(4)闡明基因變化與胰臟癌的臨床病理特徵關係。

在第一年的計劃中，吾人首先以回溯性及前瞻性共收集一系列包括胰臟及胰臟附近的癌症病例，共有胰臟癌 33 例(表 1)，十二指腸乳頭癌 30 例(表 2)，非胰管腺癌 16 例(表 3)及 5 例慢性胰臟炎(表 4)，這些案例目前將用免疫組織化學染色法分析細胞周期蛋白及常見基因變化。另外為了取得較純檢體做進一步的 DNA 和 RNA 分析，有 10 例胰臟癌以雷射捕捉顯微切割系統(LCM)取得癌細胞(圖 1)，並順利抽取 DNA 分析 k-ras 基因變化，結果發現有 5 例在 codon 12 上發生 GGTǦGAT 的點突變(圖 2)，証實此套系統可成功運用於胰臟癌的研究。

在第二年的計劃中，吾人除了繼續收集更多樣化的檢體(組織、血和不同部位及病理亞型)外，將利用 LCM 取得胰臟癌細胞的 DNA 或 RNA，以過去吾人在胃癌已建立的微衛星體分析和比較基因雜交法(參考文獻 13-18)等技術探討更詳細的基因變化及其在胰臟癌的臨床病理的意義。

關鍵詞：胰臟癌，基因變化，雷射捕捉顯微切割系統，比較基因雜交法，微衛星體分析

Table1. Summary of 33 patients of pancreatic cancer

Name	Chart No.	Sex	Age	Location
盧鏗鎗	3087376	M	67	head
廖黃菜根	3202462	F	62	head
王永聰	2125577	M	36	head
林正雄	3251066	M	51	head
張漢鼎	3250594	M	70	head
楊樹木	3319656	M	67	head
于越周	3362512	M	76	head
張嬌	3371800	F	64	head
陳成枝	3444090	M	66	head
林富田	3470824	M	56	head
潘金枝	1571702	F	69	head
鄭書壤	3483369	M	62	head
林蔡淑如	2940848	F	59	head
劉長庚	3533061	M	60	head
許林紡	3641883	F	67	head
謝茂潭	3550074	M	60	head
杜文祥	2920646	M	73	head
蔡梅喜英	3481448	F	67	head
王源雄	3344365	M	54	head
劉忠城	3605820	M	30	head
王錦和	3363773	M	52	head
林榮德	1412474	M	63	head
陳阿雲	3709969	F	71	head
鄧于文	3620283	F	46	head
沈月	3613250	F	66	head
黃郭阿秀	2950092	F	64	head
林王阿員	2645699	M	74	head
張樹枝	2551476	M	62	head
沈秀鑾	3203006	F	78	head
林國勢	3255368	M	65	head
蔡義隆	3319413	M	54	head
李張阿敏	1696782	F	54	head
游鳳鑾	3580171	F	51	head & tail

Table 2. Summary of 30 patients of papilla Vater cancer

Name	Chart No.	Sex	Age	Location
何振	2361707	M	69	papilla
劉月潔	3069541	F	41	papilla
劉陳花	3132970	F	54	papilla
曾國斌	3133076	M	59	papilla
葉根祥	2085458	M	71	papilla
李春良	3392004	M	76	papilla
楊黃玉英	3271206	F	61	papilla
蔡李玉蘭	3450853	F	67	papilla
陳森穆	3453751	M	37	papilla
方志堅	2559461	M	71	papilla
官呂勉	3515541	F	63	papilla
王金蓮	3552157	F	49	papilla
陳阮秀鸞	3553113	F	59	papilla
李文賓	2502350	M	69	papilla
周詩群	3688742	M	70	papilla
游秋鳳	3738613	F	35	papilla
陳劉玉嬌	2960715	F	66	papilla
郭土	2172381	M	63	papilla
林秀英	3045247	F	47	papilla
呂愛	2009826	F	59	periampullar
黃泉五	1525924	M	74	papilla
黃麗嬪	3063307	F	66	papilla
周金台	2807991	M	33	papilla
羅曾火妹	2861192	F	67	papilla
郭萬得	3274963	M	70	papilla
蔡金元	3247489	M	47	papilla
沈慶芳	3304797	M	67	papilla
王菊	2495740	F	58	papilla
朱麗珠	3594280	F	42	papilla
莊忠勇	2968680	M	60	papilla

Table 3. Summary of 16 patients with non-ductal tumor

Name	Chart No.	Sex	Age	Location or Patholgoy
張嬌	3371800	F	64	Head ?
黃克政	0847599	M	66	Duodenal ?
李照蔭	3594771	F	65	CBD
孟琦慧	3578731	F	42	Solid & papillary
何沛	3301873	F	68	Mucinous adeno CA
吳明國	3558791	M	52	Acinar cell ca
鄭馬碧霞	3663831	F	81	Meta. Adeno
黃志士	2977598	M	47	Acinar cell ca
曾鳳蘭	3103589	F	43	Meta. Adeno
許麗玉	3657940	F	48	Diffuse large B cell lymphoma
陳成福	3691771	M	63	Small cell carcinoma
陳鄭月娥	3707015	F	74	Papilla, small cell carcinoma
賴織美	0407089	F	66	Insulinoma
陳承良	3101165	M	85	Meta adeno CA (LN)
巫蕭梅	3714177	F	66	Islet cell tumor
張學峰	3698489	F	30	Diffuse large B cell lymphoma

Table 4. Summary of 5 patients with chronic pancreatitis

Name	Chart No.	Sex	Age
盧鏗鎗	3087376	M	67
林瑩忠	3377542	M	44
蔡李玉蘭	3450853	F	67
蕭南	2589078	M	73
謝茂潭	3550074	M	60

Abstract

About 90% of pancreatic tumors are adenocarcinomas with a ductal phenotype. They differ from adenocarcinoma of the distal bile duct, ampulla of Vater and mucinous cystic carcinoma in terms of clinical manifestations and prognosis. Increasing evidence indicates that pancreatic cancer (PC) development is a multistep event proceeding from normal, preneoplastic lesions, to highly malignant tumor accompanied by accumulations of multiple genetic alterations. Collectively, it was believed that variability in the biologic characteristics of PC may be related to the profile of genetic alterations. Delineating genes involved in development and progression of PC can reflect the heterogeneity of their causes and subtypes. However, genetic changes underlying the initiation and progression of PC lack systemic data. This is partly attributable to the limitation of current research techniques such as manual microdissection and conventional cytogenetics. This project has been designed to further investigate the chromosomal aberrations of PC, identify target genes for DNA amplifications and losses in PC, clarify different genetic alterations in the development of PC, and elucidate the relationship between genetic abnormalities and different subtypes of PC.

In the first year grant period, we have collected the following cases retrospectively and prospectively: 33 patients with PC (Table 1), 30 patients with papilla vater cancer (Table 2), 16 patients with non-ductal pancreatic tumors (Table 3), and 5 patients with chronic pancreatitis (Table 4). These cases have been subjected for immunohistochemical analysis of cell cycle proteins and common genetic alterations. To obtain pure cancer cells for further DNA and RNA analysis, laser capture microdissection (LCM) was performed in 10 cases with PC (Fig 1). K-ras mutations were investigated in the subsequently extracted DNA and disclosed GGTǴGAT point mutation at codon 12 in 5 cases (Fig 2). These results indicated LCM could be successfully applied to investigate PC.

In addition to continuously enrolling more versatile specimens (blood and tissues for different location and histological subtypes), LCM-procured pure DNA or RNA of pancreatic cancer will be further analyzed in the second year period. The microsatellite analysis and comparative genomic hybridization which we have established in gastric cancer (references 13-18) will be applied to study the genetic profiles of these samples and elucidated the clinicopathologic significance of genetic abnormalities in the second year grant period.

Keywords: Pancreatic cancer, Genetic alterations, Laser capture microdiseection, Comparative genomic hybridization, Microsatellite analysis

參 考 文 獻

1. Department of Health, Executive Yuan, Repulic of China. Annual report of vital statistics, 1997. Taipei: Department of Health, Executive Yuan 1998.
2. Warshaw AL, del Castillo CF. Pancreatic carcinoma. N Engl J Med 1992;326:455-465.
3. Rosewicz S, Wiedenmann B. Pancreatic carcinoma. Lancet 1997;349:485-489.
4. Rozenblum E, Schutte M, Goggins M, et al. Tumor suppressive pathways in pancreatic carcinoma. Cancer Res 1997;57:1731-1734.
5. Hahn SA, Schmiegel WH. Recent discoveries in cancer genetics of exocrine pancreatic neoplasia. Digestion 1998;59:493-501.
6. Bramhall SR. The use of molecular technology in the differentiation of pancreatic cancer and chronic pancreatitis. Int J Pancreatol 1998;23:83-100.
7. Pasricha PJ. ERCP meets c-K-ras: towards an improved diagnosis of pancreatic cancer. Gastroenterology 1996;110:311-313.
8. Iwao T, Hiyama E, Yokoyama T, et al. Telomerase activity for the preoperative diagnosis of pancreatic cancer. J Nat Cancer Inst 1997;89:1621-1623.
9. Rodriguez E, Sreekantaiah C, Chaganti RSK. Genetic changes in epithelial solid neoplasia. Cancer Res 1994;54:3398-3406.
10. Hahn SA, Seymour AB, Hoque ATMS, et al. Alleotype of pancreatic adenocarcinoma using xenograft enrichment. Cancer Res 1995;55:4670-4675.
11. Kallioniemi A, Kallioniemi OP, Sudar D,Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818-821.
12. Kuukasjarvi T, Tanner M, Pennanen S, Karhu R, Visakorpi; T, Isola J, Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridization. Genes Chromosom Cancer 1997;18:94-101.
13. Lin JT, Wu MS, Shun CT, Lee WJ, Wang JT, Wang TH, Sheu JC. Microsatellite instability in gastric carcinoma with special reference to histopathology and cancer stages. Eur J Cancer 1995;31:1879-1882.
14. Lin JT, Wu MS, Shun CT, Lee WJ, Sheu JC, Wang TH. Occurrence of microsatellite instability in gastric carcinoma is associated with enhanced expression of erbB-2 oncoprotein. Cancer Res 1995;55:1428-1430.

15. Wu MS, Shun CT, Wang HP, Sheu JC, Lee WJ, Wang TH, Lin JT. Genetic alterations of gastric cancer relation to histological subtypes, tumor stage, and *Helicobacter pylori* infection. Gastroenterology 1997;112:1457-1465.
16. Wu MS, Sheu JC, Wang HP, Shun CT, Lee WJ, Cheng AL, Wang TH, Lin JT. Infrequent hMSH2 mutations in sporadic gastric adenocarcinoma with microsatellite instability. Cancer Lett 1997;112:161-166.
17. Wu MS, Lee WC, Shun CT, Wang HP, Lee WJ, Shen JC, Lin JT. Clinicopathologic significance of altered loci of replication error and microsatellite instability associated mutations in gastric cancer. Cancer Res 1998;58:1494-1497.
18. Wu MS, Chang MC, Huang SP, Tseng CC, Shen JC, Lin YW, Shun CT, Lin MT, Lin JT. Correlation of histologic subtypes and replication error phenotype with comparative genomic hybridization in gastric cancer. (submitted)

即將發表之論文

1. Chang MC, Chang YT, Wu MS, Shun CT, Tien YW, Lin JT. K-ras mutatioin at codon 12 of pancreatic adenocarcinoma in Taiwan: analysis by laser capture microdissection and direct sequencing (submitted).