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(3/3)

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

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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，將可能反映其致癌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方式有系統地收集包括胰臟及胰臟附近的癌症病例，為了取得較純檢體做進一步的 DNA，有 15 例胰臟癌以雷射捕捉顯微切割系統(LCM)取得癌細胞，並順利抽取 DNA 分析 k-ras 基因變化，結果發現有 9 例在 codon 12 上發生 GGT→GAT 的點突變(表一)，証實此套系統可成功運用於胰臟癌的研究(詳見已發表論文 1)。而 k-ras 的基因變化在不同的研究報告中(參考文獻 13)，由於所使用的方法與檢體不同，所得之結果亦異。尤其在胰臟癌的腫瘤當中，有許多的基質細胞(stromal cell)可能干擾到對胰臟癌基因研究的結果，所以目前之趨勢，均建議應以“純粹”的癌細胞來研究。而吾人之報告，即為文獻中首次應用雷射捕捉顯微系統(LCM)來分析 k-ras 在胰臟癌之變化情形。此外，在西方的文獻中，也注意到可能有一類以野生型(wildtype) ras 基因之突變為主的胰臟癌，名之為 medullary carcinoma of pancreas，暗示可能由於不同的致癌過程，k-ras 之變化與否也有所差異(參考文獻 14)。

在第二年的計劃中，吾人運用免疫組織化學染色法分析與細胞週期蛋白(cell cycle protein)常見的基因變化，包括 p16, Rb, cylin D 與 p53。結果發現在 30 例確定為胰臟癌的檢體當中，70%(21/30)有 Rb 的變化；40%(12/30)有 p53

的突變；26.7%(8/30)有 p16 的變化；26.7%(8/30)有 cyclin D 的變化。而在 25 例確定為十二指腸乳頭癌之病例當中，68%(17/25)有 Rb 的變化，24%(6/25)有 p53 的變化，4%(2/25)有 p16 的變化 56%(14/25)有 cyclin D1 的變化(表二)。依初步之統計分析，這些細胞週期蛋白的變化，在分辨胰臟癌或十二指腸乳頭癌上，不具統計學上的差異。至於更一步的了解，將配合後續對於這些基因之變化分析予以釐清。

在第三年的計劃中，吾人以比較基因雜交法分析胰臟癌的基因變化，發現在染色體有 gain 的包括 1p (36.4%), 2p(36.4%), 2q(36.4%), 3q(45.5%), 5p(63.6%), 7p(36.4%), 8q(45.5%), 9p(36.4%), 13q(54.5%), 18q(36.4%), 21q(36.4%), Xp(31.4%)。而在染色體 1p(36.4%), 1q(27.3%), 2q(27.3%), 3p(27.3%), 7p(27.3%), 11q(36.4%), 16q(27.3%), 17p(63.6%), 17q(27.3%), 18q(45.5%), 19q(36.4%)則有不同程度的 loss(表三)。

在此計劃當中，吾人利用 LCM 取得胰臟癌細胞的 DNA，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 15-20)等技術探討更詳細的基因變化的區段及其在胰臟癌的臨床病理的意義：由於所分析的是 genomic DNA，所以在一些石蠟包埋組織或是保存包埋不妥切的組織，無法得到較好的 DNA 來分析。故吾人僅將檢體收集得宜並得到較佳品質的組織與 DNA 予以分析，亦為後續之研究建立基礎，之後亦將善用此次之經驗繼續收集並研究胰臟癌之基因變化。

關鍵詞：胰臟癌、基因變化、免疫組織化學染色法、雷射捕捉顯微切割系統、比較基因雜交法、微衛星體分析

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. To obtain pure cancer cells for further DNA analysis, laser capture microdissection (LCM) was performed in 15 cases with PC. K-ras mutations were investigated in the subsequently extracted DNA and disclosed GGT→GAT point mutation at codon 12 in 9 cases (Table 1). These results indicated LCM could be successfully applied to investigate PC. Different data were reported about the k-ras mutation rate by using different methods or material in the literature (reference 13). However, abdance of stromal cell DNA will easily contaminate the analysis of genetic alterations of pancreatic cancers. Microdisseciton is strongly suggested in investigation of pancreatic cancers nowadays. Our report is the first one which analyzed the “true” k-ras mutation rate in pancreatic cancer using laser capture microdissection. Besides, the relative lower rate of k-ras mutation may echo the newly classified category of PC, medullary caninoma of pancreas, which imply a different caninogenesis (reference 14).

In the second year grant period, we performed immunohistochemical analysis of cell cycle proteins, including p16, Rb, cyclin D and p53 in our specimens. The data showed 70%(21/30) of pc with Rb changes; 40%(12/30) with mutant p53; 26.7%(8/30) with p16 changes and 26.7%(8/30) with cyclin D changes. Besides, in the cancers of papilla of vater, 68%(17/25) was with Rb changes; 24%(6/25) with mutant p53; 4%(2/25) with p16 changes and 56%(14/25) with cyclin D changes (Table 2). There is no obvious statistical difference between above 2 groups noticed. However, further analysis of these genes is mandatory to elucidate the role of these genes in cancinogenesis of pancreatic cancer.

In addition to continuously enrolling more specimens LCM-procured pure DNA of pancreatic

cancer and noncancerous tissue were be further analyzed in the last year period. The comparative genomic hybridization which we have established in gastric cancer (references 15-20) was applied to study the genetic profiles of these samples and elucidate the clinicopathologic significance of genetic abnormalities in the third year grant period. We found that chromosomes gains occurred more frequently in 1p(36.4%), 2p(36.4%), 2q(36.4%), 3q(45.5%), 5p(63.6%), 7p(36.4%), 8q(45.5%), 9p(36.4%), 13q(54.5%), 18q(36.4%), 21q(36.4%), Xp(31.4%). In the other hands, chromosome loss occurred more frequently in chromosome 1p(36.4%), 1q(27.3%), 2q(27.3%), 3p(27.3%), 7p(27.3%), 11q(36.4%), 16q(27.3%), 17p(63.6%), 17q(27.3%), 18q(45.5%), 19q(36.4%) (Table 3).

In this project, we also noticed that some genomic DNA extracted from paraffin embedded formalin fired tissues might be well- applicated by DOP-PCR followed by CGH. However, some cases even though dealt with OCT might not be feasible in such applications. Therefore, some valuable experiences in dealing with tissue observation and management also were obtained in this grant period. We will keep on collecting suitable specimens and completing more data based on the present results in the future.

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

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表一(Table 1)曾被報告 K-ras 在胰臟癌的變化情形

Location	codon 12 (GAT→GGT): highest	
	codon 13	: occasion
	codon 61	: rare
Frequency	95-100%	western
	50-60%	Japan (non-diseases)
	60%	Taiwan (LCM-microdissected specimen)
time event in caningoeic	early	

表二(Table 2)以免疫化學組織染色法分析壺腹周圍癌細胞週期蛋白之變化

	Ductal pancreatic cancer	Cancer of papilla of Vater
P16	26.7%(8/30)	4% (2/25)*
Rb	70%(21/30)	68%(17/25)*
Cycli D	26.7%(8/30)	64%(14/25)*
P53	40%(12/30)	24%(6/25)*

P<0.05

表三(Table 3)以比較基因雜交法分析胰臟癌之染色體變化情形

gain	loss
1p (36.4%)	1p (36.4%)
2p (36.4%)	1q (27.3%)
2q (36.4%)	2q (27.3%)
3q (45.5%)	3p (27.3%)
5p (63.6%)	7p (27.3%)
7p (36.4%)	11q (36.4%)
8q (45.5%)	16q (27.3%)
9p (36.4%)	**17p (63.6%)**
13q (54.5%)	17q (27.3%)
18q (36.4%)	**18q (45.5%)**
21q (36.4%)	19q (36.4%)
Xp (31.4%)	