計畫成果報告

計畫名稱:利用微小衛星來做肝癌的基因變化之細微定位

Fine Mapping of the Genetic Changes of Hepatocellular Carcinoma by Microsatellite Analysis

計畫編號: **NSC89-2315-B-002-018**

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執行期間:88 年 8 月 1 日至 89 年 7 月 31 日

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中文摘要

關鍵詞：肝細胞癌，微小衛星，雜合子丟失

肝細胞癌是全世界最好發的癌症之一，在台灣也是國人癌症死因的第一位，目前真正的致病機轉仍不清楚。肝癌的發生通常伴隨基因的變化，其中包括致癌基因的活化、或癌抑制基因的不活化。目前已知有多種人類腫瘤均與癌抑制基因之變異有關。近年來利用染色體中的微小衛星可以有系統而快速的分析比較每一染色體中基因的變化，更可進一步尋找可能的癌抑制基因。

在本研究中，我們選用 88 例肝癌的病例，有系統地比較染色體 16q 上腫瘤與非腫瘤部份基因的變化，從而得知雜合子丟失情形(LOH)，對每對 primer 來說，雜合子丟失情形(LOH)的範圍從 2.5%到 45%(平均 17.5%)。.頻率最高的 primer 包括 D16S496, D16S504, D16S3034, D16S3039, D16S517, D16S409, D16S514, D16S419, D16S3080, D16S3018, D16S3137, D16S415, D16S390, D16S515, D16S3048, D16S408, D16S266,和 D16S422。雜合子丟失(LOH)的頻率似乎與 B 型肝炎帶原有關，有統計上的意義。

我們的研究結果顯示肝癌病患在 16q 常有雜合子丟失(LOH)的情形，且細微定位的結果靠近 16q12.1,16q21-22 和 16q24.3。因此表示這些位置可能有癌抑制基因的存在。目前，我們利用位於 16q12.1 的標記(D16S415, D16S419, D16S409, D16S3080 及 D16S3034) 開始篩選人類之細菌人工染色體基因庫(Bacterial Artificial Chromosome Library, BAC Library)，並配合表現序列捕捉系統(exon trapping system)找到表現序列的 clones。總共選殖到 10 個可能的表現序列，與 NCBI (national center for Biotechnology Information)的資料庫比對的結果，有二個序列與最近被發現的 KIAA1005 基因序列完全相同。而 KIAA 1005 基因在肝癌組織中有同源性丟失的現象，反轉錄-聚合酶連鎖反應(RT-PCR)的結果也顯示 mRNA 的表現有差異，因此我們推測 KIAA1005 基因可能是位於 16q12.1 附近的假想抑癌基因

Abstract

Key words: hepatocellular carcinoma, micorsatellite analysis, tumor suppressor gene

Hepatocellular carcinoma (HCC) is one of the most common cancer in the world and is the leading cause of cancer death in Taiwan. The prognosis of this cancer is extremely poor with survival of only several months after symptoms occurred. Elucidation of the basic genetic changes of HCC is important for the understanding and treatment of this cancer. Cancer is usually accompanied with genetic alternations either through the activation of cellular oncogene or the inactivation of cancer suppressor gene. The recently identified short tandem repeat, the microsatellite, which is widely distributed throughout the whole human genome. Identification of disease genes as well as tumor suppressor genes by microsatellite polymorphism. have been published recently. In this study we use microsatellite marker to analyze 88 cases of HCCs, most of them are small in size, in order to study the genetic changes of HCC and to further narrow down the common LOH sites in 16q.

A total of 36 microsatellite markers in chromosome 16q were used to study the LOH of HCC. The average number of microsatellite markers included in 16q was about 3-5 cM. Range of LOH for each primer was from 2.5 to 40.5% and the average was 17.5%. The highest percentage of LOH was shown for loci at D16S496(40.5%), D16S504(33.9%), D16S3034(30.2%), D16S3039(30%), D16S517(27%), D16S409(27%), D16S514(26%), D16S419(25%), D16S3080(24.7%), , D16S3018 (24%), D16S3137(23.4%), D16S415(22%), D16S390(22%), D16S515(21%), D16S3048(20.5%), D16S408(20%), D16S266(18%), and D16S422(18%). The frequency of LOH seemed to have relationship with HBV carrier. The HBsAg positive group seemed to have higher LOH frequency at 16q12.1, and the difference was statistatically significant.

In this study, we have used 35 microsatellite markers for further fine mapping of LOH. We have confirmed the most frequent regions of LOH for HCC are 16q12.1, 16q22, and 16q24. After analyzing these information, we started to screen the human BAC(Bacterial Artificial chromosome)library by these markers at 16q12.1 and we identified 15 clones. Exon trapping system is used to search the putative exon sequences of BAC genomic clones. Two exon-like sequences are identical to the KIAA1005 gene. Homozygous deletion of KIAA1005 was found in 37%(10 /27) HCCs. These data suggested that the KIAA1005 might be the putative tumor suppressor genes at chromosome 16q12.1.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancer in the world [1] and is the leading cause of cancer death in Taiwan [2-11]. Chronic hepatitis B and recently the hepatitis C viral infection is thought related to the development of HCC [2-13]. However, the basic molecular mechanism remained to be clarified. Cancer is usually accompanied with genetic alternations either through the activation of cellular oncogene or the inactivation of cancer suppressor gene [14-25]. Traditionally, cytogenetics was used to detect genetic change of cancer[26-32]. However, only major genetic changes resulting in chromosomal translocation, deletion or other changes can be identified by this method. Another method to detect the cancer suppressor gene is the use of RFLP (restriction fragment length polymorphism). However, this method is tedious, laborious and need a lot of DNA samples. Microsatellite, which is short tandem sequences is widely distributed throughout the whole human genome. The target DNA sequences can be amplified by polymerase chain reaction and the fragment was run on gel. Only small sample of starting DNA sample was needed. These markers were rather complete evenly distributed in the whole chromosome. Using the microsatellite as markers, we can study the loss of heterozygosity(LOH) of tumor[33-92].Identification of disease gene including tumor suppressor gene by the linkage analysis of comparison of tumor and non-tumor part had been published recently[58,59].

In the past years, we have using 232 microsatellite markers to study the LOH of HCC. We included 30 cases of HCC and we have found the most common site of LOH for HCC is: 16q(43.3%), 13q(36.7%), 17p(30%), 5q(20%), 11p(20%), 11q(16.7%). These data provides as a basis for further fine mapping of the common site of LOH and to identify the putative tumor suppressor gene.

In this proposal, we will further study the LOH of HCC by narrowing down the common site of LOH and to identify the putative tumor suppresser gene. Since the 16q has the highest rate of LOH, we will start from this chromosome in the first year. Our preliminary study indicated that the most minimal commonly affected region of 16q was deduced to be between 16q12.1 and 16q12.2. Another small region was determined to be 16q24.1. Therefore, in this project, we used more micorsatellite markers in these 3 regions to narrow down the common site of LOH and to identify the putative tumor suppressor gene.

Materials and Methods

Methods

LOH analysis

Genomic DNA was extracted from tumor and non-tumor part with classical methods, then these samples subjected to study by using the microsatellite markers in the aforementioned 2 region. All the markers available from the literature will be used. Each reaction was amplified in a total volume 25 ul containing 25 ng of genomic DNA; 75 uM concentrations each of dGTP, dCTP,

and dTTP; 2.5 uM dATP; 2 uCi[a-35S]dATP; 1x PCR buffer (10 mM Tris-Cl pH 8.3, 1.5 mM MgCl2, 0.01% gelatin); 0.2 uM for each primer; and 0.5 u of Taq polymerase. The PCR was performed in 94°C for 30 sec, 55°C for 75 sec, 72°C for 15 sec. A total of 27 cycles were performed. The product was further incubated in 72°C for another 6 min. After completion of PCR, 6 microliter products were subjected to electrophoresis in a 6% PAGE gel followed by exposure of the gel to X ray film for 1 to 2 days.

The band pattern between tumor and non-tumor part was compared. The LOH was analyzed and the common site of LOH was deduced.

BAC library screening

After analyzing data, we started to screen the human BAC(Bacterial Artificial chromosome) by these markers at 16q12.1 and we identified 15clones.

Exon trapping system

We applied exon amplification strategy to isolate genes from complex BAC genomic DNA (Figure 1).

Results

LOH and fine mapping analysis of 16q in HCC

In this study we use microsatellite marker to analyze 88 cases of HCCs, most of them are small in size, in order to study the genetic changes of HCC and to further narrow down the common LOH sites in 16q.

A total of 36 microsatellite markers in chromosome 16q were used to study the LOH of HCC. The average number of microsatellite markers included in 16q was about 3-5 cM. Range of LOH for each primer was from 2.5 to 40.5% and the average was 17.5%. The highest percentage of LOH was shown for loci at D16S496(40.5%), D16S504(33.9%), D16S3034(30.2%), D16S3039(30%), D16S517(27%), D16S409(27%), D16S514(26%), D16S419(25%), D16S3080(24.7%), D16S3018(24%), D16S3137(23.4%), D16S415(22%), D16S390(22%), D16S515(21%), D16S3048(20.5%), D16S408(20%), D16S266(18%), and D16S422(18%) (Figure2). The frequency of LOH seemed to have relationship with HBV carrier. The HBsAg positive group seemed to have higher LOH frequency at 16q12.1, and the difference was statistatically significant.

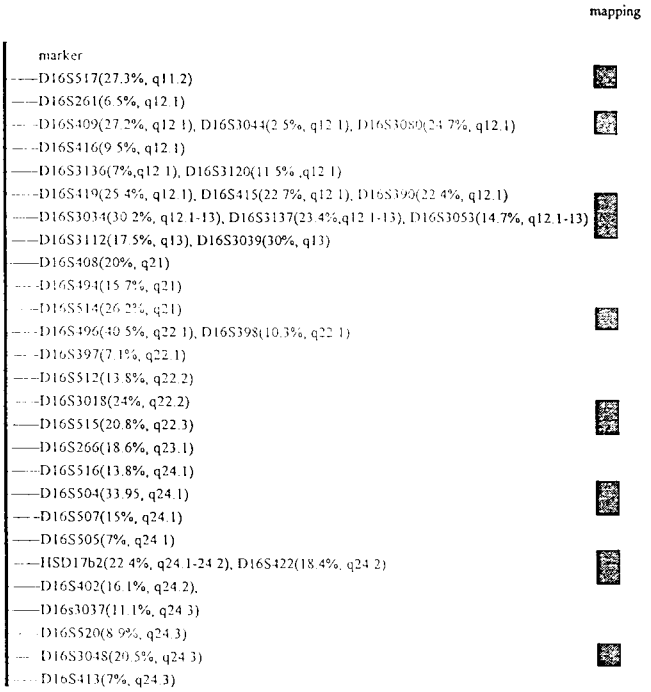
In the past year, we started to screen the human BAC(Bacterial Artificial chromosome)library by these markers at 16q12.1 and we identified 15 clones(Table 1). Exon trapping system is used to search the putative exon sequences of BAC genomic clones. Two exon-like sequences are identical to the KIAA1005 gene. Homozygous deletion of KIAA1005 was found in 37%(10 /27) HCCs. These data suggested that the KIAA1005 might be the putative tumor suppressor genes at chromosome 16q12.1.

Discussion

Our preliminary result shows that HCCs in Taiwan have frequent LOH in chromosome 16q and the common LOH regions were narrowed down to D16S409 (16q12.1), D16S419-D16S408 (16q12.1), D16S514-D16S496 (16q21-22), and D16S422-D16S402 (16q24.3). Furthermore, familial HCC seems to have common LOH sites at 16q12.1 and 16q21-22. These data suggested that the putative tumor suppressor genes might lie in these regions and we identified 15 BAC clones near these regions.

In addition to construct the physical map of 16q, we also intend to search the possible exon sequence near these regions. Exon trapping system is a good approach to search the possible exon sequences. Now we have applied the system to our project. Furthermore, we try to clone full-length cDNA using RACE system and search for the putative tumor suppressor genes.

圖一 染色體 16q 共同丟失區域及其 physical map 總覽



表一：微小衛星標記篩選人類 BAC 基因庫的結果

Microsatellite loci used for screening	BAC clone
D16S409	106K13
D16S419	107F06
D16S415	29M12
	60F14
	72M15
D16S390	211D06
D16S3034	71E17
	35D12
	216N13
	250G9
D16S3044	25L24
	26L24
	39O04
D16S3080	46B1
	58M17

表二：質體 pSPL3 中含有表現序列片段的 clone

微小衛星標記 (Microsatellite marker)	BAC clone	含表現序列的 clone	相似百分比 (% similarity)	比對結果 BLASTN
D16S409	106K13	P1(P9)		
		P8(E40)		
D16S415	029M12	P9(E13)	100	KIAA1005(nt2654-2837)
		P15		
		P16	98	KIAA1005(nt3057-3216)
	072m15	Bp16		
		E3		
		P10		
	060F14	B5		
D16S419	107F06	E1		

圖二：KIAA1005 基因在肝癌組織及肝癌細胞株的變化情形