行政院國家科學委員會補助專題研究計畫成果報告

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※　　　**尋找與人類肝癌有關的抑癌基因之研究**　　　※

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC89－2323－B－002－011－

執行期間：八十九年八月一日至九十年七月三十一日

執行單位：國立台灣大學醫學院內科

計畫主持人：許金川

共同主持人：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

■出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

中　華　民　國 90 **年** 10 **月** 17 **日**

年度研究成果報告內容

中文摘要

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。

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 15 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　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。

在 microarray 的研究方面,則分析了 4 對 HCC 的病人,找到數十個 upregulate 與 downregulate 的基因,將進一步利用定量 PCR 來確認結果。

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 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.

In microarray study, we have assayed 4 pairs of HCCs patients to compare differential gene expression between nontumor part and tumor part. Of the informative genes, 0.3%-0.6% had an

expression level in cancer that was 2 fold lower or higher than that in the non-tumor liver samples , designated as differentially expressed genes. The clustering analysis is in process in our lab. As for these up-regulated or down regulated genes, we will apply quantitative PCR to prove these differences in HCCs.

一、計畫緣起。

Hepatocellular carcinoma (HCC) is the leading cause of cancer death in Taiwan. Chronic hepatitis B and recently the hepatitis C viral infection are thought related to the development of HCC. However, the basic molecular mechanism remained to be clarified. Recent studies have suggested that accumulation of these genetic changes, which affect the expression of oncogenes and tumor suppressor genes, occur in a stepwise manner and during the development of HCC. Many tumor suppressor genes are inactivated by intragenic mutations in one allele accompanied by the loss of a chromosomal region containing the other allele, termed loss of heterozygosity (LOH). Mapping of homozygous deletions within regions showing a high frequency of LOH has been a critical step in the discovery of several tumor suppressor genes.

In the past years, we have using 231 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 basis for further fine mapping of the common site of LOH. In our previous project, we further study the LOH of HCC by narrowing down the common site of LOH on chromosome 16q. The most frequent regions of LOH for HCC are 16q12.1, 16q22, and 16q24. Fifteen clones were identified by screening the human BAC (Bacterial Artificial chromosome) by microsatellite markers at 16q12.1. Exon trapping system is used to search the putative exon sequences of BAC genomic clones. One putative tumor suppressor gene on chromosome 16q12.1 is found.

In this proposal, We will prepare the tissue sample by LCM and extract the DNA or RNA. Then, we will apply microarray system to monitor the expression of ESTs and search the putative tumor suppressor genes on chromosome 16q (16q12.1, 16q22, and 16q24) in HCC patients. Furthermore, we hope that functional assay of putative tumor suppressor genes *in vitro* will be established. Specific genes that are differentially expressed between tumor and non-tumor part will be analyzed to elucidate gene function and their role in hepatocarcinogenesis.

Since HCC is a very common cancer in Taiwan, study the molecular mechanism in important for prevention and improvement of treatment. We hope through this project, we can have a better understanding of the genetic change of HCC and wishing to identify possible tumor suppressor genes of this cancer.。

二、計畫目的

Specific aims

Because HCC is a very common cancer in Taiwan, study the molecular mechanism in important for prevention and improvement of treatment. We hope through this project, we can have a better understanding of the genetic change of HCC and wishing to identify possible tumor suppressor genes of this cancer.

- Objectives (long term):

1. Improvement of liver cancer treatment.
2. As cancer diagnostic marker

- Focus (short term)

1. ESTs on chromosome 16q will be analyzed between non-tumor parts and tumor parts by microarray. Deletion mapping, BAC library screening, exon trapping system will be applied to analysis the other chromosomes regions with high frequency LOH.(first year)
2. Functional assay of candidate genes will be established.(second year)

三、執行進度，包括：

1. 研究成果

ª cDNA microarray analysis.

Method

Microarray membrane

The Human Foundation 1 gene expression microarray, part of Incyte's Human Genome Set of microarrays, contains more than 9,600 verified clones representing both known genes and Incyte proprietary ESTs (Incyte’s company).

cDNA Probes preparation and microarray hybridization

The LifeArray technology uses a color coding technique to discover the differences in gene expression between two mRNA samples. First, the messenger RNA (mRNA) is extracted from the notumor sample, and a fluorescent labeled cDNA probe (cy 3) is generated. Next, the mRNA is extracted from tumor sample. The fluorescent labeling step is repeated to generate a second cDNA probe using a different color fluorescent molecule(cy 5) Fluorescence-labeled cDNA probes were made from 2-µg aliquots of mRNA samples from cells by oligo(dT)-primed reverse transcription using Superscript II reverse transcriptase (Life Technologies, Rockville, MD). The nucleotide concentrations were 10 µmol/L each for dATP, dCTP, and dGTP, and 4 µmol/L for dTTP and 0.1 mmol/L for cy 3 or cy5 dUTP. The reaction mixture contained 3 µg of oligo-dT primers, first-strand buffer (Life Technologies), 0.01 mol/L dithiothreitol (Life Technologies) and 1 µL of RNase inhibitor (Promega, Madison, WI). One microliter of Superscript II reverse transcriptase was added to initiate labeling reaction and incubated at 42°C for 2 hours. The probes were purified by passing twice through a Microcon 30 column (Millipore, Bedford, MA). The blocking reagent, 10 µg of yeast tRNA (SIGMA, St. Louis, MO), 4 µg of poly dA polymer (SIGMA), and 15 µg of human Cot DNA (Life Technologies) were added to probes after the first round of purification. The purified and concentrated fluorescence-labeled cDNA from non-tumor and tumor samples were mixed, and hybridization mixture was prepared. The hybridization mixture was incubated at 100°C for 2 minutes and then at room temperature for 30 minutes, and mounted onto the microarray glass slide. After hybridization and washing, the slides were dried by a brief spin at 500 rpm for 1 minute and immediately subjected to image processing.

Imaging and image analysis

Fluorescence intensities generated by the Cy5 or Cy3 immobilized at the target sequence on the microarray slides were measured by a laser confocal microscope equipped scanning system (ScanArray 3000, General Scanning, Watertown, MA) with appropriate excitation and emission filters. The 2 fluorescent images (Cy5 and Cy3) were scanned separately and stored for further image analysis. Signals from each immobilized DNA target on a microarray slide were localized and the expression ratio between experimental and reference (Cy5/Cy3 ratio) was determined.

Results

The cDNA microarray was applied to analyze the expression patterns of 4 sets of HCC patients, each including paired and distal liver tissue from the same patients. Of the informative genes, 0.3%-0.6% had an expression level in cancer that was 2 fold lower or higher than that in the non-tumor liver samples , designated as differentially expressed genes. Table 1 and Table 2 shows the differentially expressed gene in 4 sets of HCC. The clustering analysis is in process in our lab. As for these up-regulated or down regulated genes, we will apply quantitative PCR to prove these differences in HCCs.

Table 1. Representative list of candidate genes that expressed up-regulated in 4 pairs of HCC by microarray

Human RNA for NADH dehydrogenase.
Human moesin mRNA, complete cds.
Human cDNA: FLJ21171 fis, clone CAS10962.
Human IL-17B receptor (IL17BR) mRNA, complete cds.
Human mRNA for titin protein (clone hsk1-hsk19).
Human cytoplasmic dynein light chain 1 (hdlc1) mRNA, complete cds.
Human S19 ribosomal protein mRNA, complete cds.
Human gene for phosphate carrier.
Human ADP-ribosylation factor 1 gene, exons 2-5.
Human (chromosome X) glypican (GPC3) mRNA, complete cds.
Human HALPHA44 gene for alpha-tubulin, exons 1-3.
Human mRNA for putative p64 CLCP protein.
Human 1-8D gene from interferon-inducible gene family.
Human HSPC224 mRNA, complete cds.
Human CpG island DNA genomic Mse1 fragment, clone 97h11, reverse read cpg97h11.rt1a.
Human Cctg mRNA for chaperonin.
Human myelin basic protein (MBP) mRNA, complete cds.
Sequence 28 from Patent WO9954448.
Human liver glucose transporter-like protein (GLUT2), complete cds.
Human protein phosphatase 2A B56-delta (PP2A) mRNA, complete cds.
Human mRNA for polyA binding protein.
Human ubiquitin gene.
Human DNA for ribosomal protein S13, complete cds, U14 small nucleolar RNA, complete sequence.
Human mRNA for KIAA1293 gene, complete cds.
Human mitochondrial carrier homolog 1 isoform a mRNA, complete cds; nuclear gene for mitochondrial product.
Human ADP-ribosylation factor 1 gene, exons 2-5.
Incyte EST
Human 90-kDa heat-shock protein gene, cDNA, complete cds.

Human mRNA for cytochrome oxidase subunit VIb.
Human cDNA: FLJ22916 fis, clone KAT06406, highly similar to HSCYCR Human mRNA for T-cell cyclophilin.
Human sin3 associated polypeptide (SAP18) mRNA, complete cds.
Human mRNA for diubiquitin.
Human cDNA: FLJ22980 fis, clone KAT11387, highly similar to HSCYC1 Human mRNA for cytochrome c1.
Human mRNA for non-muscle type cofilin.
Human mRNA for lipocortin II, complete cds.
Human lysosomal membrane glycoprotein CD63 mRNA.
Human MHC class I HLA-C-alpha-2 chain and alternative mRNA, complete cds, clones 4 and 10.
Human genomic DNA, chromosome 21q, section 37/105.
Human nuclear protein SDK3 mRNA, complete cds.
Human PRO1608 mRNA, complete cds.
Human flotillin-1 mRNA, complete cds.
Human hum-a-tub2 alpha-tubulin mRNA, complete cds.
Human mRNA for myosin regulatory light chain.
Human mRNA for KIAA0703 protein, complete cds.
Human colon carcinoma laminin-binding protein mRNA, complete cds.
Human mRNA for Nop10p, complete cds.
Human cDNA: FLJ23506 fis, clone LNG03055.
Human sorcin CP-22 mRNA, complete cds.
Human nonmuscle/smooth muscle alkali myosin light chain gene, complete cds.
Human ADP/ATP carrier protein mRNA, complete cds.
Human mRNA for ribosomal protein S18.
Human cDNA: FLJ22915 fis, clone KAT06354, highly similar to Human Wilm's tumor-related protein (QM) mRNA.
Human profilin mRNA, complete cds.
Human FK506-binding protein (FKBP) mRNA, complete cds.
Human full length insert cDNA clone YB24D08

Table 2. Representative list of candidate genes that expressed down-regulated in 4 pairs of HCC by microarray

Human mineralocorticoid receptor (MLR), exon 8.
Incyte EST
Incyte EST
Human aldehyde oxidase (hAOX) mRNA, complete cds.
Human flavin-containing monooxygenase 3 (FMO3) gene, exon 7.
Human cytochrome P450IIE1 (ethanol-inducible) gene, complete cds.
Human afamin mRNA, complete cds.
Human 4q13 genomic sequence.
Human nicotinamide N-methyltransferase gene, exon 1 and 5' flanking region.

Human HSPC062 mRNA, complete cds.
Sequence 13 from Patent WO9951727.
cytochrome P450 2C [Macaca fascicularis=monkeys, liver, mRNA, 1901 nt].
Sequence 92 from Patent WO9955858.
RhoE=26 kda GTPase homolog [Human, HeLa cell line, mRNA, 833 nt].
Human class II alcohol dehydrogenase (ADH4) pi subunit mRNA, complete cds.
Human histidine-rich glycoprotein mRNA, complete cds.
Human cytochrome P450IIA3 (CYP2A3) mRNA, complete cds.
Human mRNA for thrombospondin.
Human 11-beta-hydroxysteroid dehydrogenase (HSD11) gene, exon 6.
Human mRNA for hBD-1 protein.
Human cytokine (GRO-beta) mRNA, complete cds.
Sequence 12 from Patent WO9954460.
Human pre-B-cell stimulating factor homologue (SDF1b) mRNA, complete cds.
Human fetal liver cytochrome P450 (P-450 HFLa), complete cds.
Human mRNA for organic cation transporter, liver.
Human putative glycine-N-acyltransferase mRNA, complete cds.
Human coagulation factor IX mRNA, complete cds.

[a] Exon-like sequences on chromosome 16q12-13

Method

LCM

Unstained thin (about 10 μm) frozen tissue sections was dissected under microscopic visualization. A laser beam and special transfer film are used to lift the desired cells out of the tissue section, leaving all of the contaminating or unwanted cells behind. At this precise location the film melts and fuses with the underlying cells of choice. When the film is removed, the chosen cell(s) remain stuck to its undersurface, while the rest of the tissue is left behind.

RNA extraction

RNA will be extracted from the tumor and non-tumor liver tissues using the single step extraction method with guanidinium thiocyanate-phenol-chloroform.

LOH and deletion mapping

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[α-^{33}p]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^{o}C for 30 sec, 55^{o}C for 75 sec, 72^{o}C for 15 sec. A total of 27 cycles were performed. The product was further incubated in 72^{o}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 16 hours.

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

The BAC human library is housed in 288 microtiter dishes. Genome Systems, Inc. has prepared DNA from each dish individually and combined them in blocks of ten to create the upper assay pools. A total of 29 upper pools of DNA are located in microtiter dish #1. Three rounds of PCR experiments are required in order to identify the complete clone address of you clones. The first round PCR is to identify the plate pools that correspond to upper pool that positive by PCR. The second round PCR is to identify plate that house your clone. The third round PCR is to identify the position in the dish.

Exon trapping system

The exon trapping system provides a simplified means for isolating exons from cloned genomic DNA. The exon trapping vector, pSPL3, reagents, and protocols for isolating exons can be used to screen subcloned genomic DNA. This systetm provides an exon control plasmid DNA that consists of pSPL3 with a cloned human DNA fragment containing a functional exon.

Genomic DNA to be screened or exons is subcloned into the exon trapping vector, pSPL3. The multiple cloning site is flanked by functional splice donor and acceptor sites. The pSPL3 derivatives are propagated in E. coli, and the DNA is then isolated and transfected into COS-7 cells. When the cloned DNA contains an exon in the proper orientation, splicing can occur between the vector and insert sequences. The transfected cells are cultured, and total RNA is isolated and used for the synthesis of first-strand cDNA. The cDNA is amplified by PCR using primers that are complementary to the splicing vector. A unique DNA fragment is produced when splicing has occurred between splice sites from the subcloned genomic DNA and pSPL3.

After primary amplification, BstXI is used to reduce preservation of vector-vector splicing or cryptic splicing products. Secondary amplification is then performed using oligonucleotides SD2 and SA4. The amplification products can be subcloned by TOPO-TA cloning kit. Sequence analysis is performed on these positive clones.

RACE system

The SMART^{TM} RACE cDNA Amplification Kit provides a novel method for performing both 5'- and 3'-rapid amplification of cDNA ends (RACE) using SMART technology. SMART technology eliminates the need for problematic adaptor ligation and lets you use first-strand cDNA directly in RACE PCR, benefit that makes RACE far less complex and much faster. The SMART RACE kit also includes recent advances in PCR technology that both increase the sensitivity and reduce either poly A+ or total RNA as starting material for constructing full-length cDNAs of even very rare transcripts.

ClONTECH's SMART technology provides a mechanism for generating full-length cDNAs in reverse transcription reactions. This is made possible by the joint action of the SMART II oligonucleotide and MMLV reverse transcriptase (RT). When certain MMLV RT variants reach the end of an RNA template, they exhibit a terminal transferase activity that adds 3-5 residues (predominantly dC) to the 3'end of the first-strand cDNA (Figure 1). After RT switches templates from the mRNA molecule to the SMART oligo, a complete cDNA copy of the original RNA is synthesized with the additional SMART sequence at the end of the RNA template, the SMART sequences is typically added only to complete first-strand cDNAs. This process guarantees that the use of high quality RNA will result in the formation of a set of cDNA s that has a maximum amount of 5' sequence.

Poly A+ RNA

5' ----------poly A 3'

5'-------GGG ------- oligo (dT) primer

SMARTII oligo

↓ first-strand synthesis coupled with (dC) tailing by RT

↓

5'-------**GGG** 5' ----------poly A 3'

CCC----------

↓template switching and extension by RT

↓

5’-------**GGG** 5’ ----------poly A 3’

--------**CCC**----------

Figure 1

Following reverse transcription, the first-strand cDNA is used directly in 5’- and 3’- RACE PCR reactions, without the need for tedious second-strand synthesis and adaptor ligation. The incorporation of SMART technology also permits the use of universal priming in the RACE PCR amplification. The only requirement for SMART RACE cDNA amplification is that you know at least 23-28 nucleotides (nt) of sequence information in order to design gene-specific primers for the 5’- and 3’- RACE reactions.

Functional analysis of candidate genes

PCR amplification of target gene

HCC samples will be examined for deletions of specific genes. The primers used for PCR amplification will be designed from the published sequences.

Sequence analysis

To investigate whether somatic mutation of target genes is present in any of HCCs, we screened the coding region of target genes using automated fluorescence-based sequencing analysis. Sequence analysis of PCR products was performed to screen for gene mutation of HCC samples. The PCR products were directly sequenced using a cycle sequencing protocol and reagents supplied with the Big Dye Terminator Cycle Sequencing Kit (ABI, Foster City, California, U.S.A).

Southern blot analysis

Southern blot was performed for further confirmation of homozygous deletions of CDKN2B gene. Ten microgram of DNA was digested with optimum restriction enzyme(BM, GmbH, Germany), separated by electrophoresis in 1.0% agarose gel and transferred to a nylon membrane. PCR-derived probes of target genes are used individually for hybridization.

Expression vector construction

In order to understand the function of cloned candidate genes, we will construct the eukaryotic expression vector and analyze the phenotype after transfection.

We intend to analyze the localization of expressed candidate genes, cell growth rate, relation with cell cycle, soft agar assay, focus formation assay, anti-transformation activity etc.

Results

DNA and RNA preparation from LCM

In this study we have succeeded to prepare the DNA or RNA (Figure 1 and Figure 2) from microdissected tissues from OCT slide.

LOH and fine mapping analysis of 16q in HCC

A total of 5 microsatellite markers near chromosome 16q12-13 were used to reconfirm the LOH pattern of HCC samples, which DNA is prepared by LCM. The highest percentage of LOH was shown for loci at, D16S409, D16S419, D16S415, D16S390, D16S3034 (Table1). The minimal deleted region was mapped to 16q12.1, and the LOH frequency was higher than previous data without LCM preparation.

Exon trapping and full length cDNA cloning

In the past ten moths, we started to screen the human BAC(Bacterial Artificial chromosome)library by these markers at 16q12.1 and we identified 10 clones(Table 2). Then we used exon trapping system to search the putative exon sequences of BAC genomic clones. Several clones containing exon-like sequences have been sequenced. In the next year, we will continue analysis these clones by sequencing and sequence analysis using Blast network service of the NCBI. Now, we are going to clone full-length cDNA using RACE system and search for the putative tumor suppressor genes.

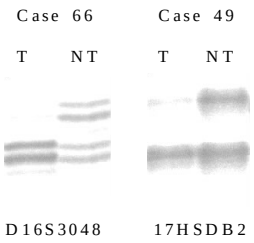

Figure 1. Representative results of loss of heterozygosity observed with 16q24 microsatellite markers in hepatocellular carcinoma. N and T, matched DNA sample isolated from non-tumor and tumor tissues, respectively. Two tumors with distinct patterns of 16q24. LOH are depicted: arrows, lost alleles. Case 66 showed LOH at markers *D13S3046* and case 49 had evidence of LOH at *D17HSDB2*.

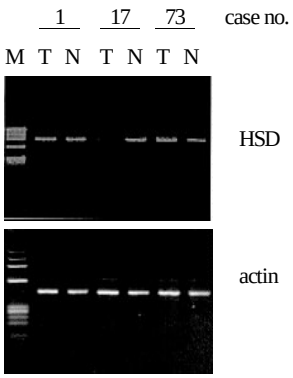

Figure 2 RT-PCR analysis for mRNA expression of *17 -HSD*. Case 17 showed no visible band on a agarose gel. To control for cDNA quality, β-actin was used as a control. T: tumor; N: non-tumor part.

Table 1 Loss of heterozygosity at 5 microsatellite loci on chromosome 16q in HCCs

Locus Symbol	*Chromosomal location*	*(LOH/informative cases)*	*Frequency of LOH(%)*
D16S409	16q12.1	7/15	47
D16S419	16q12.1	9/20	45
D16S415	16q12.1-q13	11/17	65
D16S390	16q12.1	11/22	50
D16S3034	16q12.1-q13	3/8	38

Table 2. Exon-like sequence trapped by pSPL3 splicing vector

(Microsatellite marker)	BAC clone	clone with Exon-like sequences	(% similarity)	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		

附件

心得報告

今年在布拉格所舉行的是第 36 屆歐洲肝病醫學會的年會，4 天的議程對於 B 型肝炎 、C 型肝炎的報告較多，同時也有很多有關肝硬化及門靜脈高血壓的研究。

在 B 肝的免疫學研究方面，B 型肝炎病毒本身可能會感染樹突狀細胞，使得樹突狀細胞的成熟受到干擾，因而影響宿主對 B 型肝炎的免疫力。對 B 型肝炎的治療性疫苗，目前有數篇初步的報告，不過正式的人體試驗仍有待進一步的觀察。治療 B 型肝炎的另一種新藥 entecavir 在美國剛做完 phase –II 的 study，初步的報告認為 entecavir 似忽比拉美夫錠效果還要好一些，不過還必需等 phase –III 的 study 之後才能下結論。

在肝癌的研究方面，有研究報告顯示利用 DNA 疫苗，以胎兒蛋白作為腫瘤標記來控制會分泌胎兒蛋白的肝細胞癌。不過值得注意的是，用胎兒蛋白 DNA 疫苗免疫過的老鼠，可能在肝再生過去中產生比較嚴重的肝細胞壞死，這種對肝細胞的破壞與胎兒蛋白特異性的 T 細胞是有相關的，可以說是一種自體免疫性肝炎，所以用胎兒蛋白作為免疫療法的抗原標記，是不是適宜，仍有待以後的探討。

攜回資料:

第 36 屆歐洲肝病醫學會年會論文摘要 CD 一片。

發表論文:

Topic: Deletion mapping of chromosome 16q24 in Hepatocellular Carcinoma in Taiwan and Mutational Analysis of the *17-beta-HSD* gene localized to the region

Objectives: Human chromosome band 16q24 commonly undergoes loss of heterozygosity (LOH) in human hepatocellular carcinoma (HCC). To further localize the region of deletion on 16q24 and to evaluate a genetic role of *17-beta-HSD*, which is near 16q24, in HCC, we examined the pattern

of loss of heterozygosity in 88 HCC patients,

Methods: DNA from 88 pairs of HCCs and corresponding non-tumor parts was prepared. Loss of heterozygosity on chromosomes 16q24 was investigated by 11 sets of microsatellite markers. Mutation analysis of type II *17-beta-HSD* was performed by automatic sequencing.

Results: LOH on 16q24 at least one locus was found in 46 of the 88 tumor DNAs (52.3%). Three independent regions of frequent LOH were defined by the 46 tumors with partial deletions. The first region was between *D16S516* loci and *D16S507*, encompassed by a 1-cM region, defined by the *D16S504*. The second region was defined by the *17HSDB2* locus between *D16S505* and *D16S422*, encompassed approximately by a 1-cM region. The third region was between *D16S520* and *D16S413*, defined by *D16S3048*, encompassed approximately by a 4-cM region. Homozygous deletions of any exons in *17HSDB2* gene were identified in 7 of 27 cases (26%). Automated sequencing analysis of *17HSDB2* failed to demonstrate mutations in any of these specimens.

Conclusions: Our data suggest that the *17HSDB2* locus is a frequent target of deletion in HCC but the inactivation of *17HSDB2* may not involve sequence mutations. Furthermore, the presence of the other two frequent LOH regions suggest that the putative tumor suppressor genes at these locations might be involved in the development of HCC.