MOLECULAR KARYOTYPING OF HUMAN HEPATOCELLULAR CARCINOMA CELL LINES USING SINGLE-NUCLEOTIDE POLYMORPHISM ARRAYS

A THESIS SUBMITTED TO
THE DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS
AND THE INSTITUTE OF ENGINEERING AND SCIENCE OF
BILKENT UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

BY
KUBİLAY DEMİR
AUGUST 2007

I certify that I have read this thesis and that in my opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

_____

Assist. Prof Cengiz YAKICIER

I certify that I have read this thesis and that in my opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

_____

Assist. Prof Uygar TAZEBAY

I certify that I have read this thesis and that in my opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

_____

Assist. Prof Ayşe Elif ERSON

Approved for the Institute of Engineering and Science

_____

Prof. Dr. Mehmet BARAY

Director of Institute of Engineering and Science

ABSTRACT

MOLECULAR KARYOTYPING OF HUMAN HEPATOCELLULAR CARCINOMA CELL LINES USING SINGLE-NUCLEOTIDE POLYMORPHISM ARRAYS

KUBİLAY DEMİR

M.Sc. in Molecular Biology and Genetics

Thesis Supervisor: Assist. Prof. Cengiz Yakıcıer

August 2007, 110 Pages

Hepatocellular carcinoma (HCC) etiology is genetically heterogeneous; multiple different mechanisms have been shown to promote hepatocarcinogenesis. However, chromosomal aberrations (CAs) and signaling pathways that they alter are still poorly understood. Changes in chromosome number (aneuploidies) or structural chromosomal aberrations, such as; amplifications, deletions, loss of heterozygosity and recessive mutations are important mechanisms for tumor evolution.

Recently developed single nucleotide polymorphism (SNP) microarrays provide high-throughput quantitative and qualitative screening of genomic DNA with higher resolution compared to conventional methods such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH). In cancer research, SNP arrays ease the screening of structural changes as well as aneuploidies with exact physical position.

In the framework of this study, we aimed to detect DNA copy number alterations in a panel of 14 HCC cell lines. We screened all the autosomal chromosomes and the X-chromosome and found previously undescribed novel regions that harbor homozygous and hemizygous deletions at 13q12 and Xq21; amplifications at 8p23, 8q13, 8q24, 9p22-21, 12p1, 14q12, 15q21, 16q23, 17p12-p11, 17q11, 22q11 and Xp22. In our knowledge, our results are the first comprehensive high-throughput screen of commonly used HCC cell lines.

ÖZET

İNSAN HEPATOSELÜLER KARSİNOM HÜCRE HATLARININ TEK NÜKLEOTID POLİMORFİZM YONGALARI KULLANILARAK KARYOTİPLENDİRİLMESİ

KUBİLAY DEMİR

Moleküler Biyoloji ve Genetik Bölümü Yüksek Lisansı

Tez Yöneticisi: Yard. Doç. Cengiz Yakıcıer

Ağustos 2007, 110 Sayfa

Hepatoselüler karsinom (HSK) etiyolojisi çeşitli genetik özellikler göstermektedir ve HSK oluşumuna sebebiyet veren birçok değişik işleyiş şekli daha önce gösterilmiştir. Ancak, kromozomsal bozukluklar ve düzensizliğe sebebiyet verdikleri sinyal yolakları halen tamamiyle açıklığa kavuşmamıştır. Koromozom sayısındaki değişimler (aneuploidik) veya yapısal kromozom bozuklukları, örneğin; amplifikasyonlar, delesyonlar, tek kopya kaybı ve resesif mutasyonlar tümör evrimi için önemli mekanizmalardandır.

Yakın bir zaman önce kullanılmaya başlanan tekli nükleotid polimorfizm (SNP) mikroarraylari yüksek çıktılı nitelik ve nicelikte genomik DNA taranmasında kullanılmakta ve geleneksel yöntemlere göre, örneğin florasan in sitü hibridizasyon (FISH) ve karşılaştırmalı genomik hibridizasyon (CGH), daha yüksek çözünürlük sağlamaktadır. Kanser araştırmalarında SNP mikroarraylari yapısal kromozom değişimlerini ve aneuploidileri tam fiziksel genomik pozisyonları ile birlikte vermektedir.

Bu çalışma çerçevesinde, 14 HSK hücre hattı panelinde DNA kopya sayısı değişimlerini ortaya çıkarmayı hedefledik. Tüm otozomal kromozomları ve X-kromozomunu taradık ve daha önce tanımlanmamış olan 13q12 ve Xq21 homozigot ve hemizgot kayıplarını ve 8p23, 8q13, 8q24, 9p22-21, 12p1, 14q12, 15q21, 16q23, 17p12-p11, 17q11, 22q11 ve Xp22 amplifikasyonlarını bulduk. Sonuçlarımız bilgilerimiz dahilinde, yaygın HSK hücre hatlarının en kapsamlı, yüksek çıktılı tarama çalışmasıdır.

Aileme…

ACKNOWLEDGEMENTS

First of all, I wish to express my greatest thanks to Assist. Prof. Dr. Cengiz Yakıcıer for his invaluable guidance, endless patience, and true scientific support. This thesis would not be complete without his valuable critics and inspiring comments, support and encouragement for personal development in research.

I am deeply thankful to my thesis jury members Assist. Prof. Dr. Uygar Tazebay and Assist. Prof. Dr. Ayşe Elif Erson for their invaluable comments and discussions.

I am grateful to Prof. Dr. Mehmet Öztürk and all other MBG Faculty Members for their efforts and help during my growth as a young scientist and in providing us an effective and pleasant atmosphere for doing research.

I am also indebted to Prof. Dr. Neşe Atabey, Assist. Prof. Dr. Esra Erdal and Assist. Prof. Dr. Hilal Özdağ for their kindness in providing expression microarray data of cell lines.

I would like to thank Tolga Acun for his help in my work and friendship.

I would like to thank Biter Bilen who helped me in improving my computational skills.

I would like to thank all MBG family for their help, suggestions and friendships.

I am also indebted to Assoc. Prof. Dr. Mehmet Alikaşifoğlu and Hacettepe Üniversitesi Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Ana Bilim Dalı Genetik Ünitesi & Çocuk Sağlığı Enstitüsü Temel Bilimler Ana Bilim Dalı Genetik Bilim Dalı Faculty Members for giving me the opportunity to use their microarray experiment equipments.

I am also indebted to Ay-Ka Ltd; namely to Ayşegül Akman & Kayhan Akman, for providing Affymetrix SNP chips and kits used in this work; and people at Ay-Ka Ltd for technical assistance and help in performing microarray experiments.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AFB1	Aflatoxin B1
AML	Acute Myeloid Leukemia
APC	Apolipoprotein C
BMP	Bone Morphogenetic Protein
bp	Base Pair
BRCA	Breast Cancer
BRCA2	Breast Cancer 2
BWS	Beckwith-Wiedemann Syndrome
CA	Chromosomal Aberrations
Cdk	Cyclindependent Kinase
cDNA	Complementary DNA
CGH	Comparative Genomic Hybridization
CHEK	Chk Checkpoint Homolog (S.Pompe)
dChip	DNA Chip Analyzer
ddH2O	Double Distilled Water
DM	Double Minutes
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleicacid
dNTP	Deoxyribonucleotide Triphosphate
dsDNA	Double-Stranded DNA
EDTA	Ethylene Diamine Tetra-Acetic Acid
EGF	Epidermal Growth Factor
eIF2a	Translation Initiation Factor 2 Alpha
ERBB2	V-Erb-B2 Erythroblastic Leukemia Viral Oncogene
EtBr	Ethidium Bromide
FBS	Fetal Bovine Serum
FGF	Fibroblast Growth Factor
FISH	Fluorescent in situ Hybridization

g	Gram
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GEO	Gene Omnibus
GSK-3ß	Glycogensynthasekinase-3 Beta
HBV	Hepatis B Virus
HBX	Hepatitis Virus Protein
HCC	Hepatocellular Carcinoma
HCV	Hepatis C Virus
HER2	Erbb2
HGF	Hepatocyte Growth Factor
HMM	Hidden Markov Model
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
HSR	Homogenously Staining Regions
IGF	Insulin-Likegrowthfactor
KCl	Potassium Chloride
LOH	Loss of Heterozygosity
MB	Mega base-pairs
mg	Miligram
MIN	Microsatellite Instability
min	Minute
ml	Mililiter
mm	Milimeter
mM	Milimolar
MMR	Mismatchrepair
MPF	Mitosispromotingfactor
mRNA	Messenger RNA
MYC	Myelocytomatosis Viral Oncogene Homolog (Avian)
NaCl	Sodium Chloride
NFKB	Nuclear Factor Kappa B
P21/CIP1	Cyclin Dependent Kinase Inhibitor 1A
PBS	Phosphate Buffered Saline

PCR	Polymerase Chain Reaction
PI3K	Protein3 Kinase
Rb	Retinoblastoma
RDA	Representational Difference Analysis
RFU	Relative Fluorescence Unit
RLGS	Restriction Landmark Genome Scanning
RNA	Ribo Nucleic Acid
Rpm	Revolutions Per Minute
RT	PCR Reverse Transcription Pcr
RTK	Receptor Tyrosine Kinase
SCLC	Small Cell Lung Cancer
Sec	Second
SNP	Single Nucleotide Polymorphism
TAE	Tris Acetate Edta Buffer
TBE	Tris Boric Acid Edta
TGFß	Transfrominggrowthfactorbeta
Tm	Melting Temperature
TP53	Tumor Protein P53
Tris	Tris(Hydroxymethyl)-Methylamine
UV	Ultraviolet
v/v	Volume/Volume
VC	Vinyl Chloride
VEGF	Vascular Endothelial Growth Factor
w/v	Weight/Volume
WHV	Woodchuck Hepatitis Virus
Wnt	Wingless
XC	Xylenecyanol
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromolar

1. INTRODUCTION

1.1 Epidemiology and Etiology of Hepatocellular Carcinoma

1.1.1 Epidemiology of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the most common primary epithelial malignancy of the liver and is one of the most common malignancies in the world. It is the fifth most prevalent carcinoma worldwide and the third cause of mortality among deaths from cancer with an annual number of 600 thousand (Parkin et al. 2001).

It is well described that HCC shows a characteristic geographic distribution. High-incidence areas (defined as those with more than 20 cases per year per 100,000 populations) include Sub-Saharan Africa, Southeast Asia, China, Taiwan, Japan, and Hong Kong. Low-incidence areas (less than 5 cases per year per 100,000 populations) include most of the Western Europe, the United Kingdom, the United States, and Canada. However, the incidence of HCC has substantially increased in the United States and Western Europe over the past 25 years. In the United States, the incidence of HCC increased from 1.4 to 2.4 cases per 100,000 populations between 1976 and 1995 (El-Serag and Mason 1999). The incidence and mortality rates of HCC are expected to double over the next 10–20 years (El Serag and Mason, 1999; Davila et al. 2004; El Serag, 2004).

Like many other cancers, the incidence of HCC increases progressively with age. This probably reflects the time for accumulation of genetic alterations required for HCC development. Younger age of onset is observed in countries endemic for viral hepatitis, and this may be due to increased risk of generating genome alterations during rapid liver regeneration (Stroffolini et al. 1998). Another interesting future of HCC is that it has a male predominance, regardless of geographical differences (Ng et al. 1995; Chen et al. 1997). In HCC-prevalent regions, such as Africa, China, and Hong Kong, the male: female ratio is even higher. In Hong Kong, the male to female ratio for HCC is about 6 to1 (Ng et al. 1995; Chen et al. 1997).

1.1.2 Etiology of Hepatocellular Carcinoma

HCC is one of the few human cancers with clearly established causal etiologies in most of the cases. The etiology of HCC is multi-factorial and consists of chronic viral hepatitis (caused by hepatitis B and C viruses), cirrhosis, aflatoxin B1 intake, alcohol abuse, and inherited metabolic disorders.

1.1.2.1 Hepatitis B Virus (HBV)

The etiologic association between HBV infection and HCC was first demonstrated by epidemiological studies. The incidence of HCC worldwide parallels the incidence of HBV infection. Variations in HCC incidence within a region generally relate to differences in HBV carrier rates. For chronic hepatitis B (hepatitis B surface antigen [HBsAg] carriers), the life-long risk of developing HCC has been estimated to be up to 40-50% (Beasley 1988). Animal studies have provided additional evidence to support the role of HBV infection in HCC development. Persistent infection of woodchucks with woodchuck hepatitis virus (WHV), which is a HBV-like hepadnavirus, resulted in HCC in almost all animals (Snyder et al. 1982). However, the molecular mechanisms underlying HBV-induced HCC remained obscure.

HBV infection has been shown to promote carcinogenesis by at lest three different mechanisms. First, integration of the viral DNA in the host genome can induce chromosomal instability (Aoki et al. 1996). Persistent HBV infection may provide a cellular environment for hepatocarcinogenesis through non-specific mechanisms such as increase of mutation rate and genome instability associated with rapid cell turnover caused by liver injury and subsequent regeneration. Second, insertional mutations at HBV integration sites may disrupt cellular genes and result in activation of endogenous genes such as retinoic acid β-receptor (Dejean et al. 1986), cyclin A (Wang et al. 1990) and mevalonate kinase (Graef et al. 1994). Recently, more than 10 genes have been found to be altered by HBV integration in tumors. These genes are involved in

controlling cell proliferation, viability and differentiation suggesting that HBV integration at particular sites are mechanisms frequently involved in HBV hepatocarcinogenesis (Ferber et al. 2003; Horikawa and Barrett. 2003; Paterlini-Brechot et al. 2003). But, unlike WHV-induced HCC, HBV-DNA integration is not specific and is not frequently associated with activation of any cellular proto-oncogenes (Brechot et al. 2000). Third, expression of viral protein HBX has been shown to modulate cell proliferation and viability (Andrisani and Barnabas, 1999; Diao et al. 2001). HBX binds to p53 which results in abnormal p53-dependent activities such as p53-mediated apoptosis (Feitelson et al. 1993). There are also additional studies suggesting HBX can activate NF-κB signaling pathway, as well as other growth regulatory genes such as c-fos, c-jun, c-myc, and EGF (Feitelson 1999; Brechot et al. 2000; Yeh 2000). In addition, HBV 'X' gene transgenic mice frequently develop HCC (Di Bisceglie et al. 1998; Yu et al. 1999). Sequencing of HBV DNA from HCC and adjacent nontumorous liver tissues has shown a high rate of mutations (Di Bisceglie et al. 1998). Recent evidence has shown that mutations in the HBV 'X' gene in HCC can abolish both HBX-induced growth arrest and apoptosis. These naturally occurring mutations might therefore render the hepatocytes susceptible to uncontrolled growth and contribute to multi-step hepatocarcinogenesis associated with HBV-infection (Sirma et al. 1999).

There are increasing bodies of evidence supporting that HBV itself may also play a direct oncogenic role in hepatocarcinogenesis. HBV-DNA has been shown to be integrated into the genomes of HCC cell lines and of liver cells of long-term asymptomatic HBsAg carriers. In woodchuck model, WHV genome was found to be frequently integrated into the cellular *N-myc* gene (Wei et al. 1992). Insertional activation of this proto-oncogene was believed to be responsible for the transformation phenotype. However, HBV-mediated HCC does not follow a similar pattern. Unlike WHV-induced HCC, HBV-DNA integration is usually not specific and not associated with activation of any cellular proto-oncogenes (Brechot et al. 2000).

Previous studies have shown that HBX (a viral protein encoded by the 'X' gene in HBV genome) physically binds to and inactivates the wild-type p53 tumor suppressor protein (Wang et al. 1994; Ueda et al. 1995; Greenblatt et al. 1997). There are also additional

studies suggesting that HBX can activate NF-κB signaling pathway, as well as other growth regulatory genes such as c-fos, c-jun, c-myc, and EGF (Feitelson 1999; Brechot et al. 2000; Yeh 2000). In addition, HBV 'X' gene transgenic mice frequently develop HCC (Di Bisceglie et al. 1998; Yu et al. 1999). On the other hand, some reports have indicated that HBX expression can induce G1 cell cycle arrest and apoptosis through a p53-independent pathway (Terradillos et al. 1998; Sirma et al. 1999).

1.1.2.2 Hepatitis C Virus (HCV)

In a series of HCV epidemiology studies, HCV has been detected in 6-75% of patients with HCC, and chronic HCV infection was found to be the major etiological factor for HCC in Japan, Europe, and the United States (Colombo et al. 1989; Chen et al. 1990; Hasan et al. 1990; Saito et al. 1990; Vargas et al. 1990; Yu et al. 1990; Kaklamani et al. 1991; Nishioka et al. 1991). A prospective follow-up study indicated that the incidence of HCC in patients with chronic hepatitis C was 2.7 times higher than patients with chronic hepatitis B (Takano et al. 1995).

The molecular mechanism of HCV-related hepatocarcinogenesis is still obscure. Genome instability and mutations, occurring in regenerating hepatocytes associated with immune-mediated turnover during chronic inflammation and cirrhosis remains a leading hypothesis for HCV-related hepatocarcinogenesis.

Some recent experimental data suggest that HCV may be directly involved in hepatocarcinogenesis. The core protein of HCV is a likely oncogenic candidate. HCV core protein was found to cooperate with Ras in cellular transformation. Primary rat embryo fibroblast cells co-transfected with HCV core gene and H-ras exhibited rapid proliferation, anchor-independent growth, and tumor formation in athymic nude mice (Ray et al. 1996). Other data suggest that amino acid residue 80-122 of HCV core protein may repress the transcriptional activity of the p53 promoter (Ray et al. 1997). The oncogenic role of HCV core protein was further supported by transgenic mouse

model. The incidence rate of HCC in transgenic mice harboring HCV core gene was significantly higher than that in non-transgenic mice (Moriya et al. 1998). Interestingly, HCC developed in these transgenic mice followed a stepwise transformation and closely resembled the histopathological characteristics of the early stages of HCC in patients with chronic hepatitis C. The neoplastic lesions first appeared as adenomas, and then HCC developed from the adenomas, presenting a 'nodule-in-nodule' manner (Moriya et al. 1998).

1.1.2.3 Aflatoxin B1

Aflatoxins are mycotoxins produced by the common fungus *Aspergillus flavus*. Aflatoxins are powerful carcinogens for animals. Field studies have shown a close association between aflatoxin intake and high incidence of HCC in poor countries, where fungal contamination in food is common. In geographies where AFB1 exposure level is high, such as Qidong-China and Mozambique, G-T transversion at codon 249 has been reported in more than 50% of the cases (Hsu et al. 1991; Bressac et al. 1991). This mutation at codon 249 of TP53, leading to the amino-acid substitution R249S, is exceptionally found in HCC from geographical regions without AFB1 exposure supporting the hypothesis that this mutagen has a causative role in hepatocarcinogenesis. Molecular mechanisms of AFB1–DNA binding and mutagenesis have been elucidated in human tumors, animal models and in vitro (Smela et al. 2001). These results contrast with p53 mutations reported in other regions of China and Japan where aflatoxin is not the risk factor of HCC (Hayashi et al. 1993; Li et al. 1993a; Fujimoto et al. 1994). Thus, this mutation specificity can be considered as a finger print of aflatoxin B1 exposure.

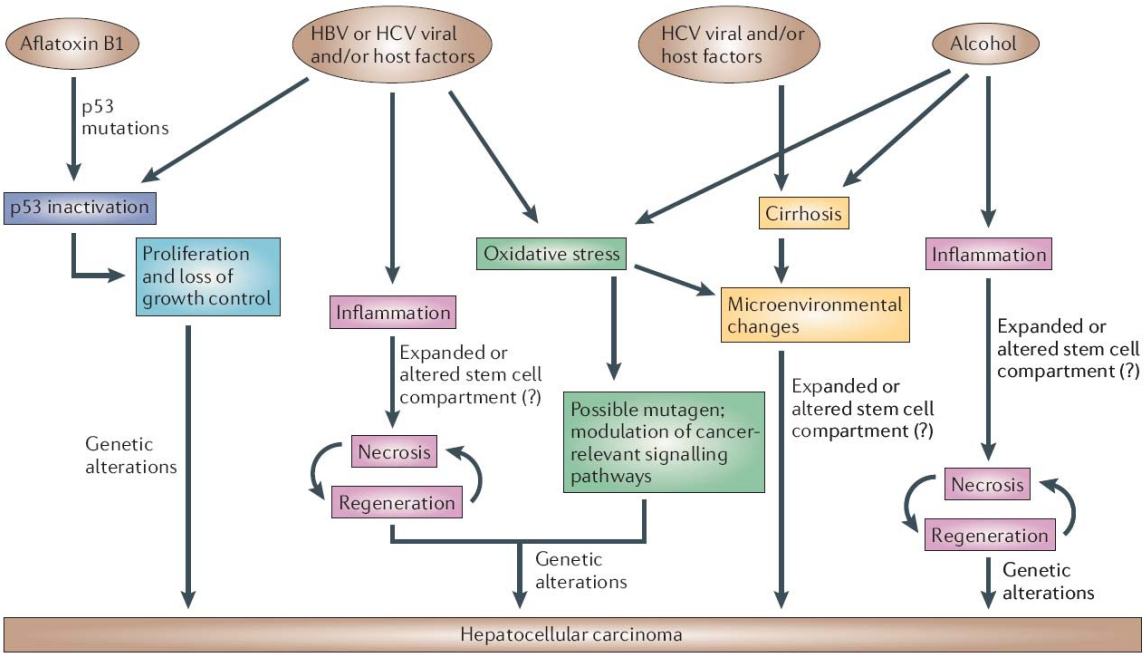


Figure 1.1: Mechanisms of Hepatocarcinogenesis for different risk factors. Commonalities are shown in the same color (Farazi and DePinho, 2006)

1.2 Genetic and Epigenetic Changes in Hepatocellular Carcinoma

Cancer is a DNA disease which emerges through accumulation of genetic alterations in the genes controlling cell cycle, proliferation, differentiation and apoptosis; hepatocellular carcinoma is no exception.

HCC has been extensively studied in terms of genetic alterations in the past ten years which resulted in an increase in our knowledge of altered pathways in hepatocarcinogenesis. Likewise in other solid tumors, a large number of genetic alterations accumulate during the hepatocarcinogenesis process. Genetic and epigenetic alterations have been observed in cirrhotic nodules and half of them have been found to have a monoclonal origin by examining the X-chromosome methylation pattern (Piao et al. 1997; Paradis et al. 1998; Yeh et al. 2001). Chromosome aberrations with loss of alleles are found in half of cirrhotic nodules and more frequently in nodules with small

cell dysplasia (Yeh et al. 2001). Various genetic alterations have been described in primary liver tumors including activating mutations of oncogenes and inactivating mutations of tumor suppressor genes have been only found in HCC and liver adenomas but not in cirrhosis.

1.2.1 Chromosomal Abnormalities

Human cancers are characterized by the presence and accumulation of genetic alterations which target genes or genomic loci. Chromosomal aberrations (CA) are changes in chromosome structure and morphology which are indicators of genetic damage in cancer. CAs are involved in tumor genesis and progression by altering the functions of genes that positively or negatively regulate several aspects of cell proliferation, apoptosis, genome stability, angiogenesis, invasion and metastasis. Their pattern varies between malignancies, ranging from simple balanced rearrangements to complex abnormalities affecting both chromosome structure and euploidy. Subchromosomic abnormalities are often related with genetic alterations, including formation of fusion gene products and swapping of promoter elements which consequently lead to dysregulated gene expression (Aman et al. 1999). The majority of malignant solid tumors, however, exhibit a complex pattern of chromosomal abnormalities, rarely showing any direct association with specific morphological or prognostic subgroups. Many common aggressive epithelial tumors, such as high-grade pancreatic, ovarian, and lung cancer, fall within this category (Pejovic et al. 1992; Johansson et al. 1995; Gorunova et al. 1998), so do many sarcomas, such as osteosarcoma, leiomysorcoma, and malignant peripheral nerve sheath tumor (Mandahl 1996). The molecular genetic alterations corresponding to these complex cytogenetic anomalies are not well characterized, although abnormal activation of oncogenes and losses of tumor suppressor genes are common. These changes are rarely subtype specific. However, the total number of chromosomal aberrations is roughly proportional to the risk of metastasis (Mitelman et al. 1997).

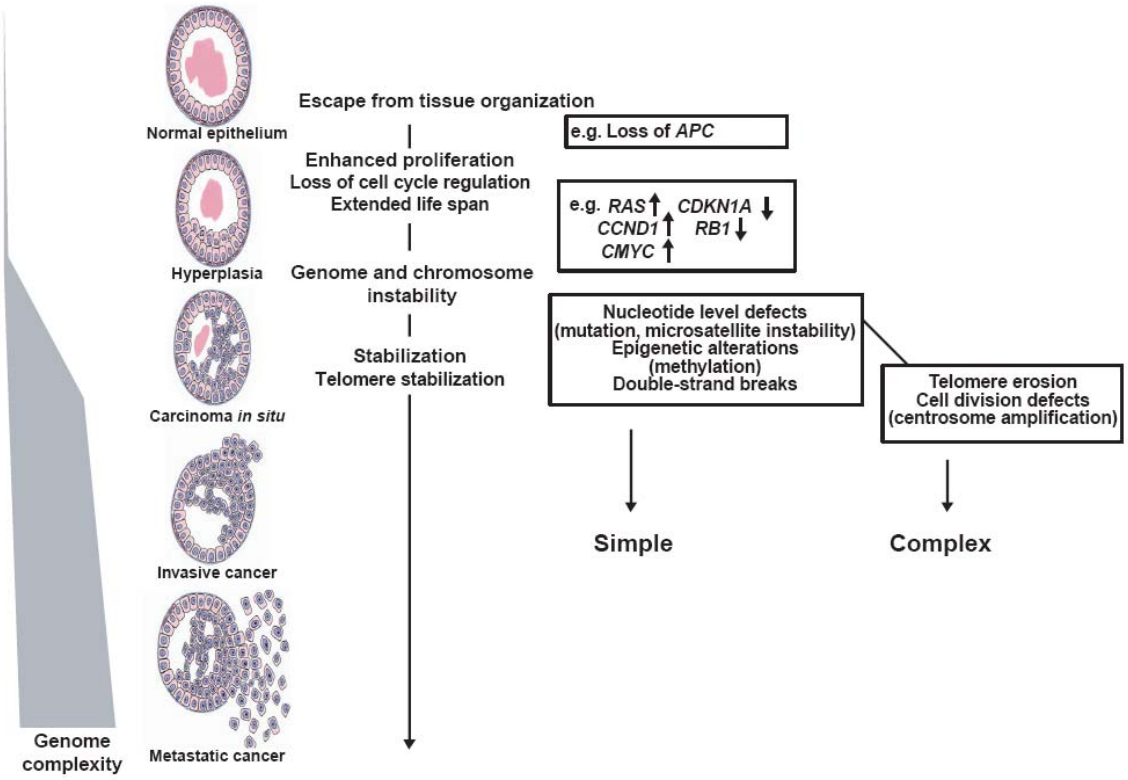
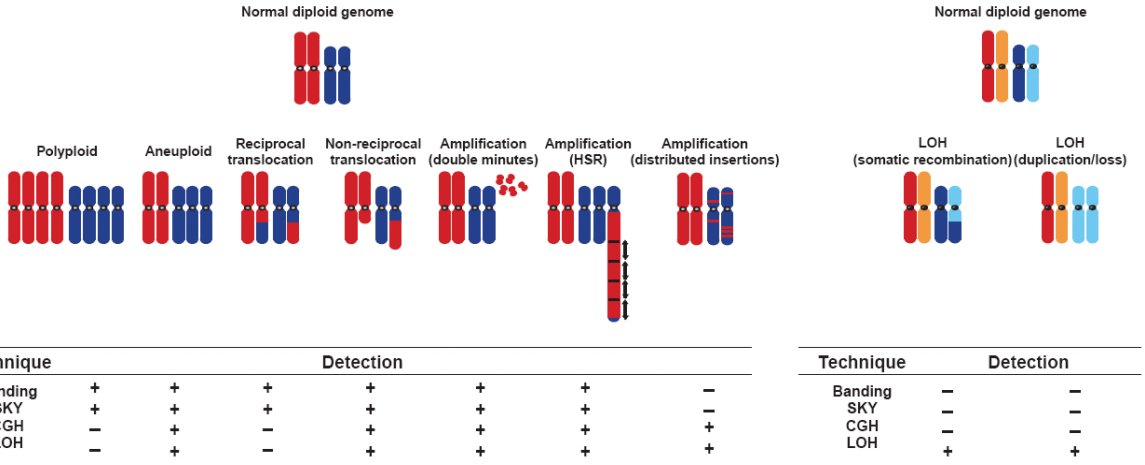


Figure 1.2: Chromosomal evolution in human solid tumor progression: Cells may begin to proliferate excessively owing to loss of tissue architecture, abrogation of checkpoints and other factors. Relatively few aberrations occur before development of in situ cancer and the incidence of genomic aberrations increases during the development of in situ disease (Albertson et al. 2003)

CAs can be studied with an increasing number of large-scale genomic and molecular genetic technologies such as chromosome banding (Mitelman Database of Chromosome Aberrations in Cancer), high-throughput analysis of loss of heterozygosity (LOH) analysis (Hampton et al. 1996), comparative genomic hybridization (CGH) (Pinkel et al. 1998), fluorescence in situ hybridization (FISH) (Schrock et al. 1996), restriction landmark genome scanning (RLGS) (Imoto et al. 1994), representational difference analysis (RDA) (Lisitsyn et al. 1993) and recently introduced SNP microarrays. These techniques differ in which they detect whether balanced or unbalanced aberrations. RLGS, analysis of LOH, RDA and SNP arrays detect allelic imbalances that occur by somatic recombination or copy number change. FISH and CGH are sensitive to unbalanced physical structure of the genome or copy number such as altered ploidy, gain

or loss of chromosomes and chromosome portions and structural rearrangements. SNP arrays differ from FISH and CGH in detecting unbalanced rearrangements only. Structural changes involve equal exchange of material between two chromosome regions (balanced) or non-reciprocal, such as portions of the genomes are gained or lost. These methods analyze genome-wide DNA content and provide clear information about sporadic and recurrent chromosomal aberrations. The most frequently deleted chromosome arms are 17p, 8p, 16q, 16p, 4q, 9p, 13q, 1p and 6q; and the most frequent gains are observed at 1q, 7q, 8q and 17q (Fujimoto et al. 1994; Boige et al. 1997; Marchio et al. 1997; Nagai et al. 1997; Piao et al. 1998; Guan et al. 2000; Wong et al. 2000; Balsara et al. 2001; Laurent-Puig et al. 2001; Nishimura et al. 2002). Today, Mitelman Database of Chromosome Aberrations in Cancer and University of Helsinki's Laboratory of Cytomolecular Genetics harbor extensive catalog chromosomal abnormalities in a wide range of tumors.



Technique	Detection						
Banding	+	+	+	+	+	+	–
SKY	+	+	+	+	+	+	–
CGH	–	+	–	+	+	+	+
LOH	–	+	–	+	+	+	+

Technique	Detection	
Banding	–	–
SKY	–	–
CGH	–	–
LOH	+	+

Figure 1.3: Mechanisms by which chromosomal aberrations result in aneuploidy and common techniques used in detection (Albertson et al. 2003)

Amplification is likely to be initiated by a DNA double-strand break. It can occur only in cells that are able to progress inappropriately through the cell cycle with this damaged DNA whereas normal cells would arrest due to activation of cell-cycle checkpoints. A segment of the chromosomes are copied many times and result in extra copies of genetic material. If extra copies are fused head-to-tail in long tandem arrays within a

chromosomal segment, it is called homogeneously staining regions (HSRs). A segment may also be detached from a chromosome and replicate as an autonomous extrachromosomal entity. Such formations result in subchromosomal fragments termed "double minutes" (DMs). HSRs and DMs increase the copy number of genes they carry and resulting in gene amplifications and are unbalanced. In cancer, amplified regions are likely to carry genes with oncogenic character that bypass cellular control barriers and favor proliferation. Gene amplifications can result in copy number increases from duplications to high level amplifications (700 copies) (Schwab et al. 1999). Today, there is a wide agreement that only less than half of the amplifications result in overexpression of the genes that they harbor. In a recent study with a panel of breast cancer cell lines, 40% of the amplified genes were overexpressed (Hyman et al. 2002).

Interstitial deletions occur when a segment in the middle of a chromosome arm is discarded and the flanking chromosomal regions are rejoined. Interstitial deletions may be rare but they dramatically affect cellular behavior. Such deletions may originate by chromosome breakage and subsequent loss of acentric segment or unequal crossover between misaligned homologous chromosomes or sister chromatids. Chromosome losses are frequent mechanisms of inactivation of one allele of a tumor suppressor gene in solid tumors, recurrent losses at precise loci may point the presence of tumor suppressor genes. In HCC, LOH events have been reported targeting loci in 17p, 13q, 16p, 9p and 6q and inactivating tumor suppressor genes TP53, RB1 (retinoblastoma 1), AXIN1 (axis inhibition protein 1), CDKN2A (cyclin-dependent kinase inhibitor 2A) and IGF2R (insulin-like growth factor 2 receptor), respectively. On the other hand, no tumor suppressor genes have been identified on 1p, 4q, 8p and 16q although high-resolution methods have been used to define consensus boundaries of deletions in these regions (Koyama et al. 1999; Piao et al. 1999; Pineau et al. 1999; Balsara et al. 2001; Yakicier et al. 2001; Bluteau et al. 2002a).

Recurrent genomic aberrations are observed on several occasions in a series of independently arising human tumors. They are likely to contain genes that are important for tumor development. In many cases these regions contain with oncogenic or tumor

suppressive character whose expression levels are altered by genomic changes. In solid tumors, amplification of ERBB2, MYC and CCND1 have been reported (Slamon et al. 1989; Hinds et al. 1994). Amplification also plays an important role in the development of drug resistance. Cultured cells selected for resistance to N-(phosho-nacetyl)-L-aspartate frequently amplify CAD (Wahl et al. 1979; Schimke et al. 1978) and DHFR is amplified in cultured cells with methotrexate resistance (Banerjee et al. 2002). Similarly, BCR-ABL is amplified in individuals resistant to STI571 (Gorre et al. 2001). Other aberrations include loss of specific regions of the genome. Tumor suppressor genes such as PTEN, CDKN2A have been reported to be lost by homozygous deletions (Li et al. 1997; Orlow et al. 1995). Recessive mutations along with LOH have been shown in the elimination of the functions RB1, BRCA1, BRCA2, PTPRJ and TP53 (Nagai et al. 1994; Cavenee et al. 1983; Baker et al. 1990; Ruivenkamp et al. 2002).

Cytogenetic studies have identified many chromosomal changes in tumors but relatively few of them are recurrent and are involved in tumorigenesis. On the other hand, recurrent abnormalities are frequent transforming events in sarcomas, leukemias and lymphomas (Rowley et al. 1998). Identification of driver genes in the disturbed regions is not easy because these regions often contain multiple genes and more than one gene may important in tumor formation. For example, growth factors FDF19, FGF4, FGF3 and actin-binding oncogene EMS1 are in close proximity to CCND1 and they are amplified together with CCND1 (Bekri et al. 1997). Similarly, growth factor receptor-bound protein GRB7 maps in close proximity to ERBB2 and amplified together. Additionally, cancer genomes may involve many disturbed regions with tens of genes resulting in a complex alteration of different signaling pathways. In such cases, it is harder to establish the driver mechanisms in tumor formation. Finally, the presences of extra copies of individual chromosomes have been reported to be associated with higher cancer risk (Willenbucher et al. 1999).

Genomes of tumor hepatocytes in HCC accumulate a large number of chromosome rearrangements leading to highly abnormal karyotypes, like in other solid tumors. Cytometric analyses have been reported that most HCC cases acquire a global gain of

genetic material (Ezaki et al. 1988; Fujimoto et al. 1991; Chiu et al. 1992). Hyperploidy is also seen in nearly half of the dysplastic lesions observed in cirrhotic disease (Thomas et al. 1992) and its incidence increases in higher grade dysplastic lesions suggesting that chromosome losses followed by endomitosis are early steps in hepatocarcinogenesis.

As we already mentioned HCC is genetically heterogeneous and mostly these changes are related to etiological factors. Even though there are several studies addressing chromosomal changes in HCC, new studies with techniques providing higher resolution will probably reveal unknown genetic alterations in HCCs.

1.2.2 Mutations

In human cancers, the most frequently altered gene is the TP53 located at 17p13.1 (Hollstein et al. 1991, Isobe et al. 1986; Miller et al. 1986). Li-Fraumeni syndrome was described as germline mutations of this gene which results in predisposition to cancer in some individuals (Malkin et al. 1990). P53 is a multifunctional transcription factor involved in the control of the cell cycle, apoptosis, senescence, differentiation and development, transcription, DNA replication, DNA repair and maintenance of genome integrity. In HCC, the specific TP53 mutation R249S is found in about 50% of tumors in populations exposed to AFB1 (Bressac et al. 1991; Hsu et al. 1991). In contrast, patients who have not been exposed to this carcinogen have a lower prevalence of TP53 gene mutations (10–30%) and codon 249 is rarely altered. Another frequent mutation in HCC is the hereditary hemochromatosis at codon 220 (Vautier et al. 1999).

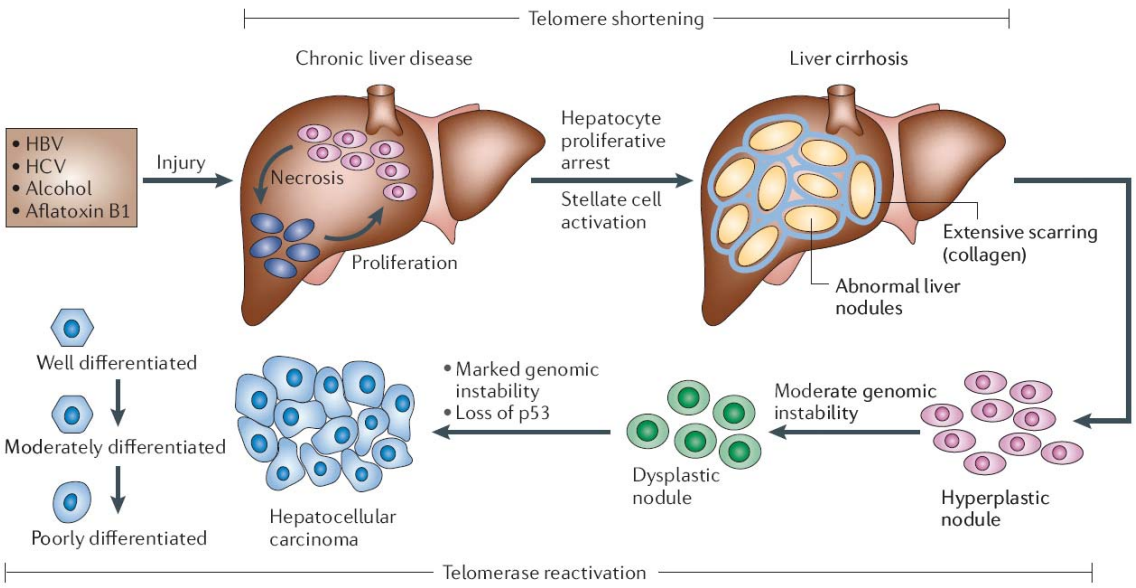


Figure 1.4: Histopathological progression and molecular features of HCC: After hepatic injury incurred by any one of several factors (hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol and aflatoxin B1), there is necrosis followed by hepatocyte proliferation. Continuous cycles of this destructive–regenerative process foster a chronic liver disease condition that culminates in liver cirrhosis. Cirrhosis is characterized by abnormal liver nodule formation surrounded by collagen deposition and scarring of the liver. Subsequently, hyperplastic nodules are observed, followed by dysplastic nodules and ultimately hepatocellular carcinoma (HCC), which can be further classified into well differentiated, moderately differentiated and poorly differentiated tumours — the last of which represents the most malignant form of primary HCC. Telomere shortening is a feature of chronic liver disease and cirrhosis. Telomerase reactivation has been associated with hepatocarcinogenesis (its activation in the early versus late stages of disease is still a point of debate, and is discussed in the text). Loss and/or mutation of p53 and genomic instability also characterize hepatocarcinogenesis. p53 loss and/or mutation is shown to occur during progression to HCC, however, there is some evidence that loss and mutation of p53 might also occur in the initial stages of hepatocarcinogenesis (Farazi and DePinho, 2006)

β-catenin is the ortholog of armadillo in Drosophila melanogaster. It is both involved in cell – cell adhesion and Wnt signaling. β-catenin forms complexes with E-cadherin and catenins in adherent junctions. In Wnt signaling, β-catenin may acquire oncogenic character by dominant gain of function mutations in its N-terminus (Morin et al. 1997). These mutations result in the loss of phosphorylation sites in its negative regulation by GSK3β/APC/axin complex. The inhibition of its negative regulation results in higher levels of β-catenin in the cytoplasm and in nuclei leading to abnormal activation of Wnt target genes GLP1 and GRP49. In HCC, β-catenin activating mutations have been

reported in human and mouse models (de La Coste et al. 1998; Miyoshi et al. 1998). In hepatoblastomas and hepatocellular adenomas, β-catenin has also been reported to carry mutations (Koch et al. 1999; Wei et al. 2000; Chen et al. 2002).

AXIN1 maps to 16p13 and this region is frequently (~30%) deleted in HCC (Laurent-Puig et al. 2001). This gene encodes a protein of the GSK3β/APC/axin complex and negatively regulates Wnt pathway. In HCC, LOH events along with mutations and homozygous deletions have been reported in biallelic inactivation of AXIN1 (Satoh et al. 2000; Laurent-Puig et al. 2001). These mutations prevent phosphorylation of β-catenin leading to accumulation of hyperactivation of Wnt target genes.

RB1 locus maps to 13q14 region which is frequently involved in LOH events (Boige et al. 1997; Nagai et al. 1997; Laurent-Puig et al. 2001). RB1 plays major roles in cell division, differentiation and apoptosis. Point mutations and epigenetic regulations along with LOH have been reported in RB1 inactivation (Zhang et al. 1994; Lin et al. 1996).
P16INK4 codes for cyclin D-dependent kinase inhibitor 2(CDKN2) and ARF which are involved in p53 mediated apoptosis. These gene products function as tumor suppressors in the RB pathway (Hickman et al. 2002). P16INK4A maps to 9p21 which has been reported to show LOH in 20% of HCC cases (Boige et al. 1997; Nagai et al. 1997; Laurent-Puig et al. 2001). Epigenetic silencing of the p16INK4A promoter has been reported in 30-70% of the tumors (Liew et al. 1999; Matsuda et al. 1999; Jin et al. 2000; Weihrauch et al. 2001). Homozygous deletions of this gene have been reported in HCC, as well (Biden et al. 1997; Jin et al. 2000).

TGF-β pathway is altered in 10-30 % of HCC cases. In TGF-β signaling pathway, inactivating mutations of mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) have been reported in HCC (Motyka et al. 2000). IGFR also have been shown to carry amino acid substations in two HCC screens (De Souza et al. 1995; Oka et al. 2002). Amino acid substitutions have also been reported in MADH2/Smad2 and MADH4/Smad4 which are involved in TGF-β/BMP-2/4 signaling pathway (Yakicier et al. 1999). Recently, activating mutations have been reported in PIK3CA

(phosphatidylinositol 3-kinase) in HCC which leads to activation of AKT pathway (Lee et al. 2005).

Vinyl chloride (VC) exposure has been reported be involved in KRAS mutations in hepatocellular carcinoma. VC is a carcinogen associated with the development of liver angiosarcomas and rarely with HCC. Recently, the presence of KRAS2 mutations was observed in 33% of 18 vinyl chloride-associated HCCs and three mutations were found in adjacent non-neoplastic liver tissue (Weihrauch et al. 2001). KRAS mutations are rarely observed in HCCs that are not associated with vinyl chloride exposure which suggest that KRAS2 mutations play an important role in the carcinogenetic pathway linked to vinyl chloride exposure.

Recent reports showed that TCF1 gene (12q24.2) carry biallelic mutation in 60% of a sample of liver cell adenoma cases (Bluteau et al. 2002). TCF1, transcription factor 1, encodes hepatocyte nuclear factor 1α (HNF1α) and function in hepatocyte differentiation and involved in liver specific expression of various genes including β-fibrinogen, albumin and α1-antitrypsin (Frain et al. 1989; Baumhueter et al. 1990; Cereghini et al. 1990; Chouard et al. 1990). In liver cell adenomas, inactivation of both TCF1/HNF1α alleles is usually observed; in 90% of the cases both mutations are of somatic origin.

Most of the hepatoblastomas are of sporadic origin. Hepatoblastomas have been reported in Beckwith–Wiedemann syndrome (BWS) and in the familial adenomatous polyposis (FAP). In most hepatoblastomas, β-catenin N-terminal domain harbor interstitial deletions or missense mutations in the GSK3β phosphorylation motif (Koch et al. 1999; Wei et al. 2000; Buendia, 2002). In other hepatoblastoma cases, hyperactivity of the Wnt/β-catenin pathway is related with AXIN2 mutations (Koch et al. 2004)

1.2.3 Epigenetic Alterations

Aberrant DNA methylation patterns have been reported in HCC (Thorgeirsson et al. 2002; Kanai et al; 1996,1999 & 2000; Yu et al. 2003). Methylation has been reported in the earliest stages of hepatocarcinogenesis and extensively in tumor progression. Molecular analysis of human HCC has shown many epigenetic alterations that result in the deregulation of several oncogenes and tumor suppressor genes including TP53, β – catenin, ErbB receptor family member, MET and its ligand hepatocyte growth factor (HGF), p16 (INK4A), E-cadherin and cyclooxygenase 2 (COX2), apoptosis – associated speck- like – kinase (ASC) and deleted in liver cancer 1 (DLC1) (Feitelson et al. 2002; Wong et al. 1999; Matsuda et al. 1999; Liew et al. 1999; Murata et al. 2004, Kubo et al. 2004; Wong et al. 2003; Maeta et al. 2005). Recently, secreted frizzled-related protein 1 gene (SFRP1) has been reported to be epigenetically silenced in HCC cell lines and primary tumors along with LOH events (Shih et al. 2007). Phosphatase and tensin homologue (PTEN) has been shown to be downregulated by promoter methylation and other epigenetic mechanism in HCC tissues (Wang et al. 2007). Zinc fingers and homeoboxes protein 2 (ZHX2), glutathione S-transferase pi (GSTP1), Ras association domain family 1 (RASSF1), methylation-induced silencing 1 (TMS1), tissue factor pathway inhibitor-2 (TFPI-2), spleen tyrosine kinase (SYK) and LINE-1 type transposase domain containing 1 are other genes that have recently been shown to be downregulated in HCC by methylation (Lv et al. 2006; Wang et al. 2006; Di Gioia et al. 2006; Zhang et al. 2007; Wong et al. 2007; Yuan et al. 2007; Tangkijvanich et al. 2007). Suppressor of cytokine signaling 1 (SOCS1), which is a negative regulator of the JAK/STAT pathway, has been shown to be silenced by methylation in HCC (Yoshikawa et al. 2001).

2. HYPOTHESIS

Amplifications and deletions are common genetic alterations in epithelial cancers. Numerous oncogenes and tumor suppressor genes located in these regions have been identified in cancers. New techniques which provide higher resolution may reveal unknown small chromosomal alterations where important genes for carcinogenesis may be located. DNA copy number changes in HCC still have not been studied with recently available high – throughput molecular methods which provide higher resolution.

In the framework of this study, we aimed to screen HCC cell lines for their DNA copy number changes. We think cell lines are ideal models for this study because their genomic DNAs are available as homogenous and high – quality (intact) which are crucial requirements for SNP microarray analysis. Unlike tissue samples, their genomic DNAs are pure, without any contamination of neighboring normal cells or infiltrating blood DNA which gives better estimates for low – copy number changes. Moreover, screening a panel of commonly used HCC cell lines may provide us independent abnormalities as well as recurrent ones. Thus, our results may reveal new regions of abnormality in HCC genome, in which oncogenes or tumor suppressor genes may reside. Analysis of these new candidates may contribute to our understanding of hepatocarcinogenesis by introducing new mechanisms and related pathways.

3. METHODOLOGY

3.1 Materials

3.1.1 Hepatocellular Carcinoma Cell Lines

In the framework of this study, 14 Hepatocellular carcinoma (HCC) cell lines were used as shown in Table 3.1

Cell Lines	Origin	Sex & Age	HBV-DNA	Tumorigenity in Nude Mice	Chromosome Ploidy
Focus	US	Female	Positive	Yes	Hypotriploid
HepG2	Argentina	Male, 15	Negative	Yes	Hyperdiploid
Hep3B	US	Male, 8	Positive	Yes	Hyperdiploid
Hep40	China	Male,	Positive	No Data	Hyperdiploid
Huh-7	Japan	Male,	Negative	Yes	Hypotetraploid
Mahlavu		Female			
PLC/PRF/5	South Africa	Male	Positive	Yes	Hyperdiploid
SK-Hep-1	US	Male, 52	Negative	Yes	Hyperdiploid
SNU182	Korea	Male, 24	Positive	No Data	Hypertriploid
SNU387	Korea	Female, 41	Positive	No Data	Hypertriploid
SNU398	Korea	Male, 42	Positive	No Data	Hypertriploid
SNU423	Korea	Male, 40	Positive	No Data	Hypertriploid
SNU449	Korea	Male, 52	Positive	No Data	Hypertriploid
SNU475	Korea	Male, 43	Positive	No Data	Hypertriploid

Table 3.1: Characteristics of the HCC cell lines.

3.1.2 Reagents

Ethidium Bromide (EtBr);
10mg/ml in water (stock solution)
30ng/ml (working solution)

10X TBE Buffer Solution;

108g	Tris
55g	Boric Acid
8.3g	EDTA

Dissolved in 1lt of deionized water.

6X Loading Buffer Solution

30%	Glycerol
0.04%	Bromphenolblue
0.04%	Xylene Cyanol

ΔdH2O

10x Phosphate-Buffered Saline (PBS)

80 g	NaCl
2 g	KCl
14.4 g	Na2HPO4
2.4 g	KH2PO4

Dissolved in 1 lt of water and pH is adjusted to 7.4.

50x TAE Buffer (Tris-Acetate-4EDTA)

242 g	Tris Base
57.1 ml	Acetic Acid
100ml	0.5M EDTA

ddH2O was added to 1 liter and adjust pH to 8.5

Wash A: Non-Stringent Wash Buffer

(6X SSPE, 0.01% Tween 20)

For 1000 mL:

300 mL of 20X SSPE

1.0 mL of 10% Tween-20

699 mL of water, filtered through a 0.2 μm filter.

Wash B: Stringent Wash Buffer
(0.6X SSPE, 0.01% Tween 20)
For 1000 mL:
30 mL of 20X SSPE
1.0 mL of 10% Tween-20
969 mL of water, filtered through a 0.2 μm filter

0.5 mg/mL Anti-Streptavdin Antibody
Resuspend 0.5 mg in 1 mL of water

12X MES Stock Buffer
(1.22M MES, 0.89M [Na+])
For 1,000 mL:
70.4g of MES hydrate
193.3g of MES Sodium Salt
800 mL of Molecular Biology Grade water
Mix and adjust volume to 1,000 mL.
The pH should be between 6.5 and 6.7.
Filtered through a 0.2 μm filter

1X Array Holding Buffer
(Final 1X concentration is 100 mM MES, 1M [Na+], 0.01% Tween-20)
For 100 mL:
8.3 mL of 12X MES Stock Buffer
18.5 mL of 5M NaCl
0.1 mL of 10% Tween-20
73.1 mL of water

Stain Buffer
H2O 666.7 µL
SSPE (20X) 300 µL 6X
Tween-20 (3%) 3.3 0.01%
Denhardt's (50X) 20 1X
Subtotal 990 µL
Subtotal / 2 495 µL

SAPE Solution Mix
Stain Buffer 495 µL 1X
1 mg/mL Streptavidin Phycoerythrin (SAPE) 5.0 µL 10 µg/mL
Total 500 µL

Antibody Solution Mix
Stain Buffer 495 µL 1X
0.5 mg/mL biotinylated antibody 5 µL 5 µg/mL
Total 500 µL

3.2 Methods

3.2.1 Tissue Culture

All cell lines were cultured in 75ml flasks (Greigner-Bio) as monolayers. Cell lines were either grown in RPMI-1640 (Biological Industries) or Dulbecco's Modified Eagle Medium (DMEM) (Biochrom AG) supplied with 10% FBS (Sigma), 50mg/ml penicillin / streptomycin and non-essential amino acids (Biochrom AG). Cell lines were culture at 37°C incubator with 5% CO2 (Heto-Holten, Surrey, UK). Cells were handled in sterile laminar hoods (Heto-Holten, Surrey, UK). Medias and solutions were kept at 4°C and preheated to 37°C before use.

3.2.1.1 Cyropreservation of Cell Lines

Exponentially growing cells were harvested with trypsin and fresh medium was added to inactivate trypsin through neutralization. The numbers of cells were counted with hemocytometer and precipitated at 250g for 5 minute at room temperature. Following, cells were resuspended with freezing media at a concentration of 5 million / ml in one vial. Freezing medium was prepared as 90% FBS and 10%DMSO (Sigma). Cryotubes were incubated at -20°C for 1 hour, following -80°C overnight and kept in liquid nitrogen tank for long term storage.

3.2.1.2 Culturing of Cell Lines

After removal from liquid nitrogen tanks, cells were thawed at 37°C and 5ml of growth medium was added. Following centrifugation at 1500 rpm for 5 minutes, supernatant was discarded and fresh medium was added. Cells were then grown in 25ml flasks in the incubator.

3.2.1.3 Subculturing of Cell Lines

Cells were grown at a confluency of app 80%. Old medium was removed from the flasks with vacuum and the cells were washed with PBS twice. Trypsin was added to flasks and cells were incubated for 2-3 minutes with trypsin/EDTA solution. After detaching from the flask surface, fresh medium was added to inactivate the trypsin. Collected cells were then transferred to new plates.

3.2.1.4 Preparation of Cell Pellets

Cell pellets were prepared for gDNA and RNA isolation. When the cells reached 80% confluency, medium was removed and cells were washed with PBS twice. Cells were detached with trypsin and fresh medium was added. After centrifugation for 5 minutes at 1500 rpm, supernatant was discarded and the pellet was washed with PBS twice. Pellets were immediately placed -80°C refrigerator.

3.2.1.5 Genomic DNA Isolation

Frozen cell pellets were thawed at room temperature and gDNAs were isolated by using Qiagen DNeasy Tissue Kit according to manufacturer's recommendation. Isolated genomic DNAs (gDNA) were either dissolved in manufacturer's buffer or in water. Quality of gDNA was checked on 0.75 % agarose gel and concentration was measured with Nanodrop Spectrophotometer (Nanodrop Technologies). gDNAs were stored at-20°C for long term.

3.2.2 SNP Microarray Assay

Probe preparation for SNP microarray hybridization experiments were done according to manufacturer's manual (Affymetrix, 10K2.0 Assay). The overall assay is shown in Figure 3.1

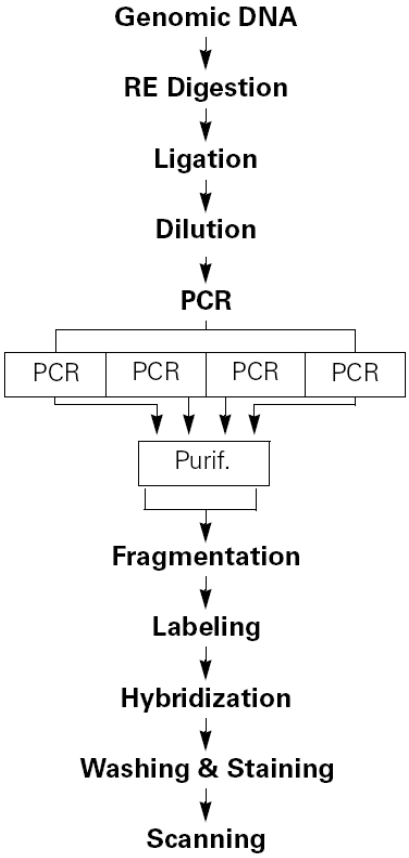


Figure 3.1: Outline of SNP microarray assay.

Briefly, genomic DNAs were diluted to 50ng/µl in water. 250ng of each gDNA was subjected to restriction digestion with XbaI enzyme (New England Biolabs) for two hours at 37°C in thermal cycler (Tech e), in replicate. One replicate was run on a 0.75% agarose gel to check if the digestion assay performed well; the other replica was continued with adaptor ligation. T4 DNA ligase (New England Biolabs) was used to attach adaptor Xba (Affymetrix) to XbaI restriction sites. The ligation assay was done at 16°C for 2 hours. Adaptor Xba contains a binding site for Xba Primer (Affymetrix). Later, ligated restriction fragments were diluted to 4 fold with H2O and used in whole genome PCR as template. Hot Star Taq Plus polymerase (Qiagen) was used in the amplification process. The thermo profile was as follows: 94°C for 2 minutes and 30 seconds, denaturation at 94°C for 30 seconds, annealing temperature (varies) for 45 seconds, extension at 72°C for 30 seconds, and final extension for 5 minutes and 30

seconds. PCR amplicons were purified with Qiagen Qiaquick PCR purification kit. 20µg of PCR was fragmented to a range between 35bp – 200bp using DNAseI (Affymetrix). The fragment sizes were checked by electrophoresis on 4% agarose gel. Following, the fragmented PCR products were end labeled with Biotin-labeled reagent (Affymetrix) using Terminal Deoxynucleotidyl Transferase (Affymetrix). Probe DNA was then denatured at 95°C in hybridization buffer containing TMACL (Sigma), DMSO (Sigma), Denhardt's Solution (Sigma), MES (Sigma), Herring Sperm DNA (Promega), EDTA (Ambion), Tween-20 (Sigma), Human Cot-1 DNA (Invitrogen) and Oligonucletide control (Affymetrix). Following, denatured probe was injected to the array (Affymetrix 10K2.0). The hybridization was done at 48°C, 60 rpm for 16 hours in the hybridization oven (Affymetrix). After hybridization, the probe mix was discarded and the array was washed with Wash A and B buffers (6X SSPE, 0.01% Tween 20, 0.6X SSPE, 0.01% Tween 20, respectively) in fluidics station (Affymetrix). Then, the chips were stained with buffers containing Biotinylated Anti-Streptividin antibody (Vector), SAPE (Streptavidin, R-phycoerythrin conjugate), Acetylated Bovine Serum Albumin, 20X SSPE, Denhardt's Solution and Tween-20. The stained chip was scanned at the scanner (Affymetrix) and pre-analyzed with GeneChip Operating Software (GCOS) and GeneChip DNA Analysis Software (GDAS) software bundle (Affymetrix). All these steps above are briefly shown in figure 3.2.

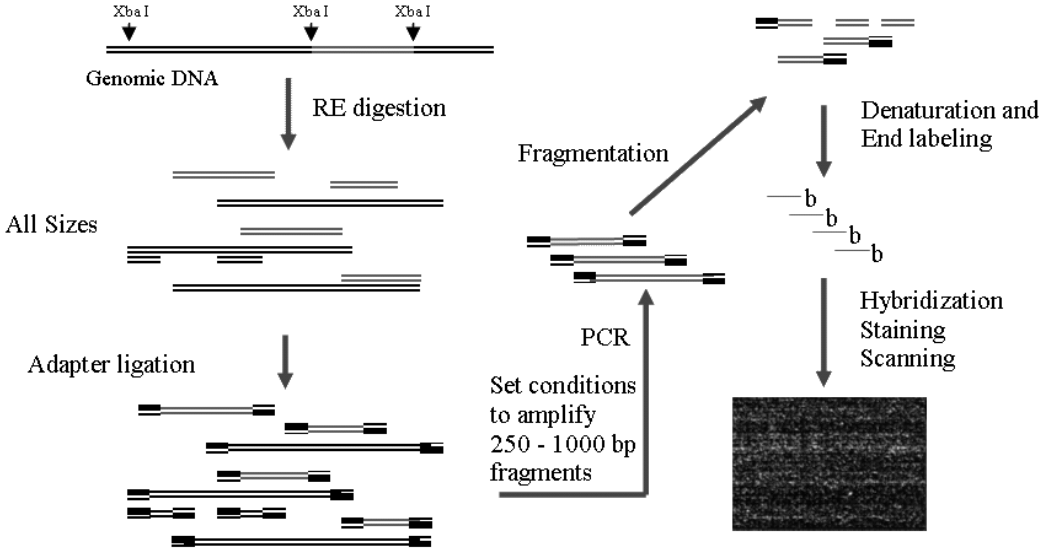


Figure 3.2: Preparation of target from genomic DNA.

3.2.3 Microarray Analysis

3.2.3.1 Pre-Analysis

Pre-analysis of the SNP microarrays were performed with GCOS and GDAS software bundle. The bundle operates the scanner and builds the raw data captured by the sensor. It uses the specific library files containing the information about the probesets on the array using pre-defined settings and algorithms (Affymetrix 10K2.0 Manual). The bundle then extracted probeset information from raw data and generates CEL files containing the signal intensity of each probeset along with a chip report file. The chip report file provided information about the performance of the hybridization, such as; average signal intensity of probesets, background and oligonucleotide controls along with a pseudo-image of the chip. Later, using the CEL files, genotype calls of the each probeset was calculated with their intensities.

3.2.3.2 Advance-Analysis

Advance analysis was performed with DNA-Chip Analyzer (dChip) (Harvard University) Software freely available for academic users at www.dchip.org. dChip is a Windows software package for probe-level and high-level analysis of gene expression microarrays and SNP microarrays (Li and Wong 2001, Lin et al. 2004). At the probe level, dChip can display and normalize the CEL files, and the model-based approach allows pooling information across multiple arrays and automatic probe selection to handle cross-hybridization and image contamination. High-level analysis in dChip includes comparing samples, hierarchical clustering, view expression and SNP data along chromosome, LOH and copy number analysis of SNP arrays, and linkage analysis. In these functions the gene information and sample information are correlated with the analysis results. In the analysis, model-based expression was selected with perfect match-mismatch background correction. Normalization was performed according to the median chip with median intensity using invariant set and smoothed with running median

method. Hidden Markov Model and median smoothing were used in inferred copy number analysis.

3.2.4 Genomic DNA PCR

All PCR reactions were performed using Techne-512 equipment (Techne Inc). Primers were first checked for their optimal conditions by altering magnesium levels in a thermo-gradient PCR.). A reaction mixture of 2.5µl 10X reaction buffer, 2.5µl MgCl2 (25mM), 1µl dNTP (10µM), 1µl of each primer (10pmol), and 0.5µl Taq DNA polymerase (5u/µL) was prepared per 250ng of gDNA. The thermo profile was 94°C for 2 minutes and 30 seconds; denaturation at 94°C for 30 seconds, annealing temperature (differs) for 45 seconds, extension at 72°C for 30 seconds, and final extension for 5 minutes and 30 seconds.

3.2.4.1 Oligonucleotide Design

All oligonucleotide primers were designed by using Primer3 algorithm available at http://frodo.wi.mit.edu/. Oligonucletides were purchased from Iontek (Iontek) as lyophilized. Primer sequences are listed in Table 3.2

TPTE2_1F	ATGGACACATTTAGTTCGACTTC
TPTE2_1R	CAGCCTTCTCATCAGCTTTT
HSA_MIR_31_F	ATACACAGCAATACACGAAGGACT
HSA_MIR_31_R	GGTGAAAGGAAAAATTTTGGAA
GAPDH_070228_cDNA_F	GGCTGAGAACGGGAAGCTTGTCAT
GAPDH_070228_cDNA_R	CAGCCTTCTCCATGGTGGTGAAGA
Mir124a1-F	GTCGGTCGCTCCTTCCTT
Mir124a1-R	TCTACCCACCCCTCTTCCTT
SATL1_F	GGGGACAATCCCCTTTTCTAC

SATL1_R	AAAGTACCTTGCCAGTCCATGA
NUBPL_gDNA_F	AGTTCCGATTTTGTTTCTTTCCA
NUBPL_R	ACAATTGGCTGGCCTGTATCT

Table 3.2: Primers used in the PCR assays.

3.2.4.2 PCR Purification

All PCR products were purified by using the Qiagen Qiaquick PCR purification system according to manufacturer's recommendation except a few modifications. After washing the membrane containing the PCR products with ethanol containing was buffer, an additional step of centrifugation was performed at 20,000g for 5 minutes with caps open. This allowed complete evaporation of PCR products and then H20 was used for reconstitution of the PCR products.

3.2.8.3 Agarose Gel Electrophoresis

2µl of 6X DNA loading dye was added to 10µl of each PCR product. PCR products were then loaded in 30ng/µl ethidium bromide containing 1% (w/v) agarose gels and were run in horizontal gel electrophoresis equipment in 1X TAE buffer under 90V for 30 minutes. Gene Ruler DNA ladder (Fermentas) was used as DNA size marker. Transilluminator equipment (Bio – Rad) was used for visualization at 340nm wavelength UV along with MultiAnalyst software (Bio – Rad).

3.2.4.4 Sequencing

Selected amplified PCR products were purified by using the Qiagen Qiaquick PCR purification kit and quantified with Nanodrop spectrophotometer. Required amounts of purified PCR products were sent to Iontek along with the PCR primers for sequencing. Received sequence data was analyzed with Mutation Explorer Software (Softgenetics).

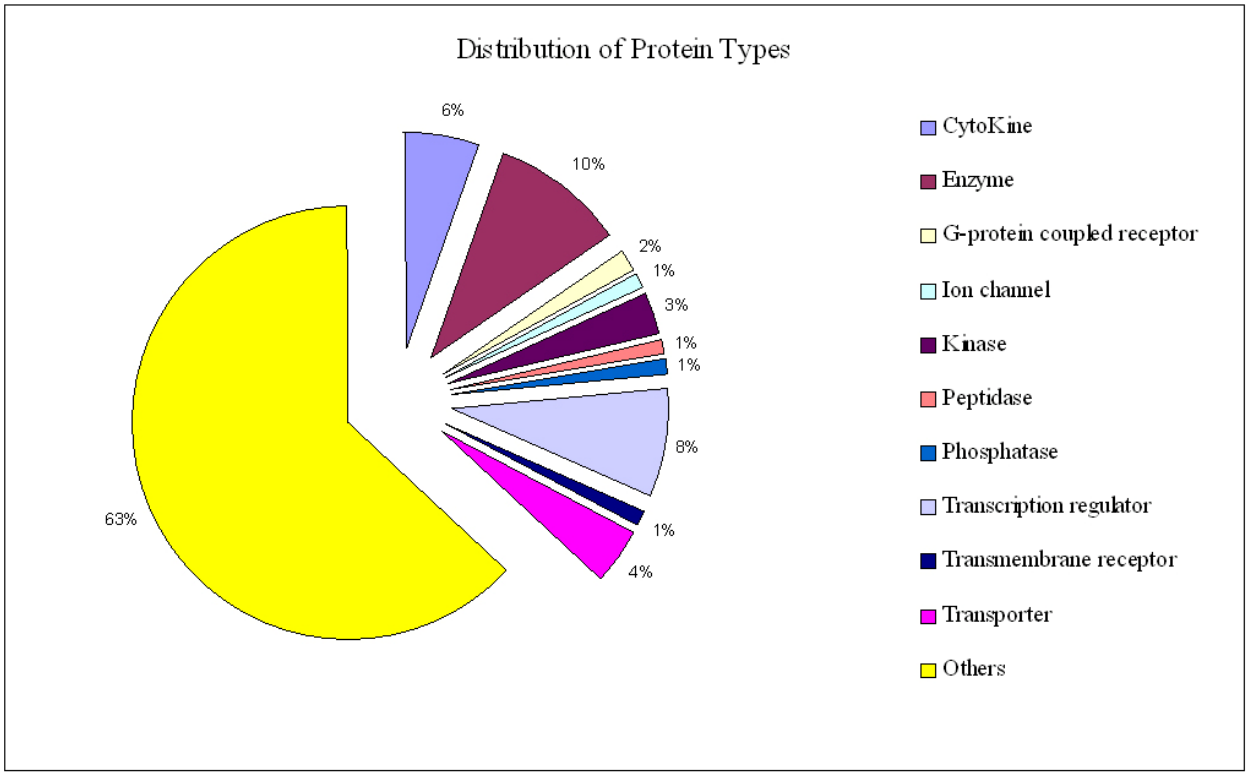
4. RESULTS

In the framework of this study, we have detected two deletions and 12 amplifications which are novel in hepatocellular carcinoma. These disturbed regions harbor more approximately 570 transcripts. Some these genes are well described in cell cycle and tumorigenesis, other's role are still poorly understood. Among the described ones, a high percentage of these genes code for enzymes, transcription regulators, cytokines, transporters and kinases. Concordantly, most of the gene products of these trascipts are found in cytoplasm, nucleus and extracellular spaces. Below are the figures that show overall results along with protein functions and cellular distribution.

	Hep40	Hep3B	HepG2	Huh7	Plc	Focus	Mahlavu	SkHep1	Snu182	Snu387	Snu398	Snu423	Snu449	Snu475
Chr1														
Chr2														
Chr3														
Chr4														
Chr5														
Chr6														
Chr7														
Chr8	p23.1-q13.3-q21.11-q24.13				p23		p23	p23-p22.1-p21.2	p23	p23-p22.1-p21.2	p22.1-p21.2	p23	p22.1-p21.2	
Chr9														
Chr10														
Chr11														
Chr12														p11.21-p11.1
Chr13				q12.11				q12.11						
Chr14				q12-q13.1										
Chr15	q21.3													
Chr16	q21.3								p13.1-q11.1					p13.1-q11.1,q21.2
Chr17														
Chr18						q13.31-q13.32	q13.31-q13.32							
Chr19														
Chr20														
Chr21														
Chr22									q11.21-q11.22					
ChrX				q21.1-21.33					p22.11					

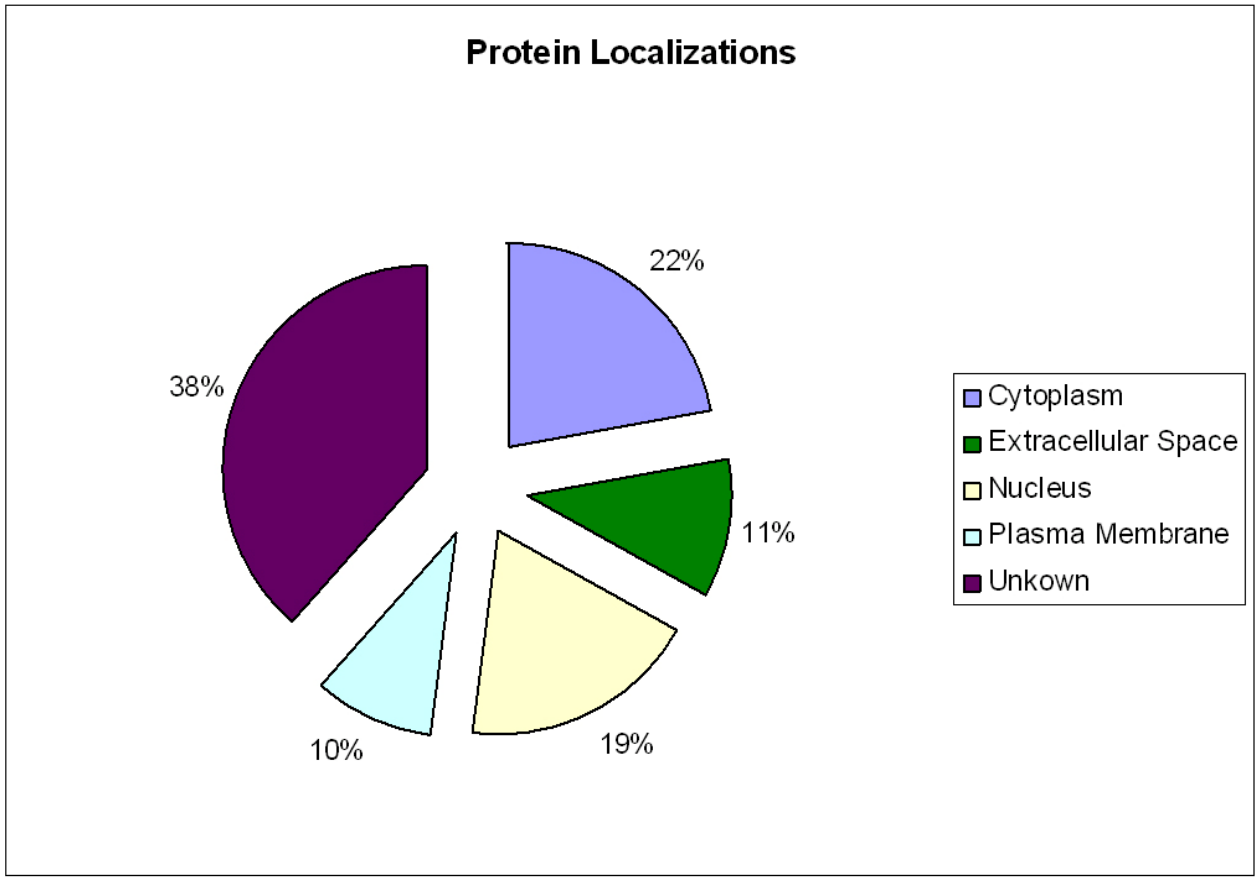
Table 4.a: Overall disturbances in HCC cell lines; red and green represent amplifications and deletions, respectively.

Table 4.b: Types of gene products mapping to disturbed regions.



Type of Protein	Number	%
CytoKine	32	5.6
Enzyme	57	9.9
G-protein coupled receptor	10	1.7
Ion channel	6	1
Kinase	17	3
Peptidase	7	1.2
Phosphatase	6	1
Transcription regulator	47	8.2
Transmembrane receptor	6	1
Transporter	24	4.2
Others	361	63
Total	573	

Table 4.c: Localization patterns of proteins in disturbed regions.



Protein Localizations	Number	%
Cytoplasm	127	22.2
Extracellular Space	62	10.8
Nucleus	108	18.8
Plasma Membrane	55	9.6
Unkown	221	38.6
Total	573	

4.1 Homozygous and Hemizygous Deletions

We have observed three homozygous and one hemizygous deletions. Homozygous deletions are located at 9p23 in Mahlavu, PLC, SkHep1, Snu182, Snu387 and Snu423; 9p22.1-p21.2 in SkHep1, Snu387 and Snu449; 13q12.11 in Huh7 and SkHep1; hemizygous deletion maps to Xq21.1-21.33 in Huh7 (male origin). All the deletions are in concordance with the microarray expression data (not shown) and they have also been confirmed by PCR.

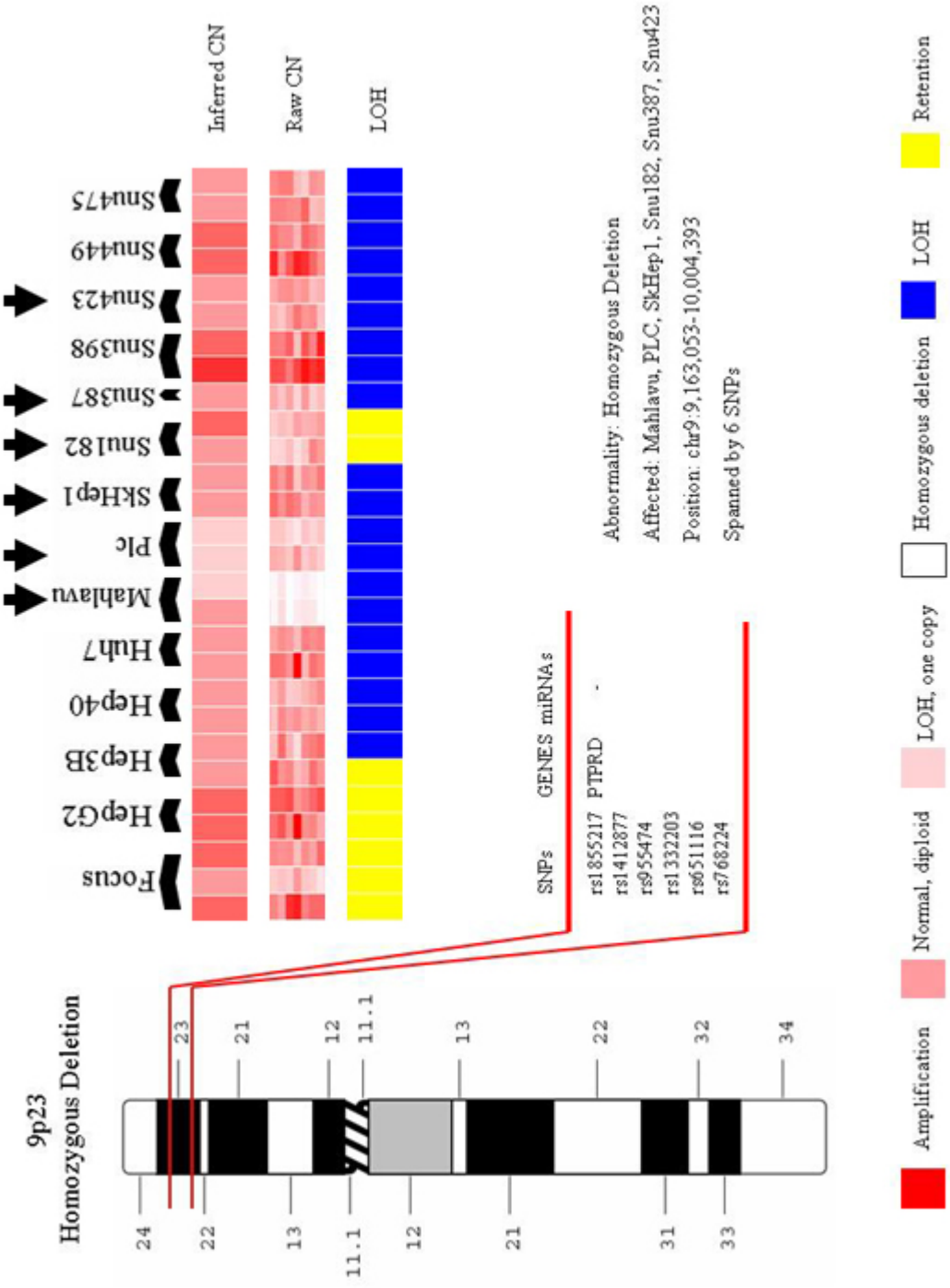


Figure 4.1.1: Homozygous deletion at 9p23 in Mahlavu, PLC, SkHep1, Snu182, Snu387 and Snu423.

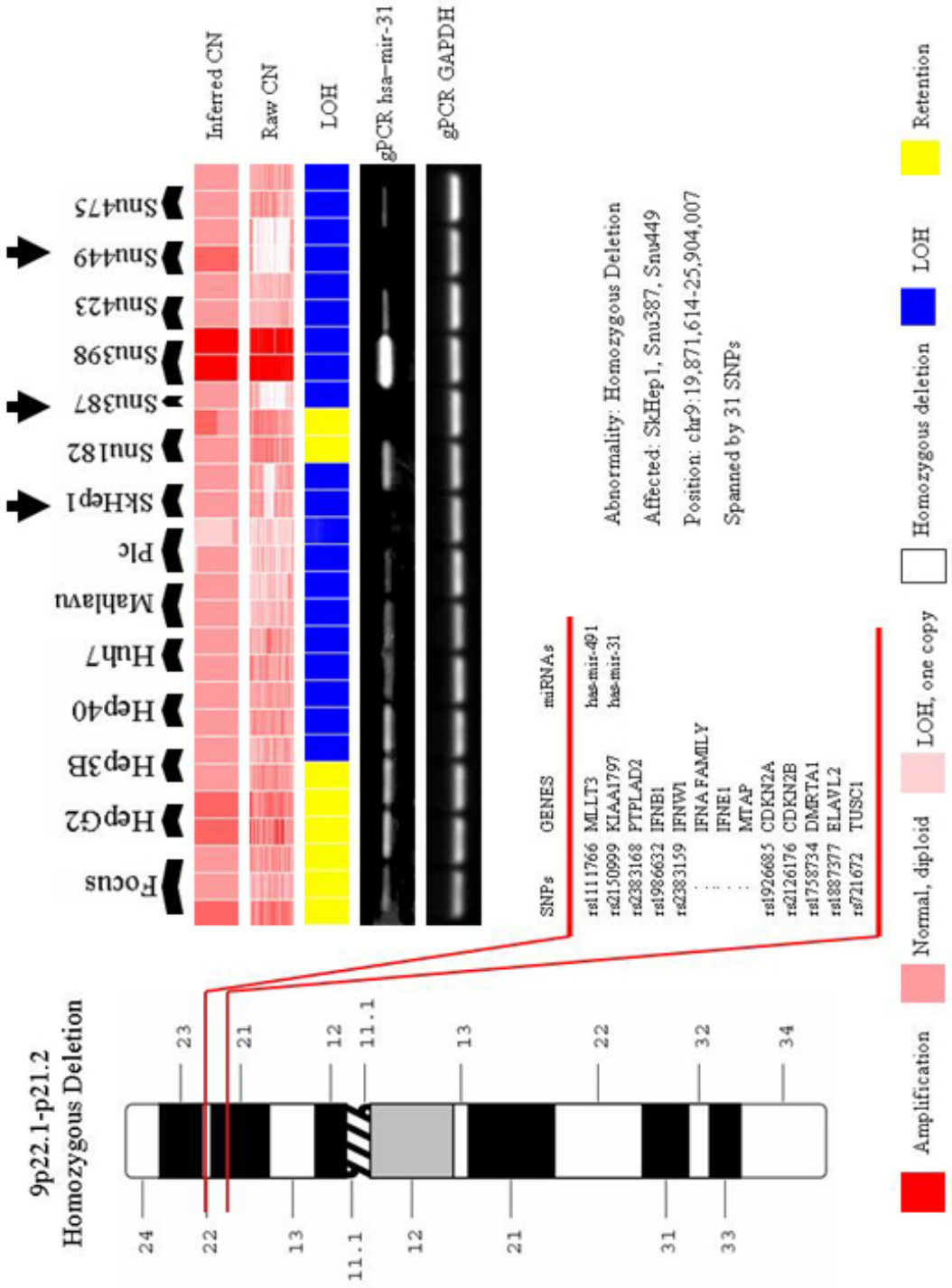


Figure 4.1.2: Homozygous deletion at 9p22.1-p21.2 in SkHep1, Snu387 and Snu449.

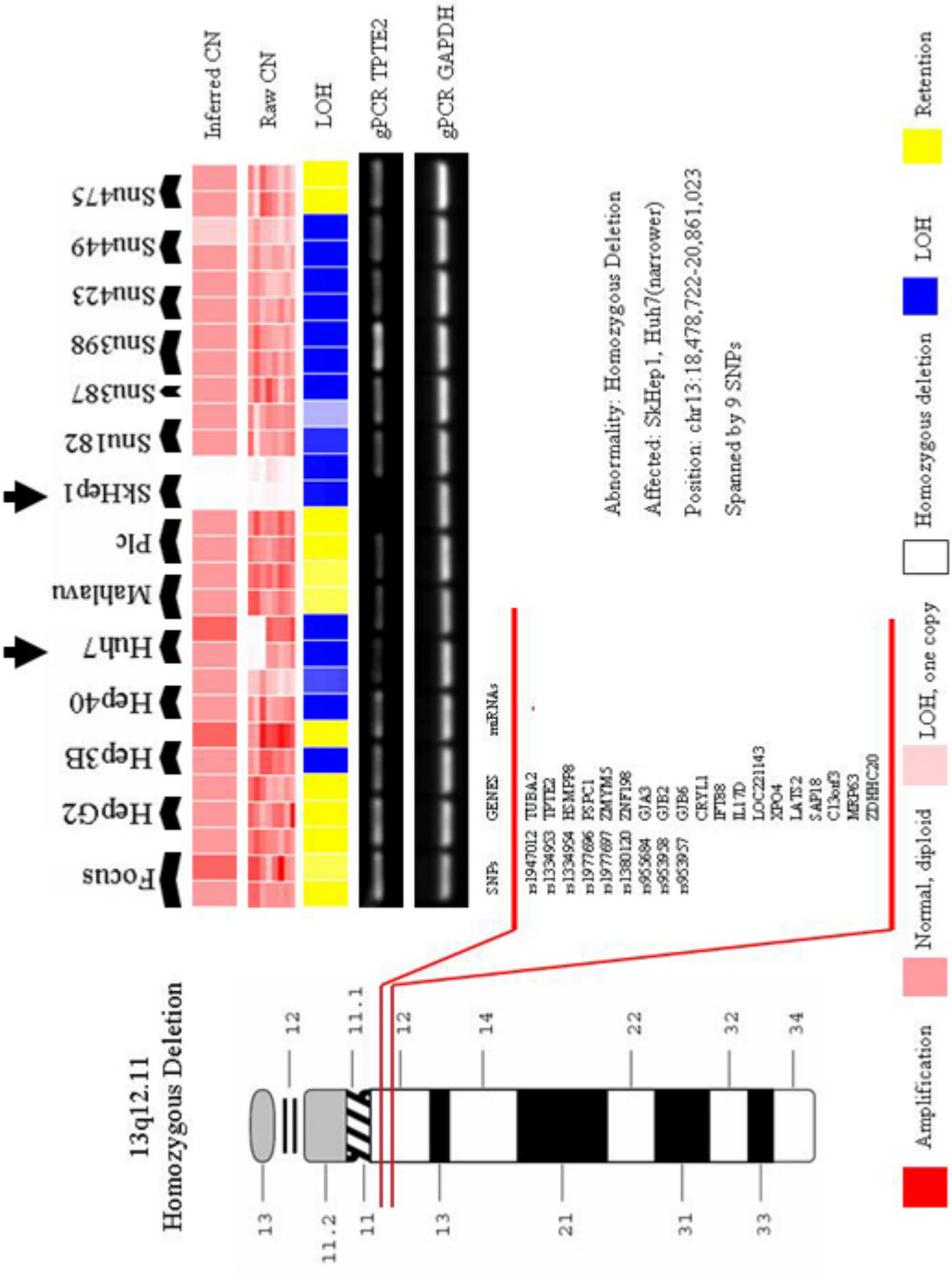


Figure 4.1.3: Homozygous deletion at 13q12.11 in Huh7 and SkHep1.

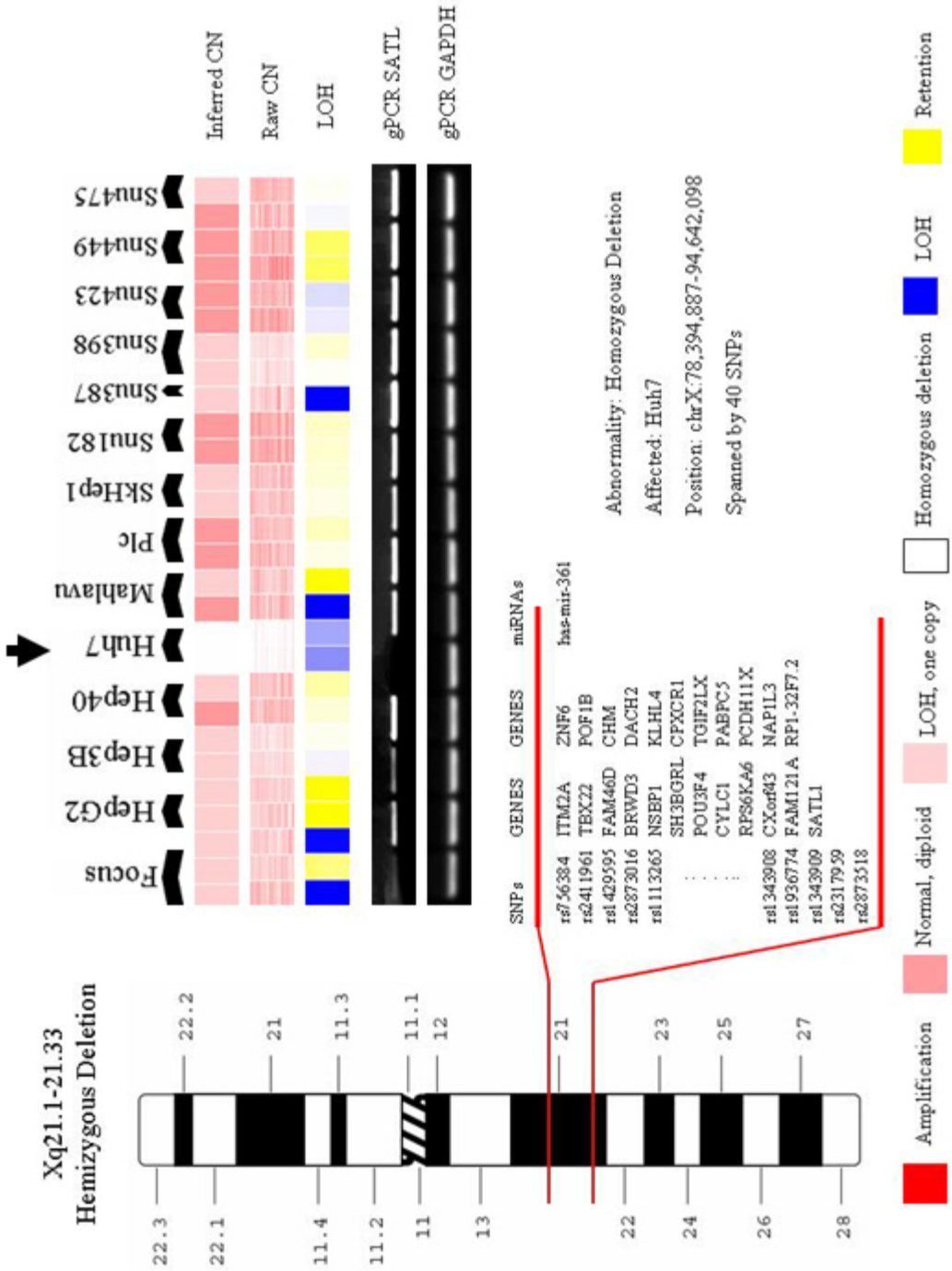


Figure 4.1.4: Hemizygous deletion at Xq21.1-21.33 in Huh7.

Region	Aberration	Cell Line	Gene	Description	Location	Type
9p23	Homozygous Deletion	Mahlavu, PLC, SkHep1, Snu182, Snu387, Snu423	PTPRD	protein tyrosine phosphatase, receptor type, D	Plasma Membrane	phosphatase
9p22.1-p21.2	Homozygous Deletion	SkHep1, Snu387, Snu449	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	Nucleus	transcription regulator
"	"	"	CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	Nucleus	transcription regulator
"	"	"	DMRTA1	DMRT-like family A1	Unknown	other
"	"	"	ELAVL2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	Cytoplasm	other
"	"	"	IFNA1	interferon, alpha 1	Extracellular Space	cytokine
"	"	"	IFNA10	interferon alpha 10	Unknown	other
"	"	"	IFNA10	interferon, alpha 10	Extracellular Space	cytokine
"	"	"	IFNA13	interferon, alpha 13	Extracellular Space	cytokine
"	"	"	IFNA14	interferon, alpha 14	Extracellular Space	cytokine
"	"	"	IFNA16	interferon, alpha 16	Extracellular Space	cytokine
"	"	"	IFNA17	interferon, alpha 17	Extracellular Space	cytokine
"	"	"	IFNA2	interferon, alpha 2	Extracellular Space	cytokine
"	"	"	IFNA21	interferon, alpha 21	Extracellular Space	cytokine
"	"	"	IFNA4	interferon, alpha 4	Extracellular Space	cytokine
"	"	"	IFNA5	interferon, alpha 5	Extracellular Space	cytokine
"	"	"	IFNA6	interferon, alpha 6	Extracellular Space	cytokine
"	"	"	IFNA7	interferon, alpha 7	Extracellular Space	cytokine
"	"	"	IFNA8	interferon, alpha 8	Extracellular Space	cytokine
"	"	"	IFNB1	interferon, beta 1, fibroblast	Extracellular Space	cytokine
"	"	"	IFNE1	interferon epsilon 1	Extracellular Space	cytokine
"	"	"	IFNW1	interferon, omega 1	Extracellular Space	cytokine
"	"	"	KIAA1797	KIAA1797	Unknown	other
"	"	"	KLHL9	kelch-like 9 (Drosophila)	Unknown	other
"	"	"	MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	Nucleus	other
"	"	"	MTAP	methylthioadenosine phosphorylase	Nucleus	enzyme
"	"	"	PTPLAD2	protein tyrosine phosphatase-like A domain containing 2	Unknown	other
"	"	"	TUSC1	tumor suppressor candidate 1	Unknown	other
"	"	"	MAP2	microtubule-associated protein 2	Cytoplasm	other
"	"	"	MAP4	microtubule-associated protein 4	Cytoplasm	other
"	"	"	MAP6	microtubule-associated protein 6	Cytoplasm	other
"	"	"	MAP7	microtubule-associated protein 7	Cytoplasm	other
"	"	"	MAP9	microtubule-associated protein 9	Unknown	other
"	"	"	IFI44	interferon-induced protein 44	Cytoplasm	other
"	"	"	MAP1A	microtubule-associated protein 1A	Cytoplasm	other
"	"	"	MAP1B	microtubule-associated protein 1B	Cytoplasm	other
"	"	"	MAP1S	microtubule-associated protein 1S	Cytoplasm	enzyme
"	"	"	MTAP2K	microtubule-associated protein 2 kinase	Unknown	other
"	"	"	IFNA13 PREDICTED	interferon alpha family, gene 13 (predicted)	Unknown	other
"	"	"	IFNA5 PREDICTED	interferon alpha family, gene 5 (predicted)	Unknown	other
"	"	"	MTAP PREDICTED	methylthioadenosine phosphorylase (predicted)	Unknown	enzyme

Table 4.1.1: Hom. del. at 9p23 and 9p22.1-p21.2 in Mahlavu, PLC, SkHep1, Snu182, Snu387, Snu423 and Snu449.

Region	Aberration	Cell Line	Gene	Description	Location	Type
13q12.11	Homozygous Deletion	Huh7, SkHep1	C13ORF3	chromosome 13 open reading frame 3	Unknown	other
"	"	"	CRLF2	cytokine receptor-like factor 2	Plasma Membrane	transmembrane receptor
"	"	"	CRYL1	crystallin, lambda 1	Unknown	other
"	"	"	GJA3	gap junction protein, alpha 3, 46kDa	Plasma Membrane	transporter
"	"	"	GJB2	gap junction protein, beta 2, 26kDa	Plasma Membrane	transporter
"	"	"	GJB6	gap junction protein, beta 6	Plasma Membrane	transporter
"	"	"	HSMPP8	M-phase phosphoprotein, mpp8	Nucleus	transcription regulator
"	"	"	IFT88	intraflagellar transport 88 homolog (Chlamydomonas)	Cytoplasm	other
"	"	"	IL17D	interleukin 17D	Extracellular Space	other
"	"	"	LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)	Nucleus	kinase
"	"	"	MRP63	mitochondrial ribosomal protein 63	Unknown	other
"	"	"	PSPC1	paraspeckle component 1	Nucleus	other
"	"	"	SAP18	Sin3A-associated protein, 18kDa	Nucleus	transcription regulator
"	"	"	TPTE2	transmembrane phosphoinositide 3-phosphatase and tensin homolog 2	Cytoplasm	phosphatase
"	"	"	TUBA1A	tubulin, alpha 1a	Cytoplasm	other
"	"	"	TUBA1B	tubulin, alpha 1b	Cytoplasm	other
"	"	"	TUBA3C	tubulin, alpha 3c	Unknown	other
"	"	"	XPO4	exportin 4	Nucleus	transporter
"	"	"	ZDHHC11	zinc finger, DHHC-type containing 11	Unknown	other
"	"	"	ZDHHC20	zinc finger, DHHC-type containing 20	Unknown	other
"	"	"	ZMYM2	zinc finger, MYM-type 2	Nucleus	other
"	"	"	ZMYM5	zinc finger, MYM-type 5	Nucleus	other
"	"	"	MRP63 PREDICTED	mitochondrial ribosomal protein 63 (predicted)	Unknown	other

Table 4.1.2: Homozygous deletion at 13q12.11 in Huh7 and SkHep1.

Region	Aberration	Cell Line	Gene	Description	Location	Type
Xq21.1-21.33	Hemizygous Deletion	Huh7	APOOL	apolipoprotein O-like	Extracellular Space	other
"	"	"	BRWD3	bromodomain and WD repeat domain containing 3	Unknown	other
"	"	"	CHM	choroideremia (Rab escort protein 1)	Cytoplasm	enzyme
"	"	"	CPXCR1	CPX chromosome region, candidate 1	Unknown	other
"	"	"	CXORF43	chromosome X open reading frame 43	Unknown	other
"	"	"	CYLC1	cylicin, basic protein of sperm head cytoskeleton 1	Cytoplasm	other
"	"	"	DACH2	dachshund homolog 2 (Drosophila)	Nucleus	other
"	"	"	FAM46D	family with sequence similarity 46, member D	Unknown	other
"	"	"	ITM2A	integral membrane protein 2A	Plasma Membrane	other
"	"	"	KLHL4	kelch-like 4 (Drosophila)	Unknown	other
"	"	"	MPZ	myelin protein zero (Charcot-Marie-Tooth neuropathy 1B)	Plasma Membrane	other
"	"	"	NAP1L3	nucleosome assembly protein 1-like 3	Nucleus	other
"	"	"	NSBP1	nucleosomal binding protein 1	Nucleus	transcription regulator
"	"	"	PABPC5	poly(A) binding protein, cytoplasmic 5	Cytoplasm	other
"	"	"	PCDH11X	protocadherin 11 X-linked	Plasma Membrane	other
"	"	"	PCDH11Y	protocadherin 11 Y-linked	Plasma Membrane	other
"	"	"	POF1B	premature ovarian failure, 1B	Plasma Membrane	other
"	"	"	POU3F4	POU domain, class 3, transcription factor 4	Nucleus	transcription regulator
"	"	"	RP1-32F7.2	hypothetical protein FLJ37639	Unknown	other
"	"	"	RPS6KA6	ribosomal protein S6 kinase, 90kDa, polypeptide 6	Cytoplasm	kinase
"	"	"	SATL1	spermidine/spermine N1-acetyl transferase-like 1	Unknown	other
"	"	"	SH3BGRL	SH3 domain binding glutamic acid-rich protein like	Unknown	other
"	"	"	TBX22	T-box 22	Nucleus	transcription regulator
"	"	"	TGIF2LX	TGFB-induced factor homeobox 2-like, X-linked	Nucleus	transcription regulator
"	"	"	ZNF711	zinc finger protein 711	Nucleus	other
"	"	"	LECT1	leukocyte cell derived chemotaxin 1	Extracellular Space	other
"	"	"	LECT2	leukocyte cell-derived chemotaxin 2	Extracellular Space	other
"	"	"	TNMD	tenomodulin	Plasma Membrane	other

Table 4.1.3: Hemizygous deletion at Xq21.1-21.33 in Huh7.

4.2 Amplifications

We have observed 11 amplifications at 8p23.1 in Hep40; 8q13.3-q21.11 in Hep40; 8q24.13 in Hep40; 9p22.1-p21.2 in Snu398; 12p11.21-p.11 in Snu475; 14q12-q13.1 in Huh7, 15q21.3 in Hep40; 16q21.3 in Hep40; 17p13.1-q11.1 in Snu182 and Snu475; 17q21.2 in Snu475; 19q13.31-q13.32 in Focus and Mahlavu; 22q11.21-q11.22 in Snu182 and Xp22.11 in Huh7. Expression analysis results mostly did not reflect any signs of amplifications in this amplicons. We confirmed selected regions by PCR methods.

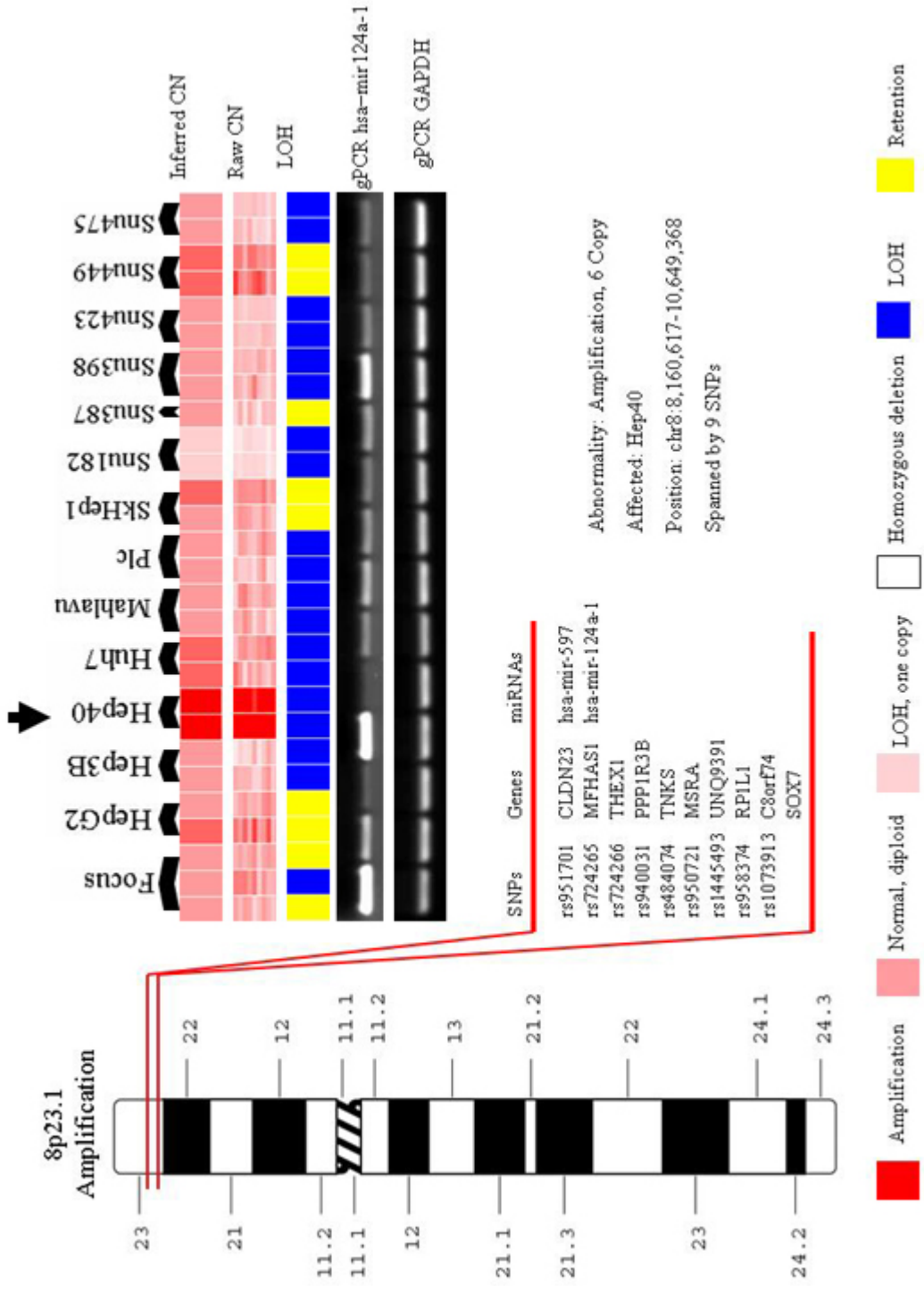


Figure 4.2.1: Amplification at 8p23.1 in Hep40.

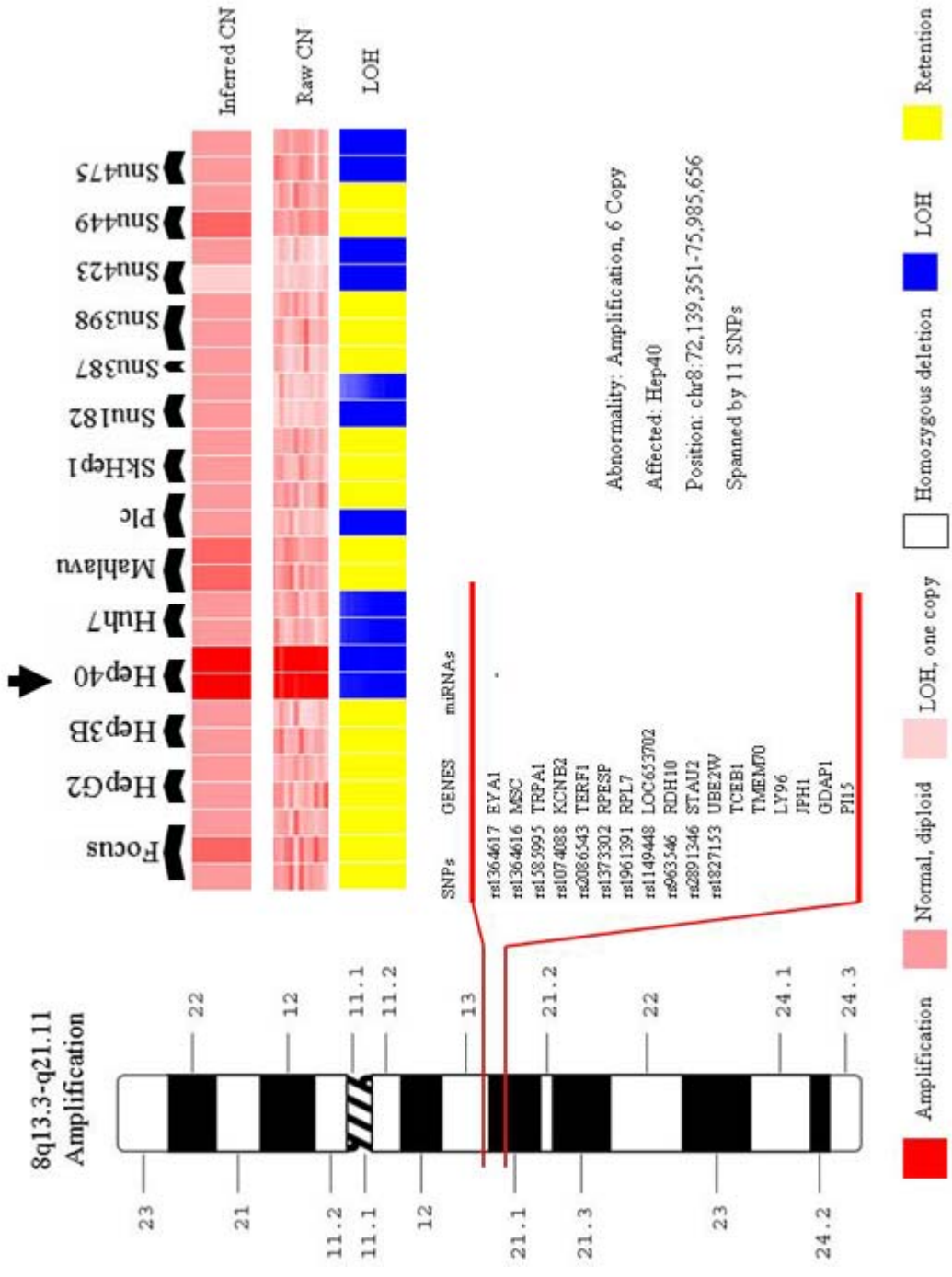


Figure 4.2.2: Amplification at 8q13.3-q21.11 in Hep40.

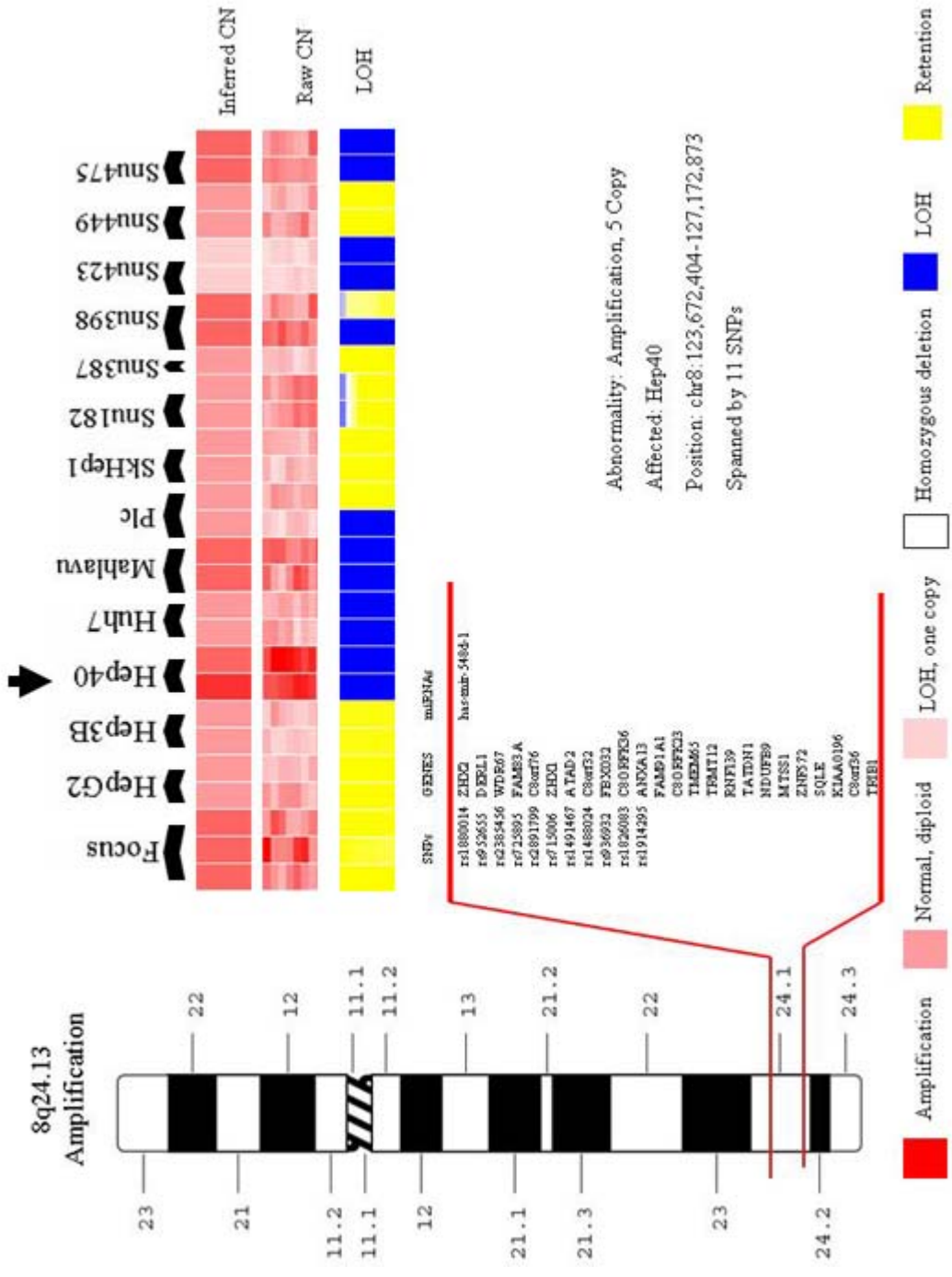


Figure 4.2.3: Amplification at 8q24.13 in Hep40.

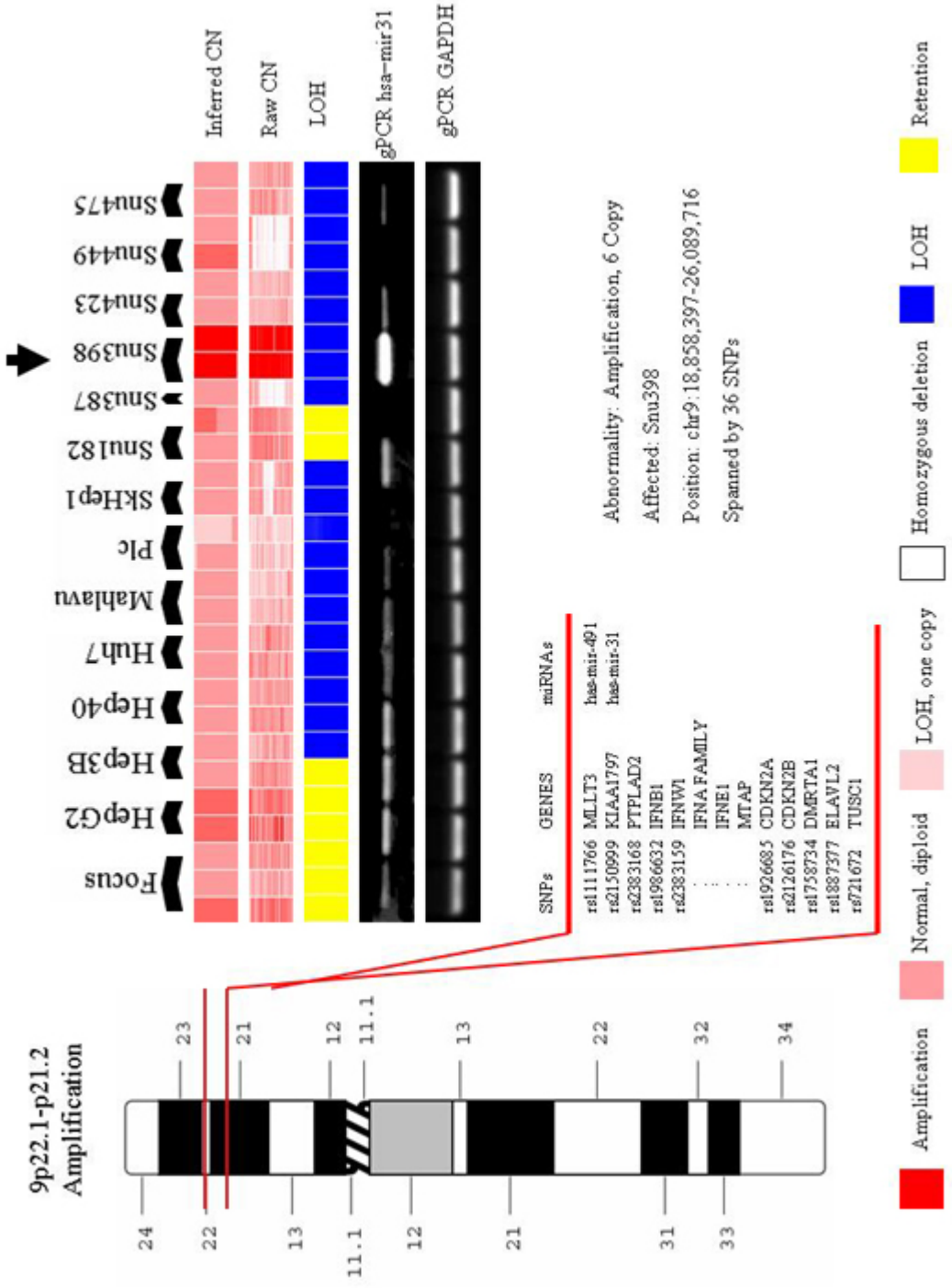


Figure 4.2.4: Amplification at 9p22.1-p21.2 in Snu398.

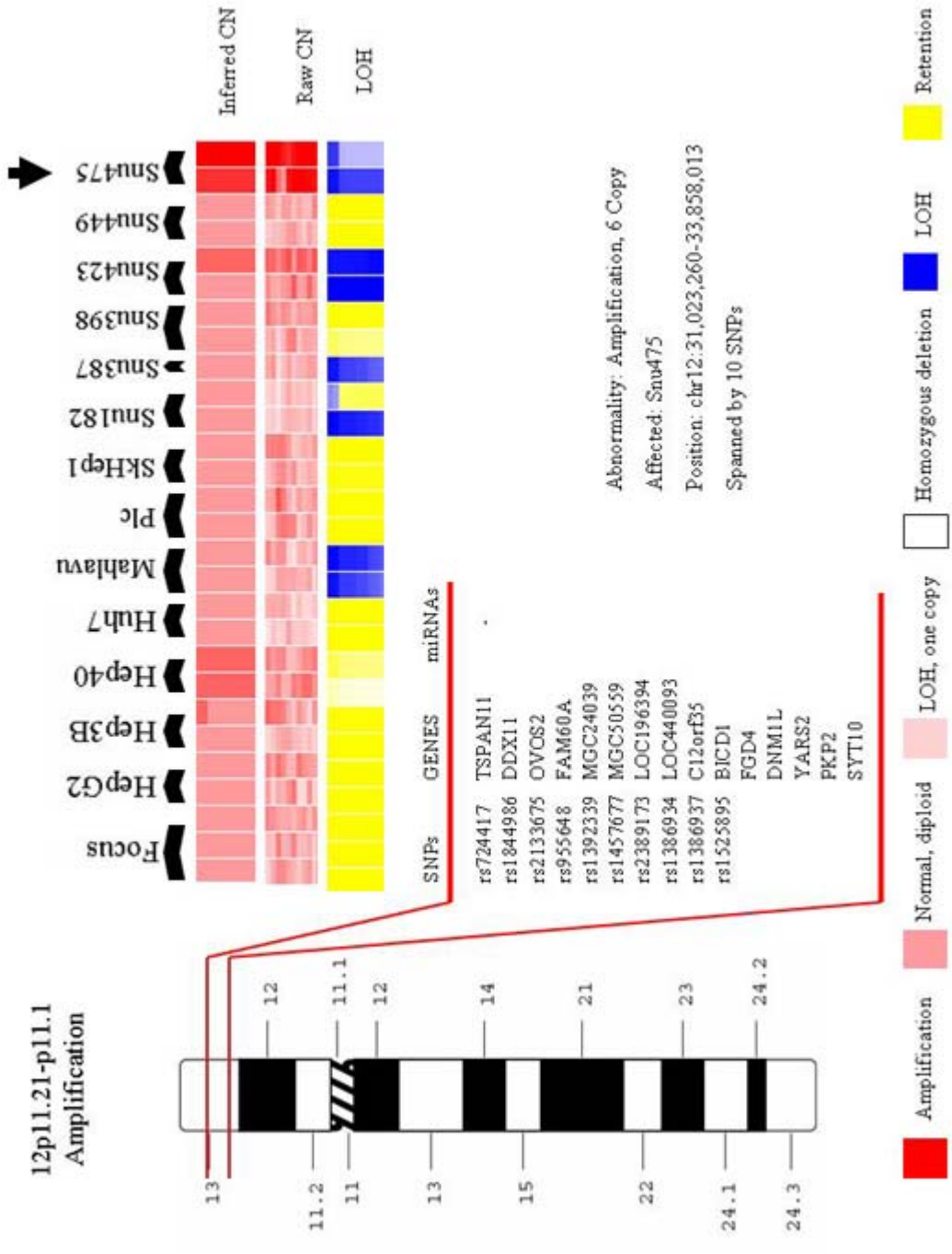


Figure 4.2.5: Amplification at 12p11.21-p11 in Snu475

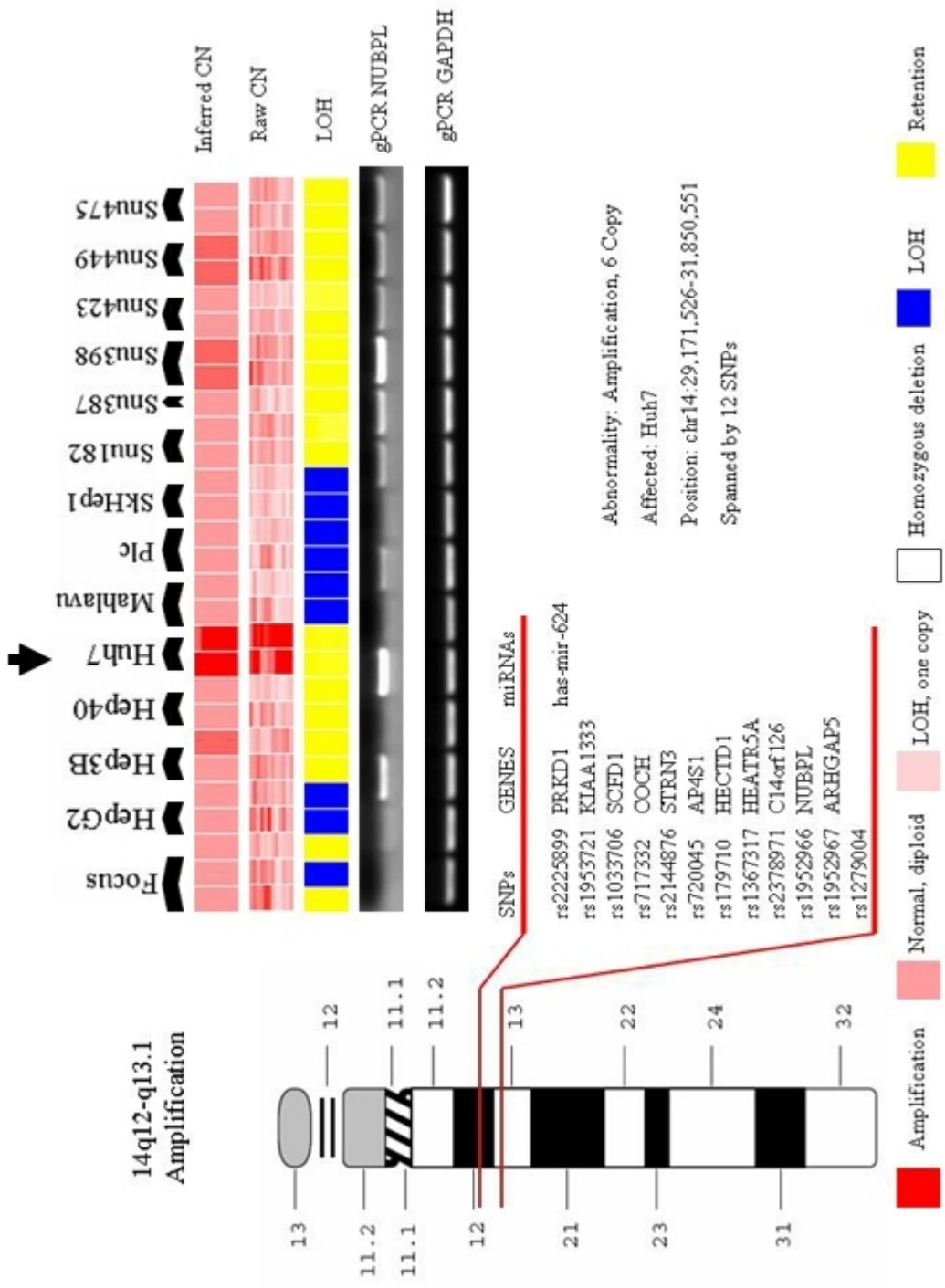


Figure 4.2.6: Amplification at 14q12-q13.1 in Huh7.

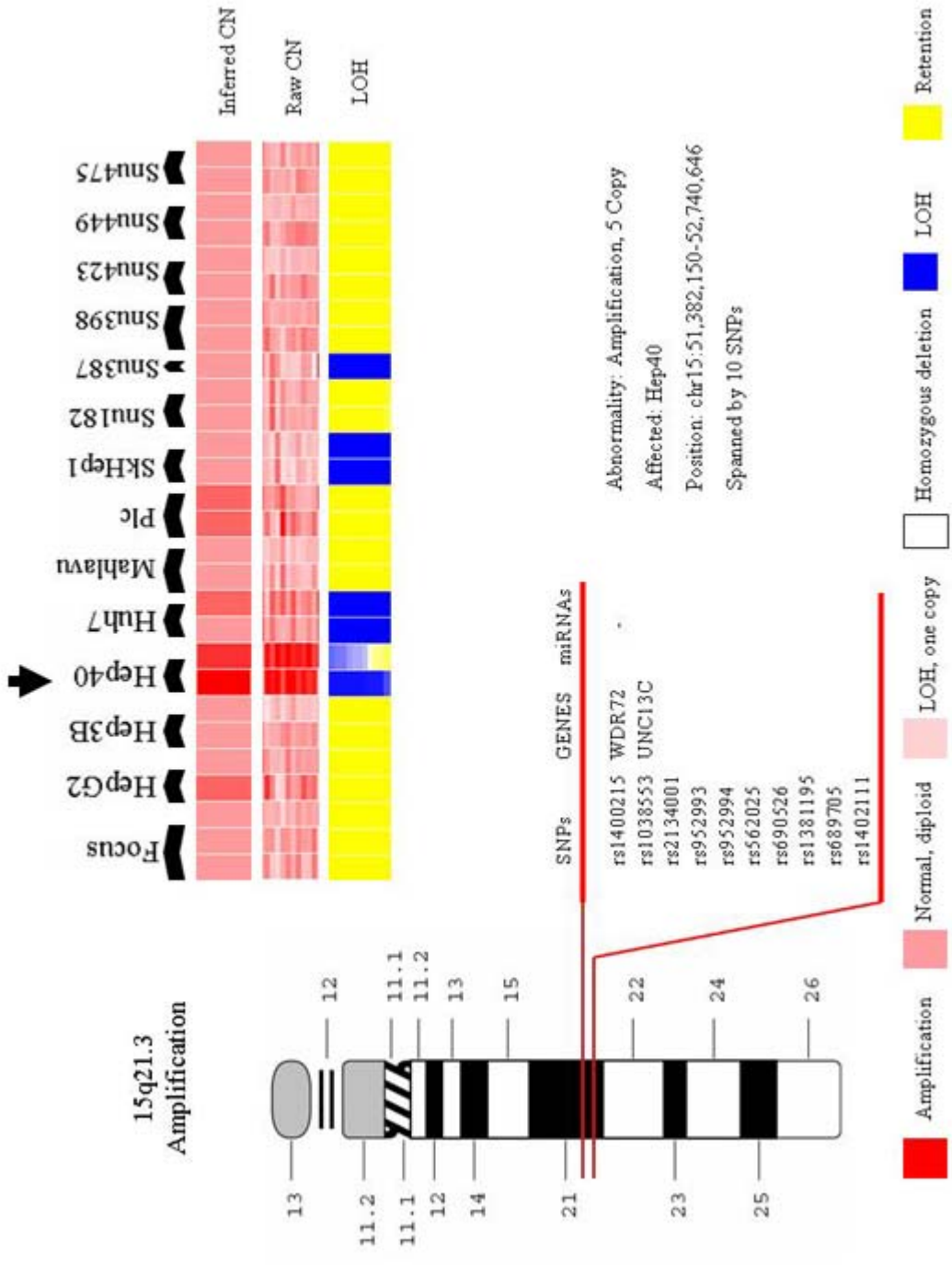


Figure 4.2.7: Amplification at 15q21.3 in Hep40.

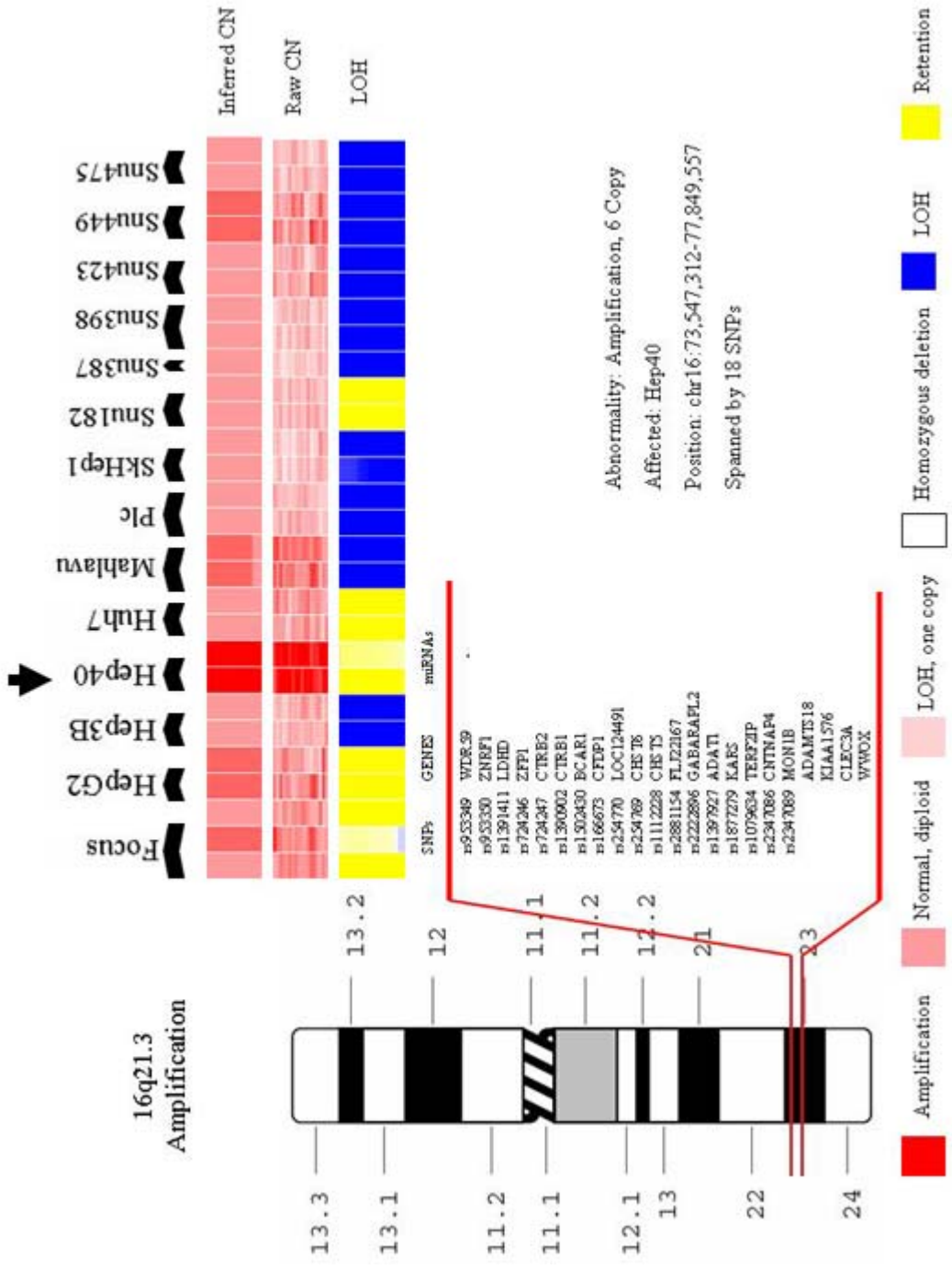


Figure 4.2.8: Amplification at 16q21.3 in Hep40.

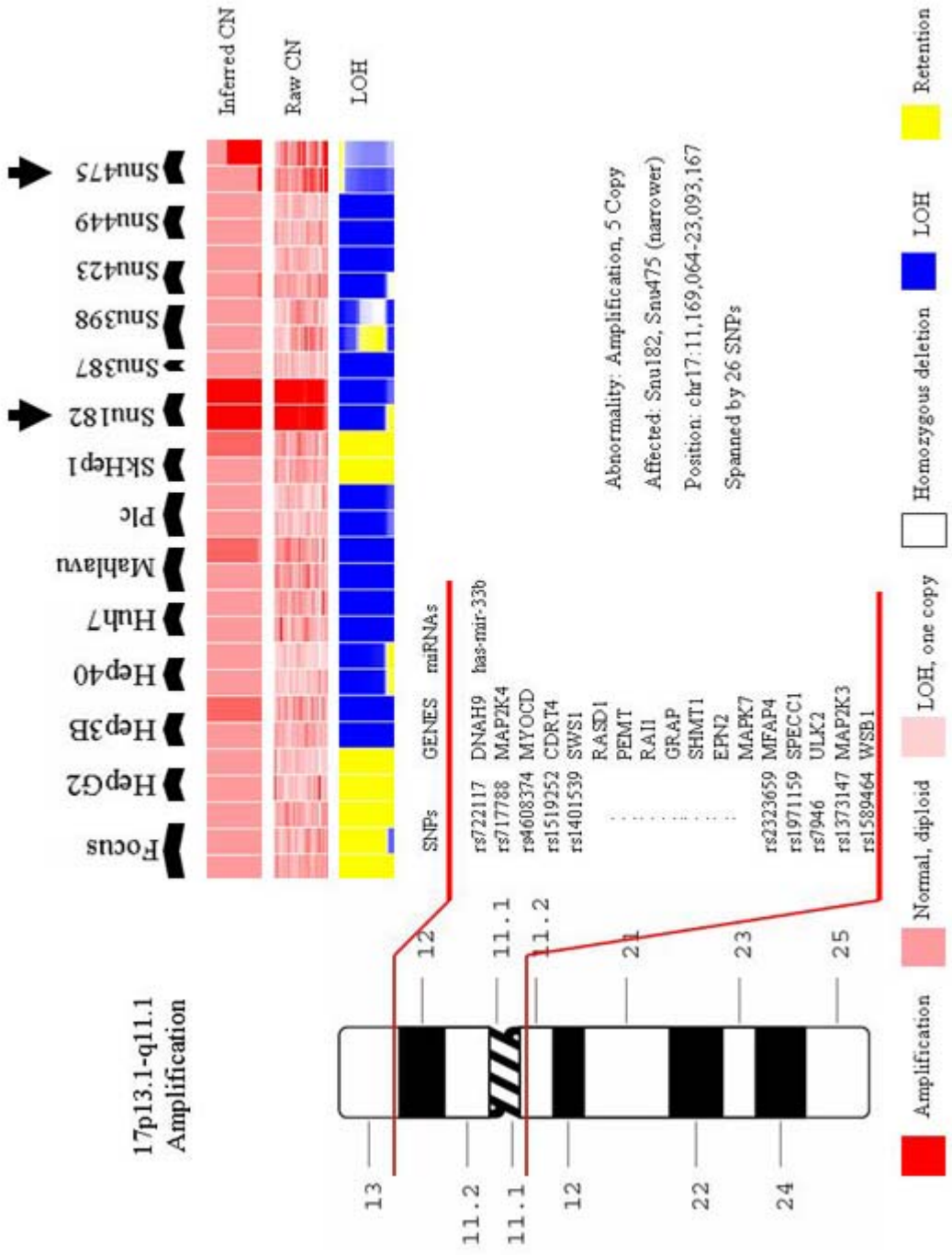


Figure 4.2.9: Amplification at 17p13.1-q11.1 in Snu182 and Snu475.

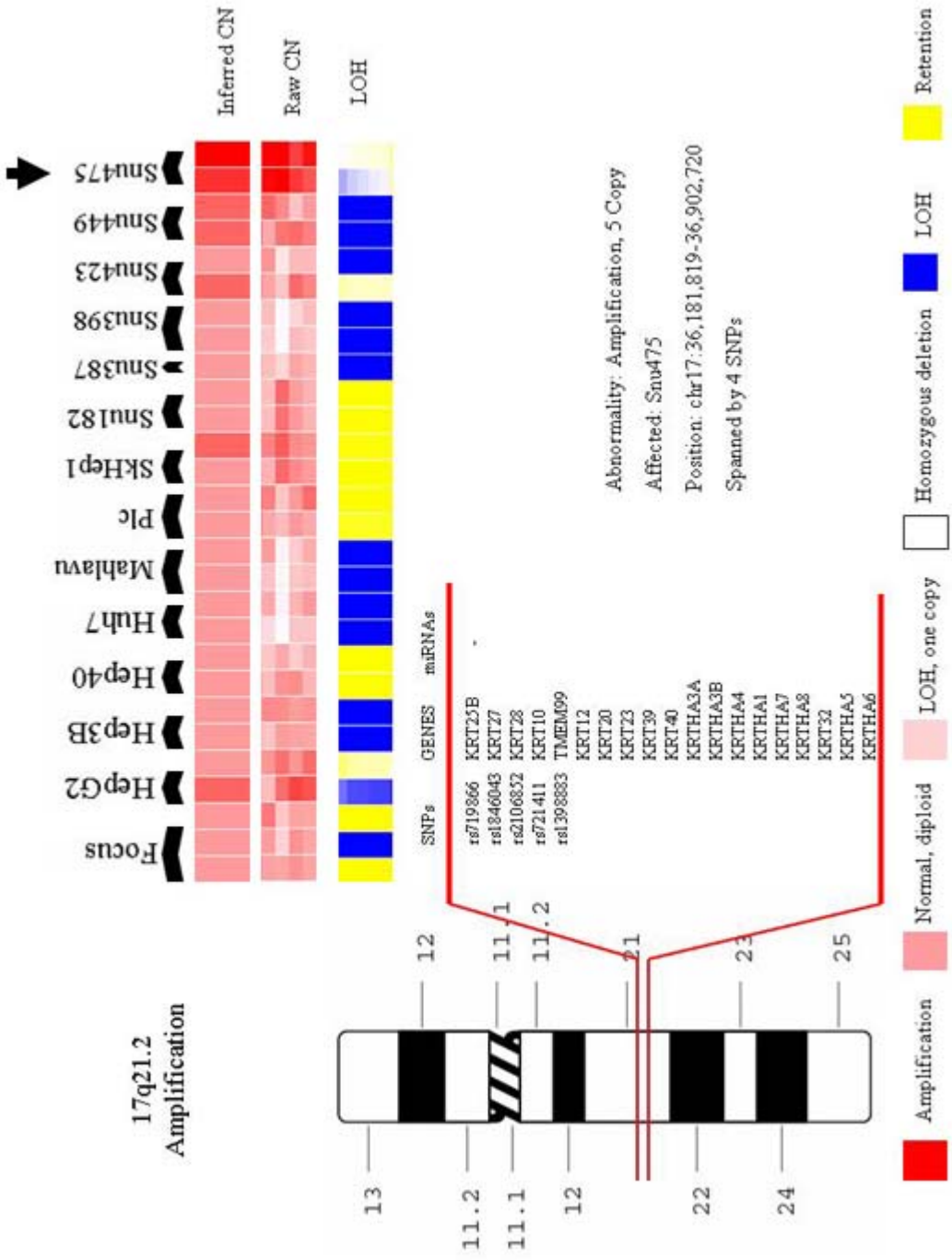


Figure 4.2.10: Amplification at 17q21.2 in Snu475.

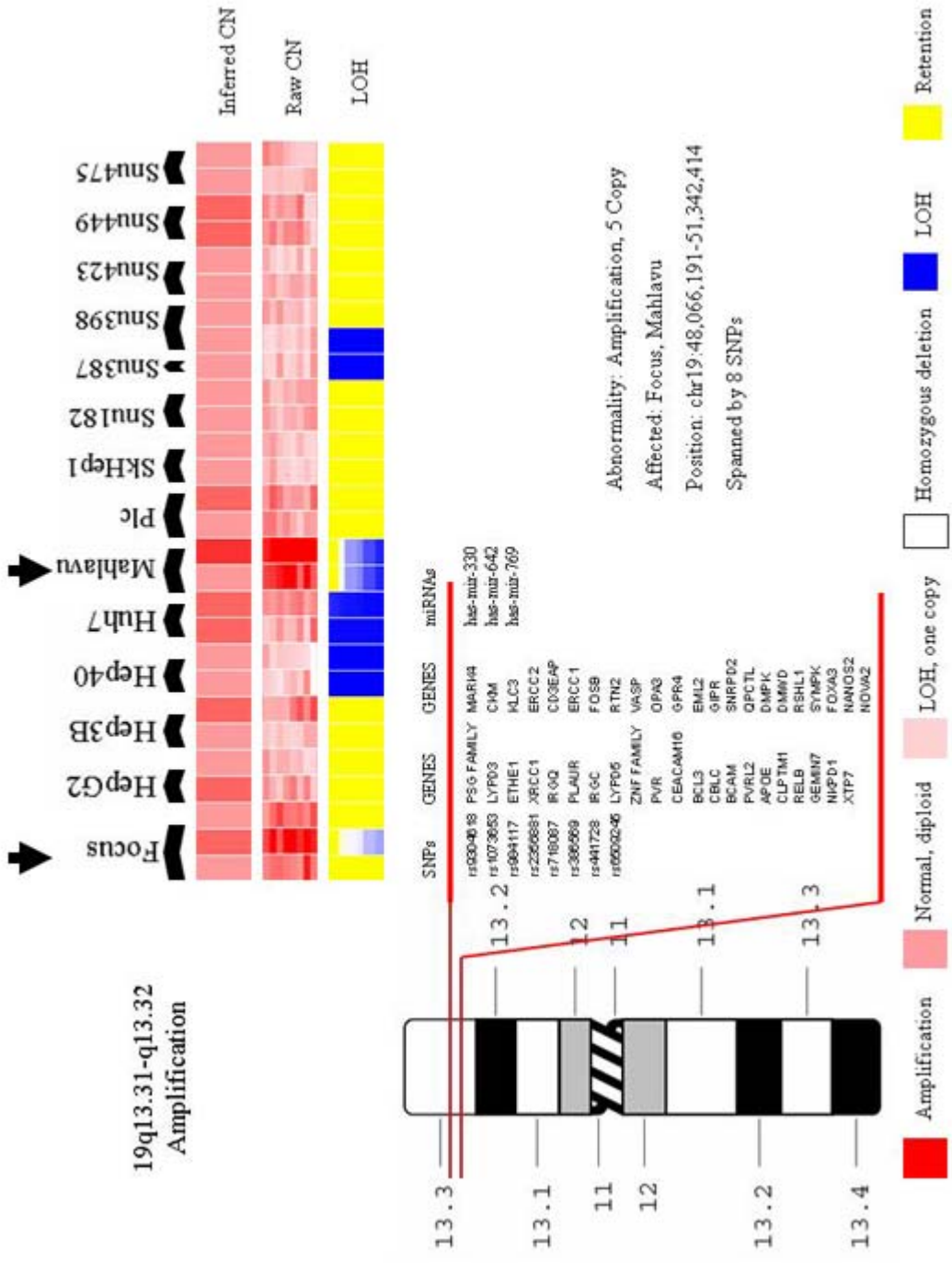


Figure 4.2.11: Amplification at 19q13.31-q13.32 in Focus and Mahlavu.

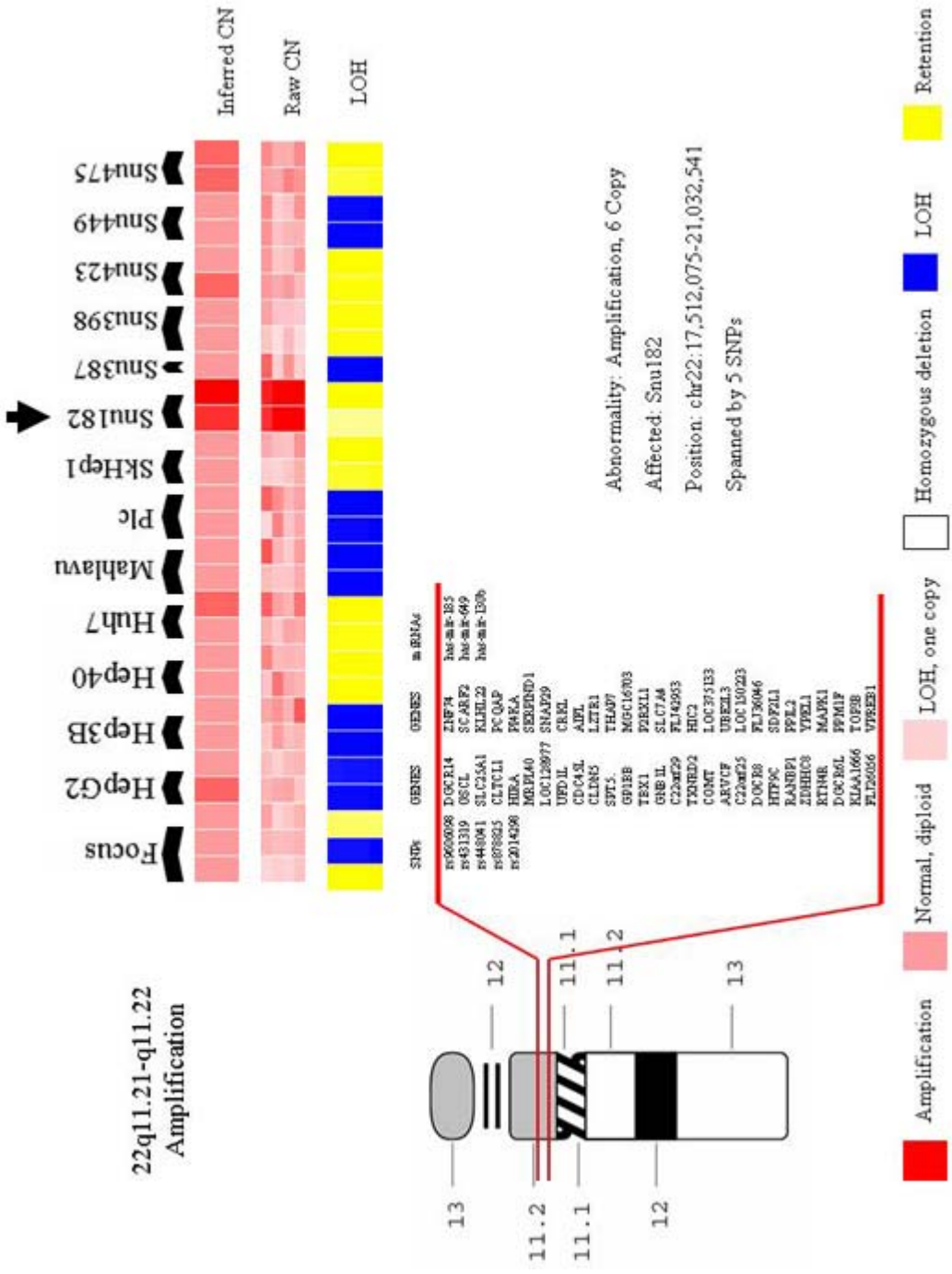


Figure 4.2.12: Amplification at 22q11.21-q11.22 in Snu182.

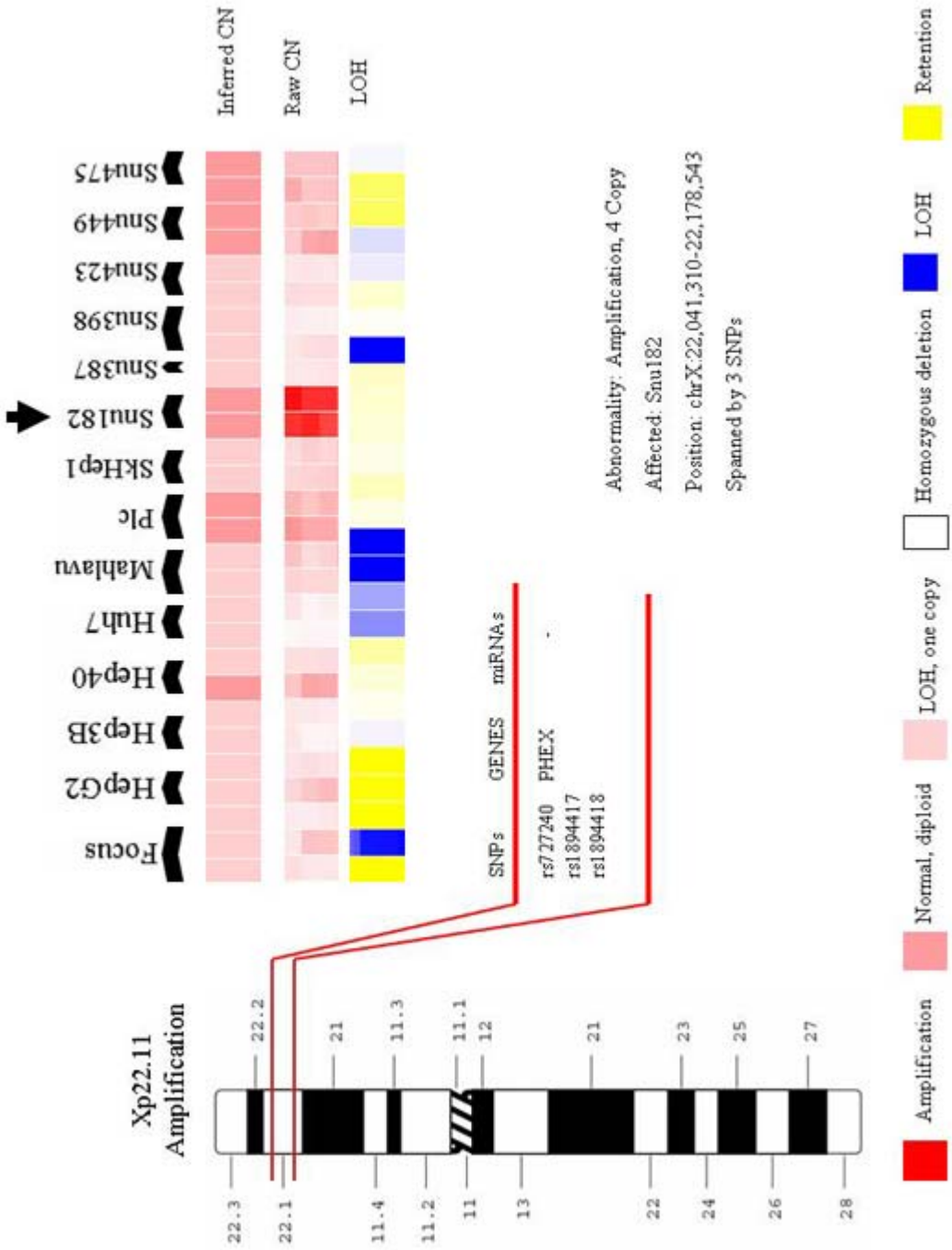


Figure 4.2.13: Amplification at Xp22.11 in Snu182.

Region	Aberration	Cell Line	Gene	Description	Location	Type
8p23.1	Amplification	Hep40	C8ORF74	chromosome 8 open reading frame 74	Unknown	other
"	"	"	CLDN23	claudin 23	Plasma Membrane	other
"	"	"	MFHAS1	malignant fibrous histiocytoma amplified sequence 1	Cytoplasm	other
"	"	"	MSRA	methionine sulfoxide reductase A	Cytoplasm	enzyme
"	"	"	PPP1R3B	protein phosphatase 1, regulatory (inhibitor) subunit 3B	Unknown	other
"	"	"	RP1L1	retinitis pigmentosa 1-like 1	Unknown	other
"	"	"	SOX7	SRY (sex determining region Y)-box 7	Nucleus	transcription regulator
"	"	"	THEX1	three prime histone mRNA exonuclease 1	Unknown	enzyme
"	"	"	TNKS	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase	Nucleus	enzyme
"	"	"	UNQ9391	tryptophan/serine protease	Unknown	other
"	"	"	TNKS2	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2	Nucleus	enzyme
"	"	"	RP1L1 PREDICTED	retinitis pigmentosa 1-like 1 (predicted)	Unknown	other
8q13.3-q21.11	Amplification	Hep40	EYA1	eyes absent homolog 1 (Drosophila)	Nucleus	phosphatase
"	"	"	GDAP1	ganglioside-induced differentiation-associated protein 1	Cytoplasm	other
"	"	"	JPH1	junctophilin 1	Plasma Membrane	other
"	"	"	KCNB2	potassium voltage-gated channel, Shab-related subfamily, member 2	Plasma Membrane	ion channel
"	"	"	LY96	lymphocyte antigen 96	Plasma Membrane	other
"	"	"	MSC	musculin (activated B-cell factor-1)	Cytoplasm	transcription regulator
"	"	"	PI15	peptidase inhibitor 15	Unknown	other
"	"	"	RDH10	retinol dehydrogenase 10 (all-trans)	Unknown	enzyme
"	"	"	RPESP	RPE-spondin	Unknown	other
"	"	"	RPL7	ribosomal protein L7	Cytoplasm	transcription regulator
"	"	"	SLC25A37	solute carrier family 25, member 37	Cytoplasm	transporter
"	"	"	STAU2	staufen, RNA binding protein, homolog 2 (Drosophila)	Cytoplasm	other
"	"	"	TCEB1	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	Nucleus	transcription regulator
"	"	"	TERF1	telomeric repeat binding factor (NIMA-interacting) 1	Nucleus	other
"	"	"	TMEM70	transmembrane protein 70	Unknown	other
"	"	"	TRPA1	transient receptor potential cation channel, subfamily A, member 1	Plasma Membrane	transporter
"	"	"	UBE2W	ubiquitin-conjugating enzyme E2W (putative)	Unknown	enzyme
"	"	"	GDAP1L1	ganglioside-induced differentiation-associated protein 1-like 1	Unknown	other
"	"	"	SKP1A	S-phase kinase-associated protein 1A (p19A)	Nucleus	transcription regulator
"	"	"	TCEB1P	transcription elongation factor B (SIII), polypeptide 1 pseudogene	Unknown	other
"	"	"	RPL7A	ribosomal protein L7a	Nucleus	other
"	"	"	EYA1 PREDICTED	eyes absent 1 homolog (Drosophila) (predicted)	Unknown	phosphatase

Table 4.2.1: Amplifications at 8p23.1 and 8q13.3-q21.11 in Hep40.

Region	Aberration	Cell Line	Gene	Description	Location	Type
8q24.13	Amplification	Hep40	ANXA13	annexin A13	Plasma Membrane	other
"	"	"	ATAD2	ATPase family, AAA domain containing 2	Unknown	other
"	"	"	C8ORF32	chromosome 8 open reading frame 32	Unknown	other
"	"	"	C8ORF76	chromosome 8 open reading frame 76	Unknown	other
"	"	"	DERL1	Der1-like domain family, member 1	Cytoplasm	other
"	"	"	FAM83A	family with sequence similarity 83, member A	Unknown	other
"	"	"	FAM91A1	family with sequence similarity 91, member A1	Unknown	other
"	"	"	FBXO32	F-box protein 32	Cytoplasm	enzyme
"	"	"	KIAA0196	KIAA0196	Unknown	other
"	"	"	MTSS1	metastasis suppressor 1	Cytoplasm	other
"	"	"	NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	Cytoplasm	enzyme
"	"	"	NSMCE2	non-SMC element 2, MMS21 homolog (S. cerevisiae)	Unknown	other
"	"	"	RNF139	ring finger protein 139	Cytoplasm	enzyme
"	"	"	SQLE	squalene epoxidase	Cytoplasm	enzyme
"	"	"	TATDN1	TatD DNase domain containing 1	Unknown	other
"	"	"	TMEM65	transmembrane protein 65	Unknown	other
"	"	"	TRIB1	tribbles homolog 1 (Drosophila)	Unknown	kinase
"	"	"	TRMT12	tRNA methyltransferase 12 homolog (S. cerevisiae)	Unknown	other
"	"	"	WDR67	WD repeat domain 67	Unknown	other
"	"	"	ZHX1	zinc fingers and homeoboxes 1	Nucleus	transcription regulator
"	"	"	ZHX2	zinc fingers and homeoboxes 2	Nucleus	transcription regulator
"	"	"	ZNF572	zinc finger protein 572	Unknown	other
"	"	"	ATAD2B	ATPase family, AAA domain containing 2B	Unknown	other
			TATDN1 PREDICTED	TatD DNase domain containing 1 (predicted)	Unknown	other

Table 4.2.2: Amplification at 8q24.13 in Hep40.

Region	Aberration	Cell Line	Gene	Description	Location	Type
9p22.1-p21.2	Amplification	Snu398	ADAMTSL1	ADAMTS-like 1	Extracellular Space	other
"	"	"	ADFP	adipose differentiation-related protein	Plasma Membrane	other
"	"	"	ASAH3L	N-acylsphingosine amidohydrolase 3-like	Unknown	other
"	"	"	C9ORF138	chromosome 9 open reading frame 138	Unknown	other
"	"	"	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	Nucleus	transcription regulator
"	"	"	CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	Nucleus	transcription regulator
"	"	"	DENND4C	DENN/MADD domain containing 4C	Unknown	other
"	"	"	DMRTA1	DMRT-like family A1	Unknown	other
"	"	"	ELAVL2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	Cytoplasm	other
"	"	"	FAM29A	family with sequence similarity 29, member A	Unknown	other
"	"	"	IFNA1	interferon, alpha 1	Extracellular Space	cytokine
"	"	"	IFNA10	interferon alpha 10	Unknown	other
"	"	"	IFNA13	interferon, alpha 13	Extracellular Space	cytokine
"	"	"	IFNA14	interferon, alpha 14	Extracellular Space	cytokine
"	"	"	IFNA16	interferon, alpha 16	Extracellular Space	cytokine
"	"	"	IFNA17	interferon, alpha 17	Extracellular Space	cytokine
"	"	"	IFNA2	interferon, alpha 2	Extracellular Space	cytokine
"	"	"	IFNA21	interferon, alpha 21	Extracellular Space	cytokine
"	"	"	IFNA4	interferon, alpha 4	Extracellular Space	cytokine
"	"	"	IFNA5	interferon, alpha 5	Extracellular Space	cytokine
"	"	"	IFNA6	interferon, alpha 6	Extracellular Space	cytokine
"	"	"	IFNA7	interferon, alpha 7	Extracellular Space	cytokine
"	"	"	IFNA8	interferon, alpha 8	Extracellular Space	cytokine
"	"	"	IFNB1	interferon, beta 1, fibroblast	Extracellular Space	cytokine
"	"	"	IFNE1	interferon epsilon 1	Extracellular Space	cytokine
"	"	"	IFNW1	interferon, omega 1	Extracellular Space	cytokine
"	"	"	KIAA1797	KIAA1797	Unknown	other
"	"	"	KLHL9	kelch-like 9 (Drosophila)	Unknown	other
"	"	"	MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3	Nucleus	other
"	"	"	MTAP	methylthioadenosine phosphorylase	Nucleus	enzyme
"	"	"	PTPLAD2	protein tyrosine phosphatase-like A domain containing 2	Unknown	other
"	"	"	RPS6	ribosomal protein S6	Cytoplasm	other
"	"	"	RRAGA	Ras-related GTP binding A	Cytoplasm	enzyme
"	"	"	SLC24A2	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	Plasma Membrane	transporter
"	"	"	SLC24A4	solute carrier family 24 (sodium/potassium/calcium exchanger), member 4	Unknown	transporter
"	"	"	TUSC1	tumor suppressor candidate 1	Unknown	other
"	"	"	MAP2	microtubule-associated protein 2	Cytoplasm	other
"	"	"	MAP4	microtubule-associated protein 4	Cytoplasm	other
"	"	"	MAP6	microtubule-associated protein 6	Cytoplasm	other
"	"	"	MAP7	microtubule-associated protein 7	Cytoplasm	other
"	"	"	MAP9	microtubule-associated protein 9	Unknown	other
"	"	"	IFI44	interferon-induced protein 44	Cytoplasm	other
"	"	"	MAP1A	microtubule-associated protein 1A	Cytoplasm	other
"	"	"	MAP1B	microtubule-associated protein 1B	Cytoplasm	other
"	"	"	MAP1S	microtubule-associated protein 1S	Cytoplasm	enzyme
"	"	"	MTAP2K	microtubule-associated protein 2 kinase	Unknown	other
"	"	"	IFNA13 PREDICTED	interferon alpha family, gene 13 (predicted)	Unknown	other
"	"	"	IFNA5 PREDICTED	interferon alpha family, gene 5 (predicted)	Unknown	other
"	"	"	MTAP PREDICTED	methylthioadenosine phosphorylase (predicted)	Unknown	enzyme

Table 4.2.3: Amplification at 9p22.1-p21.2 in Snu398.

Region	Aberration	Cell Line	Gene	Description	Location	Type
12p11.21-p11.1	Amplification	Snu475	BICD1	bicaudal D homolog 1 (Drosophila)	Cytoplasm	other
"	"	"	C12ORF35	chromosome 12 open reading frame 35	Unknown	other
"	"	"	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)	Nucleus	enzyme
"	"	"	DNM1L	dynamin 1-like	Cytoplasm	enzyme
"	"	"	FAM60A	family with sequence similarity 60, member A	Unknown	other
"	"	"	FGD4	FYVE, RhoGEF and PH domain containing 4	Cytoplasm	other
"	"	"	MGC24039	hypothetical protein MGC24039	Unknown	other
"	"	"	MGC50559	hypothetical protein MGC50559	Unknown	other
"	"	"	OWOS2	ovostatin 2	Unknown	other
"	"	"	PKP2	plakophilin 2	Plasma Membrane	other
"	"	"	PKP2L	plakophilin 2-like	Unknown	other
"	"	"	SYT10	synaptotagmin X	Cytoplasm	transporter
"	"	"	TSPAN11	tetraspanin 11	Unknown	other
"	"	"	YARS2	tyrosyl-tRNA synthetase 2, mitochondrial	Cytoplasm	enzyme
"	"	"	DDX11P	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 like 1	Unknown	other
14q12-q13.1	Amplification	Huh7	AP4S1		Cytoplasm	transporter
"	"	"	ARHGAP5		Cytoplasm	enzyme
"	"	"	C14orf126		Unknown	other
"	"	"	COCH		Extracellular Space	other
"	"	"	HEATR5A		Unkown	other
"	"	"	HECTD1		Unkown	enzyme
"	"	"	KIAA1333		Unkown	other
"	"	"	NUBPL		Unkown	other
"	"	"	PRKD1		Cytoplasm	kinase
"	"	"	SCFD1		Unkown	transporter
"	"	"	STRN3		Nucleus	other
15q21.3	Amplification	Hep40	UNC13C		cytoplasm	other
"	"	"	WDR72		unkown	other

Table 4.2.4: Amplifications at 12p11.21-p11 in Snu475, 14q12-q13.1 in Huh7, 15q21.3 in Hep40

Region	Aberration	Cell Line	Gene	Description	Location	Type
16q21.3	Amplification	Hep40	ADAMTS18	ADAM metallopeptidase with thrombospondin type 1 motif, 18	Extracellular Space	peptidase
"	"	"	ADAT1	adenosine deaminase, tRNA-specific 1	Unknown	enzyme
"	"	"	BCAR1	breast cancer anti-estrogen resistance 1	Plasma Membrane	other
"	"	"	CFDP1	craniofacial development protein 1	Extracellular Space	other
"	"	"	CHST5	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 5	Extracellular Space	enzyme
"	"	"	CHST6	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6	Cytoplasm	enzyme
"	"	"	CLEC3A	C-type lectin domain family 3, member A	Unknown	other
"	"	"	CNTNAP4	contactin associated protein-like 4	Plasma Membrane	other
"	"	"	CTRB1	chymotrypsinogen B1	Extracellular Space	peptidase
"	"	"	CTRB2	chymotrypsinogen B2	Unknown	peptidase
"	"	"	FA2H	fatty acid 2-hydroxylase	Unknown	enzyme
"	"	"	GABARAPL2	GABA(A) receptor-associated protein-like 2	Cytoplasm	transporter
"	"	"	KARS	lysyl-tRNA synthetase	Cytoplasm	enzyme
"	"	"	KIAA1576	KIAA1576 protein	Unknown	enzyme
"	"	"	LDHD	lactate dehydrogenase D	Unknown	enzyme
"	"	"	MON1B	MON1 homolog B (yeast)	Unknown	other
"	"	"	TERF2IP	telomeric repeat binding factor 2, interacting protein	Nucleus	other
"	"	"	Transferase		Unknown	group
"	"	"	WDR59	WD repeat domain 59	Unknown	transporter
"	"	"	WWOX	WW domain containing oxidoreductase	Cytoplasm	enzyme
"	"	"	ZFP1	zinc finger protein 1 homolog (mouse)	Nucleus	other
"	"	"	ZNRF1	zinc and ring finger 1	Cytoplasm	other
"	"	"	sulfotransferase		Unknown	group
"	"	"	ADAT1 PREDICTED	adenosine deaminase, tRNA-specific 1 (predicted)	Unknown	enzyme

Table 4.2.5: Amplification at 16q21.3 in Hep40.

Region	Aberration	Cell Line	Gene	Description	Location	Type
17p13.1-q11.1	Amplification	Snu182, Snu475	ADORA2B	adenosine A2b receptor	Plasma Membrane	G-protein coupled receptor
"	"	"	AKAP10	A kinase (PRKA) anchor protein 10	Cytoplasm	other
"	"	"	ALDH3A1	aldehyde dehydrogenase 3 family, memberA1	Cytoplasm	enzyme
"	"	"	ALDH3A2	aldehyde dehydrogenase 3 family, member A2	Cytoplasm	enzyme
"	"	"	ALKBH5	alkB, alkylation repair homolog 5 (E. coli)	Unknown	other
"	"	"	ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2	Cytoplasm	other
"	"	"	C17ORF39	chromosome 17 open reading frame 39	Unknown	other
"	"	"	C17ORF45	chromosome 17 open reading frame 45	Unknown	other
"	"	"	C17ORF76	chromosome 17 open reading frame 76	Unknown	other
"	"	"	C20ORF191	chromosome 20 open reading frame 191	Nucleus	transcription regulator
"	"	"	CDRT1	CMT1A duplicated region transcript 1	Unknown	other
"	"	"	CDRT15	CMT1A duplicated region transcript 15	Unknown	other
"	"	"	CDRT4	CMT1A duplicated region transcript 4	Unknown	other
"	"	"	COPS3	COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis)	Cytoplasm	other
"	"	"	COX10	COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast)	Cytoplasm	enzyme
"	"	"	DENR	density-regulated protein	Unknown	other
"	"	"	DHRS7B	dehydrogenase/reductase (SDR family) member 7B	Unknown	other
"	"	"	DNAH9	dynein, axonemal, heavy chain 9	Cytoplasm	other
"	"	"	DRG2	developmentally regulated GTP binding protein 2	Cytoplasm	other
"	"	"	EFHD2	EF-hand domain family, member D2	Unknown	other
"	"	"	ELAC2	elaC homolog 2 (E. coli)	Nucleus	enzyme
"	"	"	EPN2	epsin 2	Cytoplasm	other
"	"	"	FAM18B	family with sequence similarity 18, member B	Unknown	other
"	"	"	FAM18B2	family with sequence similarity 18, member B2	Unknown	other
"	"	"	FAM27L	family with sequence similarity 27-like	Unknown	other
"	"	"	FAM83G	family with sequence similarity 83, member G	Unknown	other
"	"	"	FBXW10	F-box and WD repeat domain containing 10	Unknown	other
"	"	"	FLCN	folliculin	Unknown	other
"	"	"	FLII	flightless I homolog (Drosophila)	Nucleus	other
"	"	"	FLJ10847	hypothetical protein FLJ10847	Unknown	other
"	"	"	FLJ36492	hypothetical protein FLJ36492	Unknown	other
"	"	"	FLJ40244	hypothetical protein FLJ40244	Unknown	other
"	"	"	FLJ45455	FLJ45455 protein	Unknown	other
"	"	"	FLJ45831	FLJ45831 protein	Unknown	other
"	"	"	GRAP	GRB2-related adaptor protein	Cytoplasm	other
"	"	"	HS3ST3A1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1	Cytoplasm	enzyme
"	"	"	HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	Cytoplasm	enzyme
"	"	"	KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	Plasma Membrane	ion channel
"	"	"	KERATINOCYTE SPECIFIC RECEPTOR 1		Cytoplasm	other
"	"	"	KIAA0565	KIAA0565 gene product	Unknown	other
"	"	"	KSR1	kinase suppressor of ras 1	Cytoplasm	kinase
"	"	"	LGALS9	lectin, galactoside-binding, soluble, 9 (galectin 9)	Extracellular Space	other
"	"	"	LLGL1	lethal giant larvae homolog 1 (Drosophila)	Cytoplasm	other
"	"	"	LOC201164	similar to CG12214 gene product	Unknown	other
"	"	"	LRRC48	leucine rich repeat containing 48	Unknown	other
"	"	"	M-RIP	myosin phosphatase-Rho interacting protein	Cytoplasm	other
"	"	"	MAP2K3	mitogen-activated protein kinase kinase 3	Cytoplasm	kinase
"	"	"	MAP2K4	mitogen-activated protein kinase kinase 4	Cytoplasm	kinase
"	"	"	MAPK7	mitogen-activated protein kinase 7	Cytoplasm	kinase
"	"	"	MED9	mediator of RNA polymerase II transcription, subunit 9 homolog (S. cerevisiae)	Nucleus	other
"	"	"	MFAP4	microfibrillar-associated protein 4	Extracellular Space	other
"	"	"	MGC33894	transcript expressed during hematopoiesis 2	Unknown	other

Table 4.2.6: Amplification at 17p13.1-q11.1 in Snu182 and Snu475.

Region	Aberration	Cell Line	Gene	Description	Location	Type
17p13.1-q11	Amplification	Snu182, Snu475	MGC51025	hypothetical protein MGC51025	Unknown	other
"	"	"	MGC87631	similar to hypothetical protein FLJ36492	Unknown	other
"	"	"	MUC1	mucin 1, cell surface associated	Plasma Membrane	other
"	"	"	MYO15A (includes EG:445447)	myosin XVA	Extracellular Space	other
"	"	"	MYO15A (includes EG:51168)	myosin XVA	Cytoplasm	other
"	"	"	MYOCD	myocardin	Nucleus	transcription regulator
"	"	"	NCOR1	nuclear receptor co-repressor 1	Nucleus	transcription regulator
"	"	"	NT5M	5',3'-nucleotidase, mitochondrial	Cytoplasm	phosphatase
"	"	"	PEMT	phosphatidylethanolamine N-methyltransferase	Cytoplasm	enzyme
"	"	"	PIGL	phosphatidylinositol glycan anchor biosynthesis, class L	Cytoplasm	enzyme
"	"	"	PMP22	peripheral myelin protein 22	Plasma Membrane	other
"	"	"	PRPSAP2	phosphoribosyl pyrophosphate synthetase-associated protein 2	Unknown	other
"	"	"	PRR6	proline rich 6	Nucleus	other
"	"	"	PXMP2	peroxisomal membrane protein 2, 22kDa	Cytoplasm	other
"	"	"	Phosphatidylethanolamine N-methyltransferase		Unknown	group
"	"	"	RAI1	retinoic acid induced 1	Cytoplasm	other
"	"	"	RASD1	RAS, dexamethasone-induced 1	Cytoplasm	enzyme
"	"	"	SHMT1	serine hydroxymethyltransferase 1 (soluble)	Cytoplasm	enzyme
"	"	"	SLC5A10	solute carrier family 5 (sodium/glucose cotransporter), member 10	Unknown	transporter
"	"	"	SLC5A11	solute carrier family 5 (sodium/glucose cotransporter), member 11	Unknown	transporter
"	"	"	SMCR7	Smith-Magenis syndrome chromosome region, candidate 7	Unknown	other
"	"	"	SMCR8	Smith-Magenis syndrome chromosome region, candidate 8	Unknown	other
"	"	"	SPECC1	sperm antigen with calponin homology and coiled-coil domains 1	Nucleus	other
"	"	"	SREBF1	sterol regulatory element binding transcription factor 1	Nucleus	transcription regulator
"	"	"	TEKT3	tektin 3	Unknown	other
"	"	"	TMEM11	transmembrane protein 11	Plasma Membrane	G-protein coupled receptor
"	"	"	TNFRSF13B	tumor necrosis factor receptor superfamily, member 13B	Plasma Membrane	transmembrane receptor
"	"	"	TOM1L2	target of myb1-like 2 (chicken)	Unknown	transporter
"	"	"	TOP3A	topoisomerase (DNA) III alpha	Nucleus	enzyme
"	"	"	TRIM16	tripartite motif-containing 16	Nucleus	transcription regulator
"	"	"	TRIM16L	tripartite motif-containing 16-like	Unknown	other
"	"	"	TRPV2	transient receptor potential cation channel, subfamily V, member 2	Plasma Membrane	ion channel
"	"	"	TTC19	tetratricopeptide repeat domain 19	Unknown	other
"	"	"	UBB	ubiquitin B	Cytoplasm	other
"	"	"	ULK2	unc-51-like kinase 2 (C. elegans)	Cytoplasm	kinase
"	"	"	USP22	ubiquitin specific peptidase 22	Unknown	peptidase
"	"	"	WSB1	WD repeat and SOCS box-containing 1	Unknown	other
"	"	"	ZNF179	zinc finger protein 179	Nucleus	transcription regulator
"	"	"	ZNF18	zinc finger protein 18	Nucleus	transcription regulator
"	"	"	ZNF286A	zinc finger protein 286A	Nucleus	other
"	"	"	ZNF287	zinc finger protein 287	Nucleus	transcription regulator
"	"	"	ZNF624	zinc finger protein 624	Nucleus	other
"	"	"	ZSWIM7	zinc finger, SWIM-type containing 7	Unknown	other
"	"	"	SPECC1L	SPECC1-like	Unknown	other
"	"	"	ZNF286B	zinc finger protein 286B	Unknown	other
"	"	"	ZNF286L	zinc finger protein 286-like	Unknown	other
"	"	"	SMCR7L	Smith-Magenis syndrome chromosome region, candidate 7-like	Unknown	other
"	"	"	USP27X	ubiquitin specific peptidase 27, X-linked	Unknown	peptidase
"	"	"	GRAP2	GRB2-related adaptor protein 2	Cytoplasm	other
"	"	"	UBB1	ubiquitin 1	Unknown	other
"	"	"	UBB3	ubiquitin 3	Unknown	other
"	"	"	COX10 PREDICTED	COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast) (predicted)	Unknown	other
"	"	"	HSD17B1	hydroxysteroid (17-beta) dehydrogenase 1	Cytoplasm	enzyme
"	"	"	DRG2		Unknown	other

Table 4.2.7: Amplification at 17p13.1-q11.1 in Snu182 and Snu475 (cont.).

Region	Aberration	Cell Line	Gene	Description	Location	Type
17q21.2	Amplification	Snu475	KRT10	keratin 10 (epidermolytic hyperkeratosis; keratosis palmaris et plantaris)	Cytoplasm	other
"	"	"	KRT12	keratin 12 (Meesmann corneal dystrophy)	Cytoplasm	other
"	"	"	KRT20	keratin 20	Cytoplasm	other
"	"	"	KRT23	keratin 23 (histone deacetylase inducible)	Unknown	other
"	"	"	KRT26	keratin 26	Unknown	other
"	"	"	KRT27	keratin 27	Unknown	other
"	"	"	KRT28	keratin 28	Unknown	other
"	"	"	KRT31	keratin 31	Cytoplasm	other
"	"	"	KRT32	keratin 32	Cytoplasm	other
"	"	"	KRT33A	keratin 33A	Cytoplasm	other
"	"	"	KRT33B	keratin 33B	Cytoplasm	other
"	"	"	KRT34	keratin 34	Cytoplasm	other
"	"	"	KRT35	keratin 35	Cytoplasm	other
"	"	"	KRT36	keratin 36	Cytoplasm	other
"	"	"	KRT37	keratin 37	Cytoplasm	other
"	"	"	KRT38	keratin 38	Cytoplasm	other
"	"	"	KRT39	keratin 39	Unknown	other
"	"	"	KRT40	keratin 40	Unknown	other
"	"	"	KRTAP1-1	keratin associated protein 1-1	Unknown	other
"	"	"	KRTAP1-3	keratin associated protein 1-3	Unknown	other
"	"	"	KRTAP1-5	keratin associated protein 1-5	Unknown	other
"	"	"	KRTAP17-1	keratin associated protein 17-1	Unknown	other
"	"	"	KRTAP2-4	keratin associated protein 2-4	Unknown	other
"	"	"	KRTAP3-1	keratin associated protein 3-1	Unknown	other
"	"	"	KRTAP3-2	keratin associated protein 3-2	Unknown	other
"	"	"	KRTAP3-3	keratin associated protein 3-3	Unknown	other
"	"	"	KRTAP4-10	keratin associated protein 4-10	Unknown	other
"	"	"	KRTAP4-12	keratin associated protein 4-12	Unknown	other
"	"	"	KRTAP4-14	keratin associated protein 4-14	Unknown	other
"	"	"	KRTAP4-2	keratin associated protein 4-2	Unknown	other
"	"	"	KRTAP4-3	keratin associated protein 4-3	Unknown	other
"	"	"	KRTAP4-4	keratin associated protein 4-4	Unknown	other
"	"	"	KRTAP4-5	keratin associated protein 4-5	Unknown	other
"	"	"	KRTAP9-2	keratin associated protein 9-2	Unknown	other
"	"	"	KRTAP9-3	keratin associated protein 9-3	Unknown	other
"	"	"	KRTAP9-4	keratin associated protein 9-4	Unknown	other
"	"	"	KRTAP9-8	keratin associated protein 9-8	Unknown	other
"	"	"	TMEM99	transmembrane protein 99	Unknown	other
"	"	"	KRT10L_MAPPED	K51 keratin-like locus (mapped)	Unknown	other
"	"	"	KRTAP3-1_PREDICTED	keratin associated protein 3-1 (predicted)	Unknown	other

Table 4.2.8: Amplification at 17q21.2 in Snu475.

Region	Aberration	Cell Line	Gene	Description	Location	Type
19q13.31-q13.32	Amplification	Focus, Mahlavu	APOA4	apolipoprotein A-IV	Extracellular Space	
"	"	"	APOC1	apolipoprotein C-I	Extracellular Space	transporter
"	"	"	APOC2	apolipoprotein C-II	Extracellular Space	transporter
"	"	"	APOC4	apolipoprotein C-IV	Extracellular Space	transporter
"	"	"	APOE	apolipoprotein E	Extracellular Space	transporter
"	"	"	BCAM	basal cell adhesion molecule (Lutheran blood group)	Plasma Membrane	transporter
"	"	"	BCAT2	branched chain aminotransferase 2, mitochondrial	Cytoplasm	transmembrane receptor
"	"	"	BCL3	B-cell CLL/lymphoma 3	Nucleus	enzyme
"	"	"	BLOC1S3	biogenesis of lysosome-related organelles complex-1, subunit 3	Cytoplasm	transcription regulator
"	"	"	C19ORF61	chromosome 19 open reading frame 61	Unknown	other
"	"	"	CADM4	cell adhesion molecule 4	Plasma Membrane	other
"	"	"	CBLC	Cas-Br-M (murine) ecotropic retroviral transforming sequence c	Nucleus	other
"	"	"	CD177	CD177 molecule	Cytoplasm	enzyme
"	"	"	CD3EAP	CD3e molecule, epsilon associated protein	Nucleus	other
"	"	"	CEACAM16	carcinoembryonic antigen-related cell adhesion molecule 16	Unknown	other
"	"	"	CEACAM19	carcinoembryonic antigen-related cell adhesion molecule 19	Unknown	transcription regulator
"	"	"	CEACAM20	carcinoembryonic antigen-related cell adhesion molecule 20	Unknown	other
"	"	"	CKM	creatine kinase, muscle	Cytoplasm	other
"	"	"	CLPTM1	cleft lip and palate associated transmembrane protein 1	Plasma Membrane	kinase
"	"	"	DMPK	dystrophia myotonica-protein kinase	Cytoplasm	other
"	"	"	DMWD	dystrophia myotonica, WD repeat containing	Nucleus	kinase
"	"	"	EGR1	early growth response 1	Nucleus	other
"	"	"	EML2	echinoderm microtubule associated protein like 2	Cytoplasm	transcription regulator
"	"	"	ERCC1	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	Nucleus	other
"	"	"	ERCC2	excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)	Nucleus	enzyme
"	"	"	ETHE1	ethylmalonic encephalopathy 1	Cytoplasm	enzyme
"	"	"	EXOC3L2	exocyst complex component 3-like 2	Unknown	other
"	"	"	FBXO46	F-box protein 46	Unknown	other
"	"	"	FLJ40125	hypothetical protein FLJ40125	Unknown	other
"	"	"	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	Nucleus	other
"	"	"	FOXA3	forkhead box A3	Nucleus	transcription regulator
"	"	"	GEMIN7	gem (nuclear organelle) associated protein 7	Nucleus	transcription regulator
"	"	"	GIPR	gastric inhibitory polypeptide receptor	Plasma Membrane	other
"	"	"	GPR4	G protein-coupled receptor 4	Plasma Membrane	G-protein coupled receptor
"	"	"	IGFL3	IGF-like family member 3	Extracellular Space	G-protein coupled receptor
"	"	"	IGFL4	IGF-like family member 4	Extracellular Space	other
"	"	"	IRF2BP1	interferon regulatory factor 2 binding protein 1	Nucleus	other
"	"	"	IRGC	immunity-related GTPase family, cinema	Unknown	transcription regulator
"	"	"	IRGQ	immunity-related GTPase family, Q	Unknown	other
"	"	"	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	Plasma Membrane	other
"	"	"	KLC3	kinesin light chain 3	Unknown	ion channel
"	"	"	LOC339344	hypothetical protein LOC339344	Nucleus	enzyme
"	"	"	LYPD3	LY6/PLAUR domain containing 3	Plasma Membrane	transcription regulator
"	"	"	LYPD5	LY6/PLAUR domain containing 5	Unknown	other
"	"	"	MARK4	MAP/microtubule affinity-regulating kinase 4	Cytoplasm	other
"	"	"	NANOS2	nanos homolog 2 (Drosophila)	Unknown	kinase
"	"	"	NKPD1	NTPase, KAP family P-loop domain containing 1	Unknown	other
"	"	"	NOVA2	neuro-oncological ventral antigen 2	Nucleus	other
"	"	"	NR2C1	nuclear receptor subfamily 2, group C, member 1	Nucleus	other
"	"	"	OPA3	optic atrophy 3 (autosomal recessive, with chorea and spastic paraplegia)	Unknown	transcription regulator
"	"	"	PGLYRP1	peptidoglycan recognition protein 1	Plasma Membrane	other
"	"	"	PHLDB3	pleckstrin homology-like domain, family B, member 3	Unknown	transmembrane receptor
"	"	"	PLAUR	plasminogen activator, urokinase receptor	Plasma Membrane	other
"	"	"	PPP1R13L	protein phosphatase 1, regulatory (inhibitor) subunit 13 like	Nucleus	transmembrane receptor
"	"	"	PSG1	pregnancy specific beta-1-glycoprotein 1	Extracellular Space	transcription regulator
"	"	"	PSG11	pregnancy specific beta-1-glycoprotein 11	Extracellular Space	other
"	"	"	PSG2	pregnancy specific beta-1-glycoprotein 2	Extracellular Space	other

Table 4.2.9: Amplification at 19q13.31-q13.32 in Focus and Mahlavu.

Region	Aberration	Cell Line	Gene	Description	Location	Type
19q13.31-q13.32	Amplification	Focus, Mahlavu	PSG4	pregnancy specific beta-1-glycoprotein 4	Extracellular Space	other
"	"	"	PSG5	pregnancy specific beta-1-glycoprotein 5	Extracellular Space	other
"	"	"	PSG6	pregnancy specific beta-1-glycoprotein 6	Extracellular Space	other
"	"	"	PSG7	pregnancy specific beta-1-glycoprotein 7	Extracellular Space	other
"	"	"	PSG8	pregnancy specific beta-1-glycoprotein 8	Extracellular Space	other
"	"	"	PSG9	pregnancy specific beta-1-glycoprotein 9	Extracellular Space	other
"	"	"	PVR	poliovirus receptor	Plasma Membrane	other
"	"	"	PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)	Plasma Membrane	G-protein coupled receptor
"	"	"	QPCTL	glutaminyl-peptide cyclotransferase-like	Unknown	other
"	"	"	RELB	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)	Nucleus	other
"	"	"	RNF141	ring finger protein 141	Unknown	transcription regulator
"	"	"	RSHL1	radial spokehead-like 1	Unknown	other
"	"	"	RTN2	reticulon 2	Cytoplasm	other
"	"	"	SFRS16	splicing factor, arginine/serine-rich 16	Nucleus	other
"	"	"	SIX5	sine oculis homeobox homolog 5 (Drosophila)	Nucleus	other
"	"	"	SNRPD2	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	Nucleus	transcription regulator
"	"	"	SYMPK	symplekin	Cytoplasm	other
"	"	"	TEX101	testis expressed 101	Unknown	other
"	"	"	TOMM40	translocase of outer mitochondrial membrane 40 homolog (yeast)	Cytoplasm	other
"	"	"	TRAPPC6A	trafficking protein particle complex 6A	Cytoplasm	ion channel
"	"	"	VASP	vasodilator-stimulated phosphoprotein	Plasma Membrane	other
"	"	"	XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1	Nucleus	other
"	"	"	ZNF155	zinc finger protein 155	Nucleus	other
"	"	"	ZNF180	zinc finger protein 180	Nucleus	transcription regulator
"	"	"	ZNF221	zinc finger protein 221	Nucleus	transcription regulator
"	"	"	ZNF222	zinc finger protein 222	Nucleus	other
"	"	"	ZNF223	zinc finger protein 223	Nucleus	other
"	"	"	ZNF224	zinc finger protein 224	Nucleus	other
"	"	"	ZNF225	zinc finger protein 225	Nucleus	other
"	"	"	ZNF226	zinc finger protein 226	Nucleus	other
"	"	"	ZNF227	zinc finger protein 227	Nucleus	transcription regulator
"	"	"	ZNF228	zinc finger protein 228	Nucleus	transcription regulator
"	"	"	ZNF229	zinc finger protein 229	Nucleus	other
"	"	"	ZNF230	zinc finger protein 230	Nucleus	other
"	"	"	ZNF233	zinc finger protein 233	Unknown	other
"	"	"	ZNF234	zinc finger protein 234	Nucleus	other
"	"	"	ZNF235	zinc finger protein 235	Nucleus	other
"	"	"	ZNF283	zinc finger protein 283	Nucleus	other
"	"	"	ZNF284	zinc finger protein 284	Unknown	other
"	"	"	ZNF285A	zinc finger protein 285A	Nucleus	other
"	"	"	ZNF313	zinc finger protein 313	Unknown	other
"	"	"	ZNF342	zinc finger protein 342	Nucleus	other
"	"	"	ZNF404	zinc finger protein 404	Unknown	other
"	"	"	ZNF428	zinc finger protein 428	Unknown	other
"	"	"	ZNF45	zinc finger protein 45	Nucleus	other
"	"	"	ZNF575	zinc finger protein 575	Nucleus	transcription regulator
"	"	"	ZNF576	zinc finger protein 576	Nucleus	other
"	"	"	CLPTM1L	CLPTM1-like	Unknown	other
"	"	"	TOMM40L	translocase of outer mitochondrial membrane 40 homolog (yeast)-like	Unknown	other
"	"	"	CDC42BPG	CDC42 binding protein kinase gamma (DMPK-like)	Cytoplasm	other
"	"	"	ADCYAP1R1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	Plasma Membrane	kinase
"	"	"	ADCYAP1R2 MAPPED	adenylate cyclase activating polypeptide 1 receptor 2 (mapped)	Plasma Membrane	G-protein coupled receptor
"	"	"	ADCYAP1R3 MAPPED	adenylate cyclase activating polypeptide 1 receptor 3 (mapped)	Unknown	G-protein coupled receptor
"	"	"	VIPR1	vasoactive intestinal peptide receptor 1	Plasma Membrane	other
"	"	"	VIPR2	vasoactive intestinal peptide receptor 2	Plasma Membrane	G-protein coupled receptor
"	"	"	BLOC1S3 PREDICTED	biogenesis of lysosome-related organelles complex-1, subunit 3 (predicted)	Unknown	G-protein coupled receptor
"	"	"	MARK4 PREDICTED	MAP/microtubule affinity-regulating kinase 4 (predicted)	Unknown	other
"	"	"	NKPD1 PREDICTED	NTPase, KAP family P-loop domain containing 1 (predicted)	Unknown	kinase

Table 4.2.10: Amplification at 19q13.31-q13.32 in Focus and Mahlavu (cont.).

Region	Aberration	Cell Line	Gene	Description	Location	Type
22q11.21-q11.22	Amplification	Snu182	AIFM3	apoptosis-inducing factor, mitochondrion-associated, 3	Unknown	
"	"	"	ARVCF	armadillo repeat gene deletes in velocardiofacial syndrome	Plasma Membrane	enzyme
"	"	"	C22ORF25	chromosome 22 open reading frame 25	Unknown	other
"	"	"	C22ORF29	chromosome 22 open reading frame 29	Unknown	other
"	"	"	CALM3	calmodulin 3	Unknown	other
"	"	"	CCDC116	coiled-coil domain containing 116	Unknown	other
"	"	"	CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)	Nucleus	other
"	"	"	CLDN5	claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)	Plasma Membrane	other
"	"	"	CLTCL1	clathrin, heavy chain-like 1	Plasma Membrane	other
"	"	"	COMT	catechol-O-methyltransferase	Cytoplasm	other
"	"	"	CRKL	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	Cytoplasm	enzyme
"	"	"	DGCR14	DiGeorge syndrome critical region gene 14	Nucleus	kinase
"	"	"	DGCR6L	DiGeorge syndrome critical region gene 6-like	Nucleus	other
"	"	"	DGCR8	DiGeorge syndrome critical region gene 8	Unknown	other
"	"	"	FLJ26056	hypothetical protein LOC375127	Unknown	other
"	"	"	FLJ42953	FLJ42953 protein	Unknown	other
"	"	"	GNB1L	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	Unknown	other
"	"	"	GP1BB	glycoprotein Ib (platelet), beta polypeptide	Plasma Membrane	other
"	"	"	GSCL	goosecoid-like	Nucleus	G-protein coupled receptor
"	"	"	HIC2	hypermethylated in cancer 2	Nucleus	transcription regulator
"	"	"	HIRA	HIR histone cell cycle regulation defective homolog A (S. cerevisiae)	Nucleus	other
"	"	"	HTF9C	Hpall tiny fragments locus 9C	Unknown	transcription regulator
"	"	"	KIAA1666	KIAA1666 protein	Unknown	kinase
"	"	"	KLF8	Kruppel-like factor 8	Nucleus	other
"	"	"	KLHL22	kelch-like 22 (Drosophila)	Unknown	other
"	"	"	LITAF	lipopolysaccharide-induced TNF factor	Nucleus	other
"	"	"	LOC128977	hypothetical protein LOC128977	Unknown	transcription regulator
"	"	"	LOC150223	LOC150223 protein	Unknown	other
"	"	"	LOC375133	similar to phosphatidylinositol 4-kinase alpha	Unknown	other
"	"	"	LZTR1	leucine-zipper-like transcription regulator 1	Cytoplasm	other
"	"	"	MAPK1	mitogen-activated protein kinase 1	Cytoplasm	transcription regulator
"	"	"	MGC16703	alpha tubulin-like	Unknown	kinase
"	"	"	MRPL40	mitochondrial ribosomal protein L40	Cytoplasm	other
"	"	"	P2RXL1	purinergic receptor P2X-like 1, orphan receptor	Plasma Membrane	other
"	"	"	PCQAP	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein	Nucleus	ion channel
"	"	"	PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2	Nucleus	transcription regulator
"	"	"	PPM1F	protein phosphatase 1F (PP2C dcmain containing)	Cytoplasm	enzyme
"	"	"	RANBP1	RAN binding protein 1	Nucleus	phosphatase
"	"	"	RTN4R	reticulon 4 receptor	Plasma Membrane	other
"	"	"	SCARF2	scavenger receptor class F, member 2	Plasma Membrane	other
"	"	"	SDF2L1	stromal cell-derived factor 2-like 1	Cytoplasm	transmembrane receptor
"	"	"	SEPT5	septin 5	Cytoplasm	other
"	"	"	SERPIND1	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	Extracellular Space	enzyme
"	"	"	SLC25A1	solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1	Plasma Membrane	other
"	"	"	SLC7A4	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	Plasma Membrane	transporter
"	"	"	SNAP29	synaptosomal-associated protein, 29kDa	Cytoplasm	transporter
"	"	"	TBX1	T-box 1	Nucleus	transporter
"	"	"	THAP7	THAP domain containing 7	Nucleus	transcription regulator
"	"	"	TOP3B	topoisomerase (DNA) III beta	Nucleus	other
"	"	"	TXNRD2	thioredoxin reductase 2	Cytoplasm	enzyme
"	"	"	UBE2L3	ubiquitin-conjugating enzyme E2L 3	Cytoplasm	enzyme
"	"	"	UFD1L	ubiquitin fusion degradation 1 like (yeast)	Cytoplasm	enzyme
"	"	"	VPREB1	pre-B lymphocyte gene 1 (mapped)	Unknown	peptidase
"	"	"	YPEL1	yippee-like 1 (Drosophila)	Nucleus	other
"	"	"	ZDHHC8	zinc finger, DHHC-type containing 8	Cytoplasm	enzyme
"	"	"	ZNF74	zinc finger protein 74	Nucleus	other
"	"	"	ZFP520	zinc finger protein 520	Unknown	other
"	"	"	Catechol O-methyltransferase		Unknown	other
"	"	"	GNB1L PREDICTED	guanine nucleotide binding protein (G protein), beta polypeptide 1-like (predicted)	Unknown	group
"	"	"	CRK/CRKL		Unknown	other
Xp22.11	Amplification	Snu182	PHEX	X-linked phosphate regulating endopeptidase	Cytoplasm	peptidase

Table 4.2.11: Amplification at 22q11.21-q11.22 and Xp22.11 in Snu182.

5. DISCUSSION

In the framework of this study, we searched for DNA copy number changes in the genomic DNAs of 14 HCC cell lines. We used commercially available SNP microarrays consist of approximately 10 thousand SNP markers representing the whole genome with a mean physical inter-marker distance of 210KB and 0.32 cM of genetic distance. These SNP markers spanned all the autosomal chromosomes and the X-chromosome. We performed two biological replicates for each cell line except Focus (three) and Snu387 (one).

SNP markers, in principle, provide two types of information which can be classified as qualitative and quantitative. Qualitative information refers to genotyping of the DNA to be investigated. Each SNP marker is chosen from a pool of highly heterozygous SNPs (0.37 on average) representing Caucasian, Asian and Afro-American populations. High heterozygosity values of these bi-allelic markers enable genotyping of genomic DNA. Briefly, each allele of the SNP markers are spotted as different probesets on the array and genotyping is performed based on the hybridization efficiencies of each allele's probesets. Genotyping calls can be used in two ways; first, "no calls" which theoretically refer to non or mis-hybridization, may point homozygous deletions; second, homozygous calls of a number of consecutive SNPs may suggest loss of heterozygosity regions. The former can be used as a deletion marker if they include at least three consecutive SNPs. In such deleted regions, inter-SNP marker distances should also be checked. In the latter, the unlikely probability of homozygous calls for consecutive SNPs is calculated as the possibility of LOH events. Moreover, in the analysis of SNP array data, the source of specimen to be investigated (such as cell lines or peripheral blood DNA etc.) and copy number neutral events should also be concerned while drawing conclusions.

Quantitative information is described as the percentage of saturation of each probeset by the interrogated DNA during hybridization. Briefly, amplified regions saturate probesets more than normal (diploid) regions; likewise, LOH regions saturate less and homozygously deleted regions are expected to have signal values close to background

and mismatch probesets. Similar to the genotype values, in the quantitative analysis of SNP data, a number of consecutive SNP markers are expected to behave similarly to conclude as copy number gains or losses. Finally, qualitative and quantitative values for each SNP are expected to be in accordance to obtain significant results.

In the present study, we benefited from both genotype and copy number values of the probesets to achieve significant results with minimal regions and maximum confidentiality. For genotype calls, we expected to have at least three consecutive SNP markers to be present as no calls in order to represent homozygous deletions; therefore our resolution is expected to be around 600-KB. We also considered the possibility of failure in the restriction enzyme digestion and subsequent whole genomic DNA PCR amplification. This may cause under-representation of target and result in false-positive deletions. This type of false-positive errors can be batch specific, observed as common no calls at particular SNP markers in most of the samples and they usually behave unlike adjacent SNPs. Some SNPs with no call values may have normal copy number values in contrast to deletions, therefore we checked raw copy number values each no call SNPs and excluded the ones with values higher than 0.5.

For quantitative measurement, we used dChip Software to analyze saturation values (Li and Wong 2001, Lin et al. 2004). We first tried peripheral blood genomic DNA results of four healthy individuals as reference controls to obtain copy number values since we had no chance to use match-controls for our cell lines. We noticed that these individuals have characteristic copy number polymorphisms in their genome and behaved differently than the nature of the cell lines' gDNA. Therefore, we excluded these controls in the analysis and performed no-reference analysis by introducing all the cell lines as normal to the software. This approach significantly reduced the noise and disturbance. Moreover, we also considered the concordance of genotype data with copy number data whether they are in accordance with homozygous deletion and LOH regions. Noteworthy, we also checked inter-marker distances and saturation signatures at the raw copy number values for an additional level of evaluation of the significance and to set the physical margins. We mapped the physical positions of disturbances using UCSC Genome

Browser Build March 2006. In some chromosomal regions, SNP markers can be very few and the distance between SNP markers in an imbalanced region and the neighboring normal region SNP marker can be as large as a few MB. On such occasions, the disturbed regions might exceed the imbalance region defined by the borderline SNP markers. To be on the confident side, we preferred to use the last SNPs as the margins of imbalance and neglect if there are any genes neighboring, but we checked if these regions contain interesting genes.

In this study, we preferred to report only homozygous deletions and amplifications with copy number values equal to or greater than four. We excluded LOH profiles based on genotype calls due to cell line's nature. The cell lines used in this study are hyperploid; therefore using genotyping calls as a qualitative marker would be erroneous. Although qualitative use of genotype calls from SNP chips are invaluable information in linkage and association studies in which the target DNA is usually from blood or tissue (Ozturk et al. 2006). When we analyzed our cell lines for their LOH profile, we observed LOH in less than half of the whole genomes of the cell lines; therefore we preferred not to report them. On the other hand, we also did not include copy number changes smaller than 4 in our report although we observed quite few hemizygous duplications which can be as large as whole chromosomes.

Furthermore, we compared our copy number data with the available microarray expression data of the cell lines and primary tumors. We accessed the raw expression data (Affymetrix U133 Plus 2.0 Platform) of primary tumors (GSE6764, Wurmbach et al. 2007), HepG2 (GSE6368, Wang et al. 2006) through Gene Omnibus (GEO). Huh7, SkHep1 and Hep40 cell lines' expression data were obtained through personal communications. Although the aims of these experiments were quite different than ours, we used them only with purpose of comparing the expression signatures in regions of our interest. One advantage of these expression data is that it has higher resolution compared to our SNP array. The expression arrays we analyzed have more probesets and cover all most all the genes in human genome. Therefore, in regions where SNP markers are not available, we used expression signatures to define and check imbalance margins.

Basically, we expected no expression of consecutively mapped genes in the deleted regions. For copy number gains, we did not expect abnormally high expression of consecutive genes in all of amplified regions, because not all the amplifications result in overexpression of the genes they contain. Therefore, the expression data of the available cell lines and the primary tumors allowed us to check and confirm our findings in gDNA at transcript level and gave us a chance to correlate it with primary tumors.

5.1 Homozygous Deletions

Our results showed three homozygous deletions on chromosomes 9, 13 and X. In 9p23, Mahlavu, Plc, Skhep1, Snu182, Snu387 and Snu423 contain a homozygous deletion site within Mahlavu and Snu182 the largest. This region spans 1-MB and maps to a part of protein tyrosine phosphatase, receptor type, D gene (PTPRD) which is a large gene and spans a region of 2.3-MB. This gene has partially been shown to be deleted in other cancers and no data is available for HCC (Sato et al. 2005). At present, the pathogenic significance of PTPRD deletion is unclear, but, frequent deletions at this locus indicates that the inactivation of this gene may have a major role in tumorigenesis. Expression array results also support our findings that this gene is downregulated in cell lines, cirrhotic and HCC tumor tissue compared to normal liver.

Another homozygous deletion maps to 9p21.3-p21.2 region in SkHep1, Snu387 and Snu449 and it spans 6-MB. Genomic DNA PCR targeting hsa-mir-31 region in this deletion confirmed our SNP array results. This region harbors important tumor suppressor locus of cyclin-dependent kinase inhibitors 2 (CDKN2A/p14ARF/CDKN2B) which encode negative regulators of cell growth. The region has been shown to be frequently inactivated by homozygous deletions in HCC, lung and other cancers (Liew et al. 1999; Liggett and Sidransky, 1998). In addition to deletions, this locus is also inactivated by epigenetic regulation, LOH and mutations (Lukas et al. 1995). Among the three cell lines, SkHep1 has the narrowest deletion targeting this locus with a span of 1.4-MB, while the span of deleted regions in Snu387 and Snu449 are 3.5 and 5-MB,

respectively. SkHep1 also shows no transcriptional activity for this locus in the expression array supporting our findings. In addition to CDKN2A/p14ARF/CDKN2B locus, other interesting genes such as Ras-related GTP binding A (RRAGA) and tumor suppressor candidate 1 (TUSC1) also map to these disturbed regions. It is suggested that since LOH ratio over detected mutations ratio is different, this region may contain other tumor suppressor genes.

In Skhep1 and smaller in Huh7, we detected another homozygous deletion mapping to 13q12.11 region which has a length of 1.5 MB. We also confirmed this deletion by PCR methods with genomic DNA and cDNA targeting TPTE2 locus. This region spans 2-MB in length and harbors genes TPTE and PTEN homologous inositol lipid (TPTE2) and large tumor suppressor homolog 2 (LATS2). TPTE2 is a member of a large class of membrane-associated phosphatases with substrate specificity for the 3-position phosphate of inositol phospholipids and LATS2 is an essential mitotic regulator required for the coordination of cell division (Yabuta et al. 2007). TPTE2 and LATS2 can be candidate tumor suppressor genes in hepatocarcinogenesis. A close region has previously shown to be deleted in HCC cell lines and tissues using micro-satellite markers but no genes were reported to be significant for HCC (Chen et al. 2005). Moreover, microarray expression data supports our findings for SkHep1 and Huh7, but we could not observe and abnormality in tumor samples.

Interestingly, Huh7 harbors a large hemizygous deletion of 16-MB at Xq21.1-21.33 region. We confirmed this deletion with PCR targeting spermidine/spermine N1-acetyl transferase-like 1 (SATL1) gene in this region at the genomic DNA and transcript level. This region contains more than 20 genes. Considering the lower incidence of HCC in females, this region may contain genes with tumor suppressor character in hepatocarcinogenesis. There is no study showing a deletion in HCC cell lines and tissues. Expression platform supports our findings for Huh7 and it also suggest nucleosomal binding protein 1(NSBP1) as an interesting candidate in this region since it is downregulated in tumors and cell lines with respect to normal liver. This gene is

overexpressed in cervical cancer cell lines (Shirakawa et al. 2000) but may have different roles in HCC.

Recently, miRNA genes were found to play a critical role in cell growth, death, and differentiation (Tsuchiya et al. 2006). In our study, three microRNA genes, hsa-mir-491 and hsa-mir-31 at 9p22.1-p21.2 region and hsa-mir-361 at Xq21.1-21.33 region are lost. These miRNAs has previously shown to be involved in colorectal carcinoma (Bandres et al. 2006), pancreatic ductal adenocarcinoma and in HCC (Szafranska et al. 2007; Bandres et al. 2006). The inactivation of these microRNAs through deletion may play a role in hepatocarcinogenesis.

Our scanning at a 600-KB resolution led to the identification of three deleted regions. Our results are in complete concordance with the expression data; the transcripts in these regions had signal intensities close to background level. These regions contained approximately 90 genes, including well-known tumor suppressor genes and three miRNA genes. The authenticity of most of these genes as HCC tumor suppressors is unknown at present; however, some of them have been indicated as being involved in cell signaling, polarity, motility and adhesion. Inactivation of these genes by deletion might have given more malignant phenotypes to cancer cells by changing their ability to proliferate, survive and metastasize. We also observed small deletions involving no genes. The significance of such deletions is unknown. In cancer cells, not all the deleted regions contain genes (Cox et al. 2005), instead, they may contain regulatory sequences. Further analysis of these regions may lead to identification of new tumor suppressors.

Working with cancer cell lines has the advantage of re-expressing the genes which are found to be deleted to study phenotypic effects. If such a deleted gene is important in carcinogenesis, an effect should be observed in the deleted cell line but not in the cell line still expressing the endogenous gene. On the contrary, loss of expression can be achieved in cell line models through shRNA knockdowns (Sato et al. 2006)

Lastly, it should be noted that we bypassed several possible deletions due to our stringent analysis parameters. Since SNP markers are not equally distributed over the genome, some of the true-positive deletions may be represented by only one or two markers. Moreover, in some deletions sites, there may exist no SNPs, therefore we might have overlooked several deletions which might have previously shown by other methods. We also caution that all the deletions in the present study were detected in cultured HCC cells Primary HCC tumors often show intra-tumoral heterogeneity, and it is possible that some of the deletions detected in the present study were present only in small subsets of cancer cells. Tissue cultivation during the establishment of cell lines might have selected cancer cells with deletions giving cells advantages in in-vitro growth, and this fact might have led to over-representation of certain deletions in our set of HCC cell lines. Therefore, it is possible that the incidence of homozygous deletions in the cell lines does not necessarily represent that in HCC tumors. Thus, further studies should also focus on the identification of homozygous deletions in HCC tissues to further elucidate their prevalence and significance.

5.2 Amplifications

In our results, copy number gains were more frequent than losses. We have observed copy number gains on chromosomes 8, 9, 12, 14, 15, 16, 17, 19, 22 and X. Although raw copy numbers of the SNP markers mapping the peak of the amplicons reach over 10 copies, the inferred copy numbers of the regions were mostly rounded around five and six by Hidden Markov Model.

Chromosome 8 harbors three amplicons. First, in segment 8p23.1, we observed amplification in Hep40 with an inferred copy number of six and confirmed this amplification with semi-quantitative genomic DNA PCR. This region spans 2.5-MB and its peak value is over 11. There are 10 genes mapping this region. One of the remarkable genes is malignant fibrous histiocytoma amplified (MFHAS1) which is a potential oncogene. Its expression is enhanced in malignant fibrous histiocytomas (Sakabe et al.

1999). PIN2-interacting protein 1 (PINX1) and tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase (TNKS) are also interesting. PINX1 is a liver-related putative tumor suppressor and its overexpression results in the inhibition of telomerase activity (Liao et al. 2000). In addition, contradictory studies have also been reported in its tumor suppressive activity in HCC suggesting it is not related with HCC but instead, regulates telomere length (Oh et al. 2004). TNKS may regulate vesicle trafficking and modify telomere repeat binding factor 1 (TERF1) and negatively regulates the telomere length. A recent report showed that TNKS can positively regulate telomere length and it is upregulated in some human cancers (Gelmini et al. 2007). To conclude, these candidates have not been studied in detail and contradictory results exist. When we looked at the expression microarray data, we observed no significant abnormal signatures in Hep40 and tumor samples due to amplification; only histone mRNA 3' end-specific exonuclease (THEX1) and PINX1 has higher transcription values in Hep40 compared to other cell lines.

We have also observed a second amplification in Hep40 at 8q13.3-q21.11. This region has an amplification value of six. It spans approximately 3.75-MB, has a peak value of seven and homes 17 genes. This region has also been shown to be amplified in a similar screen in HCC tissue but no candidates were reported (Midorikawa et al. 2004). One of these genes mapping this region is the telomeric repeat binding factor 1 isoform 1 (TERF1), which negatively regulates telomere length. Microarray expression results show that this gene has increased transcription in Hep40 (and in advanced HCC tissues) compared to other cell lines and normal tissue which can be due to amplification. It is noteworthy that, co-amplification of 8p23.1 and 8q13.1-p21.11 which harbor three not extensively characterized genes related with telomere maintenance can point a mechanism for Hep40 cells to overcome replicative senescence through telomere regulation. Staufen homolog 2 (STAU2) and ganglioside-induced differentiation-associated (GDAP1) are other interesting genes mapping this amplicon which are double-stranded RNA-binding protein (Buchner et al. 1999) and ganglioside-induced differentiation-associated protein (Cuesta et al. 2002), respectively. Expression array

results show that both these genes are upregulated in Hep40 and to an extent, in advanced HCC tumors.

A third amplicon on chromosome 8 maps to q24.13 region. This segment spans 3.5-MB and is duplication. 8q23-q24 region has been shown to be frequently amplified in HCC; PTK2 and EIF3S3 have been reported as driver genes in these amplicons (Okamoto et al. 2003). Amongst several genes in this amplicon, two AAA domain containing protein (ATAD2) and zinc fingers and homeoboxes 1 (ZHX1) are other interesting genes to be further studied. ATAD2 has been shown to be upregulated in breast, uterus, colon, ovary, and stomach tumors and amplified in other cancers (Van Duin et al. 2005, Cheng et al. 2006). ZHX1 is a transcriptional repressor and has been shown to be amplified and upregulated in multiple myeloma cell lines (Largo et al. 2006). Expression array results show that ATAD2 transcript is highly abundant in Hep40 and this gene shows increased expression value in tumor tissue.

Interestingly, in 9p22.1-p21.2 region, in contrast to SkHep1, Snu387 and Snu449 which show homozygous deletions, Snu398 show a copy number increase of six. Our semi-quantitative gPCR targeting hsa-mir-31 confirmed amplification of this region. This amplicon spans a region of 7-MB, has a peak of eight and approximately coincides with Snu449's deleted region. This region is a well characterized tumor suppressor loci and deleted in many human cancers. Copy number gains have not been reported for this region before and this amplicon supports the notion that there are other genes involved in tumorigenesis besides loss of function mechanisms in 9p22. In this amplicon 5'-methylthioadenosine phosphorylase (MTAP) and myeloid/lymphoid or mixed-lineage leukemia (MLLT3) are interesting. MTAP is involved in the growth of breast cancer cell lines and its expression has been observed in many epithelial cancers (Tang et al. 2000). Overexpression of this gene due to amplification and other possible mutations may play a role in hepatocarcinogenesis, as well. MLLT3 is involved in growth, apoptosis, differentiation, cell death, cell cycle progression and expansion. In U-2 OS and TK6 cells, MLLT3 protein is involved in the decrease of acetylation of p53 to acetylated p53 that is mediated by p300 protein and damage of DNA (Wiederschain et al. 2005).

MLLT3 is also involved in histone methylation. Abnormal expression of this gene because of amplification may infer proliferative advcantage to cancerous cells.
In Snu475, 12p11.21-p11.1 region harbors an amplicon of six in value. This region spans a region of 2.5-MB, has a maxima of nine and contains over 10 genes. This segment has previously shown to be to amplified in HCC and lung carcinomas (Marchio et al. 1997; Zhao et al. 2005). Antagonist of mitotic exit network 1 homolog (AMN1) maps this amplicon. It acts as a switch that helps cells exit from mitotic exit and reset the cell cycle in yeast (Wang et al. 2003). AMN1 exerts its affects through inhibition of G protein signaling and results in inhibition of Cdc14. This in turn leads to helping the cells be competent for S-phase entry. Overexpression of this gene because of amplification may cause bypassing G1 to S phase controls and may result in genomic mutations. Another gene in this amplicon is DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 gene (DDX11) that functions as both ATPase and DNA helicase activities in cellular growth and division. Loss of DDX11 helicase in mouse has been shown to cause lethality due to the accumulation of aneuploid cells which may suggest it plays a role in genome stability (Inoue et al. 2007). Another interesting gene in this region is FYVE, RhoGEF and PH domain containing 4 (FGD4) which activates cell division cycle 42 (CDC42). Expression array results showed no differentially regulated genes in this region for the tumor samples.

Huh7 cell line has an amplicon at 14q12-q13.1 spanning a region of 2.3 MB. This region displays a 7 fold-increase with a peak of 14 and harbors 10 genes. Microarray expression data for Huh7 cell line shows that most of these genes in this region are constitutively and consecutively overexpressed. This expression signature is clearly in accordance with amplification. Although this region is not touched in the literature, there exist two discordant reports; one study shows in HCC tissues, amplifications are related with HCV infection (Sakakura et al. 1999) other shows in HCC cell lines that 14q12-q13 region is subject to LOH (Zimonjic et al. 1999). Both studies used low resolution methods therefore might have skipped our findings. Amongst the genes in the amplicon, nucleotide-binding protein-like (NUBPL) and protein kinase D1 (PRKD1) are interesting. NUBPL has been shown to be involved in translocations and upregulated in acute

myeloid leukemia by SAGE analysis (Lee et al. 2005). Another interesting study on massive amplification of rolling-circle transposons in the lineage of the bat Myotis lucifugus shows that a subfamily of these nonautonomous transposons, HeliBatN3, display high homology with NUBPL (Pritham et al. 2006). The product of PRKD1 is a calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme. Missense mutations have been described in colorectal and breast cancers (Sjoblom et al. 2006). PRKD1 has been shown to be involved in LOH events but with high expression values in hepatoblastomas (Adesina et al. 2007). Another study shows PRKD1 is highly expressed in pancreatic ductal adenocarcinoma cell lines that are highly resistant to chemotherapeutic drugs (Ammerpohl et al. 2007). Our semi-quantitative PCR targeting NUBPL gene confirms the amplification in Huh7 and to an extent in Snu398. On the contrary, we have not observed any significant differentially expressed genes in tumor samples in the expression analysis.

In Hep40, we detected amplification at 15q21.3 with five in copy number. This region is 2-MB in length and contains only two genes, WD repeat domain 72 (WDR72) and unc-13 homolog C (UNC13C) map here. WDR72 has also shown to be amplified in melanoma with a similar approach to ours (Stark et al. 2007). Since functions of these genes' products are not well characterized, we can not conclude the significance of this amplicon. The only interesting information, dog ortholog of WDR72 has been found to be similar to TGF-β resistance-associated protein. Therefore, amplification of this gene may result in high expression and overcome inhibitory affects of TGF-β signaling in cell cycle. Moreover, expression data provides no significant differentially expression for these genes.

We have also seen another amplicon in Hep40 which maps 16q23.1 region. This region is 3-MB in size and six in copy number with a peak of nine. In the literature, no amplifications have been reported for this region. Among the genes mapping here, cell recognition protein CASPR4 isoform 1 (CNTAP4) and WW domain-containing oxidoreductase isoform 1 (WWOX) are the most interesting ones. CNTAP4 gene belongs to the neurexin family, members of which function in the vertebrate nervous

system as cell adhesion molecules and receptors. This protein, like other neurexin proteins, contains epidermal growth factor repeats and laminin G domains. Contradictorily, this gene has been shown to be deleted in prostate tumors in a similar study (Liu et al. 2006). WWOX plays an important role in the regulation of a wide variety of cellular functions such as protein degradation, transcription, and RNA splicing. Tumor suppressive role of this gene has been reported; WVOX is frequently involved in LOH and its function is lost in various cancers and tumor cell lines (Qin et al. 2006; Iliopoulos et al. 2005). Expression analyses showed that amplification of this region is not in concordance with the expression signatures of Hep40 and other tumor samples; we failed to observe any amplification trend in this region.

A large amplicon, 11.5-MB in size, maps to 17p13.1-q11.1 region in Snu182 region. Its copy number is six in number and harbors more than 20 genes. This region has also shown to be amplified in sarcomas (Kaur et al. 2007). The expression analysis showed no aberrant amplification signature in this region. Therefore we looked at the most amplified sub-region using raw copy number values. Myocardin (MYOCD) maps to the center of one of the amplification peaks in this regions. MYOCD is a transcription factor that uses the canonical single or multiple CArG boxes DNA sequence. Inactivation of myocardin and p16 during malignant transformation has been shown to contribute to a differentiation defect (Milyavsky et al. 2007), but its overexpression has not been studied and it may also play a role in carcinogenesis. Phosphatidylethanolamine N-methyltransferase (PEMT) maps to the second peak in the region. This gene encodes an enzyme which converts phosphatidylethanolamine to phosphatidylcholine by sequential methylation in the liver. Contradictorily, PEMT expression has been found to be reduced in HCC (Tessitore et al. 2003). RAS, dexamethasone-induced 1 (RASD1) is another gene mapping close to the center of the second peak. This gene encodes a Ras-related protein that is stimulated by dexamethasone with exact function unknown. Although RASD1 is a member of the Ras superfamily of small G-proteins that often promotes cell growth and tumor expansion, plays an active role in preventing aberrant cell growth, anti-growth function has been reported (Vaidyanathan et al, 2004). To sum up all, this region

is quite large and further analysis required for finding out the driver gene or genes in this amplicon.

In chromosome 17, we have also observed another amplicon at p11.2-q11.1. This region spans a 1.75-MB and is six in copy number with a peak value of eight. This region can be larger than we expect since the neighboring SNPs with normal copy numbers are further than one MB. Analysis of expression data of primary tumor did not reveal any significant upregulation of consecutive genes. There are 13 genes mapping this region and among them, mitogen-activated protein kinase kinase 3 (MAP2K3) and kinase suppressor of ras (KSR1) are the most interesting candidates to be the driver genes of the amplicon. MAP2K3 encodes a dual specificity protein kinase that belongs to the MAP kinase family and activates MAPK14/p38-MAPK (Derijard et al. 1995). Expression of RAS oncogene is found to result in the accumulation of the active form of this kinase. Therefore amplification of this region may result in abnormal activity of MAP2K3 resulting in oncogenic character. KSR1 functions downstream of Ras and is required for MAP kinase activation (Kornfeld et al. 1995; Ohmachi et al. 2002). Although KSR1 is not well characterized and its metastasis suppressive roles have been reported (Hartsough et al. 2002), abnormal expression of this gene may result in disturbances in MAPK and other pathways since positive and negative signaling pathways regulate tumor metastasis, including multiple metastasis suppressor genes (Steeg , 2003)

We also detected another amplicon on chromosome 17 mapping to q21.2 region. It spans a region of 0.7-MB and is five in copy number with a maximum value of eight. This amplicon is likely larger than we can detect because of the absence of SNP markers in the region. This amplicon contains members of keratin family which encode intermediate filament proteins responsible for the structural integrity of epithelial cells. ErbB-2 isoform a (ERBB2) has not escaped from our eyes that it maps very close to this region and it is may be involved in this amplification. ERBB2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Amplification and overexpression of this gene has been reported in numerous cancers, as well as in HCC (Zimonjic et al. 1999). Thyroid hormone receptor, alpha isoform 1

(THRA) is another interesting gene in this region. This gene is a proto-oncogene and it has been reported be involved in human cancers including breast and thyroid papillary tumors (Silva et al. 2002; Puzianowska-Kuznicka et al. 2002). RARA protein (RARA), C-terminal tensin-like (TNS4), chemokine (C-C motif) receptor 7 precursor (CCR7) and breast cancer 1, early onset isoform 1 (BRCA1) are other interesting genes in this region that have been shown to be related with human cancers. Expression analysis showed that only keratin 23 (KRT23) is significantly highly expressed in tumor tissue.

19q13.2-13.32 is a very gene rich region with more than 20 genes and is amplified in Mahlavu and Focus. This region spans 3.25-MB, has an amplification value of six and it is also amplified in other cancers (Dekken et al. 1999). Amongst several genes, reticuloendotheliosis viral oncogene homolog B (RELB), B-cell CLL/lymphoma 3 (BCL3) and malignancy-associated protein (MAG) are quite interesting. Analysis of expression data gave no clues about highly expressed genes in advanced tumors compared to normal. RELB is a member of Rel/NF-κB transcription factor family and stimulates promoter activity in the presence of p49- and p50-NF-kappa-B (Suhasini et al. 1997) and minor sporadic amplifications has been reported (Rayet et al. 1999). NF-κB signaling is important in HCC (Pikarsky et al. 2004); therefore amplification of this gene may result in abnormal activity. BCL3 is a proto-oncogene candidate; act as transcription factor and is involved in NF-κB signaling (Karin et al. 2002). BCL3 locus has been shown to be involved in recurrent translocations in Hodgkin and peripheral T-cell lymphoma (Michaoux et al. 2004). MAG is expressed in various malignant tumors including glioblastomas and HCC and in tumor preexisting conditions such as hepatitis C virus- and hepatitis B virus-induced liver cirrhosis (Ljubimova et al. 1998). Although our expression analysis results do not support this evidence, this gene may play a role in progression of premalignant conditions and in the development of HCC and other cancers.

Another amplification maps to 22q11.21-22 region. This region contains over 20 genes and spans 3.5-MB. This amplicon is present only in Snu182 and has a value of six. In expression analysis, we could not detect any amplification expression signature in

primary tumors. This region contains interesting genes such as v-crk sarcoma virus CT10 oncogene homolog (CRKL), phosphatidylinositol 4-kinase, catalytic, alpha (PIK4CA), hypermethylated in cancer 2 (HIC2) and mitogen-activated protein kinase 1 (MAPK1). CRKL maps to the center of the amplicon and can be the driver gene. CRKL has been shown to activate the RAS and JUN kinase signaling pathways and transform fibroblasts in a RAS-dependent fashion. It is a substrate of the BCR-ABL tyrosine kinase and plays a role in fibroblast transformation by BCR-ABL. In addition, CRKL has oncogenic potential (Ten Hoeve et al. 1993; Senechal et al. 1996, 2002). PIK4CA encodes a phosphatidylinositol (PI) 4-kinase which catalyzes the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate (Wong et al. 1994). Although HIC2 has reported to be a putative tumor suppressor (Deltour et al. 2002), overexpression of this gene may have other unexpected roles. The protein encoded by MAPK1 gene is a member of the MAP kinase family and is involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK1 (Meloche and Pouyssegur 2007).

Last, on X-chromosome, we found amplification in Snu182 cell line. This cell line has male origin and shows a copy number of four. This region maps p22.12-p22.11 and spans more than 1 MB. Since there are not so many SNP markers in this region, we can not map the center of the amplicon and the size of the disturbed regions can be larger than we detected. With the SNP markers available, connector enhancer of kinase suppressor of Ras (CNKSR2) maps close to the amplicon. This gene is a necessary element in receptor tyrosine kinase pathways, possibly as a tyrosine phosphorylation target. It is involved in regulation of RAF in the MAPK pathway and may also play a role in a MAPK-independent pathway (Lanigan et al. 2003). It is highly expressed cervical cancer cell lines, embryonic cell lines, epithelial cell lines; kidney cell lines (Jaffe et al. 2004). Cyclin-dependent kinase-like 5 (CDKL5) is another gene which may reside in the amplicon. CDKL5 is a member of Ser/Thr protein kinase family and encodes a phosphorylated protein with protein kinase activity. It interacts with MECP2 and it is expressed in breast carcinoma and prostate cancer cell lines (Mari et al. 2005; Bertucci et

al. 2004; Lin et al. 2005). In expression analysis, we observed no abnormal expression attributable to amplification.

Besides genes encoding proteins, we also have showed 13 microRNAs mapping the amplified regions above. These include hsa-mir-597 and hsa-mir-124a-1 at 8p23.1; hsa-mir-548d-1 at 8q24.13; hsa-mir-491 and hsa-mir-31 at 9p22.1-p21.2; hsa-mir-624 at 14q12-q13.1; hsa-mir-33b at 17p13.1-q11.1; hsa-mir-330, hsa-mir-642 and hsa-mir-769 at 19q13.31-q13.32; hsa-mir-185, hsa-mir-649 and hsa-mir-130b at 22q11.21-q11.22 regions. Among these miRNAs, experimental studies showed that upregulation of hsa-mir-31 and hsa-mir-330 are associated with colorectal and breast cancer, respectively (Bandres et al. 2006; Mattie et al. 2006). Expression of hsa-mir-130b has also been detected in pancreatic cancer cells (Mattie et al. 2006)

Our screen led to the identification of more than ten amplified regions. These regions contained over 400 genes, including 13 miRNA genes. Our results are mostly not in concordance with the expression data since usually less than 40% of amplicons result in overexpression. Some of these genes have been indicated as being involved in cell signaling, polarity, motility and adhesion. Overexpression of these genes due to amplification might have given more malignant phenotypes to cancer cells by changing their ability to proliferate, survive and metastasize. Further analysis of these regions may lead to identification of new oncogenes. Working with cancer cell lines has the advantage of silencing the genes with RNAi methods which are found to be amplified and overexpressed to study phenotypic effects. If such an amplified gene is important in carcinogenesis, an effect should be observed in the amplified cell line. Noteworthy, we skipped several possible focal amplificons due to our stringent analysis parameters. Since SNP markers are not equally distributed over the genome, some of the true-positive amplicons may be represented by only one or two markers. Moreover, in some amplicons, there may exist no SNPs, therefore we might have overlooked several copy number gains which might have previously shown by other methods. We also caution that all the amplifications in the present study were detected in cultured HCC cells. It is possible that the incidence of amplifications in the cell lines does not necessarily

represent that in HCC tumors. Thus, further studies should also focus on the identification of amplifications in HCC tissues to further elucidate their prevalence and significance.

In this study, we screened copy number changes in a panel of 14 HCC lines at a resolution of 0.6-MB. In addition, we tried to correlate the copy number changes with the available microarray expression data of the cell lines and primary tumors to compare and correlate losses and gains. We also confirmed selected disturbed regions using conventional methods. Consequently, we prepared a list of candidate genes which can be directly or indirectly related to tumorigenesis. Further study of these genes through genetic, epigenetic and functional analysis may provide new insights in our understanding of HCC biology.

6. REFERENCES

Adesina,A.M., Nguyen,Y., Guanaratne,P., Pulliam,J., Lopez-Terrada,D., Margolin,J., and Finegold,M. (2007). FOXG1 is overexpressed in hepatoblastoma. Hum. Pathol. *38*, 400-409.

Albertson,D.G., Collins,C., McCormick,F., and Gray,J.W. (2003). Chromosome aberrations in solid tumors. Nat. Genet. *34*, 369-376.

Altabef,M., Garcia,M., Lavau,C., Bae,S.C., Dejean,A., and Samarut,J. (1996). A retrovirus carrying the promyelocyte-retinoic acid receptor PML-RARalpha fusion gene transforms haematopoietic progenitors in vitro and induces acute leukaemias. EMBO J. *15*, 2707-2716.

Aman,P. (1999). Fusion genes in solid tumors. Semin. Cancer Biol. *9*, 303-318.

Ammerpohl,O., Trauzold,A., Schniewind,B., Griep,U., Pilarsky,C., Grutzmann,R., Saeger,H.D., Janssen,O., Sipos,B., Kloppel,G., and Kalthoff,H. (2007). Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells. Br. J. Cancer *96*, 73-81.

Andrisani,O.M. and Barnabas,S. (1999). The transcriptional function of the hepatitis B virus X protein and its role in hepatocarcinogenesis (Review). Int. J. Oncol. *15*, 373-379.

Aoki,H., Kajino,K., Arakawa,Y., and Hino,O. (1996). Molecular cloning of a rat chromosome putative recombinogenic sequence homologous to the hepatitis B virus encapsidation signal. Proc. Natl. Acad. Sci. U. S. A *93*, 7300-7304.

Baker,S.J., Preisinger,A.C., Jessup,J.M., Paraskeva,C., Markowitz,S., Willson,J.K., Hamilton,S., and Vogelstein,B. (1990). p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. *50*, 7717-7722.

Balsara,B.R., Pei,J., De,R.A., Simon,D., Tosolini,A., Lu,Y.Y., Shen,F.M., Fan,X., Lin,W.Y., Buetow,K.H., London,W.T., and Testa,J.R. (2001). Human hepatocellular carcinoma is characterized by a highly consistent pattern of genomic imbalances, including frequent loss of 16q23.1-24.1. Genes Chromosomes. Cancer *30*, 245-253.

Bandres,E., Cubedo,E., Agirre,X., Malumbres,R., Zarate,R., Ramirez,N., Abajo,A., Navarro,A., Moreno,I., Monzo,M., and Garcia-Foncillas,J. (2006). Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol. Cancer *5*, 29.

Bandres,E., Cubedo,E., Agirre,X., Malumbres,R., Zarate,R., Ramirez,N., Abajo,A., Navarro,A., Moreno,I., Monzo,M., and Garcia-Foncillas,J. (2006). Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol. Cancer *5*, 29.

Banerjee,D., Mayer-Kuckuk,P., Capiaux,G., Budak-Alpdogan,T., Gorlick,R., and Bertino,J.R. (2002). Novel aspects of resistance to drugs targeted to dihydrofolate reductase and thymidylate synthase. Biochim. Biophys. Acta *1587*, 164-173.

Baumhueter,S., Mendel,D.B., Conley,P.B., Kuo,C.J., Turk,C., Graves,M.K., Edwards,C.A., Courtois,G., and Crabtree,G.R. (1990). HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF. Genes Dev. *4*, 372-379.

Beasley,R.P. (1988). Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer *61*, 1942-1956.

Bekri,S., Adelaide,J., Merscher,S., Grosgeorge,J., Caroli-Bosc,F., Perucca-Lostanlen,D., Kelley,P.M., Pebusque,M.J., Theillet,C., Birnbaum,D., and Gaudray,P. (1997). Detailed

map of a region commonly amplified at 11q13-->q14 in human breast carcinoma. Cytogenet. Cell Genet. *79*, 125-131.

Bertucci,F., Borie,N., Ginestier,C., Groulet,A., Charafe-Jauffret,E., Adelaide,J., Geneix,J., Bachelart,L., Finetti,P., Koki,A., Hermitte,F., Hassoun,J., Debono,S., Viens,P., Fert,V., Jacquemier,J., and Birnbaum,D. (2004). Identification and validation of an ERBB2 gene expression signature in breast cancers. Oncogene *23*, 2564-2575.

Biden,K., Young,J., Buttenshaw,R., Searle,J., Cooksley,G., Xu,D.B., and Leggett,B. (1997). Frequency of mutation and deletion of the tumor suppressor gene CDKN2A (MTS1/p16) in hepatocellular carcinoma from an Australian population. Hepatology *25*, 593-597.

Bluteau,O., Beaudoin,J.C., Pasturaud,P., Belghiti,J., Franco,D., Bioulac-Sage,P., Laurent-Puig,P., and Zucman-Rossi,J. (2002). Specific association between alcohol intake, high grade of differentiation and 4q34-q35 deletions in hepatocellular carcinomas identified by high resolution allelotyping. Oncogene *21*, 1225-1232.

Boige,V., Laurent-Puig,P., Fouchet,P., Flejou,J.F., Monges,G., Bedossa,P., Bioulac-Sage,P., Capron,F., Schmitz,A., Olschwang,S., and Thomas,G. (1997). Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotype. Cancer Res. *57*, 1986-1990.

Boige,V. and Laurent-Puig,P. (1997). [Genetic alterations associated with hepatocellular carcinoma]. Gastroenterol. Clin. Biol. *21*, 34-44.

Brechot,C., Gozuacik,D., Murakami,Y., and Paterlini-Brechot,P. (2000). Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin. Cancer Biol. *10*, 211-231.

Bressac,B., Kew,M., Wands,J., and Ozturk,M. (1991). Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature *350*, 429-431.

Buchner,G., Bassi,M.T., Andolfi,G., Ballabio,A., and Franco,B. (1999). Identification of a novel homolog of the Drosophila staufen protein in the chromosome 8q13-q21.1 region. Genomics *62*, 113-118.

Buendia,M.A. (2002). Genetic alterations in hepatoblastoma and hepatocellular carcinoma: common and distinctive aspects. Med. Pediatr. Oncol. *39*, 530-535.

Cavenee,W.K., Dryja,T.P., Phillips,R.A., Benedict,W.F., Godbout,R., Gallie,B.L., Murphree,A.L., Strong,L.C., and White,R.L. (1983). Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature *305*, 779-784.

Cereghini,S., Yaniv,M., and Cortese,R. (1990). Hepatocyte dedifferentiation and extinction is accompanied by a block in the synthesis of mRNA coding for the transcription factor HNF1/LFB1. EMBO J. *9*, 2257-2263.

Chen,C.F., Yeh,S.H., Chen,D.S., Chen,P.J., and Jou,Y.S. (2005). Molecular genetic evidence supporting a novel human hepatocellular carcinoma tumor suppressor locus at 13q12.11. Genes Chromosomes. Cancer *44*, 320-328.

Chen,C.J., Yu,M.W., and Liaw,Y.F. (1997). Epidemiological characteristics and risk factors of hepatocellular carcinoma. J. Gastroenterol. Hepatol. *12*, S294-S308.

Chen,Y.W., Jeng,Y.M., Yeh,S.H., and Chen,P.J. (2002). P53 gene and Wnt signaling in benign neoplasms: beta-catenin mutations in hepatic adenoma but not in focal nodular hyperplasia. Hepatology *36*, 927-935.

Cheng,T.C., Manorek,G., Samimi,G., Lin,X., Berry,C.C., and Howell,S.B. (2006). Identification of genes whose expression is associated with cisplatin resistance in human ovarian carcinoma cells. Cancer Chemother. Pharmacol. *58*, 384-395.

Chouard,T., Blumenfeld,M., Bach,I., Vandekerckhove,J., Cereghini,S., and Yaniv,M. (1990). A distal dimerization domain is essential for DNA-binding by the atypical HNF1 homeodomain. Nucleic Acids Res. *18*, 5853-5863.

Colombo,M., Kuo,G., Choo,Q.L., Donato,M.F., Del,N.E., Tommasini,M.A., Dioguardi,N., and Houghton,M. (1989). Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet *2*, 1006-1008.

Cox,C., Bignell,G., Greenman,C., Stabenau,A., Warren,W., Stephens,P., Davies,H., Watt,S., Teague,J., Edkins,S., Birney,E., Easton,D.F., Wooster,R., Futreal,P.A., and Stratton,M.R. (2005). A survey of homozygous deletions in human cancer genomes. Proc. Natl. Acad. Sci. U. S. A *102*, 4542-4547.

Cuesta,A., Pedrola,L., Sevilla,T., Garcia-Planells,J., Chumillas,M.J., Mayordomo,F., LeGuern,E., Marin,I., Vilchez,J.J., and Palau,F. (2002). The gene encoding ganglioside-induced differentiation-associated protein 1 is mutated in axonal Charcot-Marie-Tooth type 4A disease. Nat. Genet. *30*, 22-25.

Davila,J.A., Morgan,R.O., Shaib,Y., McGlynn,K.A., and El-Serag,H.B. (2004). Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology *127*, 1372-1380.

de La,C.A., Romagnolo,B., Billuart,P., Renard,C.A., Buendia,M.A., Soubrane,O., Fabre,M., Chelly,J., Beldjord,C., Kahn,A., and Perret,C. (1998). Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc. Natl. Acad. Sci. U. S. A *95*, 8847-8851.

De Souza,A.T., Hankins,G.R., Washington,M.K., Orton,T.C., and Jirtle,R.L. (1995). M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. Nat. Genet. *11*, 447-449.

Deltour,S., Pinte,S., Guerardel,C., Wasylyk,B., and Leprince,D. (2002). The human candidate tumor suppressor gene HIC1 recruits CtBP through a degenerate GLDLSKK motif. Mol. Cell Biol. *22*, 4890-4901.

Derijard,B., Raingeaud,J., Barrett,T., Wu,I.H., Han,J., Ulevitch,R.J., and Davis,R.J. (1995). Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. Science *267*, 682-685.

Di Bisceglie,A.M., Carithers,R.L., Jr., and Gores,G.J. (1998). Hepatocellular carcinoma. Hepatology *28*, 1161-1165.

Di,G.S., Bianchi,P., Destro,A., Grizzi,F., Malesci,A., Laghi,L., Levrero,M., Morabito,A., and Roncalli,M. (2006). Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver. BMC. Cancer *6*, 89.

Diao,J., Garces,R., and Richardson,C.D. (2001). X protein of hepatitis B virus modulates cytokine and growth factor related signal transduction pathways during the course of viral infections and hepatocarcinogenesis. Cytokine Growth Factor Rev. *12*, 189-205.

El-Serag,H.B. and Mason,A.C. (1999). Rising incidence of hepatocellular carcinoma in the United States. N. Engl. J. Med. *340* , 745-750.

El-Serag,H.B. (2004). Hepatocellular carcinoma: recent trends in the United States. Gastroenterology *127*, S27-S34.

Ezaki,T., Kanematsu,T., Okamura,T., Sonoda,T., and Sugimachi,K. (1988). DNA analysis of hepatocellular carcinoma and clinicopathologic implications. Cancer *61*, 106-109.

Farazi,P.A. and DePinho,R.A. (2006). The genetic and environmental basis of hepatocellular carcinoma. Discov. Med. *6*, 182-186.

Feitelson,M.A., Zhu,M., Duan,L.X., and London,W.T. (1993). Hepatitis B x antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. Oncogene *8*, 1109-1117.

Feitelson,M.A. (1999). Hepatitis B virus in hepatocarcinogenesis. J. Cell Physiol *181*, 188-202.

Feitelson,M.A., Sun,B., Satiroglu Tufan,N.L., Liu,J., Pan,J., and Lian,Z. (2002). Genetic mechanisms of hepatocarcinogenesis. Oncogene *21*, 2593-2604.

Ferber,M.J., Montoya,D.P., Yu,C., Aderca,I., McGee,A., Thorland,E.C., Nagorney,D.M., Gostout,B.S., Burgart,L.J., Boix,L., Bruix,J., McMahon,B.J., Cheung,T.H., Chung,T.K., Wong,Y.F., Smith,D.I., and Roberts,L.R. (2003). Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. Oncogene *22*, 3813-3820.

Frain,M., Swart,G., Monaci,P., Nicosia,A., Stampfli,S., Frank,R., and Cortese,R. (1989). The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. Cell *59*, 145-157.

Fujimoto,Y., Ishizaka,Y., Tahira,T., Sone,H., Takahashi,H., Enomoto,K., Mori,M., Sugimura,T., and Nagao,M. (1991). Possible involvement of c-myc but not ras genes in hepatocellular carcinomas developing after spontaneous hepatitis in LEC rats. Mol. Carcinog. *4*, 269-274.

Fujimoto,Y., Hampton,L.L., Wirth,P.J., Wang,N.J., Xie,J.P., and Thorgeirsson,S.S. (1994). Alterations of tumor suppressor genes and allelic losses in human hepatocellular carcinomas in China. Cancer Res. *54*, 281-285.

Gelmini,S., Quattrone,S., Malentacchi,F., Villari,D., Travaglini,F., Giannarini,G., Della,M.A., Pazzagli,M., Nicita,G., Selli,C., and Orlando,C. (2007). Tankyrase-1 mRNA expression in bladder cancer and paired urine sediment: preliminary experience. Clin. Chem. Lab Med. *45*, 862-866.

Gorre,M.E., Mohammed,M., Ellwood,K., Hsu,N., Paquette,R., Rao,P.N., and Sawyers,C.L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science *293*, 876-880.

Gorunova,L., Hoglund,M., ndren-Sandberg,A., Dawiskiba,S., Jin,Y., Mitelman,F., and Johansson,B. (1998). Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations. Genes Chromosomes. Cancer *23*, 81-99.

Graef,E., Caselmann,W.H., Wells,J., and Koshy,R. (1994). Insertional activation of mevalonate kinase by hepatitis B virus DNA in a human hepatoma cell line. Oncogene *9*, 81-87.

Greenblatt,M.S., Feitelson,M.A., Zhu,M., Bennett,W.P., Welsh,J.A., Jones,R., Borkowski,A., and Harris,C.C. (1997). Integrity of p53 in hepatitis B x antigen-positive and -negative hepatocellular carcinomas. Cancer Res. *57*, 426-432.

Guan,X.Y., Fang,Y., Sham,J.S., Kwong,D.L., Zhang,Y., Liang,Q., Li,H., Zhou,H., and Trent,J.M. (2000). Recurrent chromosome alterations in hepatocellular carcinoma detected by comparative genomic hybridization. Genes Chromosomes. Cancer *29*, 110-116.

Hampton,G.M., Larson,A.A., Baergen,R.N., Sommers,R.L., Kern,S., and Cavenee,W.K. (1996). Simultaneous assessment of loss of heterozygosity at multiple microsatellite loci using semi-automated fluorescence-based detection: subregional mapping of chromosome 4 in cervical carcinoma. Proc. Natl. Acad. Sci. U. S. A *93*, 6704-6709.

Hartsough,M.T., Morrison,D.K., Salerno,M., Palmieri,D., Ouatas,T., Mair,M., Patrick,J., and Steeg,P.S. (2002). Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. J. Biol. Chem. *277*, 32389-32399.

Hasan,F., Jeffers,L.J., De,M.M., Reddy,K.R., Parker,T., Schiff,E.R., Houghton,M., Choo,Q.L., and Kuo,G. (1990). Hepatitis C-associated hepatocellular carcinoma. Hepatology *12*, 589-591.

Hayashi,H., Sugio,K., Matsumata,T., Adachi,E., Urata,K., Tanaka,S., and Sugimachi,K. (1993). The mutation of codon 249 in the p53 gene is not specific in Japanese hepatocellular carcinoma. Liver *13*, 279-281.

Hickman,E.S., Moroni,M.C., and Helin,K. (2002). The role of p53 and pRB in apoptosis and cancer. Curr. Opin. Genet. Dev. *12*, 60-66.

Hinds,P.W., Dowdy,S.F., Eaton,E.N., Arnold,A., and Weinberg,R.A. (1994). Function of a human cyclin gene as an oncogene. Proc. Natl. Acad. Sci. U. S. A *91*, 709-713.

Hollstein,M., Sidransky,D., Vogelstein,B., and Harris,C.C. (1991). p53 mutations in human cancers. Science *253*, 49-53.

Horikawa,I. and Barrett,J.C. (2003). Transcriptional regulation of the telomerase hTERT gene as a target for cellular and viral oncogenic mechanisms. Carcinogenesis *24*, 1167-1176.

Hsu,I.C., Metcalf,R.A., Sun,T., Welsh,J.A., Wang,N.J., and Harris,C.C. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature *350*, 427-428.

Hyman,E., Kauraniemi,P., Hautaniemi,S., Wolf,M., Mousses,S., Rozenblum,E., Ringner,M., Sauter,G., Monni,O., Elkahloun,A., Kallioniemi,O.P., and Kallioniemi,A. (2002). Impact of DNA amplification on gene expression patterns in breast cancer. Cancer Res. *62*, 6240-6245.

Iliopoulos,D., Guler,G., Han,S.Y., Druck,T., Ottey,M., McCorkell,K.A., and Huebner,K. (2006). Roles of FHIT and WWOX fragile genes in cancer. Cancer Lett. *232*, 27-36.

Imoto,H., Hirotsune,S., Muramatsu,M., Okuda,K., Sugimoto,O., Chapman,V.M., and Hayashizaki,Y. (1994). Direct determination of NotI cleavage sites in the genomic DNA of adult mouse kidney and human trophoblast using whole-range restriction landmark genomic scanning. DNA Res. *1*, 239-243.

Inoue,A., Li,T., Roby,S.K., Valentine,M.B., Inoue,M., Boyd,K., Kidd,V.J., and Lahti,J.M. (2007). Loss of ChlR1 helicase in mouse causes lethality due to the accumulation of aneuploid cells generated by cohesion defects and placental malformation. Cell Cycle *6*, 1646-1654.

Isobe,M., Emanuel,B.S., Givol,D., Oren,M., and Croce,C.M. (1986). Localization of gene for human p53 tumour antigen to band 17p13. Nature *320*, 84-85.

Jaffe,A.B., Aspenstrom,P., and Hall,A. (2004). Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. Mol. Cell Biol. *24*, 1736-1746.

Jin,M., Piao,Z., Kim,N.G., Park,C., Shin,E.C., Park,J.H., Jung,H.J., Kim,C.G., and Kim,H. (2000). p16 is a major inactivation target in hepatocellular carcinoma. Cancer *89*, 60-68.

Johansson,B., Mertens,F., and Mitelman,F. (1995). Cytogenetic evolution patterns in non-Hodgkin's lymphoma. Blood *86*, 3905-3914.

Kaklamani,E., Trichopoulos,D., Tzonou,A., Zavitsanos,X., Koumantaki,Y., Hatzakis,A., Hsieh,C.C., and Hatziyannis,S. (1991). Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. JAMA *265*, 1974-1976.

Kanai,Y., Ushijima,S., Tsuda,H., Sakamoto,M., Sugimura,T., and Hirohashi,S. (1996). Aberrant DNA methylation on chromosome 16 is an early event in hepatocarcinogenesis. Jpn. J. Cancer Res. *87*, 1210-1217.

Kanai,Y., Hui,A.M., Sun,L., Ushijima,S., Sakamoto,M., Tsuda,H., and Hirohashi,S. (1999). DNA hypermethylation at the D17S5 locus and reduced HIC-1 mRNA expression are associated with hepatocarcinogenesis. Hepatology *29*, 703-709.

Kanai,Y., Ushijima,S., Tsuda,H., Sakamoto,M., and Hirohashi,S. (2000). Aberrant DNA methylation precedes loss of heterozygosity on chromosome 16 in chronic hepatitis and liver cirrhosis. Cancer Lett. *148*, 73-80.

Karin,M. and Lin,A. (2002). NF-kappaB at the crossroads of life and death. Nat. Immunol. *3*, 221-227.

Kaur,S., Larramendy,M.L., Vauhkonen,H., Bohling,T., and Knuutila,S. (2007). Loss of TP53 in sarcomas with 17p12 to approximately p11 gain. A fine-resolution oligonucleotide array comparative genomic hybridization study. Cytogenet. Genome Res. *116*, 153-157.

Koch,A., Denkhaus,D., Albrecht,S., Leuschner,I., von,S.D., and Pietsch,T. (1999). Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. Cancer Res. *59*, 269-273.

Koch,A., Weber,N., Waha,A., Hartmann,W., Denkhaus,D., Behrens,J., Birchmeier,W., von,S.D., and Pietsch,T. (2004). Mutations and elevated transcriptional activity of conductin (AXIN2) in hepatoblastomas. J. Pathol. *204*, 546-554.

Kornfeld,K., Hom,D.B., and Horvitz,H.R. (1995). The ksr-1 gene encodes a novel protein kinase involved in Ras-mediated signaling in C. elegans. Cell *83*, 903-913.

Koyama,M., Nagai,H., Bando,K., Ito,M., Moriyama,Y., and Emi,M. (1999). Localization of a target region of allelic loss to a 1-cM interval on chromosome 16p.13.13 in hepatocellular carcinoma. Jpn. J. Cancer Res. *90*, 951-956.

Kubo,T., Yamamoto,J., Shikauchi,Y., Niwa,Y., Matsubara,K., and Yoshikawa,H. (2004). Apoptotic speck protein-like, a highly homologous protein to apoptotic speck protein in the pyrin domain, is silenced by DNA methylation and induces apoptosis in human hepatocellular carcinoma. Cancer Res. *64*, 5172-5177.

Lanigan,T.M., Liu,A., Huang,Y.Z., Mei,L., Margolis,B., and Guan,K.L. (2003). Human homologue of Drosophila CNK interacts with Ras effector proteins Raf and Rlf. FASEB J. *17*, 2048-2060.

Largo,C., Alvarez,S., Saez,B., Blesa,D., Martin-Subero,J.I., Gonzalez-Garcia,I., Brieva,J.A., Dopazo,J., Siebert,R., Calasanz,M.J., and Cigudosa,J.C. (2006). Identification of overexpressed genes in frequently gained/amplified chromosome regions in multiple myeloma. Haematologica *91*, 184-191.

Laurent-Puig,P., Legoix,P., Bluteau,O., Belghiti,J., Franco,D., Binot,F., Monges,G., Thomas,G., Bioulac-Sage,P., and Zucman-Rossi,J. (2001). Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology *120*, 1763-1773.

Lee,J.W., Soung,Y.H., Kim,S.Y., Lee,H.W., Park,W.S., Nam,S.W., Kim,S.H., Lee,J.Y., Yoo,N.J., and Lee,S.H. (2005). PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene *24*, 1477-1480.

Li,C. and Wong,W.H. (2001). Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc. Natl. Acad. Sci. U. S. A *98*, 31-36.

Li,D., Cao,Y., He,L., Wang,N.J., and Gu,J.R. (1993). Aberrations of p53 gene in human hepatocellular carcinoma from China. Carcinogenesis *14*, 169-173.

Li,J., Yen,C., Liaw,D., Podsypanina,K., Bose,S., Wang,S.I., Puc,J., Miliaresis,C., Rodgers,L., McCombie,R., Bigner,S.H., Giovanella,B.C., Ittmann,M., Tycko,B., Hibshoosh,H., Wigler,M.H., and Parsons,R. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science *275*, 1943-1947.

Liao,C., Zhao,M., Song,H., Uchida,K., Yokoyama,K.K., and Li,T. (2000). Identification of the gene for a novel liver-related putative tumor suppressor at a high-frequency loss of heterozygosity region of chromosome 8p23 in human hepatocellular carcinoma. Hepatology *32*, 721-727.

Liew,C.T., Li,H.M., Lo,K.W., Leow,C.K., Chan,J.Y., Hin,L.Y., Lau,W.Y., Lai,P.B., Lim,B.K., Huang,J., Leung,W.T., Wu,S., and Lee,J.C. (1999). High frequency of p16INK4A gene alterations in hepatocellular carcinoma. Oncogene *18*, 789-795.

Liggett,W.H., Jr. and Sidransky,D. (1998). Role of the p16 tumor suppressor gene in cancer. J. Clin. Oncol. *16*, 1197-1206.

Lin,C., Franco,B., and Rosner,M.R. (2005). CDKL5/Stk9 kinase inactivation is associated with neuronal developmental disorders. Hum. Mol. Genet. *14*, 3775-3786.

Lin,M., Wei,L.J., Sellers,W.R., Lieberfarb,M., Wong,W.H., and Li,C. (2004). dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. Bioinformatics. *20*, 1233-1240.

Lin,S.C., Skapek,S.X., and Lee,E.Y. (1996). Genes in the RB pathway and their knockout in mice. Semin. Cancer Biol. *7*, 279-289.

Lisitsyn,N.A., Rosenberg,M.V., Launer,G.A., Wagner,L.L., Potapov,V.K., Kolesnik,T.B., and Sverdlov,E.D. (1993). A method for isolation of sequences missing in one of two related genomes. Mol. Gen. Mikrobiol. Virusol. 26-29.

Liu,W., Chang,B., Sauvageot,J., Dimitrov,L., Gielzak,M., Li,T., Yan,G., Sun,J., Sun,J., Adams,T.S., Turner,A.R., Kim,J.W., Meyers,D.A., Zheng,S.L., Isaacs,W.B., and Xu,J. (2006). Comprehensive assessment of DNA copy number alterations in human prostate cancers using Affymetrix 100K SNP mapping array. Genes Chromosomes. Cancer *45*, 1018-1032.

Ljubimova,J.Y., Wilson,S.E., Petrovic,L.M., Ehrenman,K., Ljubimov,A.V., Demetriou,A.A., Geller,S.A., and Black,K.L. (1998). Novel human malignancy-associated gene (MAG) expressed in various tumors and in some tumor preexisting conditions. Cancer Res. *58*, 4475-4479.

Lukas,J., Parry,D., Aagaard,L., Mann,D.J., Bartkova,J., Strauss,M., Peters,G., and Bartek,J. (1995). Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature *375*, 503-506.

Lv,Z., Zhang,M., Bi,J., Xu,F., Hu,S., and Wen,J. (2006). Promoter hypermethylation of a novel gene, ZHX2, in hepatocellular carcinoma. Am. J. Clin. Pathol. *125*, 740-746.

Maeta,Y., Shiota,G., Okano,J., and Murawaki,Y. (2005). Effect of promoter methylation of the p16 gene on phosphorylation of retinoblastoma gene product and growth of hepatocellular carcinoma cells. Tumour. Biol. *26*, 300-305.

Malkin,D., Li,F.P., Strong,L.C., Fraumeni,J.F., Jr., Nelson,C.E., Kim,D.H., Kassel,J., Gryka,M.A., Bischoff,F.Z., Tainsky,M.A., and . (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science *250*, 1233-1238.

Mandahl,N. (1996). Cytogenetics and molecular genetics of bone and soft tissue tumors. Adv. Cancer Res. *69*, 63-99.

Mandahl,N., Limon,J., Mertens,F., Arheden,K., and Mitelman,F. (1996). Ring marker containing 17q and chromosome 22 in a case of dermatofibrosarcoma protuberans. Cancer Genet. Cytogenet. *89*, 88-91.

Marchio,A., Meddeb,M., Pineau,P., Danglot,G., Tiollais,P., Bernheim,A., and Dejean,A. (1997). Recurrent chromosomal abnormalities in hepatocellular carcinoma detected by comparative genomic hybridization. Genes Chromosomes. Cancer *18*, 59-65.

Mari,F., Azimonti,S., Bertani,I., Bolognese,F., Colombo,E., Caselli,R., Scala,E., Longo,I., Grosso,S., Pescucci,C., Ariani,F., Hayek,G., Balestri,P., Bergo,A., Badaracco,G., Zappella,M., Broccoli,V., Renieri,A., Kilstrup-Nielsen,C., and Landsberger,N. (2005). CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. Hum. Mol. Genet. *14*, 1935-1946.

Matsuda,Y., Ichida,T., Matsuzawa,J., Sugimura,K., and Asakura,H. (1999). p16(INK4) is inactivated by extensive CpG methylation in human hepatocellular carcinoma. Gastroenterology *116*, 394-400.

Mattie,M.D., Benz,C.C., Bowers,J., Sensinger,K., Wong,L., Scott,G.K., Fedele,V., Ginzinger,D., Getts,R., and Haqq,C. (2006). Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol. Cancer *5*, 24.

Meloche,S. and Pouyssegur,J. (2007). The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene *26*, 3227-3239.

Michaux,L., Wlodarska,I., Theate,I., Stul,M., Scheiff,J.M., Deneys,V., Ferrant,A., and Hagemeijer,A. (2004). Coexistence of BCL1/CCND1 and CMYC aberrations in blastoid mantle cell lymphoma: a rare finding associated with very poor outcome. Ann. Hematol. *83*, 578-583.

Midorikawa,Y., Tsutsumi,S., Nishimura,K., Kamimura,N., Kano,M., Sakamoto,H., Makuuchi,M., and Aburatani,H. (2004). Distinct chromosomal bias of gene expression signatures in the progression of hepatocellular carcinoma. Cancer Res. *64*, 7263-7270.

Miller,C., Mohandas,T., Wolf,D., Prokocimer,M., Rotter,V., and Koeffler,H.P. (1986). Human p53 gene localized to short arm of chromosome 17. Nature *319*, 783-784.

Milyavsky,M., Shats,I., Cholostoy,A., Brosh,R., Buganim,Y., Weisz,L., Kogan,I., Cohen,M., Shatz,M., Madar,S., Kalo,E., Goldfinger,N., Yuan,J., Ron,S., MacKenzie,K., Eden,A., and Rotter,V. (2007). Inactivation of myocardin and p16 during malignant transformation contributes to a differentiation defect. Cancer Cell *11*, 133-146.

Mitelman,F., Johansson,B., Mandahl,N., and Mertens,F. (1997). Clinical significance of cytogenetic findings in solid tumors. Cancer Genet. Cytogenet. *95*, 1-8.

Miyoshi,Y., Iwao,K., Nagasawa,Y., Aihara,T., Sasaki,Y., Imaoka,S., Murata,M., Shimano,T., and Nakamura,Y. (1998). Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res. *58*, 2524-2527.

Morin,P.J., Sparks,A.B., Korinek,V., Barker,N., Clevers,H., Vogelstein,B., and Kinzler,K.W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science *275*, 1787-1790.

Moriya,K., Fujie,H., Shintani,Y., Yotsuyanagi,H., Tsutsumi,T., Ishibashi,K., Matsuura,Y., Kimura,S., Miyamura,T., and Koike,K. (1998). The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat. Med. *4*, 1065-1067.

Motyka,B., Korbutt,G., Pinkoski,M.J., Heibein,J.A., Caputo,A., Hobman,M., Barry,M., Shostak,I., Sawchuk,T., Holmes,C.F., Gauldie,J., and Bleackley,R.C. (2000). Mannose 6-phosphate/insulin-like growth factor II receptor is a death receptor for granzyme B during cytotoxic T cell-induced apoptosis. Cell *103*, 491-500.

Murata,H., Tsuji,S., Tsujii,M., Sakaguchi,Y., Fu,H.Y., Kawano,S., and Hori,M. (2004). Promoter hypermethylation silences cyclooxygenase-2 (Cox-2) and regulates growth of human hepatocellular carcinoma cells. Lab Invest *84*, 1050-1059.

Nagai,H., Pineau,P., Tiollais,P., Buendia,M.A., and Dejean,A. (1997). Comprehensive allelotyping of human hepatocellular carcinoma. Oncogene *14*, 2927-2933.

Nagai,M.A., Yamamoto,L., Salaorni,S., Pacheco,M.M., Brentani,M.M., Barbosa,E.M., Brentani,R.R., Mazoyer,S., Smith,S.A., Ponder,B.A., and . (1994). Detailed deletion mapping of chromosome segment 17q12-21 in sporadic breast tumours. Genes Chromosomes. Cancer *11*, 58-62.

Ng,I.O., Ng,M.M., Lai,E.C., and Fan,S.T. (1995). Better survival in female patients with hepatocellular carcinoma. Possible causes from a pathologic approach. Cancer *75*, 18-22.

Nishimura,T., Nishida,N., Itoh,T., Kuno,M., Minata,M., Komeda,T., Fukuda,Y., Ikai,I., Yamaoka,Y., and Nakao,K. (2002). Comprehensive allelotyping of well-differentiated human hepatocellular carcinoma with semiquantitative determination of chromosomal gain or loss. Genes Chromosomes. Cancer *35*, 329-339.

Nishioka,K., Watanabe,J., Furuta,S., Tanaka,E., Iino,S., Suzuki,H., Tsuji,T., Yano,M., Kuo,G., Choo,Q.L., and . (1991). A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. Cancer *67*, 429-433.

Oh,B.K., Lee,C.H., Park,C., and Park,Y.N. (2004). Telomerase regulation and progressive telomere shortening of rat hepatic stem-like epithelial cells during in vitro aging. Exp. Cell Res. *298*, 445-454.

Ohmachi,M., Rocheleau,C.E., Church,D., Lambie,E., Schedl,T., and Sundaram,M.V. (2002). C. elegans ksr-1 and ksr-2 have both unique and redundant functions and are required for MPK-1 ERK phosphorylation. Curr. Biol. *12*, 427-433.

Oka,Y., Waterland,R.A., Killian,J.K., Nolan,C.M., Jang,H.S., Tohara,K., Sakaguchi,S., Yao,T., Iwashita,A., Yata,Y., Takahara,T., Sato,S., Suzuki,K., Masuda,T., and Jirtle,R.L.

(2002). M6P/IGF2R tumor suppressor gene mutated in hepatocellular carcinomas in Japan. Hepatology *35*, 1153-1163.

Okamoto,H., Yasui,K., Zhao,C., Arii,S., and Inazawa,J. (2003). PTK2 and EIF3S3 genes may be amplification targets at 8q23-q24 and are associated with large hepatocellular carcinomas. Hepatology *38*, 1242-1249.

Orlow,I., Lacombe,L., Hannon,G.J., Serrano,M., Pellicer,I., Dalbagni,G., Reuter,V.E., Zhang,Z.F., Beach,D., and Cordon-Cardo,C. (1995). Deletion of the p16 and p15 genes in human bladder tumors. J. Natl. Cancer Inst. *87*, 1524-1529.

Ozturk,N., Erdal,E., Mumcuoglu,M., Akcali,K.C., Yalcin,O., Senturk,S., rslan-Ergul,A., Gur,B., Yulug,I., Cetin-Atalay,R., Yakicier,C., Yagci,T., Tez,M., and Ozturk,M. (2006). Reprogramming of replicative senescence in hepatocellular carcinoma-derived cells. Proc. Natl. Acad. Sci. U. S. A *103*, 2178-2183.

Paradis,V., Laurendeau,I., Vidaud,M., and Bedossa,P. (1998). Clonal analysis of macronodules in cirrhosis. Hepatology *28*, 953-958.

Parkin,D.M. (2001). Global cancer statistics in the year 2000. Lancet Oncol. *2*, 533-543.

Paterlini-Brechot,P., Saigo,K., Murakami,Y., Chami,M., Gozuacik,D., Mugnier,C., Lagorce,D., and Brechot,C. (2003). Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. Oncogene *22*, 3911-3916.

Pejovic,T., Heim,S., Mandahl,N., Baldetorp,B., Elmfors,B., Floderus,U.M., Furgyik,S., Helm,G., Himmelmann,A., Willen,H., and . (1992). Chromosome aberrations in 35 primary ovarian carcinomas. Genes Chromosomes. Cancer *4*, 58-68.

Pejovic,T., Himmelmann,A., Heim,S., Mandahl,N., Floderus,U.M., Furgyik,S., Elmfors,B., Helm,G., Willen,H., and Mitelman,F. (1992). Prognostic impact of chromosome aberrations in ovarian cancer. Br. J. Cancer *65*, 282-286.

Piao,Z., Kim,H., Jeon,B.K., Lee,W.J., and Park,C. (1997). Relationship between loss of heterozygosity of tumor suppressor genes and histologic differentiation in hepatocellular carcinoma. Cancer *80*, 865-872.

Piao,Z., Park,C., Park,J.H., and Kim,H. (1998). Allelotype analysis of hepatocellular carcinoma. Int. J. Cancer *75*, 29-33.

Pikarsky,E., Porat,R.M., Stein,I., Abramovitch,R., Amit,S., Kasem,S., Gutkovich-Pyest,E., Urieli-Shoval,S., Galun,E., and Ben-Neriah,Y. (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature *431*, 461-466.

Pineau,P., Nagai,H., Prigent,S., Wei,Y., Gyapay,G., Weissenbach,J., Tiollais,P., Buendia,M.A., and Dejean,A. (1999). Identification of three distinct regions of allelic deletions on the short arm of chromosome 8 in hepatocellular carcinoma. Oncogene *18*, 3127-3134.

Pinkel,D., Segraves,R., Sudar,D., Clark,S., Poole,I., Kowbel,D., Collins,C., Kuo,W.L., Chen,C., Zhai,Y., Dairkee,S.H., Ljung,B.M., Gray,J.W., and Albertson,D.G. (1998). High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat. Genet. *20*, 207-211.

Pritham,E.J. and Feschotte,C. (2007). Massive amplification of rolling-circle transposons in the lineage of the bat Myotis lucifugus. Proc. Natl. Acad. Sci. U. S. A *104*, 1895-1900.

Puzianowska-Kuznicka,M., Krystyniak,A., Madej,A., Cheng,S.Y., and Nauman,J. (2002). Functionally impaired TR mutants are present in thyroid papillary cancer. J. Clin. Endocrinol. Metab *87*, 1120-1128.

Qin,H.R., Iliopoulos,D., Semba,S., Fabbri,M., Druck,T., Volinia,S., Croce,C.M., Morrison,C.D., Klein,R.D., and Huebner,K. (2006). A role for the WWOX gene in prostate cancer. Cancer Res. *66*, 6477-6481.

Ray,R.B., Lagging,L.M., Meyer,K., and Ray,R. (1996). Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. J. Virol. *70*, 4438-4443.

Ray,R.B., Steele,R., Meyer,K., and Ray,R. (1997). Transcriptional repression of p53 promoter by hepatitis C virus core protein. J. Biol. Chem. *272*, 10983-10986.

Rayet,B. and Gelinas,C. (1999). Aberrant rel/nfkb genes and activity in human cancer. Oncogene *18*, 6938-6947.

Rowley,J.D. (1998). The critical role of chromosome translocations in human leukemias. Annu. Rev. Genet. *32*, 495-519.

Ruivenkamp,C.A., van,W.T., Zanon,C., Stassen,A.P., Vlcek,C., Csikos,T., Klous,A.M., Tripodis,N., Perrakis,A., Boerrigter,L., Groot,P.C., Lindeman,J., Mooi,W.J., Meijjer,G.A., Scholten,G., Dauwerse,H., Paces,V., van,Z.N., van Ommen,G.J., and Demant,P. (2002). Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers. Nat. Genet. *31*, 295-300.

Saito,I., Miyamura,T., Ohbayashi,A., Harada,H., Katayama,T., Kikuchi,S., Watanabe,Y., Koi,S., Onji,M., Ohta,Y., and . (1990). Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc. Natl. Acad. Sci. U. S. A *87*, 6547-6549.

Sakabe,T., Shinomiya,T., Mori,T., Ariyama,Y., Fukuda,Y., Fujiwara,T., Nakamura,Y., and Inazawa,J. (1999). Identification of a novel gene, MASL1, within an amplicon at 8p23.1 detected in malignant fibrous histiocytomas by comparative genomic hybridization. Cancer Res. *59*, 511-515.

Sakakura,C., Hagiwara,A., Taniguchi,H., Yamaguchi,T., Yamagishi,H., Takahashi,T., Koyama,K., Nakamura,Y., Abe,T., and Inazawa,J. (1999). Chromosomal aberrations in human hepatocellular carcinomas associated with hepatitis C virus infection detected by comparative genomic hybridization. Br. J. Cancer *80*, 2034-2039.

Sato,F., Kurokawa,M., Yamauchi,N., and Hattori,M.A. (2006). Gene silencing of myostatin in differentiation of chicken embryonic myoblasts by small interfering RNA. Am. J. Physiol Cell Physiol *291*, C538-C545.

Sato,M., Takahashi,K., Nagayama,K., Arai,Y., Ito,N., Okada,M., Minna,J.D., Yokota,J., and Kohno,T. (2005). Identification of chromosome arm 9p as the most frequent target of homozygous deletions in lung cancer. Genes Chromosomes. Cancer *44*, 405-414.

Satoh,S., Daigo,Y., Furukawa,Y., Kato,T., Miwa,N., Nishiwaki,T., Kawasoe,T., Ishiguro,H., Fujita,M., Tokino,T., Sasaki,Y., Imaoka,S., Murata,M., Shimano,T., Yamaoka,Y., and Nakamura,Y. (2000). AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat. Genet. *24*, 245-250.

Schimke,R.T., Kaufman,R.J., Alt,F.W., and Kellems,R.F. (1978). Gene amplification and drug resistance in cultured murine cells. Science *202*, 1051-1055.

Schrock,E., du,M.S., Veldman,T., Schoell,B., Wienberg,J., Ferguson-Smith,M.A., Ning,Y., Ledbetter,D.H., Bar-Am,I., Soenksen,D., Garini,Y., and Ried,T. (1996). Multicolor spectral karyotyping of human chromosomes. Science *273*, 494-497.

Schwab,M. (1999). Oncogene amplification in solid tumors. Semin. Cancer Biol. *9*, 319-325.

Senechal,K., Halpern,J., and Sawyers,C.L. (1996). The CRKL adaptor protein transforms fibroblasts and functions in transformation by the BCR-ABL oncogene. J. Biol. Chem. *271*, 23255-23261.

Senechal,K., Maury,O., Le,B.H., Ledoux,I., and Zyss,J. (2002). Zinc(II) as a versatile template for the design of dipolar and octupolar NLO-phores. J. Am. Chem. Soc. *124*, 4560-4561.

Shih,Y.L., Hsieh,C.B., Lai,H.C., Yan,M.D., Hsieh,T.Y., Chao,Y.C., and Lin,Y.W. (2007). SFRP1 suppressed hepatoma cells growth through Wnt canonical signaling pathway. Int. J. Cancer *121*, 1028-1035.

Shirakawa,H., Landsman,D., Postnikov,Y.V., and Bustin,M. (2000). NBP-45, a novel nucleosomal binding protein with a tissue-specific and developmentally regulated expression. J. Biol. Chem. *275*, 6368-6374.

Silva,J.M., Dominguez,G., Gonzalez-Sancho,J.M., Garcia,J.M., Silva,J., Garcia-Andrade,C., Navarro,A., Munoz,A., and Bonilla,F. (2002). Expression of thyroid hormone receptor/erbA genes is altered in human breast cancer. Oncogene *21*, 4307-4316.

Sirma,H., Giannini,C., Poussin,K., Paterlini,P., Kremsdorf,D., and Brechot,C. (1999). Hepatitis B virus X mutants, present in hepatocellular carcinoma tissue abrogate both the antiproliferative and transactivation effects of HBx. Oncogene *18*, 4848-4859.

Sjoblom,T., Jones,S., Wood,L.D., Parsons,D.W., Lin,J., Barber,T.D., Mandelker,D., Leary,R.J., Ptak,J., Silliman,N., Szabo,S., Buckhaults,P., Farrell,C., Meeh,P., Markowitz,S.D., Willis,J., Dawson,D., Willson,J.K., Gazdar,A.F., Hartigan,J., Wu,L., Liu,C., Parmigiani,G., Park,B.H., Bachman,K.E., Papadopoulos,N., Vogelstein,B., Kinzler,K.W., and Velculescu,V.E. (2006). The consensus coding sequences of human breast and colorectal cancers. Science *314*, 268-274.

Slamon,D.J., Godolphin,W., Jones,L.A., Holt,J.A., Wong,S.G., Keith,D.E., Levin,W.J., Stuart,S.G., Udove,J., Ullrich,A., and . (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science *244*, 707-712.

Smela,M.E., Currier,S.S., Bailey,E.A., and Essigmann,J.M. (2001). The chemistry and biology of aflatoxin B(1): from mutational spectrometry to carcinogenesis. Carcinogenesis *22*, 535-545.

Snyder,R.L., Tyler,G., and Summers,J. (1982). Chronic hepatitis and hepatocellular carcinoma associated with woodchuck hepatitis virus. Am. J. Pathol. *107*, 422-425.

Stark,M. and Hayward,N. (2007). Genome-wide loss of heterozygosity and copy number analysis in melanoma using high-density single-nucleotide polymorphism arrays. Cancer Res. *67*, 2632-2642.

Steeg,P.S. (2003). Metastasis suppressors alter the signal transduction of cancer cells. Nat. Rev. Cancer *3*, 55-63.

Stroffolini,T., Andreone,P., Andriulli,A., Ascione,A., Craxi,A., Chiaramonte,M., Galante,D., Manghisi,O.G., Mazzanti,R., Medaglia,C., Pilleri,G., Rapaccini,G.L., Simonetti,R.G., Taliani,G., Tosti,M.E., Villa,E., and Gasbarrini,G. (1998). Characteristics of hepatocellular carcinoma in Italy. J. Hepatol. *29*, 944-952.

Stroffolini,T., Andreone,P., Andriulli,A., Ascione,A., Craxi,A., Chiaramonte,M., Galante,D., Manghisi,O.G., Mazzanti,R., Medaglia,C., Pilleri,G., Rapaccini,G.L., Simonetti,R.G., Taliani,G., Tosti,M.E., Villa,E., and Gasbarrini,G. (1998). Characteristics of hepatocellular carcinoma in Italy. J. Hepatol. *29*, 944-952.

Suhasini,M., Reddy,C.D., Reddy,E.P., DiDonato,J.A., and Pilz,R.B. (1997). cAMP-induced NF-kappaB (p50/relB) binding to a c-myb intronic enhancer correlates with c-myb up-regulation and inhibition of erythroleukemia cell differentiation. Oncogene *15*, 1859-1870.

Szafranska,A.E., Davison,T.S., John,J., Cannon,T., Sipos,B., Maghnouj,A., Labourier,E., and Hahn,S.A. (2007). MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene *26*, 4442-4452.

Takano,S., Yokosuka,O., Imazeki,F., Tagawa,M., and Omata,M. (1995). Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology *21*, 650-655.

Tang B, Li YN, Kruger WD. (2000). Defects in methylthioadenosine phosphorylase are associated with but not responsible for methionine-dependent tumor cell growth. Cancer Res. 2000 Oct 1;60(19):5543-7.

Tangkijvanich,P., Hourpai,N., Rattanatanyong,P., Wisedopas,N., Mahachai,V., and Mutirangura,A. (2007). Serum LINE-1 hypomethylation as a potential prognostic marker for hepatocellular carcinoma. Clin. Chim. Acta *379*, 127-133.

ten,H.J., Morris,C., Heisterkamp,N., and Groffen,J. (1993). Isolation and chromosomal localization of CRKL, a human crk-like gene. Oncogene *8*, 2469-2474.

Terradillos,O., Pollicino,T., Lecoeur,H., Tripodi,M., Gougeon,M.L., Tiollais,P., and Buendia,M.A. (1998). p53-independent apoptotic effects of the hepatitis B virus HBx protein in vivo and in vitro. Oncogene *17*, 2115-2123.

Tessitore,L., Marengo,B., Vance,D.E., Papotti,M., Mussa,A., Daidone,M.G., and Costa,A. (2003). Expression of phosphatidylethanolamine N-methyltransferase in human hepatocellular carcinomas. Oncology *65*, 152-158.

Thomas,R.M., Berman,J.J., Yetter,R.A., Moore,G.W., and Hutchins,G.M. (1992). Liver cell dysplasia: a DNA aneuploid lesion with distinct morphologic features. Hum. Pathol. *23*, 496-503.

Thorgeirsson,S.S. and Grisham,J.W. (2002). Molecular pathogenesis of human hepatocellular carcinoma. Nat. Genet. *31*, 339-346.

Tsuchiya,S., Okuno,Y., and Tsujimoto,G. (2006). MicroRNA: biogenetic and functional mechanisms and involvements in cell differentiation and cancer. J. Pharmacol. Sci. *101*, 267-270.

Ueda,H., Ullrich,S.J., Gangemi,J.D., Kappel,C.A., Ngo,L., Feitelson,M.A., and Jay,G. (1995). Functional inactivation but not structural mutation of p53 causes liver cancer. Nat. Genet. *9*, 41-47.

Vaidyanathan,G., Cismowski,M.J., Wang,G., Vincent,T.S., Brown,K.D., and Lanier,S.M. (2004). The Ras-related protein AGS1/RASD1 suppresses cell growth. Oncogene *23*, 5858-5863.

van,D.H., Geelen,E., Dinjens,W.N., Wijnhoven,B.P., Tilanus,H.W., Tanke,H.J., and Rosenberg,C. (1999). Comparative genomic hybridization of cancer of the gastroesophageal junction: deletion of 14Q31-32.1 discriminates between esophageal (Barrett's) and gastric cardia adenocarcinomas. Cancer Res. *59*, 748-752.

van,D.M., van,M.R., Watson,J.E., Paris,P.L., Lapuk,A., Brown,N., Oseroff,V.V., Albertson,D.G., Pinkel,D., de,J.P., Nacheva,E.P., Dinjens,W., van,D.H., and Collins,C. (2005). Construction and application of a full-coverage, high-resolution, human

chromosome 8q genomic microarray for comparative genomic hybridization. Cytometry A *63*, 10-19.

van,D.M., van,M.R., Vissers,K., Watson,J.E., van Weerden,W.M., Schroder,F.H., Hop,W.C., van der Kwast,T.H., Collins,C., and van,D.H. (2005). High-resolution array comparative genomic hybridization of chromosome arm 8q: evaluation of genetic progression markers for prostate cancer. Genes Chromosomes. Cancer *44*, 438-449.

Vargas,V., Castells,L., and Esteban,J.I. (1990). High frequency of antibodies to the hepatitis C virus among patients with hepatocellular carcinoma. Ann. Intern. Med. *112*, 232-233.

Vautier,G., Bomford,A.B., Portmann,B.C., Metivier,E., Williams,R., and Ryder,S.D. (1999). p53 mutations in british patients with hepatocellular carcinoma: clustering in genetic hemochromatosis. Gastroenterology *117*, 154-160.

Wahl,G.M., Padgett,R.A., and Stark,G.R. (1979). Gene amplification causes overproduction of the first three enzymes of UMP synthesis in N-(phosphonacetyl)-L-aspartate-resistant hamster cells. J. Biol. Chem. *254*, 8679-8689.

Wang,G., Chen,H., Huang,M., Wang,N., Zhang,J., Zhang,Y., Bai,G., Fong,W.F., Yang,M., and Yao,X. (2006). Methyl protodioscin induces G2/M cell cycle arrest and apoptosis in HepG2 liver cancer cells. Cancer Lett. *241*, 102-109.

Wang,J., Chenivesse,X., Henglein,B., and Brechot,C. (1990). Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature *343*, 555-557.

Wang,L., Wang,W.L., Zhang,Y., Guo,S.P., Zhang,J., and Li,Q.L. (2007). Epigenetic and genetic alterations of PTEN in hepatocellular carcinoma. Hepatol. Res. *37*, 389-396.

Wang,X.W., Forrester,K., Yeh,H., Feitelson,M.A., Gu,J.R., and Harris,C.C. (1994). Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc. Natl. Acad. Sci. U. S. A *91*, 2230-2234.

Wang,Y., Shirogane,T., Liu,D., Harper,J.W., and Elledge,S.J. (2003). Exit from exit: resetting the cell cycle through Amn1 inhibition of G protein signaling. Cell *112*, 697-709.

Wei,Y., Fourel,G., Ponzetto,A., Silvestro,M., Tiollais,P., and Buendia,M.A. (1992). Hepadnavirus integration: mechanisms of activation of the N-myc2 retrotransposon in woodchuck liver tumors. J. Virol. *66* , 5265-5276.

Wei,Y., Fabre,M., Branchereau,S., Gauthier,F., Perilongo,G., and Buendia,M.A. (2000). Activation of beta-catenin in epithelial and mesenchymal hepatoblastomas. Oncogene *19*, 498-504.

Weihrauch,M., Benicke,M., Lehnert,G., Wittekind,C., Wrbitzky,R., and Tannapfel,A. (2001). Frequent k- ras -2 mutations and p16(INK4A)methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. Br. J. Cancer *84*, 982-989.

Wiederschain D, Kawai H, Shilatifard A, Yuan ZM. Multiple Mixed Lineage Leukemia (MLL) Fusion Proteins Suppress p53-mediated Response to DNA Damage. J Biol Chem 2005 Jul 01;280(26):24315-21.

Willenbucher,R.F., Aust,D.E., Chang,C.G., Zelman,S.J., Ferrell,L.D., Moore,D.H., and Waldman,F.M. (1999). Genomic instability is an early event during the progression pathway of ulcerative-colitis-related neoplasia. Am. J. Pathol. *154*, 1825-1830.

Wong,C.M., Lee,J.M., Ching,Y.P., Jin,D.Y., and Ng,I.O. (2003). Genetic and epigenetic alterations of DLC-1 gene in hepatocellular carcinoma. Cancer Res. *63*, 7646-7651.

Wong,C.M., Ng,Y.L., Lee,J.M., Wong,C.C., Cheung,O.F., Chan,C.Y., Tung,E.K., Ching,Y.P., and Ng,I.O. (2007). Tissue factor pathway inhibitor-2 as a frequently silenced tumor suppressor gene in hepatocellular carcinoma. Hepatology *45*, 1129-1138.

Wong,I.H., Lo,Y.M., Zhang,J., Liew,C.T., Ng,M.H., Wong,N., Lai,P.B., Lau,W.Y., Hjelm,N.M., and Johnson,P.J. (1999). Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res. *59*, 71-73.

Wong,K. and Cantley,L.C. (1994). Cloning and characterization of a human phosphatidylinositol 4-kinase. J. Biol. Chem. *269*, 28878-28884.

Wong,K.S., Li,Y.J., Howard,J., and Ben-David,Y. (1999). Loss of p53 in F-MuLV induced-erythroleukemias accelerates the acquisition of mutational events that confers immortality and growth factor independence. Oncogene *18*, 5525-5534.

Wong,N., Lai,P., Pang,E., Fung,L.F., Sheng,Z., Wong,V., Wang,W., Hayashi,Y., Perlman,E., Yuna,S., Lau,J.W., and Johnson,P.J. (2000). Genomic aberrations in human hepatocellular carcinomas of differing etiologies. Clin. Cancer Res. *6*, 4000-4009.

Wurmbach,E., Chen,Y.B., Khitrov,G., Zhang,W., Roayaie,S., Schwartz,M., Fiel,I., Thung,S., Mazzaferro,V., Bruix,J., Bottinger,E., Friedman,S., Waxman,S., and Llovet,J.M. (2007). Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. Hepatology *45*, 938-947.

Yabuta,N., Okada,N., Ito,A., Hosomi,T., Nishihara,S., Sasayama,Y., Fujimori,A., Okuzaki,D., Zhao,H., Ikawa,M., Okabe,M., and Nojima,H. (2007). Lats2 is an essential mitotic regulator required for the coordination of cell division. J. Biol. Chem. *282*, 19259-19271.

Yakicier,M.C., Irmak,M.B., Romano,A., Kew,M., and Ozturk,M. (1999). Smad2 and Smad4 gene mutations in hepatocellular carcinoma. Oncogene *18*, 4879-4883.

Yakicier,M.C., Legoix,P., Vaury,C., Gressin,L., Tubacher,E., Capron,F., Bayer,J., Degott,C., Balabaud,C., and Zucman-Rossi,J. (2001). Identification of homozygous deletions at chromosome 16q23 in aflatoxin B1 exposed hepatocellular carcinoma. Oncogene *20*, 5232-5238.

Yeh,C.T., Shen,C.H., Tai,D.I., Chu,C.M., and Liaw,Y.F. (2000). Identification and characterization of a prevalent hepatitis B virus X protein mutant in Taiwanese patients with hepatocellular carcinoma. Oncogene *19*, 5213-5220.

Yeh,S.H., Chen,P.J., Shau,W.Y., Chen,Y.W., Lee,P.H., Chen,J.T., and Chen,D.S. (2001). Chromosomal allelic imbalance evolving from liver cirrhosis to hepatocellular carcinoma. Gastroenterology *121*, 699-709.

Yoshikawa,H., Matsubara,K., Qian,G.S., Jackson,P., Groopman,J.D., Manning,J.E., Harris,C.C., and Herman,J.G. (2001). SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat. Genet. *28*, 29-35.

Yu,D.Y., Moon,H.B., Son,J.K., Jeong,S., Yu,S.L., Yoon,H., Han,Y.M., Lee,C.S., Park,J.S., Lee,C.H., Hyun,B.H., Murakami,S., and Lee,K.K. (1999). Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. J. Hepatol. *31*, 123-132.

Yu,J., Zhang,H.Y., Ma,Z.Z., Lu,W., Wang,Y.F., and Zhu,J.D. (2003). Methylation profiling of twenty four genes and the concordant methylation behaviours of nineteen genes that may contribute to hepatocellular carcinogenesis. Cell Res. *13*, 319-333.

Yuan,J.M., Lu,S.C., Van Den,B.D., Govindarajan,S., Zhang,Z.Q., Mato,J.M., and Yu,M.C. (2007). Genetic polymorphisms in the methylenetetrahydrofolate reductase and thymidylate synthase genes and risk of hepatocellular carcinoma. Hepatology.

Zhang,C., Li,H., Zhou,G., Zhang,Q., Zhang,T., Li,J., Zhang,J., Hou,J., Liew,C.T., and Yin,D. (2007). Transcriptional silencing of the TMS1/ASC tumour suppressor gene by an epigenetic mechanism in hepatocellular carcinoma cells. J. Pathol. *212*, 134-142.

Zhang,X., Xu,H.J., Murakami,Y., Sachse,R., Yashima,K., Hirohashi,S., Hu,S.X., Benedict,W.F., and Sekiya,T. (1994). Deletions of chromosome 13q, mutations in Retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. Cancer Res. *54*, 4177-4182.

Zhang,X.K., Egan,J.O., Huang,D., Sun,Z.L., Chien,V.K., and Chiu,J.F. (1992). Hepatitis B virus DNA integration and expression of an erb B-like gene in human hepatocellular carcinoma. Biochem. Biophys. Res. Commun. *188*, 344-351.

Zhao,X., Weir,B.A., LaFramboise,T., Lin,M., Beroukhim,R., Garraway,L., Beheshti,J., Lee,J.C., Naoki,K., Richards,W.G., Sugarbaker,D., Chen,F., Rubin,M.A., Janne,P.A., Girard,L., Minna,J., Christiani,D., Li,C., Sellers,W.R., and Meyerson,M. (2005). Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. Cancer Res. *65*, 5561-5570.

Zimonjic,D.B., Keck,C.L., Thorgeirsson,S.S., and Popescu,N.C. (1999). Novel recurrent genetic imbalances in human hepatocellular carcinoma cell lines identified by comparative genomic hybridization. Hepatology *29*, 1208-1214.