Original Article

Genomic alterations across six hepatocellular carcinoma cell lines by panel-based sequencing

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Background: Current sequencing efforts have revealed the mutational landscape of patients with hepatocellular carcinoma (HCC). However, genetic alterations in human HCC cellular models have remained unclear.

Methods: We present a panel-based sequencing method to identify somatic mutations in six human hepatoma cell lines (HuH-7, Hep3B, SK-HEP-1, MHCC97, HepG2 and HepG2.2.15). Target enrichments from a genomic library of captured exons of 325 mutated genes in various types of cancer were then used for paired-end sequencing.

Results: This method exhibited a 99.7% average coverage rate in target regions with an approximately 1,000 read depth. We discovered 344 somatic non-synonymous variations in 100 genes. Among these genes, 38 significantly altered cancer-related genes were identified and enriched in the following five oncogenic pathways: chromatin remodeling, Notch, MAPK, p53 cell cycle and Wnt/β-catenin. Four cell lines (HuH-7, Hep3B, SK-HEP-1 and HepG2) established from different HCC individuals had different mutational patterns. However, genomic alterations in two series of cell lines from parent MHCC97 and HepG2 cells both showed similarities and some minor discrepancies.

Conclusions: Our panel-based sequencing analysis of HCC cell lines identified genomic alterations in HCC experimental cellular models as well as the mutational patterns of cells from different and same clone origins. These investigations of HCC cell lines provide new insights into the understanding the genetic heterogeneity and clonal evolution of liver cancer.

Keywords: Hepatocellular carcinoma (HCC); cell lines; targeted sequencing; somatic mutation; clone origin

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Introduction

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is the third leading cause of cancer-related death globally (1). Hepatocarcinogenesis is a complex multistep process driven by the accumulation of genetic alterations in oncogenic genes and pathways (2,3). In recent years, multicenter and multi-omic cancer genome projects based on next-generation sequencing (NGS) have exposed the comprehensive mutational landscape and core oncogenic network of HCC patients, including

aberrant signaling cascades, such as the p53 cell cycle, Wnt/β-catenin and MAPK pathways (4,5). A number of cell lines established from HCC tumors have been extensively used as experimental models for this disease, with the advantage of a relative homogeneity of the genetic traits within a cell colony compared to tissues. However, little attention has focused on the mutational patterns in HCC cell lines.

Conventional methods, such as whole-genome sequencing, provide extensive variation landscapes of the cancer genome; however, these results are obtained at the expense of relatively low coverage and a large amount of redundancy data, which are obstacles to the application of these methods in clinical practice (6). Gene panel testing is an emerging option for searching for genetic variants of specific genes of interest (7-9). Gene panel testing has several remarkable advantages compared to full-genome sequencing, including deeper sequencing, higher coverage for rare variant identification and the generation of more manageable data (6).

Therefore, we established a panel-based sequencing platform targeting 325 genes mutated in various types of cancer to identify somatic mutations in six HCC cell lines. This study aimed to test the performance of the targeted sequencing platform for identification of genetic variants in cancer-related genes and, more importantly, to explore the somatic variations in HCC experimental cellular models compared to previous knowledge of mutation status in HCC tissues.

Methods

Cell line culture

A total of six human hepatoma cell lines were used. The HepG2 cell line was originally obtained from the American Type Culture Collection (Manassas, VA, USA). HuH-7, Hep3B and SK-HEP-1 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The MHCC97 series cells (MHCC97H and MHCC97L) cell lines were kindly provided by the Institutes of Biomedical Sciences Fudan University (Shanghai, China). The HepG2.2.15 cell line was kindly provided by Prof. J. T. Guo, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine (PA, USA). The complete growth medium for the cells was supplemented with 10% fetal bovine serum and penicillin-streptomycin antibiotics (Gibco, Grand Island, NY, USA). The cells were incubated in a humidified atmosphere containing 5% CO_2 at 37 ℃.

Targeted NGS Panel design

Genes included in our panel were selected according to the following criteria: cancer-related genes involved in core signaling processes and pathways collected from scientific literature and genes with high somatic mutation frequency from the Catalogue of Somatic Mutations in Cancer database (COSMIC, http://www.sanger.ac.uk/cosmic) (10). With these criteria, the panel consisted of the protein coding regions of 325 genes (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-1.pdf) with a total length of 901 kb of target regions, using the hg19/GRCh37 genome build (Ensembl version 75). A custom capture reagent containing predefined probe sets was constructed using the SeqCap EZ Choice Library according to the NimbleDesign guidelines (Roche NimbleGen, Madison, WI, USA).

Target enrichment of genomic DNA and sequencing

Genomic DNA was extracted from the cell lines using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA sample library was prepared using a KAPA Library Preparation Kit (KAPA Biosystem, Woburn, MA, USA) according to the manufacturer's guidelines. Each sample was fragmented to 180–220 bp, followed by end-repair, A-tailing and Illumina adapter ligation (Life Technologies, Carlsbad, CA, USA). After dual-SPRI size selection (250–450 bp), the libraries were amplified by polymerase chain reaction (PCR) and quantified using Qubit 3.0 (Life Technologies) and an Agilent 2100 Bioanalyzer DNA 1000 Kit (Agilent Technology, California, USA). The amplified sample library was used to enrich target regions by hybrid capture for the 325 gene target regions, using a solution-based SeqCap EZ Choice Library Kit (Roche NimbleGen). Finally, the amplified and captured multiplex DNA samples were subjected to paired-end 150-bp read length sequencing using a HiSeq X Ten platform (Illumina, San Diego, CA, USA) following the manufacturer's instructions.

Bioinformatics analysis

The bioinformatics analysis flowchart was developed as follows (*Figure S1*). The captured sequencing data were mapped against the hg19 human reference genome using the Burrows-Wheeler Aligner (BWA) (11). SAMtools was

Table 1 Summary of human HCC cell line backgrounds

Cell lines	Establishment background	Growth medium
HuH-7	In 1982, Nakabayshi *et al*. established the cell line from the hepatoma tissue of a 57-year-old Japanese male with well-differentiated HCC (14)	DMEM
Hep3B	Knowles *et al*. established the cell line from the biopsy of an 8-year-old black juvenile with HCC (15)	EMEM
SK-HEP-1	The cell line was established from the ascitic fluid of a patient with liver adenocarcinoma of HCC origin (16)	EMEM
MHCC97H	In 1999, Tian *et al*. established a human HCC cell line (MHCC97) with highly metastatic potential from HCC mice model (LCI-D20) (17). In 2001, Li *et al*. isolated MHCC97H cell line with high metastatic potential from parent cell line MHCC97 (18)	DMEM
MHCC97L	Li *et al*. isolated the cell line with low metastatic potential through *in vivo* selection of MHCC97 (18)	DMEM
HepG2	In 1980, Knowles *et al*. established HepG2 from the biopsy of a 15-year-old male Caucasian with HCC. The cell line is negative for HBV genome integration (15)	DMEM/F-12
HepG2.2.15	In 1987, Sells *et al*. established HepG2.2.15 by transfecting the HepG2 cells with pDolTHBV-1 vector followed by G418 screening. The cell line is integrated with HBV DNA sequences chromosomally (19)	DMEM/F-12

HCC, hepatocellular carcinoma.

used to generate pileup files and VarScan2 was used to call mutations, including single nucleotide variants (SNV) and short insertions/deletions (indels) (12). To identify somatic mutations in the exome region, only variants located in the coding DNA sequence (CDS) regions were used for further analysis. We filtered out known polymorphisms documented in dbSNP129. To minimize the potential errors in base calling, we defined the following filtering conditions: (I) variants of the candidate position with an alteration frequency no less than 5% and (II) sites with at least a 100 read depth.

Pathway analysis

We performed both computation-based and literature-based pathway analyses for annotating the candidate mutated genes in HCC cell lines. We used Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) for BBID, BioCarta and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping (13). Well-studied pathways, including the p53 cell cycle, Wnt/β-catenin, chromatin remodeling, MAPK and Notch, were surveyed by referring to prior knowledge and literature.

Results

Cell line characteristics

The establishment background of the HCC cell lines is summarized (14-19) (*Table 1*), including three cell lines derived from biopsies of HCC patients (HuH7, Hep3B and HepG2), one derived from ascitic fluid of liver adenocarcinoma patient (SK-HEP-1), one from HepG2 cells (HepG2.2.15) and MHCC97 series cells (MHCC97L and MHCC97H). MHCC97L and MHCC97H had similar genetic backgrounds but different metastatic potentials as a result of *in vivo* selection of MHCC97, which provided metastatic human HCC models (20). The cell line HepG2.2.15 is a widely used hepatitis B viral (HBV) replication model that was established by stably transfecting an HBV genome into HepG2 cells (19).

Sequencing profile and overall mutations

On average, each library yielded 4.5 Gb of sequence, and the mean read depth was approximately 1,000. The average coverage rate in target regions was 99.7%, and the targeted regions covered by at least 100 reads accounted for 99.4% (*Table S1*).

By using bioinformatics analysis and filtering from the raw data, we yielded 100 genes mutated in at least one cell line with an alteration frequency of no less than 5%. A total of 344 non-synonymous mutations, 325 silent mutations, 8 stop-codon-gain mutations, and 69 indels were identified, corresponding to 49 non-silent mutations per cell line (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-2.pdf; *Table S2*). To further identify significantly altered genes in HCC cell lines, we performed a literature-based summary of cancer-related genes from ten

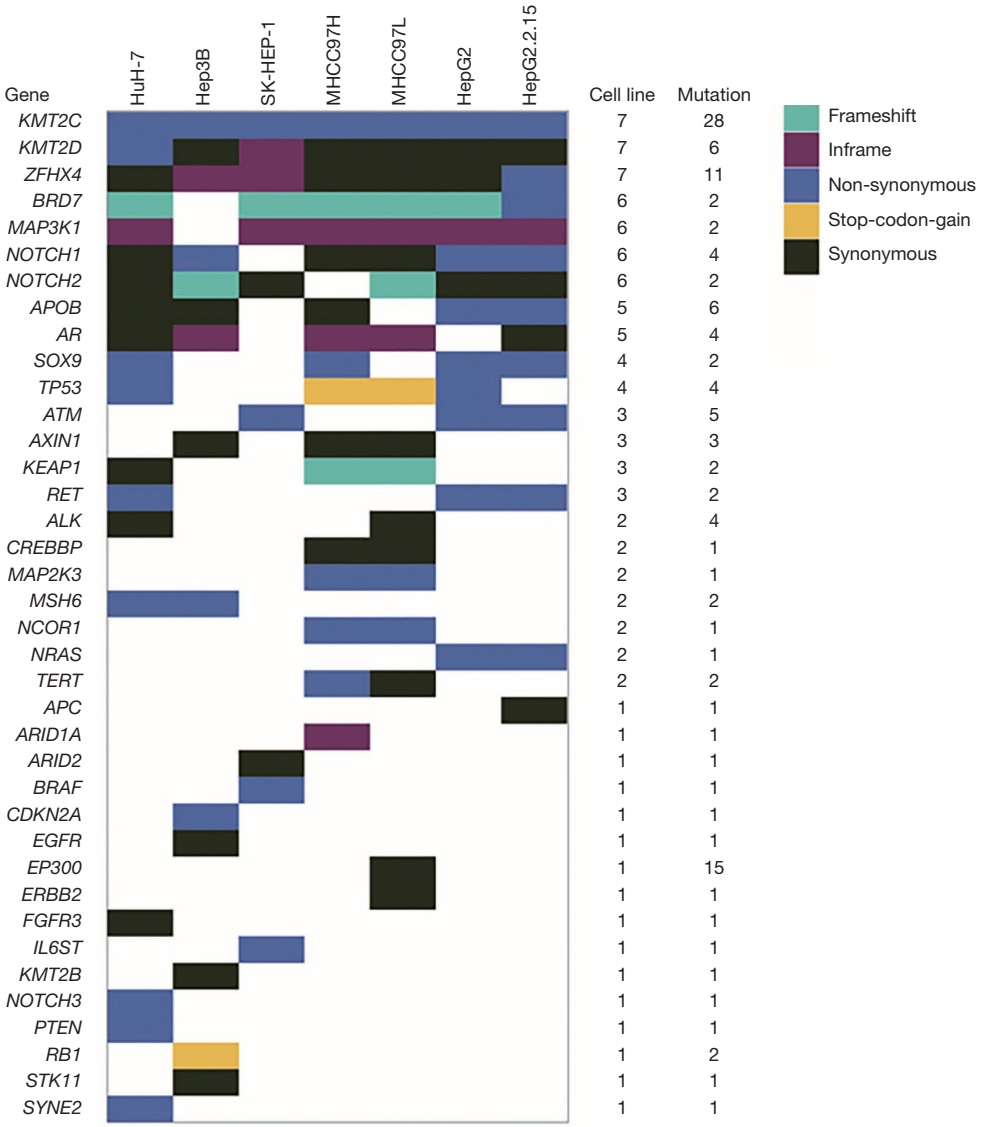

Figure 1 Significantly mutated cancer-related genes in HCC cell lines identified by panel-based sequencing. HCC, hepatocellular carcinoma.

high-quality papers published in the last 5 years (4,5,21-28). These research articles and reviews represent comprehensive genomic profiling of HCC and common forms of other human cancers. The aberrant genes that were reported at least once in the above papers were included. A total of 552 HCC and cancer mutated genes were summarized (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-3.pdf) and further compared to the 100 mutated genes we identified. We annotated the overlapping 52 genes using both DAVID functional annotation tools (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-4.pdf) and eleven major HCC major signaling pathways from the literature (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-5.pdf) (4,5,22). A total of 38 candidate genes were identified to be involved in cancer core cellular processes and signaling pathways, such as cell survival, cycle and apoptosis (*Table S3*).

Mutated cancer-related genes and pathways in HCC cell lines

We investigated the 38 genes mutational patterns in the HCC cell lines (*Figure 1*). Half of the altered genes (27/54)

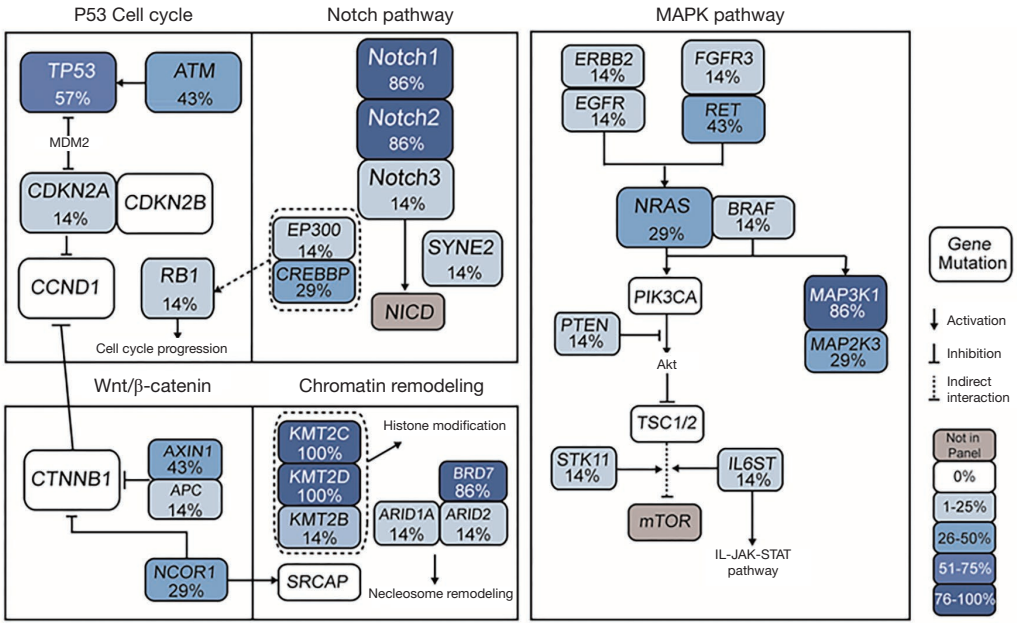

Figure 2 Somatically altered pathways in HCC cell lines. Genes are indicated by rectangles along with the percentage of their somatic mutation frequencies and the activating or inhibiting interactions among them. Ascending alteration frequency is colored from white to dark blue. Several key genes in a pathway that are not in the targeted NGS panel are added in grey rectangles. HCC, hepatocellular carcinoma.

in 11 major HCC signaling pathways (4,5), which are also included in the gene panel, were identified as sequence variations in HCC cell lines, though with different mutational rates to some extent. The mutated genes were significantly enriched in four major HCC signaling pathways (chromatin remodeling, Wnt/β-catenin, MAPK and p53 cell cycle) and one cancer-related pathway (Notch) (Available online: http://tcr.amegroups.com/public/addition/tcr/supp-tcr.2018.02.14-5.pdf; *Figure 2*). The four major HCC signaling pathways are well-acknowledged as having a high somatic alteration frequency. We identified six significantly mutated genes in the tumor-suppressor p53 cell cycle cascade (*TP53*, *ATM*, *CREBBP*, *EP300*, *CDKN2A* and *RB1*), two genes in the Wnt/β-catenin pathway (*AX1N1* and *APC*) and 11 genes in the MAPK pathway (*MAP3K1*, *MAP2K3*, *NRAS*, *RET*, *FGFR3*, *ERBB2*, *EGFR*, *PTEN*, *STK11*, *IL6ST* and *BRAF*). It is worth mentioning that, compared to HCC tissues, more genetic alterations were identified in chromatin modulators (*KMT2C*, *KMT2D*, *ZFHX4*, *BRD7* and *NCOR1*) and the Notch pathway (*Notch1* and *Notch2*). Our results identified three new cancer-related genes that were not significantly mutated in HCC cohorts. These three genes included two tumor suppressors *MSH6* (c.2582A>T and c.3005G>A) and *SOX9* (c.724A>C and c.715A>C), and one oncogene *ALK* (c.3600C>G, c.678G>T, c.831G>A and c.931G>C). In short, we have identified the genetic alterations in cancer-related genes and pathways in HCC cell lines by referring to HCC cohorts in previous reports.

Mutational patterns in cell lines from the same parent cells

It was obvious that the four specimen-derived HCC cells from different individuals, including HuH-7, Hep3B, SK-HEP-1 and HepG2, showed different mutational patterns. In contrast, the MHCC97 and HepG2 series of cells showed similar mutational patterns (*Figure 1*). Therefore, we further analyzed the mutations in these two series of cell lines.

There were 44 mutated genes identified in the MHCC97 series cells (MHCC97H and MHCC97L), and 68% of these genes (30 genes) were found in both cell lines (*Figure 3A*). We defined genes between which the mutation ratio difference value was less than 5% as "shared mutated genes". Among the above 38 cancer-related genes, the shared mutated genes between MHCC97L and MHCC97H cells were enriched in oxidative stress (*KEAP1*), chromatin modification (*NCOR1*, *KMT2D* and *ARID1A*), the Wnt/β-catenin pathway (*AXIN1*, *SOX9*) and the MAPK (*MAP3K1* and *MAP2K3*) pathway. However, the genes had several individual somatic variations. *ALK* and *ERBB2*

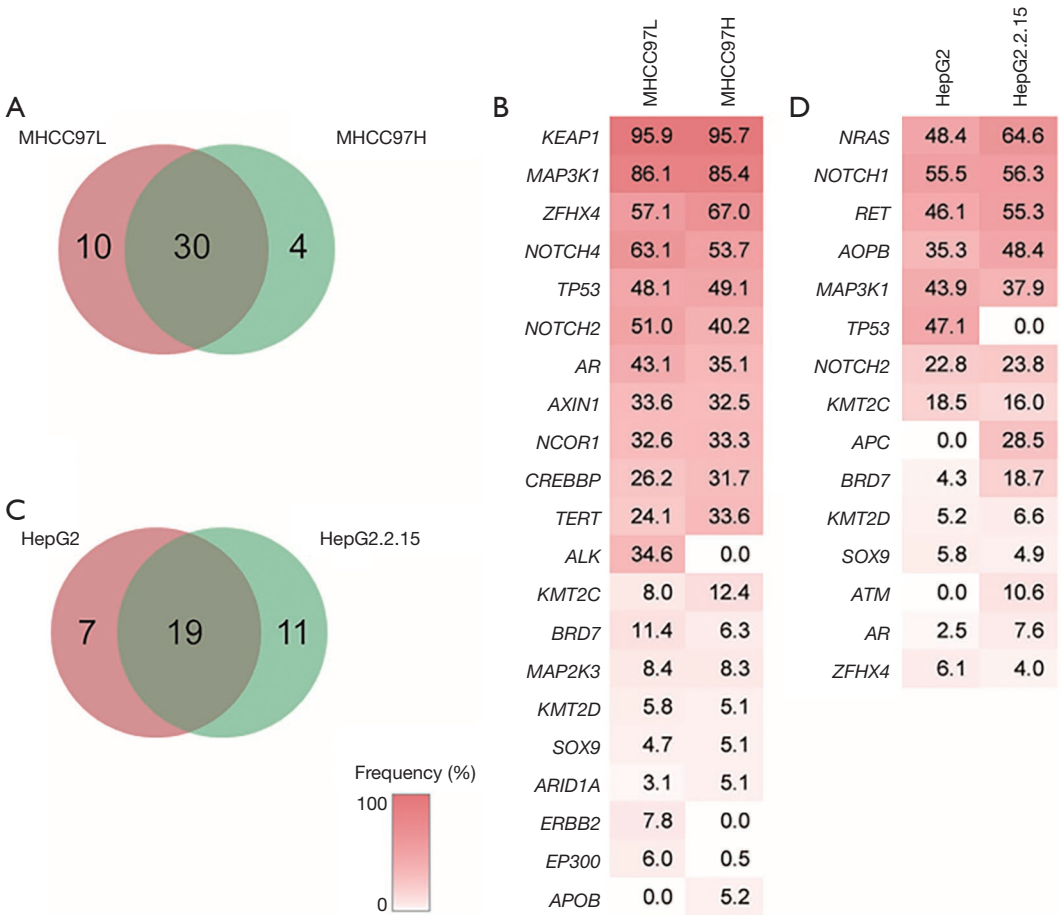

Figure 3 Mutational patterns in MHCC97 and HepG2 series of cell lines. (A,C) Overlapping of all mutated genes in cell lines from their parent MHCC97 and HepG2 cells, respectively; (B,D) the similarities and differences of mutated genes within the 38 genes in MHCC97 and HepG2 series of cells, respectively.

were only mutated in MHCC97L cells, and *APOB* was only mutated in MHCC97H cells. MHCC97L cells had higher mutation frequency in Notch pathway genes (*Notch1* and *Notch2*) than MHCC97H cells. TERT had higher mutation frequency in MHCC97H cells compared to MHCC97L cells (*Figure 3B*).

HepG2 and HepG2.2.15 cells showed similar genetic alteration patterns with MHCC97 cells, and 37 mutated genes were detected, with 51% (19 genes) shared among the cell lines (*Figure 3C*). Two Notch pathway genes (*Notch1* and *Notch2*) and two chromatin regulators (*KMT2D* and *ZFHX4*) were identified to have similar alteration frequencies at the same mutation site. Among the 38 genes, there were several individual somatic variations. *TP53* was specifically mutated in HepG2 cells, while *APC* and *ATM* were specifically mutated in HepG2.2.15 cells (*Figure 3D*).

Discussion

In our present study, to achieve deep sequencing for rare variant identification in cancer-related genes, we performed a panel-based targeted sequencing method to identify somatic mutations in six widely used HCC cell lines. Our method exhibited a 99.7% mean coverage rate in target regions at an approximately 1,000× read depth, which demonstrated the reliable quality of our custom targeted gene sequencing. We have established a targeted sequencing platform using Roche NimbleGen capture paired with Illumina Hiseq sequencing technology, allowing identification of genetic variants in cancer-related genes for HCC genomic samples.

By comparing the mutated genes in eleven major HCC signaling pathways, we identified a consistency in somatic

mutations between HCC tissues and cell lines. To further reveal the critical genomic variations, we focused on 38 significantly mutated genes, selected by pathway enrichment and prior knowledge, that are enriched in five oncogenic pathways. Genetic mutations in these critical genes and pathways may be the molecular mechanisms underlying their carcinogenic characteristics in HCC cell lines. A large proportion of the frequently mutated genes encode multiple chromatin modulators. *KMT2C*, *KMT2D* and *ARID1A* are all potential driver genes in HCC cell lines that are involved in the nucleosome structure remodeling and histone modification of their target genes (5,29). Mutated genes *BRD7*, *ARID1A* and *ARID2* are core subunits making up the SWI/SNF nucleosome-remodeling complex (30). Aberrant activation of the Notch pathway leads to oncogenesis by promoting the self-renewal of liver cancer stem cells and modification of the inflammatory environment, which may be the cause of HCC (31,32).

In addition to the carcinogenic pathways, we identified three new cancer-related genes that are frequently mutated in HCC cell lines (*MSH6*, *SOX9* and *ALK*). Deleterious mutations of the mismatch-repair gene MSH6 leads to mismatch repair (MMR) deficiency and microsatellite instability (MSI). MMR deficiency has recently been proved to be a biomarker for predicting clinical benefit of immune checkpoint programmed death 1 (PD-1) blockade for multiple solid tumors, including liver cancer (33,34). It was reported that HCCs have MSI-high (MSI-H) frequency (16%) and MSI-low (MSI-L) frequency (27%), and MSI status might affect HCC patients' responses to immune checkpoint inhibitors (35,36). Thus, the consequences of MSH6 substitutions in HuH7 and Hep3B (p.Glu861Val and p.Arg1002Lys) remain to be examined further. SOX9 regulates the expression of stemness genes in tumor initiation and invasion in a Wnt/β-catenin-dependent manner (37). ALK mutation is one of the major oncogenic drivers and therapeutic targets in lung cancer, which may also have a potential impact on HCC (38,39). In brief, we identified genetic alterations in HCC cell lines similar to those previously reported in HCC tissues, and we also found several genes and pathways not previously known to be frequently mutated in HCC.

We further observed the different mutational patterns across the four specimen-derived HCC cells from different individuals, Hep3B, HuH-7, SK-HEP-1 and HepG2. These patterns validate that genetic variations exist among different individual tumors of the same tumor type from the perspective of eliminating the distractions associated with tissue complexity (40). In contrast, major similarities and some minor discrepancies in genomic alterations were observed among cell lines from the same clone origin. By comparing the genomic alterations between MHCC97L and MHCC97H, cell strains from the same clone origin but with different metastatic potentials, we found that 68% of shared mutated genes were significantly enriched in core cancer signaling pathways and also identified several differentially mutated genes. Although no specific gene mutations were identified to directly explain their different metastatic potentials, we here verified this type of tumor evolution in cell lines, which is consistent with previous knowledge that HCC primary tumors and metastatic subclones evolve from the same origin in patients (41,42). The two HepG2 series cells had 51% shared mutated genes and several individual somatic variations. The mutational differences between cell lines from the same clone origin may mainly be ascribed to subclones that have been selectively mutated due to their metastatic characteristics or continuous viral replication. Additionally, dynamic mutation accumulation may occur in the long-term selection of in-vitro culture due to genomic instability of tumor cells. In summary, our results validate the theory prevalent in recent years that extensive genetic heterogeneity exists within individual tumors from the perspective of cell lines and that HCC cells from the same clone origin have great similarities in genomic alterations, which may be used to trace HCC cell clone origins.

The genetic variation pattern of HCC cell lines provides insight into these extensively used experimental models. However, HCC cell lines of other clone origins and the detailed molecular mechanism for their mutational similarities and differences need to be further explored to validate the mutation patterns in our study. In future studies, we will further optimize our targeted sequencing platform and explore its application for the research of tumor heterogeneity and clonal evolutionary processes in clinical setting. In summary, we identified altered genes and pathways in HCC cell lines through targeted sequencing technology, which could have implications for the genetic heterogeneity of liver cancer and for tracing tumor origins based on genetic background.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.02.14). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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Supplementary

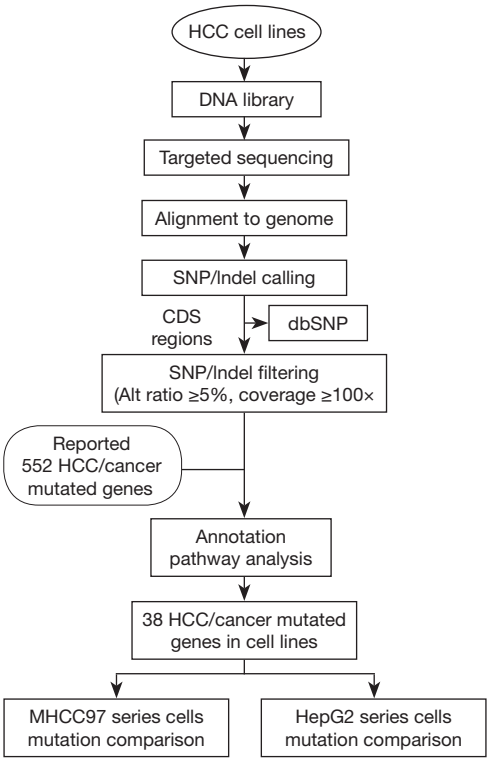

Figure S1 Study workflow. HCC, hepatocellular carcinoma.

Table S1 Summary of targeted sequencing statistics

Cell lines	Raw reads (M)	Filtered bases (Gb)	Total coverage (×)	Mapped to genome reads (M)	Align rate to genome reads	Total target base	Mapped to genome base (Mb)	Covered target base	Coverage rate	Targeted base with at least 100 reads	Coverage rate with at least 100 reads
HuH-7	40.3	5.9	1,325	40.1	99.7%	901,218	3378.8	898,865	99.7%	898,007	99.6%
Hep 3B	24.0	3.5	747	23.9	99.8%	901,218	2029.5	898,642	99.7%	894,659	99.3%
SK-HEP-1	25.3	3.7	753	25.3	99.7%	901,218	1962.1	897,719	99.6%	890,435	98.8%
MHCC97H	29.2	4.3	1,080	29.1	99.8%	901,218	2321.0	898,865	99.7%	896,584	99.5%
MHCC97L	46.4	6.8	1,752	46.2	99.7%	901,218	3749.6	898,865	99.7%	897,802	99.6%
HepG2	26.1	3.8	853	26.0	99.8%	901,218	2173.5	898,840	99.7%	896,654	99.5%
Hep2.2.15	25.6	3.8	848	25.5	99.7%	901,218	2178.8	898,865	99.7%	896,916	99.5%

Table S2 Summary of mutations in HCC cell lines

Cell lines	Total	Substitution			Indel	
		Non-synonymous	Synonymous	Stopgain	Frameshift	Inframe
HuH-7	115	64	41	0	9	1
Hep 3B	126	63	52	4	4	3
SK-HEP-1	80	38	30	1	6	5
MHCC97H	81	36	33	1	6	5
MHCC97L	163	53	97	1	8	4
Hep G2	89	45	34	1	6	3
Hep2.2.15	92	45	38	0	6	3

HCC, hepatocellular carcinoma.

Table S3 Thirty-eight significantly mutated genes in core cancer processes and pathways

Gene symbol	Gene name	Function and process	Core pathway
ALK	Anaplastic lymphoma receptor tyrosine kinase	Control cell survival	PI3K; RAS
APC	Adenomatous polyposis coli	Control cell migration, adhesion and apoptosis	Wnt/β-catenin
APOB	Apolipoprotein B	Lipoprotein catabolic process, cell motion	Hepatic differentiation
AR	Androgen receptor	Control cell death	MAPK
ARID1A	AT-rich interaction domain 1A	ATP-dependent chromatin remodeling	Chromatin modification
ARID2	AT-rich interaction domain 2	Cell Fate	Chromatin modification
ATM	ATM serine/threonine kinase	Control cell cycle and maintain genome stability	P53 cell cycle, genome maintenance
AXIN1	Axin 1	Induce apoptosis	Wnt/β-catenin
BRAF	B-Raf proto-oncogene	Affect cell division, differentiation, and secretion	MAPK
BRD7	Bromodomain containing 7	Prevent tumor growth by interacting with p53	Chromatin Modification
CDKN2A	Cyclin dependent kinase inhibitor 2A	Control cell cycle	P53 cell cycle
CREBBP	CREB binding protein	Control embryonic development	P53 cell cycle
EGFR	Epidermal growth factor receptor	Regulate cell proliferation	MAPK, PI3K
EP300	E1A binding protein p300	Control cell proliferation and differentiation	P53 cell cycle, chromatin modification
ERBB2	Erb-b2 receptor tyrosine kinase 2	Regulate cell proliferation	MAPK, PI3K
FGFR3	Fibroblast growth factor receptor 3	Control cell survival	MAPK, PI3K
IL6ST	Interleukin 6 signal transducer	Control cell cycle	MAPK
KEAP1	Kelch like ECH associated protein 1	Sense oxidative stress	Oxidative stress (NRF2/KEAP1)
KMT2B	Lysine methyltransferase 2B, also known as MLL4	Possess histone methylation activity	Chromatin modification
KMT2C	Lysine methyltransferase 2C, also known as MLL3	Possess histone methylation activity	Chromatin modification
KMT2D	Lysine methyltransferase 2D, also known as MLL2	Possess histone methylation activity	Chromatin modification
MAP2K3	Mitogen-activated protein kinase kinase 3	Control cell survival	MAPK
MAP3K1	Mitogen-activated protein kinase kinase kinase 1	Control cell survival	MAPK
MSH6	Muts homolog 6	Mismatch repair	Genome maintenance
NCOR1	Nuclear receptor corepressor 1	Promote chromatin condensation	Chromatin modification, Wnt/β-catenin
NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	Control cell fate decisions	NOTCH
NOTCH2	Notch 2	Control cell fate decisions	NOTCH
NOTCH3	Notch 2	Control cell fate decisions	NOTCH
NRAS	Neuroblastoma RAS viral oncogene homolog	Control cell cycle and differentiation	RAS, MAPK
PTEN	Phosphatase and tensin homolog	Control cell survival	MAPK, PI3K, P53 cell cycle
RB1	RB transcriptional corepressor 1	Negatively regulate the cell cycle	P53 cell cycle
RET	Ret proto-oncogene	Control cell growth and differentiation	MAPK, PI3K
SOX9	SRY (sex determining region Y)-box 9	Control cell survival and apoptosis	Wnt/β-catenin
STK11	Serine/threonine kinase 11	Regulate cell polarity	MAPK, mTOR
SYNE2	Spectrin repeat containing nuclear envelope protein 2	Maintain nucleus structural integrity	NOTCH
TERT	Telomerase reverse transcriptase	Control cellular senescence and keep the telomere integrity	Telomere maintenance
TP53	Tumor protein p53	Control cell cycle, apoptosis, DNA repair and metabolism	P53 cell cycle
ZFHX4	Zinc finger homeobox 4	Regulate chromatin-remodeling complex and tumor cell state	Chromatin modification