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TOPIC HIGHLIGHT

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Target genes discovery through copy number alteration analysis in human hepatocellular carcinoma

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Abstract

High-throughput short-read sequencing of exomes and whole cancer genomes in multiple human hepatocellular carcinoma (HCC) cohorts confirmed previously identified frequently mutated somatic genes, such as *TP53*, *CTNNB1* and *AXIN1*, and identified several novel genes with moderate mutation frequencies, including *ARID1A*, *ARID2*, *MLL*, *MLL2*, *MLL3*, *MLL4*, *IRF2*, *ATM*, *CDKN2A*, *FGF19*, *PIK3CA*, *RPS6KA3*, *JAK1*, *KEAP1*, *NFE2L2*, *C16orf62*, *LEPR*, *RAC2*, and *IL6ST*. Functional classification of these mutated genes suggested that alterations in pathways participating in chromatin remodeling, Wnt/β-catenin signaling, JAK/STAT signaling, and oxidative stress play critical roles in HCC tumorigenesis. Nevertheless, because there are few druggable genes used in HCC therapy, the identification of new therapeutic targets through integrated genomic approaches remains an important task. Because a large amount of HCC genomic data genotyped by high density single nucleotide polymorphism arrays is deposited in the public domain, copy number alteration (CNA) analyses of these arrays is a cost-effective way to reveal target genes through profiling of recurrent and overlapping amplicons, homozygous deletions and potentially unbalanced chromosomal translocations accumulated during HCC progression. Moreover, integration of CNAs with other high-throughput genomic data, such as aberrantly coding transcriptomes and non-coding gene expression in human HCC tissues and rodent HCC models, provides lines of evidence that can be used to facilitate the identification of novel HCC target genes with the potential of improving the survival of HCC patients.

Key words: Copy number alteration; High-density single nucleotide polymorphism arrays; Driver genes; Hepatocellular carcinoma

Core tip: In addition to detecting somatic mutations in cancer genomes with high-throughput short-read sequencing technologies, analysis of copy number alteration in hepatocellular carcinoma (HCC) cancer genomes genotyped by high density single nucleotide polymorphism arrays is a cost-effective approach to reveal genome-wide somatic alterations accumulated during tumorigenesis. Integration with other genomic data from HCC tissues derived from high-throughput short-read sequencing, proteomics, epigenomics and transcriptomics could provide lines of evidence to identify common and novel HCC genes for potential clinical applications.

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INTRODUCTION

Human hepatocellular carcinoma (HCC) is the fifth leading cause of cancer mortality, causing an estimated half a million deaths annually[1,2]. Risk factors for developing HCC include hepatitis infection, obesity, alcoholism and consumption of aflatoxin-contaminated food. Due to the rising incidence of hepatitis C infection, HCC is one of the fastest-growing cancers in the United States and Western countries, and the incidence is expected to continue to increase[3]. Surgical resection is the most successful treatment for early stage HCC. However, fewer than 30% of HCC patients are qualified for curative resection owing to liver dysfunction and cirrhosis. Moreover, frequent tumor recurrence is observed even after curative resection.

Recent successes in cancer targeted therapy arising from the identification of somatic alterations and their specific inhibitors are associated with reduced side effects and prolonged patient survival. Many of these FDA-approved inhibitors are small molecules or monoclonal antibodies against cancer-specific tyrosine kinase mutations, including Imatinib mesylate (Gleevec) for fusion oncogene Bcr/Abl-positive chronic myelogenous leukemia[4], Gefitinib (Iressa) or Erlotinib (Tarceva) for epidermal growth factor receptor mutated non-small cell lung cancer[5] and Trastuzumab (Herceptin) for HER2/neu receptor amplified and overexpressed breast cancer patients[6]. Although no specific drug target has been identified for HCC, FDA approved the multi-kinase inhibitor sorafenib for treatment of advanced HCC, due to a favorable overall patient survival[7]. However, HCC patients receiving sorafenib showed marginal benefits, with a prolonged survival of 3-4 mo on average[8,9]. With limited improvement of HCC patient survival, identification of recurrent and altered somatic genes through integrated genomic approaches is vital to better understand HCC molecular tumorigenesis, to develop early diagnostic markers and methods, and to find additional druggable targets for the improvement of HCC management.

MUTATED HCC GENES WITHIN RECURRENT ALTERED CHROMOSOME LOCI

In HCC, many tumor suppressor genes and oncogenes were identified based on recurrent genetic lesions, including loss of *TP53* (17p13)[10], *RB* and *BRCA2* (13q)[11], and amplification of *c-myc* (8q24)[12] and *ERBB2* (17q12-q21)[13]. Epigenetic mechanisms also contribute to HCC progression, such as CpG island hyper- methylation of *p16* (*INK4a*) and *COX2*[14-16], as well as altered expression of microRNAs[17,18]. Conventional point mutation is another common mechanism to alter cancer gene functions. In HCC, frequent point mutations of *TP53* and *β-catenin* are involved in key pathways of hepatocarcinogenesis[19,20]. Other studies have reported mutations in *M6P/IGF2R*[21], *BRCA2*[22], *Smad2/4*[23], *HCCS1*[24], *PTEN*[25] and *Axin1*[26].

Recently developed high-throughput short-read sequencing technologies were used to identify somatic mutations in HCC cancer genomes at genome-wide scales. These studies confirmed that *TP53* and *CTNNB1* (encoding for β-catenin) are the most frequent recurrent mutations in human HCC. In addition, moderate mutation frequencies were identified in multiple HCC cohorts for several novel genes, including epigenetic and chromatin remodeling genes (*ARID1A*, *ARID2*, *MLL* and *MLL3*) and members of a number of oncogenic pathways (*RPS6KA3*, *JAK1* and *KEAP1*)[27-32]. These results suggested that aberrant pathways involved in cell cycle regulation, oxidative stress, chromatin remodeling and oncogenic signaling, such as Wnt/β-catenin, JAK/STAT and Akt/mTOR, play critical roles in the process of HCC tumorigenesis. Nevertheless, HCC remains a highly lethal cancer due to the lack of biomarkers for early diagnosis, molecular classification and efficient therapeutic interventions. Efforts to develop specific inhibitors for these aberrant pathways and reveal better therapeutic targets in HCC are urgently needed.

HIGH DENSITY SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS FOR ANALYSIS OF RECURRENT COPY NUMBER ALTERATIONS

Copy number alterations (CNAs), distinguished from germ line transmitted copy number variations, account for some of the genetic diversity of populations, in addition to the accumulated genomic DNA changes during tumor progression. CNAs are important subclasses of somatic mutations, with aberrant chromosomal regions of amplification or deletion commonly associated with overexpressed oncogenes or loss of tumor suppressor genes, respectively[33]. With the comprehensive annotation of human genome in the last decade, the mutated cancer genes could be aberrant protein-coding and non-coding genes such as small microRNAs or long non-coding RNAs within the CNA regions[34].

Copy number alterations in cancer cells can be detected by conventional karyotyping and chromosomal in situ hybridization technologies. To profile CNAs in cancer genomes compared to the genomes of adjacent normal cells, comparative genome hybridization (CGH) technology was used to identify copy number changes in karyotypes from breast cancer cell lines and primary blad-

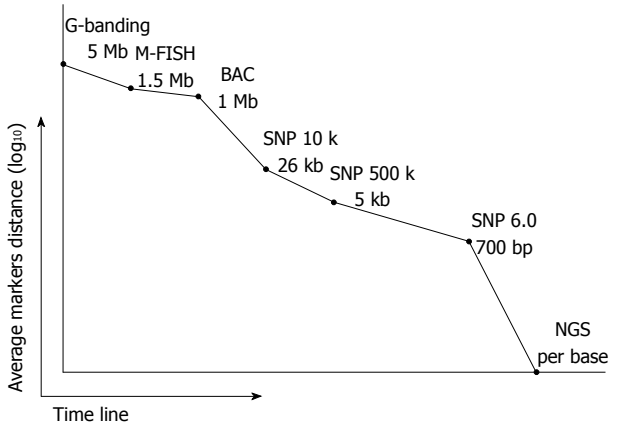

Figure 1 Timeline and average marker distance of technologies for the detection of copy number alterations.

der tumors[35]. With the availability of genomic resources (*e.g.*, BAC clones) and array technologies (*e.g.*, high density oligonucleotide probes), array-based CGH (aCGH) technologies not only examine genome-wide CNAs in high resolution but also allow researchers to pinpoint and profile the non-random CNAs for identification of novel aberrant cancer genes (Figure 1)[36-38]. The recently developed high-throughput short-read sequencing technology might become an alternative and effective approach to simultaneously detect CNAs and other classes of somatic mutations at the single nucleotide level[39,40]. Nevertheless, the availability of thousands of cancer genomes genotyped by high-density SNP arrays from various tumor samples and cancer cell lines at both NCBI GEO (Gene Expression Omnibus) and EBI ArrayExpress databases is a critical resource for *in silico* analysis of CNAs[41,42]. Moreover, integrated genomic analysis with both high-throughput short-read sequencing technology and high-density SNP genotyping arrays to comprehensively profile and validate recurrent CNAs of cancer genomes are promising approaches for the identification of novel cancer genes[40].

DIFFERENT TYPES OF CANCER MUTATIONS EMBRACED IN CNAS LOCI

To identify novel diagnostic and therapeutic target genes, CNA analysis of cancer genomes genotyped using commercial high-density SNP arrays from your own experiments or downloaded from public domains is a powerful and cost-effective approach. First, to discover putative tumor suppressor genes, we overlapped homozygous deleted regions from multiple samples to narrow down the common deleted regions by using high-density SNP genotyping arrays. As shown in Figure 2, the homozygous deleted region at chromosome 13q12.11 in SK-hep1 cells could be refined from 1.88 to 1.46 Mb to facilitate the identification of candidate tumor suppressor genes[38,43]. Second, for the identification of candidate oncogenes in HCC, the most common approach is to integrate data

Table 1 Copy-number altered regions in genomes of hepatocellular carcinoma cell lines

Cytoband	Start (Mb)	End (Mb)	Known cancer genes	Novel candidates
Amplicons				
1q21.2-22	150.07	151.89	*SHC1, CKS1B, ADAM15*	*CREB3L4, RAB1, mir190b, S100A14*
3p26.1-25.3	6.90	9.43		*LMCD1*
3q26.2-26.31	170.07	170.24		
	170.28	170.99	*EVI1, MDS1, TERC*	
	171.21	173.50		*FNDC3B*
5p15.33-12	0.40	45.14	*TRIO, AMACR, DAB2*	*LPCAT1, SEMA5A, CDH12*
7p22.2-14.3	4.15	32.10	*RAC1, ETV1, CHN2*	
7p12.1-11.2	52.79	55.17	*EGFR*	
	56.00	56.53		
8p11.21	40.44	40.62		
8q24.21	129.21	129.29		
11q13.2-13.3	65.85	66.44	*RIN1, BRMS1*	*SLC29A2*
	67.58	67.71		
	67.91	69.35	*LRP5, CCND1, ORAOV1*	*FGF4, FGF3*
12p12.1	24.36	25.54	*BCAS1, K-ras*	
20q13.31	53.94	53.96		
Homozygous deletions				
2q22.1	141.72	141.80	*LRP1B*	
7q21.11	77.96	78.04		*MAGI2*
9p23	9.42	9.46		*PTPRD*
	11.90	12.00		
9p21.3	21.85	21.90	*MTAP, CDKN2A*	
	24.27	24.84		
13q12.11	18.98	20.44	*TPTE2, Tg737*	

from genomic experiments in order to reveal genes residing in overlapping amplicons with up-regulated gene expression. For instance, *FNDC3B*, *SLC29A2*, *Ago2*, *IER3* and many others were identified as putative oncogenes due to their genomic DNA amplification and mRNA overexpression in HCC tissues[38,44-47]. When ectopically expressed, these putative oncogenes in HCC cells commonly show malignant phenotypes using various functional assays and facilitated tumor progression *in vitro* and *in vivo*.

Third, CNA analysis allows the identification of HCC genes with attributes of genomic DNA amplification, mRNA overexpression and recurrent point mutations, such as the putative metastatic HCC oncogene with LMCD1 mutations at E135K (in 3/48 cases) and K237R (in PLC/PRF/5 cells)[44]. When these mutations were expressed in HCC cells, HCC cell migration capability was enhanced in association with cortical actin accumulation and lamellipodial extension. Moreover, the overexpression of the LMCD1 E135K mutation in HCC cells significantly promoted systemic lung metastasis in a murine tail vein injection model. Table 1 summarizes some novel HCC genes in association with overlapping amplicons and homozygous deletions in HCC cell lines. Finally, CNA analysis detects differences in copy number (*i.e.*, dosages), such as amplifications and deletions. Therefore,

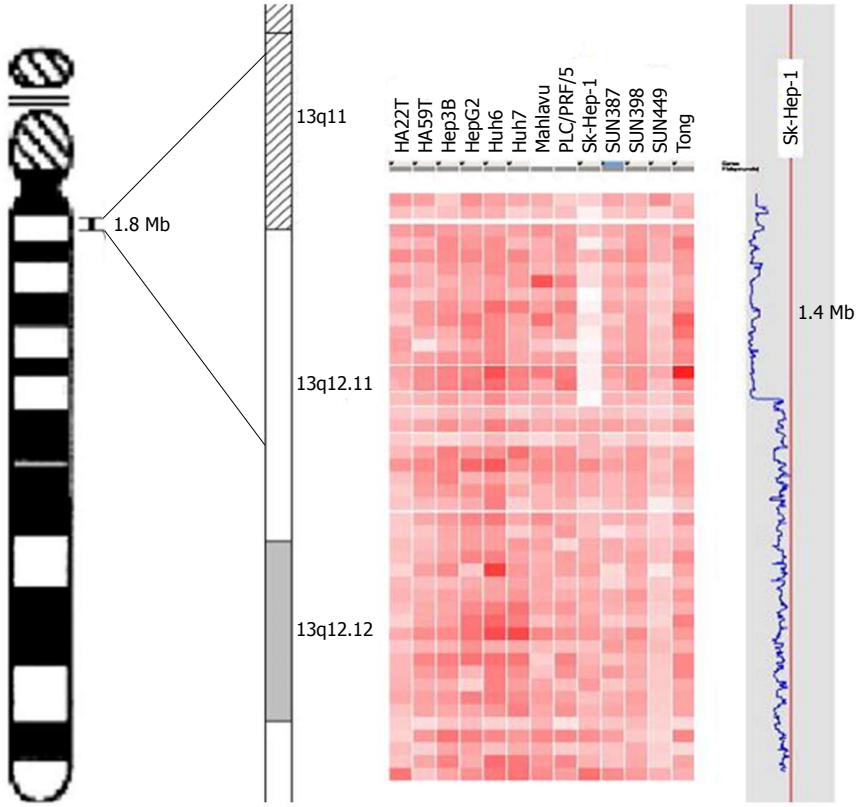

Figure 2 Refinement of homozygous deletion by copy number alteration analysis at chromosome 13q12.11 in hepatocellular carcinoma cells.

Table 2 List of some integrated cancer genomic databases

Database	Project	Website	Ref.
cBioPortal for cancer genome	Project provides visualization, analysis and download of large-scale cancer genomic data sets	http://www.cbioportal.org/public-portal/	Cerami *et al*[57]
COSMIC	Catalogue of somatic mutations in cancer	http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/	Forbes *et al*[58]
ICGC	International Cancer Genome Consortium provides tools for visualizing, querying and downloading the data.	http://dcc.icgc.org/	Joly *et al*[59]
TCGA data portal	A platform for researchers to search, download, and analyze data sets generated by TCGA	https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp	
Tumorscape	High-resolution copy number data collected from multiple cancer types	http://www.broadinstitute.org/tumorscape/pages/portalHome.jsf	
UCSC cancer genome browser	A set of web-based tools to display, investigate and analyze cancer genomic data and associated clinical information	https://genome-cancer.ucsc.edu/proj/site/hgHeatmap/	Goldman *et al*[60]

COSMIC: Catalogue of somatic mutations in cancer.

it will not reveal balanced translocation but will detect sudden dosage changes for unbalanced translocation. Using CNA analysis and high-density SNP arrays, *PAX5* fusion genes were identified with a variety of partner genes, including *ETV6*, *FOXP1*, *AUTS2*, and *C20orf112*, in pediatric acute lymphoblastic leukemia (ALL)[48].

INTEGRATED HCC CANCER GENOMIC DATABASES WITH CNAS

Integrated data derived from multiple genomic approaches could potentially avoid pitfalls of data inconsistency usual with the single genomic approach and provide lines of evidence to validate target genes embraced in the aberrant genomic loci from the level of DNA and RNA to protein. For these advantages, several user-friendly HCC databases were constructed, including OncoDB.HCC, HCCnet, dbHCCvar, CellMinerHCC, HCC-M, and EHCO[49-54]. However, only OncoDB.HCC integrated genomic alteration data to prioritize HCC cancer genes for further expression and functional validations in HCC cell lines and tissues. Nevertheless, recent international efforts at applying high-throughput short-read sequencing technologies and CNA analysis of cancer genomes in multiple cancer types, including HCC, comprehensively cataloged different types of somatic mutations and revealed genetic heterogeneity even from the same subtype

of cancer. Table 2 lists common open-access integrated cancer genome databases for downloading and visualizing cancer genomic data[55,56].

CONCLUSION

As discussed in this review article, an integrated genomic approach is an effective and essential method of identifying novel HCC genes. With the availability of a tremendous amount of high-throughput short-read sequencing data and SNP array data from cancer genomes deposited in the public domain, integrated genomic approaches, including CNA analysis, are the most cost-effective approach for revealing HCC driver genes for improving HCC therapy.

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