Recent updates of genetic and genomic alterations in hepatocellular carcinoma

Zhang Zhao, Jian Huang
Liver Research Center, Experimental Center, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

ABSTRACT
Hepatocellular carcinoma (HCC) is one of the most common malignant cancers worldwide. However, the molecular mechanisms underlining the development and progression of HCC remain unclear. Genetic and genomic alterations are common events in various types of cancers including HCC. With the development and application of next generation sequencing technology, novel genetic and genomic alterations in HCC have been identified. Here, the article reviews recent updates on the genetic and genomic alterations in HCC.

Key words: Hepatocellular carcinoma; genetics; genomics; next generation sequencing

INTRODUCTION
Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide, with a prevalence of more than 50% in China. Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, ingestion of food contaminated with aflatoxin B1, and alcohol consumption are considered major risk factors for HCC development.[1] Despite well-established risk factors, the specific molecular mechanisms underlying pathogenesis of HCC remain unclear. Genetic and genomic alterations are common events in various types of cancers including HCC, and may be associated with the development and progression of cancer. With the development of the technology of next generation sequencing, that is, whole-genome sequencing, novel genetic and genomic alterations have been identified. Recent studies on whole-genome sequencing of HCC confirmed the important roles of previously reported genetic and genomic alterations in the development and progression of HCC.[2] However, the fact that the most frequently mutated genes were generally previously reported, and that few novel mutated genes with high frequency were identified by the whole-genome sequencing suggests the complexity regarding the role of genetic mutations in the pathogenesis of HCC. In this paper, we review recent updates on genetic mutations and genomic imbalances in HCC.

GENETIC ALTERATIONS: MUTATION AND SINGLE NUCLEOTIDE POLYMORPHISM

Somatic mutation
Previous studies have demonstrated that the most significantly mutated genes in HCC include tumor
Only a few genes were identified such as AT-rich interactive domain 1A (ARID1A) and genes of chromatin-remodeling complex. However, studies have also reported United States and Europe, 18.2% of HCV-associated transcriptional activation by nuclear receptors. In the complex, which facilitates ligand-dependent ARID2 is a subunit of the PBAF chromatin-remodeling complex, which facilitates ligand-dependent transcriptional activation by nuclear receptors. In the United States and Europe, 18.2% of HCV-associated HCC cases were identified with ARID2-inactivating mutations. However, studies have also reported mutation frequencies of approximately 5-10% for ARID2 in HCC and truncation of ARID2 leads to loss of protein function and chromatin dysregulation.

With the development of whole-genome sequencing technology, the next generation sequencing of genome DNA provides the possibility that more novel genetic and genomic alterations may be discovered and may provide new insights for understanding the pathogenesis of HCC. However, several recent studies using next generation sequencing for analysis of mutation in HCC showed that the most frequent mutations with mutated rate over 10% were mainly genes reported previously such as TP53, β-catenin, and genes of chromatin-remodeling complex such as AT-rich interactive domain 1A (ARID1A) (14/110). Only a few genes were identified with mutation rates over 10%, for example, the low-density lipoprotein receptor-related protein 1B gene, reported by Kan et al. to have a mutation rate of 11.4% in patients with family hypercholesterolemia. Notably, several components of the chromatin-remodeling complex, such as ARID1A and ARID2 were mutated in over 10% HCC specimens, similar to previous reports, confirming the important role of chromatin-remodeling in the pathogenesis of HCC. In addition, Janus kinase 1 (JAK1) mutation was identified with mutation rate of 9.1% through the whole-genome sequencing of 88 HCC cases, and the JAK/STAT pathways were altered in 45.5% of cases, inconsistent with a previous study which reported low frequency (1/84, 1.2%) of JAK1 mutation in HCC, implying that the JAK/STAT pathways may act as major oncogenic drivers in HCC. However, the fact that the most frequently mutated genes were generally previously reported, and that few novel mutated genes with high mutation frequencies were identified by whole-genome sequencing suggests the complexity regarding the role of genetic mutations in the pathogenesis of HCC.

It has been reported that genomic instability is a characteristic of most cancers. Genomic instability results from mutations in DNA repair genes and drives cancer development in hereditary cancers. However, in sporadic cancers, previous studies and recent high-throughput sequencing studies suggested that mutations in DNA repair genes were infrequent. Instead, the mutation patterns of the tumor suppressor TP53, ataxia telangiectasia mutated (ATM), and cyclin-dependent kinase inhibitor 2A (CDKN2A) support the oncogene-induced DNA replication stress model, which attributes genomic instability and TP53 and ATM mutations to oncogene-induced DNA damage, that is, high frequency of TP53 mutations in human cancers could be in response to oncogene-induced DNA damage. The hypothesis was confirmed by several studies showing that deletion of the TP53 gene in mouse models and human cells did not lead to aneuploidy, and that in human precancerous lesions, genomic instability was present before the establishment of TP53 mutations. Consistent with the above studies, previous studies and recent whole-genome sequencing of HCC also showed that mutations in DNA repair genes in HCC were infrequent suggesting there may be similar mechanisms of genetic mutations in somatic HCC, that is, high frequency of TP53 mutations and additional genetic mutations favoring cancer development in somatic HCC could be in response to oncogene-induced DNA damage.

Single nucleotide polymorphism
Single nucleotide polymorphism (SNP) is the most common genetic variation in the human genome.
Genome-wide association study (GWAS) was also applied for SNP analysis of HCC in recent years. In a GWAS of HCC in Japanese population, one intronic SNP (rs1012068) in the DEP domain containing 5 gene was identified to be associated with HCC risk.[21] In a GWAS of HCC in chronic HBV carriers of Chinese ancestry, one intronic SNP (rs17401966) in kinesin family member 1B was identified to be highly associated with HBV-related HCC.[22] In addition, SNP (rs9679162) in polypeptide N-acetylgalactosaminyl transferase 14 (GALNT14) have been shown to be associated with chemotherapy response in patients with advanced HCC; for advanced HCC patients treated with FMP (fluorouracil oxantrone cisplatin) chemotherapy, GALNT14 genotype (rs9679162) was an effective predictor of the therapeutic outcome.[23,24]

**GENOMIC ALTERATION: GENOMIC IMBALANCES**

**Copy number variation-genomic gain or loss**
Chromosomal abnormalities in HCC have been well reported, and comparative genomic hybridization (CGH) has revealed a consistent pattern of genomic gains and losses involved in the development and progression of HCC. The most prominent changes are partial or entire gains of chromosome arms 1q, 8q, and 2q; and losses of 1, 4q, 8p, 13q, 16q, and 17p. In one meta-analysis, using conventional CGH analysis with low resolution (approximately 2 Mb) from several studies, it was revealed that the most prominent changes were gains of 1q (57.1%), 8q (46.6%), 6p (23.3%), and 17q (22.2%); and losses of 8p (38%), 16q (35.9%), 4q (34.3%), 17p (32.1%), and 13q (26.2%).[25] Using array CGH analysis from four studies, it was revealed that loci with genomic gains with a prevalence of more than 25% included 1q, 6p, 8q, 17q, 20p, 5p15.33, and 9q34.2-34.3; and loci with genomic losses with prevalence of more than 25% comprised 4q, 6q, 8p, 9p, 13q, 14q, 16q, and 17p; and were associated with 31 classical molecular pathways, particularly the antiviral immunological pathway.[25] A series of tumor suppressor genes have been identified in these regions, such as PR domain containing 5 (PRDM5, 4q26), TP53 (17p13.1), retinoblastoma 1 (RB1, 13q14), and cadherin 1, type 1 (CDH1, 16q22.1).[26-28] Some clinicopathological associations have been noted with specific abnormalities: Losses of 4q, 13q, and 16q are associated with HBV infection,[25] loss of 4q has been associated with elevated α-fetoprotein levels, TP53 mutations,[29] tumor size, and vascular invasion[30] while 9p and 6q losses have been reported to be independent predictors of poor outcome of HCC patients,[31] and that losses of 4q, 13q, and 16q are associated with HBV infection.

Similar to the finding reported by the previous array CGH based study, a recent whole-genome sequencing study on HCC showed similar patterns of genomic imbalances: The copy number variation in HCC genomes is dominated by large-scale amplifications and deletions of chromosomal arms or entire chromosomes including gain at 1q, 5p, 6p, 8q, 17q, and 20q; and deletion at 4q or loss at 4p/4q, 8p, 13p/13q, 16p/16q, 17p, 21p/21q, and 22q.[22]

**Loss of heterozygosity**
Loss of heterozygosity (LOH) refers to one of two polymorphic alleles on a tumor chromosome. Zhang et al.[32] identified a high frequency of LOH 4q (48.1%) in HCC, in which the caspase-6 and ras-related C3 botulinum toxin substrate 1 pseudogene 5 in the region 4q24-26 may be related with tumor growth. Additionally, inhibitor of growth family, member 2 (ING2) in the region 4q34.3-4q35 was found to be down-regulated frequently in HCC, and its gene expression was also significantly decreased, suggesting that ING2 might be a tumor-specific glycoprotein of HCC.[32] In a variety of human tumors, the most common chromosomal changes were 8p allelic loss, suggesting that there might be one or several tumor suppressor genes on the short arm of chromosome 8. LOH was frequently observed on chromosomes 8p22-23, but the gene closely related with HCC was still unknown. However, Peng et al.[33] identified that LOH of zinc finger, DHHC-type containing 2 (in 8p22-23) was associated with early metastatic recurrence of HCC after liver transplantation.

**Gene amplification and deletion**
Gene amplification in certain regions of chromosomes plays a crucial role in the development and progression of human malignancies. Recently, researchers found amplification of the ecotropic viral integration site 1 (EVII) gene at the chromosomal region 3q26 in the HCC cell line JHH-1.[34] A copy number gain of EVII was observed in 36% (24/66) of primary HCC tumors. EVII antagonizes TGF-β-mediated growth inhibition in HCC cells, suggesting the EVII may be a potential molecular target for the development of novel therapies to treat HCC.[34] In another study, granulin-epithelin precursor, a secretary growth factor, was identified with gene amplification in 20% of HCC cases, and this amplification was correlated with enhanced expression levels in the same HCC.
cases.[33] Human epithelial growth factor receptor-2 (HER2) and topoisomerase II alpha (TOP2A) have been identified to be co-amplified in breast and some other cancers,[36] but the HER2 gene status and HER2 protein expression in HCC has been controversial.[37] However, no correlation was shown between TOP2A amplification and TOP2A overexpression in HCC.[38]

Gene deletion of tumor suppressor genes in certain regions of chromosomes also plays a crucial role in the pathogenesis of cancer. CDKN2A is a tumor suppressor gene that encodes for p16 and p14ARF. In a recent whole-genome sequencing study, CDKN2A deletion was identified in 10.2% HCC cases.[2] Protein tyrosine phosphatase, receptor type D, a tumor suppressor gene, which was previously identified to be co-amplified in breast and some other neoplasms.[39-41] was also identified with homozygous deletion in human HCCs.[42]

**PROSPECTS**

In summary, multiple lines of evidence have shown that the genetic and genomic alterations play important roles in the development and progression of HCC. The next generation sequencing of genomic DNA provides the possibility that more novel genetic and genomic alterations may be discovered and may provide new insights for understanding of the pathogenesis of HCC. However, further studies on the role of genetic mutation and genomic imbalances in the pathogenesis of HCC, as well as related functional and mechanistic studies are also urgently needed.

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**Conflicts of interest**

There are no conflicts of interest.

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