Editorial

# Genomic alterations in hepatocellular carcinoma and their clinical application to genomic medicine

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Liver cancer remains a global health problem and its incidence is growing worldwide. Liver cancer is an epidemiologically biased disease and is one of the most common cancers, especially in East Asia. Hepatocellular carcinoma (HCC) is the dominant histological type of liver cancer and the principal etiological factor for HCC in Japan is hepatitis C virus (HCV), while that in other Asian countries is hepatitis B virus (HBV) (1). Other known risk factors include alcohol abuse, autoimmune hepatitis, diabetes, and several metabolic diseases. However, the precise molecular events underlying hepatocarcinogenesis are still not fully understood, and the genetic characteristics associated with the etiologies still remain unclear. Recent large-scale genomic analyses have revealed the overall landscape and there are active attempts to apply the results to clinical practice. Based on the latest findings, we will describe the overall landscape of genomic alterations in HCC and their application to genomic medicine.

## Overview of HCC driver genes

Totoki *et al.* reported a Japan-U.S. collaborative study on HCC whole exon sequencing (2). A total of 503 cases of HCC, including 413 Japanese cases and a portion of the TCGA data (90 United States cases) were enrolled. The study identified 30 driver genes, among which telomerase (*TERT*) promoter mutations were the most frequent (>50%), and *TP53* and *CTNNB1* mutations were found in approximately 30% of all cases. In addition, although the frequency is less than 10% of the total cases, the study identified other driver genes, such as the SWI/SNF complex components related to chromatin remodeling (*ARID1A*, *ARID1B*, *ARID2*), Wnt pathway regulators (*AXIN*, *APC*), oxidative stress adaptation regulators (*NRF2*, *KEAP1*), and the mTOR pathway (*TSC2*, *RPS6*, and *KA3*).

The TCGA group in the U.S. reported the results of an integrative molecular analysis of 363 HCC cases, including whole exon sequencing and RNA sequencing as well as methylation and proteomic analyses (3). Twenty-six driver genes were identified, which were almost identical to those reported by Totoki *et al.* Consistent with this, *TERT* promoter mutations (44%), *TP53* (31%), *CTNNB1* (27%), and chromatin regulatory molecules were frequently aberrant.

Fujimoto *et al.* reported the whole genome sequencing of 300 Japanese HCC cases (4). Nine genes, including *TFP12*, *MEDWE6*, and *WDR74* showed the statistically significant accumulation of promoter mutations, but *TERT* promoter mutations were also identified as the most frequent mutations in whole genome sequencing. In addition, long non-coding RNAs, *NEAT1* and *MALAT1*, were mutated in 22% and 6% of the HCCs, respectively, and are reported as novel driver genes.

## Cancer-associated pathways and therapeutic development in HCC

### *Telomerase*

Telomerase is an enzyme that locates at the ends of chromosomes and elongates telomere structures, which

consist of specific repeats. The telomere length shortens with each cell division, inducing cellular senescence. In cancer cells, telomerase activation is known to be one of the essential molecular mechanisms in carcinogenesis. The known mechanisms in HCC include amplification of *TERT* itself and activation by insertion of the HBV genome. In addition to these mechanisms, mutations in the promoter region also enhance the expression of *TERT* by forming new transcription factor binding sequences. Totoki *et al.* (2) found mutations in 58% of cases in Japan, Wang *et al.* (5) found mutations in 45% of cases in China, and the TCGA (3) found mutations in 44% of cases in the U.S. There was no difference in the rate of *TERT* mutations, regardless of the rate of HBV infection.

Telomerase inhibitors as anticancer agents, drugs targeting telomeric DNA, viral vectors carrying the promoter of *TERT*, and immunotherapy targeting *TERT* have been developed (6). Although the high frequency of *TERT* promoter mutations make agents targeting the increased expression of *TERT* promising in HCC, clinical trials of immunotherapy using *TERT*-based vaccines have demonstrated only limited efficacy. Currently, immunotherapy targeting *TERT* is expected to be effective for HCC as a personalized immunotherapy when used in combination with immune checkpoint inhibitors (ICIs) (7).

### *P53 pathway*

P53 functions as a transcription factor in the regulation of genome stability, the cell cycle, and cell death. Mutations have been reported in more than 30% of HCCs. When *MDM2* and *MDM4* amplification (upstream negative regulators), *IRF2* mutation (positive regulator), and downstream *ATM* mutations are included, the number of cases with P53 pathway defects is reported to be 37–72% (2,3,5,8,9). A number of inhibitors that block the p53-MDM2 interaction and agents that restore the function of the mutated p53 protein by acting as molecular chaperones are in development (10). However, approaches that take advantage of the p53 pathway to produce therapies with either p53 mutant or wildtype proteins remain to be established. The use of synthetic mutations and the restoration of a mutant p53 protein to a wildtype protein by a small molecular reactivator remains to be explored. Further research, which employs some of the candidates, is required to address these questions.

### *Wnt pathway*

Wnt is one of the humoral factors that regulate hepatocyte proliferation and differentiation. The signal induces CTNNB1 (β1 catenin) protein stability, and the accumulated CTNNBI migrates into the cell nucleus and binds to TCF4, enhancing its transcriptional activity. When combined with mutations in *AXIN* or *APC*, which degrade CTNNB1 and negatively regulate its activity, Wnt pathway abnormalities occur in approximately 37–66% of all HCC cases (2,3,5,8,9). These abnormalities induce the expression of its downstream genes, *MYC* and *CCND1*, and may act to promote the proliferation of HCC cells. The Wnt pathway is involved in cell survival, proliferation, migration, and invasion, and is therefore associated with an aggressive phenotype in HCC. Thus, due to their potential efficacy as therapeutic agents against HCC, Wnt signaling inhibitors are currently being developed. Importantly, however, because the Wnt pathway is essential for tissue and liver regeneration and stem cell self-renewal, Wnt pathway inhibitors can induce substantial and long-term side effects, including anemia, immunosuppression, and gastrointestinal tract damage. The safety and efficacy of Wnt signaling inhibitors are currently being evaluated in clinical trials.

### *Chromatin-regulating molecules*

The transcription or repair of DNA processes requires changes in chromatin structure, and ARID1A/1B/2 are important components of the SWI/SNF molecular complex that initiates such chromatin remodeling. Loss-of-function mutations in *ARID1A/1B/2* play an important role in hepatocarcinogenesis and cancer progression. In particular, *ARID1A* mutations have been reported to interfere with mismatch repair mechanisms and to enhance the sensitivity of ICIs. *ARID1A* mutation is expected to be useful as a biomarker for predicting the response to ICI therapy (11).

### *PIK3CA/mTOR pathway*

The PIK3CA/mTOR pathway is involved in metabolic pathways as well as cell proliferation and survival. In HCC, deletion of *PTEN* and mutation of *RPS6KA3* have been reported in 7% and 4% of cases, respectively and inactive mutations of *TSC1/2*, which negatively regulate mTOR activity, have been reported in a few percent of cases.

Amplification of *FGF19* and amplification of *CCND1* are also found in approximately 10% of cases. It has been reported that 27–45% of all cases have some alterations in the PIK3CA/mTOR pathway (2,3,5,8,9). In a phase I/II study of everolimus, an mTOR inhibitor, in HCC, the disease control rate was 44% at a maximum dose of 10 mg/day (12). Although phase III trials failed to prove the usefulness of everolimus as second-line therapy after sorafenib (13), the PIK3CA/mTOR pathway is expected as a promising therapeutic target in HCC.

## Utility as a biomarker for genomic alterations

Among the genomic alterations, those that directly lead to targeted therapies, so-called actionable alterations, are limited. Harding *et al.* (14) reported that cases with genomic abnormalities in the PI3K/mTOR pathway were significantly less sensitive to the multi-kinase inhibitor sorafenib, than those without mutations, and that these cases showed significantly shorter overall survival. Furthermore, in the subgroup treated with ICIs, cases with alterations in the Wnt pathway had a significantly shorter recurrence-free period than those without alterations.

ICIs are expected to be effective for HCC as well as other cancers, but there are no established genomic or immune markers that predict their efficacy. Fujita *et al.* performed subtyping of HCCs by an integrative analysis of mutation, DNA methylation, and gene expression data, and found that the subgroup with *CTNNB1* mutation were so-called "cold tumors", in which anti-cancer immunity is suppressed and these tumors may not be a promising target for ICIs (15). Currently, the most promising drug therapy for HCC is the combination of molecular targeted therapy and ICIs, and the combination of lenvatinib and the anti-PD-1 antibody pembrolizumab is approved by the FDA for the primary treatment of unresectable HCC in the U.S. A number of clinical trials are still ongoing to explore the usefulness and biomarkers for combination therapies of molecular targeted therapy and ICIs.

## Conclusions

Large-scale genome sequencing studies of HCC have identified many potential therapeutic targets in HCC. This means that a single drug cannot effectively treat HCC, suggesting the need to construct a library of drugs that specifically target different therapeutic targets. The establishment of personalized genomic medicine also requires the development of biomarkers to predict sensitivity to each of these agents along with the development of therapeutic drugs.

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