

# Comprehensive characterization of hepatitis B virus-associated multifocal hepatocellular carcinoma using a multi-omics strategy

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Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide (1). Although numerous studies have examined the molecular mechanisms involved in hepatocarcinogenesis, powerful diagnostic and/or prognostic factors as well as an efficient therapeutic target for HCC have yet to be developed.

In the recent investigative study by Miao *et al.* (2), "Identification of prognostic biomarkers in hepatitis B virus (HBV)-related HCC and stratification by integrative multi-omics analysis", the authors addressed the characteristics of multifocal HCCs using a global genome and transcriptome analyses approach, and identified novel biomarkers predicting survival duration. In their study, the authors compared the genetic profiles of multifocal HCCs from two patients with different outcomes after surgery. Patient I (PI) had multiple liver tumors (poorly differentiated HCC) with portal vein tumor thrombus and a very short survival, whereas patient II (PII) had multi-centric nodules in the liver (well-differentiated HCC) and achieved a long survival.

Miao *et al.* (2) reported differences in the HCC features between the two patients in several aspects. They found that the HBV genome was integrated in a particular region in all tumors in PI, but the integration sites differed between nodules in PII. Whole-genome sequencing analysis demonstrated differences in the pattern of copy-number variations between the two patients; that is, some amplifications or deletions in particular regions were detected in all nodules in PI, whereas the alteration patterns varied between nodules in PII. According to the phylogenetic tree that was established based on the analyses of gene mutations, copy number variations, and structure variations, in PI the intrahepatic metastatic tumors were most distant from the putative germline compared to portal

invasion or primary tumors, whereas in PII multiple tumors located the same distance from the germline and each other. These findings suggest that all of the nodules that formed in the liver of PI originated from the primary tumor, whereas in PII the liver tumors developed in an independent molecular process.

Next, the authors analyzed the gene expression profiles of the PI and PII tumors. Every tumor in PI shared a similar gene expression pattern, consistent with findings from the genomic approach. Pathway analysis clarified that all the tumors in PI had a deregulated function in common, whereas each of the tumors in PII had a distinct transcriptomic dysregulation pattern. In addition, the functional changes essential for metastasis, including cell migration and proliferation, were remarkable in PI tumors compared with PII tumors, suggesting an association with tumor aggressiveness and patient prognosis.

Lastly, the authors selected seven candidate genes with highly differential expression between the two patients, followed by validation studies using paired tumor/non-tumor tissues of 174 HBV-HCC patients to confirm the specificity of the gene expression in HCC tissue. Among the candidate genes, they identified the expression of *TKK*, a dual-specific protein kinase participating in the p53 pathway, as being significantly correlated with tumor grade, recurrence-free survival, and overall survival; emphasizing the possible applicability of *TKK* as a novel adverse prognostic factor of HBV-HCC.

In their article, the Miao *et al.* (2) described the different molecular features of tumors that developed in the two patients with multiple HCCs using various methods, including identification of the HBV integration sites, somatic mutation pattern, copy-number variations, and

phylogenetic analysis. Especially, in patients with aggressive HCC having a poor prognosis, analyses of vascular invasion and metastatic nodules might enable us to speculate the types of genetic alterations necessary for the primary tumor to acquire the ability to metastasize. The authors demonstrated that aggressive tumors acquired malignant potential, including functional somatic mutations and gene signatures related to functional changes. Through their multi-omics analyses, the authors identified the contribution of a novel prognostic biomarker, *TKK*, to tumorigenesis in various human cancers by modulating the mitotic checkpoint, which is worthy of special mention.

Recently, omics technologies driven by advanced mass spectrometry have been applied as powerful tools to improve the understanding of the pathogenesis and treatments of various diseases, including human cancers (3,4). In general, omics technologies are defined as concurrent high-throughput methods that are specifically utilized for global profiling of *in vivo* molecules (DNAs, RNAs, proteins, and metabolites) present in various biologic matrices. Introducing omics technologies to analyses of the pathogenesis or treatments of human diseases might contribute not only to the identification of molecular biomarkers used in a clinical context, but also to exploration of the pathogenesis of various diseases in an experimental setting. Indeed, multiple omics technologies have been broadly utilized in cancer research. In particular, these technologies are often used to dissect different biologic aspects of tumors for the purpose of biomarker discovery, developing a better understanding of the pathogenesis, and for therapeutic discovery.

Recent outstanding advances in analysis technology, including ultra-deep sequencing, enabled us to perform multi-omics analyses more rapidly, precisely, and easily. Several studies have examined exome-sequencing or whole genome sequencing to identify driver gene mutations in HCC (5-7). In addition, several gene-expression profiling studies have suggested a variety of multi-gene scoring systems that are useful for classifying HCC and predicting overall survival and/or recurrence-free survival (8,9). These previous studies focused on various genes as putative prognostic markers or predictors of the malignant potential of HCCs. In the near future, additional cohort studies on prognostic factors of HCC patients will be conducted using omics technologies and it is expected that potential candidate genes associated with prognosis or recurrence of patients with HCC will be identified.

Although the perspectives mentioned above contribute

to elucidating the pathogenesis of HCC, the abundant data obtained using those strategies could be confusing due to the magnitude and complexity of the information. In Miao *et al.* (2), the authors focused on *TTK* as a representative adverse prognostic marker, but the relationship between high expression of the gene *TTK* and the poor prognosis of HCC patients was demonstrated only by univariate analysis, such as Kaplan-Meier curves and log-rank test. It is important to note that several other factors could also be associated with a patient's prognosis, including other genomic and transcriptomic information as well as the clinicopathologic background. Multivariate analyses of these other co-factors, such as by the Cox proportional hazard model, might further clarify the significance of *TKK* as a prognostic factor. In the post-genome era, integration of the information obtained from global genetic analysis and understanding how to incorporate the large amount of data into the clinical data, including survival information, is extremely complex, but important. Furthermore, quality control and validation studies of the results obtained from ultra-deep sequencing are critical (10).

HCC is an extremely heterogeneous tumor. To date, only a couple of studies have classified patients with HCC into several subgroups based on their gene expression profiles. The molecular features of HCC, such as driver mutations, genetic profiles, and prognostic biomarkers differ between subgroups. On the other hand, HCC etiology could also influence the molecular profiles of the tumor. It is a challenging task to identify the prognostic biomarkers from multi-omics analyses that can be applied to other cohorts with other etiologies, such as hepatitis C virus infection, alcoholic liver disease, and nonalcoholic steatohepatitis (11).

To benefit the various populations of HCC patients, the establishment of diagnosis and treatment methodologies according to the pathogenesis and tumor status of each patient is warranted. Application of the currently rapidly progressing omics technologies will facilitate the development of strategies for diagnosis and treatment based on multi-omics rather than clinicopathology. In this new paradigm, an important topic will be how we will utilize significant information among the enormous amounts of omics data, so-called "big data", and how we will apply the large amounts of information to the advancement of clinical practice.

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