Commentary

Next generation sequencing, inter-tumor heterogeneity and prognosis of hepatitis B related hepatocellular carcinoma

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Submitted Dec 03, 2014. Accepted for publication Dec 05, 2014.
doi: 10.3978/j.issn.1000-9604.2014.12.11
View this article at: http://dx.doi.org/10.3978/j.issn.1000-9604.2014.12.11

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related death in Asia and Africa (1). It reflects the high burden of hepatitis B virus (HBV) infection in these areas. Curative treatments of HCC as radiofrequency ablation and resection are impaired by a high rate of tumor recurrence. However, most of the time, HCC is frequently diagnosed at advanced stages where only palliative treatment as transarterial chemoembolization or sorafenib are recommended (1). Major advances have been performed in the field of molecular classification and identification of driver genes of liver carcinogenesis (2). Several signaling pathways are deregulated in HCC including the Wnt/β-catenin, cell cycle regulator, chromatin remodeling, ras/raf/map kinase and oxidative stress pathway (3,4). Moreover, *TERT* (coding for the telomerase reverse transcriptase) promoter mutations are the most frequent somatic genetic alterations observed both in cirrhotic premalignant lesions and in HCC. These mutations lead to reactivation of telomerase and telomere synthesis (4,5). Additional mechanisms related to HBV infection have been already described including insertional mutagenesis, oncogenic role of viral proteins and induction of genetic instability by the virus himself (6,7). However, few data are available about genetic heterogeneity of HCC. In addition of the classical intra-tumor heterogeneity described in most of solid cancer, the inter-tumor heterogeneity observed in HCC adds a new level of complexity (8). Multiple HCC could reflect a metastatic process or the development of independent tumors ("multicentric carcinogenesis"). However, the inter-tumor genetic heterogeneity has been rarely studied using next generation sequencing. The study of Miao R *et al*., published in *Journal of Hepatology*, focused on multifocal HCC and analyzed the results of whole genome sequencing and whole transcriptome sequencing (RNA sequencing) in multiples tumors of two patients (9). First, they described the presence of HBV insertion in the tumor genome, a phenomenon known as insertional mutagenesis (6). In patient 1 they identified the same HBV insertion in all the lesions suggesting an intrahepatic metastatic process. In contrast, they identified different insertion sites among the tumors of patient 2. Interestingly, in one case, they found a HBV insertion in the *TERT* gene, a recurrent site of HBV insertion previously described (10). The author also analyzed somatic mutations and copy number variations and confirmed the intrahepatic metastatic process in patient 1 and the multicentric carcinogenesis in patient 2.

Moreover, implementation of genetic knowledge in clinic remains to be performed (11). In the field of curative treatments, we need to better stratify patients according to their prognosis to assist the clinician for decision-making. In this line, Miao R *et al*. compared the HCC expression profile between HCC of patient 1 and HCC of patient 2 arguing that the metastatic process observed in patient 2 is associated with poor prognosis and could be used to identify prognostic biomarkers (9). Seven genes differentially expressed between tumors of patient 1 and patient 2 were selected for validation in a larger set of patients. The expression of these seven genes was assessed in a cohort of 174 patients with HCC related to HBV infection and treated by liver resection. Finally, a high *TTK* mRNA level was associated with short overall survival and recurrence

free survival. Consequently, *TTK* could be considered as an "easy-to-use" prognostic biomarker for HCC treated by liver resection. *TTK* codes for a protein kinase involved in P53 pathway and regulation of mitotic checkpoint through modulation of AURKB (12).

However, several molecular signatures have already been described and were able to predict prognosis (11). Recently, a tumor 5-gene score could accurately predict prognosis in HCC patients treated by liver resection (13). Moreover, a signature including 186 genes derived from the non-tumor cirrhotic liver was also able to predict survival and tumor recurrence (13,14). These two signatures capture different molecular features and clinical events: early recurrence mostly due to intra-hepatic metastasis of the primary tumor for the 5-gene score and late recurrence mostly due to new HCC development for the 186 genes signature. *TTK* as a prognostic biomarker should be compared to the other molecular signatures and it independent value should be compared to classical and histological features (15). In addition, the prognostic value of *TTK* should be externally validated, especially in HCC related to HCV, alcohol or metabolic syndrome.

In conclusion, in the study of Miao R *et al*. (9), next-generation sequencing has allowed to dissect the inter-tumor heterogeneity and identify new prognostic biomarkers. The main goal of post genomic area will be to translate this knowledge in clinic and move from a technological revolution into a revolution in clinical cancer care.

Acknowledgements

This work was supported by HECAM (BPI), EBCI, INCa (WntHCC project). J-C.N. was supported by a fellowship from INCa.
Disclosure: The author declares no conflict of interest.

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Cite this article as: Nault JC. Next generation sequencing, inter-tumor heterogeneity and prognosis of hepatitis B related hepatocellular carcinoma. Chin J Cancer Res 2014;26(6):730-731. doi: 10.3978/j.issn.1000-9604.2014.12.11

