Multi-omics in prognosis of hepatocellular carcinoma

Anna Lena Ress[1], Rishi Wagle[2], Martin Pichler[1,2]

[1]Division of Oncology, Department of Internal Medicine, Medical University of Graz, Austria; [2]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, USA

Correspondence to: Dr. Martin Pichler. Division of Oncology, Department of Internal Medicine, Medical University of Graz, Augenbruggerplatz 15, 8010 Graz, Austria. Email: martin.pichler@medunigraz.at.

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The hepatocellular carcinoma (HCC), a malignant tumor of liver parenchymal cells, is one of the most common malignances in the world. Currently, it ranks among the top ten leading cancer types for estimated deaths in both sexes and its incidence continues to increase. In the majority of all liver cancer cases (three-quarters), a chronic infection of hepatitis B virus (HBV) or hepatitis C virus (HCV) leads to chronic liver damage and plays an important role in hepatocarcinogenesis (1-3).

The current therapy and management of HCC follows staging, grounded on clinical practical guidelines. The management of local diseases varies from resection, with local ablation and liver transplantation dependent upon other terms from the number and size of lesions and clinical performance status. Unfortunately, most HCC patients attain locally advanced or metastatic diseases and reduced liver function due to the underlying cirrhosis, which commonly impairs curative treatment considerations. The only currently approved palliative agent, sorafenib, targets different growth signals and angiogenesis by blocking RAF and other kinases. Despite the effects of this approved drug, some HCC cells are initially resistant to it (primary resistance) or become resistant (secondary resistance) after long-term exposure to the drug. The need for sufficiently therapeutic options is highlighted by the prognosis, which drops from ≥36 to ≤16 months of median survival for patients with advanced or metastatic diseases (4-6). The ongoing clinical development of new targeted agents along with the identification of clinically relevant biomarkers might provide further advances (7). Biomarkers are a helpful tool to prognosticate patients' clinical outcome and might help to improve the stratification of patients with similar clinical or pathological stages. The commonly used and established HCC tumor marker alpha-fetoprotein is rather unspecific and often results in false positives or positives due to known prepositions (liver cirrhosis, chronically hepatitis) (8). This shows the urgent need for specific and significant markers and a screening test for HCC including the individuality and complexity of every patient and HCC (9-11). In this context, a recently published paper by Aleksandrova *et al.* illustrates the complexity and range of this topic. The authors showed that a higher risk of HCC is also associated with elevated levels of biomarkers of inflammation and hyperinsulinemia (interleukin-6, CRP, Adiponektin, C-Peptid) independent of obesity and established liver cancer risk factors (12). Recent technological advances, especially next generation sequencing (NGS) strategies, have enabled a completely new view of the underlying molecular mechanisms in cancer genomics, bringing the level of information from the single parametric level to the multi-genomic area (13,14).

Since the genomics era, our understanding of cancer biology has greatly improved, while simultaneously the complexity of the cancer genome was pictured. The success in translating cancer genomics offered potential targets, which can extends the lives of many cancer patients. In the context of HCC, frequently diagnosed as multifocality tumors, the prognosis is quite different for patients. One reason for this disparity is that the differentiation between synchronous developed multi-focal lesions and intra-hepatic metastatic spread was nearly impossible to observe with classical diagnostic tools. The latter has a significantly poor clinical outcome, making a stratification of these two multi-focal events important. Recently, a very interesting paper by Miao and colleagues showed the power and impact of this possible approach prior the above discoveries. Through

the use of multi-omics profiling of HCC tissue specimen (tumour and normal) and integration with detailed clinico-pathological information the HCC type, clonality and aggressiveness could be described and promising prognostic markers were found (15). In their study, they selected two multifocal HBV-related HCC patients as following: patient I (PI) with cirrhosis and poorly differentiated HCC died of recurrences three month after resection and patient II (PII) was non-cirrhotic and well-differentiated multifocal HCC, no recurrences appeared within 2 years after surgery. Based on this clinical presentation, they hypothesized that PI had intrahepatic metastases in contrast to PII whom they assumed had synchronous primary tumour development, without spread or metachronous lesions. NGS was carried out for each patient with different tissues from multiple lesions, non-cancerous liver controls and peripheral blood, and the results were validated by independent PCR analysis.

The different manifestations of the multiple tumours in these two patients were first explained with the HBV integration data that suggested different pattern of tumour clonality. Whereas the HBV integration was associated with a 3,209 bp event in the intergenic region of 3q26.1 in all tumours of PI (monoclonal origin of metastases), the PII tumours had completely different HBV integration sites (different tumour-initiating clones). This finding was validated by the following four different experiments. Somatic mutations including substitutions and small insertions/or deletions were studied. Through analysis of whole-genome sequencing and SNP genotyping data, copy number variations were assessed. Additionally, genomic structural variations were analyzed and a phylogenetic tree was constructed. Together, these findings clearly indicate the genomic similarities of all PI tumours and distinct mutation profiles of PII (13,14).

The transcriptomic analyses supports the genetic alterations identified at the genomic level.

Moreover, based on the multi-omic results, potential biomarkers for prognosis were validated in an independent cohort of 174 HBV-related HCC patients. Genes were evaluated for pathway enrichment and in parallel associated with clinico-pathological characteristics. In the correlation of gene expression with postsurgical prognosis, Miao *et al*. found out that *TKK* expression might be an independent prognostic indicator for metastatic potential, postsurgical recurrence, and survival of HCC patients (15). Interestingly, the median recurrence-free survival was 3.53 months in *TKK*-high group compared to 12.48 months in *TKK*-low group (P=0.0122). In spite of this encouraging data, independent confirmation in different cohorts should be the next steps to enable a generalizability of this result. For instance, a further independent evaluation of *TKK* expression and prognostic relevance in HCC can be done by using publicly available data and also by comparing their findings in non-HBV-related HCC.

Additionally, the HBV X protein (HBx), one of four overlapping open-reading frames of the double-stranded DNA genome of HBV, is known to influence the development of HCC in different processes including metastasis and involvement in p53 signaling (16). While performing the transcriptomic analyses and validation of biomarkers of multifocal HCC, it would have been of interest to connect the generated data with HBx expression (17).

For the first time, the authors were able to explain and define on the levels of genomic and transcriptomic studies the different multi-focal tumour development models in HCC (metastatic versus synchronous primary). With this work, new mechanisms in HCC development were found and consequently HCC biomarkers could be identified and validated in HCC patient cohorts, which offers new and helpful therapy planning options and may influence clinical decision making.

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