Molecular classification of hepatocellular carcinoma: the view from metabolic zonation

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Words:1493 (including references); Figures: 1

Hepatocellular carcinomas (HCCs) arise from diverse etiological backgrounds and display remarkable biological and clinical heterogeneity. Unsupervised analyses of gene expression profiles variably subdivide HCCs into between two and six subclasses with distinct transcriptomic profiles and clinicopathologic features.(1-5) Nevertheless, HCC molecular subclassification has not been implemented in the clinic, hampered in part by a lack of consensus between the various classifications and their prognostic and, in particular, predictive implications. Sorafenib, the only approved systemic treatment, on average prolongs patient survival by 2.8 months.(6) However, robust predictive biomarkers for sorafenib response have not been defined. Indeed, prognostication and treatment decisions for HCC patients are currently made primarily based on the Barcelona Clinic Liver Cancer (BCLC) staging system.

Nonetheless, several recurrent themes have emerged from the molecular analyses. HCCs can be broadly classified as proliferative and non-proliferative in roughly equal proportions. Compared to non-proliferative HCCs, proliferative HCCs are typically more aggressive and less differentiated,(3, 5) and are frequently associated with higher serum α-fetoprotein (AFP) levels, *TP53* mutations and poor outcome.(1, 3, 4) Furthermore, proliferative HCCs may display TGF-β, MET, AKT, and/or IGF2 pathway activation(1-3) and/or the progenitor cell phenotype.(3) By contrast, the non-proliferative subgroup appears to be heterogeneous with less certain biological significance. While a subset (~30%) of the non-proliferative HCCs harbor mutations in β-catenin (encoded by *CTNNB1*) and show β-catenin pathway activation,(1-3, 5) there is no consensus on the distinguishing features of the remaining non-proliferative HCCs. In the current issue of Hepatology, Désert *et al.*(7) further our understanding of non-proliferative HCCs through the lens of the metabolic zonation program of the liver.

Liver parenchymal cells display a gradient of metabolic processes along the porto-central axis relative to the vascular structure of the liver (Figure 1). For instance, gluconeogenesis

and urea synthesis are primarily performed by hepatocytes near the portal vein ("periportal") whereas lipogenesis and glycolysis are increased on the central end ("perivenous" or "pericentral"). β-catenin-mediated Wnt signaling and HNF4A-regulated gene networks play important roles in governing metabolic zonation,(8) where in the periportal hepatocytes, TCF4 induces the transcription of HNF4A-regulated genes in the absence of β-catenin and in the periportal hepatocytes, β-catenin allows TCF4 to bind to Wnt-response elements thus inducing the transcription of β-catenin-induced genes.(9) Through a meta-analysis of 1,113 HCCs previously profiled using gene expression microarrays, Désert *et al.*(7) report that non-proliferative HCCs are divided into two distinct subclasses, each preserving the periportal or perivenous phenotypes of the metabolic zonation that is critical for normal liver functioning.

To enable the prediction of *CTNNB1* mutation status in the microarray metadata set, the investigators first defined a robust 5-gene signature using independent training and validation datasets with known *CTNNB1* mutation status. The signature consists of three (*GLUL*, *LRG5* and *ODAM*) and two (*HAL* and *VNN1*) genes whose expression positively and negatively, respectively, correlates with *CTNNB1* mutation status. While *GLUL* and *LRG5* are well-known Wnt target genes, the remaining three constitute novel HCC biomarkers. Using HCC cell lines, the authors demonstrate that activating β-catenin signaling using GSK3β inhibitor 6-bromoindirubin-3′-oxime upregulates odontogenic ameloblast-associated protein (*ODAM*) and attenuates histidine ammonia lyase (*HAL*) and vanin 1 (*VNN1*) expression. Indeed, a network analysis of mouse liver periportal and perivenous signatures showed that, although *ODAM*, *HAL* and *VNN1* were not present in the signatures, they were highly connected to the genes in the signatures. The 5-gene signature was found to have accuracies of 87% and 93% in the training and validation sets and was used to predict *CTNNB1* mutations status for the meta-analysis.

The investigators report that hierarchical clustering of the 1,1113 HCCs revealed four subclasses with different prognoses. “ECM-type” (extracellular matrix) and “STEM-type” HCCs display signatures of high tumor cell proliferation and are associated with the S1/S2 (Wnt/TGF-β, poor prognosis) subclasses.(3) Additionally, the “ECM-type” subclass is characterized by signatures of ECM modelling, integrin signaling, epithelial-to-mesenchymal transition, while the “STEM-type” subclass is enriched for cancer stem cell, metastasis, cell cycle progression and p53 mutation signatures.

The remaining two subclasses display signatures of low proliferation and favorable prognosis and both show little intra-cluster variability. What is interesting, however, is that, gene set enrichment analyses revealed that these two classes reflect the tightly regulated metabolic zonation program of the liver (Figure 1). These two subclasses were named “perivenous-type” (PV) and “periportal-type” (PP) HCCs to reflect their resemblance to the phenotypes at the two ends of the metabolic zonation spectrum. The PV subclass is highly enriched for predicted *CTNNB1* mutations and Wnt activation, the G6 subclass,(1) and perivenous hepatocyte gene signatures, including lipid and bile salt metabolism signatures. By contrast, the PP subclass is enriched for periportal hepatocyte gene signatures, such as those of gluconeogenesis and amino acid catabolism, and was enriched for the S3 subclass.(3) Finally, given the role of HNF4A, the authors demonstrated that the expression profiles of PP-type HCCs show a strong enrichment of HNF4A-regulated genes. Clinically, PP, PV, ECM and STEM types form a continuum of increasingly aggressive clinical behavior in terms of tumor aggressiveness, TNM staging, BCLC stages, vascular invasion, serum AFP concentrations, overall survival and disease-free survival.

The current study by Désert *et al.*(7) highlights the importance of interpreting HCC molecular classification in the context of basic liver biology and anatomy. Previous studies invariably identified a transcriptomically heterogeneous subset of well differentiated HCCs that display features consistent with or suggestive of hepatocytes. While some of these non-proliferative

HCCs harbor *CTNNB1* mutations and show β-catenin pathway activation,(1-3, 5) there is no agreement on the biological significance of the *CTNNB1*-wild-type subset. When viewed from the perspective of metabolic zonation, Désert *et al.*(7) demonstrate that non-proliferative HCCs recapitulate the phenotypes at the two distinct ends of the zonation program. In this context, the less aggressive clinical behavior of non-proliferative HCCs can be interpreted as their higher degree of preservation of normal liver phenotype. In particular, the lack of β-catenin activation in the PP-type HCCs would also be consistent with their least aggressive clinical and biological behavior. Notably, on the periportal vs perivenous spectrum, the only subclass that leans towards a perivenous phenotype is the PV-like, underscoring its unique biology compared to the other subclasses. Finally, the current study also provides a plausible explanation for the lack of prognostic difference between patients with *CTNNB1*-mutant or -wild-type HCCs,(10) as PV-type HCCs driven by β-catenin activation sit between proliferative HCCs and PP-type HCCs in terms of prognosis and highlights the molecular heterogeneity within the *CTNNB1*-wild-type subset. A more speculative interpretation of the current results raises the intriguing question of the cell of origin of HCCs. It remains to be seen whether the metabolic signatures observed in the various HCC subclasses merely reflect the metabolic programming along the porto-central axis or are indicative of their spatial origin.

A contextualized view of HCC molecular subclassifcation is crucial towards understanding HCC biology and identifying drug targets that consider the unique biology of liver cancer. The study by Désert *et al.*(7) represents a significant step towards a unifying molecular classification of HCCs. Going forward, it will be of interest to validate the results from the current study on biopsy and resected HCC samples, aiming not only to obtain prognostic indicators but also to discover predictive biomarkers.

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Figure 1

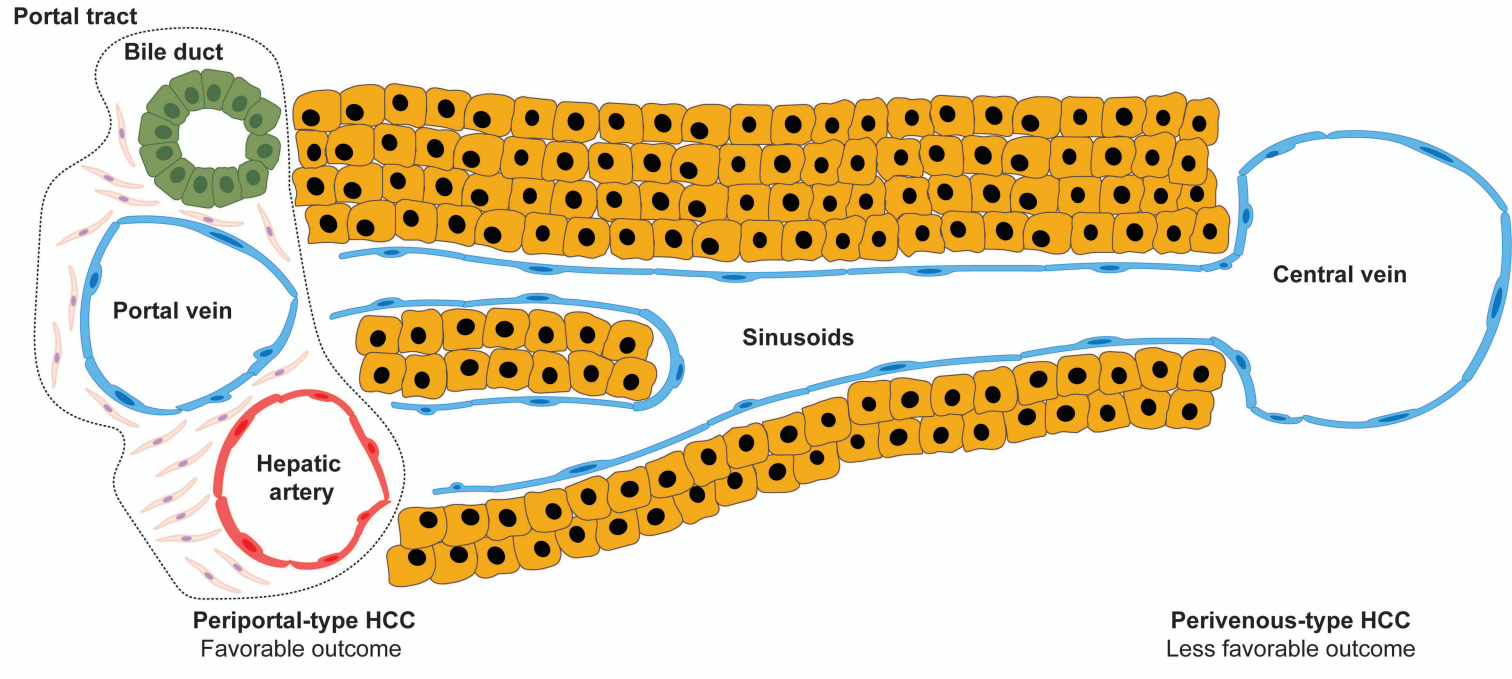
Portal tract
Bile duct
Portal vein
Hepatic artery
Sinusoids
Central vein
Periportal-type HCC
Favorable outcome
Perivenous-type HCC
Less favorable outcome
CTNNB1 mutations, G6 subclass, BCLC, TNM staging, vascular invasion, metastasis signature, fatty acid and bile salt metabolism, glycolysis, lipogenesis
Well differentiated, S3 subclass, HNF4A regulated networks, amino acid catabolism, biliary acid transport, gluconeogenesis, urea cycle

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FIGURE LEGEND

Figure 1: **Non-proliferative HCCs recapitulate metabolic zonation.** The metabolic zonation program of the liver stipulates that the metabolic functions of hepatocytes form a spatial gradient in relation to their proximity to either the portal vein ("periportal") or the central vein ("perivenous"). Periportal hepatocytes are involved in functions such as gluconeogenesis and amino acid catabolism, whereas perivenous hepatocytes perform functions such as glycolysis and lipogenesis. Non-proliferative hepatocellular carcinomas (HCCs) exhibit molecular features that are reminiscent of either the periportal ("periportal-type HCC") or the perivenous ("perivenous-type HCC") hepatocytes. Periportal-type HCCs are associated with favorable outcome, well differentiated HCC and HNF4A-associated gene networks. Perivenous-type HCCs are associated with less favorable outcome, increased frequency of *CTNNB1* mutations and β-catenin activation, and increased expression of metastasis gene signature.