COMMENTARY

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Prognostic signatures from hepatocellular carcinoma biopsy

Hepatic Oncology

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“It is very important to evaluate hepatocellular carcinomas not only statically (as simple number and size of nodules) but also dynamically, in other words, as space-occupying lesions with extremely different growth patterns. This has a molecular counterpart: fast hepatocellular carcinomas are biologically aggressive and have a severe prognosis. A simple liver biopsy at presentation is able to predict the hepatocellular carcinoma subsequent course.”

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Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor worldwide and the second cause of cancer-related death in men and the sixth in women [1]. Around 85% of HCCs develop as a result of chronic hepatitis caused by hepatitis B virus, hepatitis C virus, nonalcoholic steatohepatitis or alcohol use [2] and only 15% of HCCs occur in noncirrhotic livers.

HCC can be categorized as an inflammation-related cancer. Chronic-unresolved inflammation is associated with persistent hepatic damage, nonregulated growth of hepatocytes and concurrent regeneration, leading to regenerative nodules, dysplasia and eventually HCC.

Unlike all solid tumors, HCC prognosis is related not only to tumor stage but also to the underlying chronic liver disease. This has favored the creation of staging systems that include both clinical- and tumor-related features like number and size of tumor. The most commonly used are the tumor, node, metastases (TNM) staging, the Barcelona Clinic Liver Cancer (BCLC) systems and the Hong Kong Liver Cancer staging. These systems are typically based on systemic reviews of the literature and/or expert opinion. Other systems, such as and the Cancer of the Liver Italian Program score (CLIP), the Model to Estimate Survival in Ambulatory HCC patients (MESIAH) and Japanese Integrated Staging (JIS) scores, are prognostic scores that incorporate variables that were significant in multivariable Cox survival analyses [3–10]. However, all these tumor stagings are inaccurate in determining the individual prognosis, especially on the short term. One possible reason for this inaccuracy is their application in patients dissimilar from those used to set up the staging system. For example, etiology, therapeutic strategies and outcome are very different in Western and Eastern cohorts. Although these staging systems are particularly useful to stratify the HCC patients into appropriate risk categories, their predictive accuracy remains limited for the absence of biological prognostic factors. Not surprisingly, the recently published ITA.LI.CA staging system has a better ability to predict individual survival. Indeed, the ITA.LI.CA staging system includes, apart from clinical

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and tumor-related features, also α -fetoprotein, which is known to relate with more aggressive HCC and to predict outcome in the individual patient, when greatly elevated [11].

A definite progress in prognostication of HCC patients would be expected by refining the molecular signature so far developed, in other words, by using liver tissue not only derived from resection but also from consecutive prospectively enrolled patients, undergoing any type of therapy, in order to have a wider possibility to relate molecular characteristics to outcome. We will briefly compare in this report the available data on resection-derived signatures with those obtained from liver biopsy.

Prognostic signatures from resection

The advent of high-throughput technologies has greatly changed the ways in which scientists study the pathogenesis of diseases. Although initially restricted to fresh frozen samples, current technologies allow genomic profiling of partially degraded samples, such as formalin-fixed paraffin-embedded tissue. These new technologies find the application in oncology and have been used to evaluate DNA structural damage (e.g., point mutation, chromosomal rearrangements), functional genomics (e.g., mRNAs and noncoding RNAs), epigenetics (e.g., aberrant methylation and histone deacetylation) and metabolic profiles.

HCC is a highly heterogeneous disease and this heterogeneity represents the major obstacle for understanding the mechanisms underlying its onset and its progression [12]. During the last 20 years, the development of these new technologies has allowed to identify several signatures able to stratify HCCs in several subgroups that differ according to etiological factors [13,14], rate of recurrence [15,16], metastasis [17,18], vascular invasion [19–21] and survival [22,23]. Despite the limitations due to the source tissue, genomic profiling allowed to identify especially patients with poor prognosis, likely for their ability to identify risk of developing *de novo* tumor or recurrence. Although most prior studies were only able to stratify HCC tumors into two subgroups, Boyault *et al.* found that HCC tumors clustered into six distinct subgroups or classes (named G1–G6), closely associated with various clinical and genetic alterations, in this classification, the most important variable being chromosome stability status. HCCs from group G1 to G3 were chromosome instable, whereas HCCs from G4 to G6

were chromosome stable [24]. Subsequently, in an attempt to describe a common molecular classification, Hoshida *et al.* performed the first biostatistical meta-analysis of nine different transcriptomic studies in HCC [25]. Goossens *et al.* [26] also performed a detailed analysis of most of the available studies, including microarray, and proposed a comprehensive molecular classification of HCC. According to these last two studies, HCC tumors can be first classified into two major subgroups. The first group is characterized by aggressive biological/clinical features (S1 and S2). S2 subclass has a vascular invasion gene signature more strongly represented than S1 subclass. Subclass S3 is characterized by less aggressive features. The proposed classification encompasses all the most relevant studies performed; however, it is hardly applicable in the routine clinical practice, as it would require a time-consuming and costly workup for each single patient.

Similarly, Nault *et al.* [27] identified a score based on five genes (*HN1*, *RAN*, *RAMP3*, *CK19* and *TAF9*) that were associated with poor survival in HCC patients undergoing liver resection.

The complexity of molecular classification of HCC has recently increased following the identification of specific miRNA and epigenetic changes able to predict clinical outcomes. MiRNA-based signatures were shown to be useful for HCC classification and for the definition of risk of recurrence, response to therapy and prognostic stratification [28]. The expression of miR-122, miR-221 and miR-26 [29] was found, for example, to be associated with a poor prognosis in human HCC, while miR-125b expression was correlated with good survival [30]. Kim *et al.* identified and validated a risk score based on expression patterns of 65 genes that can quantify the likelihood of overall survival in HCC patients who have undergone surgical resection as primary treatment. The risk score correctly identified cases with significantly more severe prognosis of HCC, not recognized by current staging criteria. For example, the risk score can identify high-risk patients in both patients with early-stage HCC (BCLC stage A) and those with intermediate or advanced stage (BCLC stages B and C) and identify patients with poor prognosis without vasculature invasion, who are typically considered as patients with good prognosis [31].

All these features, together with the already mentioned bias of resection as the only source of tumor tissue and with the technical complexity of many of these signatures (often composed of

dozens/hundreds of genes), have so far prevented their wide application in the clinical practice. Last but not least, the real predictive value in prognostication at time of HCC diagnosis has not been prospectively validated. In this contest, it becomes essential to evaluate the possibilities of biopsy-based tests in HCC characterization.

Prognostic signature from HCC biopsy

Pathologic examination of solid tumor tissues has always been a mainstay of oncology. This has allowed to clarify the underlying molecular mechanisms and to identify targeted therapies much earlier than in hepatology. In HCC field, ultrasound-guided liver biopsy has been scarcely used, especially after the availability of extremely accurate imaging tools. While the combination of two second-level imaging techniques is usually sufficient to accurately define the nature of a space-occupying lesion in the liver, they are not informative on its biologic characteristics apart from indirect factors, like vascularization, necrosis, presence of a capsule. To date, therefore, there are only few studies, which used liver biopsy to investigate molecular HCC characteristics.

Makowska *et al.* [32] studied by microarray tumor and paired nontumor liver biopsies performed under ultrasound guidance in a series of 60 HCC patients and five normal liver tissue samples, aiming to develop a molecular classification system not biased by the stage of the HCC and, at the same time, to make a comparison with the existing molecular classifications of HCC in an unselected patient population. The authors were able to validate previously reported HCC classifications. However, they concluded that although gene expression profiling of HCC biopsies can identify subgroups with different prognosis, it has limited capacity to direct therapies. Although this study is interesting, it suffers from several potential biases that can have influenced the conclusions. HCC cohort was collected in a period of 10 years, during which HCC staging criteria and therapeutic strategies have greatly changed. In addition, although the identified clusters related with survival, the numerosity of the genes included in each of them prevents a routine use. Furthermore, apart from survival, it was not identified a HCC feature (like growth speed, microvascular density, etc.) that could be used to stratify HCCs in more homogenous groups in order to magnify the molecular differences.

In a recent prospective study, we choose to use growth speed of the tumor (evaluated as doubling time of the HCC lesion in two CT scans performed at 6-week interval) as a distinctive feature that could be strictly related with outcome. We identified a transcriptomic signature (including *ANGPT2*, *DLL4*, *NETO2*, *ESM1* and *NR4A1*), which was able to predict growth speed but, most importantly, survival in a series of HCC at first presentation [33]. This study underlines the importance of identifying a leading factor (in our case growth speed) that can shed light on the molecular data found. As a further demonstration of the strength of the described transcriptomic signature, we have characterized the microenvironment of these cases and the presence of regulating miRNA, all in the material derived from the US-guided liver biopsy [34]. These further data indicate that fast and slow HCCs are two subgroups with definitely very distinctive characteristics and that the transcriptomic signature is a potent tool for prognostication.

Conclusion & future perspective

HCC is not a 'standard' tumor: it is the end result of a longstanding inflammatory process that not only is able to give rise to the tumor but also determines a chronic liver disease, with ensuing functional failure. Thus, the diagnostic and therapeutic approach to HCC must take into account its complexity.

It is very important to evaluate HCCs not only statically (as simple number and size of nodules) but also dynamically, in other words, as space-occupying lesions with extremely different growth patterns. This has a molecular counterpart: fast HCCs are biologically aggressive and have a severe prognosis. A simple liver biopsy at presentation is able to predict the HCC subsequent course. Hopefully, increasingly refined techniques in the future will be able to further improve HCC prognostication at the individual level.

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