Reply to: "Network-based discovery of gene signature for vascular invasion prediction in HCC"

To the Editor:

Liu and colleagues raise some issues regarding our recently published study [1] to which we would like to make the following comments. We acknowledge the limitations that a gene-expression-based biomarker could have, and that our signature is not unique. Certainly, previous attempts to find such a signature have been published in the past [2]. We also know that, as in other gene expression studies, potential bias could occur. In fact, reported prognostic signatures are often not reproducible, in most of the cases due to suboptimal study design, small sample sizes, and also because many of them have been based on retrospectively collected tissue samples [3]. Even after taking into account these sources of bias or inconsistencies, it so happens that only a small minority of the reported signatures truly retain prognostic significance. In fact, our recent outcome analysis including 22 gene signatures with prognostic significance in HCC (18 from the tumor, and four from the non-tumoral adjacent tissue) showed that only two signatures retained independent prognostic value [4].

In our work, we select a training cohort based upon a homogeneous etiology to minimize the risk of molecular heterogeneity and to identify a clean and distinct signature. Patients with HCV-related HCC were selected, since this is the most common etiology in the Western countries. Then, we validate the signature in an independent multi-etiologic cohort of patients and the accuracy remained stable when an etiology-dependent subgroup analysis was performed [1]. The study was aimed at providing a gene-set to ease the preoperative diagnosis of vascular invasion, but was not designed for defining outcome prediction. Nonetheless, we have data indicating that the presence of a vascular invasion signature correlates with poor outcome, since the signature was found to be associated with early recurrence ($p = 0.057$), and was enriched in patients sharing signatures of poor prognosis [4].

Even considering that the question posed is simple (to identify a gene-signature capturing vascular invasion) the characteristics of patients, sample collection, sampling issues, technical variation, validation of results, and bioinformatics approaches are certainly heterogeneous, and thus the results might vary. In most instances, however, the different signatures seem to be able to capture common oncogenic mechanisms, as reflected by their capacity to adequately allocate patients into a poor or good prognosis group [5]. By applying a different methodological approach (weighted gene co-expression network analysis) to our data, Liu *et al.* provide a 9-gene signature with similar accuracy and no overlap with our 35-gene signature. The method applied is based on systems biology to find clusters of highly correlated genes across microarray samples, identify hubs of each module and correlate them with clinical traits [6]. This analysis is based on the hypothesis that information on signaling pathways is crucial to understand how genes are connected to each other and how they influence cellular functions in both normal and cancer conditions. This result further underlines the need for integrating the vast amount of available data and the development of powerful bioinformatics resources (annotation, methodologies, technical platforms, etc.).

A more relevant question is when can our signature-alone or in combination with tumor size- be translated into clinical practice. Strict rules have been proposed recently by Simon and colleagues [7]. Following this proposal, the EASL-EORTC guidelines on management of HCC have outlined a list of requirements in order to adopt molecular signatures in the clinical practice [8], which are as follows:

1. First, the signature should be generated in the setting of randomized studies or in case of cohort studies, it should follow the training/validation approach.
2. The signature should retain independent prognostic value when tested along known clinico-pathological variables.
3. The results should be confirmed by independent investigators in a separate set of samples.

Thus, according to these rules, in order to implement our signature in the decision-making process, for instance in the waiting list of liver transplantation, it should be validated by independent investigators in a novel set of samples. Ideally, the signature has to be reproduced in a device, which should give similar results. Only then, data is ready for acceptance in guidelines. It is a long path, but the only one for translation of genomic results into our practice.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Beatriz Mínguez
Mount Sinai Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA

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Liver Unit, Department of Internal Medicine, Hospital Vall d'Hebron, Barcelona, Spain
Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto Carlos III, Spain

Daniela Sia
HCC Translational Research Laboratory, Barcelona-Clinic Liver Cancer Group, Institut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), Liver Unit, Hospital Clinic, Barcelona, Catalonia, Spain
Gastrointestinal Surgery and Liver Transplantation Unit, National Cancer Institute, Department of Experimental Oncology, Milan, Italy

Josep María Llovet*
Mount Sinai Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA
HCC Translational Research Laboratory, Barcelona-Clinic Liver Cancer Group, Institut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), Liver Unit, Hospital Clinic, Barcelona, Catalonia, Spain
Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto Carlos III, Spain
Institució Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Catalonia, Spain
**Corresponding author.*
E-mail addresses: Josep.Llovet@mssm.edu, jmllovet@clinic.ub.es

Deregulation of microRNAs expression occurs in stages of multistep hepatocarcinogenesis: Why is it different?

To the Editor:

HBV is a major cause of acute and chronic liver infection, and can lead to hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1], which is the fifth most common cancer and the third leading cause of cancer death worldwide. More than 80% of HCC patients are in developing countries, especially in Southeast Asia and sub-Saharan Africa. However, the incidence of HCC has been rising in Western countries in recent years [2]. The current standard of care and therapy for patients with advanced HCC are not satisfactory. Surgical resection or liver transplantation remains the most effective treatment options for HCC, but few patients are fortunate to get the treatment, for all kinds of reasons. Therefore, the analysis of the molecular mechanisms of oncogenesis is badly needed to uncover novel targets for specific systemic therapy and to discover novel biomarkers for early diagnosis of HCC.

MicroRNAs (miRNAs), which are endogenous, small non-coding RNAs consisting of 20–25 nucleotides, have been shown to play important roles in various cellular and physiological processes, including cellular development, apoptosis, proliferation, and differentiation [3,4]. Typically, they modulate gene expression by regulating the mRNA at a posttranscriptional stage, base-pairing with sequences in the 3′-untranslated region [3,5]. There are currently 940 identifiable human miRNAs (The miRBase Sequence Database-Release ver. 15.0), which can recognize hundreds of target genes with incomplete complementarity and over one third of human genes appear to be conserved miRNA targets [6]. Recent evidence clearly shows that deregulation of miRNAs may contribute to aberrant activation of oncogenes and inactivation of tumor suppressor genes in human carcinogenesis. Several miRNAs have been linked to the initiation and progression of human cancers, including HBV-associated HCC [7–10]. Thus, great efforts have been put on miRNA mimetics and anti-sense miRNA as potential therapeutics for hepatocellular carcinoma, due to their stability and predominant uptake by the liver.

In the study by Gao *et al.* [7], a panel of seven miRNAs (miR-10b, miR-21, miR-122, miR-145, miR-199b, miR-221, and miR-224) was selected to examine miRNA deregulation during early stages of hepatocarcinogenesis. Among these miRNAs, only miR-145, miR-199b, and miR-224 were found to be significantly deregulated during the multistep hepatocarcinogenesis. While other miRNAs' expression changes seldom reached statistically significant levels among non-tumorous livers, dysplastic nodules and small HCC, after Bonferroni correction for multiple comparison tests [7]. This conclusion was quite different from other study results. Pineau *et al.* concluded that among the miRNAs present in the progression signature, miR-221 was capable of stimulating tumor growth *in vivo* possibly through p27 and/or DDIT4 downregulation [8]. The findings of Kutay *et al.* suggested that miR-122 downregulation was associated with hepatocarcinogenesis and could be a potential biomarker for liver cancer [9]. Connolly *et al.* initial analysis of cirrhotic livers suggested that upregulation of miR-17-92 and miR-21 occurred in pre-cancerous stages of liver disease [10]. Then, which conclusion is more convincing? And what makes them into controversies?

Despite the difference in the methods used by the researchers, the development of hepatocellular carcinoma in individuals with chronic hepatitis B is a multistage, multifactorial process including the interaction between host and environmental factors [11]. Risk factors for chronic HBV-related hepatocellular carcinoma include HBV DNA level, sex, age, cigarette smoking, alcohol consumption, chemical carcinogens, hormonal factors, and genetic susceptibility [12]. In a previous study, Zhang *et al.* [13] demonstrated that perturbations of miRNA expression during HBV infection were significantly correlated with those in HCC, and that aberrant expression of miR-199a, miR-199a*, miR-200a, and miR-200b was associated with liver fibrosis progression. In addition, the work by Liu *et al.* [14] suggested that microRNA-18a prevented estrogen receptor expression, promoting proliferation of hepatocellular carcinoma cells, which could be a mechanism explaining the sex disparity observed in HCC. However, the relationship between miRNA expression and other risk factors for chronic HBV-related HCC remains elusive. In the past studies, scientists paid most of their attention on the miRNA expression changes during the process of HCC, without taking other risk factors for chronic HBV-related HCC into consideration. Even though more and more miRNAs are being reported to be frequently deregulated in human cancers and may play a role in liver carcinogenesis, it is difficult to judge