

Vascular invasion leaves its mark in hepatocellular carcinoma

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Survival after either resection or liver transplantation for hepatocellular carcinoma (HCC) is related intimately to tumour recurrence. To reduce that risk, patients are selected for liver transplantation currently on the basis of tumour size and number, which are both crude, indirect measures of tumour biology. Despite careful radiological assessment, often based on multiple modalities and further review whilst on the waiting list, recurrence rates remain at 10–20%, even in the best centres [1]. A critical view of transplanting patients with such a high risk of tumour recurrence might conclude that this was wasteful in an era of extreme shortage of donor organs and a vital area for research focused on improved recipient selection. The much higher rate of HCC 'recurrence' after liver resection in patients with cirrhosis (as high as 70% after 5 years) [2], almost certainly reflects the combination of new primary tumour evolution as well as recurrence of the original tumour.

The pathological feature associated most closely with HCC recurrence is vascular invasion, divided conventionally into macroscopic (invasion of large blood vessels identified radiologically) or microscopic, by definition a histological diagnosis. A targeted biopsy of the lesion prior to resection or transplantation could help determine the value of subsequent surgery if vascular invasion was observed, thereby reducing futile transplantation. There are caveats: the hallmark lesion of microscopic vascular invasion is patchy and can be missed while a targeted biopsy may be difficult technically and there may be multiple lesions. Moreover, the risk of tumour tracking down the biopsy needle track is often cited as a reason not to perform a pre-treatment staging biopsy. That is not a view we share and published series (likely to estimate risk to be at the higher end of the spectrum) reveal a low risk of needle-track seeding, up to 2.7% overall [3,4].

Microscopic vascular invasion can only be confirmed as definitely present or absent when the whole tumour is available for careful histological examination after surgery. Thus, there is a real need for surrogate markers of vascular invasion in HCC that can be determined before resection or liver transplantation. Such a marker would have a profound effect on clinical practice, allowing patients with larger tumours without vascular invasion

access to curative therapies that are currently denied to them, or selection of patients with early-stage but more aggressive disease for clinical trials of new adjuvant agents, whilst avoiding futile surgery or liver transplantation, with implications for the restricted donor pool.

Minguez *et al.*, [5] in the current issue of the *Journal*, address this important question using genome-wide gene expression microarrays to derive a gene expression signature associated with macroscopic and microscopic vascular invasion. The authors used fresh-frozen tissue from 79 patients with hepatitis C virus related HCC as a 'training set' to define the 'vascular invasion signature', which comprised 14 genes that were over-expressed and 21 genes that were under-expressed in HCC with vascular invasion compared to HCC without vascular invasion. The 'vascular invasion signature' was then validated using formalin fixed paraffin embedded tissue in a further set of 135 patients with HCC and various causes of liver injury including hepatitis B virus and alcohol as well as those with hepatitis C virus infection. The 'vascular invasion signature' identified the presence or absence of vascular invasion in the validation set correctly in 69%. Following univariate and multivariate logistic regression analyses, both tumour size and the 'vascular invasion signature' were associated independently with vascular invasion, while ROC analysis showed that the 'vascular invasion signature' in combination with tumour size improved the sensitivity for the prediction of vascular invasion, but had little additive benefit over size alone in identification of those without vascular invasion (their Supplementary Table 1).

Their study has several limitations. Firstly, there are differences in the methods used for the training and validation sets. The training set used fresh-frozen tissues on the Affymetrix U133Plus2.0 array, containing probes from >50,000 transcripts and the validation set used formalin-fixed, paraffin embedded tissues in the Illumina DASL assay, covering 29,000 genes. The platforms use different probe sets, which are unlikely to be directly comparable; arguably, this is also a strength of the study, since successful validation of the 'vascular invasion signature' with a different platform suggests that the results are more applicable generally. However, gene expression profiles are influenced by mRNA quality and that extracted from formalin-fixed, paraffin embedded tissue is generally more degraded and of lower quality than that extracted from fresh frozen tissue. Nevertheless, a number of studies suggest equivalent gene expression profiles can be obtained using fresh-frozen or formalin-fixed, paraffin embedded tissue on the *same* platform, but in this study design the use of

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different tissue preservation methods on different platforms might have introduced bias.

Secondly, there are a number of important clinical differences between the training and validation groups. All the patients in the training set with HCC had HCV-related HCC, while in contrast the validation set had HCC with liver disease of mixed aetiology. While the validation set better reflects the 'real world', the single aetiology of the training set might have missed some genes important for prognosis of non-HCV-related HCC. Perhaps more importantly, all 8 cases with macroscopic vascular invasion were in the training set. The heatmap (their Fig. 1) shows a dominant effect of these cases on the gene signature. Thus, gene expression profiles of macroscopic vascular invasion appear to have a strong influence on the genes selected for the signature, which appears less consistent among the group with microscopic vascular invasion, which of course has clinical implications for tumour recurrence.

Finally, the follow-up period for the training set is much shorter than the validation set (median 21 months vs. 34 months), thus there are likely to be more individuals in the training set yet to develop HCC recurrence but allocated currently to the 'wrong' group.

Microscopic vascular invasion, which cannot be determined readily or reliably before surgery, is such an important risk factor for the long-term outcome that many previous studies have sought to identify tumour derived molecular markers to predict vascular invasion and the risk of tumour recurrence (reviewed in [6]). The ideal marker would be easy to measure, reproducible and have high negative and positive predictive value for vascular invasion in HCC arising on a background of liver disease of any aetiology. Candidate markers so far range from cell cycle regulators, oncogenes, tumour suppressors, angiogenesis, markers of chromosome instability [6], and more recently, microRNA expression [7]. Rationally, a gene expression signature is a strong candidate as it reflects global phenotypic change in a complex genetic disease such as HCC and indeed, previous studies have also proposed gene signatures that are related to vascular invasion [8–14]. However, this study highlights the limited reproducibility of gene expression microarrays between different platforms, centres and populations, as two of the gene signatures identified previously in different laboratories [8,9] were tested in the current cohort and were not predictive of vascular invasion in their hands. This question has been further addressed by the same group in a separate study [15] assessing the concordance of 22 published gene expression signatures. The current 'vascular invasion signature' clustered with a number of poor prognosis gene expression signatures [15].

The use of tumour gene expression to predict vascular invasion still requires a targeted pre-operative biopsy, just as conventional histological assessment of microvascular invasion and exactly the same caveats apply. Comparison of conventional histological assessment and tumour related gene signatures from the same sample could be instructive. The authors address the risks associated with a targeted biopsy and conclude that this approach is safe and on balance, it is a risk that would be outweighed if the biopsy findings were to improve patient selection for liver resection or liver transplantation. However, this and other studies investigating molecular markers have defined molecular markers in resection specimens. It remains to be shown that any molecular marker determined in a pre-operative biopsy correlates sufficiently well with the same marker in the surgical specimen.

An accurate circulating marker for microscopic vascular invasion would be even better than a tissue marker as this would

obviate the need for a pre-operative biopsy. Traditional serum markers, such as alphafetoprotein, have limitations. Novel markers such as circulating tumour cells, circulating microRNA or serum proteomic profile, are areas of active research and may provide predictive markers in the future.

In conclusion, this study has defined a gene expression signature that correlates well with vascular invasion in resected HCC samples, but there is a long way to go before we have an accurate surrogate marker of vascular invasion, available before surgery, that could be introduced into clinical 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.

References

- [1] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907–1917.
- [2] Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European association for the study of the liver. *J Hepatol* 2001;35:421–430.
- [3] Silva MA, Hegab B, Hyde D, Guo B, Buckels JAC, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008;57:1592–1596.
- [4] Chang S, Kim SH, Lim HK, Lee WJ, Choi D, Lim JH. Needle tract implantation after sonographically guided percutaneous biopsy of hepatocellular carcinoma: evaluation of doubling time, frequency, and features on CT. *AJR Am J Roentgenol* 2005;185:400–405.
- [5] Minguez B, Hoshida Y, Villanueva A, Toffanin S, Cabellos L, Thung S, et al. Gene-expression signature of vascular invasion in hepatocellular carcinoma. *J Hepatology* 2011;55:1325–1331.
- [6] Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007;43:979–992.
- [7] Sato F, Hatano E, Kitamura K, Myomoto A, Fujiwara T, Takizawa S, et al. MicroRNA profile predicts recurrence after resection in patients with hepatocellular carcinoma within the Milan Criteria. *PLoS One* 2011;6:e16435.
- [8] Ho MC, Lin JJ, Chen CN, Chen CC, Lee H, Yang CY, et al. A gene expression profile for vascular invasion can predict the recurrence after resection of hepatocellular carcinoma: a microarray approach. *Ann Surg Oncol* 2006;13:1474–1484.
- [9] Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, et al. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med* 2003;9:416–423.
- [10] Villanueva A, Minguez B, Forner A, Reig M, Llovet JM. Hepatocellular carcinoma: novel molecular approaches for diagnosis, prognosis, and therapy. *Ann Rev Med* 2010;61:317–328.
- [11] Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, et al. Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. *Lancet* 2003;361:923–929.
- [12] Kurokawa Y, Matoba R, Takemasa I, Nagano H, Dono K, Nakamori S, et al. Molecular-based prediction of early recurrence in hepatocellular carcinoma. *J Hepatol* 2004;41:284–291.
- [13] Wang SM, Ooi LL, Hui KM. Identification and validation of a novel gene signature associated with the recurrence of human hepatocellular carcinoma. *Clin Cancer Res* 2007;13:6275–6283.
- [14] Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, et al. Unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. *Cancer Res* 2010;70:10202–10212.
- [15] Villanueva A, Hoshida Y, Battiston C, Tovar V, Sia D, Alsinet C, et al. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011;140:1501–1512.