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Gene-expression signature of vascular invasion in hepatocellular carcinoma

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Abstract

Background & Aims—Vascular invasion is a major predictor of tumor recurrence after surgical treatments for hepatocellular carcinoma (HCC). While macroscopic vascular invasion can be detected by radiological techniques, pre-operative detection of microscopic vascular invasion, which complicates 30–40% of patients with early tumors, remains elusive.

Methods—A total of 214 patients with hepatocellular carcinoma who underwent resection were included in the study. By using genome-wide gene-expression profiling of 79 hepatitis C-related hepatocellular carcinoma samples (training set), a gene-expression signature associated with vascular invasion was defined. The signature was validated in formalin-fixed paraffin-embedded tissues obtained from an independent set of 135 patients with various etiologies.

Results—A 35-gene signature of vascular invasion was defined in the training set, predicting vascular invasion with an accuracy of 69%. The signature was independently associated with the presence of vascular invasion (OR 3.38, 95% CI 1.48–7.71, $p = 0.003$) along with tumor size (diameter greater than 3 cm, OR 2.66, 95% CI 1.17–6.05, $p = 0.02$). In the validation set, the signature discarded the presence of vascular invasion with a negative predictive value of 0.77, and significantly improved the diagnostic power of tumor size alone ($p = 0.045$).

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Conclusions—The assessment of a gene-expression signature obtained from resected biopsied tumor specimens improved the diagnosis of vascular invasion beyond clinical variable-based prediction. The signature may aid in candidate selection for liver transplantation, and guide the design of clinical trials with experimental adjuvant therapies.

Keywords

Hepatocellular carcinoma; Vascular invasion; Gene-expression signature; Surgical resection; Liver transplantation; Prediction; Diagnosis

Introduction

Hepatocellular carcinoma is a major global health problem representing the third cause of cancer-related mortality and the most common cause of death among cirrhotic patients [1]. Resection and liver transplantation are considered as the first-line therapeutic options able to potentially cure the disease [2]. However, resection is hampered by a recurrence rate of 70% at 5 years, while relapses complicate liver transplantation in 10–20% of cases. It is known that micro- and macrovascular invasion of the tumor is a major predictive factor of recurrence due to tumor cell dissemination and poor survival [3–7]. While preoperative imaging techniques are feasible to diagnose macroscopic vascular invasion, documenting microscopic invasion is still challenging, as it is detected only at pathological examination of surgically resected specimen.

Liver transplantation currently offers acceptable long-term survival rates (70% at 5 years) in well-selected hepatocellular carcinoma patients with the use of widely accepted Milan criteria, which are based on the number and size of tumors [8]. Recent studies have suggested that the criteria could be expanded in patients without vascular invasion to achieve a similar survival benefit [4,9,10]. Previous efforts have estimated the presence of vascular invasion without relying on postoperative pathological examination by utilizing clinical and/or molecular information [6,11–14]. However, validation of these predictors is still lacking.

In the current study, we have developed a 35-gene signature that predicts the presence of vascular invasion in patients with surgically treated hepatocellular carcinoma. The signature is shown to be more sensitive in detecting vascular invasion than previously reported predictors.

Patients and methods

Patients and samples

We analyzed a total of 214 hepatocellular carcinoma samples from patients consecutively treated with surgical resection between 1995 and 2006 in three centers integrating the *HCC Genomic Consortium*: Mount Sinai School of Medicine (New York), Hospital Clínic (Barcelona), and Istituto Nazionale dei Tumori (Milan). Seventy-nine fresh frozen samples (New York, n = 29; Barcelona, n = 24; Milan, n = 26), were used to define the signature (training set) (Fig. 1). All samples were obtained after acquisition of written informed consent. The training set represents a subset of patients from among 91 patients profiled and reported in a previous study of molecular classification of hepatitis C-related hepatocellular carcinoma [15]. An independent cohort of 135 formalin-fixed paraffin-embedded hepatocellular carcinoma tissues (New York, n = 59; Barcelona, n = 20; Milan, n = 56), archived as part of routine pathology diagnosis after resection, was used to evaluate predictive performance of the signature (validation set). Samples included in the validation

set were obtained from patients with various etiologies, including HCV, HBV, and alcohol, aiming to test real-world applicability of the signature in heterogeneous patient population.

Vascular invasion is identified either as macroscopic, when the invasion of the vessel is visible on gross examination, or as microscopic, when the invasion is visible only on microscopy. Microscopic vascular invasion was defined as tumoral cells within a vascular space lined by endothelium that was visible only on microscopy, and was assessed by several sections of non-tumoral hepatic parenchyma 1 cm away from the tumor. More detailed differences between macro and microscopic vascular invasion are further described in previous reports of our group [16]. Confirmation of reported vascular invasion from local pathologist of the three centers was centrally confirmed by an independent expert pathologist (S.T.).

Genome-wide gene-expression profiling

RNA extraction was performed using TRIzol reagent (Invitrogen Ltd., Paisley, Scotland, UK) as previously described [17,18]. Genome-wide gene-expression profiling of fresh frozen tissues and formalin-fixed paraffin-embedded tissues was performed using HG-U133 plus 2 array (Affymetrix) and whole-genome DASL assay [19,20] (Illumina, GPL8432), respectively, according to the manufacturer's instruction. Data were normalized using RMA and cubic spline method, respectively, using GenePattern analysis toolkit [21] (www.broadinstitute.org/genepattern). Microarray datasets are publicly available through NCBI's Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) (Accession No.: GSE20238). We sought to use frozen specimens for an initial extensive molecular characterization of HCC and to use a larger number of fixed tissues with longer follow-up data available to validate our key findings. Besides, DASL assay has been extensively evaluated for frozen and fixed tissue comparison as reported in several publications including ours [18]. These studies confirmed that the assay gene expression measurements obtained are consistent with other types of assay, such as qPCR. Testing the signature in a cross-platform setting further ensures that the signature did not overfit a specific assay platform and is ready for potential clinical applications.

Statistical analysis

Genes differentially expressed between tumors with and without vascular invasion were selected using standard random permutation test (GenePattern, ComparativeMarkerSelection module) based on significance criteria of false discovery rate less than 0.001 and absolute difference of groups' mean signal intensity greater than 100 units. Prediction of vascular invasion in the validation set was performed using the nearest template prediction method (GenePattern, NearestTemplatePrediction module) [18,22]. Prediction of the presence of vascular invasion was made based on a significance threshold of false discovery rate less than 0.05.

Clinicopathological variables predicting vascular invasion were first assessed in the univariate analysis and then included in a logistic regression analysis. Variables tested were tumor related (size, multinodularity, differentiation degree), demographics (age, gender), etiology of cirrhosis, and liver function status (AFP, serum bilirubin, serum albumin and platelet count) (Table 1).

Continuous and categorical variables were tested using Mann–Whitney's U-test and Fisher's exact test, respectively. The association between vascular invasion and the signature, together with the clinical variables, was evaluated using univariate and multivariate logistic regression modeling. Subgroup analysis was performed for patients infected with hepatitis C virus or with a single tumor nodule. Receiver-operating-characteristic (ROC) curve was

generated based on the prediction made in the validation set, with the use of the logistic regression model defined in the training set to evaluate diagnostic value of the signature. Difference in area-under-ROC curve (AUROC) was tested using DeLong's method [23]. Sensitivity, specificity, positive and negative predictive values, likelihood ratio and overall accuracy – defined as proportion of true positive and true negative results – of the gene signature was calculated as previously reported [17]. All analyses except as indicated above were done using the R statistical packages (www.r-project.org) or SPSS package (SPSS 15.0, Chicago, IL).

Results

Patient characteristics

Table 1 summarizes clinical characteristics of the patients analyzed in the study. The training set includes mostly Caucasian patients; whereas the validation set comprise a larger number of Asian patients (20%). By study design, all patients in the training set were hepatitis C-related in contrast to the validation set which included tumors of different etiologies, with one-third of patients infected with hepatitis B virus. Tumor characteristics were similar in both cohorts, although tumors in the training set presented with more macrovascular invasion (18% vs. 0%). These heterogeneities in clinical characteristics between the training and validation sets may help in evaluating the applicability of the signature to diverse patient populations seen in actual clinical setting while the use of different platforms for the two data sets further ensures gene signature reproducibility, excluding the risk of overfitting with one specific assay.

Gene-expression signature associated with vascular invasion

In the training set, we identified a 35-gene signature associated with the presence of vascular invasion: 14 over-expressed and 21 under-expressed genes in tumors with vascular invasion (Table 2, Fig. 2). We observed prominent vascular invasion-associated over-expression of *CD24*, an adhesion receptor of activated endothelial cells and platelets which has been implicated in metastasis and poor prognosis of epithelial cancers, including hepatocellular carcinoma [24–26]. Among the signature genes, *YY1API* was reported previously as a gene over-expressed in hepatocellular carcinoma [27]. Liver metabolism-related genes like *GLYAT*, *UGT2B15*, *CYP3A4*, and *ADH4* were under-expressed in the tumors with vascular invasion, consistent with their dedifferentiated phenotype.

Validation of vascular invasion signature

The identified 35-gene signature was next tested in an independent dataset including 135 FFPE tissues. Prediction of vascular invasion was made with an accuracy of 69%, high specificity (79%) and negative predictive value (77%). A subgroup analysis was performed in order to test if the signature behaved equally when applied to specific underlying etiologies. Accuracy of the signature showed similar performance for HCV-related HCC (n = 54) 70% and non-HCV related HCC (n = 81) 68%, and also in the specific subclass of HBV-related HCC (n = 45) 69%.

These results confirmed the applicability of this gene signature in FFPE tissues. Univariate logistic regression modeling revealed a significant association of the signature with the presence of vascular invasion ($p = 0.006$) (Table 3). Larger tumor size (>3 cm in diameter), a well-known predictor of vascular invasion [11], was also significantly associated with vascular invasion ($p = 0.03$). We also assessed previously reported gene-expression signatures of vascular invasion defined in small sets of patients [13,14], although none of them provided prediction with statistically significant confidence in our validation set (Supplementary Fig. 1).

Multivariate analysis

Multivariate analysis revealed that the signature remained significantly associated with vascular invasion ($p = 0.003$) after adjustment for the tumor size (Table 3). Subgroup analyses confirmed that the association of the signature with vascular invasion was still significant in patients with hepatitis C infection or a single tumor nodule (Table 4). The ROC curve analysis showed that the signature adds statistically significant information beyond tumor size (improvement of AUROC from 0.60 to 0.69, $p = 0.045$) (Fig. 3, Supplementary Table 1).

Discussion

The therapeutic field of hepatocellular carcinoma has changed considerably during the last decade. Outcomes after curative therapies (surgical resection, liver transplantation and local ablation) have improved due to better selection of candidates and surgical techniques [28]. Transcatheter arterial chemoembolization enhances survival in patients with tumors limited to the liver [29], whereas the multikinase inhibitor sorafenib has demonstrated survival benefits for patients with advanced disease [30]. However, recurrence after surgical treatments and the lack of effective adjuvant therapy are still unsolved challenges hampering the cure of hepatocellular carcinoma [2,31].

The ability to recognize patients with vascular invasion and at higher risk of recurrence, would aid the clinical decision making in the HCC therapeutic area [2]. Preoperative knowledge could guide the physicians in choosing the right surgical procedure or even adjuvant therapy and would be helpful for designing and refining clinical trials for HCC. Currently no established standard adjuvant care for HCC is available [2], with large trials testing novel drugs such as sorafenib or retinoids at the time.

Liver transplantation has been accepted as a treatment option for hepatocellular carcinoma with the establishment of candidate patient selection criteria (Milan criteria) based on tumor size and number [3,8]. Subsequent studies have been attempting to expand the criteria to identify more patients who are likely to benefit from liver transplantation [32], however, liver transplantation is constrained by the shortage of donors, and expansion of the conventional criteria has led to an ongoing debate in the field. This remains very controversial with many societal implications. Nonetheless, recent data suggest that some patients currently excluded from these criteria might achieve good results if properly selected [9]. Certainly, patients with single large tumors (i.e. 6–7 cm) without vascular invasion or even those with multiple small tumors (up-to-7 criteria) achieve acceptable 5-year survival rates if vascular invasion is discarded prior to transplantation [4,9]. Thus, size, number, and vascular invasion are critical variables for predicting outcome and for strategic clinical decisions, but the latter cannot be defined at the time of diagnosis [10].

In both circumstances, resection and liver transplantation, vascular invasion plays a critical role, since it is the main predictor of recurrence and poor outcome after these procedures. Despite some reported efforts for predicting recurrence [13,33], there is no established tool to identify the presence of vascular invasion prior surgical or loco-regional procedures. This represents a clear unmet medical need.

A recent large scale retrospective study analyzing around 1500 hepatocellular carcinoma patients revealed that the presence of vascular invasion is a critical prognostic factor, and the candidate selection criteria based on tumor size and number could be expanded according to the status of vascular invasion. In this study, 2 charts were developed to estimate patient survival after liver transplantation according to presence or absence of vascular invasion (Metroticket calculator) [4]. The hazard of death was doubled by the presence of vascular

invasion, and 5-year outcomes varied by 20% according to the status of this complication even with a similar tumor stage. This wide range of outcomes can be refined by applying the diagnostic model proposed herein. The signature-based preoperative assessment of vascular invasion may guide which chart to use in prognosis estimation, and help physicians in establishing whether liver transplantation is indicated. The high negative predictive value may indicate its usefulness in identifying patients eligible for liver transplantation who exceed Milan criteria. Given the shortage of donors and high medical cost in liver transplantation, preoperative diagnosis of vascular invasion has a huge socioeconomic impact in the clinical management of this neoplasm.

From a public health and cost-benefit perspective, it is of utmost importance to refine outcome prediction prior to establishing criteria for liver transplantation in HCC patients. The economic burden of changing the current criteria is substantial, and involves academic centers, regulatory agencies, providers, and governments alike. During the last two decades, the effort of expansion of criteria has been based on the refinement of the combination of size and number of nodules. While prognostic molecular data defining poor outcome subclasses are awaited [34], we are herein providing a model that complements the information by describing the status of microvascular invasion before surgery. Based on our recent study, ~20% of patients meet the up-to-7 expansion rule, but only half of them are ideal candidates for transplantation due to the absence of vascular invasion [4]. Patients presenting with single tumors of 6 or 7 cm are suboptimal candidates for liver transplantation, with a median 5-year survival of 50% that varies from 40% to 60% depending upon the vascular invasion status [4]. Although it is apparent that microvascular invasion is a striking prognostic factor, it can only be detected by pathological evaluation of surgically resected specimens, which it is unlikely to be available for decision making prior to liver transplantation. We have confirmed that survival estimation using the signature is well comparable with that made based on pathological evaluation of microvascular invasion (see Supplementary Table 2).

Vascular invasion is described as intrahepatic dissemination that initiates when tumor cells have developed an advanced phenotype to invade blood vessels and begin the metastatic process. Consequently, it is associated with poor prognosis after surgical treatments. Identification of patients with vascular invasion will also help to select patients indicated for experimental adjuvant therapies targeting potential residual tumor cells after the surgical and ablative treatments. The application of the herein proposed signature to daily medical practice might represent an easy tool to better refine patients stratification and enable more efficient clinical trial design by enrolling a subset of patients selected by the signature-based assessment (i.e., targeted clinical trial) [2,35].

Gene signatures identified from genome-based assays have been expected to fulfill the unmet clinical needs in hepatocellular carcinoma stratification [34]. To date, studies have attempted to define gene signatures predicting vascular invasion or recurrence due to tumor cell dissemination using mostly small set of patients [13,14,34,36–38]. However, reliable validation employing sufficient number of independent set of patients has been lacking. In fact, previously reported gene signatures failed to show robust predictive performance in our patient series, posing serious concern about validity of these signatures. In addition, it has to be noted that the novel signature can be applied both in fresh frozen and FFPE tissue, an advance that has clinical implications. We recently reported molecular profiling from mRNA obtained from fixed tissue [18]. This feature enables interrogating the whole transcriptome in samples routinely achieved in the pathology department. The signature was reproduced in 135 samples with FFPE tissues, demonstrating the principle that it can be translated to clinical practice without the need for access to a fresh frozen tissue bank. Other efforts reported that exploring molecular markers of vascular invasion have not been tested

in this setting [13,33]. Hopefully, pre-operative biopsied tumor specimens might represent the eligible material to appropriately apply the herein developed gene signature. We need to take into account, though, the potential risks of the biopsy procedure. In a recent meta-analysis including thousands of patients from eight studies, risk of tumor seeding has been estimated to be 2.7% [39,40]. Nonetheless, the size of the lesion being biopsied was not specified in most studies. Risk of bleeding is estimated not to be different than for biopsy of the liver in general. Having said that, the potential benefits of collecting liver tissues prior surgical procedures are threefold. First, to ensure a positive diagnosis of HCC prior transplantation outweighs the cost of transplanting candidates with misleading diagnosis. Second, tissue specimens will allow testing molecular markers for prognosis and lead toward a more personalized treatment approach. Finally, in our scenario, assessing the signature predicting vascular invasion can guide the decision-making process since 5-year outcome based on size and number of nodules after transplantation can vary by 20% according to vascular invasion status [4].

In conclusion, the present study proposes a reliable predictive model of vascular invasion by combining our 35-gene signature with clinical data. In the light of the reported importance of vascular invasion in prognosis, we suggest that this finding can aid in the decision-making of therapeutic process in HCC patients by providing more accurate estimation of prognosis for each patient. Particularly, clinical implications can be expected in the setting of selecting the target population of liver transplantation and the design of adjuvant therapies after resection and local ablation. Our results suggest that the signature warrants further clinical assessment for its value in clinical management of hepatocellular carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HBV	hepatitis B virus
FFPE	formalin-fixed paraffin-embedded
PPV	positive predictive value
NPV	negative predictive value
DASL	cDNA-mediated annealing, selection, extension, and ligation

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Fig. 1. Study design

A gene-expression signature associated with vascular invasion was defined in the training set (left) and evaluated in the independent validation set (right).

Fig. 2. Expression pattern of the 35-gene signature of vascular invasion in the training set
 Expression pattern of 14 overexpressed and 21 under-expressed genes in tumors with vascular invasion is shown. Patients are ordered according to the presence of macro- and micro-vascular invasion of hepatocellular carcinoma. Red indicates high expression; blue indicates low expression. VI, vascular invasion.

Fig. 3. Comparison of diagnostic performance of tumor size and the vascular invasion signature Receiver-operating-characteristic (ROC) curves are shown for prediction of vascular invasion (VI) using the vascular invasion signature, tumor size (>3 cm in diameter), and combination of both variables. The signature improves predictive performance of tumor size with statistical significance ($p = 0.045$).

Table 1

Clinical characteristics of patients included in the training and validation set.

	Training set (n = 79)	Validation set (n = 135)	<i>p</i>
Demographics			
Gender			
Male, n (%)	52 (65.8%)	102 (75.6%)	0.138
Age, years			
Median (range)	68 (42–80)	67 (36–83)	0.205
Race, n (%)			
Caucasian	72 (91%)	102 (75.6%)	0.001
African-American	3 (4%)	4 (3%)	
Asian	4 (5%)	28 (20.7%)	
Etiology of cirrhosis, n (%)			
Hepatitis C	79 (100.0)	54 (40.0)	<0.001
Hepatitis B		45 (33.3)	
Alcohol		9 (6.7)	
Others		24 (17.8)	
Tumor features			
Tumor diameter, cm			
Median (IQR)	3.5 (2.5–5.6)	3.5 (2.3–5)	0.234
>3 cm, n (%)	42 (53.2)	75 (55.6)	0.737
Tumor number, n (%)			
Single (≤ 3 cm)	32 (40.5)	50 (37.0)	0.727
Single (>3 cm)	35 (44.3)		
Multiple	12 (15.2)	18 (13.3)	
Vascular invasion, n (%)			
Microvascular	45 (57.0)	40 (29.6)	<0.001
Macrovascular	37 (82.0)	40 (100.0)	
	8 (18.0)	0 (0.0)	
Blood test			
Platelets (×1000/ml)	156 (131–179)	160 (120–209)	0.44
Bilirubin (mg/dl)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.347
Albumin (g/dl)	4.1 (3.6–4.4)	4 (3.8–4.4)	0.904
Alpha-fetoprotein, n (%)			
<10 mg/dl	25 (35%)	53 (41.4%)	0.70
10–100 mg/dl	26 (36.1%)	41 (32%)	0.02
>100 mg/dl	34 (47.2%)	21 (16.4%)	0.28
Events			
Recurrence	43 (54.4%)	80 (59.3%)	0.560
Early Recurrence	32 (40.5%)	60 (44.4%)	0.629

	Training set (n = 79)	Validation set (n = 135)	<i>p</i>
Deaths	23 (29%)	32 (23.7%)	0.393
Median Follow up (months)	21 (13–42)	34 (25–47)	0.011

Table 2

35-Gene signature of vascular invasion.

Gene ID	Gene symbol	Gene name	Over-expressed in	Signal-to-noise ratio
100133941	<i>CD24</i>	CD24 molecule	VI	0.50
25796	<i>PGLS</i>	6-phosphogluconolactonase	VI	0.41
3069	<i>HDLBP</i>	high density lipoprotein binding protein (vigilin)	VI	0.40
26003	<i>GORASP2</i>	golgi reassembly stacking protein 2, 55 kDa	VI	0.38
7298	<i>TYMS</i>	thymidylate synthetase	VI	0.37
11065	<i>UBE2C</i>	ubiquitin-conjugating enzyme E2C	VI	0.37
1362	<i>CPD</i>	carboxypeptidase D	VI	0.37
11260	<i>XPOT</i>	exportin, tRNA (nuclear export receptor for tRNAs)	VI	0.36
55249	<i>YY1API</i>	YY1 associated protein 1	VI	0.36
1033	<i>CDKN3</i>	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	VI	0.34
26502	<i>NARF</i>	nuclear prelamin A recognition factor	VI	0.33
10945	<i>KDELRL1</i>	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	VI	0.33
283820	<i>NOMO2</i>	NODAL modulator 2	VI	0.33
4728	<i>NDUFS8</i>	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23 kDa (NADH-coenzyme Q reductase)	VI	0.25
5053	<i>PAH</i>	phenylalanine hydroxylase	no VI	-0.32
10891	<i>PPARGC1A</i>	peroxisome proliferative activated receptor, gamma, coactivator 1, alpha	no VI	-0.32
10747	<i>MASP2</i>	mannan-binding lectin serine peptidase 2	no VI	-0.33
91614	<i>DEPDC7</i>	DEP domain containing 7	no VI	-0.34
10249	<i>GLYAT</i>	glycine-N-acyltransferase	no VI	-0.34
7366	<i>UGT2B15</i>	UDP glucuronosyltransferase 2 family, polypeptide B15	no VI	-0.34
7763	<i>ZFAND5 (ZA20D2)</i>	zinc finger, AN1-type domain 5	no VI	-0.35
687	<i>KLF9</i>	Kruppel-like factor 9	no VI	-0.35
1576	<i>CYP3A4</i>	cytochrome P450, family 3, subfamily A, polypeptide 4	no VI	-0.36
55089	<i>SLC38A4</i>	solute carrier family 38, member 4	no VI	-0.36
5295	<i>PIK3R1</i>	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)	no VI	-0.36
5444	<i>PON1</i>	paraoxonase 1	no VI	-0.38
1807	<i>DPYS</i>	dihydropyrimidinase	no VI	-0.39
54407	<i>SLC38A2</i>	solute carrier family 38, member 2	no VI	-0.40
92292	<i>GLYATL1</i>	glycine-N-acyltransferase-like 1	no VI	-0.40
5105	<i>PCK1</i>	phosphoenolpyruvate carboxykinase 1 (soluble)	no VI	-0.40
4638	<i>MYLK</i>	myosin, light chain kinase	no VI	-0.40
10157	<i>AASS</i>	aminoadipate-semialdehyde synthase	no VI	-0.41
4143	<i>MAT1A</i>	methionine adenosyltransferase I, alpha	no VI	-0.44
127	<i>ADH4</i>	alcohol dehydrogenase 4 (class II), pi polypeptide	no VI	-0.46
10171	<i>RCL1</i>	RNA terminal phosphate cyclase-like 1	no VI	-0.48

VI, vascular invasion.

Gene ID indicates National Center for Biotechnology Information's Entrez GeneID.

Table 3

Association of vascular invasion signature and tumor diameter with presence of vascular invasion.

	Univariate analysis		Multivariate analysis	
	Odds ratio (95%CI)	<i>p</i>	Odds ratio (95%CI)	<i>p</i>
Vascular invasion signature	3.07 (1.39–6.79)	0.006	3.38 (1.48–7.71)	0.003
Tumor diameter >3 cm	2.38 (1.08–5.23)	0.03	2.66 (1.17–6.05)	0.02

Table 4

Association of vascular invasion signature and tumor diameter with presence of vascular invasion (multivariate subgroup analysis).

	<u>Hepatitis C infection (n = 54)</u>		<u>Single tumor nodule (n = 116)</u>	
	Odds ratio (95%CI)	<i>p</i>	Odds ratio (95%CI)	<i>p</i>
Vascular invasion signature	12.58 (1.30–122.00)	0.03	3.12 (1.29–7.51)	0.01
Tumor diameter >3 cm	18.56 (2.16–159.21)	0.008	2.93 (1.21–7.13)	0.02