Research Article

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Progenitor cell markers predict outcome of patients with hepatocellular carcinoma beyond Milan criteria undergoing liver transplantation

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Background & Aims: In patients with hepatocellular carcinoma (HCC), liver transplantation (LT) is an excellent therapy if tumor characteristics are within the Milan criteria. We aimed to define genomic features enabling to identify HCC patients beyond Milan criteria who have acceptable transplant outcomes.

Methods: Among 770 consecutive HCC patients transplanted between 1990 and 2013, 132 had tumors exceeding Milan criteria on pathology and were enrolled in the study; 44% of the patients satisfied the 'up-to-7 rule' [7 = sum of the size of the largest tumor and the number of tumors]. Explant tumors were assessed for genomic signatures and immunohistochemical markers associated with poor outcome.

Results: At a median follow-up of 88 months, 64 patients had died and 45 recurred; the 5-year overall survival (OS) and recurrence rates were 57% and 35%, respectively. Cytokeratin 19 (CK19) gene signature was independently associated with recurrence [Hazard ratio (HR) = 2.95, p <0.001], along with tumor size (HR = 3.37, p = 0.023) and presence of satellites (HR = 2.98, p = 0.001). S2 subclass signature was independently associated with poor OS (HR = 3.18, p = 0.001), along with tumor size (HR = 5.06, p <0.001) and up-to-7 rule (HR = 2.50, p = 0.002). Using the presence of progenitor cell markers (either CK19 or S2 signatures) patients were classified into poor prognosis (n = 58; 5-year recurrence 53%, survival 45%) and good prognosis (n = 74; 5-year recurrence 19%, survival 67%) (HR = 3.16, p <0.001 for recurrence, and HR = 1.72, p = 0.04 for OS).

Conclusions: HCC patients transplanted beyond Milan criteria without gene signatures of progenitor markers (CK19 and S2) achieved survival rates similar as those within Milan criteria. Once prospectively validated, these markers may support a limited expansion of LT indications.

Keywords: Gene expression; Prognosis; Stem cell; Gene signature; Survival.
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Abbreviations: HCC, hepatocellular carcinoma; LT, liver transplantation; CK19, cytokeratin protein 19; HR, hazard Ratio; EpCAM, epithelial cell adhesion molecule; OS, overall survival; miVI, microvascular invasion; TGFb, transforming growth factor-beta; CT, computed tomography; MRI, magnetic resonance imaging; AFP, alpha-fetoprotein; IHC, immunohistochemical; RPS6, ribosomal protein S6; FDR, false discovery rate; H&E, hematoxylin and eosin; PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; HBV, hepatitis B virus; VIF, variance inflation factor.

Introduction

Liver transplantation (LT) is an effective treatment option for hepatocellular carcinoma (HCC) when disease is defined by the widely accepted Milan criteria [1,2]. Transplantation for patients within the Milan criteria generally yields a 5-year overall survival (OS) of 70% and a recurrence rate less than 15% [1–3]. Several efforts have been made to expand the criteria based on tumor size and number [4–8]. Although downstaging of tumors beyond Milan criteria is accepted by some UNOS regions, it has not yet been adopted by international consensus guidelines of LT [9] or guidelines of management of HCC [2]. Mazzaferro *et al.* proposed the up-to-7 rule [the sum of the number of tumor nodule(s) and the maximum diameter of the nodule(s) must not exceed the value of 7] which, in the absence of microvascular invasion (miVI) results in 5-year OS above 70% [8]. This study along with previously published data confirmed miVI to be a key predictor of

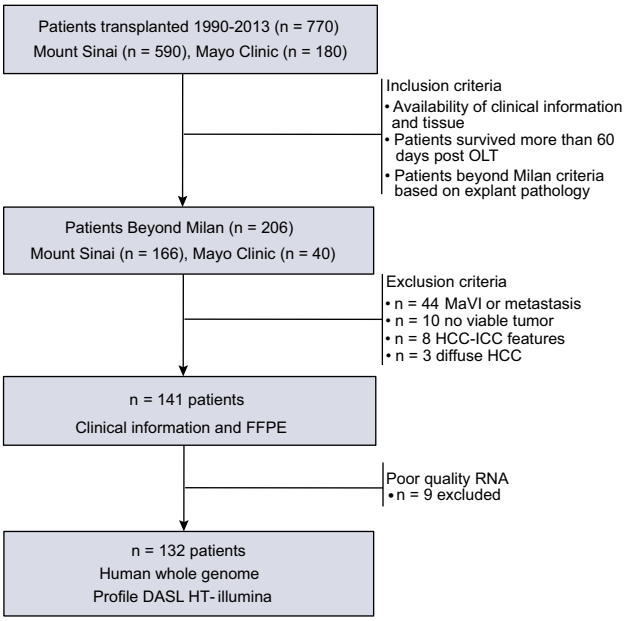

Fig. 1. Flow chart of the study. The initial cohort included 770 patients that were consecutively transplanted for HCC at Mount Sinai Hospital, New York (n = 590) and Mayo Clinic, Scottdale Arizona (n = 180). A total of 206 patients were beyond Milan criteria based on the explant pathology. Tumors with HCC-ICC features, diffused pattern, necrotic tissue, macro-vascular invasion or metastasis were excluded. From the remaining 141 cases, six tumors with poor quality RNA were discarded. Total RNA from 135 tumors was subjected to transcriptome profiling. Three samples had poor quality profile and were excluded and eventually 132 tumors were tested for the presence of previously reported outcome-associated gene signatures. OLT, orthotopic liver transplantation; ICC, intrahepatic cholangiocarcinoma; FFPE, Formalin Fixed Paraffin Embedded; DASL HT, cDNA-mediated Annealing, Selection, Extension, and Ligation (DASL) High Throughput (HT) Assay.

recurrence in patients with HCC [10]. However, this information cannot be used in pre-transplant decision-making as an indication of transplantation since miVI is only diagnosed based on post-surgical histological assessment. This highlights the limitations of the current image-based prognostic algorithm for selecting HCC patients for LT.

Genome-wide transcriptome profiling has identified several key deregulated genes, molecular pathways and signatures associated with disease progression and prognosis in HCC [11,12]. Activation of specific molecular pathways such as transforming growth factor-beta (TGFb) as well as the presence of progenitor cell markers such as cytokeratin 19 (CK19) and epithelial cell adhesion molecule (EpCAM) have been associated with more aggressive biological tumor characteristics and rapid disease spread [13–35].

In a previous study, we identified gene signatures that significantly improved prediction of HCC recurrence after surgical resection [21]. Here we sought to define if gene signatures are also able to identify patients with HCC beyond Milan criteria who nevertheless may have acceptable outcomes with LT. In addition, we are providing the transcriptomic landscape of patients at more advanced states of the disease compared with those undergoing resection or transplantation according to Milan criteria, as per AASLD/EASL guidelines [2,36].

Materials and methods

Patient cohorts and tissue samples

A total of 770 patients were transplanted for HCC between 1990 and 2013 at Mount Sinai Hospital, New York (n = 590) and Mayo Clinic, Scottsdale-Arizona (n = 180). Among these, we selected 132 patients (Mount Sinai: n = 94, Mayo: n = 38) with HCC beyond Milan criteria by pathological assessment, for whom base-line and follow-up clinical information and archived fixed tissue of viable tumor were available (Fig. 1). The study protocol was approved by the respective Institutional Review Boards. See Supplementary methods.

Genome-wide transcriptome profiling

Total RNA was subjected to transcriptome profiling using Whole-Genome DASL-HT (Illumina). See Supplementary methods. GEO accession number: GSE62743.

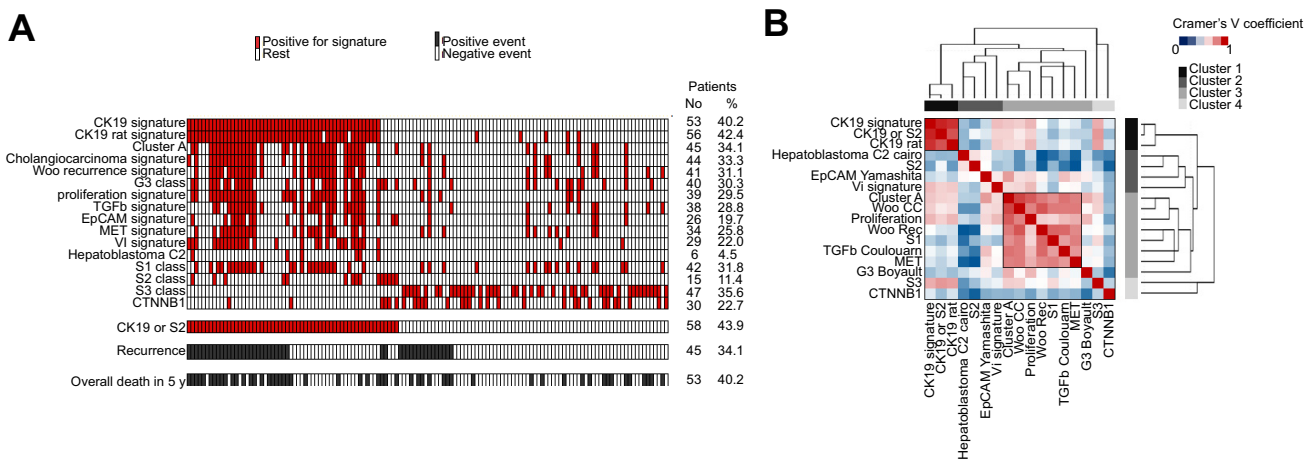

Fig. 2. Prediction of gene expression signatures. Only signatures that were able to assign patients into good and poor prognosis groups with FDR >0.05 were included. (A) Each column represents different sample and each row different signature. Positivity of each signature is represented by red bars. Events (recurrence or 5-year death) are shown with black bars. (B) Visualization of cramer's V coefficient for the pair-wise comparison of gene signatures. The scale from blue to red represents the strength of correlation (red represents the highest correlation). The signatures are clustered according to their correlation.