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Liver transplantation for hepatocellular carcinoma: Extension of indications based on molecular markers☆

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***Background/Aims*: Liver transplantation usually cures hepatocellular carcinoma when the Milan selection criteria are applied, whereas there is substantial risk of posttransplant recurrence with tumors beyond these criteria. This study uses molecular data to identify a subgroup of patients who, despite having hepatocellular carcinoma beyond Milan criteria, have favorable outcomes.**

***Methods*: Allelic imbalance of 18 microsatellites was analyzed in 70 consecutive patients (35 within Milan, 35 beyond Milan criteria) transplanted for hepatocellular carcinoma of whom 24 had recurrence and 46 survived at least 5 years recurrence-free. Fractional allelic imbalance (the fraction of significant microsatellites that demonstrated allelic imbalance) and relevant clinical / pathological variables were tested for correlation with time to recurrence.**

***Results*: Allelic imbalance in 9/18 microsatellites correlated with recurrence. Fractional allelic imbalance >0.27 and macrovascular invasion were independent predictors of recurrence in patients with tumors beyond Milan criteria; the probability of recurrence at 5 years was 85% with fractional allelic imbalance $\geqslant 0.27$ vs. 10% when <0.27 ($p = 0.0002$). An algorithm including Milan criteria and fractional allelic imbalance status is 89% accurate in predicting tumor recurrence after transplantation.**

***Conclusion*: Analysis of allelic imbalance of 9 microsatellites identifies a subgroup of patients who, despite having hepatocellular carcinoma beyond Milan criteria, have a low risk of posttransplant recurrence.**

***Keywords*: Hepatocellular carcinoma; Allelic imbalance; Liver transplantation extended indications; Biomarkers**

1. Introduction

Hepatocellular carcinoma (HCC) is a major health problem worldwide [1], and liver transplantation (LT) has come to play an important role in its management. Risk estimation of postsurgical tumor recurrence is an essential element in selecting patients with HCC for LT. In current clinical practice, this estimation is based on the size and number of tumor nodules and the presence of macroscopic vascular invasion (VI) as defined on preoperative imaging studies. The empirical rule pro-

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Abbreviations: HCC, hepatocellular carcinoma; LT, liver transplant; VI, vascular invasion; MS, microsatellite; AI, allelic imbalance; FAI, fractional allelic imbalance; AFP, alphafetoprotein; ROC, receiver operating characteristic.

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posed by the Milan group for selection of patients – one nodule ⩽5 cm or 2–3 nodules all ⩽3 cm, and without macroscopic VI – has been shown to provide survival rates above 70% at 5 years with ~10% likelihood of recurrence [2]. These so-called Milan criteria have been validated by several groups and are widely employed for candidate selection in the US and Europe alike [3,4]. Despite their proven utility, however, it is well-recognized that some patients with tumors that exceed these criteria are also potentially curable by LT [5].

Efforts to refine recurrence prediction based on preoperatively available clinical and radiological variables [6] have led to the current realization that tumor size and number are imperfect surrogates for predicting HCC metastatic potential. Increasingly, attention is being turned to molecular data for insights into tumor behavior. Gene expression studies using microarray analysis to define global expression patterns are beginning to yield potentially useful information, but remain preliminary [7–10].

Losses and/or gains of chromosomal DNA at tumor suppressor and/or oncogene loci have long been recognized as an important element in tumorigenesis and cancer progression [11], and analysis of allelic imbalance (AI) of microsatellites (MS) situated near these loci is a method to detect such copy number change. This study, based on the detection of AI of a set of previously defined MS [12], defines molecular markers that can predict tumor recurrence in patients with HCC beyond Milan criteria, thus providing a rationale for expanding the conventional indications published in HCC management guidelines [13].

2. Materials and methods

Between September 1990 and December 1997, 160 patients with HCC underwent LT at Mount Sinai. Patients who died in <5 years without evidence of HCC recurrence ($n = 45$) were excluded. Among the 115 patients remaining, 70 were included in the present study (35 within Milan, and 35 beyond Milan criteria), while the remainder were excluded for technical reasons including lack of sufficient pathological material or adequate tumor DNA suitable for analysis due to pre-LT chemoembolization.

The primary aim of the study was to identify molecular markers of HCC recurrence after LT among the 18 MS chosen as informative markers in our previous analyses [12]. The 18 MS analyzed represent loci from 9 chromosomes within 17 distinct cytogenetic bands.

This study was approved by the Institutional Review Boards at The Mount Sinai Hospital and the University of Pittsburgh.

2.1. Clinical data

Patient characteristics are summarized in Table 1. Age, sex, underlying liver disease, tumor size and number, within vs. beyond Milan criteria (based on pathology; many cases predated the availability of helical computerized tomography), alpha-fetoprotein (AFP), VI (none vs. micro- vs. macroscopic), and histologic grade (well vs. moderately vs. poorly-differentiated; data unavailable in two cases), as well as date of LT, date of recurrence, and date of death, were recorded for all patients.

Table 1
Patient and tumor characteristics ($n = 70$)

Age	55.3 ± 10.4
Sex	
Male	54
Female	16
Underlying disease	
Hepatitis C	37
Alcohol	9
Cryptogenic/NASH	9
Hepatitis B	8
Other	7
Number of tumor nodules	
1	31
2	15
3	6
>3	18
Vascular invasion	
None	26
Microscopic	32
Macroscopic	12
Largest tumor diameter	
⩽5 cm	54
> 5 cm	16
Mean	4.0 ± 2.8 cm
Histologic differentiation grade*	
Well	37
Moderate	23
Poor	8
Milan criteria	
Within criteria	35
Beyond criteria	35

* 2 Cases with missing histologic differentiation data.

2.2. DNA analysis

Microdissection and AI experiments were done according to previously published methods [12,14]. Briefly, paired samples of tumor and non-tumor liver were prepared from each patient. Genomic DNA was isolated from tissue microdissected manually under stereomicroscopic observation and amplified via PCR with fluorescent-labeled oligonucleotides that flank the MS. PCR products were separated by capillary electrophoresis (ABI 310: Applied Biosystems) and the ratio of the two alleles for each MS was determined using GeneScan software (Applied Biosystems, Foster City, CA). Manual microdissection was employed in preference to the more cumbersome laser-capture technology based on side-by-side comparison in our laboratory that failed to demonstrate any advantage to LCM in this setting. In addition to analysis of HCC [12,14,15], the techniques employed in the current work have been previously validated in a variety of other tumors including lung [16], ovary [17], bone [18], hemangioblastoma [19], thyroid [20], pancreas [21], meningioma [22], melanoma [23], neuroendocrine [24], and esophagus [25].

In each patient, MS that were found to be homozygous in non-tumor liver were designated as noninformative for that patient. MS demonstrating heterozygosity in non-tumor liver were scored as showing AI in the tumor when the ratio of the individual allele peaks in the tumor fell outside the range of 0.66 to 1.50. Replicate analysis was performed in every case, with concordance of 85–100% and a standard deviation varying among patients from 0.06 to 0.2.