c-MET and *HGF* mRNA Expression in Hepatocellular Carcinoma: Correlation with Clinicopathological Features and Survival

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Abstract. *Background/Aim: Data on the clinicopathological features and prognostic impact of c-N-Methyl-N'-nitro-N-nitroso-guanidine HOS Transforming gene (c-MET) and hepatocyte growth factor (HGF) in hepatocellular carcinoma (HCC) are inconsistent. We assessed c-MET and HGF expression in 49 patients with early-stage HCC and correlated the results with disease characteristics and survival. Materials and Methods: Expression of c-MET and HGF mRNA in tumor (T) and non-tumor (NT) tissues was assessed. Results were correlated with patient characteristics and overall and recurrence-free survival. Results: Median relative tumor c-MET and HGF expressions were 3.23 (T/NT ratio 6.46) and 9.07 (T/NT ratio 0.77), respectively. c-MET and HGF were overexpressed in early-stage disease with favorable characteristics although there was no association with survival. Conclusion: Contrary to other studies, in our series increased tumor c-MET and HGF expressions were associated with favorable disease attributes but not with survival. The prognostic and therapeutic applications of this knowledge to HCC are under active investigation.*

The *c-MET* receptor and its cognate ligand, hepatocyte growth factor (*HGF*), play key roles in hepatogenesis, hepatohomeostasis and regeneration following injury (1, 2). *c-MET/HGF* binding activates intracellular signaling transduction pathways such as the PI3K/AKT/mTOR and the mitogen activated protein (MAP) kinase cascades, thus promoting cell proliferation, survival, morphogenesis and scattering (dissociation and motility) (3). Dysregulation of *c-MET/HGF* signaling has been implicated in the development and progression of multiple human malignancies (3).

Transgenic mice that overexpress *c-MET* in hepatocytes develop hepatocellular carcinoma (HCC). Inactivation of the transgene leads to regression of even highly advanced tumors suggesting that *c-MET* plays a role in both the genesis and maintenance of HCC in animal models (4, 5). Recent clinical trials of *c-MET* inhibitors showed therapeutic promise for advanced HCC (6, 7). Furthering our understanding of the *c-MET* and *HGF* signatures in HCC may help to characterize patient subpopulations which are likely to benefit from therapeutic targeting of this axis.

The objective of this study was to investigate the expression patterns of *c-MET* and *HGF*, their associated clinicopathological features and impact on survival among patients with early-stage HCC who were treated at the Memorial Sloan-Kettering Cancer Center (MSKCC).

Materials and Methods

Patients. Institutional Review Board approval was obtained (Request for Waiver of Authorization, WA0056-07). Patients who underwent liver resection for HCC at MSKCC and who had fresh-frozen tumor and non-tumor tissues available for analysis were identified. Patients with mixed HCC-cholangiocarcinoma and fibrolamellar carcinoma were excluded. Clinical, pathological and laboratory data from the time of surgery were extracted from electronic medical records.

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Laboratory methods. Samples were processed using a hand homogenizer, trizol/chloroform, and low-speed centrifugation. Total RNA was isolated using the Qiagen RNeasy mini kit (cat no. 74104; Valencia, CA, USA). The quantity and purity of the extracted RNAs were assayed using a Nanodrop ND1000 spectrophotometer (Thermoscientific, Wilmington, DE, USA). All extracted RNAs had an optical density 260/280 ratio between 1.8 and 2.0. Total RNA (1 μg) from each sample was transcribed with the Applied Biosystem's Taqman Reverse Transcription kit (part no. N808-0234; Carlsbad, CA, USA) to synthesize cDNA.

Pre-designed Taqman Gene Expression Assays containing primers and probes for *c-MET* (Cat# Hs00179845_m1), HGF (Cat# Hs00300159_m1) and 18s RNA (Cat# Hs99999901_s1; all Applied Biosystems) were used. Tumor and non-tumor cDNA samples each were analyzed in triplicate for *c-MET* and *HGF* gene expression by quantitative real-time polymerase chain reaction (qRT-PCR) using Applied Biosystems' 7900HT Sequence Detection System, in a total volume of 20 μl with 2X Taqman Master Mix (Applied Biosystems) according to the manufacturer's instructions. Thermal cycler variables included 2 min at 50˚C, 10 min at 95˚C, and 40 cycles involving denaturation at 95˚C for 15 s and annealing/extension at 60˚C for 1 min.

Samples were all processed in a blinded fashion with regard to clinical characteristics. *c-MET* and *HGF* mRNA expression were normalized to 18S rRNA expression using the following equations (8): *c-MET* mRNA expression=(mean *c-MET* RNA/mean 18S RNA) X100 and *HGF* mRNA expression=(mean HGF RNA/mean 18S RNA) ×1000.

The relative expression of *c-MET* and *HGF* in tumor (T) compared to non-tumor (NT) liver tissue was calculated as the ratio of expression.

Statistical methods. Correlation of clinicopathological characteristics with *c-MET* and *HGF* RNA expression was performed using the Kruskal-Wallis test and linear models for categorical and continuous variables, respectively. Kaplan-Meier overall survival (OS) was calculated from the date of surgery to the date of death or last follow-up. Recurrence-free survival (RFS) was calculated from the date of surgery to the date of radiographic recurrence, global clinical deterioration or death. To account for patients who died without documented recurrence or clinical deterioration, sensitivity analyses censoring patients at the date of their last scan, defining death as the recurrence event, and defining the last scan as a recurrence event were performed. Logarithmic transformation of normalized values and ratios of tumor *c-MET* and *HGF* expression were used.

Results

Demographic, clinical and pathological features for the 49 patients included are summarized in Table I. The median age at diagnosis was 65 years, and the majority of patients were Caucasian and male. Alcohol and hepatitis C were the predominant causes of liver disease. Child-Pugh score was determined in 37 patients, 35 of whom had Child-Pugh A disease. Sixty-nine percent of patients had symptoms at diagnosis mainly consisting of abdominal pain, fatigue, weight loss and digestive symptoms. All patients had normal transaminases and liver function tests. The median alpha-fetoprotein (AFP) was 11.2 ng/dl (range 1.2 - 62,608 ng/dl).

Table I. *Demographic, laboratory and clinicopathological characteristics of patients in this study.*

Demographics & clinical presentation	% out of 49 patients
Median age (range)	65 (41-85)
Gender (male/female)	65/35
Ethnicity (Caucasian/Black/Asian)	80/2/18
Cirrhosis	18
Hepatitis C	9
Hepatitis B	29
Alcohol	26
Symptomatic	69
Pathology	
Number of lesions (1/>1)	73/27
Satellite lesions (yes/no)	29/71
Bilobar disease (yes/no)	24/76
Portal vein thrombosis	2/98
Edmondson grade (1/2/3/4)	15/59/22/4
Vascular invasion (yes/no)	40/60
Perineural invasion (yes/no)	5/95
Margin (+/–)	10/90
T Stage (1/2/3/4)	41/14/33/12
N Stage (0/1)	100
M Stage (0/1)	96/4
Laboratory	Median value
Total bilirubin [mg/dl]	0.6
Albumin [g/dl]	4.3
Prothrombin (INR)	1
Platelets [103/μl]	244
Alkaline phosphatase [U/l]	115
Alpha fetoprotein (mean) ng/ml	3020.46
Creatinine [mg/dl]	1.1
Lactate dehydrogenase [U/l]	174
Calcium [mg/dl]	9.6
Aspartate aminotransferase [U/l]	35
Alanine aminotransferase [U/l]	39

Radiographically, 73% of patients had unifocal HCC, 24% had bilobar disease and 2% had portal vein thrombosis. The median tumor diameter was 10.5 cm (range 2-26 cm). Primary tumor stage using the AJCC TNM 6th edition (9) was T1, T2, T3 and T4 in 41%, 14%, 33% and 12% of patients, respectively. No patients had lymph node metastases. One patient had lung metastases. Seventy percent of patients had an Edmondson tumor grade of I or II. Vascular invasion was present in 40% of tumors, 5% had perineural invasion, and surgical margins were positive in 10%.

c-MET and HGF expression. The median relative *c-MET* mRNA level in tumor was significantly higher than that in non-tumor tissue (3.23 *vs*. 0.5, $p<0.01$) but the median relative *HGF* mRNA levels were not significantly different (9.07 *vs*. 11.73, $p=0.14$) (Figure 1). The corresponding c-MET and HGF T/NT ratios were 6.46 and 0.77, respectively.

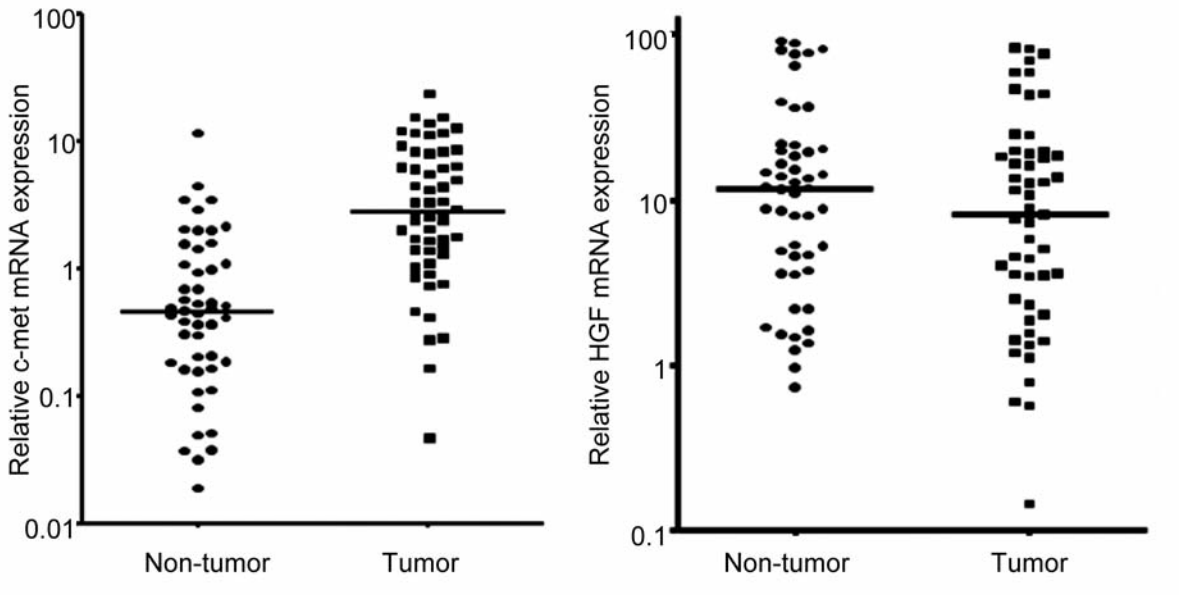

Figure 1. *Distribution of c-MET and HGF mRNA expression in tumor and non-tumor liver tissues.*

Table II. *Correlation of tumor c-MET and HGF mRNA expression with clinicopathological features.*

Characteristic	Correlation with *c-MET*	*p*-Value	Correlation with *HGF*	*p*-Value
T Stage	Inverse	0.002	Inverse	0
TNM	Inverse	0.01	Inverse	0.01
Grade	None		None	
Largest tumor diameter	Inverse	0.05	Inverse	0.01
Vascular invasion	Inverse	0.02	Inverse	0
Positive margin	Inverse	0.004	Inverse	0.01
Abdominal pain	Inverse	0.03	Inverse	0.03
Satellite nodules	Inverse	0.04	Inverse	0.04
Albumin	None		Positive	0.04
Aspartate aminotransferase	None		Inverse	0

Table III. *Correlation of clinicopathological factors with survival outcomes.*

	OS	
	HR (95% CI)	*p*-Value
Abdominal pain present *vs*. absent	1.37 (0.70-2.70)	0.35
Albumin (>4 *versus* <4 g/dl)	1.32 (0.53-3.32)	0.55
AST (>35 U/L *versus* <35 U/l)	1.31 (0.60-2.88)	0.49
Tumor diameter (>10 *versus* <10 cm)	1.15 (0.57-2.32)	0.68
Vascular invasion (present *versus* absent)	2.10 (1.03-4.29)	0.04
Margin (positive *versus* negative)	2.42 (0.91-6.38)	0.07
T3/4 *versus* T1/2	2.64 (1.32-5.27)	0.005

OS=Overall survival.

Correlation of c-MET and HGF with clinicopathological characteristics. Correlation of tumor *c-MET* and *HGF* mRNA expression with stage, tumor size, Edmondson grade, vascular invasion, surgical margin status, abdominal pain, serum aspartate aminotransferase (AST) and albumin levels revealed a significant inverse relationship; high levels of both markers correlated with earlier stage disease and less abdominal pain (Table II). Increased *HGF* correlated with preserved liver function. Tumor *c-MET* and *HGF* levels appeared to differ according to risk factors. Patients with hepatitis B tended to exhibit higher tumor *c-MET* levels than those without hepatitis B or any other risk factor. Patients with hepatitis C had lower tumor *HGF* levels than those without hepatitis C. Patients with alcoholic liver disease or cirrhosis had higher levels of tumor *HGF* mRNA than those who did not.

Correlation of c-MET and HGF with survival. At the time of data censoring in December 2010, 36 (73%) patients had died. The median OS was 44.8 months, with a median follow-up of 62.8 months for living patients. Vascular invasion (hazard ratio (HR)=2.1, 95% confidence interval (CI)=1.03-4.29, p=0.04) and stage T3/4 *versus* T1/2 (HR=2.64, 95% CI=1.32-5.27, p=0.005) were significantly associated with poorer OS on univariate analysis (Table III). Tumor *c-MET* and *HGF* levels did not correlate with survival.

Five patients died of unknown causes without documented recurrence or progression. Kaplan-Meier RFS for the entire cohort, censoring these five patients at the time of their last scan, was 11.6 months. When the date of death or last scan was counted as an events, RFS was 11.6 and 9.9 months, respectively. On univariate analysis, higher T stage (T3/4 *versus* T1/2) was strongly and consistently associated with poorer RFS across all sensitivity analyses. Vascular invasion was significantly associated with poorer RFS when the last scan was the recurrence event. Neither tumor nor non-tumor *c-MET* and *HGF* mRNA levels correlated with RFS, irrespective of the sensitivity analyses. Multivariate analyses were not performed due to the small sample size.

Discussion

In this study, we observed a significant increase in HCC tumor *c-MET* mRNA expression relative to non-tumor tissue. Conversely, tumor *HGF* mRNA content was slightly lower compared to non-tumor tissue. Our findings are consistent with the majority of other studies that have demonstrated a trend of maintained *c-MET* overexpression in hepatitis, cirrhosis and HCC relative to normal liver controls along with a parallel decline in tissue *HGF* concentration (10, 11). Given that *HGF* has been shown to exert anti-mitogenic properties (12), the shifting pattern of *c-MET* and *HGF* expression across the pathological spectrum suggests a disturbance of the normal dynamics between the two, causing them to shed their roles as guardians of liver integrity, and become enablers of malignant transformation.

A key finding of our study was that HCC expression of *c-MET* and *HGF* mRNA exhibited a generally uniform relationship with early stage disease and favorable clinicopathological characteristics though there was no impact on survival. Our observations contrast with other series (13, 14) as well as a recent prospective trial (6) which have reported an adverse relationship between *c-MET* overexpression, clinicopathological features and survival. Although no clear relationship between tumor *HGF* levels and clinical features has been defined (11, 14) high serum *HGF* has been associated with more aggressive disease behavior and reduced survival (15, 16). Serum *HGF* levels were not measured in this study, but would have been an interesting correlate.

How can these divergent results be explained? Firstly, this was a small monocentric retrospective series subject to selection bias, therefore limiting the applicability of our findings. The selection of mRNA as the analyte for this study is another possible pitfall. Differential processing of *c-MET* and *HGF* mRNA generates an assortment of transcripts (17) and protein product isoforms, each with potentially unique effects (18-20). As such, there are limitations as to how mRNA levels can be directly linked with a particular biological outcome. Confirmation of mRNA expression patterns with protein levels was not possible due to the lack of sufficient material, but would have been informative.

Another possibility is that the variables influencing the observed outcomes lie beyond the *c-MET*/*HGF* axis. Pre-clinical studies suggest that the range of biological effects mediated by *c-MET*/*HGF* signaling is context-dependent; different intracellular circuits may be activated depending on the signaling milieu, and cross-communication between these can also alter disease behavior (21-25). Furthermore, *c-MET* can be activated by molecules other than HGF such as osteopontin, the epidermal growth factor receptor and cell-cell adhesions to induce tumorigenesis and proliferation (26-28). Altogether, these findings indicate that *c-MET* and *HGF* do not act in isolation but are part of a much larger signaling network.

Therapeutic targeting of the *c-MET* axis in HCC is now a clinical reality. A recent randomized phase II trial showed that the small molecule tyrosine kinase inhibitor tivantinib may improve survival in patients with high tumor *c-MET* expression after sorafenib progression (6). Continued efforts to enrich the patient population most likely to benefit from these agents and define the appropriate clinical contexts in which to use them should be prioritized.

In conclusion, this study implicates altered *c-MET* and *HGF* expression in HCC. Our observation that increased tumor *c-MET* mRNA correlated with favorable disease characteristics diverges from other studies reporting a negative association with clinical phenotype and outcome. The clinical relevance of the differences in tumor and non-tumor expression of these biomarkers remains a subject of debate. A better understanding of the role of *c-MET* and *HGF* in HCC will help to guide rational therapeutic intervention.

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