**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.
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# Hs0030159_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 Biosystem’s 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 RNA 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) x100.

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 mRNA 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).

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.
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.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Correlation with c-MET</th>
<th>p-Value</th>
<th>Correlation with HGF</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Stage</td>
<td>Inverse</td>
<td>0.002</td>
<td>Inverse</td>
<td>0</td>
</tr>
<tr>
<td>TNM</td>
<td>Inverse</td>
<td>0.01</td>
<td>Inverse</td>
<td>0.01</td>
</tr>
<tr>
<td>Grade</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Largest tumor diameter</td>
<td>Inverse</td>
<td>0.05</td>
<td>Inverse</td>
<td>0.01</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>Inverse</td>
<td>0.02</td>
<td>Inverse</td>
<td>0</td>
</tr>
<tr>
<td>Positive margin</td>
<td>Inverse</td>
<td>0.004</td>
<td>Inverse</td>
<td>0.01</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Inverse</td>
<td>0.03</td>
<td>Inverse</td>
<td>0.03</td>
</tr>
<tr>
<td>Satellite nodules</td>
<td>Inverse</td>
<td>0.04</td>
<td>Inverse</td>
<td>0.04</td>
</tr>
<tr>
<td>Albumin</td>
<td>None</td>
<td>Positive</td>
<td>Positive</td>
<td>0.04</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>None</td>
<td>None</td>
<td>Inverse</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

OS=Overall survival.

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

**References**


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