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Molecular co-expression of the c-Met oncogene and hepatocyte growth factor in primary colon cancer predicts tumor stage and clinical outcome

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Abstract

Introduction/hypothesis: Over-expression of the c-Met receptor tyrosine kinase has been described in a variety of cancers and implicated in tumor progression. Unlike some solid tumors, current evidence indicates that c-Met activation in colon cancer is unrelated to gene mutation, is ligand dependent, and occurs via a paracrine fashion. We hypothesize that over-expression of the c-Met receptor and its ligand, hepatocyte growth factor (HGF) in the tumor microenvironment is associated with tumor progression and metastases.

Methods: Primary tumor c-Met and HGF mRNA expression was analyzed in 60 colon adenocarcinomas. Receptor and ligand expression was analyzed for correlation and association with clinicopathologic features and outcome.

Results: Compared to adjacent normal mucosa, 69% and 48% of tumors showed a greater than 2- and greater than 10-fold elevation in c-Met mRNA, respectively. Elevated HGF mRNA was noted in 47% of tumors with 19% having a greater than 10-fold increase. Tumor c-Met expression was correlated with HGF expression, and a cohort of 33 patients could be defined with both low c-Met and HGF expression. Compared with the 27 tumors with either high c-Met or HGF, the cohort with low c-Met and HGF expression had fewer nodal and distant metastases as well as improved overall survival (HR = 2.3, $p < 0.05$).

Conclusion: Evaluation of the c-Met receptor in context of ligand, HGF, allows identification of a metastatic phenotype that correlates with advanced stage and poor survival. c-Met and HGF co-expression in the tumor microenvironment could be useful in the molecular staging of colon cancer and viable therapeutic targets.

Keywords: Colon cancer; c-Met; Hepatocyte growth factor; Scatter factor; HGF; Tumor stage; Survival; Outcome; Metastasis; Tumor progression

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1. Introduction

Worldwide, colorectal cancer (CRC) afflicts over 1 million individuals annually [1], and in industrialized countries CRC is the most common gastrointestinal malignancy [2]. For localized disease, surgical resection is the foundation of treatment. However, distant micrometastatic disease is present in over 50% of patients that have undergone curative surgery. Since low volume metastatic disease may be responsive to chemotherapy, patients at risk for harboring clinically inapparent distant disease are offered postoperative chemotherapy. Randomized trials have shown that patients receiving the most contemporary chemotherapy regimen after curative resection have 40–50% reduction in recurrence and improved survival when compared to patients not receiving adjuvant therapy [3,4]. The importance of identifying those patients with micrometastatic disease is further evidenced by the fact that when relapse becomes clinically apparent, cure is almost never achieved even with the most effective combinations of cytotoxic and biologic therapy [5].

This study investigates the role of the *c-met* oncogene and its ligand, hepatocyte growth factor (HGF) in CRC progression and metastases. The *c-met* encoded protein is a receptor tyrosine kinase (RTK) which, under normal circumstances, binds HGF produced by stromal cells. A fundamental gene required for embryogenesis, *c-met* is also a critical oncogene for tumor metastasis, enabling cellular proliferation, motility, and invasion [6–15]. Previous studies have generally examine c-Met overexpression in CRC with semiquantitative techniques such as immunoblotting [9–13] or immunohistochemistry [16]. Few studies have reported more accurate quantitative methodology such as gene expression analysis [17,18]. There has been comparably minimal emphasis placed on quantitation of the c-Met ligand, HGF. Although ligand independence as a result of activating mutation has been noted in select hereditary renal cell cancers, childhood liver tumors, and head and neck cancers, the majority of solid gastrointestinal tumors appear to be ligand-responsive to HGF [19]. Therefore, we hypothesize that evaluating the co-expression of both c-Met and HGF is critical to better understand the role of this RTK and its natural ligand in the behavior of primary CRC.

In this series of human CRC specimens, we report that c-Met and HGF co-expression predicts tumor phenotype, specifically propensity for nodal and distant metastases. Co-expression is also associated with poor outcome. These findings lend credence to the theory that *c-met* is a critical oncogene for colorectal cancer progression and that co-expression of ligand and receptor can be used for molecular staging. Further, the data supports investigation of c-Met and HGF as therapeutic targets in CRC.

2. Patients and methods

2.1. Patients and samples

Tissue specimens were analyzed from 63 randomly selected patients who underwent standard of care resection of primary colon adenocarcinoma at Memorial Sloan-Kettering Cancer Center (MSKCC) between 1991 and 1993. Patients consented to enrollment in a prospective tissue procurement protocol that was approved by the Institutional Review Board of the MSKCC. Immediately after surgical resection, samples of tumor tissue and adjacent normal colonic mucosa were harvested and placed in liquid nitrogen until RNA extraction. Matched normal samples were taken from the end of the resected specimen which generally corresponded to greater than 5 cm from the tumor tissue. All specimens were reviewed by the department of pathology at MSKCC. Tumors were histologically confirmed to have greater than 70% tumor cellularity.

2.2. RNA isolation and complementary DNA synthesis

Total RNA isolation from primary colon tumors and normal mucosal specimens was performed with RNeasy minikits using manufacturer's instructions (Qiagen, Santa Clarita, CA). For complementary DNA (cDNA) synthesis, about 1 μg of total RNA was transcribed with cDNA Transcription Reagents (Applied Biosystems, Foster City, CA) with the use of random hexamers.

2.3. Primers and probes

c-Met and 18S ribosomal RNA (18S rRNA) sequence specific primers and probe were obtained as Assay-on-Demand Gene Expression Products (Applied Biosystems, Foster City, CA). HGF primers and *Taq*Man probe (Custom Oligonucleotide Factory, Foster City, CA) were designed using Primer Express Software (Applied Biosystems, Foster City, CA) to span an exon–intron junction to prevent amplification of genomic DNA and also to result in an amplicon of fewer than 150 base pairs to enhance efficiency of PCR amplification. HGF primer sequences