Somatic Mutations in the Kinase Domain of the \textit{Met}/Hepatocyte Growth Factor Receptor Gene in Childhood Hepatocellular Carcinomas

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Abstract

The \textit{MET} protooncogene encodes a transmembrane tyrosine kinase identified as the receptor of a polypeptide known as hepatocyte growth factor/scatter factor. We performed PCR-based single-strand conformational polymorphism and sequencing analysis of the tyrosine kinase domain of the \textit{MET} gene (exon 15–19) in 75 primary liver cancers. Three missense mutations were detected exclusively in 10 childhood hepatocellular carcinomas (HCCs), while no mutations were detected in 16 adult HCCs, 21 cholangiocarcinomas, or 28 hepatoblastomas. The extremely short incubation period from hepatitis B virus infection to the genesis of childhood HCC as compared with the adult HCC suggests that there may be an additional mechanism that accelerates the carcinogenesis of childhood HCC. Our results indicate that mutations of the tyrosine kinase domain of the \textit{MET} gene may be involved in the acceleration of the carcinogenesis in childhood HCC.

Introduction

The \textit{MET} protooncogene encodes a transmembrane tyrosine kinase identified as the receptor of a polypeptide known as HGF/SF\textsuperscript{1} (1). Binding with HGF/SF triggers tyrosine autophosphorylation of the intracellular domain in the \textit{MET} receptor and induces a pleiotropic response in epithelial cells, including: (a) mitogenesis; (b) stimulation of cell motility; (c) dissociation of epithelial sheets; and (d) promotion of extracellular matrix invasion (2). In a normal liver, HGF/SF has been detected in bile duct epithelia, Ito cells, and endothelial cells of both the central lobular vein and the portal tract vessels, whereas MET receptor has been identified only in normal mature hepatocyte and in the “facultative stem cells” often referred to as oval cells (3). The latter are considered to be bipotential progenitor cells because they can be differentiated toward either bile duct epithelial cells or hepatocytes. Overexpression of \textit{MET} protein has been documented in numerous human solid tumors including primary liver carcinomas such as HCC, hepatoblastoma, and intrahepatic cholangiocarcinoma (4–6). Activation of an oncogene is a frequent mechanism of tumor promotion, whereas childhood HCC shows a minimum time period of 3 years (12); the reason for this short malignant transformation period after perinatal HBV infection in childhood HCC is still open to question (12, 13).

Here, we performed PCR-based SSCP and sequencing analysis of the tyrosine kinase domain of the \textit{MET} gene (exon 15–19) in 75 malignant liver tumors; 10 childhood HCCs, 16 adult HCCs, 21 intrahepatic cholangiocarcinomas, and 28 hepatoblastomas. Our results indicate that mutations of the tyrosine kinase domain of the \textit{MET} gene play an important role in the carcinogenesis of childhood HCC but not in adult HCC, hepatoblastoma, or intrahepatic cholangiocarcinoma of the liver.

Materials and Methods

Materials. This study was conducted on 75 primary liver carcinomas diagnosed at the Department of Pathology of the Catholic University Medical College or of Seoul National University Medical College, both in Seoul, Korea. We examined 26 HCCs (10 childhood and 16 adult-type), 28 hepatoblastomas, and 21 intrahepatic cholangiocarcinomas. Histological and DNA studies were performed on formalin-fixed, paraffin embedded tissue samples.

Microdissection. Tumor cells were selectively procured from H&E-stained slides using a 30 G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a microdissection device (SPEM II; Simple, Precise and Economical Microdissection device, Sam Jung Co., Seoul, Korea), as described previously (14–16). We also obtained inflammatory cells or surrounding normal liver cells for corresponding normal DNA from the same slides in all cases.

DNA Extraction. DNA extraction was performed by a modified single-step DNA extraction method, as described previously (14–16).

SSCP and DNA Sequencing. Seven sets of primers covering the kinase domain of the \textit{MET} gene (exon 15–19) were designed by using the OLGIO software program (version 5.0, National Bioscience Inc., Plymouth, MN) according to the cDNA and partial intron-exon boundary sequences of \textit{MET} gene. The
PCR primer pairs for the amplification of the kinase domain of the MET gene exon 15–19 were as follows: (a) 5'-CAGGGTGACGTAGTACTGTA-3' and 5'-TTCTATACCATGCAAAGAG-3' for exon 15; (b) 5'-GTAGGAGGTCTGCAAGCT-3' and 5'-GTGCTCACTGTTTACCC-3' for exon 16; (c) 5'-ATGAACTGTCCCTCCACCTG-3' and 5'-TTCACTGAGGAGGATTATT-3' for exon 17a; (d) 5'-CGAGGAGAAAACTCATTGAAGAAA-3' and 5'-GAATCTTTTGCAGGCTGTT-3' and 5'-TGATTTGTTGCAGAGTAGTCT-3' for exon 18; (f) 5'-TATTTCTTTAGCCGAGCGGTAAT-3' and 5'-CCA-AAGGATCCATCCACTC-3' for exon 19a; and (g) 5'-CAGGACGATGATGTAATT-3' and 5'-AGGAGAAACTCAG-3' for exon 19b. Tumor and corresponding normal DNA from each slide were amplified in a thermal cycler (MJ Research Instrument, Watertown, MA) with primers. Each PCR reaction was generally performed under standard conditions in a 10 μl reaction mixture containing 1 μl template DNA, 0.4 μm of each primer, 125 μM each dNTP, 1.5 mM MgCl2, 0.4 units Taq polymerase, 0.5 μCi [32P]dCTP (Amersham, Buckinghamshire, United Kingdom), and 1 μl of 10× buffer. The reaction mixture was denatured for 5 min at 95°C and incubated for 35 cycles (denaturing at 95°C for 50 s, annealing at 50°C for 90 s and extending at 72°C for 90 s) with some variations in the annealing temperature. Final extension was continued for 10 min. The amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM/liter EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). The samples were denatured for 5 min at 95°C and loaded onto a MDE gel (AT Biochem, Malvern, PA) with 10% glycerol. After electrophoresis, the gels were transferred to 3 MM Whatmann paper, and sequencing was done using Amplicycle Sequencing Kit (Perkin-Elmer, Branchburg, NJ). Normal and tumor DNA sequences were compared. All of the mutations were verified by repeated PCR and gel analyses using different SSCP gel conditions.

Results

Demographic Data. Among 16 cases of histologically proven adult HCCs, 11 patients were HBV-seropositive, 3 patients were Hepatitis C virus-seropositive, and the remaining 2 were anti-HBs-positive by the RIA method. The average age at diagnosis was 55.5 years (range, 42–72 years), and 13 patients had cirrhotic background. For the childhood HCC cases, HBs status and relevant clinical information are summarized in Table 1. HBV seropositivity was seen in 8 of 10 children with a 90% HBV-positivity rate in maternal serum; in all of 16 adult HCCs, 11 patients were HBV-seropositive, 3 patients were negative by the RIA method. The average age at diagnosis was 55.5 years (range, 42–72 years), and 13 patients had cirrhotic background.

Table 1: Demographic data and MET mutations in 10 childhood HCCs

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Maternal HBV</th>
<th>HBV</th>
<th>Grade</th>
<th>Cirrhosis</th>
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<tr>
<td>1</td>
<td>M</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>15</td>
<td>+</td>
<td>–</td>
<td>II–III–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>I–II</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>II–III–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>III–IV</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>13</td>
<td>+</td>
<td>+</td>
<td>III–IV</td>
<td>–</td>
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<tr>
<td>10</td>
<td>F</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>II–III</td>
<td>–</td>
</tr>
</tbody>
</table>

Discussion

It is currently believed that gene amplification, rearrangement, or constitutive activating mutation of protooncogene encoding growth factor receptors may play an important role in the development of neoplasia by conferring a selective growth advantage. The MET gene is a receptor-type tyrosine kinase oncogene, originally identified as a transforming gene activated by rearrangement in cells treated with a chemical carcinogen (17). Increased levels of expression of HGF/SF and its specific receptor MET have been shown in several primary liver cancers such as HCC, cholangiocarcinoma, and hepatoblastoma (3–6). Recently, missense mutations—predicted to cause constitutive activation of the MET kinase in the absence of ligand—have also been identified in MET in patients with a hereditary or sporadic form of papillary renal cell carcinoma, which suggests that mutant MET might also be involved in other tumor development and progression (9, 18). It is not clear, however, whether activating mutations of MET are involved in the process of carcinogenesis of primary liver cancers in spite of its name, hepatocyte growth factor receptor. These facts led us to look for somatic mutations of the kinase domain of the MET gene in primary liver cancers. We designed seven sets of primers covering the kinase domain in MET and performed mutational analyses using PCR-based SSCP on our 75 primary liver cancer samples.

Mutations that activate receptor tyrosine kinases generally fall into two classes: (a) those that are localized to the extracellular or transmembrane domain and that promote receptor dimerization/oligomerization; and (b) those that are localized to the kinase domain and that alter catalytic activity or substrate specificity (8). We examined only those exons covering the kinase domain (exon15 to 19) because the sheer size of the MET coding region (consisting of 21 exons) makes the identification of the entire mutation labor-intensive and costly. We detected three missense mutations (T1191I, K1262R, and M1268I) in the kinase domain, and all of these mutations were exclusively in childhood HCC (3 of 10). There were no mutations in 21 cholangiocarcinomas, 28 hepatoblastomas, and 16 adult HCCs. Of these mutations, the K1262R and M1268I mutations fall within the COOH-terminal lobe of the kinase domain. This region of the molecule is believed to act as an intramolecular substrate that, in the absence of ligand, functions to inhibit enzymatic activity by blocking the active site (18, 19). It is possible that some of the mutations stimulate the kinase activity of MET by altering the structure of the intramolecular substrate such that it is constitutively disengaged from the active site.
In addition, the M1268 codon is highly conserved in a number of other tyrosine kinase receptors including c-Kit, Ret, PDGF-R, FGF-R, Ins-R, and EGFR-R (18, 19). In fact, Ret receptor tyrosine kinase has been shown to be activated by amino acid change at methionine (M918), corresponding to the position of M1268 of MET, which leads to multiple endocrine neoplasia type 2B and sporadic medullary carcinoma of the thyroid gland (9). Additionally, this is the same location for a naturally occurring MET mutation found in papillary renal cell carcinoma, which was shown to be constitutively activating and transforming in NIH3T3 cells (18). Mutant T1191I is located in the subdomain span between NH2- and COOH-terminal lobes of kinase domain (19), and the hydrophilic amino acid residue threonine is replaced by hydrophobic isoleucine.

The close relationship between HBV infection and HCC has been well documented by epidemiological case control and molecular biological studies (11–13). Because these infections are widespread in the Asian population, HCC occurs with a high incidence in Asia (11). In Korea, HCC is one of the most common malignant neoplasms, with an age-standardized incidence rate of 16.3% in male cancer patients and 5.6% in female patients, but remains an uncommon tumor in children (1.9% of all childhood malignant cases (20)). In the present study, adult HCC patients were found to have an 87.5% HBV and/or Hepatitis C virus. An 80% serum HbsAg positivity has been demonstrated in our childhood HCC patients, with a 90% positivity rate in maternal serum, which indicates that HBV transmission from the mother during perinatal period or early childhood is the most important mode of HBV infection in HCC patients in Korea. However, questions still remain to be answered concerning the relationship between HBV infection and the carcinogenesis of HCC. According to general opinion concerning the role of HBV in the oncogenesis of HCC, the incubation period is generally believed to be a minimum of 20 years (11, 12). This contrasts markedly to the average incubation period between the time of infection and the development of malignancy reported to be about 7 to 8 years in childhood HCC (12, 13). In our study, the mean age of childhood HCC is 10.4 years and the youngest child with HCC associated with HBV infection reported in the literature was 3 years old (12). In addition, a cirrhosis accompanying HCC in children is not common (2 of 10 in present study). Thus, this extremely short duration from HBV infection to the genesis of childhood HCC and the low incidence of cirrhosis in childhood HCC suggest that there may be an additional mechanism by which carcinogenesis in childhood HCC is accelerated when compared with adult HCC. We detected three mutations in the kinase domain of the MET gene that were exclusively observed in childhood HCC. These results suggest that mutant MET may be involved in the acceleration of the carcinogenesis process in childhood HCC.

Despite the small number of cases, the mutations in the kinase domain of the MET gene observed exclusively in childhood HCC suggest that mutant MET may play an important role in the carcinogenesis process of childhood HCC. However, additional studies in a large patient population and screening of the whole coding region of MET gene will be needed to verify these initial observations, and the identification of the biological function of MET will certainly broaden our understanding of the role of the MET oncogene in the pathogenesis of not only childhood HCC but also other tumors deserving consideration.

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References


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