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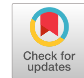
Original contribution

MET amplification, expression, and exon 14 mutations in colorectal adenocarcinoma☆,☆☆



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Summary *MET* amplification, expression, and splice mutations at exon 14 result in dysregulation of the MET signaling pathway. The aim of this study was to identify the relationship between *MET* amplification, protein or mRNA expression, and mutations in colorectal cancer (CRC). MET immunohistochemistry was used for MET protein expression analysis, and fluorescence in situ hybridization was used for *MET* amplification detection. Both analyses were performed in tissue microarrays containing 294 colorectal adenocarcinoma tissue samples and 131 samples of adjacent normal epithelial tissue. MET mRNA expression was examined by real-time quantitative polymerase chain reaction in 72 fresh colorectal adenocarcinoma tissue samples and adjacent normal colon tissue. Polymerase chain reaction sequencing was performed to screen for *MET* exon 14 splice mutations in 59 fresh CRC tissue samples. Our results showed that MET protein expression was higher in colorectal tumor tissue than in adjacent normal intestinal epithelium. Positive

Abbreviations MET, MET proto-oncogene, receptor tyrosine kinase; FISH, fluorescence in situ hybridization; EGFR, epidermal growth factor receptor; PCR, polymerase chain reaction; TCGA, The Cancer Genome Atlas; VEGF, vascular endothelial growth factor; CEP7, chromosome 7

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MET protein expression was associated with significantly poorer overall survival and disease-free survival. Multivariate analysis revealed that positive MET protein expression was an independent risk factor for disease-free survival but not for overall survival. MET mRNA expression was upregulated in tumor tissues compared with the adjacent normal tissues. The incidence of *MET* amplification was 4.4%. None of the patients was positive for *MET* mutation. Collectively, MET was overexpressed in colorectal adenocarcinoma, and its positive protein expression predicted a poorer outcome in CRC patients. Furthermore, according to our results, *MET* amplification and exon 14 mutation are extremely rare events in colorectal adenocarcinoma.
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1. Introduction

In the past decades, targeted agents, such as vascular endothelial growth factor–targeted antibodies and epidermal growth factor receptor (EGFR)–targeted antibodies, were added to the therapeutic armamentarium of colorectal cancer (CRC) together with the traditional chemotherapies, increasing the median overall survival of metastatic patients from 18 to 30 months [1,2]. However, the development of acquired (secondary) resistance, which typically occurs within 3 to 12 months after the beginning of the therapy, limits the clinical efficacy of EGFR inhibitors [3,4]. The evolution of targeted therapies in CRC has been characterized by the slow and gradual recognition of a number of biomarkers that predict negative responses to anti-EGFR agents [2].

MET is a heterodimeric tyrosine kinase receptor which acts as a cell surface receptor for hepatocyte growth factor [5,6]. The binding of hepatocyte growth factor to MET stimulates downstream signaling pathways, such as the extracellular regulated protein kinase/mitogen-activated protein kinase and phosphatidylinositide 3-kinases/protein kinase B pathways [6]. Previous studies have reported overexpression, amplification, or mutation of MET as the most common ways of MET pathway dysregulation [7]. In addition, *MET* amplification has been reported to be related to acquired resistance in patients without KRAS mutations during anti-EGFR therapy [8]. Therefore, to better define an adequate target population and selection strategy for treatment with anti-EGFR therapy, it is also essential to understand the role of MET alterations in the acquired resistance. Based on these findings, our study aimed to determine the relationship between *MET* amplification, protein and mRNA expression, and mutations in CRC.

2. Materials and methods

2.1. Patients and specimens

A total of 294 patients were selected for this retrospective study from those newly diagnosed as having colorectal adenocarcinoma who have undergone surgical resection without any preoperative therapy at Fudan University Shanghai Cancer Center (FDUSCC) between 2008 and 2009. Written informed consent was obtained from all patients. All specimens were reviewed by 2 pathologists to confirm the diagnosis, and they selected the representative tumor region from the primary formalin-fixed, paraffin-embedded blocks. Corresponding fresh tumor tissue samples were obtained from the Tissue Bank of Fudan University Shanghai Cancer Center. All specimens were obtained from surgical resection samples. This study was conducted according to the Declaration of Helsinki and approved by the institutional review board of Fudan University Shanghai Cancer Center.

Clinicopathological features of CRC patients which were recorded included age, sex, American Joint Committee on Cancer (AJCC) stage, vascular invasion, Ki67 index, tumor invasion depth (T stage), lymph node metastasis (N stage), distant metastasis (M stage), and tumor differentiation.

2.2. Immunohistochemistry and fluorescence in situ hybridization

For immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) analysis, tissue microarrays (TMAs) were created from tissue samples of recruited patients. The CRC TMAs were comprised of 294 colorectal adenocarcinoma samples and 131 adjacent normal epithelial tissues. The median age of the 294 patients was 57 years (range, 27-81 years), and male to female ratio was 1.4:1. For antigen retrieval, TMA sections were incubated with 0.1 mol/L citrate solution (pH 6.0) for 3 minutes of high-pressure retrieval. Next, slides were incubated with MET (D1C2) XP rabbit monoclonal antibody (#8198; Cell Signaling Technology, Danvers, MA) overnight at 4°C. Twenty-four hours later, MET protein was visualized by EnVision Detection Systems (K5007; Dako, Santa Clara, CA) for 30 minutes at room temperature.

The IHC score was based on a scoring system for both the intensity and extent of staining [9] and was defined as follows: intensity score (cytoplasmic and/or membranous staining of MET, 0; no staining; 1, weak staining; 2, moderate staining; 3, strong staining), extent score (the percentage of the positive staining cells: 0, 0%; 1, <10%; 2, 10%-50%; 3, >50%), and IHC score (the sum of intensity score and extent score: 0-2, negative; 3-6, positive). The MET H score was calculated by adding the multiplication of the intensity score in 4 gradations with each percentage of positive cells [10]. Finally, a continuous score from 0 to 300 was obtained for Spearman correlation analyses.

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