

MET Is Highly Expressed in Advanced Stages of Colorectal Cancer and Indicates Worse Prognosis and Mortality

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Abstract. *The aim of the present study was to evaluate by immunohistochemistry the prognostic meaning of the tumor marker MET (hepatocyte growth factor) in patients submitted to surgical resection due to primary colorectal adenocarcinoma. Patients and Methods: A retrospective study was carried out that included 286 consecutive patients with colorectal adenocarcinoma, submitted to surgical resection at Barretos Cancer Hospital, from 1993 to 2002. The histopathological expression of the MET tumor marker was evaluated using an anti-protein monoclonal antibody against MET by the streptavidin-biotin-peroxidase technique. The expression of the tumor marker was semi-quantitative, and the slide samples were independently analyzed by three pathologists unaware of patient clinical and histopathological data. Results: The tumor marker expression was positive in 236 (79%) out of a total of 286 patients. This expression was statistically significantly different between stages I and IV ($p=0.004$), for overall survival ($p=0.009$), and for cancer-related mortality rates ($p=0.022$). However, no association between the tumor marker and recurrence ($p=0.89$) or disease-free interval ($p=0.91$) was observed. Conclusion: MET has shown significant expression at advanced stages of the disease, as well as for overall survival and cancer-related mortality rates demonstrating to be a valuable marker for poor prognosis in colorectal cancer patients.*

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Colorectal cancer is the most important cause of cancer death among gastrointestinal cancer patients (1). In the United States of America, 106,680 new colorectal cancer cases were expected for 2006 and 41,930 cases of rectal cancer (2). Similarly in Brazil, colorectal cancer is one of the five more prevalent types of cancer among women and men, mainly in individuals 50 to 70 years old (3).

Several clinical, pathological and epidemiological factors can influence the patient's prognosis (4, 5) but the classification of tumor stage is the most significant parameter used to evaluate clinical behavior. Consequently, the presence of lymph node metastasis indicates that only 30% of patients survive for five years, and for those with hepatic metastasis, life expectancy is severely limited. However, tumor stage alone is far from comprehensive in terms of prognosis which seriously limits its use for this purpose. Theoretically, patients staged I or II should demonstrate good prognosis; however, one third of these patients die in five years with distant metastasis or local relapse (6). Conversely, there are patients with very voluminous tumoral masses involving contiguous tissues and organs without lymph node invasion or distant metastasis, and benevolent clinical behavior (7-10). Consequently, it is crucial to find one or more parameters informative for prognosis.

Among a plethora of tumoral markers, the activity of mesenchymal-epithelial transition factor, a proto-oncogene that encodes a protein MET, known as MET hepatocyte growth factor receptor (HGFR), is believed to be particularly important in cancer prognosis. HGF is the unique ligand known for MET which is involved in carcinogenesis of several types of tumors, invasion, differentiation and tumoral angiogenesis (11-19). Perceptibly, the expression of MET protein is believed to be crucial to determine prognosis. It has already been demonstrated that MET can enhance colorectal

tumoral cell motility, facilitating invasion and metastasis (13). Interestingly, HGF is not detectable in normal hepatic cells but its expression is enhanced in hepatocytes neighboring the malignant colorectal tumor cells metastasized to the liver (20). High expression of MET in malignant transformation is also associated with an augmented capacity for invasion and metastasis (21). In fact, MET was reported to be expressed in more than 50% of colorectal lesions from dysplastic adenoma to invasive carcinoma, which suggests that MET influence occurs from the early stages of malignant disease and is highly associated with advanced disease (22). Despite its well-documented role in the metastatic potential of colorectal cancer (23), MET did not demonstrate any independent value as a prognostic factor (17). Indeed, the alleged participation of MET amplification in colorectal cancer development is not unequivocal (24), but the majority of investigations have reported data supporting the participation of MET in colorectal cancer. Increased MET expression is frequently associated with concomitant augmentation of HGF expression, which infers a paracrine effect that optimizes the cellular mass expansion (18). Moreover MET/HGF is more significantly expressed in Dukes' C than Dukes' B tumors. Both conditions are limited to the organ but differ in lymph node status, which infers that MET/HGF expression can predict metastasis (11).

The aim of this work was to verify if HGFR was more highly expressed or not in advanced stages of colorectal cancer and to correlate these findings with prognosis and mortality.

Patients and Methods

All 286 patients enrolled in this study were consecutively examined and treated by surgery at Barretos Cancer Hospital, in São Paulo State, Brazil, between 1993 and 2002 due to colorectal adenocarcinoma. Clinical and pathological data were retrospectively obtained from the files of the hospital medical archive. Cases with history of any previous cancer treatment were excluded. General information included size of the tumor, histological classification of the tumor, including type and degree of histopathological differentiation, invasion of colonic wall, lymph node invasion and distant metastasis, time to recurrence, survival rates and cause of death when death occurred.

Immunohistochemistry reaction. Immunohistochemistry was performed according to the avidin-biotin-peroxidase complex principle (Dako Co., San Diego, CA, USA), using a primary antibody raised against MET oncoprotein (HGTR), NCL-MET (Dako Co.) diluted at 1:1500.

Briefly, deparaffinized and rehydrated sections were immersed in EDTA (pH 8.0), heated to 98°C in a water-bath for 15 minutes and washed in phosphate-buffered saline (PBS). Endogenous peroxidases were then inactivated using 3% hydrogen peroxide in methanol for 10 minutes, followed by washing in PBS. Tissue sections were then incubated with blocking solution for 10 minutes and incubated at room temperature with the primary antibody for 2 hours. Sections were then sequentially washed in PBS and incubated

with biotinylated goat anti-polyvalent antibody for 10 minutes, streptavidin peroxidase for 10 minutes, and developed with 3,3'-diaminobenzidine (DAB+ Substrate System; Dako, Carpinteria, CA, USA) for 10 minutes. Tissue sections were counterstained with hematoxylin and permanently mounted.

Immunohistochemical evaluation. The immunoreaction was scored semi-quantitatively according to Wielenga *et al.* (25): the reactions were considered augmented when $\geq 50\%$ of the malignant cells were positively stained; (Figure 1) and diminished when the positive cells were between $>10\%$ to $<50\%$ (Figure 2). Cases were considered negative for MET when $\leq 10\%$ of cells gave positive reactions (Figure 3).

Evaluation of MET immunohistochemical expression was performed blindly by two independent observers and discordant results were reassessed using in a double-head microscope and a final score was agreed.

Statistical analysis. Data were stored and analyzed using SPSS statistical software (version 14.0; SPSS Inc., Chicago, IL, USA). The comparison of MET expression between tumor and normal cells, as well as the relationship between MET expressions and the clinicopathological parameters, were examined for statistical significance using Pearson's chi-square (χ^2) test, using a threshold for significance of p -values <0.05 . Survival curves were plotted using the method of Kaplan and Meier and data compared using the log-rank test.

Ethics. The study was approved by the local Committees of Ethics on Research (No. 0436/04).

Results

A total of 286 patients were enrolled in the study, with mean age of 63 years (ranged from 28 to 93): 154 (50.3%) male and 142 (49.7%) female. Adenocarcinoma without other specification was the predominant histopathological type with 251 (88%) cases, followed by 24 (8%) mucinous type, 9 (3%) tubular type and 2 (1%) squamous adenocarcinoma. In 210 cases (73%), the colonic wall had been invaded and the tumor reached the serosal membrane or adjacent structures (T4); in 43 (15%), the tumor had invaded the subserosal zone (T3); in 28 (9%) the muscular propria, and in 5 (2%) cases the tumor was restricted to the submucosal (T1). There were 195 (68%) cases exhibiting lymph node invasion and 91 (32%) cases without lymph node invasion. Distant metastases were not observed in 217 patients (76%) at first examination and 69 (24%) patients already showed signs of metastatic disease. TNM classification showed 40 (14%) stage I, 110 (39%) stage II; 67 (23%) stage III and 69 (24%) stage IV tumors.

MET reaction was positive in 236 (79%) and negative in 50 (21%) cases. The expression of MET according tumor stage is depicted in Table I. A statistically significant difference ($p=0.004$) in MET expression between stages was observed, with stage IV associated with augmented and stage I with diminished MET expression. Table II shows the comparison between MET expression and recurrence. Stage

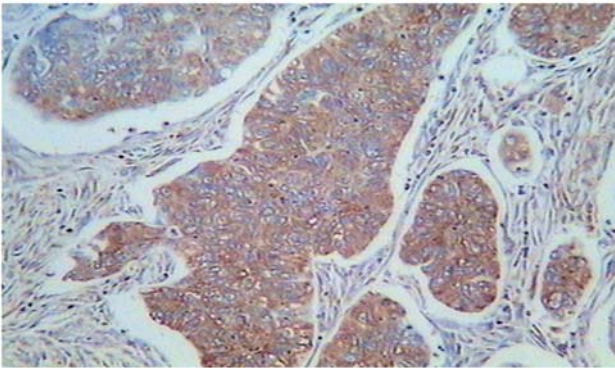


Figure 1. Highly augmented MET expression ($\times 100$).

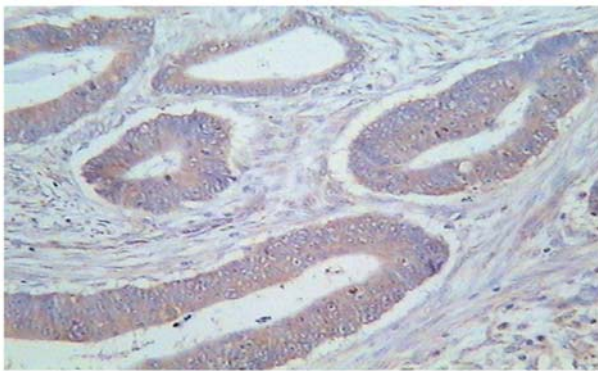


Figure 2. Diminished MET expression ($\times 100$).

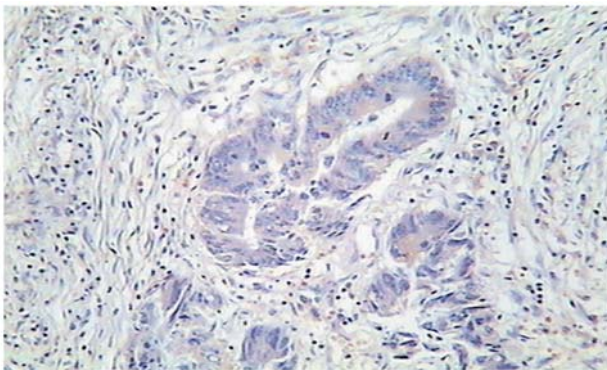


Figure 3. Negative case, without MET expression ($\times 100$).

IV patients and patients lost from follow-up were excluded from this analysis, but no significant difference was found. Figure 4a shows the Kaplan-Meier survival curves and by log-rank test, no statistical difference was observed among the three groups ($p=0.96$), which implies that the disease-free interval is not associated with MET expression.

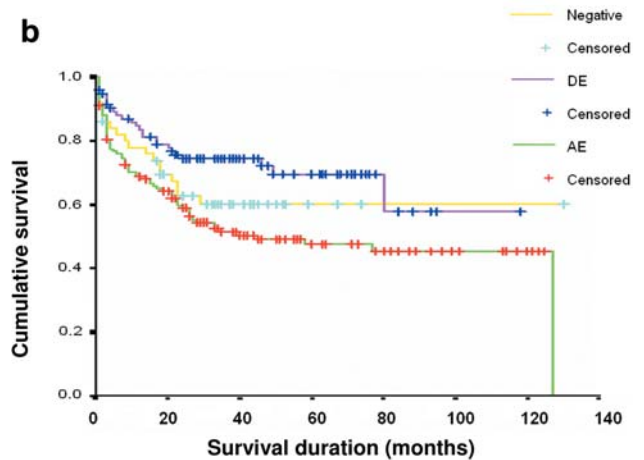
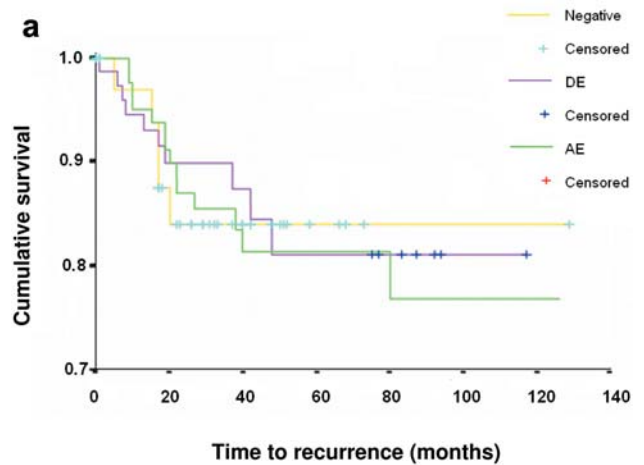


Figure 4. Recurrence-free (a) and overall (b) survival according to MET expression. DE, Diminished expression; AE, augmented expression. Log-rank test a, $p=0.96$; b, $p=0.009$.

Figure 4b shows the curve of general survival rates according to MET expression. Interestingly, longer survival rates ($p=0.009$) were observed for the MET diminished group. The overall correlation of MET and mortality is given in Table III, which shows that 49.3% of the patients with augmented expression of MET were dead by the end of this study, whilst the majority of the MET-negative group were not ($\chi^2=14.82$; $p=0.022$).

Discussion

We sought to investigate MET expression as a possible tool to indicate better or worse prognosis in cases of colorectal cancer. As previously mentioned there is a progressive augmentation of MET expression in the early stages of adenoma/carcinoma transformation, but this pattern is not useful for predicting

Table I. *MET* expression distributed according to clinical stage of colorectal cancer.

Stage	Expression of MET							
	Augmented		Diminished		Negative		Total	
	No.	%	No.	%	No.	%	No.	%
I	12	8.5	22	23.4	6	12.0	40	14.0
II	50	35.2	41	43.6	19	38.0	110	38.5
III	35	24.6	19	20.2	13	26.0	67	23.4
IV	45	31.7	12	12.8	12	24.0	69	24.1
Total	142	100.0	94	100.0	50	100.0	286	100.0

$p=0.004$

Table II. *MET* immunohistochemical expression according to the recurrence of colorectal cancer.

Recurrence	Expression of MET							
	Augmented		Diminished		Negative		Total	
	No.	%	No.	%	No.	%	No.	%
Absent	82	84.6	72	87.8	33	86.8	187	86.2
Present	15	15.4	10	12.2	5	13.2	30	13.8
Total	97	100.0	82	100.0	38	100.0	217	100.0

$p=0.89$

recurrence or the disease-free interval. For this reason, TNM classification is still believed to be a better option in evaluating prognosis. However, we observed that MET was proportionally less expressed in incipient carcinomas than in advanced stages of colorectal cancer. Our data are in part corroborated by some data already published where MET was indeed demonstrated to be a good marker for predicting the metastatic potential of colorectal tumors (11, 26). Despite its utility in demonstrating tumor aggressiveness, there was no valid indication for any association between MET expression and survival rates (27). Conversely, the data obtained herein significantly demonstrated that the patients with diminished expression of MET had greater survival rates when compared with the group of patients with high expression of MET, which endorses the basis of our study that highly expressed MET is more commonly related to worse prognosis; however, this assumption is not consensual (27). Moreover, we found that MET correlated to TNM stages with a perceptible and progressive increase of MET expression from stage I to stage IV (22, 23, 26). Additionally, we opted for immunohistochemical evaluation of MET because this method is reproducible and easily applicable in routine of pathology.

Table III. *MET* immunohistochemical expression according to mortality caused by colorectal cancer.

Mortality	Expression of MET					
	Augmented		Diminished		Negative	
	No.	%	No.	%	No.	%
No	72	50.7	68	72.3	31	62.0
Yes	70	49.3	26	27.7	19	38.0
Total	142	100.0	94	100.0	50	100.0

$p=0.003$

Despite controversies, we demonstrate that immunohistochemical expression of MET is useful in colorectal cancer prognostic evaluation.

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