ANTICANCER RESEARCH *29*: 4807-4812 (2009)

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

ANTÔNIO TALVANE TORRES DE OLIVEIRA[1], DELCIO MATOS[2], ANGELA FLÁVIA LOGULLO[3], SANDRA REGINA MORINI DA SILVA[4], RICARDO ARTIGIANI NETO[3], ADHEMAR LONGATTO FILHO[4,5] and SARHAN SYDNEY SAAD[2]

[1]Departments of Surgery and Pathology of Barretos Cancer Hospital, Pio XII Foundantion, Barretos, São Paulo; Departments of [2]Gastroenterology and [3]Pathology, School of Medicine, Federal University of São Paulo, São Paulo, Brazil; [4]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; [5]Laboratory of Medical Investigation (LIM) 14, Department of Pathology, University of São Paulo School of Medicine, São Paulo, Brazil

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.*

Correspondence to: Antônio Talvane Torres de Oliveira, Rua Antenor Duarte Vilella, 1331, Bairro Dr. Paulo Prata, CEP 14784-400, Hospital de Câncer de Barretos, Fundação Pio XII, Barretos, SP, Brazil. e-mail: netto123@uol.com.br

Key Words: Colorectal carcinoma, immunohistochemistry, MET, prognosis, survival, carcinogenesis.

Colorectal cancer is the most important cause of cancer dead 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 the 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 ≥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 ≤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

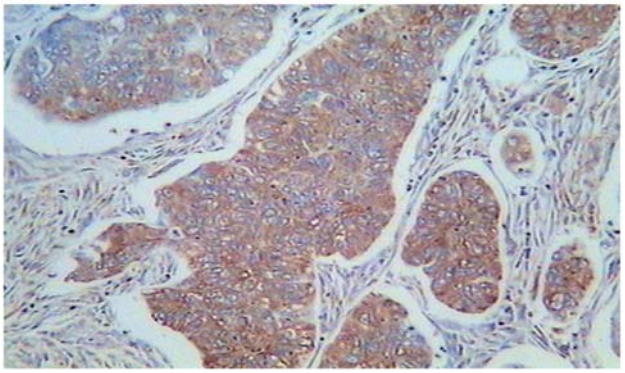


Figure 1. *Highly augmented MET expression (×100).*

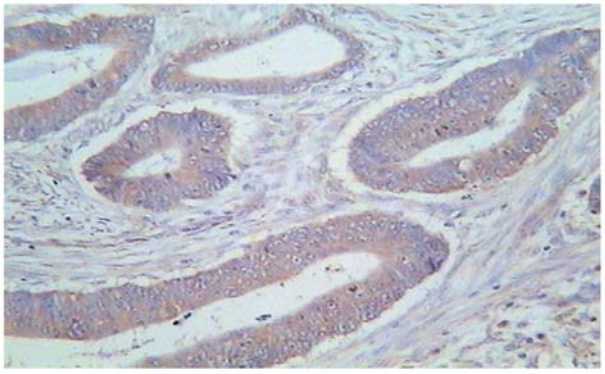


Figure 2. *Diminished MET expression (×100).*

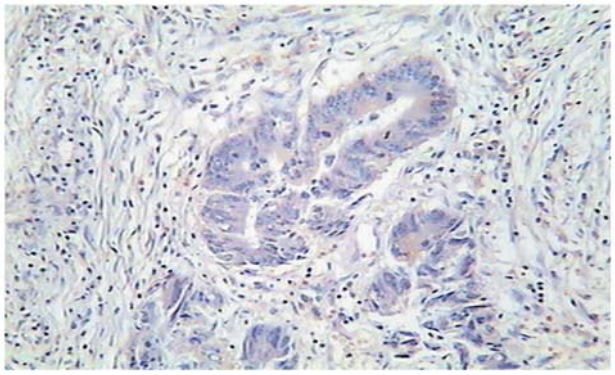
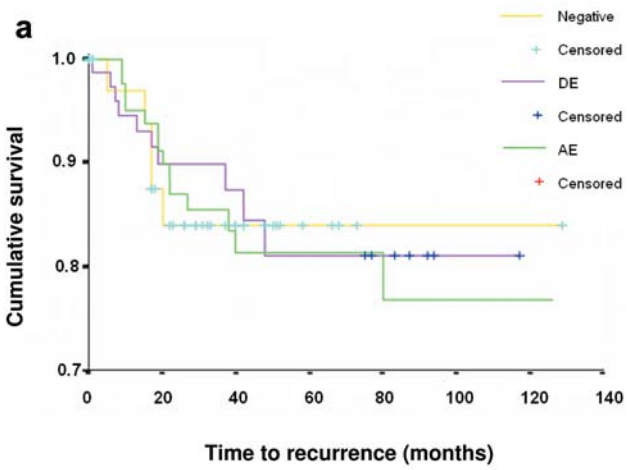


Figure 3. *Negative case, without MET expression (×100).*

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.



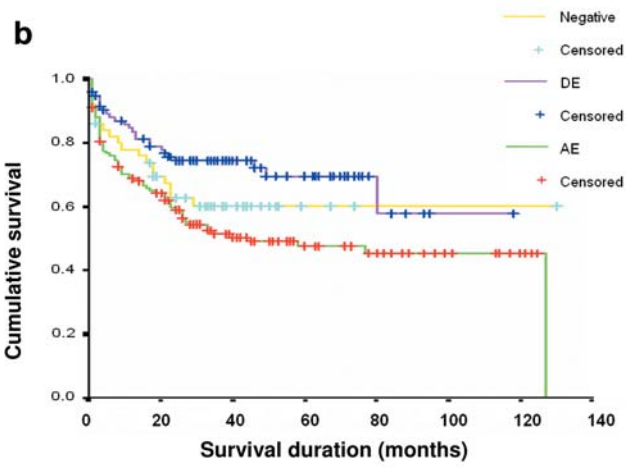


Figure 4. *Recurrence-free (a) and overall (b) survival according to MET expression. DE, Diminished expression; AE, augmented expression. Log-rank test a, p=0.96; b, p=0.009.*

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 (χ^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.*

	Expression of MET							
	Augmented		Diminished		Negative		Total	
Stage	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.*

	Expression of MET							
	Augmented		Diminished		Negative		Total	
Recurrence	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.*

	Expression of MET					
Mortality	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.

References

1 Landis SH, Murray T, Bolden S and Wingo PA: Cancer statistics, 1998. CA Cancer J Clin *48*: 4-12, 1998.

2 American Cancer Society. Cancer statistics 2006. Georgia: American Cancer Society, 2006. Available at: http://www.cancer.org/downloads/STT/Cancer_Statistics_2006_Presentation.ppt#256,1,Cancer Statistics 2006. Accessed 15 march 2006.

3 Instituto Nacional do Câncer. Estimativas de câncer no Brasil 2006. Rio de Janeiro: INCA, 2006. Available at: http://www.inca.gov.br/estimativa/2006/ Accessed 20 February 2006.

4 Hermanek P, Wiebelt H, Staimmer D and Riedl S: Prognostic factors of rectum carcinoma: experience of the German Multicentre study SGCRC. German Study Group Colorectal Carcinoma Tumori *81*: 60-64, 1995.

5 Benson AB 3rd. New approaches to assessing and treating early-stage colon and rectal cancers: cooperative group strategies for assessing optimal approaches in early-stage disease. Clin Cancer Res *13*: 6913s-20s, 2007.

6 Frati L and Codacci-Pisanelli G: Chemotherapy and immunotherapy in metastatic colorectal cancer. N Engl J Med *360*: 2134-2135, 2009.

7 Vieira RAC. Prognostic factors related to the patients submitted to colon cancer surgery. Master Thesis – Faculty of Medicine, São Paulo University, 1999.

8 Zhao DB, Wu YK, Shao YF, Wang CF and Cai JQ: Prognostic factors for 5-year survival after local excision of rectal cancer. World J Gastroenterol *15*: 1242-1245, 2009.

9 Ivanov K, Kolev N, Tonev A, Nikolova G, Krasnaliev I, Softova E and Tonchev A: Comparative analysis of prognostic significance of molecular markers of apoptosis with clinical stage and tumor differentiation in patients with colorectal cancer: a single institute experience. Hepatogastroenterol *56*: 94-98, 2009.

10 Golfinopoulos V, Salanti G, Pavlidis N and Ioannidis JP: Survival and disease-progression benefits with treatment regimens for advanced colorectal cancer: a meta-analysis. Lancet Oncol *8*: 898-911, 2007.

11 Fazekas K, Csuka O, Köves I, Rásó E and Tímár J: Experimental and clinicopathologic studies on the function of the HGF receptor in human colon cancer metastasis. Clin Exp Metastasis *18*: 639-649, 2000.
12 Herynk MH, Tsan R, Radinsky R and Gallick GE: Activation of c-MET in colorectal carcinoma cells leads to constitutive assossiation of tyrosine-phosphorylated beta-catenin. Clin Exp Metastasis *20*: 291-300, 2003.
13 Jiang WG, Lloyds D, Puntis MC, Nakamura T and Hallett MB: Regulation of spreading and growth of colon cancer cells by hepatocyte growth factor. Clin Exp Metastasis *11*: 235-242, 1993.
14 Kataoka H, Hamasuna R, Itoh H, Kitamura N and Koono M: Activation of hepatocyte growth factor/scatter factor in colorectal carcinoma. Cancer Res *60*: 6148-6150, 2000.
15 Kermorgant S, Aparicio T, Dessirier V, Lewin MJ and Lehy T: Hepatocyte growth factor induces colonic cancer cell invasiveness *via* enhanced motility and protease overproduction: evidence for Pl3 kinase and PKC involvement. Carcinogenesis *22*: 1035-1042, 2001.
16 Nabeshima K, Shimao Y, Inoue T, Itoh H, Kataoka H and Koono M: Hepatocyte growth factor/scatter factor induces not only scattering but also cohort migration of human colorectal-adenocarcinoma cells. Int J Cancer *78*: 750-759, 1998.
17 Hiscox SE, Hallett MB, Puntis MC, Nakamura T and Jiang WG: Expression of the HGF/SF receptor, c-MET, and its ligand in human colorectal cancers. Cancer Invest *15*: 513-521, 1997.
18 Otte JM, Schmitz F, Kiehne K, Stechele HU, Banasiewicz T, Krokowicz P, Nakamura T, Fölsch UR and Herzig K: Functional expression of HGF and its receptor in human colorectal cancer. Digestion *61*: 237-246, 2000.
19 Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL and Vande Woude GF: The met proto-oncogene receptor and lumen formation. Science *257*: 1258-1261, 1992.
20 Yoshinaga Y, Matsuno Y, Fujita S, Nakamura T, Kikuchi M, Shimosato Y and Hirohashi S: Immunohistochemical detection of hepatocyte growth factor/scatter factor in human cancerous and inflammatory lesions of various organs. Jpn J Cancer Res *84*: 1150-1158, 1993.
21 Baldus SE, Kort EJ, Schirmacher P, Dienes HP and Resau JH: Quantification of MET and hepatocyte growth factor/scatter factor expression in colorectal adenomas, carcinomas and non-neoplastic epithelia by quantitative laser scanning microscopy. Int J Oncol *31*: 199-204, 2007.
22 Zeng ZS, Weiser MR, Kuntz E, Chen CT, Khan SA, Forslund A, Nash GM, Gimbel M, Yamaguchi Y, Culliford AT 4th, D'Alessio M, Barany F and Paty PB: *c-Met* gene amplification is associated with advanced stage colorectal cancer and liver metastases. Cancer Lett *265*: 258-269, 2008.
23 Fujita S and Sugano K: Expression of *c-MET* proto-oncogene in primary colorectal cancer and liver metastases. Jpn J Clin Oncol *27*: 378-383, 1997.
24 Umeki K, Shiota G and Kawasaki H: Clinical signifiance of *c-MET* oncogene alterations in human colorectal cancer. Oncology *56*: 314-321, 1999.
25 Wielenga VJ, van der Voort R, Taher TE, Smit L, Beuling EA, van Krimpen C, Spaargaren M and Pals ST: Expression of *c-MET* and heparan-sulfate proteoglycan forms of CD44 in colorrectal cancer. Am J Pathol *157*: 563-573, 2000.
26 Takeuchi H, Bilchik A, Saha S, Turner R, Wiese D, Tanaka M, Kuo C, Wang HJ and Hoon DS: *c-MET* expression level in primary colon cancer: a predictor of tumor invasion and lymph node metastases. Clin Cancer Res *9*: 1480-1488, 2003.
27 Resnick MB, Routhier J, Konkin T, Sabo E and Pricolo VE: Epidermal growth factor receptor, *c-MET*, β-catenin, and p53 expression as prognostic indicators in stage II colon cancer: A tissue microarray study. Clin Cancer Res *10*: 3069-3075, 2004.

Received June 4, 2009
Revised September 14, 2009
Accepted September 22, 2009