SPANDIDOS PUBLICATIONS

c-Met expression in primary tumors and their corresponding distant metastases

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Abstract. c-Met is a receptor tyrosine kinase that has been implicated in the pathogenesis and growth of a wide variety of human malignancies, including CRC, but its role in metastasis is largely unknown. We compared c-Met expression in primary human colorectal carcinomas and distant metastases from the same patients. Formalin-fixed paraffin-embedded tissue samples from 69 colorectal cancer patients were obtained. The protein expression of c-Met was evaluated immunohistochemically using a commercial antibody. The difference in expression between primary tumors and their corresponding distant metastases was analyzed using the Wilcoxon signed-rank test. c-Met expression was statistically significantly lower in the distant metastases compared to their corresponding primary tumors ($p<0.001$), whereas no difference was found between lymph node metastases and their corresponding primary tumors ($p=0.957$). The degree of c-Met expression was not related to clinicopathological characteristics such as tumor grade and Dukes' stage at the time of primary tumor diagnosis, or to the location of the distant metastases. We demonstrated that c-Met expression is often reduced in distant metastases compared to their corresponding primary colorectal tumors. Additional studies of c-Met activation and signal transduction will increase our knowledge about the role of c-Met in colorectal cancer metastasis.

Introduction

Colorectal cancer (CRC) is the third leading cause of death in the western world with an overall 5-year survival of <60%. Succumbing to CRC is usually due to distant metastatic spread, with the liver being the most common site. Once distant metastases have been established, the chances of long-term survival are very low. Therefore, achieving an understanding of the molecular mechanisms contributing to the metastatic ability of a colorectal carcinoma is critical to the development of effective treatments.

c-Met is a receptor tyrosine kinase that has been implicated in the pathogenesis and growth of a wide variety of human malignancies (1-3), including CRC (4-6). c-Met expression in colonic cells increases during the progression towards malignancy (7), and a higher c-Met expression has been observed in colorectal tumors compared with corresponding normal colon mucosa (8).

The heterodimeric c-Met protein (9) is activated by hepatocyte growth factor (HGF), also known as scatter factor (SF) (9). The binding of HGF/SF to c-Met is known to promote motility, morphogenesis and mitogenesis in epithelial cell lines of various origins (10,11), causing colon cancer cells to form crypt-like structures (10) and inducing angiogenesis in endothelial cells (5).

The c-Met signaling pathway is believed to stimulate tumor invasion and metastasis. The Madkin-Darby canine kidney (MDCK) cell line responds to HGF/SF by colony dispersal and epithelial-to-mesenchymal transition (EMT), increasing cell motility and the invasion of collagen matrices (12). c-Met stimulation by HGF/SF from stromal cells has been suggested to facilitate local invasion of epithelial cells (13).

In CRC, it has been suggested that c-Met is overexpressed in metastatic spread (14,15), and that it may be used as a marker of lymph node metastases (8). However, despite increasing evidence for a role of c-Met in CRC metastasis, only two small (n=6 and n=10, respectively) studies have, to our knowledge, compared c-Met expression in primary CRCs and distant metastases, with conflicting results (16,17).

This study aimed to examine the degree of c-Met expression in 69 primary human colorectal carcinomas in relation to c-Met expression in distant metastases from the same patients.

Materials and methods

Patients. Sixty-nine CRC patients with archival tissue from a primary colorectal adenocarcinoma and at least one distant metastasis were included in the study. Patients were identified using the computerized patient record database at the

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Table I. Characteristics of colorectal cancer patients and tumors at time of diagnosis.

	No. of patients	%
Age, years		
<50	10	14.5
51-60	12	17.4
61-70	26	37.7
>70	21	30.4
Sex		
Male	40	58.0
Female	29	42.0
Dukes' stage[a]		
A	1	1.4
B	23	33.3
C	43	62.3
NA	2	2.9
Grade		
High	10	14.5
Moderate	54	78.3
Low	5	7.2
Mucinous		
Yes	14	20.3
No	55	79.7

[a]Dukes' stage was not determined for 2 patients for whom only biopsy tissue was available.

Table II. Localization of primary tumors and their corresponding distant metastases.

Localization of primary tumors (%)		
Right colon	16	(23.2)
Left colon	25	(36.2)
Rectum	23	(33.3)
Recto-sigmoid junction	3	(4.3)
Unspecified	2	(2.9)
Localization of distant metastases (%)		
Liver	35	(50.7)
Lung	5	(7.2)
CNS	5	(7.2)
Skeletal	5	(7.2)
Skin	5	(7.2)
Ovarian	1	(1.4)
Peritoneum	2	(4.3)
Gall bladder	1	(1.4)
Scar in abdominal wall	10	(14.5)

Department of Clinical Pathology, University Hospital, Umeå, Sweden. Patients diagnosed between the years 1982, when the computerized database was implemented, and 2000 were included. Staging and grading of the tumors were performed by pathologists at the time of surgery or biopsy. This study was approved by the Research Ethics Committee of Umeå University, Umeå, Sweden.

Immunohistochemistry. Formalin-fixed paraffin-embedded routine diagnostic tissue blocks were used to prepare slides. Tissue sections (4-μm) were cut, mounted on objective slides, deparaffinized, rehydrated and heated in EDTA (pH 8.0) in a microwave for 3x5 min. The Dako EnVision™ system (Dako Corp., Carpinteria, CA) was used to visualize the bound c-Met antibody, which was purchased from Zymed, CA, USA.

Expression of c-Met, localized in the cytoplasm and outer cellular membrane of colorectal tumor cells, was graded based on the intensity of immunohistochemical staining in the entire tumor. A four-level scale was employed: -, negative; +/-, very weak; +, medium; and ++, strong positive staining, according to Takeuchi *et al* (8). Samples were evaluated twice by the same observer, who was blinded to the details of the patients and samples. Intra-observer disagreements were reviewed a third time, followed by a conclusive judgment.

Statistical analysis. The Wilcoxon signed-rank test, a non-parametric paired test, was used to compare c-Met expression in primary tumors and their corresponding metastasis. Tests were two-sided, and a p-value of <0.05 was considered statistically significant.

Results

Details of the patients and tumors are presented in Table I. A total of 69 cases (40 men and 29 women), aged 36-85 years (median 64) at CRC diagnosis, met the inclusion criteria for the study. The distant metastases were diagnosed up to 6 months prior to, and up to 7 years after, diagnosis of the primary tumor. For 43 patients, tissue samples were available from lymph node metastases obtained at the surgical resection of the primary tumor.

Locations of the tumors and metastases are presented in Table II. Of the 69 primary tumors, 16 were located in the proximal colon, 25 in the distal colon and 23 in the rectum. The majority of the corresponding distant metastases were located in the liver (35 samples), followed by lung, central nervous system, bone, and skin (5 samples each). Less common sites included the peritoneum, gall bladder and ovary. In 10 patients, metastases were due to local recurrence in the surgical scar.

The distribution of primary tumors, lymph node metastases and distant metastases according to c-Met protein expression is presented in Table III, and the relationship between the primary tumors and their corresponding distant metastases is shown in Fig. 2. The degree of c-Met expression was statistically significantly lower in the distant metastases compared to their corresponding primary tumors ($p<0.001$), which was especially apparent in patients with clearly positive (+ and ++) c-Met staining in the primary tumor (Figs. 1 and 2). Out of 69, 30 (43.5%) metastases showed a decreased

Degree of immunohistochemical staining for c-Met in primary colorectal adenocarcinomas and in nodal and distant s from the same patients.

	Degree of immunohistochemical staining				
	- (%)	+/- (%)	+ (%)	++ (%)	p-value[a]
Primary tumor	1 (1.4)	12 (17.4)	51 (73.9)	5 (7.2)	
Nodal metastases	1 (2.8)	2 (5.7)	29 (82.8)	3 (8.6)	0.957
Distant metastases	3 (4.3)	32 (46.4)	33 (47.8)	1 (1.4)	<0.001

[a]Wilcoxon signed-rank test (non-parametric test for two related samples) comparing metastases with their corresponding primary tumors.

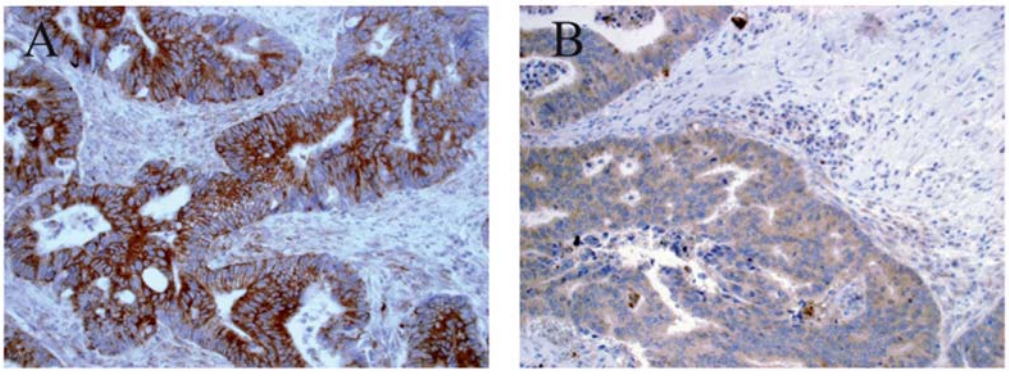

Figure 1. Representative examples of c-Met expression by immunohistochemistry on one primary tumor (A) and its corresponding liver metastasis (B).

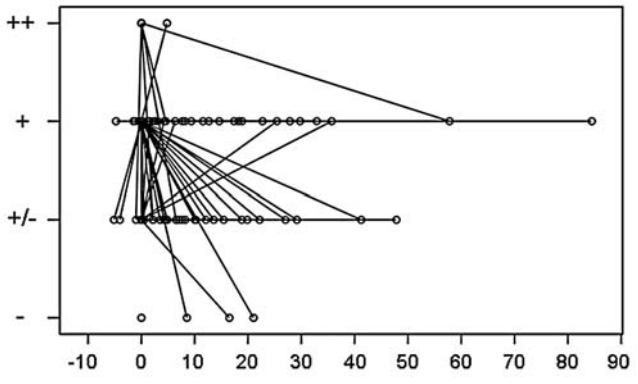

Figure 2. Schematic diagram showing the relationship between c-Met expression in primary colorectal tumors and their corresponding metastases. The X-axis shows the time in months, with 0 indicating the time of primary tumor surgery. Each primary tumor is connected via a line to the time of tissue sampling of its corresponding distant metastasis. The Y-axis shows the immunohistochemical intensity of c-Met expression.

expression compared to their corresponding primary tumors, 7 (10.1%) showed an increased expression, and 32 (46.4%) showed no change. In contrast, lymph node metastases did not differ from their corresponding primary tumors with respect to c-Met expression (p=0.957). Excluding the 10 patients with distant metastasis in the operation scar did not affect the results. The degree of c-Met expression was not related to any of the clinicopathological variables or to the location of the distant metastases (data not shown).

Discussion

In this study of 69 colorectal cancer patients with disseminated disease, we found that c-Met protein expression tended to be lower in distant metastases compared to their corresponding primary colorectal tumors.

Only two previous small studies have analyzed c-Met expression in primary colorectal tumors and metastases. A study by Otte *et al* (16) found a high c-Met expression in primary colorectal tumors, whereas only 2 out of 6 liver metastases had detectable c-Met levels. In contrast, Fujita *et al* (17) reported non-statistically significantly higher c-Met mRNA levels in liver metastasis than in the primary tumor. The down-regulation of c-Met expression in distant metastases has been associated with a more aggressive clinical phenotype in breast (18) and pancreatic cancer (19), which is in line with the findings of the present study. It is possible that c-Met initially enhances the metastatic phenotype of a cancer cell, but becomes down-regulated with the establishment of a distant metastasis, possibly due to different signals from the new microenvironment.

c-Met is a receptor tyrosine kinase which, when stimulated with HGF/SF from stromal cells, becomes autophosphorylated and is thereby activated. Active c-Met signals downstream to stimulate cell proliferation, migration and survival (11,20). The abnormal expression of c-Met and HGF/SF in most types of human cancer is associated with poor clinical outcome (11). However, although the expression of c-Met has been extensively studied in human malignancies,

the significance of phosphorylated c-Met is largely unknown. To our knowledge, only one study has addressed this topic, reporting that the phosphorylation of Y1349 on c-Met is important for tumor growth and progression in renal cell carcinoma (21). It is important to study the activity of receptors such as c-Met, since even low levels of a protein can induce a high level of signal transduction through, for example, heterodimerization or receptor mutations (22). Better phospho-specific antibodies than those currently available are a requirement for such studies. Further investigation of c-Met activation and downstream signaling in primary tumors and their corresponding metastases is needed to determine the importance of c-Met in the metastasis of colorectal cancer.

The main strength of this study is its unique design, including a comparatively large collection of tissue samples from primary colorectal carcinomas and distant metastases from the same patients. The samples were collected according to routine clinical practice over several years, but the time between collection of a primary tumor and its corresponding metastasis seldom exceeded 36 months. This short time minimizes the risk of different conditions for tumors and their metastasis due to changes in protocols for the handling of specimens.

In conclusion, we have demonstrated that c-Met expression is often reduced in distant metastases compared to their corresponding primary colorectal tumors. Additional studies of c-Met activation and signal transduction will increase our knowledge of the role of c-Met in colorectal cancer metastasis.

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