Immunohistochemical expression of HGF, c-MET and transcription factor STAT3 in colorectal tumors

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By immunohistochemistry, we have investigated the expression of hepatocyte growth factor (HGF), HGF-R or c-met and the transcritor factor STAT3 in a series of 80 colorectal tumours (40 adenomas and 40 adenocarcinomas). The expression of HGF, c-met and STAT3 was revealed in 40/40 (100%) of adenomas and in 26/40 (65%) of adenocarcinomas; the remaining 14/40 (35%) carcinomas expressed c-met but failed to express HGF and STAT3. Positive immunoreaction score was defined through the number of stained cells: low (1-10%), moderate (11-50%) and high (>51%). In adenomas, the HGF immunoreaction was high in 33 (82.5%) and moderate in 7 (17.5%); the c-met staining was high in 3 (7.5%) and moderate in 37 (92.5%); and the STAT3 reactivity was high in 25 (62.5%) and moderate in 15(37.5%). In carcinomas, the HGF immunoreaction was moderate in 21 (80.7%) and low in 5 (19.2%); the c-met staining was high in 14 (35%), moderate in 25 (62.5) and low in 1 (2.5%); and the STAT3 reactivity was moderate in 17 (65.3%) and low in 9 (34.6%). In both type of lesions, HGF and c-met showed a membranous and cytoplasmic location. In adenomas, STAT3 was detected in cytoplasm and nucleus and in carcinomas it was limited to cytoplasm. While the HGF/c-met/STAT3 expression in adenomas was significantly different from carcinomas ($\chi^2 = 17$, $p < 0.0001$), no correlation was found among HGF, c-met, or STAT3 immunostaining with histotype or degree of dysplasia in adenomas and the same for histotype, grading or staging in carcinomas. These features, suggesting a role of the HGF/c-met/STAT3 signal in colon tumorigenesis, indicate that a reduced expression of HGF and c-met is associated to progression of adenoma into carcinoma.

Key words: HGF, c-met, STAT3, colorectal, adenomas, carcinomas, immunohistochemistry.

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Hepatocyte growth factor/scatter factor (HGF/SF or HGF) is a pleiotropic cytokine secreted by mesenchymal cells in a single-chained immature form (Stoker et al., 1987). After cleavage by serine proteases and HGF/SF converting enzyme, the mature HGF is formed, a heterodimer containing a heavy α-chain, necessary for a correct binding to the receptor, and a light β-chain (Hartmann et al., 1992). HGF has been shown to promote healing of intestinal wounds by increasing epithelial cell proliferation and motility and by stimulating formation of tubular and glandular structures (Kitamura et al., 2000a; Dignass et al., 1994; Tsarfaty 1992). HGF expression has been detected in normal colonic mucosa, as well as in colorectal adenoma and carcinoma (Otte al., 2000; Kataoka et al., 2000; Wielenga et al., 2000). Additionally, a correlation between HGF serum and stage of the tumour has been found in patients with colorectal cancer (Fukuura et al., 1998).

The biological responses to HGF/SF are initiated by the interaction with a heterodimeric tyrosine-kinase receptor (HGF-R or c-met), encoded by the proto-oncogene c-met and subsequent dimerization and authophosphorylation of the receptor, which provides binding sites for molecules containing SH2 groups (Bottaro et al., 1991; Ponzetto et al., 1994). c-met is mainly expressed in normal epithelial cells of different organs such as liver, stomach and intestine, and over-expressed in a number of cancers, including thyroid and gastric carcinomas (Di Renzo et al., 1991; Trovato et al., 1998; Chowdhury et al., 1996). Along the colon adenoma-carcinoma sequence, c-met is over-expressed in both lesions without any apparent correlation (Di Renzo et al., 1995; Kitamura et al., 2000; Wielenga et al., 2000; Boon et al., 2003). The relations among c-met overexpression and the differentiation and stage of colon carcinoma remain to be unclear because contrasting results have been reported (Di Renzo et al., 1995; Fujita et al., 1997; Hiscox et

al., 1997; Umeki K et al., 1999; Fazekas K et al., 2000; Kitamura et al., 2000; Otte al., 2000; Wielenga et al., 2000, Takeuchi H et al., 2003).

The HGF/HGF-R interaction promotes scattering, growth and morphogenic responses in epithelial cells, each response being mediated by specific downstream transducing effectors (Naldini et al., 1991; Taijima et al., 1992; Bhargava et al., 1992). STAT3, a member of the signal transducers and activators of transcription (STAT), has been linked to the HGF-dependent morphogenic response (Boccaccio et al.,1998). STAT3 is a cytoplasmic protein whose monomeric form is transcriptionally inactive (Deker and Kovarik, 1999). Following interaction with c-met either directly, through the SH2 domain, or indirectly, through Gab1 bridges, STAT3 becomes phosphorylated and, as such, it is able to form homo- and heterodimers which translocate to the nucleus.

Once inside the nucleus in the active form, STAT3 induces the expression of target genes whose promoters are located in regions containing the functional regulatory sequences SIE (sis-inducible element) (Boccaccio et al., 1998). STAT3 is expressed in many malignancies such as papillary thyroid carcinoma and renal cell carcinoma (Trovato et al., 2003; Horiguchi et al., 2002). In colonic mucosa, STAT3 has been explored only in intestinal chronic inflammation where it has been demonstrated to be activated in ulcerative colitis and Crohn's disease (Suzuki et al., 2001).

In the present study, we have evaluated whether HGF, c-met and the morphogenetic effector STAT3 are expressed in colorectal adenomas and carcinomas comparing it to histopathologic findings.

Materials and Methods

Specimen collection

We examined tissue samples from 80 sporadic colorectal tumours: 40 adenomas and 40 adenocarcinomas. All adenomas were removed by endoscopy from 25 men and 15 women aged between 50 and 75 years (mean age: 64 years). They included 15 tubular adenomas (TA), 15 villous adenomas (VA) and 10 tubulo-villous adenomas (TVA). A low-grade dysplasia was observed in 7/15 TA, 8/15 VA and 5/10 TVA, and a high-grade dysplasia in 7/15 VA and 5/10 TVA. All carcinomas were resected by surgery from 22 men and 18 women aged between 40 and 81 years (mean age: 70 years). Following grade of differentiation was assessed: 12/40 well differentiated (GI), 19/40 moderately differentiated (GII) and 9/40 poorly differentiated (GIII). In addition, 5/40 showed a mucinous differentiation. According to the modified Dukes' classification system (Astler and Coller, 1954), tumor stage was A in 11/40 carcinomas, B in 22/40 and C in 7/40. All specimens were 4% formalin fixed and paraffin embedded. 4-mm sections from each specimen were stained with Hematoxylin-Eosin before performing immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed on 4-mm sequential tissue sections from paraffin embedded blocks using polyclonal rabbit antibodies against HGF-a (H-145, 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), c-met (p140 anti h-met, 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and STAT3 (h-190, 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Tissue sections were deparaffinized with xylene, rehydrated through a graded series of ethanol solutions to distilled water and treated with 0.3% (v/v) H2O2 in absolute methanol for 30 min to quench the endogenous peroxidase activity. 3,3'-diaminobenzidine (DAB, Sigma) activated with 0.05% hydrogen peroxide was used to visualize the end products. Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted.

Specificity of the binding was assessed by three kinds of controls: (i) omitting the primary antiserum, (ii) replacing it with normal rabbit serum or (iii) previously absorbing it with its homologous antigen. For each protein we evaluated the number of positive cases, the number of reactive cells per case and the cellular location. The second parameter was determined from a population of 1000 cells/case, using 50 X magnification. Based on the proportion of tumour cells that were stained, we scored expressions as low (1-10% positive cells), moderate (11-50%) and high (> 51%).

Histological and immunocytochemical evaluations were performed twice and blindly by three different pathologists (MT, MG and GB), with an inter-observer agreement of 100%.

Difference between proportions were analyzed by χ^2 setting the level of statistical significance at $p < 0.05$.

Table 1. Expression of HGF, c-met and STAT3 in colorectal adenomas and carcinomas. Tumors are stratified based on intensity of staining.

	HGF				c-met				STAT 3			
	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High
Adenomas	40/40	0	7	33	40/40	0	37	3	40/40	0	15	25
Carcinomas	26/40	5	21	0	40/40	1	25	14	26/40	9	17	0

Table 2. Expression of HGF, c-met and STAT3 in adenomas which are categorized based on histotype and grade of dysplasia.

	HGF				c-met				STAT3			
	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High
Histotype												
Tubular (n = 15)	15/15	0	3	12	15/15	0	13	2	15/15	0	6	9
Villous (n = 15)	15/15	0	2	13	15/15	0	14	1	15/15	0	5	10
Tubulovillous (n = 10)	10/10	0	2	8	10/10	0	10	0	10/10	0	4	6
Grade of dysplasia												
No-Dysplasia (n = 8)	8/8	0	1	7	8/8	0	6	2	8/8	0	2	6
Low (n = 20)	20/20	0	5	15	20/20	0	19	1	20/20	0	9	11
High (n = 12)	12/12	0	1	11	12/12	0	12	0	12/12	0	4	8

Table 3. Expression of HGF, c-met and STAT3 in carcinomas which are categorized based on histotype, differentiation and staging.

	HGF				c-met				STAT3			
	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High	Positive Cases	Staining score Low	Moderate	High
Adeno (n= 35)	22/35	4	18	0	35/35	1	23	11	22/35	7	15	0
Mucinous (n= 5)	4/5	1	3	0	5/5	0	2	3	4/5	2	2	0
GI (n = 19)	12/19	3	9	0	19/19	0	12	7	12/19	5	7	0
GII (n = 12)	7/12	1	6	0	12/12	1	6	5	7/12	1	6	0
GIII (n = 9)	7/9	1	6	0	9/9	0	7	2	7/9	3	4	0
A (n = 11)	8/11	2	6	0	11/11	0	8	3	8/11	2	6	0
B (n = 22)	13/22	2	11	0	22/22	0	14	8	13/22	4	9	0
C (n = 7)	5/7	1	4	0	7/7	1	3	3	5/7	3	2	0

Results

Data are summarized in Tables 1-3 and, representatively, in Figures 1 and 2.

Adenomas

As shown in Table 1, all 40 adenomas stained positively for HGF, c-met and STAT3 (HGF+/c-met+/STAT3+). In most of the cases, HGF expression was high (33/40 or 82.5%), c-met expression moderate (37/40 or 92.5%) and STAT3 expression high (25/40 or 62.5%). HGF reaction was confined to plasmamembrane and cytoplasm (Figure 1A) the same two locations as c-met (Figure 1B). STAT3 was detected in cytoplasm and nucleus cells (Figure1C).

HGF, c-met and STAT3 expressions were independent of tumor histotype ($\chi^2 = 0.29$, $p = 0.866$; $\chi^2 = 1.56$, $p = 0.458$ and $\chi^2 = 0.178$, $p = 0.915$, respectively) or degree of dysplasia ($\chi^2 = 1.62$, $p = 0.446$; $\chi^2 = 4.69$, $p = 0.096$ and $\chi^2 = 1.10$, $p = 0.576$, respectively) (Table 2).

Carcinomas

All 40 carcinomas expressed c-met, while 14 failed to express both HGF and STAT3, thus, only 26/40 (65%) were HGF+/c-met+/STAT3+, a proportion that was statistically different from the

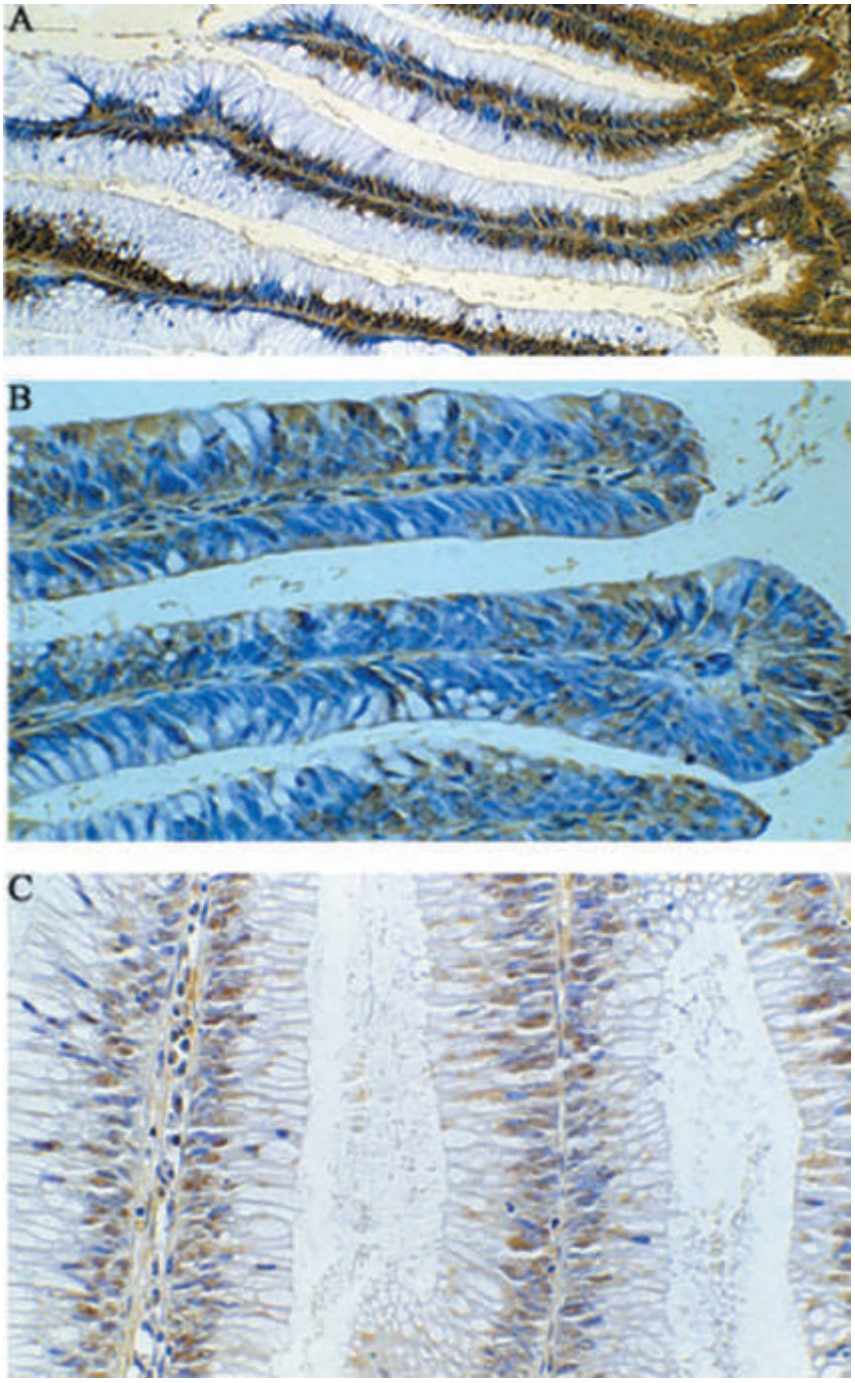

Figure 1. [original magnification 40×] A. HGF-α positive immunoreaction in villous adenoma. The intense stain is located on membrane and cytoplasm of adenomatous cells. B. c-met positive immunoreaction in villous rectal adenoma. The intense stain is detected on membrane and cytoplasm of adenomatous cells. C. STAT3 immunostaining in villous colorectal adenoma. The intense stain is encountered on cytoplasm and nucleus of adenomatous cells.

100% observed for adenomas (χ^2 = 17, p < 0.0001) (Table I). In most of the carcinomas, the expression of HGF, c-met and STAT3 was moderate: 21/26 (80.7%), 25/40 (62.5%) and 17/26 (65.3%), respectively (Table 1).

HGF and c-met immunostaining was located in the plasma-membrane and cytoplasm of tumor cells (Figures 2A and 2B) and STAT3 immunolocalization was cytoplasmic (Figure 2C).

HGF, c-met and STAT3 expressions were independent of histotype (χ^2 = 0.10, p = 0.750; c2 = 1.63, p = 0.443 and χ^2 = 0.49, p = 0.482, respectively), grade of differentiation (χ^2 = 0.48, p = 0.788; χ^2 = 3.56, p = 0.469 and χ^2 = 1.75, p = 0.416, respectively) and stage (χ^2 = 0.29, p = 0.862; χ^2 = 5.66, p = 0.226 and χ^2 = 1.83, p = 0.399, respectively) of the tumor.

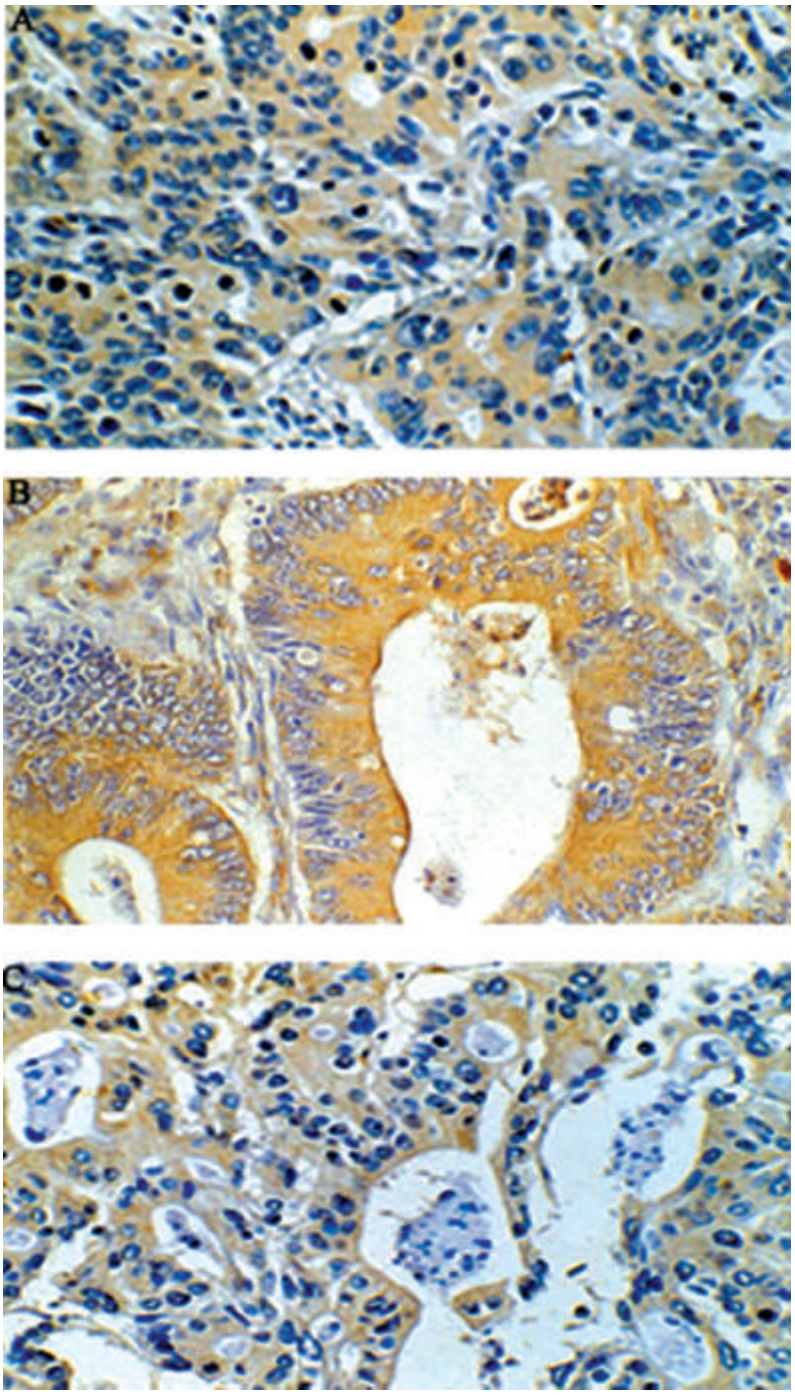

Figure 2. [original magnification 40×] A. HGF-α positive immunoreaction in rectal adenocarcinoma. The intense stain is located on membrane and cytoplasm of neoplastic cells. B. c-met positive immunoreaction in rectal adenocarcinoma. The intense stain is detected on membrane and cytoplasm of neoplastic cells. C. STAT3 immunostaining in colorectal adenocarcinoma. The intense stain is encountered on cytoplasm of neoplastic cells.

Discussion

Arising from single crypt lesions, the colorectal cancer evolves through a series of stepwise development showing the morphologic features of the adenoma-carcinoma sequence (Vogelstein et al., 1988). Growth factors may control growth and progression of tumours by binding to cell receptors and activating specific intracellular signaling pathways (Heldin and Westermark, 1984).

Here we reported a different expression of the HGF/c-met/STAT3 signal in adenomas vs carcinomas, in fact 100% of adenomas vs 65% of carcinomas showed reaction for HGF, c-met and STAT3.

Co-expression of HGF and its receptor c-met has been previously studied by three independent groups of investigators in colorectal carcinomas but not in adenomas (Hiscox et al., 1997; Otte et al., 2000, Wielenga et al., 2000). Hiscox et al. did not detect HGF in 16 c-met+ carcinomas (Hiscox

et al., 1997). On the contrary, Wielenga et al. reported HGF expression in 5/5 (100%) neoplastic colon tissues showing c-met+ (Wielenga et al., 2000). Finally, Otte et al. found HGF+ in 37 out 42 (90.4%) c-met+ carcinomas (Otte et al., 2000). These controversial results may be related either to the different number of tumour samples evaluated and/or to the different technical approach.

In our study, HGF and c-met were co-expressed in 40/40 of the adenomas but only in 26/40 of the c-met+ carcinomas suggesting that HGF and c-met have a role in early events of the natural history of colorectal tumours while the absence of the ligand HGF could be related to the malignant transformation similarly to elsewhere reported in the neoplastic progression of thyroid follicular adenomas into follicular carcinomas (Trovato et al., 1998). We have also evaluated one of the down-stream signaling activated by HGF binding to c-met through the expression of STAT3 phosphorylated. STAT3 reactivity has been exclusively observed in HGF+ adenomas and carcinomas while in 35% of carcinomas that were unstained for HGF, but expressing c-met, STAT3 was undetected. These data let us suppose that HGF carries out a main role in the activation of the STAT3 signaling and indicate that in colorectal adenoma-carcinoma sequence the HGF/c-met/STAT3 signaling is significantly more expressed in adenomas. The absence of HGF immunostaining may be related to possible losses of genetic material in HGF locus like HGF unstained follicular and anaplastic thyroid carcinomas that show loss of heterozygosity for the markers boarding 7q21 trait containing HGF gene (Trovato et al., 1999). Indeed, the long arm of chromosome 7 contains several candidate loci for tumor suppressor genes that are lost in colorectal carcinomas, even if, the HGF region has never been screened in colon cancer (Zenklusen et al., 1995). The absence of STAT3 reaction in HGF unreactive carcinomas may be explained with the lack of evidence of HGF expression.

In this study, we reported a high expression of HGF and STAT3 in most adenomas contrasting with a moderate HGF and STAT3 reactivity in most carcinomas. According to other reports (Di Renzo et al., 1995; Kitamura et al., 2000; Wielenga et al., 2000; Boon et al., 2003), we haven't found any difference of the c-met expression between adenoma and carcinoma because it was moderate in both lesions. We haven't observed any significant correlation among the expressions of HGF, c-met and STAT3 with histotype or grade of dysplasia of adenomas as well as with histotype, grade of differentiation or stage of carcinomas.

The HGF as well as c-met reaction was located on plasmamembrane and cytoplasm of epithelial cells in both rectal adenomas and carcinomas. These data, in apparent contrast with previous reports of HGF in stromal cells of colorectal carcinomas (Otte et al., 2000; Wielenga et al., 2000), could be related to the use in our study of a specific antibody against the a-chain of HGF. Without excluding a paracrine effect of HGF on cellular growth in colorectal tumours, our findings suggest that HGF dimerization may occur near the target cells expressing the cognate receptor c-met. Finally, the cytoplasmic and nuclear localization of STAT3 in adenomas, while in carcinomas it was only cytoplasmic, indicating that the nuclear translocation of transcription factor happens only in the former, also suggests that in carcinomas the HGF/c-met/STAT3 signaling is incomplete.

References

Astler VB, Coller FA. The prognostic significance of direct extension of carcinomas of the colon and rectum. Ann Surg 1954;139:846-51.

Bhargava M, Joseph A, Knesel J, Halaban R, Li Y, Pang S, et al. Scatter factor and hepatocyte growth factor: activities, properties, and mechanism. Cell Growth Differ 1992;3:11-20.

Boccaccio C, Ando M, Tamagnone L, Bardelli A, Michieli P, Battistini C, et al. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. Nature 1998;391:285-8.

Boon EM, van der Neut R, van de Wetering M, Clevers H, Pals ST. Wnt signaling regulates expression of the receptor tyrosine kinase met in colorectal cancer. Cancer Res 2003;62:5126-8.

Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science 1991;251: 802-4.

Chowdhury A, Fukuda R, Fukumoto S. Growth factor mRNA expression in normal colorectal mucosa and in uninvolved mucosa from ulcerative colitis patients. J Gastroenterol 1996;31:353-60.

Deker T, Kovarik P. Transcription factor activity of STAT proteins: structural requirements and regulation by phosphorylation and interacting proteins. Cell Mol Life Sci 1999;55:1535-46.

Di Renzo MF, Narsimhan RP, Olivero M, Bretti S, Giordano S, Medico E et al. Expression of the met/HGF receptor in normal and neoplastic human tissues. Oncogene 1991;6:1997-2003.

Di Renzo MF, Olivero M, Giacomini A, Porte H, Chastre E, Mirossay L, et al. Overexpression and amplification of the met/HGF receptor gene during the progression of colorectal cancer. Clin Cancer Res 1995;1:147-54.

Dignass AU, Lynch-Devaney K, Podolsky K. Hepatocyte growth factor/scatter factor modulates intestinal epithelial cell proliferation and migration. Biochem Biophys Res Com 1994;202:701-9.

Fazekas K, Csuka O, Koves I, Raso E, Timar J. Experimental and clinicopathologic studies on the function of the HGF receptor in human colon cancer metastasis. Clin Exp metastasis 2000;18:639-49.

Fujita S, Sugano K. Expression of c-met proto-oncogene in primary colorectal cancer and liver metastases. Jpn J Clin Oncol 1997; 27:378-83.

Fukuura T, Miki C, Inoue T, Matsumoto K, Suzuki H. Serum hepatocyte growth factor as an index of disease status of patients with colorectal carcinoma. Br J Cancer 1998;78:454-9.

Hartmann G, Naldini L, Weidner KM, Sachs M, Vigna E, Comoglio PM, et al. A functional domain in the heavy-chain of scatter factor hepatocyte growth-factor binds the c-met receptor induces cell-dissociation but not mitogenesis. Proc Natl Acad Sci USA 1992; 89:11574-8.

Heldin CH, Westermark B. Growth factors: mechanism of action and relation to oncogenes. Cell 1984;37:9-20.

Hiscox SE, Hallett MB, Puntis MC, Nakamura T, Jiang WG. Expression of the HGF/SF receptor, c-met, and its ligand in human colorectal cancers. Cancer Invest 1997;15:513-21.

Kataoka H, Hamasuna R, Itoh H, Kitamura N, Koono M. Activation of Hepatocyte growth factor/scatter factor in colorectal carcinoma. Cancer Res 2000;60:6148-59.

Kitamura S, Kondo S, Shinomura Y, Isozaki K, Kanayama S, Higashimoto Y, et al. Expression of hepatocyte growth factor and c-met in ulcerative colitis. Inflamm Res 2000;49:320-4a.

Kitamura S, Kondo S, Shinomura Y, Kanayama S, Miyazaki Y, Kiyohara T, et al. Met/HGF receptor modulates bcl-w expression and inhibits apoptosis in human colorectal cancers. Br J Cancer 2000; 85:668-73 b.

Horiguchi A, Oya M, Shimada T, Uchida A, Marumo K, Murai M. Activation of signal transducer and activator of transcription 3 in renal cell carcinoma: a study of incidence and its association with pathological features and clinical outcome. J Urol 2002;168:762-5.

Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, et al. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. EMBO J 1991;10:2867-78.

Otte JM, Schmitz F, Kiehne K, Stechele HU, Banasiewicz T, Krokowicz P, et al. Functional expression of HGF and its receptor in human colorectal cancer. Digestion 2000;6:237-46.

Ponzetto C, Bardelli A, Zhen Z, Maina F, dalla Zonca P, Giordano S, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. Cell 1994;77:261-71.

Stoker M, Gherardi E, Perryman M, Gray J. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. Nature 1987;327:239-42.

Suzuki A, Hanada T, Mitsuyama K, Yoshida T, Kamizono S, Hoshino T, et al. CIS3/SOCS3/SSI3 play a negative regulatory role in STAT3 activation and intestinal inflammation. J Exp Med 2001;193 471-81.

Taijima H, Matsumoto K, Nakamura T. Regulation of cell growth and motility by hepatocyte growth factor and receptor expression in various cell species. Exp Cell Res 1992;202:423-31.

Takeuchi H, Bilchik A, Saha S, Turner R, Wiese D, Tanaka M, et al. c-MET expression level in primary colon cancer: a predictor of tumor invasion and lymph node metastases. Clin Cancer Res 2003; 9:1480-8.

Trovato M, Villari D, Bartolone L, Spinella S, Simone A, Violi MA, et al. Expression of the hepatocyte growth factor and c-met in normal thyroid, non-neoplastic, and neoplastic nodules. Thyroid 1998; 8:125-31.

Trovato M, Fraggetta F, Villari D, Batolo D, Mackey K, Trimarchi F, et al. Loss of heterozygosity of the long arm of chromosome 7 in follicular and anaplastic thyroid cancer, but not in papillary thyroid cancer. J Clin Endocrinol Metab 1999;84:3235-40.

Trovato M, Grosso M, Vitarelli E, Ruggeri RM, Alesci S, Trimarchi F, et al. Distinctive expression of STAT3 in papillary thyroid carcinomas and a subset of follicular adenomas. Histol Histopathol 2003;18:393-9.

Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL, Vande Woude GF. The met protooncogene receptor and lumen formation. Science 1992; 257: 1258-1261.

Umeki K, Shiota G, Kawasaki H. Clinical significance of c-met oncogene alterations in human colorectal cancer. Oncology 1999; 56:314-21.

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development N Engl J Med 1988;319:525-32.

Wielenga VJ, van der Voort, Taher TE, Smit L, Beuling EA, van Krimpen C, et al. Expression of c-met and heparan-sulfate proteoglycan form of CD44 in colorectal cancer. Am J Pathol 2000; 157:1563-73.

Zenklusen JC, Thompson JC, Klein-Szanto AJP, Conti CJ. Frequent loss of heterozigosity in human primary squamous cell and colon carcinomas at 7q31.1: evidence for a broad range tumor supppressor gene. Cancer Res 1995;1347-50.