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Original Article

Expression of MACC1, HGF and Met gene in the prognosis of colorectal cancer patients with liver metastasis

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Abstract: Colorectal cancer patients are predisposed for liver metastasis after surgery. Metastasis associated factor 1 of colorectal carcinoma (MACC1), hepatocellular growth factor (HGF) and Met signal pathway are all correlated with occurrence of colorectal carcinoma. This study thus examined expressions of MACC1, HGF and Met expression, to analyze their correlation with metastasis and prognosis. A total of 50 patients diagnosed with liver metastatic colorectal carcinoma in our hospital were recruited. 30 colorectal carcinoma patients without metastasis plus 30 benign tumor patients were employed as control. ELISA was employed to measure serum MACC1, HGF and Met contents, while immunohistochemistry (IHC) staining was employed to detect expression of MACC1, HGF and Met, whose correlation with clinical features was analyzed. Metastatic group had significantly elevated MACC1, HGF and Met expression than un-metastatic group ($P<0.05$), which had higher levels than benign tumors ($P<0.05$). MACC1, HGF and Met levels were correlated with differentiation grade, number of primary lesion, size, number of liver metastatic lesions, distal metastasis, radio-/chemo-therapy and survival time ($P<0.05$). Colorectal cancer patients with liver metastasis had high expression of MACC1, HGF and Met, which are related with differentiation grade, number of primary lesions, size, liver metastatic lesion number, distal metastasis, chemo-/radio-therapy and survival times.

Keywords: Colorectal carcinoma, liver metastasis, MACC1, HGF, met

Introduction

Colorectal carcinoma is one of the most common malignant tumors worldwide, with significantly elevated incidence in China recently [1]. Colorectal carcinoma is frequently accompanied with focal lymph node metastasis, in addition to distal organ metastasis. Liver is the most common targeted organ for distal metastasis, as about 10%~25% patients already have liver metastasis at the time of diagnosis, and about 30%~50% patients had liver metastasis during disease progression [2, 3]. It is estimated that about 500 thousand colorectal cancer patients develop liver metastasis worldwide. Once having liver metastasis, unfavorable prognosis is normally occurred. A continuous dynamic process involving multiple factors and steps facilitates the dispersion of tumor cells from primary lesion to distal organs, including tumor cell adhesion, invasion, remodeling or extracellular matrix, angiogenesis, lymph tube formation and body immune response [4, 5]. Metastasis associated factor 1 of colorectal carcinoma (MACC1) is one novel gene identified by Stein et al. to be related with colorectal cancer metastasis and invasion by whole genome scan. MACC1 can be expressed in various tumor tissues including colorectal cancer, pancreatic cancer, gastric carcinoma and liver cancer, especially higher in colorectal carcinoma [6]. As one oncogene, Met has the ligand of hepatocyte growth factor (HGF). Previous study showed high expression of Met in some human tissues, and its correlation with tumor pathogenesis, progression and metastasis [7]. Met activation facilitated cell migration, potentiating separation of epithelial cells, migration of

endothelial cells and chemotaxis. After binding with HGF, Met participates in cancer cell motility. In nude mice with colorectal cancer, MACC1 can facilitate proliferation and invasion of colorectal cancer cells, thus accelerating Met-induced tumor cell metastasis and dispersion [8]. Holgren *et al.* found that elevated Met gene could significantly increase proliferation and invasion potency of Met-highly expressed tumor cells [9]. In this study, we recruited colorectal carcinoma patients with liver metastasis as research subjects, whose expression of MACC1, HGF and Met was measured to analyze the relationship between these factors and prognosis of colorectal carcinoma with liver metastasis.

Materials and methods

General information

A total of 60 colorectal carcinoma patients (30 rectal cancer and 30 colon cancer) with liver metastasis were recruited from the surgery of China-Japan Union Hospital of Jilin University from January 2015 to January 2016. There were 30 males and 30 females, aging between 30 and 70 years old (average age = 52.5±4.8 years old). Another cohort of 30 colorectal carcinoma patients without metastasis (16colon cancer, 14 rectal cancer) who received surgery at the same time were recruited, including 15 males and 15 females (aging between 35 and 70 years old, average age = 51.3±4.5 years old). Another cohort consisted of 30 samples collected from benign tumors in colon or rectal tissues. There were 15 males and 15 females in this group, with aging between 35 and 70 years old (average age = 52.6±4.2 years old).

This study has been pre-approved by the ethical committee of China-Japan Union Hospital of Jilin University. All subjects have signed the consent forms before recruitment in this study.

Inclusive criteria: All patients received surgery and diagnosed by pathology. Written consents have been obtained from all participants for completing the whole research.

Exclusive criteria: No complication with mesenchymal disease or immune disorder; No dysfunction of major organs such as heart, liver or kidney; No primary cancer in other organs or inflammation.

Reagents and equipment

ELISA kit for MACC1, HEG, and Met; Blocking reagent and primary antibody for MACC1, HGF and Met; Rabbit anti-mouse IHC secondary antibody were purchased from Sangon (China). DAB kit and 0.01% citric acid (Shanfeng Chem, China). Centrifuge (Feige, China); Microplate reader (TECNA, UK); Embedding apparatus (SAKURA, Japan); Dehydration facility (TIYODA, Japan); Microtome (Leica, Germany).

ELISA for serum MACC1, HGF and Met contents in patients

Fasted venous blood samples were collected and centrifuged to save the supernatant for storage in fridge. ELISA was used to quantify blood levels of MACC1, HGF and Met. The test kit was placed at room temperature for 30 min. Standard samples were diluted. At each concentration, 5 replicated wells were applied for adding samples, reaction buffer, washing buffer, developing reagent, and quenching solution. Absorbance values at 450 wave length were measured to plot linear regression function for calculating sample concentration.

IHC staining for tissue expression of MACC1, HGF and Met

Tissues were fixed in formalin, dehydrated, immersed in paraffin, embedded, and sectioned. Tissue slices were mounted on glass slides and dried overnight. Heated antigen retrieval was performed, followed by H_2O_2 quenching and normal goat serum blocking. 50 μl primary antibody was applied for 1 h room temperature incubation. 50 μl secondary antibody with horseradish peroxidase (HRP) was applied for 10 min room temperature incubation. Streptomycin-peroxidase was then added for 10 min incubation. DAB substrate was added for development. After quenching, hematoxylin was used for counter-staining, followed by HCl-ethanol differentiation. Following routine dehydration, the coverslip was mounted for observation under computer-assisted system. 5 fields were randomly selected from each slice for recroding.

Judgment criteria

Positive results for MACC1, HGF and Met were judged as no staining in nucleus, and brown or

Table 1. Serum MACC1, HGF and Met levels by ELISA (ng/ml)

Group	N	MACC1	HGF	Met
Colorectal carcinoma with metastasis	60	1.34±0.15*,#	1.28±0.13*,#	1.18±0.12*,#
Colorectal carcinoma without metastasis	30	0.67±0.05#	0.55±0.04#	0.41±0.03#
Benign tumor in colon-rectum	30	0.03±0.02	0.03±0.01	0.02±0.02

Note: *P<0.05 compared to colorectal carcinoma patients without metastasis; #P<0.05 compared to benign tumors in colon-rectum.

Table 2. IHC staining for tissue expression of MACC1, HGF and Met

Group	N	MACC1				HGF				Met			
		-	+-++	+++	Positive rate (%)	-	+-++	+++	Positive rate (%)	-	+-++	+++	Positive rate (%)
Colorectal carcinoma with metastasis	60	15	35	10	75*,#	18	31	11	70*,#	21	30	9	65*,#
Colorectal carcinoma without metastasis	30	18	10	2	40#	24	5	1	20	21	8	1	30#
Benign tumor in colon-rectum	30	27	3	0	10	27	3	0	10	27	3	0	10

Note: *P<0.05 compared to colorectal carcinoma patients without metastasis; #P<0.05 compared to benign tumors in colon-rectum.

brown-yellow granules on membrane or cytoplasm. Negative (-): less than 10% of positive cells; Weak positive (+): 11%~25% positive cells; Positive (++): 26%~50% positive cells; Strong positive (+++): more than 50% of positive cells. Overall positive was deduced to include weak positive, positive and strong positive results.

Statistical analysis

SPSS17.0 software was used for data processing. All data were presented as mean ± standard deviation (SD). Enumeration data were tested by chi-square test while measurement data were compared by analysis of variance (ANOVA). Multi-factor analysis was performed by Logistic regression model. A statistical significance was identified when P<0.05.

Results

Serum MACC1, HGF and Met contents in patients

Peripheral venous blood samples were collected to test serum MACC1, HGF and Met levels. Results showed significantly elevated MACC1, HGF and Met in colorectal carcinoma patients with liver metastasis (P<0.05 compared to unmetastatic group or benign tissues). Those colorectal carcinoma patients without metastasis had higher serum MACC1, HGF and Met levels than those of benign tumors (P<0.05, **Table 1**).

IHC staining for tissue expression of MACC1, HGF and Met

We further compared tissue expression of MACC1, HGF and Met levels. Results showed 10 cases of strong positive expression for MACC1, and 35 cases of positive expression, with overall positive rate at 75% in colorectal carcinoma patients with liver metastasis. HGF has 11 strong positive cases and 31 positive cases, making total positive rate as high as 70%. Met had 9 cases of strong positive expression and 30 positive cases (positive rate = 65%). All these rates were elevated compared to no metastasis or benign tumor patients (P<0.05). Those patients without metastasis had higher positive rates of MACC1, HGF and Met compared to benign tumor patients (P< 0.05, **Table 2**; **Figure 1**).

Correlation between MACC1, HGF and Met levels and clinical features of colorectal carcinoma patients with liver metastasis

We further analyzed the correlation between MACC1, HGF and Met levels and indexes including age, sex, tumor site, differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time, in colorectal carcinoma patients with liver metastasis. Results showed that MACC1, HGF and Met levels in colorectal carcinoma patients were correlated with differentiation grade, pri-

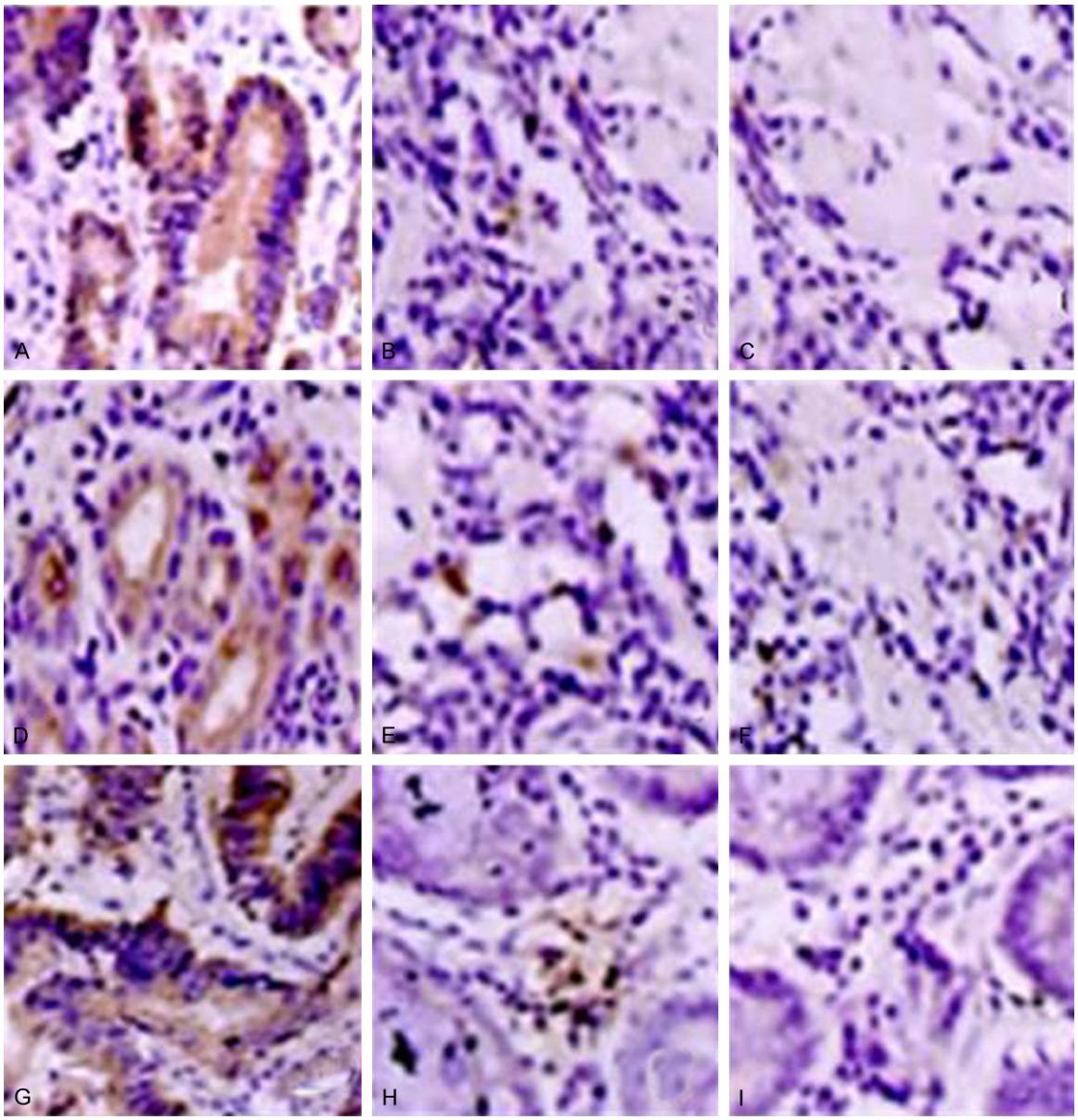

Figure 1. MACC1, HGF and Met expressions in patient tissues. A-C. MACC1; D-F. HGF; G-I. Met expression. A, D and G. Colorectal carcinoma with liver metastasis; B, E and H. Colorectal carcinoma without liver metastasis; C, F and I. Benign tumors in colon-rectum.

mary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time ($P< 0.05$) but not age, sex or tumor site ($P>0.05$). For those patients with lower differentiation grade, multiple primary tumors, larger than 3 cm primary tumor, multiple liver metastatic lesions, metastasis in other organs, no chemo-/radio therapy and survival time less than 6 months, MACC1, HGF and Met level were more significantly elevated (**Table 3**).

Multi-variant analysis between expressions of MACC1, HGF and Met and clinical features

Logistic multi-variant analysis was further performed to assess the correlation between MACC1, HGF and MET levels and clinical indices including differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time. Results indicated independent risk factors including

Table 3. Correlation between MACC1, HGF and Met levels and clinical indexes

Index	N	MACC1	HGF	Met
Age				
<45	29	21 (72.4)	20 (68.9)	18 (62.1)
≥45	31	24 (77.4)	22 (70.9)	21 (67.7)
P		>0.05	>0.05	>0.05
Sex				
M	30	22 (73.3)	21 (70)	20 (66.7)
F	30	23 (73.3)	21 (70)	19 (63.3)
P		>0.05	>0.05	>0.05
Tumor site				
Colon	28	20 (71.4)	19 (67.9)	19 (67.9)
Rectum	32	25 (78.1)	23 (71.8)	20 (62.5)
P		>0.05	>0.05	>0.05
Differentiation				
High	15	9 (60)	8 (53.3)	7 (46.7)
Moderate	20	13 (65)	11 (55)	10 (50)
Low	25	23 (92)	23(92)	22 (88)
P		<0.05 (0.03)	<0.01 (0.0071)	<0.01 (0.0067)
Primary tumor				
Single	25	16 (64)	14 (56)	11 (44)
Multiple	35	29 (82.9)	28 (80)	28 (80)
P		<0.05	<0.05	<0.05
Primary tumor size				
≤3 cm	24	14 (58.3)	14 (58.3)	11 (45.8)
>3 cm	36	31 (86.1)	28 (80)	28 (80)
P		<0.05	<0.05	<0.05
Liver metastatic lesion				
Single	28	18 (64.3)	12 (42.9)	13 (46.4)
Multiple	32	27 (84.3)	30 (93.7)	26 (81.3)
P		<0.05	<0.05	<0.05
Distal metastasis of other organs				
No	27	18 (66.7)	14 (51.8)	13 (48.1)
Yes	33	27 (81.8)	28 (84.8)	26 (78.7)
P		<0.05	<0.05	<0.05
Radio-therapy				
No	26	15 (57.7)	12 (46.2)	10 (38.5)
Yes	34	27 (79.4)	30 (88.2)	29 (85.3)
P		<0.05	<0.05	<0.05
Chemo-therapy				
No	31	15 (48.3)	16 (51.6)	15 (48.3)
Yes	29	27 (93.1)	26 (89.6)	24 (82.7)
P		<0.05	<0.05	<0.05
Survival time				
≥6 months	24	14 (58.3)	14 (58.3)	11 (45.8)
<6 months	36	31 (86.1)	28 (80)	28 (80)
P		<0.05	<0.05	<0.05

differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time (**Table 4**).

Table 4. Multi-variant analysis between expressions of MACC1, HGF and Met and clinical features

Indexes	MACC1			HGF			Met		
	Regression coefficient	*P* value	Relative risk factor	Regression coefficient	*P* value	Relative risk factor	Regression coefficient	*P* value	Relative risk factor
Differentiation grade	1.007	0.002	2.401	1.102	0.001	2.136	1.002	0.001	2.235
Primary tumor number	1.275	0.001	2.852	1.233	0.002	2.325	1.132	0.002	2.027
Size of primary lesion	1.018	0.001	2.812	1.124	0.002	2.623	1.007	0.001	2.004
Number of liver metastasis	1.004	0.001	2.730	1.015	0.001	2.457	1.113	0.002	2.892
Distal metastasis of other organs	0.952	0.003	2.376	0.878	0.002	2.054	0.748	0.002	2.156
Radiotherapy	1.008	0.002	2.502	1.104	0.001	2.273	1.005	0.001	2.247
Chemotherapy	1.203	0.001	2.104	1.116	0.002	2.211	1.153	0.002	2.034
Survival time	1.271	0.001	2.252	1. 323	0.003	2.124	1.032	0.002	2.021

Discussion

With improvement of economic condition and transition of life styles, the incidence and mortality of colorectal carcinoma are rapidly increasing worldwide. Major reasons for death include focal recurrence and distal metastasis, with predisposed metastasis in liver tissues [10]. MACC1 is one newly discovered gene associated with colorectal carcinoma metastasis [11]. Previous studies showed the close correlation between HEG/c-Met signal pathway with malignant transformation of normal tissues, motility and movement, invasion of malignant cells, epithelial-mesenchymal transition, angiogenesis, healing of wounds and tissue regeneration and repair [12]. This study thus recruited colorectal carcinoma patients with liver metastasis in our hospital, to test expressional profiles of MACC1, HGF and Met, in an attempt to analyze their correlation with patient's prognosis.

In this study, we found higher serum MACC1, HGF and Met levels in disease group compared to control group. Further tests revealed higher MACC1, HGF and Met positive rates in cancer tissues compared to those in adjacent tissues or control group. These results showed highly expressed MACC1, HGF and Met in both serum and tissues of colorectal carcinoma patients with liver metastasis. Previous study showed lower expression level of MACC1 in primary lesion of colorectal carcinoma, and higher level in metastatic lesions [13]. Stein *et al.* found significantly elevated MACC1 gene expression level in colorectal carcinoma metastatic lesion, with significant intra-nuclear translocation [14]. As one oncogene, Met is expressed on the surface of epithelial cells, and codes for HGF receptor having tyrosine kinase activity [15, 16]. Animal study showed the expression of Met in colorectal cancer might work as the marker for liver metastasis, plus its close correlation with tumor metastasis signal [17]. In transgenic mouse tissue cells, over-expression of HGF and Met significantly elevated occurrence of pulmonary metastasis [18], as consistent with our results.

To further illustrate the relationship between MACC1, HGF and Met expression and prognosis of colorectal carcinoma patients with liver metastasis, this study found the relationship between MACC1, HGF and Met expression in colorectal carcinoma patients with liver metastasis and differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time. For those patients with lower differentiation grade, multiple primary tumors, larger than 3cm primary tumor, multiple liver metastatic lesions, metastasis in other organs, no chemo-/radio therapy and survival time less than 6 months, expressions of MACC1, HGF and Met were more significantly. Multi-variant analysis showed independent risk factors of differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time. Previous study showed highly correlated relationship between MACC1 and prognosis of patients. Such expression levels are negatively correlated with total survival time, disease-free survival time and progression-free survival time of patients [19]. Takeuchi *et al.* found differential expressions of Met across colorectal cancer tissues with different size and existence or absence of metastasis [20], indicating that c-Met is important molecular marker for infiltration depth into tumors and prediction of tumor

metastasis. HGF is one known cytokine with strong effects for facilitating hepatocyte proliferation. After liver tissue resection, a regeneration status is initiated, followed by rapidly elevated HGF and reaching the peak one week after surgery. Such changes of microenvironment of liver benefit mitosis and proliferation of hepatocytes, and provide suitable micro-environment for proliferation and dispersion of micro-metastatic lesions [21-23]. For those liver cancer patients with higher expression of growth factor receptor Met, more rapid proliferation of tumor cells occurs, causing early recurrence and/or metastasis of tumors in clinics.

Conclusion

Colorectal carcinoma patients with liver metastasis had high expression of MACC1, HGF and Met in serum and tissues. These expression levels are correlated with differentiation grade, primary tumor number, size, number of liver metastatic lesion, distal metastasis of other organs, radio-/chemo-therapy and survival time. For those patients with lower differentiation grade, multiple primary tumors, larger than 3 cm primary tumor, multiple liver metastatic lesions, metastasis in other organs, no chemo-/radio therapy and survival time less than 6 months, MACC1, HGF and Met level were more significantly elevated. Further studies are required for illustrating the regulatory relationship among MACC1, HGF and Met, in addition to signal transduction pathway, in order to provide evidences for clinical treatment.

Disclosure of conflict of interest

None.

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