Original Article
Expression of MACC1-1 and c-Met in human prostatic cancer and their clinicopathological significance

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Abstract: Objective: Metastasis-associated in colon cancer-1 (MACC-1) and c-Met are associated with tumorigenesis and progression. The aim of this study is to investigate the expression of MACC-1 and c-Met in human prostatic cancer (PCa) and explore their clinical and pathological significance. Methods: The expression of MACC-1 and c-Met protein were detected in 84 cases of human PCa and 20 cases of benign prostatic hyperplasia (BPH) tissues by the immunohistochemical method. Results: The positive expression level of MACC-1 was 56.0% in human PCa which was higher than that in BPH tissues (20.0%), P=0.004. The overexpression of MACC1 protein was correlated with tumor Gleason score, PSA and TNM stage (P=0.001, P=0.027 and P<0.001, respectively). The positive expression of c-Met was 64.3% in PCa, which was higher than that in BPH tissues (30%). P=0.005. The overexpression of c-Met protein was correlated with tumor Gleason score, PSA and TNM stage (P=0.020, P=0.037 and P<0.001, respectively). The log-rank test statistical analysis suggested that patients with overexpression MACC-1 or c-Met protein had shorter overall survival time, while patients with lower expression MACC-1 or c-Met protein had better survival. Conclusion: Overexpression of MACC-1 and c-Met are markedly correlated with tumor Gleason score, PSA and TNM stage. Detection of MACC-1 and c-Met may be helpful to evaluate prognosis and infiltrative capability of PCa. In PCa tissues, the expression of MACC-1 was positively correlated with the expression of c-Met protein (r=0.540, P<0.001).

Keywords: Prostatic cancer, metastasis-associated in colon cancer-1, immunohistochemisty, c-Met, survival

Introduction

Prostate cancer (PCa) is one of the most common malignant tumors in male and the second leading cause of cancer-related death in Western countries [1]. Prostate specific antigen (PSA) is a marker widely used in the clinical diagnosis of prostate cancer, but there are some prostate cancer patients with low serum PSA level, and even in the normal range, so easy to cause leakage diagnosis and delay the treatment prostate cancer [2, 3]. To further improve prostatic cancer patient outcome, more efforts are needed to find the new molecular markers to improve diagnosis and prognosis sensitivity, and optimize therapeutic strategies.

Metastasis-associated in colon cancer-1 (MACC1) is located on human chromosome 7 (7p21.1), which is overexpressed in many kinds of tumors (such as malignant glioma, colorectal cancer, hepatocellular carcinoma, breast can-

cer, renal pelvis carcinoma and gastric cancer), and it is closely related to the invasion and metastasis of malignant tumor [4-10].

c-Met is a receptor for hepatocyte growth factor (HGF), which is overexpressed in many kinds of malignant tumors, and has influence on cellular proliferation, migration and invasion [11-13]. The studies showed that the expression of c-Met mRNA and protein was significantly inhibited when the cells were transfected with siRNA MACC1, while the expression of MACC1 was not affected when the cells were transfected with siRNA c-Met [4, 14, 15]. MACC1 is located in the upstream of the HGF/c-Met signaling pathway, and is also a key regulator of the signaling pathway, which can induce the growth, invasion and distant metastasis of cancer cells [4, 16].

Although MACC1 expression has been extensively studied in several cancers, no previous studies of MACC1 expression in PCa have been reported [5-10]. In this study, we used immuno-

Table 1. Expressions of MACC1 and c-Met in PCa and BPH tissue

Tissue type	MACC1 expression[a]		c-Met expression[a]	
	Negative (-)	Positive (+)	Negative (-)	Positive (+)
Prostate cancer tissue	37 (44.0)	47 (56.0)	30 (35.7)	54 (64.3)
BPH	16 (80.0)	4 (20.0)	14 (70.0)	6 (30.0)
P value[b]	0.004		0.005	

[a]MACC1 and c-Met protein expression, positive means IHS are ≥3, and negative means IHS are 0-2. [b]*P* values are evaluated by chi-square test.

histochemical method to assay the expression of the MACC1 and c-Met in 86 cases of PCa, which was aimed at developing a potential diagnostic and monitoring prognosis tool for it.

Materials and methods

Patients and tissue samples

The study protocol was approved by the ethics committee of the Changshu Hospital, Affiliated to Soochow University, and all tissue samples were collected from patients with appropriate informed consent. From February 2008 to February 2015, 84 patients underwent surgery or endocrine therapy. 20 cases of benign prostatic hyperplasia (BPH) were taken from the control group. Among the 84 PCa patients, 39 patients were low or equal to 65 years old, 45 patients were over 65 years old. Sections were divided into T1a-T2b (40 cases) and T2c-T4 (44 cases) according to the TNM classification system proposed by American Joint Committee on Cancer (AJCC) in 2002 by two expert pathologists [17]. 55 patients serum PSA were low or equal to 20 ng/ml, 29 patients serum PSA were over 20 ng/ml. 31 patients were divided into low score (4-7) and 53 patients were divided into high group (8-10) according to Gleason score. PCa patients in the experimental group are shown in **Tables 1**, **2**. All sections were confirmed as human PCa by pathologists. They were followed up for 3 to 60 months via telephone. None of these patients received preoperative chemotherapy or radiotherapy.

Immunohistochemical (IHC) analysis

Streptavidin-peroxidase-biotin (SP) immunohistochemical method was performed similarly as previously described [18]. In brief, specimens were cut into 4 μm sections and baked at 60°C for 60 min. then sections were deparaffinized. Then sections were submerged into a pressure cooker filed in EDTA antigenic retrieval buffer for 10 minutes and then cooled for 20 minutes,

the sections were treated with 3% hydrogen peroxide, followed by incubation with normal serum to block nonspecific binding. The sections were incubated with rabbit anti-human MACC1 antibody (1:100; Beijing Biosynthesis Biotechnology Co., Ltd.) or rabbit anti-human c-Met antibody (1:150; Beijing Biosynthesis Biotechnology Co., Ltd.), overnight at 4°C. After washing, the tissue sections were added biotinylated secondary antibody (Maixin Biotechnology Company, Fuzhou, China), incubated for 1 h at room temperature, then added the streptavidin-horseradish peroxidase, incubation for 20 minutes. After washing, DAB (Wuhan Boster Biological Technology, Ltd; Wuhan, China) was added for visualization. Haematoxylin was used to counterstain the sections. Primary antibodies were replaced with PBS as negative controls.

Evaluation of MACC1 and c-Met staining

The staining intensity score is 0 (negative), 1 (weak), 2 (medium), and 3 (strong). The integral of the rate of positive cells is 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The proportional score and the intensity score were then added to obtain a total score. The expression is defined as positive (+, high expression) when score is more than or equal to 3, and negative (-, low expression) when score is less than 3 [19].

Statistical analysis

The experimental data were expressed as mean ± standard deviation (S.D). The GraphPad Prism version 5.0 and SAS 9.2 software package were used for statistical analysis. The relationship between MACC1 and c-Met expression and clinical parameters was evaluated using Pearson χ^2 test. The overall survival rates were calculated by the Kaplan-Meier method. When P<0.05, differences were considered statistically significant.

Results

Expression of MACC-1 and c-Met protein in cancer and BPH

As is shown in **Figure 1**, MACC1 staining was predominantly observed on the cytoplasm of

Table 2. Analysis of MACC1 and c-Met positive expression and related factors

Related Factor	n	MACC1 expression[a]		P value[d]	c-Met expression[a]		P value[d]
		Negative (-)	Positive (+)		Negative (-)	Positive (+)	
Age							
>65	45	17 (37.8)	28 (62.2)	0.214	15 (33.3)	30 (66.7)	0.625
≤65	39	20 (51.3)	19 (48.7)		15 (38.5)	24 (61.5)	
Gleason score							
Low (4-7)[b]	31	21 (67.7)	10 (32.3)	0.001	16 (51.6)	15 (48.4)	0.020
High (8-10)[c]	53	16 (30.2)	37 (69.8)		14 (26.4)	39 (73.6)	
PSA							
Low (≤20 ng/ml)[b]	55	29 (52.7)	26 (47.3)	0.027	24 (43.6)	31 (56.4)	0.037
High (>20 ng/ml)[c]	29	8 (27.6)	21 (72.4)		6 (20.7)	23 (79.3)	
TNM stage							
(T1a-T2b)[b]	40	27 (67.5)	13 (32.5)	<0.001	22 (55.0)	18 (45.0)	<0.001
(T2c-T4)[c]	44	10 (22.7)	34 (77.3)		8 (18.2)	36 (81.8)	

[a]MACC1 and c-Met protein expression, positive means IHS are ≥3, and negative means IHS are 0-2. [b]low and intermediate-risk group. [c]high- risk group. [d]P values are evaluated by chi-square test.

tumor cells (**Figure 1A**). c-Met staining was predominantly observed on the cytoplasm of tumor cells (**Figure 1C**). The expression level of MACC1 in the tumor tissues was significantly increased compared with BPH tissues (P=0.004; **Table 1**). The expression level of c-Met in the tumor tissues was significantly increased compared with BPH tissues (P=0.005; **Table 1**).

Relationship of MACC-1 and c-Met expression and clinicopathological parameters

MACC-1 and c-Met protein expression and clinicopathological features of PCa were examined as was shown in **Table 2**. Overexpression of MACC1 protein were significantly correlated with tumor Gleason score, PSA and TNM stage (P=0.001, P=0.027 and P<0.001, respectively). However, MACC-1 protein expression was not associated with age. Overexpression of c-Met protein were significantly correlated with tumor Gleason score, PSA and TNM stage (P=0.020, P=0.037 and P<0.001, respectively). However, c-Met protein expression was not associated with age.

Correlations between expressions of MACC-1 and c-Met and survival

The correlations were shown in **Figure 2**. Kaplan-Meier survival curves of PCa patients based on MACC-1 or c-Met expression. Patients with overexpression of MACC-1 protein showed significantly worse survival compared with those patients with low expression (P=0.020,

log-rank test) (**Figure 2A**). Patients with overexpression of c-Met protein showed significantly worse survival compared with those patients with low expression (P=0.026, log-rank test) (**Figure 2B**).

Correlations between MACC-1 and c-Met expression in PCa tissue and clinicopathological parameters

In PCa tissues, the expression of MACC-1 protein was positively correlated with the expression of c-Met protein (r=0.540, P<0.001), **Table 3**.

Discussion

In the present study, the result showed that positive rate of MACC1 protein in PCa is significantly higher than the BPH tissue. Furthermore, overexpression of MACC1 protein was detected in PSA high level (>20 ng/ml) when compared to the lower level (≤20 ng/ml). PSA has been widely used for diagnosis and screening prostate cancer and patients with high PSA levels had a greater risk of prostate cancer [20-22]. These suggests that MACC1 may participate in PCa tumorigenesis. Shirahata et al found that the expression level of MACC1 was increased in the process of transformation from benign tumor to malignant tumor transformation, and its level also reflected the strong and weak of tumor metastasis [23]. In the study, the positive rate of MACC1 protein in high Gleason score PCa is much higher than that in low score
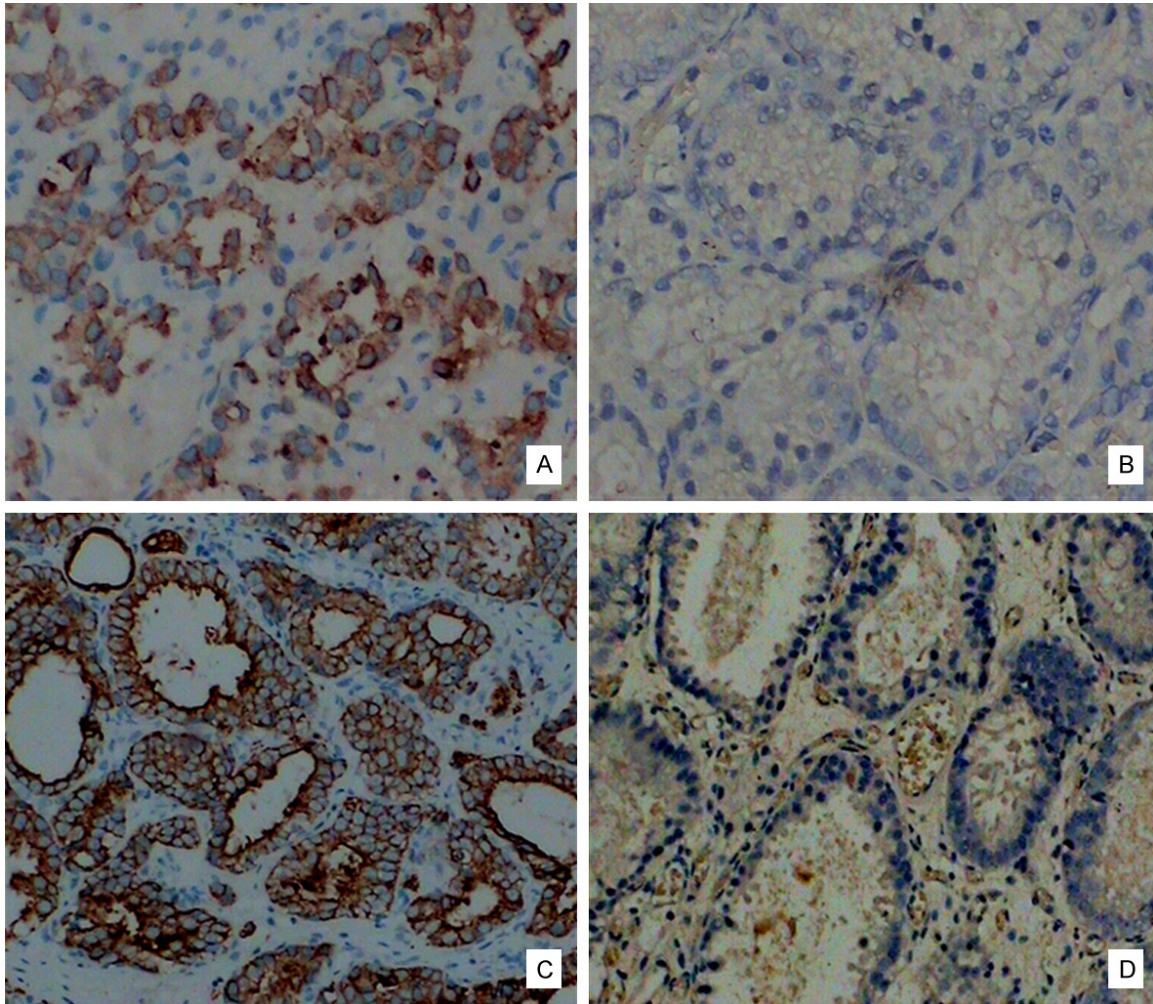
Figure 1. Immunohistochemical expression analysis of MACC-1 and c-Met protein in PCa tissue (200×). A. MACC-1 is positive staining in Gleason 8 score of PCa tissue and its cytoplasm is stained brown. B. MACC-1 is negative staining in Gleason 5 score of PCa tissue and its cytoplasm staining is weak. C. c-Met is positive staining in Gleason 6 score of PCa tissue and its cytoplasm is stained brown. D. c-Met is negative staining in Gleason 6 score of PCa tissue and its cytoplasm is not stained.
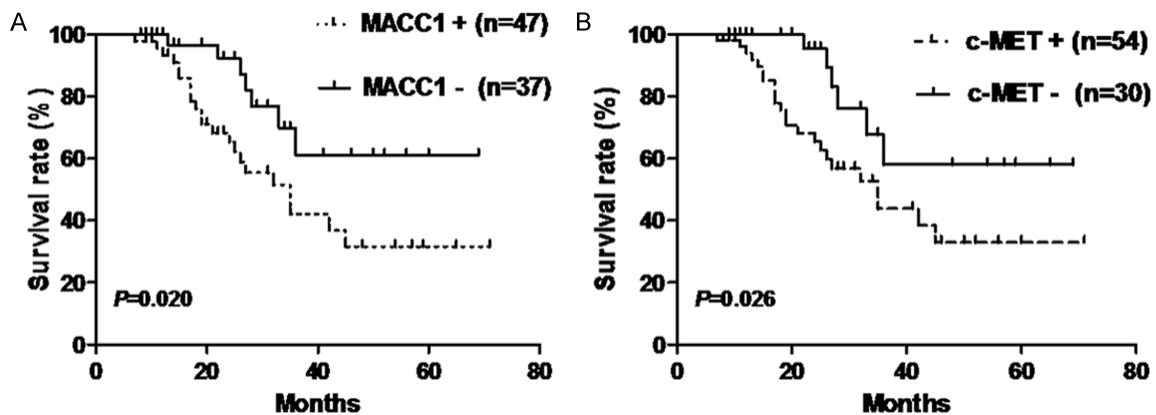
Figure 2. Kaplan-Meier survival curves of PCa patients based on MACC-1 expression (A) or c-Met expression (B).

Table 3. Correlations between MACC1 and c-Met expression in prostate cancer tissue

c-Met	MACC1 +	MACC1 -	Pearson correlation coefficient (r)	χ^2	P
+	41	13	0.540	24.475	<0.001
-	6	24			

PCa. Furthermore, the positive rate of MACC1 protein in high stage (T2c-PT4) is much higher than that in low stage (T1a-T2b). These suggest that MACC1 may play a role in the progression of PCa. T2c or higher or Gleason score >7 or PSA >20 ng/ml is well-known preoperative risk factor for prostate cancer [24]. While, MACC1 is closely related to these high risk factors, suggesting a poor prognosis.

The previous research found that the patients with high MACC1 expression had been linked to unfavorable outcomes in human breast cancer and gastric cancer [8, 19]. Our follow-up results show that the patients with overexpression of MACC-1 protein had unfavorable outcome compared with those patients with low expression by log-rank test analysis. Therefore, MACC-1 is expected to be an independent tumor prognostic factor. With more studies about proliferation and malignant mechanism of MACC-1, which as the target of gene therapy also get more and more attention. Some scholars found that the proliferation and invasion ability of tumor cells were reduced when MACC1 gene was silenced by using RNA interference [25, 26].

In combination with the previous researches, MACC1 protein may be helpful for auxiliary diagnosis of PCa and the judgment of patients' prognosis, which may become the new target for tumor gene therapy.

This study also finds that positive rate of c-Met protein in PCa tissue are significantly higher than the prostate hyperplasia tissue, suggesting that c-Met may participate in PCa tumorigenesis. Furthermore, the positive rate of c-Met protein in high Gleason score PCa is much higher than that in low score PCa. The positive rate of c-Met protein in high stage (T2c-PT4) is much higher than that in low stage (T1a-T2b). These suggest that c-Met may play a role in the progression of PCa. Many cancer patients have been found that the prognosis of patients with overexpression of c-Met protein have poor prognosis [27, 28].

Our follow-up results also show that patients with overexpression of c-Met protein showed significantly worse survival compared with those patients with low expression by log-rank test analysis. It may be considered for auxiliary diagnosis of PCa, and judging the prognosis of patients.

The studies find that MACC-1 has certain adjustment effect for c-Met protein [4, 25]. This study shows that MACC-1 protein in PCa up-regulated with higher expression of c-Met, which meant that they are positively correlated. MACC-1 gene may through the HGF/c-Met signaling pathway, which can induce the growth, invasion and distant metastasis of cancer cells.

We studied MACC1 and c-Met expression by immunohistochemistry only, which is a limitation of the present study. MACC1 and c-Met mRNA expression levels are not always congruent with the protein in the cancer cell. In future studies, we can use RNAi to reduce the expression of MACC1 mRNA, then study the changes of MACC1 and c-Met protein, and further study their role in the invasion and metastasis of prostate cancer.

Conclusion

In conclusion, protein of MACC-1 and c-Met are overexpression in PCa, and their overexpression correlate to poor prognosis in PCa patients. Detecting MACC-1 and c-Met may help for the auxiliary diagnosis of bladder cancer and judging the prognosis of patients. Further research about this is expected to be the new targets for gene therapy of PC. However, this finding should be verified in a large sample size of PC patients. Moreover, it needs to be further researched that MACC-1 and c-Met promote mechanisms of the tumorigenesis and progression in PCa.

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Disclosure of conflict of interest

None.

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