RESEARCH COMMUNICATION

Expression and Prognostic Value of Matrix Metalloproteinase-7 in Colorectal Cancer

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Abstract

The purpose of this study was to evaluate expression and prognostic value of matrix metalloproteinase-7 (MMP-7) in colorectal cancer (CRC) patients. CRC tissues and corresponding distal normal mucosa tissues of 118 CRC patients were assessed by immunohistochemistry. Correlations between MMP-7 expression, patients' clinic pathological features, and overall survival rate were analyzed. We found that positive expression of MMP-7 in CRC tissues was significantly higher than that in distal normal mucosa (61.0% vs. 39.8%, p =0.001). Poor histological differentiation, advanced clinical stage and lymph node metastasis were significantly correlated with MMP-7 expression in CRC. The overall survival rate was significantly higher in the MMP-7 negative group than the positive group (Log-rank test= 9.957, p= 0.002). MMP-7 appeared as a significant independent prognostic factor through multivariate survival analysis. Collectively, we found MMP-7 expression to be correlated with progression and metastasis of CRC and thus could be used as a predictive marker of prognosis in CRC patients.

Keywords: Colorectal carcinoma - immunohistochemistry - matrix metalloproteinase-7 - prognosis

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Introduction

Colorectal cancer (CRC) is one of the most frequent cancers in the world. According to the statistical data from International Agency for Research on Cancer (IARC), in 2008, 1.24 million new cases of colorectal cancer were clinically diagnosed, and this type of cancer killed 610,000 people. Now CRC is the fourth most common malignant tumor and the third leading cause of cancer death in both men and women in China (Long et al., 2011).

Matrix metalloproteinase-7 (MMP-7), also known as matrilysin, is the smallest member of matrix-degrading metalloproteinases (MMPs) which play important role in degradation of extracellular matrix (ECM). It is specifically expressed in epithelial tumor cells instead of interstitial cells and is associated with cancer progression (Matthew et al., 2003). The components of ECM including collagen fibers, elastin, laminin, gelatin and proteoglycan are substrates of MMP-7. Because the proteolysis of ECM is essential for the occurrence, development and progression of cancer, MMP-7 expression plays a crucial role at early stage of tumor invasion and metastasis. In early invasive colorectal carcinomas, MMP-7 expression was significantly and independently associated with distant metastasis or adverse outcome through multivariate logistic regression analysis (Masaki et al., 2001). MMP-7 is also highly expressed in early stage of pancreatic cancer (Crawford et al., 2002). Further more, tumorigenesis is a multistep process involving cell growth, invasion, metastasis, and angiogenesis. MMP-7 has been shown to play important roles not only in degradation of ECM proteins, but also in the regulation of several biochemical processes such as activation, degradation, and shedding of non-ECM proteins. MMP-7 is frequently overexpressed in human cancer tissues and is associated with in human cancer invasion, apoptosis, growth, and angiogenesis (Ii et al., 2006). Overexpression of MMP-7 correlates with tumor proliferation, and a poor prognosis in non-small cell lung cancer, oral squamous cell carcinoma and papillary thyroid carcinoma (Ito et al., 2006; de Vicente et al., 2007; Liu et al., 2008). However, the correlation between expression of MMP-7 and survival or prognosis in colorectal cancer is still inconclusive. Here, we investigated retrospectively MMP-7 expression in CRC tissues and distal normal mucosa tissues of 118 CRC patients, and analyzed the association of MMP-7 expression with clinicopathologic features and prognosis using univariate and multivariate analysis.

Materials and Methods

Ethics statement

The study was approved by the Ethics Committee of Wuhan General Hospital, Guangzhou Command of the People's Liberation Army. Written informed consent was obtained from all participants.

Patients and tissue samples

A total of 118 consecutive patients with colorectal carcinoma who underwent surgery at the Department

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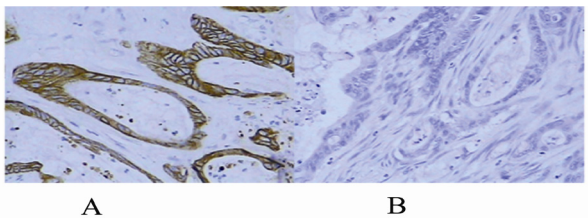
of Oncology, Wuhan General Hospital of Guangzhou Command between January 2004 and January 2007 were included in the study. Diagnosis and staging were performed according to the modified Dukes' classification (Davis et al., 1984). 61 patients were men and 57 patients were women. The mean age was 60 years with a range of 44 to 71 years. The depth of invasion was classified as mucosa and submucous membrane layer, muscular layer and serosa layer. Tumors were classified as well, moderately and poorly differentiated adenocarcinomas. Lymph node metastasis happened in 68 patients, and other 50 patients had no metastatic lymph node. All patients were followed up for survival. None of the patient underwent radiotherapy or chemotherapy before surgery. Formalin-fixed and paraffin-embedded surgical tissue samples were collected from the archives of the Department of Pathology, Wuhan General Hospital of Guangzhou Command. Histopathologically representative regions of tumor specimens were defined and marked on H&E slides.

Immunohistochemistry

The paraffin embedded CRC tissues and corresponding distal normal mucosa tissues were cut at 4 μm and mounted on glass slides. Then the slides were dewaxed in xylene and rehydrated in ethanol, and treated with a solution of peroxidase-blocking reagent (Dako, Glostrup, Denmark) to exhaust endogenous peroxidase activity. They were put in 0.01 mol/L citrate buffer at pH 6.0 for 15 minutes in an 800W microwave oven and then left at room temperature for 20 minutes to expose antigen hidden inside the tissue due to formalin fixation. To inhibit non-specific antigen–antibody reactions possible in immunohistochemical staining, protein blocker (Research Genetics, Huntsville, AL, USA) was used for 5 minutes and the slides were washed thoroughly with PBS buffer. Then the slides were incubated overnight with the primary antibodies against MMP-7 (1:100; rabbit polyclonal antibody, BA0571, Boster Bio-engineering Limited Company, Wuhan, China) at 4 centigrade. Biotinylated goat anti-rabbit secondary antibody (1:200; BA1003, Boster Bio-engineering Limited Company, Wuhan, China) was applied for 20 minutes at room temperature, followed by further washing with buffer to remove unbound antibody. A complex of avidin with horseradish peroxidase was then applied for 20 minutes at room temperature. For color development, the slides were stained with 3,3'diaminobenzidine (DAB, Sigma–Aldrich, St Louis, MO, USA) then were counterstained with hematoxylin. A reddish brown precipitate in the cytoplasm of cells indicated a positive reaction. In each immunohistochemistry run, the positive section provided by reagent company served as positive control and omission of the primary antibody served as negative control. Immunohistochemistry stained slides were reviewed by two investigators independently blinded to all clinical data.



A B

Figure 1. MMP-7 Expression in CRC Tissue. A: Positive MMP-7 expression in CRC tissue. The expression of MMP-7 was only found in cytoplasm by immunohistochemistry staining (×200, SP method). B: Negative MMP-7 expression in CRC tissue (×200, SP method)

Statistical analysis

Positive rates and MMP-7 expressions in colorectal carcinoma and normal mucosa were compared by χ^2 test. The χ^2 test was also used to examine the various clinicopathological characteristics and MMP-7 expression. The univariate survival analysis and cumulative survival curve were executed by Kaplan-Meier method. The difference between the curves was analyzed by Log-rank test. The multivariate survival analysis was executed by Cox proportional hazard regression model. A p value < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

MMP-7 expressions in colorectal carcinoma and distal normal mucosa

The MMP-7 expression was detected in 61.0% (72/118) CRC tissue, and in 39.8% (47/118) distal normal mucosa tissue (Figure 1). The expression of MMP-7 was only found in cytoplasm. The difference of MMP-7 expression between CRC and distal normal mucosa was statistically significant ($\chi^2 = 10.594$, p =0.001).

Table 1. The Correlation of MMP-7 Expression and Clinicopathological Features in CRC

Items	Cases	MMP-7 Positive cases	χ^2	p
Gender				
Male	61	37	0.046	0.83
Female	57	35		
Age				
<60years	29	16	0.552	0.457
≥60years	89	56		
Tumor size				
<5cm	47	33	2.172	0.141
≥5cm	71	39		
Differentiated degree				
Well differentiation	39	18	6.356	0.042*
moderate differentiation	61	40		
Poor and Undifferentiation	18	14		
Infiltrative depth				
Mucosa and submucous layer	9	4	7.048	0.029*
Muscular layer	51	38		
Serosa layer	58	30		
Metastasis of lymphnode				
Negative	50	25	4.427	0.035*
Positive	68	47		
Dukes' stage				
A	12	4	9.188	0.027*
B	38	20		
C	51	34		
D	17	14		

*Statistically significant

Table 2. Results of Survival Analysis for Individual Factors

Factor	Log rank	p
MMP-7 expression	9.957	0.002*
Gender	1.012	0.314
Age	0.032	0.858
Tumor differentiation	3.957	0.047*
Metastasis of lymphnode	5.889	0.015*
Infiltrative depth	5.168	0.023*
Dukes' stage	9.59	0.002*

*Statistically significant

Table 3. Results of Cox Multivariate Regression Factors

Factor	Wald value	p
MMP-7 expression	7.12	0.008*
Tumor differentiation	0.024	0.877
Metastasis of lymphnode	4.47	0.034*
Infiltrative depth	0.216	0.642
Dukes' stage	7.001	0.008*

*Statistically significant

Correlation of MMP-7 expressions and clinicopathological features in CRC

When comparing the MMP-7 status with clinicopathological variables, we found significant positive correlations between MMP-7 expression and degree of differentiation (p = 0.042), depth of infiltration (p = 0.029), lymph node metastasis (p = 0.035), and Dukes' stage (p = 0.027) (Table 1).

Kaplan-Meier analysis showed that the differences of survival in MMP-7 expression group and Dukes'stage group were highly statistically significant (Log-rank test, 9.957, p=0.002; Log-rank test, 9.590, p=0.002). Meanwhile, the differences of survival in metastasis of lymphnode group, infiltrative depth group, and tumor differentiation groups were also statistically significant (p<0.05, Table 2). Importantly, there was no significant difference between the two groups in terms of patient age and gender. The MMP-7 expression appeared as a significantly independent prognostic factors (p=0.008) with a relative risk of 1.745 (95% confidence interval, 1.159-2.626) in Cox multivariate analysis, which was done with the following variables for each case: MMP-7 expression, tumor differentiation, metastasis of lymphnode, infiltrative depth, and Dukes'stage (Table 3).

Relationship between MMP-7 expression and overall survival rate of CRC patients

The 5-year survival rate for 118 CRC cases was 55.1%. The 5-year survival rates for MMP-7 negative and MMP-7 positive cases were 66.1% and 43.5%, respectively. The difference of overall survival rate between MMP-7 negative group and MMP-7 positive group was statistically significant (Log-rank test= 9.957, p= 0.002) (Figure 2).

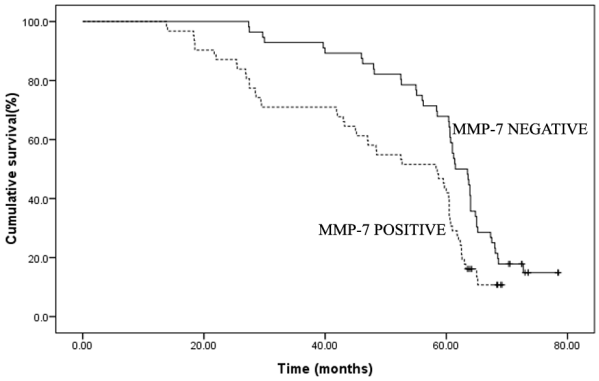


Figure 2. Kaplan-Meier Survival Plots of MMP-7 Positive Group and Negative Group. There is highly significant difference in overall survival rate between MMP-7 negative group and MMP-7 positive group (Log-rank test= 9.957, p= 0.002)

Discussion

ECM provides a structural framework to support cells and maintains cellular functions by mediating cell-cell or cell-ECM interactions. The tumor cell adhering with the ECM, then releasing proteolytic enzymes to break down the ECM and basement membrane is the first step of tumor invasion and metastasis process. Degradation of ECM components is mostly controlled by proteolytic enzymes called MMPs. MMPs belong to a family of zinc-dependent endopeptidases intrinsically responsible for the degradation of a vast number of protein targets by cleavage of internal peptide bonds (Brinckerhoff and Matrisian, 2002, Sternlicht and Werb, 2001).Currently, there are at least 25 MMPs members divided in five main groups according to their structure and substrate specificity: collagenases, gelatinases, membrane type, stromelysins and matrilysins (Lafleur et al., 2003). MMP-7 is a member of matrilysins. However, MMP-7 lacks a C-terminal hemopexin domain common to other MMP members, and it has distinctively smaller molecular weights. It locates in 11q21-->q22 and its molecular weight is 28KD (Knox et al., 1996).

In normal human tissue, MMP-7 protein localizes to secretory and ductal epithelium in the endometrium and in various exocrine glands. Meanwhile, MMP-7 is expressed in a variety of tumors ranging from adenomas to carcinomas and adenocarcinomas of the breast, colon, prostate, stomach, upper aerodigestive tract, lung, and skin, where it may be involved in tumor formation as well as the tissue degradation which accompanies tumor cell extravasation (Wilson et al., 1996).

The expression of MMP-7 in 34 non-small sell lung cancer (NSCLC) patients was investigated using a real-time PCR method. A higher expression of MMP-7 mRNA was found in the NSCLC tissue than in non-tumourous lung tissue. The expression of MMP-7 mRNA was higher in adenocarcinoma than in the epidermoid form of NSCLC (Safranek et al., 2007). The MMP-7 expression of 147 NSCLC patients was also investigated using immunohistochemistry method. 76 carcinomas (51.7%) were MMP-7-positive. The overall survival of NSCLC patients with MMP-7-positive was significantly lower than that of NSCLC patients with MMP-7-negative. A Cox regression analyses also demonstrated MMP-7 status to be a significant prognostic factor (Liu et al., 2007). In this study, we found that MMP-7 expression in colorectal cancer tissues (72/118, 61.0%) was significantly higher

than that in corresponding distal normal mucosa tissues (47/118, 39.8%), and there was statistically significant difference (χ^2 = 10.594, p =0.001) between them. Furthermore, high levels of MMP-7 expression in cancer cells correlated with poor histological differentiation, deep infiltration, lymph node metastasis and high Dukes'stage (Table 1). Therefore this finding suggested that MMP-7 was likely to play a role in promoting tumor invasion and metastasis. Meanwhile, Cox multivariate analysis suggested that MMP-7 might serve as an independent marker for poor prognosis. Although the detailed molecular mechanism involved in this process is less well defined, our study still has potentially clinical benefits. The MMP-7 expression that could be detected by immunohistochemistry may be a useful molecular marker to predict the prognosis in CRC patients.

In conclusion, our study implies that MMP-7 plays an important role in tumor development and progression process, and thus became a useful indicator for clinical assessment of tumor biological behavior and prognosis in CRC patients.

Acknowledgements

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