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RESEARCH ARTICLE

Matrilysin-2 expression in colorectal cancer is associated with overall survival of patients

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Abstract Tumor recurrence and metastasis are pressing issues of patients with colorectal cancer who receive surgery. Matrilysin-2 (MMP-26) has been proved to play an important role during invasion and metastasis of some human solid tumor. We aimed to investigate the clinical significance and prognostic value of matrilysin-2 in human colorectal cancer. Colorectal cancer and adjacent normal samples from 201 patients were collected. Matrilysin-2 expression level was investigated by immunohistochemistry assay, and its association with overall survival of patients was analyzed by statistical analysis. Results showed that matrilysin-2 expression level significantly elevated in colorectal cancer compared with adjacent normal specimens. Matrilysin-2 expression was also found to be associated with cancer invasion, lymph node metastasis, distant metastasis, and TNM stage. In addition, survival analysis showed that elevated matrilysin-2 expression was associated with poor overall survival of patients. Cox's proportional hazards model indicated that matrilysin-2 was an independent prognostic marker for patients with colorectal cancer. The present study found that the expression of matrilysin-2 increased in colorectal cancer and was associated with tumor progression. It also provided the first evidence that matrilysin-2 expression was an independent prognostic factor for patients with colorectal cancer, which might be a high specific biomarker for colorectal cancer.

Keywords Matrilysin-2 · Colorectal cancer · Immunohistochemistry · Prognosis

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Introduction

It has been proved that the degradation of the extracellular matrix (ECM) surrounding tumor cells is a crucial step in tumor invasion. The main group of enzymes involved in matrix degradation is the matrix metalloproteinases (MMPs), which are a group of zinc-dependent proteins found in the extracellular milieu of various tissues [1, 2]. They constitute a family of highly homologous enzymes sharing a similar structure [3]. To date, at least 28 known human MMPs have been discovered [4]. Based on the structure and substrate specificity, MMPs can be divided into five subgroups: collagenases, gelatinases, stromelysins, membrane-type MMPs, and other types of MMPs [3]. Human matrilysin-2, also known as MMP-26 or endometase, was isolated as an activator of pro-MMP-9 and a matrilysin (MMP-7) homolog, which shared with matrilysin the minimal domain organization required for secretion, latency, and activity [5, 6]. To date, matrilysin-2 is the smallest MMP identified which has several structural features of MMPs including the signal sequence, the prodomain involved in enzyme latency, and the catalytic domain with the zinc-binding site [7]. Moreover, matrilysin-2 is distinguished from all other known mammalian MMPs in that it does not exist in the murine genome and the catalytic domain is not so similar with other known MMPs [7]. Recent studies found that matrilysin-2 was expressed in migrating keratinocytes in normally healing skin wounds, migrating enterocytes, macrophages, and endothelial cells [8]. The specific expression of matrilysin-2 in human malignancies can degrade various components of the ECM, including type IV collagen, gelatin, fibronectin, laminin-1, and fibrinogen [7]. Investigations focused on malignant tumors proved that matrilysin-2 was expressed in endometrium cancer, breast cancer, esophageal cancer, and ovarian cancer and associated with the invasiveness of these tumors [6, 9–11]. However, to our knowledge, the protein expression of matrilysin-2 in

human colorectal cancer has not been reported yet. Efforts to better understand the biological role of matrilysin-2 in colorectal cancer may provide clinically relevant insights into gene function and efficacious cancer management.

In the present study, we have investigated the protein expression level of matrilysin-2 in clinical colorectal specimens and adjacent normal tissues, as well as analyzed its association with overall survival of patients with colorectal cancer.

Materials and methods

Patients and specimens

The present study has been approved by the Ethics Committee of Chengdu Medical College. All members involved have provided written informed consent. Fresh clinical colorectal cancer specimens and adjacent normal tissues were collected from 201 patients who underwent surgery between January 2006 and December 2008 in Shaanxi Provincial People's Hospital. None of these patients had received chemotherapy prior to surgery. In addition, normal tissue samples were taken from 18 patients who underwent surgery for reasons other than malignancy as normal control samples. The histomorphology of all tissue specimens was confirmed by the Department of Pathology, Chengdu Medical College. Specimens were collected within 10 min after surgical resection and then fixed in 10 % formaldehyde and imbedded in paraffin for histological sections. Patients' clinical information, such as age, sex, differentiation status, and TNM stage, were collected and stored into a database. Complete follow-up was made available for at least 5 years. Overall survival is defined as the time elapsed from surgery to death of patients with colorectal cancer. Follow-up information of all participants was updated every 3 months by telephone visit and questionnaire letters. Death of participants was ascertained by reports from the family and verified by review of public records.

Immunohistochemical assays

Fresh tissue specimens were deparaffinized in xylene and dehydrated through a graduated alcohol series. Endogenous peroxidase activity was blocked with 0.5 % H_2O_2 in methanol for 10 min. The antigen retrieval was performed by microwaving sections in 0.01 M sodium citrate, pH 6.0. Nonspecific binding was blocked by incubating sections with 10 % normal goat serum in phosphate-buffered saline (PBS) for 1 h at room temperature. Without being washed, these sections were incubated with rabbit anti-human matrilysin-2 antibody (1:1,000 dilution, Serotec, Oxford, UK) in PBS at 4 °C overnight in a moist box [10]. Normal rabbit immunoglobulins were substituted as negative control. The sections were incubated with biotinylated goat anti-rabbit IgG (1:400, Sigma, St. Louis, MO, USA) for 1 h at room temperature, and expression was detected with a streptavidin–peroxidase complex. The brown color indicative of peroxidase activity was developed by incubating with 0.1 % 3,3-diaminobenzidine in PBS with 0.05 % H_2O_2 for 5 min at room temperature. Appropriate positive and negative controls were performed simultaneously.

Evaluation of staining

The matrilysin-2 staining was viewed separately by two pathologists without knowing the clinical or clinicopathological status of the cases. The expression of matrilysin-2 on the slide was evaluated by scanning the entire tissue specimen under low-power magnification (×40) and then confirmed under high-power magnification (×200). An immunoreactivity score (IRS) system was applied. The extensional standard was as follows: (1) number of positive stained cells: ≤5 %, scored 0; 6~25 %, scored 1; 26~50 %, scored 2; 51~75 %, scored 3; >75 %, scored 4; (2) intensity of stain: colorless, scored 0; pallide-flavens, scored 1; yellow, scored 2; brown, scored 3. Multiply (1) and (2). The staining score was stratified as − (0 score, absent), + (1~4 score, weak), ++ (5~8 score, moderate), and +++ (9~12 score, strong) according to the proportion and intensity of positively stained cancer cells. Specimens will be rescored if the difference of scores from the two pathologists was more than 3 [12].

Statistical analysis

Associations between matrilysin-2 staining and clinicopathological characteristics were analyzed by the Mann–Whitney test and Kruskal–Wallis test, as appropriate. Survival curves were estimated using the Kaplan–Meier method, and differences in survival distributions were evaluated by the log-rank test. Cox's proportional hazards modeling of factors potentially related to survival was performed in order to identify which factors might have a significant influence on survival, controlling for age, gender, and differentiation status. Differences with a P value of 0.05 or less were considered to be statistically significant.

Results

Matrilysin-2 staining detected in colorectal cancer

In immunohistochemistry assay, 201 cases of colorectal cancer and adjacent normal specimens were investigated. Matrilysin-2 staining was found predominantly localized in the cytoplasm and/or membrane of the tumor cell. No significant matrilysin-2 staining was observed in the adjacent

normal tissue, while only weak positive staining was observed in a few cell membranes and cytoplasm. The staining pattern of matrilysin-2 was consistent with previous report [10]. Among all the 201 cases of colorectal specimens investigated, 19 cases with strong positive staining (+++) of matrilysin-2 were detected while 28 cases with moderate positive staining (++), 51 cases with weak positive staining (+), and 103 cases with negative staining (−) of matrilysin-2 were detected. On the other hand, among the same amount of cases of adjacent normal specimens, no strong positive staining (+++) of matrilysin-2 was detected while 3 cases with moderate positive staining (++), 26 cases with weak positive staining (+), and 172 cases with negative staining (−) of matrilysin-2 were detected. The staining of matrilysin-2 in colorectal cancer was significantly stronger than that in adjacent normal tissues ($P<0.05$). These results suggested that the expression of matrilysin-2 in colorectal cancer increased compared with that in normal tissues.

Relationship of matrilysin-2 staining to clinicopathological characteristics

Our investigation proved that matrilysin-2 expression increased in colorectal cancer, which indicated that matrilysin-2 might act as an oncogene in colorectal cancer. We further investigated the association of matrilysin-2 expression with clinicopathological characteristics of patients based on the IRS scores of immunohistochemical staining. Statistical analysis results (Table 1) showed that increased matrilysin-2 expression was associated with advanced colorectal cancer invasion since stronger matrilysin-2 staining was more frequently detected in T3 and T4 tumors ($P< 0.001$). As far as tumor metastasis was considered, elevated matrilysin-2 expression was related to positive lymph node and distant metastases for stronger matrilysin-2 staining was more frequently detected in tumors with positive lymph node or distant metastasis ($P< 0.001$). In addition, when we measured patients' clinical features by the TNM staging system, we found that matrilysin-2 expression was significantly associated with the TNM stage of colorectal cancer because stronger matrilysin-2 staining was more frequently detected in tumors with advanced TNM stage ($P< 0.001$). These results suggested that matrilysin-2 might play an oncogenic role in the progression of colorectal cancer. However, the positive ration of matrilysin-2 was not found to be associated with patients' sex ($P= 0.758$), age ($P= 0.855$), or differentiation status ($P= 0.991$).

Relationship of matrilysin-2 to overall survival of patients with colorectal cancer

During the whole follow-up period, 102 of the 201 patients (50.7 %) with colorectal cancer had died and the median overall survival time of all recruited patients was 49 months.

Table 1 Relationship between matrilysin-2 staining and clinicopathological characteristics

	Number	Matrilysin-2				*P* value
		−	+	++	+++	
Total	201	103	51	28	19	
Sex						0.758[a]
Male	110	56	27	15	12	
Female	91	47	24	13	7	
Age						0.855[a]
<60	118	61	30	16	11	
≥60	83	42	21	12	8	
Differentiation						0.991[b]
Poor	60	31	15	8	6	
Moderate	95	48	24	14	9	
Well	46	24	12	7	4	
Invasion						< 0.001[a]
T1+T2	98	67	19	8	4	
T3+T4	103	36	32	20	15	
Lymph node metastasis						< 0.001[a]
Negative	83	57	16	7	3	
Positive	118	46	35	21	16	
Distant metastasis						<0.001[a]
Negative	175	101	43	19	12	
Positive	26	2	8	9	7	
TNM stage						<0.001[b]
I	26	18	5	2	1	
II	57	39	11	5	2	
III	92	44	27	12	9	
IV	26	2	8	9	7	

[a] Estimated by the Mann–Whitney test

[b] Estimated by the Kruskal–Wallis test

Kaplan–Meier analysis was applied to examine the prognostic value of matrilysin-2 staining to overall survival of patients with colorectal cancer. Considering the small sample size of the matrilysin-2 strong positive staining (+++, $n=19$) group, we manually combined the strong positive (+++), moderate positive (++), and weak positive (+) staining groups into a single group described as the matrilysin-2 positive (+) staining group in order to facilitate statistical analysis. Kaplan–Meier analysis results proved that patients with colorectal cancer of positive matrilysin-2 staining tended to have worse overall survival (log rank test: $P<0.001$, Fig. 1). The median survival time of patients with negative (−) staining of matrilysin-2 cannot be estimated because more than 50 % (54.4 %, $n=56$) of patients survived during the follow-up period, while the postoperative median survival time of patients with positive (+) staining of matrilysin-2 was 23 months (95 % confidence interval (CI) 13.9–32.1). When the unadjusted hazard ratio (HR) was considered with matrilysin-2 negative (−)

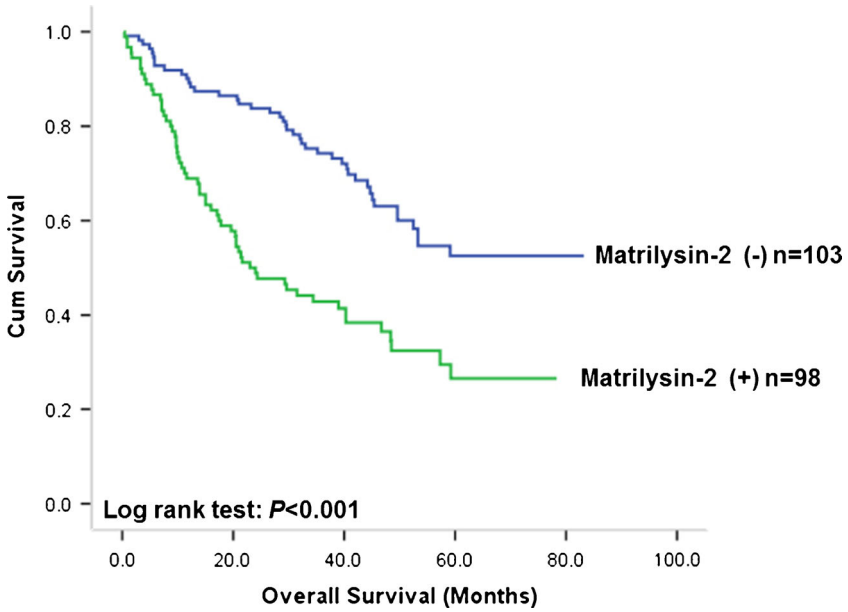

Fig. 1 Kaplan–Meier postoperative survival curve for patterns of patients with colorectal cancer and matrilysin-2 staining

staining as reference (1.00), patients with colorectal cancer of positive matrilysin-2 staining had a 2.47-fold higher risk of death (95 % CI 1.66–3.66; $P<0$ .001) compared with those with colorectal cancer of negative staining. As far as clinicopathological characteristics were considered, lymph node metastasis (log rank test: $P=0.004$), distant metastasis (log rank test: $P<0.001$), and TNM stage (log rank test: $P<0.001$) were also proved to be associated with overall survival since patients with colorectal cancer of lymph node metastasis, distant metastasis, or advanced grade tended to have worse overall survival and a higher risk of death. However, sex (log rank test: $P=0.928$) or age (log rank test: $P=0.694$) had no prognostic value on overall survival of patients with colorectal cancer.

As matrilysin-2 expression was proved to be associated with overall survival of patients in univariate survival analysis, we further investigated whether matrilysin-2 could serve as an independent prognostic marker for patients with colorectal cancer. Because lymph node metastasis and distant metastasis information were included in TNM stage data, we performed Cox's proportional hazards model, which is adjusted by sex, age, and TNM stage. Results showed that the adjusted HR of weak positive (+) matrilysin-2 staining groups was 3.16 (95 % CI 1.51–6.61; $P=0.002$). These results proved that matrilysin-2 was an independent prognostic factor for overall survival of patients with colorectal cancer. Thus, increased matrilysin-2 expression could be an indicator of poor overall survival without consideration of age, sex, or TNM stage (Table 2).

Discussion

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide [13, 14]. Recently, high colorectal cancer rates have been observed in developing countries such as China in which the risk was once low [15]. The incidence and mortality rate of colorectal cancer have been increasing simultaneously in China in the last decade, ranking it the fourth most common malignant tumor [16]. Despite earlier diagnosis and progressions in radical surgery, radiotherapy, and neoadjuvant chemotherapy, the prognosis of colorectal cancer remains unsatisfactory [17]. It has been widely accepted that the prognosis of colorectal cancer is directly correlated with the extent of local and metastatic tumor spread. Thus, finding specific and sensitive molecular markers which can identify early tumor metastasis became one of the most important topics in recent tumor studies.

MMPs are a group of structurally related proteins which can degrade ECM components and have for long been viewed as key modulators of tumor progression and metastasis. Indeed, high expression levels of several MMP members have been correlated with tumor aggressiveness of various human malignancies. Previous studies proved that several MMPs were associated with colorectal cancer invasion, metastasis, and prognosis. It has been reported that elevated MMP-1 expression in colorectal cancer was associated with cancer invasion, metastasis, and poor prognosis [18]. MMP-2 and MMP-7 were reported to be correlated with the clinical stage and could serve as prognostic markers for colorectal cancer [19, 20]. In addition, MMP-9 was found to be associated with both disease-free and overall survival of colorectal cancer [21]. Other MMPs such as MMP-13, MMP-14, and MMP-21 were also reported to be associated with the TNM stage and prognosis of colorectal cancer [22–24]. These findings indicated the strong capability of the invasive and metastatic functions of MMPs associated with the prognosis of patients

Table 2 Association of matrilysin-2 and clinical factors with overall survival of patients with colorectal cancer

	Unadjusted HR[a] (95 % CI)	*P* value	Adjusted HR[b] (95 % CI)	*P* value
Matrilysin-2				
Negative (−)	–		–	
Positive (+)	2.47 (1.66–3.66)	<0.001	3.16 (1.51–6.61)	0.002
Sex				
Female	–		–	
Male	1.02 (0.62–1.70)	0.928	1.03 (0.67–1.58)	0.908
Age				
<60	–		–	
≥60	1.08 (0.73–1.60)	0.694	1.13 (0.64–2.02)	0.672
TNM stage				
I	–		–	
II	1.30 (0.72–2.34)	0.380	1.55 (0.81–2.98)	0.192
III	3.12 (1.49–6.52)	0.004	3.72 (1.60–8.61)	0.002
IV	7.35 (3.68–14.98)	<0.001	8.12 (4.18–17.26)	<0.001

[a] Hazard ratios in univariate models

[b] Hazard ratios in multivariable models

with colorectal cancer. However, only few studies have investigated matrilysin-2 in cancer to date. To our knowledge, the expression pattern and prognostic value of matrilysin-2 in colorectal cancer are still unknown and need to be clarified.

In this study, we investigated matrilysin-2 protein expression by immunohistochemistry (IHC) assay in 201 cases of colorectal cancer from patients who had not received neoadjuvant chemotherapy. Based on the IRS score system of IHC results, we analyzed the association of matrilysin-2 with clinicopathological characteristics as well as the prognosis of patients. Results showed that matrilysin-2 expression increased in colorectal cancer compared with adjacent normal tissues for stronger positive staining of matrilysin-2 was more frequently detected in colorectal cancer specimens, which indicated the possible participation of matrilysin-2 in cancer development. It is in consistence with previous report on other kinds of human malignancies [7]. The present study also demonstrated that matrilysin-2 expression was closely related to colorectal cancer invasion, lymph node metastasis, distant metastasis, and TNM stage for stronger positive staining of matrilysin-2 was more frequently detected in tumors with deep invasion, lymph node metastasis, distant metastasis, or advanced TNM stage, suggesting its possible role in promoting tumor progression. These results were consistent with previous findings in esophageal squamous carcinoma, which found that matrilysin-2 overexpression correlates strongly with the depth of invasion and lymph node and distant metastases [10]. In pancreatic cancer, it has also been reported that matrilysin-2 expression had a trend to increase from T1 to T4, and this protease was significantly more often expressed in tumors having metastatic lymph nodes [25]. Thus, our data indicated the possible participation of matrilysin-2 in colorectal cancer invasion and metastasis. Together with the above evidence, it was thus proposed that matrilysin-2 may play an important role in colorectal cancer carcinogenesis and progression.

As matrilysin-2 expression was found to be associated with colorectal cancer invasion and metastasis, the invasion of colorectal cancer cells to nearby tissues and metastasis to distal tissues are crucial factors affecting the prognosis of patients. In order to investigate the prognostic role of matrilysin-2 in colorectal cancer, we performed Kaplan–Meier analysis on overall survival. Results showed that patients with colorectal cancer of positive matrilysin-2 staining tended to have worse overall survival in comparison to patients with tumor of negative matrilysin-2 staining, which suggested that matrilysin-2 expression might be a potential prognostic marker for patients with colorectal cancer in clinical practice. To further evaluate the prognostic value of matrilysin-2, we performed Cox's proportional hazards model which was adjusted for the gender, age, and TNM stage of patients. Results showed that increased matrilysin-2 expression was a marker for poor overall survival independent of adjusted factors; thus, matrilysin-2 could be utilized to determine patients' prognosis without considering the TNM stage. These results indicated that matrilysin-2 could constitute a molecular prognostic marker additive to TNM stage for patients with colorectal cancer, identifying high-risk individuals who are more likely to have tumor relapse, thus, good candidates to receive more aggressive treatment. In this concept, the positive linkage between matrilysin-2 overexpression and poor prognosis may be used not only for identifying colorectal cancer patients with a higher risk of early tumor relapse but also for providing valuable clues to understand the possible mechanism of colorectal cancer invasion and metastasis.

Matrilysin-2 has been reported to be a physiological and pathological activator of pro-MMP-9, and the active form of MMP-9 has been proved to play an important role in the progression and prognosis of colorectal cancer [21]. Previous investigation on human prostate carcinoma proved that cells transfected with antisense matrilysin-2, showing a significant

reduction in matrilysin-2 at the protein level, exhibited a reduction in invasive potential in vitro in addition to a significant diminution in levels of active MMP-9 protein [26]. Moreover, matrilysin-2 and MMP-9 proteins were both expressed in the same human prostate carcinoma tissue samples [26]. Thus, matrilysin-2, in concert with MMP-9, appears to play an important role in tumor progression. Based on these results, it is deduced that the activation of pro-MMP-9 by matrilysin-2 is at least one of the mechanisms that matrilysin-2 is taking part in the progression of colorectal cancer.

In conclusion, we have proved that matrilysin-2 expression increased in colorectal cancer and was associated with tumor progression. The present study also demonstrated for the first time that matrilysin-2 protein expression was an independent prognostic factor for patients with colorectal cancer. Therefore, it is possible that matrilysin-2 may play an important role in the invasiveness and metastasis of colorectal cancer. It is also possible that matrilysin-2 serves as a prognostic marker in clinical practice and even the inhibition of matrilysin-2 using specific inhibitors may become a new therapeutic method for the treatment of colorectal cancer.

Conflicts of interest None

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