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Loss of basement membrane type IV collagen is associated with increased expression of metalloproteinases 2 and 9 (MMP-2 and MMP-9) during human colorectal tumorigenesis

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Breakdown of basement membrane (BM) is believed to be an essential step for tumor invasion and metastases. We have previously demonstrated that matrix metalloproteinase-9 (MMP-9), the 92 kDa collagenase expression correlates with metastases in human colorectal cancer (CRC). This study explores the relationship between the 72 and 92 kDa type IV collagenase (MMP-2 and MMP-9) activities and pattern of type IV collagen expression during human colorectal tumorigenesis. Thirty-four CRC patients, including four synchronous adenomas and one synchronous liver metastases, were involved in this study. By immunohistochemical staining, type IV collagen expression was noted to be continuous in the BM of normal mucosa, adenoma and in two cases of carcinoma *in situ*. Limited or absent type IV collagen staining pattern was seen in 100 (19/19) and 23% (3/13) of CRC with and without metastases, respectively. By double immunostaining, MMP-9 protein expression was noted to localize within areas of limited type IV collagen staining. Similarly, type IV collagen staining was noted to be greatest in areas devoid of MMP-9 expression. Gelatin zymography detected both 92 and 72 kDa proenzyme forms in all CRC and normal mucosa extracts examined. The mean tumor/normal fold increases of the proMMP-2 and proMMP-9 enzyme forms were 1.6 ± 0.1 (mean ± SE) and 2.4 ± 0.5 in adenomas, and 2.1 ± 0.2 and 4.1 ± 0.7 in CRC, respectively. The 62 and 82 kDa bands were present in 63 (12/19) and 74% (14/19) of CRC with metastases, compared with only 20 (3/15) and 33% (5/15) of CRC without metastases, respectively. These differences were significant ($P = 0.045$ and $P = 0.030$, respectively). Our results demonstrate that loss of BM type IV collagen along with elevations in MMP-2 and MMP-9 expression, especially the activated forms, occur during colorectal tumorigenesis. Our data suggest that control of type IV collagenase activation may be beneficial in preventing human colorectal tumor progression.

Introduction

The extracellular matrix (ECM) plays an important *in vivo* role in cell migration, proliferation and differentiation (1). Proteolytic degradation of ECM is a critical event during many physiological and pathological conditions, including embryo morphogenesis, blastocyst implantation, angiogenesis, tissue remodeling, tumor invasion and metastases (2–4). The initial steps of ECM degradation include degradation of cross-linked insoluble collagen and elastin fibers. The matrix metalloproteinases (MMPs), in particular the type IV collagen specific collagenases, MMP-2 and MMP-9, participate in the degradation of ECM components including the basement membrane (BM) (2,3), which separates epithelia from stroma.

The type IV collagen specific collagenases, encoded by two distinct genes, are secreted in latent form, which require activational cleavage yielding the 62 and 82 kDa active enzymes, respectively. *In vitro* observations have independently shown that MMP-2 and MMP-9 production and type IV collagen content correlate with metastatic potential (5). In addition, increased MMP-2 and MMP-9 expression as well as loss of BM type IV collagen (6–8) have also been reported in many human solid tumors (9–21).

We have previously demonstrated a progressive increase in MMP-9 mRNA with advancing stages of human CRC (10) as well as the independent prognostic value of CRC tissue MMP-9 levels (22). Furthermore, although absent in normal liver, we demonstrated the active form of MMP-9 in liver metastasis, which suggests that proMMP-9 activation may be a pivotal event during CRC liver metastasis formation (23). The current study was undertaken in order to examine the relationship between levels of active MMP-2 and MMP-9 isoforms and type IV collagen content during CRC tumorigenesis.

Materials and methods

Patients and tissue processing

Thirty-four CRC patients were involved in this study including four patients with synchronous adenoma and CRC and one patient with synchronous CRC and liver metastases. The patients' clinicopathological characteristics are summarized in Table I.

Each case consists of primary tumor and paired adjacent normal mucosa. Surgical samples were obtained immediately after resection with the approval of the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center. They were quick-frozen in liquid nitrogen and stored at –80°C until processed. The diagnosis was confirmed by pathological assessment on hematoxylin and eosin stained paraffin sections. Tissue was thawed, weighed and homogenized in Tris buffer (50 mM Tris–HCl, pH 7.5, containing 75 mM NaCl, 1% Triton, 0.1 SDS) and centrifuged at 5000 *g* for 20 min at 4°C, as previously described (24). Protein concentration of the supernatant was determined with a protein assay reagent according to the manufacturers' instruction (Bio-Rad Laboratories, Hercules, CA). The supernatant of tumor and paired normal mucosa were used for zymography. Frozen tissue was embedded in OCT (Miles, Elkhart, IN) and frozen in 2-methylbutane cooled with liquid nitrogen.

Immunohistochemistry

Immunohistochemical staining using the standard avidin–biotin–peroxidase complex (ABC) technique was performed as previously described (24). Briefly, immediately before staining, frozen sections were fixed in 4°C acetone then treated with 1% hydrogen peroxide for 15 min. For the reduction of non-specific background staining, slides were incubated with diluted normal blocking serum for 20 min at room temperature. The serum was drained off and sections were incubated at 4°C overnight with type IV collagen monoclonal antibody CIV 22 (DAKO Corporation Glostrup, Denmark) diluted to 1:100. This monoclonal antibody is directed against collagen IV and shows characteristic staining of basement membrane in a variety of tissues and organs (25). After washing, the slides were incubated with diluted biotinylated secondary

Abbreviations: ABC, avidin–biotin–peroxidase complex; BM, basement membrane; CRC, colorectal cancer; ECM, extracellular matrix; Mab, monoclonal antibody; MMP-9, matrix metalloproteinase-9; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.

Table I. Clinicopathological characteristics of 34 patients

Parameter	No. of patients (%)
Age (years)	
Median	68
Range	41–87
Gender	
Male	16 (47)
Female	18 (53)
Tumor size (cm)	
Median	4.0
Range	1.5–10.0
Primary site	
Right colon	13 (38)
Left colon	14 (41)
Rectum	7 (21)
Dukes' stage	
A	8 (23)
B	7 (21)
C	6 (18)
D	13 (38)

antibody solution for 30 min, rinsed with phosphate-buffered saline (PBS) and then incubated with the Vectastain Elite ABC Reagent for 30 min. Following this, tissue sections were rinsed in PBS and developed by DAB (0.06% 3,3′-diaminobenzidine tetrahydrochloride) solution.

Type IV collagen and MMP-9 double immunostaining was performed using an indirect immunoperoxidase technique. The sections were first incubated with MMP-9 AB-2 (Oncogene Research Products, Cambridge, MA) overnight at 4°C. This monoclonal antibody Mab is generated by immunizing mice with MMP-9 protein partially purified from the conditioned media of PMA-stimulated HT1080 human fibrosarcoma cells (26). MMP-9 (Ab-2) only recognizes the latent (92 kDa) form of human MMP-9. The sections were incubated with biotinylated anti-mouse antibody, followed sequentially by streptavidin–peroxidase complex (Vector Laboratories Inc., Burlingame, CA). The dark brown immunostaining was developed with 3,3′-diaminobenzidine chromagen. For sequential double staining, an additional blocking step was performed for 1 h. The sections were then incubated with the type IV collagen monoclonal antibody at 4°C overnight. This was followed by incubation with peroxidase-conjugated human anti-mouse IgG and conjugated alkaline phosphatase (Vector Laboratories Inc.). Type IV collagen reactive sites were detected with Vector red (Vector Laboratories Inc). Sections were counterstained with modified Harris-hematoxylin (Fisher Scientific, Pittsburgh, PA) and 0.3% ammonia water and passed through graded alcohols and xylene to dehydrate. Slides were mounted and then observed by conventional light microscopy.

Gelatin zymography

Eight percent sodium dodecyl sulfate (SDS)–polyacrylamide electrophoretic gels copolymerized with 1 mg/ml gelatin were used to detect both latent and activated forms of gelatinase. An aliquot of 50 μg of total protein was separated by electrophoresis under non-denaturing conditions. Following electrophoresis, gels were washed twice in 2.0% Triton X-100 for 30 min at room temperature with shaking to remove SDS. Zymograms were subsequently developed by incubation overnight at 37°C in collagenases buffer [0.2 M NaCl, 5 mM $CaCl_2$, 1% (v/v) Triton X-100 and 0.02% NaN_3 in 50 mM Tris–HCl, pH 7.4]. Zymograms were stained with 1 wt/vol Coomassie blue G-250 dissolved in 30% methanol containing 10% v/v glacial acetic acid at room temperature for 60 min. Gels were destained in the same solution but without the Coomassie blue stain.

Medium conditioned by RPMI-7951 melanoma cells and by RA3-1S7 cells, which were derived from primary rat embryo fibroblasts by co-transfection with the Ha-*ras* and adenovirus *E1A* oncogenes (kindly provided by Dr R.J.Muschel), were used as 72 and 92 kDa gelatinase controls, respectively.

Gelatinolytic activity was visualized as a clear band against a dark background of stained gelatin and quantitated with a Bioimage Whole Band Analyzer. The enzyme activity levels are expressed as the fold-increase in expression of the 92, 82, 72 and 62 kDa bands in tumor relative to that measured in the corresponding adjacent normal mucosa.

Western blot analysis

These were carried out as previously described (24). The supernatant of total tissue protein (125 μg) was electrophoresed on an 8% SDS–PAGE gel using a MINIGEL apparatus (Bio-Rad, Richmond, CA). Proenzyme MMP-9 (Oncogene Research Products, Cambridge, MA) 10 ng was loaded as a positive control. Separated proteins were transferred to nitrocellulose membranes (Amersham, Buckinghamshire, UK) in Tris–glycine buffer (2.5 mM Tris, 192 mM glycine and 20% methanol) at 4°C and 100 V using a MINI system. Non-specific binding sites were blocked for 1 h at room temperature with a solution containing 4% bovine serum albumin. The blots were incubated overnight at 4°C in a solution containing MMP-9 (Ab-2), the same monoclonal antibody that was used for immunohistochemical staining. The blot was washed several times with TBS-T (10 mM Tris buffer containing 150 mM NaCl, 0.5% Tween 20), followed by an incubation step with horseradish peroxidase labeled anti-mouse antibody (1:5000 in TBS-T for 30 min at room temperature). Reactive proteins were visualized with an enhanced chemiluminescence detection system (ECL, Amersham) as described by the manufacturer.

Statistical analysis

Differences in MMP-2 and MMP-9 enzymatic activities between tumor and paired normal tissue were assessed by a paired *t*-test. The relationship between proMMP-2 and proMMP-9 activities and clinical variables between the two groups was analyzed by the Student's *t*-test. The differences among multi-groups were analyzed by ANOVA.

Results

Distribution of type IV collagen expression

Figure 1 shows the immunohistochemical staining of type IV collagen in normal colonic mucosa, and benign and malignant colorectal tumors. As shown in Figure 1A, strong and well defined type IV collagen staining is seen in the BM of normal glandular duct epithelium (head arrow) and around blood vessel walls (long arrow). The epithelial cells themselves show negative staining. In all four adenomas, type IV collagen BM is intact and continuous (Figure 1B). Various type IV collagen distributions are observed in CRC (Figure 1C–F). Figure 1C shows the expression of type IV collagen in carcinoma *in situ* where it is usually continuous (long arrow) with occasional focal interruptions (head arrow). Significant loss of type IV collagen expression, represented as discontinuous (Figure 1D) or thin staining patterns (Figure 1E) as well as limited or absent type IV collagen staining (Figure 1F), was noted in invasive CRC. Limited or absent type IV collagen staining patterns were seen in 100 (19/19) and 23% (3/13) of CRC with and without metastases, respectively. In addition, type IV collagen staining of blood vessels disappeared in CRC of advanced stages.

Decreased type IV collagen in areas of MMP-9 expression

The co-expression of MMP-9 and type IV collagen was determined by double immunostaining. MMP-9 is expressed as dark brown staining and type IV collagen as red staining. Figure 1G–I demonstrate, in a representative primary CRC specimen, strong MMP-9 staining in areas of diminished type IV collagen staining. In contrast, type IV collagen staining is strongly positive in MMP-9 negative areas.

Type IV collagenase activity

Figure 2 shows the results of zymographic analysis of six CRC and paired normal mucosa. Extracts produced zymographic lytic bands ranged in size from 62 to 180 kDa. Gelatin zymography detected the latent proforms (72 and 92 kDa) and activated enzyme (62 and 82 kDa) forms of MMP-2 and MMP-9 as well as MMP-9 dimers (150–180 kDa); these were probable complexes not separated under non-denaturing zymographic conditions (27,28). Gelatinolytic activities varied greatly amongst samples, ranging from a strong band to only trace or absent amounts. Gelatinolytic bands corresponding to 130, 92, 82, 72 and 62 kDa were confirmed as MMP activity

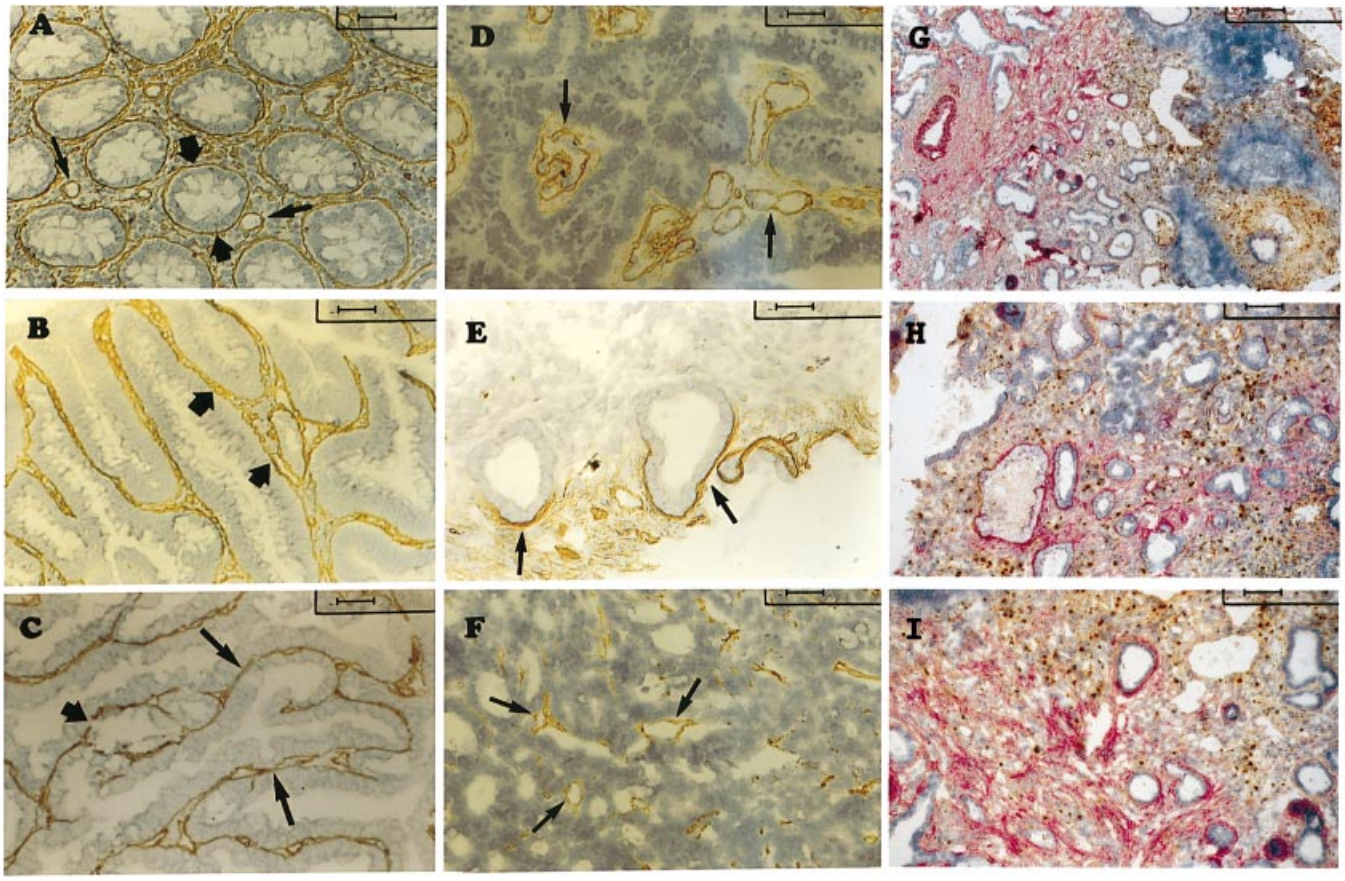

Fig. 1. Distribution of type IV collagen during human colorectal tumorigenesis. (**A**) Normal colonic mucosa. Stronger and well-defined staining of immunoreactive BM type IV collagen is seen in glandular duct epithelium (large arrow) and around the blood vessels (small arrow). (**B**) Colon adenoma. Adenomatous tubules are surrounded by a continuous layer of type IV collagen staining. (**C**) Carcinoma *in situ*. The continuity of type IV collagen staining at the interface between cancer cells and stroma is noted. Focal interruptions indicate areas of invasion (arrows). (**D–F**) Colorectal cancer. Limited or very weak type IV collagen staining is seen in CRC. Although scattered areas of BM show type IV collagen staining, there is an overall absence of type IV collagen immunoreactivity. (**G–I**) Sections were double-stained to detect MMP-9 (dark brown) and type IV collagen (red). In general type IV collagen is preferentially expressed in areas where MMP-9 expression is absent.

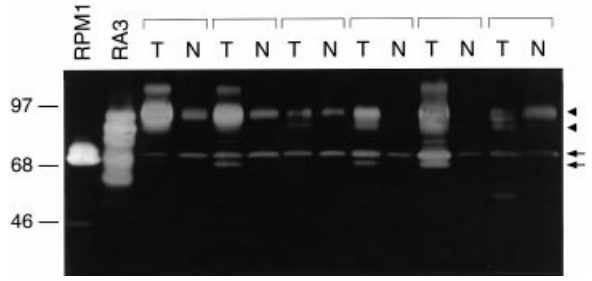

Fig. 2. Type IV collagenase activity detected by zymography. Control lane RPM1: 25 μg of medium conditioned by RPMI-7951 melanoma cells expressing 72 kDa progelatinase. Control lane RA3: medium conditioned by RA3.1S7 cells expressing both the 92 kDa progelatinase and 82 kDa activated forms. Tumor (T) and normal mucosa (N) extracts (25 μg) from each patient were separated on an 8% SDS–polyacrylamide gel containing 1 mg/ml gelatin. The positions of proenzymes 92 and 72 kDa and their activated forms 82 and 62 kDa are noted by arrows.

since they were completely inhibited by EDTA (data not shown).

Zymographic analyses demonstrated that both MMP-2 and MMP-9 proforms were present in all colonic adenomas, CRCs as well as corresponding normal mucosa extracts. As shown in Figure 2, CRC produce more MMP-9 than MMP-2. Densitometric quantitation of proMMP-2 and proMMP-9 activity in both tumor and normal tissue are summarized in Table II. The mean tumor/normal fold increase of proMMP-2 and proMMP-9 was 1.6 ± 0.1 (mean ± SE) and 2.4 ± 0.5 in adenomas, and 2.1 ± 0.2 and 4.1 ± 0.7 in CRC, respectively (Figure 3). The relationship between levels of proMMP-2 and proMMP-9 activities and clinicopathological parameters are shown in Table III. The activity of the proforms of MMP-2 and MMP-9 did not significantly correlate with any clinicopathological parameters.

In contradistinction, the activated forms of MMP-2 and MMP-9 (62 and 82 kDa bands) were absent or minimally detected in normal mucosa and adenomas ($n = 4$). However, the 62 and 82 kDa bands were present in 63 (12/19) and 74% (14/19) of CRC with metastases compared with only 20 (3/15) and 33% (5/15) of CRC without metastases, respectively (Figure 4). These differences were statistically significant ($P = 0.045$ and $P = 0.030$).

Expression of MMP-9 in colorectal adenomas, carcinomas and liver metastases

In order to investigate MMP-9 protein expression during human colorectal tumorigenesis and progression, western blot analysis was performed on total tissue protein isolated from four cases of synchronous adenoma and colon cancer samples as well as one case with synchronous liver metastases. Figure 5 is a representative sample of case no.33, a synchronous

Table II. Clinicopathological characteristics of 34 colorectal cancers and MMP-2 and MMP-9 enzymatic activities

Case	Age	Sex	Loc[a]	Diff[b]	Size (cm)	Dukes' stage	Proform activities (T/N fold increase)		Activated form ± presence	
							92 kDa	72 kDa	82 kDa	2 kDa
1	58	F	L	M	6.5	B	4.3	4.5	+	–
2	87	F	L	P	3.5	C	3.1	1.8	+	+
3	64	M	L	M	3.5	A	4.1	2.6	+	–
4	70	F	RE	M	4.5	B	1.2	1.2	–	–
5	82	F	RE	W	6.0	D	16.4	1.1	+	+
6	84	M	R	M	3.5	A	2.1	0.8	–	–
7	76	M	L	M	3.5	C	6.6	1.0	–	+
8	68	M	L	M	3.5	D	0.9	1.5	–	–
9	62	F	RE	W	7.5	A (CIS[c])	5.0	2.9	–	+
10	49	F	R	M	3.5	B	1.9	2.0	+	–
11	67	F	R	M	6.0	D	1.3	2.0	–	–
12	59	F	L	W	6.5	B	1.0	3.2	–	–
13	69	M	R	P	6.0	D	4.2	2.6	+	+
14	57	M	R	M	10.0	A	2.2	1.9	–	–
15	83	M	R	M	8.0	B	3.5	1.5	–	–
16	59	M	RE	M	4.2	D	10.1	3.9	+	+
17	81	F	R	M	3.0	C	1.3	1.9	+	+
18	69	F	L	P	2.4	D	6.3	2.1	+	–
19	54	F	L	M	4.5	D	4.1	1.0	+	–
20	74	F	R	M	4.0	D	7.0	4.1	+	+
21	52	F	L	M	2.0	A	2.4	0.6	–	–
22	60	M	L	P	7.0	D	1.8	1.7	+	+
23	64	M	L	M	3.5	C	1.4	2.8	–	–
24	61	M	R	M	5.0	D	2.4	1.6	+	+
25	73	M	R	P	5.0	D	0.9	2.4	+	+
26	72	M	L	M	3.5	C	1.7	1.8	+	+
27	62	M	RE	M	4.0	C	1.9	2.9	+	+
28	72	F	RE	M	1.5	A (CIS)	0.3	0.5	–	–
29	82	F	R	M	2.5	A	8.6	1.7	–	–
30	41	F	RE	M	3.0	A	2.7	2.3	–	–
31	58	M	R	M	7.0	B	11.6	2.0	+	–
32	71	M	R	M	3.5	D	0.6	3.6	+	+
33	77	M	L	M	5.0	B	6.1	4.0	+	–
34	47	M	L	M	5.0	D	11.1	1.1	–	–

[a]Loc refers tumor localization. R, Right colon; L, left colon; S, sigmoid colon; RS, rectum-sigmoid colon; RE, rectum.
[b]Diff refers to tumor differentiation: W, well; M, moderate; P, poor.
[c]CIS, carcinoma *in situ*.

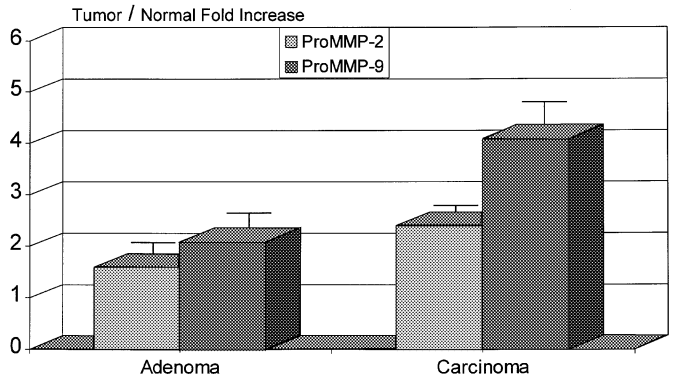

Fig. 3. Quantitation of proenzyme MMP-2 and MMP-9 levels in human colorectal cancer. Data are presented as the mean ± SE of tumor/normal mucosa fold-increase from the density signals of gelatin zymography and analyzed by a Bioimage Whole Band Analyzer.

adenoma and CRC, and case no.34, a synchronous liver metastases. In case no.33, the 92 kDa band, representative of proMMP-9 was of relatively low abundance in normal mucosa (lane N). Although the colon adenoma (lane A) samples exhibited moderate levels of MMP-9, proMMP-9 level was highly increased in CRC (lane C). In case no.34, both primary CRC (lane C) and liver metastases (lane LM) show a great increase in MMP-9 when compared with corresponding normal tissue.

Discussion

A large body of evidence supports the notion that malignant CRCs arise from pre-existing benign tumors (adenoma) through multiple steps, including normal epithelium hyperplasia, adenomatous polyp, dysplasia, carcinoma *in situ*, non-invasive cancer and ultimately invasive carcinoma. Our data demonstrate that loss of BM type IV collagen is involved in human colorectal tumorigenesis. Despite extensive architectural disorganization, benign tumors retain continuous type IV collagen staining in the BM, which separates the tumorigenic epithelium from stroma. In contrast, focal defects in BM type IV collagen staining are apparent in carcinoma *in situ* lesions. Furthermore, invasive CRC demonstrate zones devoid of type IV collagen staining around invasive tumor cells.

Although breakdown of BM is achieved by several MMPs (29), MMP-2 and MMP-9 appear to be most important for

Table III. The correlation between proMMP-2 and proMMP-9 activities and clinicopathological parameters in primary colorectal cancer

Parameters	Cases		ProMMP-2 (72 kDa)		ProMMP-9 (92 kDa)	
	No.	%	Mean ± SE	*P*-value	Mean ± SE	*P*-value
Age (years)						
<60	3	8.8	1.79 ± 0.37		5.22 ± 2.94	
60–70	18	52.9	2.28 ± 0.23		3.67 ± 0.71	
>70	13	38.3	2.01 ± 0.33	0.66	4.50 ± 1.26	0.74
Tumor location						
Right	13	38.3	1.91 ± 0.21		3.23 ± 0.90	
Left	14	41.1	2.35 ± 0.32		4.31 ± 0.77	
Rectum	7	20.6	2.11 ± 0.46	0.55	5.37 ± 2.23	0.48
Tumor size (cm)						
3.0	5	14.7	1.43 ± 0.38		4.05 ± 1.50	
3.1–5.0	15	44.1	2.18 ± 0.28		3.19 ± 0.71	
>5.0	14	41.2	2.33 ± 0.27	0.26	5.13 ± 1.26	0.934
Tumor differentiation						
Well	3	8.8	2.41 ± 0.67		7.48 ± 4.62	
Moderate	26	76.5	2.11 ± 0.22		4.08 ± 0.65	
Poor	5	14.7	2.07 ± 0.18	0.89	2.25 ± 0.61	0.16
Depth of tumor bowel wall invasion (T stage)						
T_1	3	8.8	1.34 ± 0.80		2.52 ± 1.36	
T_2	6	17.7	2.38 ± 0.44		4.25 ± 1.08	
T_3	22	64.7	2.25 ± 0.22		3.91 ± 0.73	
T_4	3	8.8	1.56 ± 0.24	0.37	6.95 ± 4.78	0.53
Metastases (lymph nodes and/or distant)						
Negative	15	44.1	2.11 ± 0.30		3.79 ± 0.79	
Positive	19	55.9	2.15 ± 0.22	0.91	4.37 ± 0.99	0.66

Student's *t*-test for comparison of two groups; *F*-test for comparison of more than two groups.

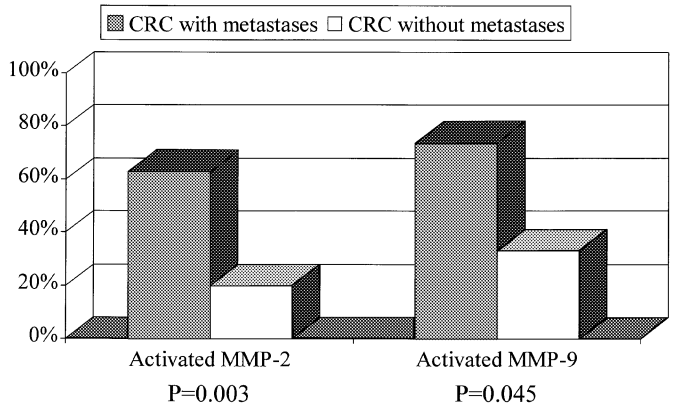

Fig. 4. Correlation between MMP-2 and MMP-9 enzymatic activity levels and colorectal cancer metastases. The activated forms of MMP-9 (left) and MMP-2 (right) are more likely to be seen in CRC with lymph nodes and/or distant metastases than in those without metastases ($P = 0.045$ and $P = 0.030$).

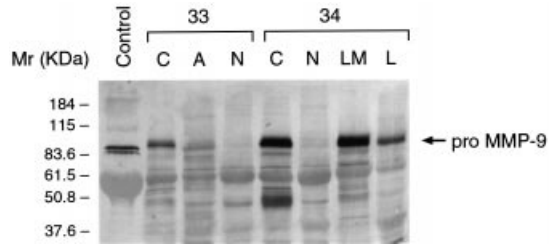

Fig. 5. Western blot analysis of MMP-9 protein in colorectal cancer tumorigenesis and progression. Tumor and paired normal tissue extracts from each patient were separated on an 8% SDS–PAGE gel and transferred to a nitrocellulose membrane, which was incubated with an antiMMP-9 monoclonal antibody and visualized as described in Materials and methods. Proenzyme, MMP-9 (10 ng) was loaded as a positive control. The position of MMP-9 is noted.

BM type IV collagen degradation (2,4,30). *In vivo* support for the importance of type IV collagenases in BM type IV collagen degradation include the localization of proMMP-9 to the tumor BM zone of skin tumors (31) as well as our previous study, which localized MMP-9 mRNA and protein in tumor stroma of human CRC rather than CRC cells themselves (24). The double immunostaining data presented in this report provide the first morphological evidence that type IV collagen degradation correlates with increased expression of MMP-9 in human CRC. Furthermore, our gelatin zymographic data demonstrate a parallel decrease in type IV collagen expression with increased proMMP-2 and proMMP-9 activities in CRC compared with paired normal mucosa and adenoma. Although both latent MMP-2 and MMP-9 are elevated in CRC, a significant correlation between metastatic phenotype and MMP-2 and MMP-9 levels was only noted with the activated forms. It therefore appears that overproduction of proMMP-2 and proMMP-9 is necessary but not sufficient for the development of the invasive phenotype whereas activation of MMP-2 and MMP-9 are required for full acquisition of the metastatic CRC phenotype. Thus, activation is a critical step in the regulation of MMP-dependent proteolytic activity.

MMP-2 and MMP-9 share structural and catalytic similarities, but their response to regulatory signals differ (32) The

mechanism by which the proMMP-2 and MMP-9 are activated in human CRC is not well understood. However, cell membrane-bound metalloproteinases (MT-MMP) have been shown to activate MMPs (33,34). The complex of proMMP-2 and TIMP-2 binds to activated MT-MMP and this binding ultimately results in activation of MMP-2 (35). which, may in turn, activate proMMP-9 to a Mr 82 000 form (35). Activated MMP-2 and MMP-9 can degrade basement membrane type IV collagen to yield 1/4 N-terminal and 3/4 C-terminal fragments (10,36). Co-expression of active MMP-2 and MMP-9 in human CRC suggest that these interactions occur *in vivo* and lead to activation and degradation of ECM. Recently, a unique interaction between proMMP-9 and the 2 chain of type IV collagen has been described, which may play a role in facilitating zymogen to cell-matrix contacts and degradation of type IV collagen network (37).

MMP-2 and MMP-9 activities are increased in CRC as well as breast and prostate cancer relative to corresponding normal tissue (11,18). However, other MMPs such as stromelysin-1 (MMP-3) and matrilysin (MMP-7) may also contribute to CRC progression (38). Since MMP-7 is expressed in both benign and invasive colorectal tumor, whereas MMP-3, stromelysin-3 (MMP-11) and MMP-2 are expressed only in invasive CRC (38), it is likely that a number of MMPs play an important role in various stages of CRC initiation and progression.

In summary, our results demonstrate that loss of BM type IV collagen occurs during colorectal tumorigenesis and is associated with elevations in the active forms of MMP-2 and MMP-9. Furthermore, by double immunostaining, our data provide the first morphological evidence that type IV collagen degradation correlates with local elevations in MMP-9 expression, thereby supporting the notion that control of type IV collagen degradation via prevention of type IV collagenase activation, may be beneficial in preventing human colorectal tumor progression.

Acknowledgements

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