Matrix Metalloprotease 2 (MMP-2) and Matrix Metalloprotease 9 (MMP-9)

Type IV Collagenases in Colorectal Cancer

Nina-Beate Liabakk,1 Ian Talbot, Rose Ann Smith, Kay Wilkinson, and Frances Balkwill2

ABSTRACT

Using quantitative zymography, we measured activity of the type IV collagenases metalloprotease 2 (MMP-2) and MMP-9 in 192 biopsies from colorectal carcinomas, adenomas, and normal bowel. The median level of MMP-9 in samples from Dukes’ stage A (n = 18) or C (n = 48) tumors was significantly higher than in stage B carcinomas (n = 65), adenomas (n = 25), and normals (n = 36; P = 0.0001). The median level of active MMP-2 was significantly higher in stage A or C compared with adenomas (P = 0.0001) and normals (P = 0.0001). The median level of inactive MMP-2 was higher in all Dukes’ stages compared with normals and adenomas (P = 0.0001). There was a significant increase in inactive MMP-2 from Jass prognostic groups I–IV (P = 0.006) but no correlation with the active enzyme. MMP activity was not related to tumor differentiation, colon versus rectal location, or disease-free, 5-year survival. All groups expressed mRNA for both enzymes, but there were quantitative and locational differences in MMP-2 mRNA expression between normal, benign, and malignant tissues. Thus MMP-2 is controlled at the level of mRNA and protein production and activation in colorectal cancer, and active MMP-2 and MMP-9 enzymes are associated strongly with Dukes’ A and C stages of the disease. Variations in MMP levels with the stage or prognostic group of colorectal cancer reflect their differing stromal content.

INTRODUCTION

Degradation of the extracellular matrix in tissue surrounding the tumor is a critical event in the process of cancer invasion and metastasis. Matrix degradation is believed to be caused by the action of proteolytic enzymes, including several types of MMPs. The MMPs are a family of highly homologous enzymes, involved in both physiological processes and in pathological conditions. To date, 11 different MMPs have been identified, which differ in molecular weight, function, localization, and substrate specificity. The first barrier for an invading epithelial tumor is the basement membrane, mainly consisting of type IV collagen. The type IV collagenases and gelatinases, MMP-2 (Mr 72,000) and MMP-9 (Mr 92,000), degrade type IV collagen, gelatin, and fibronectin. These type IV collagenases act by activating proenzymes and are activated by removal of an 80-amino acid inhibiting sequence from the NH2 terminus. The processes leading to induction and activation of MMPs are dependent on both cytokines (4, 5) and specific MMP inhibitors (1). Recently, the first membrane-bound MMP, membrane type MMP, was identified (6). Membrane type MMP is expressed on the surface of tumor cells and is thought to be involved in the activation of MMP-2 in stromal cells. In addition, a tumor cell-derived collagenase stimulatory factor, extracellular matrix metalloproteinase inhibitor, which activates fibroblasts to express metalloproteinases, has been identified (7).

MMP-2 and MMP-9 are expressed in different human epithelial cancer types such as breast (8), bladder (9), ovarian (10), and prostate cancer (11), and their levels seem to be related to malignancy and invasion. In colorectal cancer, Levy et al. (12) demonstrated increased tissue levels of MMP-2 in colorectal tumors using immunohistochemical techniques. Zucker et al. (13) demonstrated increased levels of MMP-9 activity in plasma of patients with colon cancer, and Onisto et al. (14) used RT-PCR to detect MMP-2 and MMP-9 mRNA in human colon adenocarcinomas. Although these studies indicate that MMP-2 and MMP-9 are present in colorectal cancer, they either described small numbers of samples or were not quantitative.

Colorectal cancer offers a good model to define the involvement of the type IV collagenases in tumor progression. Adenomas are well defined, and the adenocarcinomas can be classified into distinct biological and prognostic stages. Dukes’ stage A tumors are limited to the bowel wall; Dukes’ stage B tumors have penetrated the muscularis propria, and Dukes’ stage C tumors have spread to the regional lymph nodes. The more recent Jass classification system (16) uses four variables selected by means of Cox regression analysis as having an important and independent influence on survival. These are, the number of positive lymph nodes with metastatic tumors, the character of the invasive margin, peritumoral lymphocytic infiltration, and local spread. These variables are used to divide colorectal tumors into four biological and prognostic groups, with a Jass score of 1 if none or one of these variables occurs at an unfavorable level and a Jass score of 4 if most of the four factors are unfavorable.

Therefore, we have used quantitative zymography to measure enzyme levels in samples from colorectal cancer and have related these to the well-defined biological stages of this disease and 5-year, disease-free survival. MMP levels in tumor samples were compared with those in normal and adenomatous bowels. The expression of MMP-2 and MMP-9 mRNA was assayed by RT-PCR. In situ hybridization was used to assess local levels and the cellular source of MMP-2 mRNA in the tumors and the association between mRNA and protein expression further.

MATERIALS AND METHODS

Tissue Samples. All tissue samples were cryopreserved in liquid nitrogen following their removal from the patient. Samples were taken at least 5 years ago and stored at −70°C until use. The age of the sample did not seem to affect the enzyme activity. For example, enzyme levels and the number of positive samples differed between the Dukes’ stage B and C cases, but the mean age of Dukes’ stage B samples was 8.6 ± 1.3 years, and the mean age of Dukes’ stage C samples was 8.9 ± 1.2 years. Cryostat sections were cut and stored at −70°C before zymography, protein estimation, in situ hybridization, and RNA isolation. Tumor samples were obtained from patients with colorectal cancer at different stages of malignancy, classified according to Dukes (15) and Jass et al. (16). Dukes’ stage A tumors (n = 18) are tumors limited to the bowel wall. Dukes’ stage B (n = 65) denotes tumors that have penetrated the muscularis propria. In Dukes’ stage C (n = 48), the tumors have spread to involve the regional nodes. In addition, benign bowel tumors (adenomas; n = 25) and normal biopsies (n = 36) were obtained. The normal samples were obtained from colorectal cancer patients but farther away from the tumor. The classification of Jass et al. (16) was used to divide the same colorectal tumors into four groups and uses the above information combined with four variables...
selected by means of Cox regression analysis as having an important and independent influence on survival: lymph nodes with tumor deposits (none, score 0; one to four, score 1; and more than four, score 2), character of the invasive margin (expansive, score 0; and irregularly infiltrating, score 1), peritumoral lymphocytic infiltrate (conspicuous, score 0; and inconspicuous, score 1), and extent of local spread (within the bowel wall, score 0; and outside the bowel wall, score 1). Summation of the scores gives a range of possible total values from 0 to 5. The scores are divided into four prognostic groups: 0 or 1, group I; 2, group II; 3, group III; and 4 or 5, group IV. This classification incorporates a quantitative assessment of stromal inflammatory infiltrate.

Gelatin Zymography. For gelatin zymography, 5-μm cryostat tumor sections were homogenized in 50 μl SDS-PAGE sample buffer containing SDS (1% [w/v]), glyceral [10% (w/v)], and bromophenol blue, using tissue microhomogenizers (Scott Laboratory). Gelatinolytic zymography was performed as described earlier (17). The amount of type IV collagenase activity present in the sample was quantitated by computer-assisted image analysis, as described by Davies et al. (8). Conditioned media from ICRF 23 fibroblast cells and from 12-O-tetradecanoylphorbol-13-acetate-stimulated HT 1080 fibrosarcoma cells were used as collagenase standards. ICRF 23 secretes active (M, 62,000) and inactive (M, 72,000) MMP-2 constitutively, and the activity present in 13.5 μl standard stored bulk of conditioned media, as detected by zymography, was defined as 60 arbitrary units. This standard was calibrated against our original standard (8), and, thus, results from this article are comparable to our previous publications. The assay conditions allow the inactive enzyme to degrade gelatin, and, thus, it can be measured as a band discrete from the active enzyme. 12-O-tetradecanoylphorbol-13-acetate-stimulated HT 1080 secreted Mr 92,000 MMP-9, and the activity in 12 μl conditioned media was defined as 60 arbitrary units. The resolution of 11% acrylamide gels was insufficient to distinguish between the inactive proform of Mr 92,000 type IV collagenase and its Mr 81,000 activated form. The bands were verified as MMP-2 and MMP-9 using specific monoclonal antibodies (Onogene Science, Cambridge, MA) and Western blotting.

Reproducibility of Zymography. Samples from different parts of the block, at least six sections away from each other, were assayed to assess reproducibility. We expected the levels of the enzyme to vary with the ratio between stromal cells, the extracellular matrix, and the tumor cell component of the tissue in each section. However, we found a good concordance between samples from the same block in terms of high, moderate, low, or negative values. Typical data on five patients are shown in Table 1.

Protein Estimation. For protein estimation, five 5-mm cryostat sections from each tumor were homogenized in 1% (w/v) SDS and diluted 10-fold in water before measuring the protein content against BSA using the Bio-Rad protein assay reagent (18).

In Situ Hybridization. In situ hybridization was carried out on cryostat sections as described previously (8). The antisense MMP-2 riboprobe was generated from the pGEM3-72K (kindly provided by British Biotech, Oxford, United Kingdom) using SP6 RNA polymerase (Promega Biotech, Madison, WI). The negative control was sense RNA generated from the same vector linearized in the opposite direction, using T7 RNA polymerase (Promega). Cytosins from the ovarian cancer cell line SKOV were used as a positive control. In vitro transcriptions were performed using Promega Biotech transcription kits to incorporate 35SUTP (Amersham International; SJ 1303). In a detailed analysis of 16 different cases, the entire section was divided into low-power fields (×10 objective; approximately 10 fields/case). These were scored for positivity and approximate percentages of positive cells (>10, >5, >2, and <1%). A field was scored positive if any positive cell was detected within it, and then the number of positive cells was estimated.

Detection of MMP mRNA by RT-PCR. Total RNA was isolated from 25 20-μm thick cryostat tumor sections as described by Chomczynski and Sacchi (19). cDNA was synthesized from 2–10 μg RNA using the cdNA Synthesis Kit (Boehringer Mannheim AG, Mannheim, Germany) for the first-strand synthesis only. PCR reactions were performed using the GeneAmp PCR reagent kit (Perkin-Elmer Corp., Norwalk, CT) and specific primers for amplification of MMP-2 and MMP-9 type IV collagenases and GAPDH. The MMP-2 primers (kindly provided by British Biotech) gave an amplified PCR fragment of 1085 bp. The MMP-9 (14) and GAPDH primers gave specific PCR fragments of 640 and 269 bp, respectively. The following primers were used: GAPDH, 5′-TGA AGG TCG GTG TGA AGC GAT TTG G-3′ and 5′-AGC ACA TAC TCA GCA CCA GCA TCA C-3′; MMP-2, 5′-CTT CGC CCC AGG CAC TGG TG-3′ and 5′-CCT CCC TCC CAT GGG CTT CGG T-3′; and MMP-9, 5′-GTT CCC CCC ACT GCT GGC TCT ACG GCC-3′ and 5′-GTC CTC AGG CTA CTC GAT GAG GAT ATG-3′.

The cDNA was subjected to 35 cycles of PCR amplification for GAPDH, MMP-2, and MMP-9 (MMP-9 was also subjected to 70 cycles in some experiments) in a Techne (Cambridge, United Kingdom) Thermal Cycler. Each cycle consisted of 30 s of denaturation at 94°C, 1 min at a primer-specific annealing temperature (60°C for GAPDH and 68°C for MMP-2 and MMP-9), and 1 min of primer extension at 72°C. The PCR products were visualized after electrophoresis on a 1.2% agarose gel containing ethidium bromide. Total RNA isolated from the human fibrosarcoma cell line HT 1080 was used as a positive control for amplification of both MMP-2 and MMP-9. To validate the PCR, agarose gels were depurinated in 0.25 M HCl for 15 min, neutralized in 0.4 M NaOH for 5 min, and alkaline blotted onto Hybond N+ (Amersham) as described before (20). Following transfer, the membrane was hybridized to the 32P-labeled inserts of human cDNA probes of MMP-2 and MMP-9 under standard conditions as outlined earlier (21, 22). Membranes were washed subsequently to high stringency and exposed to Kodak XAR5 film at room temperature for 10 min.

Statistical Analysis. P values for comparison of median enzyme values between normals, adenomas, and Dukes’ stages A–C were calculated using the Mann-Whitney two-sample test. Median values were used in these analyses because of the large number of zero values obtained. The Mann-Whitney test was also used to assess relationships between enzyme levels and 5-year, disease-free survival. The Kruskal-Wallis test and the nonparametric trend test (23) were used to compare median enzyme levels between Jass groups I, II, III, and IV. P values for the difference in proportion of the percentage of positive samples were calculated using Fisher’s exact test.

RESULTS

Zymography. Type IV collagenase activity was assessed in 36 normal biopsies, 25 adenomas, and 131 malignant colorectal tumors of varying stages. For each tumor sample, active (M, 62,000) and inactive (M, 72,000) MMP-2 and MMP-9 (M, 92,000) were measured by their gelatinolytic activity. A computer-assisted image analysis was used to quantify the results. The results were expressed in arbitrary units/10 μg protein. Conditioned media from human tumor cell lines, which are known to produce type IV collagenases, were used as standards, as described in “Materials and Methods.”

Table 1 Reproducibility of zymography assay

<table>
<thead>
<tr>
<th>Patient</th>
<th>M, 62,000</th>
<th>M, 72,000</th>
<th>M, 92,000</th>
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<td>262</td>
<td>7.95</td>
<td>13.55</td>
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<td>0</td>
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<tr>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<tr>
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<td>1.68</td>
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<td>530</td>
<td>5.2</td>
<td>12.48</td>
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and adenomas, \( P = 0.0001 \). MMP-9: Dukes’ stages A and C versus stage B, normals, and adenomas and normals, \( P = 0.0001 \). The median level of active MMP-2 was higher in Dukes’ stage C compared with stage B, but this difference did not reach significance (5.6 versus 0; \( P = 0.06 \)). However, the levels of active MMP-2 in Dukes’ stage A were significantly different from normal samples (5.6 versus 0; \( P = 0.0001 \)) and adenomas (5.6 versus 0; \( P = 0.0001 \)). The median level of active MMP-2 was higher in Dukes’ stage C compared with stage B, but this difference did not reach significance (5.9 versus 0; \( P = 0.5 \)). The levels in stage C were significantly different from normal samples (5.9 versus 0; \( P = 0.0001 \)) and adenomas (5.9 versus 0; \( P = 0.0001 \)).

The median levels of inactive MMP-2 were higher in Dukes’ stages A–C compared with normals (\( P = 0.0001 \)) and adenomas (\( P = 0.0001 \)).

These correlations also followed through if the number of samples with detectable enzymes were analyzed. Thus, there was a significant difference in the percentage of samples with positive enzyme activity in malignant colorectal tumors from Dukes’ stages A–C compared with normals and adenomas (\( P < 0.001 \)).

Using the Dukes classification system, there was no significant increase in MMP-2 and MMP-9 levels through the Dukes stages (Fig. 2a). According to the classification system of Jass et al. (16), there was, however, a significant increase in inactive MMP-2 from Jass group I to Jass group IV (\( P = 0.006 \) for Kruskal-Wallis; \( P = 0.01 \) for trend), whereas active MMP-2 and MMP-9 showed no trend in median levels through the Jass stages (Fig. 2b).

Fig. 3 shows a representative zymogram of the standards and samples from 10 Dukes’ stage A colorectal cancers.

Samples were scored as moderately or poorly differentiated. There was no consistent or significant variation in the levels of enzymes with degrees of differentiation. There was also no significant difference in MMP-2 and MMP-9 levels between tumors located in the colon or rectum.

Follow-up data were available on a majority of Dukes’ stage B and C cases. We analyzed these for correlations between enzyme activity and 5-year, disease-free survival. In Dukes’ stage C, there were follow-up data on 39 of 48 patients. At 5 years, 12 patients were alive and disease free, and 27 patients had died. Levels of active MMP-2, inactive MMP-2, and MMP-9 were not significantly different between survivors and nonsurvivors (significance values, 0.499, 0.491, and 0.481, respectively, by the Mann-Whitney test). In Dukes’ stage B, follow-up data were available on 54 of the 65 patients. Fourteen patients had died or recurred by 5 years, and 40 patients were alive and disease free. Levels of active and inactive MMP-2 and MMP-9 were no different between the groups (significance values, 0.464, 0.488, and 0.482, respectively).

**RT-PCR.** RT-PCR was used to investigate MMP-2 and MMP-9 mRNA expression. Four samples from each group were studied. MMP-2 and MMP-9 mRNAs were expressed in Dukes’ stage A–C colorectal tumors as well as in normal and benign biopsies. Fig. 4 shows two representative samples from each group.

**In Situ Hybridization.** In situ hybridization to MMP-2 mRNA was used to confirm the RT-PCR data, to define cellular localization, and to determine the extent of mRNA expression in the different groups. The mRNA for MMP-2 was expressed in cell populations of Dukes’ stage A–C colorectal tumors as well as normal and benign biopsies, but there were quantitative and locational differences. The strong expression in Dukes’ stage C tumors showed a branching pattern that followed the stromal area of the tumors. Positive cells were either spindle shaped or stellate. In some tumors at all Dukes’ stages, the staining was extensive; in others, it was more focal (e.g., Fig. 5, a and b). mRNA for MMP-2 was also detected in adenomas. The expression was focal and weaker than in the tumors. There were fewer positive cells compared with the carcinoma samples. Positive cells in adenomas were compared with stage B, but this failed to reach significance (5.6 versus 0; \( P = 0.06 \)). However, the levels of active MMP-2 in Dukes’ stage A were significantly different from normal samples (5.6 versus 0; \( P = 0.0001 \)) and adenomas (5.6 versus 0; \( P = 0.0001 \)). The median level of active MMP-2 was higher in Dukes’ stage C compared with stage B, but this difference did not reach significance (5.9 versus 0; \( P = 0.5 \)). The levels in stage C were significantly different from normal samples (5.9 versus 0; \( P = 0.0001 \)) and adenomas (5.9 versus 0; \( P = 0.0001 \)).

<table>
<thead>
<tr>
<th>Collagenase Activity (U/mg protein)</th>
<th>Normals</th>
<th>Adenomas</th>
<th>Dukes A</th>
<th>Dukes B</th>
<th>Dukes C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5.6)</td>
<td>(36.6)</td>
<td>(5.9)</td>
<td>(33.0)</td>
<td>(5.3)</td>
<td></td>
</tr>
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**Fig. 1.** Levels of Mr 92,000 MMP-9 (a) active (Mr 62,000) MMP-2 (b), and inactive (Mr, 72,000) MMP-2 (c) in biopsies of normals (n = 36), adenomas (n = 25) and Dukes’ stage A (n = 18), B (n = 65), and C (n = 48) colorectal tumors determined by quantitative gelatinolytic zymography. The bars indicate median levels. The absence of a bar indicates that the median level is zero. Active MMP-2: Dukes’ stage A versus stage B, \( P = 0.06 \); Dukes’ stage C versus stage B, \( P = 0.05 \); Dukes’ stages A and C versus normals and adenomas, \( P = 0.0001 \). Inactive MMP-2: Dukes’ stages A–C versus normals and adenomas, \( P = 0.0001 \). MMP-9: Dukes’ stages A and C versus stage B, normals, and adenomas: \( P = 0.0001 \).
seen typically in the stroma beneath the peripheral surface of the lesion, where there was inflammation (Fig. 5c). MMP-2 expression was not seen in the mucosa of the normal bowel. However, positive cells were detected in the submucosal areas, especially the muscularis mucosae. Individual positive cells with large nuclei were detected in the outer layer of the muscularis propria and in connective tissue close to the myenteric plexus (Fig. 5d). In a detailed analysis of 16 different biopsies, the entire section was divided into low-power fields (×10 objective; approximately 10 fields/case). These were scored for positivity and approximate percentages of positive cells (>10, >5, >2, and <1%) per positive low-power field. As shown in Table 2, there were more cells expressing MMP-2 in Dukes’ stage A–C tumors compared with normals and adenomas and more microscope fields containing positive cells in the malignant samples.

**DISCUSSION**

In this study, we have shown increased levels of active MMP-2 and MMP-9 type IV collagenases in malignant colorectal tumors at Dukes’ A and C stages compared with normal bowel biopsies, benign tumors, and Dukes’ stage B tumors. Several studies have demonstrated an association between tumor spread and expression of MMP-2 and MMP-9 (8–10), indicating that MMP-2 and MMP-9 may have a prognostic potential in different epithelial cancer types and even a diagnostic potential in malignant cancers. In this study, there was, however, no clear correlation between the stage of malignancy and the levels of MMP-2 and MMP-9 type IV collagenases, using the Dukes classification system (15), because the less advanced Dukes’ stage A tumors had higher levels of active enzyme than the more invasive Dukes’ stage B tumors. The high levels seen in Dukes’ stage
A tumors may be explained by the fact that some of the patients in this group had histories of bowel disease, such as ulcerative colitis, and, therefore, may not be representative of the more common sporadic Dukes’ stage A cases. The inflammatory cells present in such cases could be a source of active MMP enzymes. However, stage A tumors are limited to the bowel wall with no extension into the serosa, whereas stage B tumors have penetrated the muscularis propria by direct continuity. It is, therefore, possible that MMP-2 and MMP-9 are necessary while the tumor is in the process of penetrating the bowel wall in Dukes’ stage A tumors but are less necessary when tumors have spread beyond the muscle into the softer surrounding adipose tissue, as in stage B.

There was also no correlation between MMP-2 and MMP-9 levels and 5-year, disease-free survival in the two groups in which sufficient patients had died or recurred to make such analysis possible, Dukes’ stages B and C. This result, perhaps, is not surprising in the Dukes B group, in which surgical intervention was sufficiently successful for there to be 40 of 54 patients alive and disease free at 5 years. Ten-year survival data may be of more interest in this group.

Several studies (e.g., Refs. 24 and 25), including this one, have localized MMP-2 mRNA to stromal elements in the tumor. There are significant differences in the stromal components of Dukes’ stage A and B tumors, and this also may account for the differences in enzyme activity. Representative samples of colorectal cancer, as in the cryostat sections we used, contain most of the same tissue components as the normal colon, although in different proportions. For example, there is a relatively lower proportion of smooth muscle and a greater proportion of fibroblasts and myofibroblasts in adenocarcinomas than in the normal colon. Epithelial cells are generally more numerous in adenocarcinomas but vary, depending on how solid the tumor tissue is. Other elements, such as inflammatory cells, are present in numbers similar to those in the normal tissues, but in subtly different proportions, due to the local tissue immunological activity that occurs in neoplasia.

The possibility that differences in stromal components of tumors may influence MMP levels was also suggested by analysis of the levels in relation to the Jass classification (16). Jass groups I and II include most of the tumors with dense stromal inflammatory cell infiltrates, which we would expect to be associated with active rather than inactive MMPs. Tumors in Jass groups III and IV do not have such stromal reactions. Because these more advanced tumors also correspond to Dukes’ stage B and C cases, this also may offer an explanation for the apparent paradox of high MMP levels in Dukes’ stage A tumors. Stage A tumors are generally in Jass group I.

The mean levels of MMP enzyme recorded in these tumor samples were comparable to those we measured in breast and bladder cancer specimens (8, 9). Mean levels of active and inactive MMP-2 in colorectal cancer samples were similar to those recorded in breast cancer and higher than those recorded in bladder cancer. Mean levels of MMP-9 were again similar but slightly, not significantly, higher than those measured in the other epithelial cancers.

Using RT-PCR, MMP-2 and MMP-9 mRNAs were found in Dukes’ stage A–C colorectal tumors as well as in normal and benign biopsies. This is a highly sensitive and nonquantitative technique, able to detect mRNA in very small numbers of cells. A more detailed study of MMP-2 expression by in situ hybridization demonstrated MMP-2 mRNA in both malignant and normal samples. However, MMP-2 mRNA was localized to different cell populations in malignant and normal and benign samples, and there were more cells expressing MMP-2 in the tumors compared with normal biopsies. In cancer samples, the positive cells were spindle shaped or stellate, with a morphology that suggested fibroblasts or myofibroblasts. The positive cells in the adenomas were found typically in inflammatory areas close to the luminal surface, and normal tissue cells with large nuclei in the submucosal areas expressed MMP-2 mRNA. Thus, our results would suggest that there is transcriptional control of MMP-2 production in colorectal malignancy, with increased mRNA and active protein in the tumor areas. In addition, this mRNA and protein is produced by different cell populations in the malignant tissues.

MMP-2 is secreted by human epithelial cell lines (26), as well as fibroblasts (4), endothelial cells (25), and macrophages (27), whereas MMP-9 is secreted by cells of mononuclear phagocyte lineage (28, 29). In situ hybridization studies from other groups have localized these enzymes in stromal tissue surrounding the tumor rather than tumor epithelial cells in colorectal cancer (24, 25). In this study, in situ hybridization confirmed that mRNA for MMP-2 was expressed not by the tumor cells themselves but, rather, by cells in the surrounding stroma. Moreover, normal and benign biopsies also expressed MMP-2 mRNA. These observations indicate that MMP-2 and MMP-9 may be expressed constitutively by stromal cells but is not translated or is at levels below the detection limits of our zymography methods in normal and adenomatous tissue. One may speculate that, in contact with tumor cells, expression may be increased. Cocultures between tumor cells and stromal cells have shown that MMPs can be induced by both cell-cell contact (30) and soluble factors (31, 32), suggesting that tumor cells and stromal cells cooperate in the production of type IV collagenases. Studies in our laboratory have identified a soluble
factor as being important in the induction of MMP-9 in macrophages during coculture with tumor cells. In this study of colorectal cancer, we have measured only two components of the cascade of proteolytic enzymes that exists in the tumor environment. Other MMPs (MMP-1, MMP-3, MMP-7, and MMP-11) have been reported in colorectal cancer (33, 34). MMPs are inhibited by a family of endogenous inhibitors known as the tissue inhibitors of metalloproteases (1). It is the balance between all these activated MMPs and the tissue inhibitors of metalloproteases, as well as other proteolytic enzymes, that modulates the invasive process. The study of MMP-2 and MMP-9 has shown significant associations with malignancy, tumor stage, and stromal components. Measurement of other enzymes and inhibitors may provide further useful correlations.

Recently, several synthetic MMP inhibitors have been designed (35), which may have a potential in inhibition of cancer invasion and metastasis. The data described here suggest that these novel therapies may be of benefit in both early and advanced colorectal cancer.

ACKNOWLEDGMENTS

We thank Gerorge Elia for help with histochemistry and section cutting, George Holt for image analysis, Sharon Love for statistical analysis, Dr. Andrew Hanby for help with photography, and Thomas Leber for technical assistance. The MMP-2 primer was kindly provided by Dr. Graham Wells (British Biotech).

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