ABSTRACT

Many studies of malignant cells or tissues have implicated cysteine proteases in the progression of malignancy. We have extended these observations by measuring quantitative and qualitative changes in the expression of cathepsin B-like and L-like cysteine proteases during the growth and development of human colorectal carcinomas. Data derived from matched pairs of normal colorectal mucosa and carcinoma tissue from 27 patients demonstrated that both cathepsin B-like and cathepsin L-like specific activities were significantly elevated ($P < 0.005$) in the carcinoma tissue, while levels of endogenous cysteine protease inhibitor remained constant. Correlation of cathepsin enzyme activities with different stages of colorectal cancer demonstrated significantly higher cysteine protease activities in individuals with Dukes' A tumors (tumors confined to the bowel wall) than in patients with more advanced tumors (Dukes' B, C, or D tumors) ($P < 0.01-0.05$). The relative proportion of activities contained in tumor epithelial and stromal elements remains to be elucidated. These results suggest an important role for cysteine proteases in the early progression of human colorectal carcinoma.

INTRODUCTION

Proteases have long been known to play a role in malignancy. Their ability to digest extracellular matrix proteins provides a rationale for the contribution of proteases to tumor invasion and metastasis. In addition, many proteases are known to act as mitogens by stimulating DNA synthesis or cell division of nonproliferating cultured fibroblasts (1, 2). Intracellular proteases have been implicated in the activation of hormones and growth factors (2, 3), as well as in protein turnover, and thus may contribute to the regulation of cell growth and metabolism.

We have studied the expression of papain-like cysteine proteases in human colorectal adenocarcinoma. These enzymes, known as cysteine cathepsins in mammalian tissues, are capable of degrading extracellular matrix components, including collagen, fibronectin, proteoglycans, and elastin (4–8). Elevated acid protease activity in the extracellular milieu of malignant cells (9) has largely been traced to a cathepsin B-like cysteine protease (10, 11). Both intracellular and extracellular cathepsin B activity is frequently increased in cultured malignant cells. It may also be present in malignant tissues in an altered phenotypic form as demonstrated by an abnormal isozyme pattern, increased stability to alkaline pH or high temperature (10), or enhancement in the plasma membrane and nuclei compared to normal cells (12–14).

Oncogene studies have also implicated the related cysteine protease, cathepsin L, in malignancy by demonstrating that this enzyme is the major induced protein of ras-transformed NIH3T3 cells (15, 16), and the “major excreted protein” of cells transformed by Kirsten, Harvey, or Maloney sarcoma viruses and by SV40 virus (14, 17, 18). In terms of ability to degrade stromal proteins, cathepsin L is more active against collagen (19, 20) and 100-fold more active against elastin (8) than is cathepsin B.

Despite this evidence from animal and cell culture models for the role of cysteine proteases in malignancy, only a few studies have attempted to correlate cathepsin activities in human tumor tissue with clinicopathological parameters. In one study of colorectal carcinoma, Durdey et al. (21) reported that of four proteases examined (cathepsin B, cathepsin H, and 2 collagenases), only cathepsin B activity was significantly correlated with local tumor invasion. We have investigated this relationship further by measuring cathepsin B-like and cathepsin L-like specific activities in matched tissue samples (normal mucosa and adenocarcinoma) from 27 patients. We have also measured levels of cysteine protease inhibitor in these tissues in order to determine whether cathepsin enzyme activities might reflect increases or decreases in protease inhibitor levels with malignancy. Utilizing an alkaline pH treatment we have also explored whether the cathepsin B-like activity in the cancer tissues is biochemically distinguishable from the enzyme activity in the normal mucosa. Finally, we have compared changes in the expression of cysteine proteases with stages of tumor development and with tumor differentiation, including mucinous/nonmucinous histological subtypes.

MATERIALS AND METHODS

Tissue Specimens. Fresh tissue from colorectal resections was obtained within 4 h of surgery at the Mallory Institute of Pathology, Boston, MA. To minimize possible differences due to genetic variation, we compared normal mucosa and tumor from the same patient. In sampling tumors, care was taken to avoid grossly necrotic areas and fat. Samples of normal colorectal tissue were obtained at least 10 cm from the tumor. The mucosa was then separated from the muscle layer, serosa, and surrounding fat. All samples were stored at $-80^\circ$C until extraction. Normal mucosa was also obtained from non-tumor-bearing colorectal resections in a similar manner. The clinical details of the patients investigated and the corresponding pathological data are summarized in Table 1.

Dukes' Classification. Individual tumors were staged according to the Dukes' classification (22) as modified by Turnbull et al. (23). Dukes' A tumors are confined to the bowel wall; Dukes' B tumors have spread beyond the wall without involving lymph nodes; Dukes' C tumors are associated with regional lymph node metastasis; and Dukes' D tumors are associated with distant metastasis.

Tissue Extraction. To minimize test variation, care was taken to extract and assay each pair of normal mucosa/tumor samples at the same time. Tissue samples (60–80 mg) were homogenized in 500 μl of distilled, deionized water, frozen and thawed three times, and centrifuged for 50 min at 4°C at 12,000 rpm (17,210 × g) in a Sorvall BB centrifuge (24). Supernatants were removed and utilized for the following assays.

Enzyme Assays. To distinguish cathepsin B-like enzyme activity from cathepsin L-like activity, the cathepsin B specific substrate, Z-Ala-Arg-
Arg-MNA (Enzyme Systems Products, CA), was used (25). A specific substrate for cathepsin L is not yet available. However, the substrate Z-Phe-Arg-MNA (Enzyme Systems Products, Dublin, CA) is cleaved much more readily by cathepsin L than by cathepsin B and so is commonly used to detect cathepsin L-like activity, with the knowledge that a lesser portion of this activity may represent cathepsin B activity in an extract containing both enzymes (26). To generate a more specific enzyme assay for cathepsin L, we have utilized pH-sensitive differences for cathepsin B-like and L-like activity against these substrates.

Cathepsin B-like activity was determined by a modification of the methods of MacGregor et al. (27) and Barrett and Kirchke (26). Cells extracts were incubated in 0.1 mM MES buffer, pH 5.8, containing 1 mM Z-Ala-Arg-Arg-MNA as substrate, 1 mM DTT, and 1 mM EDTA at 37°C. The assay was started by the addition of 20 μl of tissue extract (2–6 μg/μl protein) to the substrate/buffer mixture. At 10 min the reaction was terminated by the addition of 50 μl 1 N HCl in 2% Triton X-100. Fast Blue B (O-dianisidine tetrazotized) (Sigma Chemical Co., St. Louis, MO) was added and color developed for 10 min before reading at A550 in a Gilford spectrophotometer. For each tissue sample three extractions were done and each extract was assayed in duplicate with a mean value determined for inclusion in Table 1. For each of the enzyme assays the intraassay standard deviation was always less than or equal to the standard deviation of the interassay values.

Cathepsin L-like activity was measured by using the synthetic substrate Z-Phe-Arg-MNA. Tissue extracts (20 μl) were incubated in 0.1 mM MES buffer, pH 3.5, containing 1 mM Z-Phe-Arg-MNA, 1 mM DTT, 1 mM EDTA at 30°C for 10 min. Subsequent steps were similar to those used in the cathepsin B assay.

β-Glucuronidase activity was assayed according to the methods of Kolodny and Mumford (28) and Beaudet et al. (29). Tissue extracts identical to those prepared for cathepsin B and L assays were added (20 μl/tube) to 0.1 mM sodium acetate buffer, pH 4.4. The enzyme reaction was started by addition of 70 μl substrate (final concentration, 2.5 mM 4-methylumbelliferyl β-D-glucuronide) and the reaction mixtures were incubated in aluminum foil-wrapped tubes for 6 min at 37°C. The reaction was stopped by adding 3 ml of high pH buffer (0.1 M NH₄OH-EDTA, pH 10.5) and fluorescence was measured in a Perkin-Elmer fluorimeter.

Specific activities of cathepsin B-like and L-like enzymes and of β-glucuronidase are expressed as nmol of substrate hydrolyzed per min per mg protein. Protein content of the tissue extracts was determined by the method of Lowry et al. (30), using bovine serum albumin as a standard. The mean level of total soluble protein in our normal colorectal mucosa was 3.11 ± 1.01 (SD) compared to 3.22 ± 1.04 (SD) in the carcinoma tissue.

RESULTS

Quantitative Enzyme and Patient Data

Table 1 shows the levels of cathepsin B-like and cathepsin L-like enzyme activities measured in colorectal carcinoma and in the distant normal mucosa of the same patient for 28 adenocarcinomas from 27 individuals. Table 1 also contains the clinical data of the patient study group, including age, sex, Dukes' stage, and tumor differentiation. Only cases in which samples of both normal and tumor tissue were available for testing were included in the analysis.

Cathepsin B-like Activity

As shown by the data in Table 1, mean cathepsin B-like activity in the carcinoma tissue (79.5 ± 33.8) was significantly higher (P < 0.005) than cathepsin B-like activity in normal mucosa (57.9 ± 24.3), using a two-sample t test to calculate the difference between means (36). In 14 of 28 cases analyzed, the cathepsin B-like specific activity in tumor tissue was notably higher than that of the matched normal mucosa, as reflected by a cancer/normal ratio greater than 1.44 (see Table 1). The average ratio for the individual pairs was 1.48.

Although considerable variation is observed in cathepsin B-like enzyme levels in both normal and carcinoma tissues, only 2 of 28 normal tissues express levels of cathepsin B-like activity greater than 86 nm/min/mg protein, while 13 of 28 tumor extracts have activity in the range of 90–140 nm/min/mg protein. Among those 15 tumor tissues with relatively low enzyme activity levels (less than 86 nm/min/mg protein), 6 have a cancer/normal ratio greater than 1.44. Thus, in 19 of 28 colorectal carcinomas an abnormal expression of cathepsin B-like activity could be detected by quantitative assay as either high total activity or a high cancer/normal ratio.

Cathepsin L-like Activity

As observed for cathepsin B-like activities, cathepsin L-like activity in colorectal carcinoma tissue (13.1 ± 3.0) was significantly higher than cathepsin L-like activity in matched normal mucosa (10.4 ± 2.2), using the two-sample t test to test the difference in means (P < 0.005). In 8 of 17 pairs analyzed,
the activity in the tumor tissue was notably higher than that in the normal tissue, as reflected by a cancer/normal ratio greater than 1.28. The average cancer/normal ratio for cathepsin L-like activity in the individual pairs was 1.31 ± 0.3.

As with cathepsin B-like activity, variation is observed in both normal and carcinoma tissues, but a significant shift toward higher cathepsin L-like activity is seen in carcinoma tissues such that 9 of 17 tumor extracts express activity greater than 12.3 nm/min/mg protein, while only 3 of 17 normal extracts have activity above this level.

Cathepsin Activities in Colorectal Mucosa of Noncancer Patients

In addition to paired comparisons of normal and carcinoma tissue from specimens of patients with colorectal tumors, cathepsin B-like and L-like specific activities were also determined in colorectal mucosa from patients undergoing surgical resections for diverticular disease or abdominal trauma. Neither the cathepsin B-like activity (64.3 ± 20.9; n = 9) nor cathepsin L-like activity (10.0 ± 2.7; n = 5) levels in these nonneoplastic control tissues were significantly different from activity levels in the normal mucosa of patients with colorectal carcinoma.

Relationships between Tumor Morphology and Cathepsin Activity Levels

Enzyme data were analyzed to determine whether cathepsin activity levels correlated in any way with independently assessed (a) Dukes’ stage, (b) mucinous/nonmucinous histological subtype, and (c) tumor grade.

Dukes’ Stage. In patients with different stages of colorectal cancer, neither the cathepsin B-like nor cathepsin L-like specific activities varied significantly in normal tissue across the Dukes’ A, B, and C groups. However, in carcinoma tissue, cathepsin B-like specific activity in Dukes’ A patients was significantly higher than in Dukes’ B patients (P < 0.05), Dukes’ C patients (P < 0.05), or Dukes’ D patients (P < 0.01). For cathepsin L-like activity the pattern was similar, with Dukes’ A levels significantly higher than Dukes’ B (P < 0.01) and Dukes’ C (P < 0.05) levels of enzyme activity.

Mucinous versus Nonmucinous Carcinomas. Cathepsin B-like specific activity levels showed slightly higher cancer/normal ratios in the mucinous (1.66 ± 0.8) compared to nonmucinous cancers (1.43 ± 0.5), but the difference was not significant. Similarly, both the 6 mucinous and 22 nonmucinous tumors showed almost identical cancer/normal ratios for cathepsin L-like activity (1.31 and 1.32, respectively).

Tumor Grade. Cathepsin B-like activity in 4 poorly differentiated carcinomas showed a significantly higher (P < 0.05) cancer/normal ratio (2.07 ± 0.46) than in 23 moderately differentiated carcinomas (1.35 ± 0.58).

pH Stability of Normal and Tumor Cathepsin B-like Activities

One means of determining whether cathepsin B-like activities are not only increased in cancerous tissue but are also present in an altered form is to test the enzyme activity for changes in biochemical characteristics. One of the strongest markers of normal cathepsin B activity is its extreme lability at or above
pH 8.0 (32). However, it is also known that cathepsin B-like activities within tumor tissues or secreted by tumor tissues are often more stable to treatment at pH 8.0 than is the normal enzyme (10, 14). Thus, we treated 17 normal extracts and 17 matched carcinoma extracts for stability to pH 8.0. The results are represented in Table 2 as residual cathepsin B activity in the cancer versus normal tissue after pretreatment at pH 8.0. Even for the carcinoma extracts, treatment at pH 8.0 is remarkably destructive of cathepsin B-like activity, indicating the need for a less alkaline pH treatment (e.g., pH 7.5) to obtain more subtle comparisons in the future. Nonetheless, in 8 of the 17 pairs of extracts tested, the cathepsin B-like activity in the carcinoma extracts was 50 to 200% more stable to alkaline treatment than activity in the matched normal extracts. The mean of the individual ratios of cancer to normal residual activity is 1.42 ± 0.8, which indicates a significant increase in residual activity at pH 8.0 in the carcinoma tissues (P < 0.025).

What is perhaps most interesting to note, with respect to those pairs in which the cathepsin B-like activity is more notably stable, is that 7 of 8 were among those cases in which the absolute level of cathepsin B-like activity in the carcinoma tissue was not extremely high, falling below a value of 86 nm/min/mg, which roughly separates normal from abnormal cathepsin B-like activity in colorectal mucosa. In addition, 4 of the 9 with more alkaline-stable enzyme activity had quite low ratios of cancer/normal cathepsin B-like activity. Thus, colorectal tumor extracts which, by quantitative cathepsin B assay might look relatively normal, may be recognized as abnormal by measuring the pH stability of their cathepsin B-like activity.

Determining the Specificity of Cathepsin B-like versus Cathepsin L-like Activities

In order to measure cathepsin B-like enzyme activity, the substrate Z-Ala-Arg-Arg-MNA was used as it is highly specific for cleavage by cathepsin B but not cathepsin L (25, 26). The substrate, Z-Phe-Arg-MNA, which is cleaved more readily by L than by B, is commonly used to detect cathepsin L plus a portion of cathepsin B activity at pH 5.8 (26). To generate a more specific assay for cathepsin L, we have utilized differences in pH activity and stability for these enzymes. Cathepsin L is more active and stable in a lower pH range than cathepsin B and has a lower pH optimum for most substrates (pH 5.0–5.5) (37) compared to cathepsin B (pH 5.8–6.5) (25, 26). In addition, cathepsin B is rapidly inactivated below pH 4.0 (25) while cathepsin L retains 30% of its activity after a 1-h incubation at pH 3.5 (20). Thus, the hydrolysis of a given substrate by cathepsin L compared to cathepsin B increases from pH 6.5 to pH 3.5 (19).

As seen in Fig. 1, the ratio of colorectal mucosal extract activity against Z-Phe-Arg-MNA (cathepsin L-like) versus Z-Ala-Arg-Arg-MNA (cathepsin B-like) also increases dramatically as one moves from a pH of 6.0 to 3.5. These differences at low pH values provide a useful means of measuring cathepsin L-like specific activity in extracts containing a mixture of activities. This is demonstrated by the independent results which we have obtained for cathepsin B-like activity (hydrolysis of Z-Ala-Arg-Arg-MNA at pH 5.8) versus cathepsin L-like activity (hydrolysis of Z-Phe-Arg-MNA at pH 3.5) as seen in the matched pairs data of Table 1. Although, as a group, carcinoma tissues express higher average levels of both cathepsin B-like and L-like activities, for a given individual these enzymes do not appear to be coordinately regulated. Thus, patients with high cathepsin B-like activity will not necessarily have high cathepsin L-like activity. Similarly, it is possible to measure a dramatic increase in cathepsin B-like specific activity in cancer versus normal colorectal tissue without finding a corresponding change in cathepsin L-like specific activity in that patient’s tissues.

Table 2 Percentage of residual cathepsin B-like activity after pretreatment of paired normal mucosa and cancer extracts at pH 8.0

<table>
<thead>
<tr>
<th>Case</th>
<th>Normal</th>
<th>Cancer</th>
<th>Cancer/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7</td>
<td>4.6</td>
<td>1.24</td>
</tr>
<tr>
<td>2</td>
<td>4.6</td>
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</tr>
<tr>
<td>3</td>
<td>5.2</td>
<td>3.7</td>
<td>0.71</td>
</tr>
<tr>
<td>6</td>
<td>8.6</td>
<td>7.2</td>
<td>0.84</td>
</tr>
<tr>
<td>16</td>
<td>3.5</td>
<td>10.0</td>
<td>2.86</td>
</tr>
<tr>
<td>17</td>
<td>9.2</td>
<td>8.9</td>
<td>0.97</td>
</tr>
<tr>
<td>18</td>
<td>9.2</td>
<td>23.2</td>
<td>2.52</td>
</tr>
<tr>
<td>19</td>
<td>4.0</td>
<td>11.0</td>
<td>2.75</td>
</tr>
<tr>
<td>20 (i)</td>
<td>5.0</td>
<td>8.0</td>
<td>1.60</td>
</tr>
<tr>
<td>20 (ii)</td>
<td>5.0</td>
<td>7.7</td>
<td>1.54</td>
</tr>
<tr>
<td>23</td>
<td>3.9</td>
<td>6.0</td>
<td>1.54</td>
</tr>
<tr>
<td>32</td>
<td>10.7</td>
<td>10.4</td>
<td>0.93</td>
</tr>
<tr>
<td>33</td>
<td>9.1</td>
<td>21.1</td>
<td>2.32</td>
</tr>
<tr>
<td>34</td>
<td>18.0</td>
<td>3.6</td>
<td>0.20</td>
</tr>
<tr>
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<td>9.2</td>
<td>5.4</td>
<td>0.59</td>
</tr>
<tr>
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<td>2.9</td>
<td>2.3</td>
<td>0.79</td>
</tr>
<tr>
<td>38</td>
<td>6.6</td>
<td>12.6</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Mean ± SD 6.96 ± 3.7 8.81 ± 5.6 1.42 ± 0.8

* For details of assay, see "Materials and Methods." Mean specific activities (nm/min/mg protein) for control and treated normal mucosa were 57.9 ± 29.7 and 3.3 ± 1.2, respectively. Mean specific activities (nm/min/mg protein) for control and treated cancers were 81.3 ± 46.9 and 5.64 ± 3.2, respectively.

† (i) and (ii) represent 2 different adenocarcinomas from the same patient.

Paired Student’s t test (one-tailed) is significant at the P = 0.025 level.

Fig. 1. Comparison of pH effects on the hydrolysis of synthetic substrates (Z-Ala-Arg-Arg-MNA, O; and Z-Phe-Arg-MNA, △). Points, maximal activity of normal colorectal mucosa extracts at various pH values.
tissue was 7.49 ± 5.04 nm/min/mg protein, which is not a significant difference. Thus, in contrast to the increased protease activities, levels of β-glucuronidase in the carcinoma tissue were not different from matched normal tissues. These values for β-glucuronidase are similar to those measured in human fibroblasts (7.82 nm/min/mg protein) and leukocytes (9.37 nm/min/mg protein) (29).

Levels of Cysteine Protease Inhibitor in Matched Pairs of Tumor and Normal Colorectal Mucosa

Levels of endogenous CPI were determined in 27 matched pairs of normal mucosa and carcinoma tissue. The average content (in units of inhibitor/mg protein) of CPI in colorectal mucosa (3.85 ± 1.6) is the same as that in colorectal carcinoma (3.82 ± 1.5).

DISCUSSION

Studies using both cell culture and animal models on the role of cysteine proteases in malignancy have demonstrated that increased or altered expression of cathepsins B and L provides a marker for the transformed phenotype (4, 9–18). However, only a few studies have been done to explore whether quantitative and qualitative changes in cysteine protease activity demonstrate a reproducible pattern in human tumor tissue or a correlation with tumor staging. To obtain these types of data, we have analyzed cysteine protease activities in matched normal colorectal mucosa and carcinoma tissue from 27 patients. Colorectal cancer resections provide suitable material for examining the relationship of protease activity levels to tumor invasion. Advantages include the ability to obtain appropriate normal control mucosa with a relatively constant epithelial cell component, and the presence of a well-tested staging system (Dukes') which closely parallels the biology of tumor invasion.

Only four previous studies of human cancer have analyzed cysteine cathepsin activities in a series of matched malignant and adjacent normal tissues. Our data are consistent with those of Vasishtha et al. (38) and Watanabe et al. (39) in gastric tumors, with those of Durdey et al. (21) in colorectal carcinomas and with those of Abecassis et al. in breast tumors (40), in describing significantly elevated cysteine protease activity in tumor compared to normal tissue. However, we have examined in greater detail the relationships between altered protease expression and clinicopathological parameters. We have also extended biochemical analyses to include measurements of protease stability and endogenous protease inhibitors.

Our finding of a statistically significant increase in cathepsin B-like and L-like activities in the earliest stage (Dukes' A) of colorectal cancer compared to later stages (Dukes' B, C, and D) has not been reported previously. Durdey et al. (21) demonstrated no correlation of cysteine protease activity with Dukes' stage, although importantly, the presence of only one Dukes' A tumor in that study would have precluded finding such an association. Durdey et al. did observe surprisingly low levels of protease activity in Dukes' D tumors, an observation which our data confirm.

Elevated cysteine protease activities in early stage colorectal carcinomas suggest that tumors still in the process of invading the bowel wall (Dukes' A) may require more protease activity than tumors which have already achieved a greater degree of invasion and metastasis (Dukes' B, C, and D). This pattern of high protease activity in early tumors which decreases in later stages may provide an example of "phenotypic drift" of clonally derived tumors, with continued somatic mutation generating an admixture of distinct phenotypes (41, 42), and selection for those cells which provide advantageous characteristics at each stage.

High levels of protease activity in early tumors might also be due to a transient modification of cathepsin gene expression by other factors important in the early stages of colorectal carcinoma. For example, elevated levels of the ras oncogene protein, p21, are common to earlier stages of colon tumors (Dukes' B and C tumors) as compared to later stage primary tumors (Dukes' D) or metastatic tumor deposits (43, 44). DNA analysis has also indicated that ras gene mutations are relatively early events in colorectal tumorigenesis (45). The similarity in pattern for cathepsin B-like and L-like protease activities during colorectal tumor progression to that described for ras p21 may not be coincidental. A precursor form of cathepsin L has already been shown to be the "major induced protein" (16) and the "major excreted protein" (15, 17, 18) of ras transformed NIH 3T3 cells. However no one has yet studied the relative expression of ras p21 and cysteine protease activities in tumor tissues.

Colorectal carcinoma may provide a useful tissue type for studying a possible relationship between coordinately increased expression of cathepsin L and ras p21 during early human tumor cell growth.

In studies of gastric adenocarcinomas, Watanabe et al. (39) demonstrated significantly higher cathepsin B and L activities in poorly differentiated compared to well or moderately differentiated tumors. We have also found that the 4 poorly differentiated tumors in our series contain significantly higher cathepsin B-like activity than the moderately differentiated tumors. In addition we examined whether cysteine cathepsin activity correlated with mucinous or nonmucinous histological subtype, given that Vickery and Symonds (46) have reported that stage-matched mucinous colorectal carcinomas have a poorer 5-year survival rate than nonmucinous tumors. Our analysis demonstrates no significant difference in cathepsin B-like or L-like activities between mucinous and nonmucinous tumors. While the number of mucinous tumors we have assayed is small (six), our data support recent reports that mucinous carcinomas show a similar biological behavior, with no different prognosis, compared to their nonmucinous counterparts (47, 48).

Although we found no difference in the levels of cysteine protease inhibitor in normal versus tumor tissue, our assays do not rule out the possibility that the cysteine cathepsin activities in carcinoma tissue might somehow be less sensitive to inhibition by the tumor tissue CPI due to a qualitative change in either the tumor protease or protease inhibitors of the carcinoma tissue. This type of change has been suggested by Lah et al. (49), who found the total cysteine protease inhibitory content to be the same in liver and sarcoma tissue, but the protease/inhibitor affinity to be reduced for the sarcoma inhibitor.

Abnormalities in the expression of cathepsin B derived from malignant tissues may also be due to altered forms of the enzyme (10, 12). Our results demonstrated a significant increase in stability to pH 8.0 of cathepsin B-like activity in carcinoma tissues compared to matched normal tissues for 8 of 17 patients tested. This type of alkaline-stable cathepsin B activity in ascitic fluid of women with ovarian cancer or in culture medium of human breast tumor explants, has been shown to represent a high-molecular-weight procathepsin B which has not been fully processed to the mature lysosomal form (50). Steiner et al. (3) suggest that such precursor forms of cathepsin B may be more responsible for the activation of hormones and growth factors than the mature lysosomal enzyme. Sloane et al. (14) have also
found an atypically pH-stable cathepsin B in malignant cell lines, including colorectal carcinoma cell lines, which represents cathepsin B-like activity shifted from the lysosome to the plasma membrane (13), where it is presumed to function in the digestion of the extracellular matrix.

In summary, our observations on an increase in both the amount and stability of cysteine protease activity in colorectal carcinoma tissues indicates that cathepsins B and L should be studied further as markers of tumor progression in this type of malignancy. Work remains to be done to determine how early in the adenoma/carcinoma sequence these protease activities are altered, to explore the mechanism by which abnormal cysteine protease activities are generated, and to elucidate the predominant cell type responsible for increased cysteine protease activities in colorectal carcinoma.

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Cysteine Protease Activities and Tumor Development in Human Colorectal Carcinoma

Kieran Sheahan, Sania Shuja and Mary Jo Murnane


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