Stage-specific Increases in Cathepsin B Messenger RNA Content in Human Colorectal Carcinoma

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ABSTRACT

Cathepsin B mRNA levels and banding patterns were characterized in human colorectal mucosa and carcinoma tissues from patients with tumors of different Dukes' stages. Quantitation of mRNA content using slot blot hybridization demonstrated an increase in cathepsin B message in seven of eight tumor tissues with an average increase of 3.5-fold over patient-matched control mucosa samples. This tumor-specific increase in cathepsin B mRNA confirms and extends our previous observation that cathepsin B enzyme specific activity levels are significantly elevated in colorectal carcinomas. In fact, the increase in mRNA levels is greater and more consistent than the observed increase in enzyme activity, suggesting that posttranscriptional or posttranslational regulation of cathepsin B expression occurs in colorectal tumors. The mRNA data also support our earlier observation that cathepsin B enzyme activity levels are inversely correlated with Dukes' stage. The tumor-specific increase in cathepsin B mRNA content is almost 4 times greater in earlier stage (Dukes' A and B) tumors than in later stage (Dukes' C and D) tumors. Thus, increased cathepsin B gene expression is particularly characteristic of tumors which are in the process of invading the bowel wall or local tissues compared with tumors which have already spread to more distant sites. Northern blot data on cancer/normal pairs indicate that the increase in cathepsin B mRNA in colorectal carcinoma is due primarily to changes in the amount of the 2.2- and 4.0-kilobase transcripts which are seen in control tissue. However, low levels of two additional cathepsin B mRNA transcripts (1.5 and 3 kilobases in size) were also observed in tumor tissue.

INTRODUCTION

Abnormal expression of the cysteine protease, cathepsin B, has been reported for a variety of human and animal tumors (1, 2). Altered cathepsin B expression in tumor tissues is manifested by increased intracellular and extracellular enzyme activity as well as by the appearance of unusual forms of cathepsin B. Enzyme activity in malignant cells has been described as more stable at neutral pH or higher than normal temperature (3). It may also be secreted abnormally in a latent high molecular weight form (4) or enriched in the plasma membrane of tumor cells compared with normal tissues (2, 5, 6). These changes modify the expression of an enzyme which can degrade components of the extracellular matrix, including fibronectin, proteoglycans, elastin, laminin, and type IV collagen (1, 7–11). Thus, abnormal cathepsin B may contribute to the invasive and metastatic potential of tumor cells.

In our recent analyses of human colorectal carcinomas, we found that cathepsin B enzyme specific activity is significantly elevated in the early invasive stages of tumor growth (12). This type of increase in enzyme activity might result from abnormal expression of cathepsin B message or from changes in endogenous inhibitors for cathepsin B in early stage tumors. The endogenous cysteine protease inhibitors, stefins A and B, have been shown to be decreased in amount or altered in their affinity for cysteine cathepsins in several tumor tissues (13, 14). In human colorectal carcinoma, however, we found no decrease in the level of total cysteine protease inhibitor (12), although we have not yet analyzed the expression of individual stefins.

Abnormal patterns of cathepsin B activity in murine tumors have also been associated with increased levels of cathepsin B message or with unusual cathepsin B mRNA banding patterns (15, 16). Similar analyses of cathepsin B mRNA content and banding patterns have not yet been done on human tissues. The primary purpose of this research is to explore whether the increased level of cathepsin B activity in colorectal carcinomas (12) can be explained by a corresponding increase in the level of cathepsin B message or by the appearance of unusual cathepsin B mRNA transcripts in tumor tissues. As our previous enzyme analyses revealed a greater increase in cathepsin B activity levels in early compared with late stage colorectal tumors (12), another goal of this research is to explore whether detectable changes in cathepsin B mRNA content demonstrate this type of correlation with clinical stage.

MATERIALS AND METHODS

Tissues. Fresh tissue from colorectal resections was obtained within 4 h of surgery at the Mallory Institute of Pathology, Boston, MA. In order to perform both RNA and enzyme assays on the same tissue, samples weighing from 0.5 to 3.0 g were obtained. 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 snap frozen in liquid nitrogen and stored at —80°C until extraction.

Dukes' Classification. Individual tumors were staged according to the Dukes' classification (17) as modified by Turnbull et al. (18). 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.

Cathepsin B Enzyme Assay. Small portions of tissue (60 to 80 mg) were extracted for enzyme and protein assays with care taken to extract and assay each pair of normal mucosa/tumor samples at the same time to minimize batch variation. Cathepsin B-like activity was determined by a modification of the methods of MacGregor et al. (19) and Barrett and Kirschke (20). The assay was started by addition of 20 μl of tissue extract (2 to 6 μg of protein/μl) to a reaction mix of 0.1 M MES buffer, 0.15 μg Naclo-0.015 μl sodium citrate, pH 7.0; TE, 10 mm Tris-2.5 mm EDTA, pH 7.5, cDNA, complementary DNA; poly A+ RNA, polyadenylate-containing RNA; poly A−RNA, polyadenylate-deficient RNA.

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3 The abbreviations used are: MES, morpholinooethanesulfonic acid; CEA, carcinoembryonic antigen; E-64, l-trans-epoxyuccinyl-leucylamidod-4-guanido)butane; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl-0.015 M sodium citrate, pH 7.0, TE, 10 mm Tris-2.5 mm EDTA, pH 7.5, cDNA, complementary DNA; poly A+RNA, polyadenylate-containing RNA; poly A−RNA, polyadenylate-deficient RNA.
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pH 5.8, containing 1 mm benzoyl-alanine-arginine-arginine-4-methoxy-2-naphthylamide as substrate, 1 mm dithiothreitol and 1 mm EDTA, with incubation at 37°C for 10 min. The reaction was terminated by addition of 50 µl of IN HCl in 2% Triton X-100. Fast Blue B (O-dianisidine tetratized) (Sigma, St. Louis, MO) was added and color developed for 10 min before reading at A 520 in a Gilford spectrophotometer. Cathepsin B enzyme specific activity is expressed as nmol of substrate hydrolyzed per min per mg of protein. We have previously demonstrated by assays in the presence of the cysteine protease-specific inhibitor, E-64, that we are not measuring other types of proteolytic activity using this protocol (12).

RNA Isolation. Total RNA was isolated from 16 samples of snap-frozen tissue (8 matched pairs of carcinoma and normal mucosa) following a guanidinium isothiocyanate/cesium chloride gradient method based on that of Chirgwin et al. (21). Using a prechilled Polytron homogenizer at 4°C, frozen tissue samples (0.4 to 1.0 g) were homogenized in a volume (12 ml/g) of guanidium lysis buffer [4 M guanidine isothiocyanate (FLUKA)-0.5% sodium laurylsarcosine-25 mM sodium citrate, pH 7.0, to which 7 µl/ml of β-mercaptoethanol were added just before use]. Sample DNA was sheared by repeated passage through a 22-gauge needle before loading onto a sterile cushion of cesium chloride (5.7 M CsCl-0.1 M EDTA, pH 7.0) for an 18 h centrifugation at 35,000 rpm at 18–20°C in a Sorvall OTD65B ultracentrifuge. The supernatant was discarded, and RNA pellets were washed with ethanol before transfer to sterile Eppendorf tubes for resuspension in TE buffer. Overnight ethanol precipitation of the RNA was followed by a microcentrifuge spin (12,000 x g) at 4°C, resuspension of RNA in TE buffer, two additional ethanol precipitations, removal of the ethanol, and drying of the RNA pellets in a Speedvac centrifuge before suspension of lyophilized RNA in sterile distilled, deionized H2O. For the preparation of mRNA, crude total RNA was further purified by oligo(dT)-cellulose column chromatography, elution of the mRNA in a low-salt buffer, ethanol precipitation, drying, and resuspension in distilled, deionized water or buffer for further assay.

Molecular Probes. A 600-base pair human cathepsin B cDNA probe (CB-1) was kindly provided by D. Fong, Rutgers University (22). A 1950-base pair human β-glucuronidase probe (23) was obtained from the American Type Culture Collection/NIH Repository of Human DNA Probes and Libraries. A 1700-base pair rat “ubiquitous” β-tubulin probe (RB-T-3) was kindly provided by S. Farmer and G. Robinson, Boston University. Size markers for gels were based on a 0.24- to 9.5-kilobase RNA ladder from BRL, Inc. All of these probes were labeled with 32P-DCTP by random hexamer priming (24) for use in slot blot and Northern blot hybridizations as described below.

Slot Blot Hybridization. This modification of the “dot blot” procedure was performed with a template manifold apparatus (Minifold II; Schleicher and Schuell) (25). For the accurate interpretation of slot blots, RNA content was determined for each sample by spectrophotometric absorbance measurements. RNA samples were then adjusted by dilution so that, for each sample, a sequence of four slots was loaded with precisely 20, 10, 5, or 2 µg of total RNA. Staining of these samples with ethidium bromide after agarose gel electrophoresis served as a further control for calculations of equal RNA content. Poly A− RNA and poly A+ RNA controls were prepared from the high- and low-salt washes off an oligo-dT column which had been loaded with total cellular RNA from the CA 55 sample. These controls were used to determine whether the cathepsin B probe was binding specifically to mRNA (poly A+) and not to RNA (poly A−) in each slot. The RNA samples were diluted and denatured in formamide plus 10× SSC, incubated at 65°C for 15 min, and applied to nitrocellulose filters as described (25) before being dried under vacuum at 80°C. The dried filters were prehybridized for 3 to 5 h at 42°C in a solution of 0.05 M Tris (pH 7.5) containing 0.8 M NaCl, 50% deionized formamide, 5× Denhardt’s solution, 1% SDS, 0.1% sodium pyrophosphate (inorganic), and 1 µg/ml of salmon sperm DNA. Hybridization was done overnight at 42°C in a fresh mixture of the same solution to which 10% dextran sulfate and 32P-labeled cathepsin B cDNA probe (1 to 2 x 10⁶ dpm/ml) had been added. After hybridization, filters were put through a series of washes, including two 15-min washes at room temperature in 2× SSC/0.1% SDS, one 30-min wash at 65°C in 2× SSC/0.1% SDS, and one 30-min wash at 65°C in 0.2× SSC/0.1% SDS before being blotted dry and placed against film for autoradiography.

For sequential hybridization of filters to more than one probe, the prior probe was removed by twice pouring a boiling solution of 0.1× SSC/0.1% SDS over the filter in a Nalgene dish and incubating for 20 min in a shaking water bath at 95°C. The filter was dried by blotting on Whatman paper and was exposed to film to ensure that all radioactivity had been removed. The filter was stored in a sealed bag at 4°C until reprobing.

Northern Blot Hybridization. Total cellular RNA was electrophoresed on a 1% agarose-formaldehyde denaturing gel (20 µg/lane) as described by Alwine et al. (26). Ethidium bromide staining (0.5 µg/ml) was used to check for a 2:1 28S:18S RNA ratio as a measure of RNA quality and also to provide an indication of the relative amounts of RNA loaded in each lane. RNA was transferred to a nylon membrane (Duralon, Stratagene) (Bio-Rad) by standard blotting procedures and probed with the 32P-labeled pCB-1 probe in the hybridization solution described for slot blots. Posthybridization washes were done under stringent conditions (0.1× SSC/0.1% SDS at 65°C).

Quantitation of mRNA Levels. Scanning laser densitometry of autoradiographs was done on a Molecular Dynamics densitometer to determine the relative abundance of cathepsin B mRNA in different slot blot samples.

RESULTS

mRNA Content. Slot blot analyses indicate that cathepsin B mRNA levels are increased in 7 of 8 colorectal carcinomas compared with 8 matched normal colon mucosa samples (Fig. 1; Table 1). For these 7 pairs, the increase in cathepsin B mRNA content ranged from 1.5- to 8.2-fold, with an average

![Fig. 1. Slot blot data for cathepsin B mRNA in eight paired samples of normal human colorectal mucosa and carcinoma.](image-url)
increase of 3.9-fold ± 2.1 (P < 0.01).

Sequential hybridizations of the same slot blots to two control probes, β-glucuronidase, and tubulin, after the cathepsin B probe had been washed off were done to test the specificity of the observed increase in cathepsin B mRNA in tumor tissues. An example of the sequential hybridization of three probes to a single blot of the same patient’s tissues is seen in Fig. 2A, in which the tumor tissue (CA 52) compared with normal tissue (NCT 52) shows a 3.8-fold increase in cathepsin B mRNA, a slight decrease in β-glucuronidase mRNA (CA/NCT mRNA ratio of 0.6), and a 1.7-fold increase in tubulin mRNA. Thus, each probe is capable of detecting a unique pattern of mRNA expression in these tissues. Similar comparative data were obtained for five matched pairs of mRNA using all three probes and for three additional pairs using the cathepsin B and β-glucuronidase probes (Fig. 2B). While a range of cancer/normal ratios was observed for each probe, high increases in mRNA content (2.5- to 10-fold) were found more consistently for cathepsin B mRNA (5 of 8 cases) than for β-glucuronidase (3 of 8 cases) and tubulin (1 of 5 cases). The overall patterns were again unique to each probe. Tubulin mRNA content was not significantly different in cancer versus normal tissues, while β-glucuronidase mRNA was increased in tumor tissue, although less frequently than cathepsin B. These data are consistent with our previous observation that β-glucuronidase demonstrates a range of specific activity in colorectal carcinomas but no consistent increase in carcinoma compared with normal tissues (12).

To test for possible nonspecific hybridization of our probes to rRNA present in our RNA preparations, we separated poly A+ mRNA from poly A− RNA for the carcinoma sample, CA 55. As seen at the bottom of Fig. 1, the cathepsin B probe hybridized to poly A+ mRNA but not to poly A− RNA. Similarly, neither the β-glucuronidase nor tubulin probes hybridized to poly A+ RNA or to poly A− RNA.

Changes in Enzyme Activity Compared with Changes in mRNA Content. Cathepsin B enzyme specific activity levels were determined, and cancer/normal ratios were calculated for the same eight matched pairs of colorectal tissue which were analyzed for their mRNA content. As shown in Table 1, the pattern of increased tumor cathepsin B activity for this series was similar to that reported in our earlier study of 28 matched pairs of human colorectal tissues (12). In the earlier series, a significant tumor-specific increase in enzyme activity was demonstrated, with cancer/normal ratios of 1.44 or higher in 14 of 28 cases (P < 0.005), while in the 8 additional matched pairs, we have measured cancer/normal ratios of 1.43 or higher in 5 of 8 cases. When pooling all 36 cases, the mean cancer/normal ratio for enzyme activity was 1.45 ± 0.7 (P < 0.0005). When the 8 new matched pairs were ranked according to Dukes’ stage, increases in enzymatic activity were seen in 4 of 5 cases for Stages A and B and in one of 3 cases for Stages C and D (Table 1), confirming our prior finding that increases are higher and more consistent in earlier clinical stages. When all 36 cases were pooled according to stage, enzyme activity was significantly increased in carcinomas of Stages A and B, but not in carcinomas of Stages C and D. Compared with normal tissues, the P values for these increases in Stages A and B were <0.05 and 0.005, respectively. Compared with Stage D, the P values for Stages A and B were <0.05 and 0.05, respectively.

Table 1 also compares the data on cathepsin B enzymatic activity and mRNA content in the last 8 pairs and ranks them according to clinical stage. Tumor-specific increases in mRNA, with a mean cancer/normal ratio of 3.46, were much greater than the corresponding increases in enzyme activity (mean cancer/normal ratio of 1.35 for these 8 pairs or 1.45 for all 36 pairs). Increased mRNA content was also a more consistent change than increased enzyme activity with cathepsin B mRNA elevated in 7 of 8 (88%) matched pairs compared with elevated cathepsin B activity in 5 of 8 (62%) pairs or in 21 of 36 (58%) total pairs tested.

Ranking the 8 cancer/normal ratios for mRNA according to clinical stages showed that increases were greater for Dukes’ A...
and B carcinomas than for Dukes' C and D carcinomas, confirming the trend observed for enzyme activity. To evaluate the extent to which increases in mRNA content are negatively related to clinical stage, we assigned numerical values 1, 2, 3, and 4 to Dukes' Stages A, B, C, and D and calculated the best fitting regression line through our eight data points for cancer/normal RNA ratios. The resulting correlation coefficient \((-0.77)\) indicates a significant inverse correlation between clinical stages and cathepsin B mRNA content \((P < 0.05)\). The slope of the regression line \((-1.56)\) implies that the predicted cathepsin B mRNA level for a Dukes' A carcinoma is 4.7 times higher than the predicted value for a Dukes' D carcinoma.

Cathepsin B mRNA Banding Patterns. On a Northern gel, mRNAs isolated from 3 matched pairs of control mucosa and colorectal carcinoma demonstrate the presence of two bands for human cathepsin B in both normal and cancer tissue with the 2.2-kilobase band more strongly expressed than the 4.0-kilobase band (see Fig. 3). Both bands are increased in the carcinoma tissues, CA 47 and CA 48. The normal mucosa sample, NCT 56, demonstrates higher amounts of cathepsin B mRNA than the other control samples which brought the cancer/normal ratio to less than 1 for this pair.

The major bands of 2.2 and 4.0 kilobases which we observe in both normal and malignant human tissues are similar in size to those described previously by Moin et al. (15) for normal and malignant mouse tissues. However, two of our colorectal tumor samples, CA 47 and CA 48, expressed additional minor cathepsin B mRNA transcripts of approximately 1.5 and 3.0 kilobases. The relative amounts of the 1.5- and 3.0-kilobase transcripts in the carcinoma samples are low and do not contribute significantly to the increase in mRNA in these tumor tissues.

**DISCUSSION**

Our initial studies of cysteine protease activities in matched normal colorectal mucosa and carcinoma tissue had demonstrated a statistically significant increase in cathepsin B and L enzyme activities in colorectal carcinomas (12) and, in particular, significantly higher activities in the earlier stages of tumor growth. This study of eight new matched pairs of colorectal tissue confirms our prior research and provides new information on the regulation of cysteine protease expression during colorectal tumor progression. Our pooled data show that carcinomas of Dukes' Stages A and B have cathepsin B activity levels that are significantly increased compared with matched normal mucosa or compared with carcinomas of Dukes' Stage D. In addition, the new data show that the increases in enzyme activity are associated with corresponding increases in cathepsin B mRNA and that changes in mRNA are more striking than changes in enzyme activity, in both magnitude and frequency. As was found for cathepsin B enzyme activity, increases in mRNA content show a distinct inverse correlation with clinical stage.

A relationship between increased cathepsin B mRNA and invasiveness had been noted in two previous studies on murine tumors. In murine melanoma, Qian et al. (16) observed a better correlation of cathepsin B mRNA content with earlier stages (local invasion) than with later stages (extravasation and colonization) of the metastatic process. Moin et al. (15) reported an increase in cathepsin B mRNA in a murine hepatoma with high invasive but low metastatic potential. Our data show a similar trend in human colorectal carcinomas, with the greatest increase in mRNA and enzyme activity found in earlier stages, suggesting that elevated cathepsin B may be particularly important in tumors which are in the process of invading the bowel wall and local tissues. However, a variety of studies which have assayed the metastatic capabilities of B16 murine melanomas following tail vein injection of tumor cells demonstrate a correlation between increased cathepsin B enzyme activity or mRNA content and lung-colonizing potential (27, 28). Thus, elevated expression of cathepsin B has been shown, in different types of tumors, to contribute to either local invasion or metastatic spread. The role which cathepsin B plays in intravasation versus extravasation during the metastatic process may depend on the type of cancer being studied or on the local environment of the primary tumor. Alternatively, increased levels of cathepsin B may have a different impact on tumor progression when combined with changes in the subcellular localization of this protease. Sloane et al. (27) have suggested that changes in cathepsin B activity at later metastatic stages in animal tumors may be more qualitative than quantitative, characterized by a shift in intracellular distribution for cathepsin B with enrichment at the plasma membrane.

The increase which we have observed in mRNA content in colorectal tumor tissues is several fold higher than the average increase in enzyme specific activity in these tumor tissues. Thus, while colorectal carcinomas demonstrate a tumor-specific increase in cathepsin B mRNA, which may be due to increased transcription or stability of the cathepsin B message, other factors must exert posttranscriptional or posttranslational regulation over cathepsin B expression to result in enzyme activity levels lower than that permitted by the increase in mRNA content. In studies of mouse liver hepatoma and melanoma, a similar discrepancy between increased enzyme activity and mRNA levels has been reported for cathepsin B (15) with the authors suggesting that the presence of endogenous inhibitors may modify the final expression of tumor cathepsin B or, alternatively, that tumor tissues may synthesize higher levels of inactive, high-molecular-weight precursor forms of cathepsin B. In previous studies we did not find any change in total cysteine protease inhibitor levels in colorectal tumors compared with matched control tissues (12), but we did not rule out the possibility that levels of a single specific inhibitor might be altered or that protease/inhibitor affinities might be abnormal in colorectal carcinomas. Observation of a greater increase in a specific mRNA compared with the corresponding protein is not unique to cathepsin B but has also been reported, for example, for CEA in human colorectal cancer. Boucher et al. (29) have suggested that variations in CEA expression in human colorectal tissues result from both transcriptional and posttranscriptional control mechanisms, resulting in a lack of proportionality.
between mRNA and protein levels.

In addition to measuring quantitative changes in tumor cathepsin B mRNA, we have also analyzed the pattern of cathepsin B mRNA transcripts. Our Northern blot data demonstrate that normal human colorectal mucosa expresses a two-banded pattern for cathepsin B mRNA (2.2- and 4.0-kilobase transcripts). These data on the normal human cathepsin B mRNA banding pattern correspond to the two-banded pattern observed in normal murine liver (15) and normal rat kidney tissues (our data, not shown). Recently, Sloane and coworkers have also observed both the 2.2- and 4.0-kilobase cathepsin B transcripts in RNA isolated from normal human diploid MCF-10 breast epithelial cells. Although studies of murine melanomas by Qian et al. (16, 30) suggest that expression of the 4.0-kilobase cathepsin B message may be tumor specific, our results indicate that normal mammalian tissues typically express two cathepsin B mRNA transcripts, with the 4.0-kilobase transcript weaker and perhaps more variable in expression than the 2.2-kilobase transcript.

While the increase in cathepsin B mRNA content in colorectal carcinomas is due primarily to increases in the amount of the 2.2- and 4.0-kilobase transcripts, we have also observed minor additional bands of 1.5 and 3.0 kilobases in two of three colorectal carcinoma tissue mRNAs. No such minor bands have been reported in mouse tumor tissues, although Qian et al. (16, 30) did find a 5.0-kilobase cathepsin B mRNA transcript in mouse melanomas which was not seen in control tissues. These authors have characterized a cDNA probe for this 5.0-kilobase message as well as cDNAs for the 2.2- and 4.0-kilobase transcripts isolated from a murine melanoma cDNA library. The 4.0- and 5.0-kilobase transcripts were found to differ from the 2.2-kilobase transcript by the presence of unusually long 3' untranslated regions that are postulated to result from alternative use of 3'polyadenylation signals in tumor tissues (30). While our studies on cathepsin B mRNA patterns in colon mucosa and carcinoma specimens do not examine how longer transcripts are produced, observation of the 4.0-kilobase band in normal human tissues (this paper) and normal mouse tissues (15) indicates that the mechanism for generating the 4.0-kilobase transcript is not tumor specific. Additional research is needed to determine how frequently unusual cathepsin B mRNA transcripts occur in tumor RNA, whether certain abnormal transcripts are typical of particular types of tumors, and eventually, whether these transcripts are capable of generating a functionally abnormal form of the enzyme which may play a role in tumor cell behavior.

A number of molecular and biochemical markers appear to identify particular stages in the development of human colorectal carcinomas (31). These include hypomethylation of DNA (in very small adenomas up through carcinomas) (32, 33), somatic mutation of the ras gene (in 50% of adenomas >1 cm and in 50% of carcinomas) (34-36), loss of a cell adhesion gene region on chromosome 18 (in almost 50% of late adenomas and >70% of carcinomas) (36-39), loss of the p53 gene region on chromosome 17 (not common in adenomas but in 75% of carcinomas) (36, 38, 40), and abnormal gene expression and enzyme activities, such as tyrosine kinase activities (in carcinomas) (41-43). While no single type of abnormality is restricted to a particular stage of colorectal tumorigenesis, these changes appear to occur in a preferred order, with certain alterations more common to one stage of colorectal tumorigenesis than another. The increased levels of enzyme activity and mRNA content reported in this study for the cysteine protease, cathepsin B, are more typical of early stage than late stage colorectal carcinomas and thus may provide a useful marker for carcinoma progression. Recent investigations of another lysosomal protease, cathepsin D, suggest that high levels of the cathepsin D protein may have prognostic importance in breast cancer (44). To define further the role which increased cathepsin B enzyme activity or mRNA content may play in tumor development, we are now analyzing these changes in human colorectal carcinomas for correlation with known prognostic indicators and/or tumor recurrence or metastasis.

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