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Collagenolytic Enzymes in Human Neoplasms[1]

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SUMMARY

A variety of human neoplasms were examined for their ability to produce collagenolytic enzymes in culture. Some types of tumors of epithelial origin demonstrated a very high frequency of collagenolytic activity, while neoplasms of mesenchymal origin, nonneoplastic tissues, and other types of epithelial neoplasms only rarely produced collagenase. In particular, tumors of the colon and carcinomas of both squamous and basal cell origin displayed a high frequency of activity.

Tumor collagenases from several different sources were isolated and examined for their mode of attack on native collagen, pH optima, and inhibition of activity by EDTA, cysteine, and pooled human serum. Tumor enzymes appear to be similar to the collagenases isolated from normal human skin by these criteria.

INTRODUCTION

The notion that the capacity of some tumors to invade might be due to their ability to degrade the connective tissue structure of normal tissue has been entertained for some time. Firm experimental evidence to support this notion has not yet been established, although several publications have reported the presence of collagenolytic enzymes in some types of human and rat tumors (17–19).

Since the discovery by Gross and Lapiere (12) of a specific collagenase in tadpole tailfin, such enzymes have been reported in a large number of situations in which connective tissue remodeling occurs (7). While the evidence for collagenase involvement in pathological conditions is indirect at the present time, these enzymes have been implicated in wound healing (5), rheumatoid arthritis (13), periodontal disease (2), and alkali-burned corneas (1, 4). Recently, the presence and activity of these enzymes have been demonstrated directly *in vivo* (6, 8).

This study was undertaken in an attempt to correlate the ability of a variety of human tumors to produce, in culture, collagenase with pathological features and cell type. In addition, collagenases produced by tumors of the skin, colon, lung, and endometrium were isolated, partially characterized, and compared with normal human skin collagenase by a variety of criteria.

MATERIALS AND METHODS

Organ Culture Methods. Fresh tissue specimens were obtained from surgery and pathology laboratories of hospitals within the Texas Medical Center and were assayed for collagenolytic activity. Tissue samples were rinsed with Tyrode's medium containing penicillin and streptomycin, 150 μg/ml each, minced into fragments (1 to 2 cu mm), and incubated at 35° in a 95% O_2-5% CO_2 atmosphere for 4 days.

Assay for Collagenolytic Activity. Two major criteria were used to determine whether tissue specimens produced collagenase in culture. First, single tissue fragments were placed on 0.1% reconstituted native collagen gels in Leighton tubes (Bellco Glass, Inc., Vineland, N. J.). We prepared the gels by warming to 35° a 0.25-ml solution of acid-extracted guinea pig skin collagen. Tissue specimens were scored as positive if at least one-half of the tissue explants produced a clear zone of collagenolysis of the opaque collagen gel in 4 days at 35°.

Second, tissue fragments were incubated at 35° for 4 days in Tyrode's medium in Falcon flasks (16). At the end of this time, the medium was removed and concentrated either by ammonium sulfate precipitation to 60% saturation, or by vacuum dialysis. Concentrated medium or resuspended ammonium sulfate precipitate was dialyzed against 0.01 M Tris-HCl (pH 7.8 at 23°), 0.005 M $CaCl_2$ and assayed for collagenolytic activity viscosimetrically in Cannon-Ostwald semimicroviscometers (Cannon Instrument Co., State College, Pa.). Positive specimens produced a 40 to 60% decrease in viscosity of a solution of acid-extracted collagen in 18 hr at 24° with the use of concentrated media equivalent to two 1- to 2-cu mm tissue explants (Chart 1). In addition, these reaction mixtures were analyzed by acrylamide gel electrophoresis (15) to ascertain whether collagen degradation had occurred during incubation. All positive specimens produced the same collagen degradation products previously demonstrated for collagenases of the skin (10) and rheumatoid synovia (13). Cultures were examined routinely for bacterial or fungal contamination; such cultures were discarded and not included in the results.

RESULTS

Collagenase Production by Various Human Tissues. To date, a total of 140 human tissue specimens have been examined for their ability to produce collagenolytic enzymes by the

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techniques outlined in "Materials and Methods." The majority of these consisted of skin tumors. In addition, adenocarcinomas of the lung, colon, ovary, and breast were examined. A small number of neoplasms of mesenchymal origin are also included, as well as benign lesions such as dermatofibromas, s.c. lipomas, seborrheic keratoses, and normal skin. The results of this survey are shown in Table 1. Malignant invasive neoplasms of the skin and colonic adenocarcinomas clearly produce collagenase in culture with high frequency. On the other hand, skin neoplasms which do not show dermal invasion (actinic and seborrheic keratoses) and normal human skin do not produce collagenase with sufficient frequency to be scored as positive under our assay conditions. In addition, invasive adenocarcinomas of the lung, breast, and ovary did not produce appreciable amounts of collagenase in culture. The lack of collagenase activity in some invasive tumors may be due to either the lack of collagenase production, the presence of collagenase inhibitors (8), or to technical problems (*i.e.*, delay in transport) in handling, prior to our organ culture procedures.

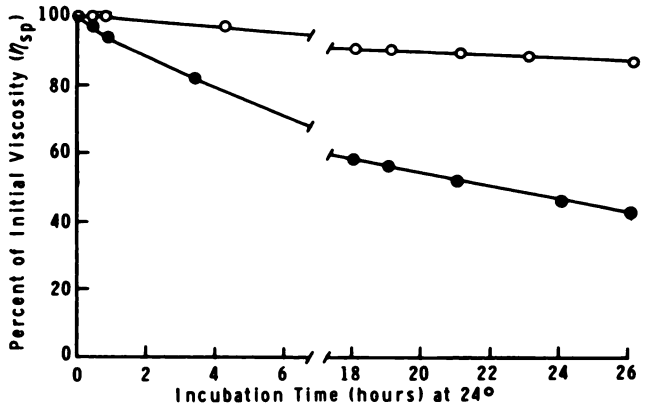

Chart 1. Viscosimetric assay of tumor collagenases. Partially purified collagenase preparations were incubated with acid-soluble guinea pig skin collagen (0.7 mg/ml) in 0.3 M NaCl, 5 mM $CaCl_2$, and 10 mM Tris-HCl, pH 7.6, at 24° in a Cannon-Ostwald semimicroviscometer. •, preparation from a colonic adenocarcinoma; ○, preparation from an adenocarcinoma of the lung.

Table 1

Correlation of tissue type and collagenase production

Tissue specimen	No. active	No. inactive
Epithelial		
Skin specimen		
Basal cell carcinoma	25	13
Squamous cell carcinoma	10	3
Keratoacanthoma	2	3
Keratoses (actinic, arsenical)	1	12
Normal skin (including fibromas and seborrheic keratoses)	1	15
Other		
Adenocarcinoma (colon)	12	6
Adenocarcinoma (breast)	0	6
Adenocarcinoma (ovary)	0	5
Adenocarcinoma (lung)	0	6
Adenocarcinoma (thyroid, 3; parathyroid, 1)	0	4
Adenocarcinoma (endometrium)	1	0
Adenocarcinoma (brain)	0	5
Mesenchymal		
Leiomyosarcoma	1	1
Astrocytoma	0	1
Chordoma	0	1
Chondrosarcoma	0	1

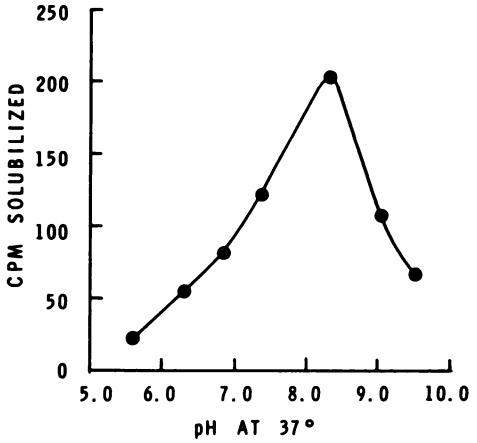

Chart 2. Activity of tumor collagenases as a function of pH. Tumor collagenases were incubated on ^{14}C-labeled glycine collagen microgels by the method of Nagai *et al.* (16). After incubation for 20 hr at 35° in the presence of 5 mM $CaCl_2$ and various Tris-HCl and Tris-maleate buffers, undigested fibrous collagen was removed by centrifugation. Solubilized, degraded collagen was counted in Bray's scintillation fluid containing 4% Ca-bo-Sil (Cabot Corp., Boston, Mass.). These data were obtained with the use of preparations derived from basal cell carcinomas. Similar results were obtained for squamous cell carcinomas and adenocarcinomas of the colon.

Partial Characterization of Tumor Collagenases. Enzymes from basal cell carcinomas, squamous cell carcinomas, colon carcinomas, and an endometrial carcinoma were isolated and concentrated by ammonium sulfate precipitation for further study. All were able to degrade both soluble collagen (as measured by a decrease in the viscosity of collagen solutions; see Chart 1) and fibrous collagen (as measured by the release of radioactivity from collagen gels) (16).

These partially purified enzymes have maximum enzyme activity in the neutral to alkaline pH range (Chart 2), with pH optima similar to that described by Eisen *et al.* (10) for the collagenase from normal human skin.

The enzyme derived from human skin is inhibited by the chelating agent EDTA, by cysteine, and by pooled human serum (10). Collagenases from both basal and squamous cell carcinomas and colon and endometrial carcinomas are similarly inhibited by these agents (Table 2).

The products of tumor collagenase action on soluble collagen were examined by acrylamide gel electrophoresis (Fig. 1). As with human skin and rheumatoid synovial enzyme, the only cleavage products found at incubation temperatures below 30° were similar to $TC_A{}^{75\%}$ [2] and $TC_B{}^{25\%}$ (Fig. 1*B*). The identity of these fragments was confirmed by electron microscopy of segment-long-spacing crystallites. Above 30°, these products undergo thermal denaturation and are degraded to dialyzable peptides by these partially purified collagenase

[2] The abbreviation used is: TC, tropocollagen.

Table 2

Effect of various inhibitors on tumor collagenases

Tumor collagenases were assayed on radioactive collagen microgels by the method of Nagai *et al.* (16). The data are expressed as percentage of *inhibition* after an 18-hr incubation period at 35°. Pooled human serum was diluted 1:10 in the reaction mixture.

Tumor type	Inhibition (%) with the following inhibitors		
	1 mM EDTA	10 mM cysteine	Human serum
Basal cell carcinoma	96	88	100
Squamous cell carcinoma	94	98	90
Colon adenocarcinoma	100	100	100
Endometrial adenocarcinoma	97	97	100

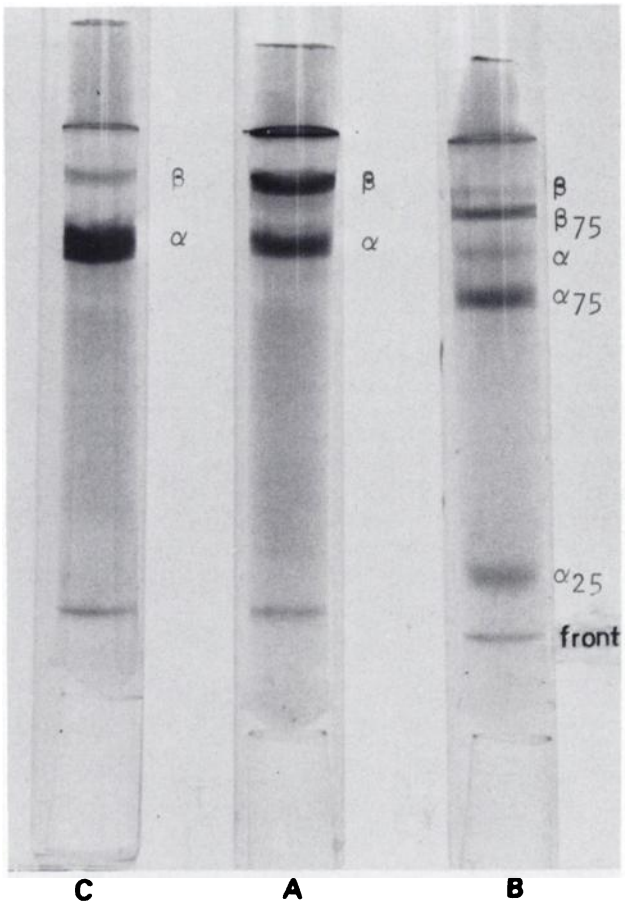

Fig. 1. Acrylamide gel electrophoresis of tumor collagenase-collagen reaction mixtures. Viscosity reaction mixtures, after 20 hr at 24°, were acidified and subjected to acrylamide gel electrophoresis by the methods of Nagai *et al.* (15). Gels were stained with Amido black. *A*, normal guinea pig skin collagen, no enzyme; *B*, guinea pig skin collagen after incubation with a preparation from an active, collagenase-producing tumor; *C*, guinea pig skin collagen after incubation with 1 of several tumor preparations. There is no significant degradation of normal β and α chains, but dimeric β chains have been partially converted to monomeric α chains by this preparation (see text for discussion).

preparations. Fig. 1*C* shows that some tumor preparations contained additional enzyme activities able to convert dimeric collagen chains (β chains) to monomeric α chains without appreciable alterations in the electrophoretic migration rate of the β and α chains, presumably by cleavage of the telopeptide regions of the cross-linked β chains. Bornstein *et al.* (3) and Eisen and Jeffrey (9) have described a similar effect on collagen by chymotrypsin and crustacean collagenase preparations, respectively. This β-splitting activity did not appear to be correlated with collagenase activity; it was not inhibited by EDTA or cysteine, but it was inhibited by phenylmethane sulfonyl fluoride, an inhibitor of chymotrypsin and trypsin (11). The occurrence and significance of this enzymatic activity is currently under investigation.

DISCUSSION

This report describes a survey of 140 human tissue specimens examined for their ability to produce collagenolytic enzymes in organ culture. The results of this survey (Table 1) demonstrate that certain types of human neoplasms produce collagenolytic activity *in vitro* with high frequency. These include basal and squamous cell carcinomas, keratoacanthomas, and carcinomas of the colon. On the other hand, nonmalignant skin lesions, *i.e.*, actinic and seborrheic keratoses, as well as normal skin, only rarely produce collagenase. A number (5) of tumors of mesenchymal and stromal origin [leiomyosarcoma (2), chordoma, chondrosarcoma, and astrocytoma] were also assayed, in view of the recent report of Taylor *et al.* (19) that indicated the presence of collagenases in tumors of mesenchymal origin. Of these, 1 tumor (a leiomyosarcoma) produced collagenase in culture. This sample is too small to permit one to draw firm conclusions.

Collagenolytic enzymes produced in culture by more than 10 basal and squamous cell carcinomas, 5 colonic carcinomas, and 1 endometrial carcinoma were isolated and further examined. All tumor enzymes are inhibited by 1 mM EDTA, 10 mM cysteine, and pooled human serum (Table 2). Similar results have been obtained for collagenases isolated from normal human skin by Eisen *et al.* (10) and by Krane and coworkers [Harris *et al.* (13)] for a collagenase from rheumatoid synovia. In addition, the relation of collagenolytic activity and pH is similar for normal and tumor enzymes. Degradation of soluble native collagen by tumor enzymes (colon as well as basal and squamous cell carcinomas) appears to yield the same products; *i.e.*, cleavage of the collagen molecule into 75% (TC_A) and 25% (TC_B) fragments, as are

those produced by collagenases from the skin (10), rheumatoid synovia (13), and human granulocytes (14).

These results indicate that, by the criteria we have used, collagenolytic enzymes from human neoplasms and normal human skin are similar. Further experiments to relate these enzymes to those of normal human tissues by biochemical and immunological criteria are in progress.

Tentatively, we conclude that these enzymes are qualitatively similar to those of normal tissue and that their enhanced presence in a small variety of human neoplasms represents overproduction of the normal enzyme.

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