Collagen and Collagen-derived Fragments Are Chemotactic for Tumor Cells

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ABSTRACT Organs that are rich in collagen such as liver, lungs, and bone are frequently sites of tumor cell metastasis. In this study, we have found that cultured tumor cells of human and rat origin migrated unidirectionally in response to collagen in vitro. Synthetic di- and tri-peptides that contained amino acid sequences found frequently in the collagen helix caused similar effects. These results are consistent with the hypothesis that collagen or collagen fragments released during connective tissue remodeling may be important in tumor cell metastasis.

INTRODUCTION

Organs rich in collagen such as liver, lung, and bone are frequently sites of tumor cell metastasis. In this study, the in vitro Boyden chamber system was used to show that cultured tumor cells are attracted unidirectionally along a concentration gradient of Types I and III human and rat collagens as well as collagen-derived fragments and synthetic di- and tri-peptides. These results are consistent with the hypothesis that release of collagen fragments during normal connective tissue remodeling may be the mechanism by which tumor cells are attracted out of the circulation into organ sites where metastatic lesions develop.

METHODS

The technique for studying both leukocyte and tumor cell chemotaxis in vitro has been described in detail previously (1–6). In brief, cultured tumor cells that are removed by mechanical dispersion are placed in the upper compartment of a modified Boyden chamber at a concentration by 5×10^5 cells/ml of culture medium. The upper and lower compartments of the Boyden chamber are separated by a nitrocellulose filter membrane with a pore size of 12 μm Diam. 1 ml of the chemotactic solution or corresponding control medium are placed in the lower compartment and the chambers are incubated for 4 h at 37°C in 5% CO_2 and air. Following this period, the filters are removed, fixed and stained with hematoxylin. Chemotaxis is measured by counting the number of cells in light microscopic high power fields (×400) that have migrated into the membrane pores to a distance of 10 μm. Three chambers are used for each chemotactic sample and in each filter five different high power fields are counted. Means and standard errors of the mean are calculated and statistical differences are analyzed using Student's *t* test for nonpaired samples.

We studied the chemotactic response of different rat and human tumor cells to collagen. We used Walker 256 rat carcinosarcoma cells (Flow Laboratories, Rockville, Md.) which were originally derived from a spontaneous rat breast tumor and are maintained by serial passage in cell culture. Chemotactic behavior of these cells has been described in detail previously (2–6). HeLa cells that were originally derived from a patient with carcinoma of the cervix were obtained from Dr. T. Schenk. MB-MDA-231 cells are cultured human breast cancer cells derived originally from the pleural effusion of a patient with disseminated carcinoma of the breast (7). Cultured rat osteosarcoma cells were provided by Dr. Gideon Rodan and Dr. Robert Majeska. Human neutrophils and monocytes were obtained from peripheral blood of normal individuals by previously described methods (8). The collagens were solubilized from cirrhotic human liver by limited pepsin digestion and purified by repetitive salt fractionation (9–11). The purity of each collagen type was >95% as assessed by electrophoresis on polyacrylamide slab gels. The free α-chains were obtained by ion exchange chromatography of lathyritic rat skin collagen (12).

RESULTS

Table I shows the chemotactic effects of purified Type I collagen on different types of cells. Walker rat tumor cells, and a subclone (clone 4), cultured human cervical cancer cells (HeLa) and cultured human breast cancer cells (MB-MDA-231) responded, whereas a subclone of the Walker rat tumor cells (clone 7) and human neutrophils did not. Human peripheral blood monocytes responded chemotactically to collagen, as has been shown previously (13).

The chemotactic effects of different types of collagen on Walker rat tumor cells were assessed (Table II). All of the intact collagens caused chemotactic responses,

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TABLE I

Chemotactic Responses of Different Cells to Solubilized Human Type I Collagen (1 μM)

Cells	Chemotactic activity (percent increase compared with chambers in which control media was placed in the lower compartment)
Walker rat tumor cells	200±30*
Clone 4	71±10*
Clone 7	1±8
HeLa human cervical cancer cells	69±11*
MB-MDA-231 human breast cancer cells	173±35*
Rat osteosarcoma cells	−4±6
Human neutrophils	−21±15
Human monocytes	134±23*

* Significantly greater than control, $P < 0.05$.
Values are mean±SE for 15 high-power fields.
The data in this table were taken from different experiments and so are expressed as percent increases in chemotactic activity compared with corresponding controls.

as did free α_1- and α_2-chains obtained by ion exchange chromatography from lathyritic rat skin collagen. The maximal effects were observed at collagen concentrations of 1 μM (Table III). There was increased movement of tumor cells into the filters only when there was a gradient between the upper and lower compartments

TABLE II

Effects of Different Types of Human Collagen, Free Chains and Synthetic Di- and Tri-peptides on Chemotactic Responses of Walker 256 Rat Carcinosarcoma Cells

Chemoattractant	Chemotactic activity (cells migrated per high-power field)
Experiment 1	
Type I collagen, 0.1 μM	20±3*
Type III collagen, 0.1 μM	21±2*
Type V collagen, 0.1 μM	19±3*
Gly-Pro,‡ 12.5 μM	16±2*
Gly-Pro-Ala, 12.5 μM	22±2*
Control media	10±1
Experiment 2	
Type I collagen, 1 μM	12±2*
α_1-chains, 1 μM	15±2*
α_2-chains, 1 μM	12±2*
Control media	8±1

Values are mean±SEM for 15 high-power fields.
* Significantly greater than corresponding control, $P < 0.05$.
Native collagen was obtained from human cirrhotic liver and free α-chains from lathyritic skin collagen according to the methods described in the text.
‡ Gly, glycine; Pro, proline.

TABLE III

Dose-response Effects of Different Concentrations of Solubilized Human Type I Collagen and the Synthetic Di-peptide Gly-Pro on Migration of Cultured Tumor Cells

Chemoattractant	Concentration	Chemotactic activity (cells migrated per high-power field)
Experiment 1		
Collagen	1 μM	17±2*
Collagen	0.1 μM	16±1*
Collagen	10 nM	12±1*
Gly-Pro	0.1 mM	18±1*
Gly-Pro	10 μM	14±1*
Gly-Pro	1 μM	13±1*
Gly-Pro	0.1 μM	14±1*
Control media		9±1
Experiment 2		
Gly-Pro	0.1 mM	8±1*
Gly-Pro	10 μM	10±1*
Gly-Pro	1 μM	11±1*
Gly-Pro	0.1 μM	10±1*
Control media		2±1
Experiment 3		
Collagen	1 μM	150±10*
Collagen	0.1 μM	75±12*
Collagen	10 nM	22±3
Collagen	1 nM	20±2
Control media		17±2

Values are mean±SEM for 15 high-power fields.
* Significantly >1.0, $P < 0.05$.

of the Boyden chamber (Table IV). There was no significant increase in cell migration in those chambers in which the collagen was present in the upper compartment with the cells as well as in the lower compartment (Table IV). Synthetic di- and tri-peptides that contained the amino acids glycine and proline or glycine and hydroxyproline in the sequence usually present in the collagen helix also caused chemotactic responses (Tables II, III, V).

DISCUSSION

The process of tumor cell metastasis has been difficult to study in vitro, since multiple mechanisms may be responsible for the cellular events that occur between the shedding of tumor cells from the primary site and the appearance of a metastasis in a selected distant site. Technical problems such as the tight adherence of tumor cells to each other and to the surfaces of cultured vessels also limit in vitro studies of tumor cell movement. The most widely used system for studying cell chemotaxis in vitro is the Boyden chamber technique (1). We have used this technique in the past to demon-

TABLE IV

Evidence that the Migration of Tumor Cells in Response to Solubilized Human Type I Collagen is Unidirectional

Upper compartment	Lower compartment	Chemotactic activity (cells migrated per high-power field)*
Experiment 1		
Collagen, 1 μM	Control media	7±1
	Collagen, 0.1 μM	8±1
	Collagen, 1 μM	7±1
Collagen, 0.1 μM	Control media	5±1
	Collagen, 0.1 μM	7±1
	Collagen, 1 μM	8±1
Control media	Control media	5±1
	Collagen, 0.1 μM	11±1‡
	Collagen, 1 μM	17±1‡
Experiment 2		
Collagen, 0.5 μM	Control media	9±1
	Collagen, 50 μM	8±1
	Collagen, 0.5 μM	7±1
Collagen, 50 μM	Control media	8±1
	Collagen, 50 μM	12±1‡
	Collagen, 0.5 μM	9±1
Control media	Control media	7±1
	Collagen, 50 μM	28±3‡
	Collagen, 0.5 μM	20±2‡

* Values are mean±SEM for 15 high-power fields.
‡ Significantly greater than corresponding control, $P < 0.05$.

TABLE V

Chemotactic Responses of Walker Tumor Cells to Synthetic Di- and Tri-peptides

Chemoattractant	Chemotactic activity (cells migrated per HPF)* Experiment 1	Experiment 2
Gly-Pro-Ala	48±3*	43±2‡
Gly-Pro-Hyp	44±3*	36±2‡
Gly-Pro	43±5*	NT
Gly-Hyp	37±2*	NT
Pro-Gly-Gly	23±1	NT
Gly-Leu-Tyr	20±2	32±2
Pro-Hyp	36±3	NT
Gly-Ile	NT	33±4
Pro-Ile	33±2	NT
Gly-Phe	26±2	33±2
Control Media	29±4	27±2

* Values are mean±SEM for high-power fields.
‡ Significantly greater than control media, $P < 0.05$.
Synthetic peptides were tested at concentrations of 12.5 μM in control media.
NT, Not tested.

strate that tumor cells migrate in response to a chemotactic factor derived from bone (2–3), and others have shown that tumor cells respond to a complement-derived chemotactic factor (4–6). Whenever tumor cells lodge in distant sites and form a metastatic nidus, they must migrate out of capillaries or sinusoids into the surrounding tissue. This process of transvascular migration of tumor cells may be a directed rather than random event. This study reports an attempt to identify signals that may be responsible for attracting tumor cells out of sinusoids as they pass through a distant organ.

Our data are consistent with the notion that the collagen molecule contains a chemotactic signal for tumor cells, and this chemotactic factor is released during the process of collagen degradation. It is likely that this chemotactic signal contains the amino acid residues glycine and proline and is at least two amino acids in length. It is possible that a whole series of fragments of the collagen molecule containing glycine and proline residues are chemotactic for tumor cells.

The molar concentration of the synthetic peptides required to produce a consistent chemotactic response was usually one order of magnitude greater than the molar concentration of intact collagen which produced a similar response. The reason for this is unclear, but similar results have been found with these peptides and fibroblast chemotaxis (14). Postlethwaite et al. (14) have suggested that the ordered helical structure of native collagen may facilitate binding of the chemotactic factor to the cellular receptor. We did not show significant loss of activity as the synthetic di-peptide was diluted beyond the lowest effective concentrations of intact collagen (Table III). This may occur because these smaller molecules are freely and rapidly diffusable across the filter membrane. The maximal effects observed with Type 1 collagen were at 1 μM, which is near the limits of its solubility.

Recently, we found that remodeling or resorbing bone produces a factor that is chemotactic for tumor cells (2–3). This bone-derived factor is nondialyzable and macromolecular and is released into the media bathing organ cultures of resorbing bones. It may provide the explanation for the selective metastasis of some tumor cells to bone. Its relationship to collagen or collagen fragments is unresolved, but since the bone matrix comprises ~95% Type I collagen, it is possible that collagen fragments that are released during the process of bone remodeling are chemotactic signals to tumor cells. In data not shown, we have found that demineralized bone exposed to bacterial collagenase also releases chemotactic activity for cultured Walker tumor cells.

It is clear that different tumors metastasize selectively to different organs. Spleen, cartilage, and skeletal muscle are rarely the site of metastasis, whereas most

tumors metastasize to the liver or lungs. Bone is a frequent site of metastasis of some tumors such as breast cancer, but is an uncommon metastatic site for carcinomas of the gastrointestinal tract or female genitalia. The reasons for these patterns of tumor metastasis are not clear. One possibility is that organs such as the lungs and liver contain a rich venous blood supply so that circulating tumor cells gain ready access to these organs. However, this cannot explain the observation that some tumors migrate selectively to bone and some do not. It is possible that the local environment of organs such as bone is unfavorable to the formation of some tumor metastases, but not to others. The data presented here suggests that another reason that tumor cells collect selectively in some organs may be due to the connective tissue remodeling of the stroma of that organ, causing the generation of fragments of collagen that may be chemo-attractants for tumor cells.

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