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Host-mediated effectors of tumor invasion: role of mast cells in matrix degradation

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The role of collagenolytic enzymes in tumor invasion and metastasis has been emphasized, but the source of enzyme activity has remained unclear. Degradation of stromal connective tissue is a common feature of invasive neoplasia, and host–tumor cell interactions are probably important for localized collagenolysis. We have examined the role of mast cells in malignant cell invasion using cells derived from the rat mammary adenocarcinoma 13762NF. Histologic studies have shown increased numbers of mast cells at the zone of tumor invasion. Mast cell products and conditioned medium from such cells stimulated the production of collagenolytic enzymes by stromal fibroblasts as well as certain subpopulations of tumor cells *in vitro*. The tumor cell response to mast cell-mediated stimulation of collagenolysis appears to be related to the metastatic potential of the tumor cell. A subpopulation of host fibroblasts derived from the invading tumor zone was also found to be more responsive to mast cell factors than normal fibroblasts, as judged by collagenase production. Thus the mast cell has the potential to induce collagenolytic activity from both host fibroblasts and tumor cells.

Introduction

Stromal changes, including extensive collagen degradation, are cardinal features of tumor invasion and metastasis [18, 36, 40]. Previous data from this laboratory and others [2, 10, 13, 18, 21, 22, 28, 40] have demonstrated that the release of relatively high levels of collagenolytic activity is associated with tumor invasive properties. However, the source of this enhanced enzyme activity has remained uncertain. Evidence has been provided that demonstrates that both stromal cells and tumor cells are likely participants in extracellular matrix degradation [2, 10, 12, 28, 38]. It has been suggested that host–tumor cell–cell interactions appear to play a significant role in mediating the release of relatively high levels of enzyme activity [2, 5, 11, 12, 28, 38]. Thus it is likely that several collagenolytic mechanisms might be functioning in facilitating the invasive growth of malignant tumors [5, 11, 38].

Histologic studies have shown that at the invading tumor edge the proportions of tumor and host cells, as well as the composition of the extracellular matrix, vary tremendously in different types of tumors [33, 35]. In response to inflammation at the periphery of growing tumors, host cells, such as macrophages and mast cells, appear to migrate to sites near the invasion zone [39]. In addition, mast cells are

unique among leukocytes in their capacities to enhance tumor proliferation [32], and mast cells have been identified at sites of connective tissue degradation [7, 30]. Mast cells have also been shown to contain soluble factors which can stimulate cultured synovial cells to produce PGE and collagenase [41]. On the basis of such studies it was suggested that mast cells may play a role in modulating collagenolysis [39].

Due to our interest in examining host–tumor cell–cell interactions during tumor invasion, we have examined the influence of mast cell and mast cell products on the collagenolytic activities of rat mammary adenocarcinoma cells and host tissue fibroblasts *in vitro*. We report here that mast cells have the potential to stimulate both tumor and host cells to produce elevated levels of collagenolytic activity which contributes to host tissue degradation at the edges of invading tumors.

Materials and methods

Tumor cell culture

Tumor cell clones (MTLn2, MTLn3 and MTF7) were isolated and cloned from rat mammary adenocarcinoma 13762NF and were characterized as previously described [23, 24, 29]. Cells obtained from stocks were grown in alpha-modified-minimum essential medium (AMEM) supplemented with 10 per cent heat-inactivated fetal calf serum (HIFCS) (Grand Island Biological Co., Grand Island, NY) without antibiotics.

Fibroblast cell culture

Normal rat skin fibroblasts (NRS) were established from skin explants of syngeneic newborn rats. Pregnant Fischer 344 pathogen- and virus-free rats were obtained from Harlan, Inc. (Indianapolis, IN). The newborn rats were sacrificed one day after birth. The skin was aseptically removed, and prepared for explant cultures. AMEM containing 10 per cent HIFCS was added to the cultures, and the cells were incubated at 37°C in a 5 per cent CO_2–95 per cent humidified atmosphere. Confluent monolayers of fibroblast cells were subcultured and stored in liquid nitrogen until needed.

Fibroblast-like cells (Ln3-F) were also isolated from primary cultures initiated by explantation of rat tumor tissue from a spontaneous lung metastasis. The tumor tissue was obtained from syngeneic female F344 rats injected subcutaneously with 5×10^5 cloned MTLn3 tumor cells. The tissue was aseptically removed 21 days after inoculation, minced and prepared for explant cultures. The resulting primary cell cultures were enzymatically dispersed and fractionated as described elsewhere [12].

Mast cell preparation (MCP)

Mast cells were isolated from 23 rats bearing 32-day-old tumors. F344 virgin female rats were each injected subcutaneously with 5×10^5 passage 18 MTLn3 (P18) tumor cells into the left posterior inguinal mammary fat pad. Mast cells were collected from the peritoneal cavity by injecting 15 ml DPBS containing 0·1 per cent gelatin (Sigma, St Louis, MO) and 10 U/ml of heparin (Abbott Laboratories, Chicago, IL) into a small incision made in the abdominal wall. The abdominal area was massaged, and the fluid was removed and pooled. The cell suspension was centrifuged at 1000 r.p.m. for 20 min and adjusted with DPBS to a cell density of $1{\cdot}5 \times 10^7$ cells/ml. The cells were purified using a 33, 39, 45, 51, 57, 63 and 69 per cent

stock Percoll density gradient as described previously [3]. The cells were centrifuged at 1200 r.p.m. for 1 h. The mast cells were collected below the 69 per cent layer (density $\rho = 1{\cdot}090$), and were present at a purity of 95 per cent and a viability of >95 per cent. The purity and viability of the isolated mast cells (MC) were determined by the toluidine blue staining procedure. The isolated mast cells were washed twice with DPBS, centrifuged, resuspended, counted and divided into two groups (one group for cell culture and one group for MCP). For cell culture, the cells were plated at a density of 1×10^6 cells/T-75 tissue culture flask in RPMI-1640 medium supplemented with 10 per cent FCS, and were incubated at 37°C in a 5 per cent CO_2–95 per cent humidified air atmosphere. For MCP, mast cells were diluted 1 : 10 with DPBS and centrifuged. The mast cells were resuspended in DPBS, and the cell density was adjusted to 2×10^6 cells/ml. This in turn was diluted 1 : 1 with a 2 M NaCl solution yielding a final concentration of 1 M NaCl and stirred overnight at 4°C. The following day, the cell suspension was sonicated for 10 s and centrifuged at 16 000 r.p.m. for 75 min. The resulting supernatant, obtained from 1×10^6 cells/ml, was collected and stored at −20°C.

Conditioned media

Mast cell conditioned medium (MCCM) was collected after 3 days of incubation in RPMI-1640 containing 10 per cent FCS. The cell-free medium was filter sterilized through a Millex-GS filter and stored at −20°C.

Tumor cell-conditioned media from MTLn2 (Ln2-cm) or MTLn3 (Ln3-cm) cells were prepared by incubating the tumor cells with AMEM containing 4 per cent HIFCS in the same manner as described above.

Enzyme activity in cell cultures

For assaying collagenase release in cell culture, the cells were seeded at 5×10^4 cells/well in multiwell trays (Corning, Corning, NY) containing AMEM and 5 per cent HIFCS and grown 24 h prior to treatment with MCCM. Culture medium from triplicate wells was removed and replaced with 0·5 ml MCCM diluted 1 : 3 (v/v). Similarly, MCP diluted 1 : 9 (v/v), or Ln2-CM and Ln3-CM diluted 1 : 1 (v/v) were applied in the same manner. Control wells received fresh AMEM plus 2 per cent HIFCS. Media were collected after 3 days and stored at −20°C. Samples were adjusted to Tris–HCl buffer (0·05 M) containing 0·01 M $CaCl_2$, 0·2 M NaCl, pH 7·5, prior to assay.

Collagenase was assayed by measuring the release of soluble radioactive peptides from 30 μl [^{14}C]glycine labeled collagen fibrils after 16 h at 35°C. One unit of collagenase activity degrades 1 μg collagen per 6 min at 35°C and data is expressed as cumulative units per 10^6 cells per 3 days of culture [11]. Azocoll was used as a substrate for protease determination. The reaction mixture consisted of 200 μl sample and 2 mg Azocoll (Calbiochem, La Jolla, CA). The mixture was incubated for 21 h at 35°C with constant agitation, filtered and the absorbance of the filtrate was measured at 520 nm.

Collagen substrate

^{14}C-Labeled collagen substrate was prepared from 16-day-old chick calvaria incubated in serum-free Dulbecco's minimal essential medium (DMEM) containing [^{14}C]glycine as described previously [12]. Acid-soluble calf skin collagen at a

concentration of 4 mg/ml was used as carrier collagen to prepare radioactive gels which served as substrates for collagenase assay.

Biochemical analysis of MCP

Histamine, heparin and prostaglandin E (PGE) contents of MCP were determined by the methods of Yoffe *et al.* [41].

Histological examination

Tumor specimens were fixed in 1 per cent formaldehyde and 0·25 per cent glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·4), embedded in JB-4 (Polysciences, Warrington, PA), and sectioned and stained according to the method of Bromley *et al.* [8].

Results

Tumor histology

Histologic examination of 13762NF rat mammary adenocarcinoma at the subcutaneous injection site revealed highly invasive, poorly differentiated tumor cells that appeared to be heterogeneous cell populations of predominantly large cells (figure 1). However, some small tumor cells were also present with no particular orientation. Arrays of tumor cells were sometimes seen in foci at the invading tumor zone, and within connective tissue adjacent to the tumor. In this region a significant number of both intact and degranulated mast cells were observed. Detailed histologic studies will be published elsewhere (Dabbous, Woolley and Haney, unpublished observations).

Mast cells

Mast cell (MC) suspensions contained more than 95 per cent mast cells as judged by metachromatic staining with toluidine blue. Histologic examination of the mast cell suspension after 2 days of culture showed round cells of various size, each containing metachromatic granules which varied in density and obscured the nucleus. The mast cells grew as single cells, but clumps of several cells were occasionally observed.

The preparation of mast cell products (MCP) used in these experiments contained heparin, histamine and prostaglandin E (PGE) (table). The concentrations of these substances are within the normal range for rat mast cell sonicates, but each preparation may show slight variation in the quantity and relative proportion of each mast cell component [20].

The collagenolytic activity of the mast cell preparation (MCP) at 1 : 10 dilution using a substrate of reconstituted fibrils of [^{14}C]collagen was negligible. Under these conditions mast cells do not appear to contain a true collagenase activity against native interstitial-type collagen.

Mast cell–stromal fibroblast interactions

Normal rat fibroblasts (NRS) released relatively low levels of collagenolytic activity into the media of subconfluent monolayer cultures. The enzyme activity was mainly present in the latent form, and could be activated by the mercurial compound

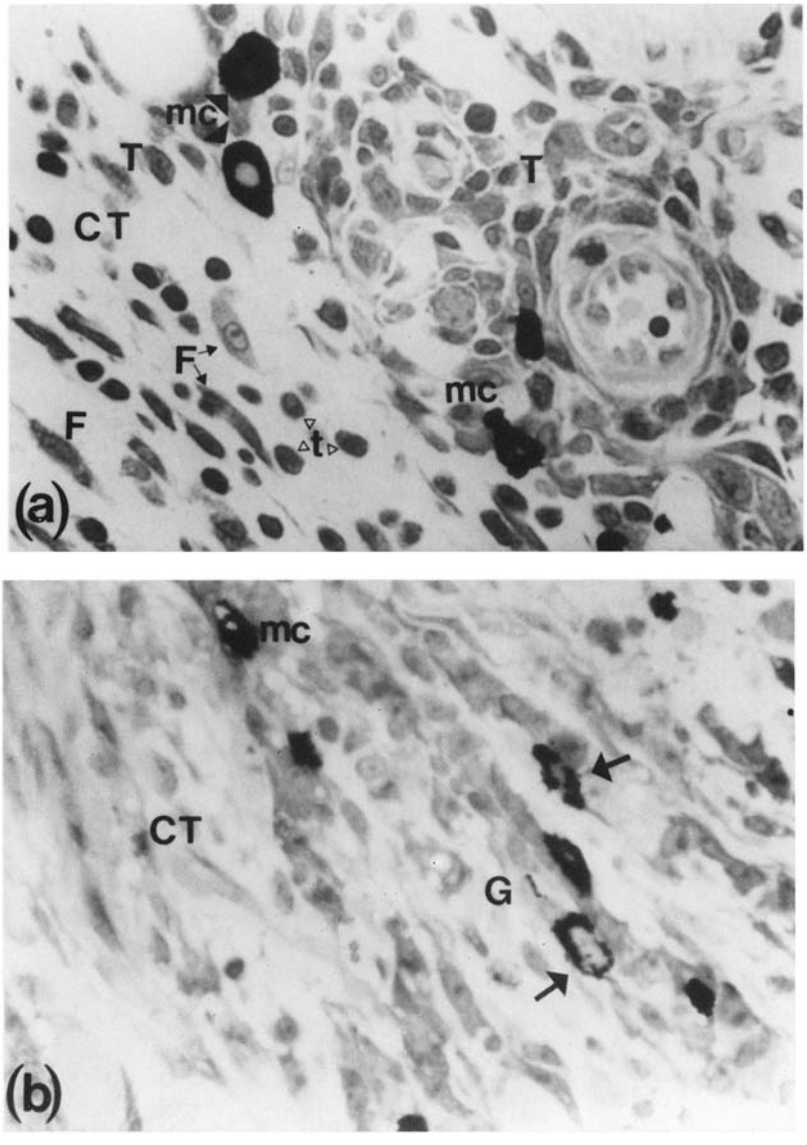

Figure 1. Mast cell (mc) at the invading tumor zone. (*a*) Mast cells in stromal connective tissue (CT) adjacent to the tumor parenchyma with large (T) and small (t) tumor cells. Some of the fibroblasts appear to be activated, enlarged or swollen (F). (*b*) shows that, at sites of active invasion, both connective tissue cells and tumor cells appeared disoriented, however, tumor cells were also seen arranged in cords within the invaded connective tissue (CT) which contained thin, frayed or fragmented collagenous fibers. Degranulating mast cells were seen in this region (arrows) and some granules (G) were visibly scattered within the extracellular matrix. ×400.

Analysis of mast cell preparation.

Component	Content (per 10^6 cells/ml)
Heparin	47 μg
Histamine	21 μg
PGE	>2·1 ng

APMA or by limited proteolysis with trypsin. The addition of MCP to NRS cultures resulted in a significant increase in the total collagenolytic activity released into the medium (figure 2). MCCM also stimulated the enzyme activity in NRS cultures, suggesting that the stimulatory activity was also present in mast cell-conditioned media. Tumor-derived, fibroblast-like cells (Ln3-F), presumably derived from stromal cells at or near the invading tumor zone, released more enzyme activity in the culture medium than NRS cells. These cells were also stimulated to produce more collagenase following exposure to both mast cell products (MCP) and MCCM. The stimulatory activity of MCP was not due to the presence of histamine or heparin, since the addition of either of these components did not significantly alter the release of enzyme into the medium.

Mast cell–tumor cell interactions

Rat 13762NF mammary adenocarcinoma cloned cell lines MTLn2 and MTLn3 were derived from lung metastases while clone MTF7 was derived from locally growing parental tumor [24, 37]. These cell clones were shown to vary in their abilities to form spontaneous pulmonary metastases. Based on the average number of lung tumor colonies and total lung tumor volume, the metastatic potential of clone MTLn3 was highest and MTLn2 was lowest, while that of MTF7 was intermediate to low [37].

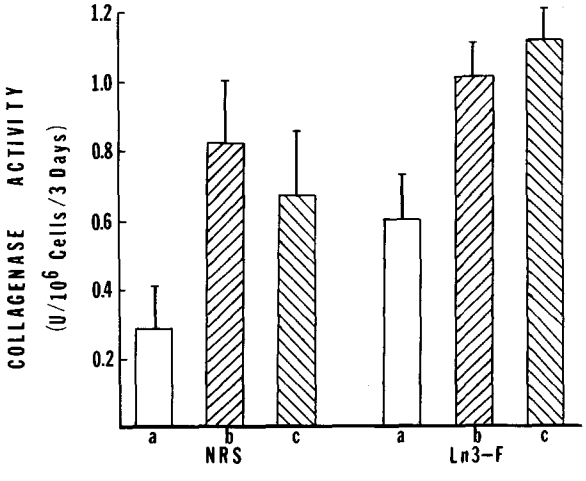

Figure 2. Effect of mast cell products (b) and mast cell conditioned medium (c) on the collagenolytic activity of normal rat fibroblasts (NRS) and tumor-derived fibroblast-like cells (Ln3-F). Control cultures contained AMEM–2 per cent HIFCS (a).

Addition of MCP to cultures of the tumor cell clone MTLn3 of high metastatic potential resulted in enhanced release of collagenolytic activity into the media of semiconfluent cultures (figure 3). Media conditioned by mast cells (MCCM) also stimulated the release of enzyme activity in MTLn3 cultures. The stimulatory activity of MCCM, however, was lower than that of MCP (figure 3).

Cell clone MTLn2, of low metastatic potential, produced relatively low levels of collagenolytic activity in the culture media. The release of enzyme activity was stimulated only slightly by either MCP or MCCM. Tumor cell clone MTF7, derived from the primary subcutaneous parental tumor growing in the mammary fat pad, showed a significant response to mast cell factors. Both MCP and MCCM markedly enhanced the release of enzyme activity by MTF7 cultures. However, whereas the mast cell factors produced an approximately three-fold stimulation of collagenase production in the MTF7 cells, a five-fold stimulation resulted from the high metastatic variant MTLn3 cells. The nature of the stimulating factor(s) are not known, and whether the stimulatory activity in MCP is the same as that in MCCM remains unclear.

The influence of mast cell–tumor cell interaction on stromal fibroblasts

Conditioned media derived from MTLn3 cells that were previously exposed to MCP at a 1 : 10 dilution (Ln3-MCP) subsequently stimulated the production of collagenolytic activity in cultures of NRS. The level of enzyme activity produced in cultures of NRS was higher in the presence of Ln3-MCP than in Ln3-CM conditioned media (figure 4). The three-fold stimulatory activity of Ln3-MCP suggest that mast cell products potentiate the tumor-mediated enhancement of enzyme release by stromal fibroblasts. The observed enhancement of enzyme production was not merely due to an additive effect of mast cell products and tumor cell-conditioned media, but rather an influence of MCP on MTLn3 tumor cells. The nature of such an interaction, however, has not been elucidated.

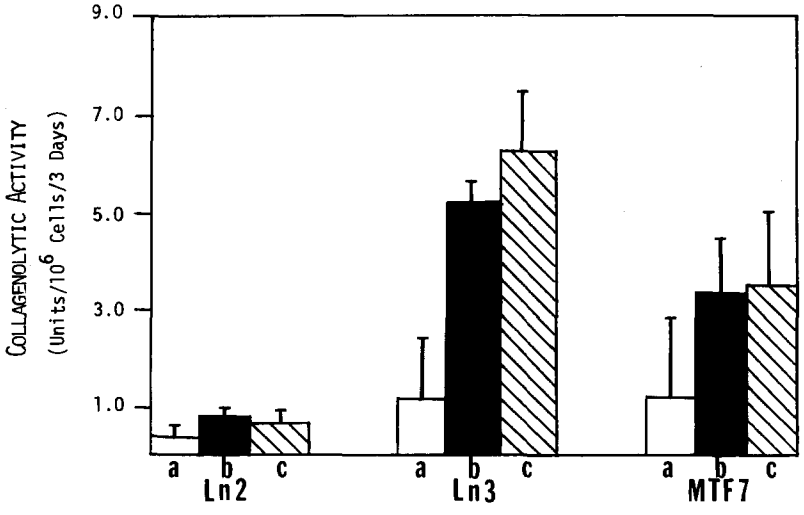

Figure 3. Effect of mast cell products (b) and mast cell conditioned medium (c) on the collagenolytic activity of tumor cell clones MTLn2, MTLn3 and MTF7. Control cultures contained AMEM–2 per cent HIFCS (a).

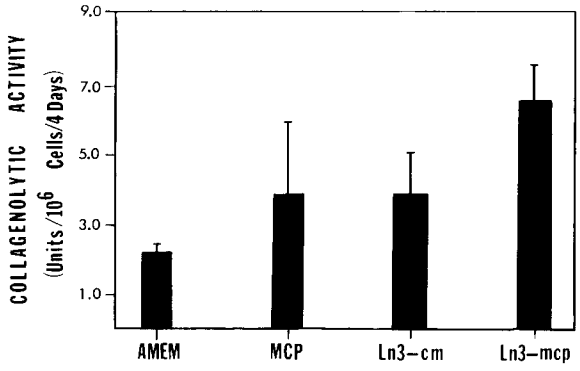

Figure 4. Stimulation of collagenolytic activity produced in cultures of normal rat skin fibroblasts following incubation with mast cell products (MCP), Ln3-conditioned medium (Ln3-CM), and conditioned medium from Ln3 cultures exposed to MCP (Ln3-MCP). Control cultures (AMEM). Vertical lines represent the mean value ±s.d.

Discussion

The role of collagenolytic enzymes in extracellular matrix degradation associated with tumor invasion and metastasis has been reported in several studies [2, 10, 13, 18, 21, 22, 28, 36, 40]. Moreover, several collagenolytic mechanisms might be functional in the invasive growth of malignant tumors [39]. Previous studies in this laboratory and in others [2, 6, 11] demonstrated the significance of host–tumor cell–cell interactions in tumor invasion. *In vitro* studies showed that epithelial-like tumor cells stimulated the release of collagenase activity in fibroblasts cultures of homologous and heterologous tissue origin [2, 6, 11]. However, at the invading tumor edges *in vivo*, other cellular interactions may be of importance in stimulating the collagenlytic mechanisms which underlie tissue stroma degradation [19, 20, 41]. Histologic studies of different types of tumors have shown significant variations in the relative proportions of tumor and host cells in the invading tumor zone [33, 35, 39].

The presence of increased numbers of mast cells at the tumor periphery was observed in the present study. Similar findings were previously reported in melanomas [39] and other tumors [32, 36]. Degranulation of mast cells and stromal metachromasia were also observed at the sites of active tumor growth and it has been suggested that cellular interactions, such as mast cell–tumor cell interactions, may enhance infiltrative growth of human and animal tumors [9, 14, 16, 17, 27].

The data presented here demonstrate that mast cell–tumor cell interactions significantly alter the collagenolytic activity of tumor cells, at least *in vitro*. The stimulatory activity appeared to be due to the presence of soluble mast cell factor(s), but apparently not histamine or heparin. The biochemical nature of such modulator(s) remains unclear. Heterogeneity of primary as well as metastatic tumors is well established [25, 26]. In order to understand fully host–tumor cellular interactions, it may be important to examine the response of subpopulations (such as cell clones) of tumor cells to host-derived effectors such as mast cell products. The present investigation provides evidence that diversity exists among different tumor cell clones, as judged by differences in phenotypic expression of collagenase activity after exposure to mast cell factors. Thus the enhanced release of collagenolytic activity in cultures of the highly metastatic clone MTLn3 cells was higher than that

of similarly stimulated low metastatic clone MTLn2 cells. The metastatic potential of clone MTF7 cells was intermediate, as was the MCP-stimulated collagenolytic activity. Therefore, one of the contributory factors that determine the metastatic potential of tumor cells may relate to their sensitivity to mast cell products, especially the subsequent expression of collagenolytic activity.

Tumor cell–stromal fibroblast interactions resulted in stimulation of collagenolytic activity in fibroblast culture media. However, prior exposure of tumor cells to mast cell products resulted in higher stimulatory activity of enzyme release by target fibroblasts in the culture media. This suggests that tumor cell-mediated stimulation of stromal fibroblasts may be potentiated by mast cell interaction with tumor cells.

The release of collagenolytic activity by fibroblasts in monolayer cultures was stimulated by MCP and also by MCCM. These results suggest that mast cell-derived soluble factor(s) may influence the rate and extent of collagen degradation by connective tissue fibroblasts. Ultrastructural studies have previously demonstrated alterations in fibroblast behaviour and connective tissue components in the presence of mast cells [15, 31, 34, 36]. Furthermore, it was shown that mast cell granules were phagocytosed by adjacent fibroblasts [26, 27], and it was proposed that the granule contents could alter fibroblast behaviour [15, 27, 31, 34, 36]. Thus fibroblast–mast cell interactions may play a significant role in matrix degradation, especially in relation to the presence of tumor and other infiltrating cells.

Tumor-derived fibroblast-like cells (Ln3-F), appeared to be more responsive to mast cells than normal fibroblasts (NRS), as shown by their higher production of collagenase following MCP exposure. Such differences were previously observed in the response of normal fibroblasts and tumor-derived fibroblasts to stimulation by VX2-carcinoma cells [12]. It is possible that a subpopulation of host fibroblasts in the vicinity of the invading tumor zone may become activated by several mechanisms, one of which could be contributed by mast cell granules or their soluble products. Mast cell-mediated activation of a select subpopulation of fibroblasts at the tumor periphery has been previously suggested [1, 15, 31, 36]. For example, histologic observations of certain fibroblast-like cells in the stromal connective tissue adjacent to the tumor periphery in the vicinity of mast cells demonstrated a larger and more swollen appearance than other resident fibroblasts (see figure 1). This observation is in agreement with previously reported data from other laboratories [36].

It is of significance that mast cells are effective modulators of collagenolytic enzyme expression by other cells. Evidence has now accumulated that suggests the importance of these cell–cell interactions in the host tissue degradation at sites of active tumor invasion in some tumors [39], and at sites of cartilage erosion in rheumatoid joints [7].

Changes in the extracellular matrix compatible with degradation of fibrous components have been observed in the vicinity of released mast cell granules [27, 36]. Although mast cell proteases may participate in matrix degradation, collagenolysis of the major type I collagen component could not be achieved by the soluble mast cell products alone. It is possible that mast cell proteases facilitate collagenolysis *in vivo* by prior activation of latent collagenases, especially as mast cell proteases have been previously shown to activate procollagenase from gingival fibroblast and macrophage cultures [4]. Mast cell products have recently been reported to stimulate the production of interleukin-1 by monocyte-macrophages, a factor that is known to stimulate fibroblast collagenase production [42]. Thus mast cells have the potential

to modulate collagenolysis, either directly or indirectly, by stimulation of effector cells of both host and tumor origin.

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References

[1] ATKINS, F. M., FRIEDMAN, M. M., SUBBA RAO, P. V., and METCALFE, D. D., 1985, Interactions between mast cells, fibroblasts, and connective tissue components. *International Archives of Allergy and Applied Immunology*, **7**, 96–102.

[2] BAUER, E. A., UITTO, J., WALTERS, R. C., and EISEN, A. Z., 1979, Enhanced collagenase production by fibroblasts derived from human basal cell carcinoma. *Cancer Research*, **39**, 4594–4599.

[3] BEELEN, H. J., and WALKER, W. S., 1986, Isolation of rat peritoneal lymphocytes, eosinophilic granulocytes, mast cells and functionally distinct macrophage subpopulations by Percoll density gradients and centrifugal elutriation. *Journal of Immunological Methods* (in the press).

[4] BIRKEDAL-HANSON, H., COBB, C. M., TAYLOR, R. E., and FULLMER, H. M., 1976, Activation of fibroblast procollagenase by mast cell proteases. *Biochimica et Biophysica Acta*, **438**, 273–286.

[5] BISWAS, C., 1982, Tumor cell stimulation of collagenase production by fibroblasts. *Biochemical and Biophysical Research Communications*, **109**, 1026–1034.

[6] BISWAS, C., 1982, Host–tumor cell interactions and collagenase activity. *Tumor Invasion and Metastasis*, edited by L. Liotta and I. R. Hart (The Hague: Martinus Nijhoff), pp. 405–425.

[7] BROMLEY, M., FISHER, W. D., and WOOLLEY, D., 1984, Mast cells at sites of cartilage erosion in the rheumatoid joint. *Annals of the Rheumatic Diseases*, **43**, 76–79.

[8] BROMLEY, M., and WOOLLEY, D. E., 1984, Histopathology of the rheumatoid lesion: identification of cell types at sites of cartilage erosion. *Arthritis and Rheumatism*, **27**, 857–863.

[9] CSABA, G., ACS, T., HORVATH, C., and MOLD, K., 1961, Genesis and function of mast cells: Mast cell and plasmacyte reaction to induced, homologous and heterogous tumors. *British Journal of Cancer*, **15**, 327–335.

[10] DABBOUS, M. KH., ROBERTS, A. N., and BRINKLEY, SR. B., 1977, Collagenase and neutral protease activities in cultures of rabbit VX-2 carcinoma. *Cancer Research*, **37**, 3537–3544.

[11] DABBOUS, M. KH., EL-TORKY, M., HANEY, L., BRINKLEY, SR. B., and SOBHY, N., 1983, Collagenase activity in rabbit carcinoma: cell source and cell interactions. *International Journal of Cancer*, **31**, 357–364.

[12] DABBOUS, M. KH., EL-TORKY, M., HANEY, L., SOBHY, N., and BRINKLEY, SR. B., 1983, Separation of VX-2 rabbit carcinoma-derived cells capable of releasing collagenase. *Experimental and Molecular Pathology*, **38**, 1–21.

[13] DRESDEN, M. H., HEILMAN, S. A., and SCHMIDT, J. D., 1972, Collagenolytic enzymes in human neoplasms. *Cancer Research*, **32**, 933–996.

[14] FARNOUSH, A., and MCKENZIE, I. C., 1983, Sequential histological changes and mast cell response in skin during chemically-induced carcinogenesis. *Journal of Pathology*, **12**, 300–306.

[15] FARNOUSH, A., and MCKENZIE, I. C., 1984, Proliferation of mast cells in normal and DMBA-treated mouse skin. *Journal of Oral Pathology*, **13**, 359–365.

[16] HARTVEIT, F., 1981, Mast cells and metachromasia in human breast cancer. Their occurence, significance and consequences: A preliminary report. *Journal of Pathology*, **134**, 7–11.

[17] HARTVEIT, F., SANDSTAD, E., 1982, Stromal metachromasia: A marker for areas of infiltrating tumor growth? *Histopathology*, **6**, 423–428.

[18] HASHIMOTO, K., YAMANISHI, Y., MAEYENS, E., DABBOUS, M. KH., and KANZAKI, T., 1973, Collagenolytic activities of squamous cell carcinoma of the skin. *Cancer Research*, **33**, 2790–2801.

[19] HENRY, N., EECKHOUT, Y., VAN LAMSWEERDE, A. L., and VAES, G., 1983, Co-operation between metastatic tumor cells and macrophages in the degradation of basement membrane (Type IV) collagen. *Federation of European Biochemical Societies Letters*, **161**, 243–246.

[20] HENRY, N., VAN LAMSWEERDE, A. L., and VAES, G., 1983, Collagen degradation by metastatic variants of Lewis lung carcinoma: Co-operation between tumor cells and macrophages. *Cancer Research*, **43**, 5321–5327.

[21] LIOTTA, L. A., TRYGGVASSON, K., GARBISA, S., HART, I., FOLTZ, C. M., and SHOFIC, S., 1980, Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature*, **284**, 64–68.

[22] MCCROSKERY, P. A., RICHARDS, J. F., and HARRIS, E. D., JR., 1975, Purification and characterization of a collagenase extracted from rabbit tumors. *Biochemical Journal*, **152**, 131–142.

[23] NERI, A., and NICOLSON, G. L., 1981, Phenotypic drift of metastatic and cell surface properties of mammary adenocarcinoma cell clones during growth *in vitro*. *International Journal of Cancer*, **28**, 731–738.

[24] NERI, A., WELCH, D. R., KAWAGUCHI, T., and NICOLSON, G. L., 1982, Development and biologic properties of malignant cell sublines and clones of a spontaneously metastasizing rat mammary adenocarcinoma. *Journal of the National Cancer Institute*, **68**, 507–517.

[25] NICOLSON, G. L., MINER, K. M., and READING, C. L., 1981, Tumor cell heterogeneity and blood-born metastasis. *Fundamental Mechanisms in Human Cancer Immunology*, edited by J. P. Saunders, S. S. Daniels, B. Cerrou, C. Rosenfield and C. B. Denney, (Amsterdam: Elsevier/North Holland Biomedical Press), pp. 31–39.

[26] NICOLSON, G. L., 1984, Generation of phenotypic diversity and progression in metastatic tumor cells. *Cancer Metastasis Reveiws*, **3**, 25–42.

[27] NORRBY, K., and ENSTROM, S., 1984, Cellular and extracellular changes following mast-cell secretion in a vascular rat mesentary. *Cell and Tissue Research*, **235**, 339–345.

[28] O'GRADY, R. L., UPFOLD, L. I., and STEPHENS, R. W., 1981, Rat mammary carcinoma cells secrete active collagenase and activate latent enzyme in the stromal via plasminogen activator. *International Journal of Cancer*, **28**, 509–515.

[29] PEARCE, V., PATHAK, S., MELLARD, D., WELSH, D. R., and NICOLSON, G. L., 1984, Chromosome and DNA analyses of rat 13762NF mammary adenocarcinoma cell lines and clones of different metastatic potentials. *Clinical and Experimental Metastasis*, **2**, 271–286.

[30] PEPYS, J., and EDWARDS, A. M., 1979, *The Mast Cell: Its Role in Health and Disease* (London: Pitman).

[31] PILLARISETTI, V., SUBBA RAO, P. V., FRIEDMAN, M. M., ATKINS, F. M., and METCALFE, D. D., 1983, Phagocytosis of mast cell granules by cultured fibroblasts. *Journal of Immunology*, **130**, 341–349.

[32] ROCHE, W. R., 1985, Mast cells and tumors: The specific enhancement of tumor proliferation *in vitro*. *American Journal of Pathology*, **119**, 57–64.

[33] STRAULI, P., 1980, A concept of invasion. *Proteases and Tumor Invasion, EORTC Monograph Series*, Vol. 6, edited by P. Strauli, A. J. Barrett and A. Baici (New York: Raven Press), pp. 1–15.

[34] TAKEDA, Y., 1985, Phagocytosis of mast cell granules by fibroblasts of the human gingiva. *Virchows Archiv A: Pathologische Anatomie*, **406**, 197–201.

[35] TARIN, D., 1976, Cellular interactions in neoplasia. *Fundamental Aspects of Metastasis*, edited by L. Weiss (Amsterdam: North Holland), pp. 151–187.

[36] VAN DEN HOOFF, A., and TIGCHELAAR-GUTTAR, W., 1983, Stromal changes in experimental carcinogenesis in the skin. 1. Ultrastructural aspects of collagen degradation. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C: Biological and Medical Sciences*, **86**, 179–190.

[37] WELCH, D. R., NERI, A., and NICOLSON, G. L., 1983, Comparison of 'Spontaneous' and 'Experimental' metastasis using Rat 13762 mammary adenocarcinoma metastatic cell clones. *Invasion and Metastasis*, **3**, 65–80.

[38] WOOLLEY, D. E., TETLOW, L. C., and EVANSON, J. M., 1980, Collagenase immunolocalisation studies of rheumatoid and malignant tissues. *Collagenase in Normal and Pathological Connective Tissues*, edited by D. E. Woolley and J. M. Evanson (Chichester: Wiley), pp. 105–125.

[39] WOOLLEY, D., 1984, Collagenolytic mechanisms in tumor cell invasion. *Cancer Metastasis Reviews*, **3,** 61–372.

[40] YAMANISHI, Y., DABBOUS, M. KH., HASHIMOTO, K., 1972, Effects of collagenolytic activity in basal cell epithelioma of the skin on reconstituted collagen and physical properties and kinetics of the crude enzyme. *Cancer Research*, **32,** 2551–2560.

[41] YOFFE, J. R., TAYLOR, D. J., and WOOLLEY, D. E., 1984, Mast cell products stimulate prostaglandin E production by cultures of adherent rheumatoid synovial cells. *Biochemical and Biophysical Research Communications*, **122,** 270–276.

[42] YOFFE, J. R., TAYLOR, D. J., and WOOLLEY, D. E., 1985, Mast-cell products and heparin stimulate the production of mononuclear-cell factor by cultured human monocyte/macrophages. *Biochemical Journal*, **230,** 83–88.