Regulation of Collagenase-3 Expression in Human Breast Carcinomas Is Mediated by Stromal-Epithelial Cell Interactions

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ABSTRACT

Collagenase-3 (MMP-13) is a recently identified member of the human matrix metalloproteinase gene family that is expressed in breast carcinomas and in articular cartilage from arthritic patients. Here, we have studied the cellular origin of this enzyme in breast carcinomas by in situ RNA hybridization, and we found that collagenase-3 is expressed by stromal cells immediately adjacent to epithelial tumor cells but not by the tumor cells themselves; nor is it expressed by the normal breast glandular epithelium. Consistent with this observation, coculture experiments using human fibroblasts and MCF-7 breast cancer cells revealed that conditioned medium from breast cancer cells stimulated the fibroblastic expression of collagenase-3 mRNA. In contrast, no stimulatory effect was observed when medium from fibroblast cells was added to breast cancer cells. These results strongly suggest that transcription of collagenase-3 in stromal cells is activated by diffusible factors released from epithelial breast cancer cells. A survey of a series of cytokines and growth factors known for their ability to induce collagenase-3 expression in human fibroblasts identified interleukin-1α and interleukin-1β as potential candidates for inducing the expression of this MMP gene in breast carcinomas. According to these results, collagenase-3 should be included among the molecular factors that are detected during the stromal reaction to invasive breast cancer and that, by concerted action, may be essential for tumor growth and progression.

INTRODUCTION

Malignant tumors have the ability to invade normal tissues and spread to distant sites, giving rise to metastasis. These processes involve the degradation of the different macromolecular components of the extracellular matrix and basement membranes and appear to require the combined action of several proteolytic enzyme systems. Among the variety of proteinases with potential involvement in facilitating tumor invasion and metastasis, the MMPs, or matrixins, have attracted particular interest due to their ability to degrade connective tissues at a neutral pH. These proteolytic enzymes form a family of structurally related endopeptidases that play critical roles not only in pathological conditions but also in normal tissue-remodeling processes, such as embryonic development, bone growth and resorption, wound healing, ovulation, and uterine involution (1–4). At present, the family of human MMPs is composed of 15 members that, according to structural and functional considerations, can be classified into four different families: collagenases, gelatinases, stromelysins, and membrane-type MMPs (Refs. 5 and 6 and references therein), although there are some enzymes, such as macrophage metalloelastase (7), stromelysin-3 (8), and MMP-19 (9), that do not belong to any of these groups. All of them are similar in that they are synthesized as latent proenzymes, contain several conserved domains with specific functions, including an activation locus and a Zn-binding site, and can be inhibited by tissue-specific inhibitors and chelating agents. However, they differ in their specificities toward extracellular matrix components such as collagens, proteoglycans, laminin, and fibronectin (2).

Recently, based on the hypothesis that samples of human tumor specimens could be appropriate materials for identifying novel proteinases with potential involvement in the spread of cancer, we cloned a new member of the MMP family of enzymes from a breast carcinoma cDNA library, which has been called collagenase-3 (MMP-13; Refs. 10 and 11). Biochemical characterization of this enzyme has revealed that it degrades the native helix of fibrillar collagens very efficiently, with preferential activity on Type II collagen, suggesting that collagenase-3 may be involved in the turnover of articular cartilage, which is particularly rich in this type of collagen (12). In fact, recent studies from different groups have demonstrated that collagenase-3 is expressed by chondrocytes during human fetal development (13) as well as in pathological conditions including osteoarthritis and rheumatoid arthritis (14–17). In addition to its degrading activity on fibrillar collagens, further analysis of the substrate specificity of collagenase-3 has revealed that this enzyme may also act as a potent gelatinase thus contributing to further degrade the initial cleavage products of collagenolysis to small fragments suitable for subsequent metabolism (12). Furthermore, very recent studies have shown that collagenase-3 is also able to degrade the large cartilage proteoglycan aggrecan and other components of the extracellular matrix and basement membranes, including type IV collagen (12, 18, 19). On the basis of these results, showing that human collagenase-3 is a potent enzyme with a broad spectrum of activity against extracellular matrix and basement membrane proteins, it is tempting to speculate that this proteolytic enzyme could play a critical role in the uncontrolled degradative processes that are associated with breast cancer progression. However, despite well-advanced studies describing activation mechanisms, substrate specificity, and inhibitor interaction of human collagenase-3 (12, 19, 20), there is currently no information available on the mechanisms controlling its expression in human breast carcinomas. Here, we provide evidence that collagenase-3 is expressed in fibroblastic cells surrounding breast carcinoma cells but not in the epithelial tumor cells themselves. Furthermore, by using cocultures of fibroblast and breast cancer cell lines, we demonstrate that interactions between both cell types are essential in modulating collagenase-3 expression by human fibroblasts. Finally, we analyze the potential role of a series of cytokines and growth factors as mediators of collagenase-3 expression in breast carcinomas.

MATERIALS AND METHODS

Materials. Samples of breast carcinoma tissues were obtained from the Department of Pathology at Karolinska Hospital (Stockholm, Sweden) and from Hospital Central de Asturias (Oviedo, Spain). According to histological analysis, all cancers examined in this study were ductal-infiltrating breast carcinomas.
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carcinomas. Histological grade was determined according to Bloom and Richardson criteria (21). All media and supplements for cell culture were obtained from Sigma Chemical Co. (St. Louis, MO), except for FCS, which was from Boehringer Mannheim (Mannheim, Germany). IL-1α, IL-1β, aFGF, bFGF, PDGF-BB, EGF, TNF-α, TGF-α, and TPA were from Sigma. Polyclonal antibodies against human IL-1β were from Genzyme (Cambridge, MA). IL-1β levels were determined using the Quantikine Immunoassay kit from R&D Systems (Minneapolis, MN). Restriction endonucleases and other reagents used for molecular cloning were from Boehringer Mannheim. Double-stranded DNA probes were radiolabeled with [α-32P]dCTP (3000 Ci/mm) from Amersham International (Buckinghamshire, United Kingdom) using a commercial random-priming kit purchased from Pharmacia Biotech Inc. (Uppsala, Sweden).

Cell Culture. Human breast cancer cells MCF-7, T-47D, ZR-75, MDA-MB 231, MDA-MB 435, and HS578T, as well as HFL-1 fibroblasts, were obtained from the American Type Culture Collection (Rockville, MD). KMST-6 cells, immortalized by γ-ray irradiation of KMS-6 human embryonic fibroblasts, were kindly provided by Dr. M. Namba (Okayama University, Okayama, Japan). Hs60 human fibroblasts were kindly provided by Dr. William Stetler-Stevenson (NIH, Bethesda, MD). Cells were routinely maintained in DMEM supplemented with 10% FCS, 100 units/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere of 5% CO2. Cells were subcultured weekly by incubation at 37°C for 2 min with 0.0125% trypsin in 0.02% EDTA, followed by addition of complete medium and washing and resuspension in fresh medium. For most experiments, approximately 5 X 10^5 cells/well were plated out in 100-mm dishes, transferred to serum-free DMEM for 24 h, and then exposed to the different growth factors, cytokines, and tumor promoters at the concentrations and for the times indicated.

Coculture Experiments. Subconfluent KMST fibroblasts in 100-mm dishes were overlayered with a variable number of MCF-7 cells in 10% (v/v) FCS/DMEM as indicated and incubated for 24 h. Then, the cells were washed twice with Ca2+/Mg2+-free PBS and cultured for a further 24 or 48 h in the same medium. MCF-7 conditioned medium was obtained from confluent MCF-7 cells grown in 75-cm² flasks, washed extensively with PBS, and then incubated in serum-free or with variable percentage of serum-DMEM for 48 h. The harvested medium was centrifuged at 1200 X g and stored at —20°C until use. To assess the influence of cell-cell contact, cell substrate culture dishes were prepared by washing confluent monolayers of each cell type twice with PBS, incubating dishes for 24 h at —20°C, and then washing each dish again with PBS. For coculture experiments involving the use of blocking antibodies, conditioned medium from breast cancer cells was incubated with 25 µg/ml polyclonal rabbit anti-human IL-1β for 3 h at 37°C. Nonimmune rabbit serum was used as a negative control for this experiment.

Isolation of RNA and Northern Blot Analysis. Total RNA from the cells isolated by the guanidinium isothiocyanate procedure according to Chomczynski and Sacchi (22), separated by electrophoresis in 1% agarose-formaldehyde gels, and blotted onto Hybond N nylon filters (Amersham International, Buckinghamshire, UK). Filters containing 20 µg of total RNA were prehybridized at 42°C for 3 h with 5 x 10^6 cpm of a 32P-labeled cDNA probe. Hybridization was then performed with a 32P-labeled cDNA probe. After hybridization, the slides were washed under stringent conditions, with PBS. For coculture experiments involving the use of blocking antibodies, conditioned medium from breast cancer cells was incubated with 25 µg/ml polyclonal rabbit anti-human IL-1β for 3 h at 37°C. Nonimmune rabbit serum was used as a negative control for this experiment.

RESULTS

Collagenase-3 Is Expressed within the Stromal Fibroblasts of Human Breast Carcinomas. Recent studies from different groups have revealed the importance of stromal-epithelial interactions in the production of proteolytic enzymes that are involved in the connective tissue degradation occurring in tumor processes (24–31). These studies have shown that some MMPs are produced by stromal cells, whereas other enzymes, like matrilysin, are preferentially expressed by the tumor cell itself. However, at present, no information is available on the cellular source of human collagenase-3. Therefore, as a preliminary step in examining the factors and conditions that could be responsible for collagenase-3 expression in human breast carcinomas, we tried to determine the cellular origin of this enzyme. For this purpose, in situ RNA hybridization analysis was performed on paraffin-embedded tissue sections prepared from human breast carcinomas. A total of 10 breast carcinomas were analyzed, and positive expression signal for collagenase-3 was found in three of these tumors. In all cases, collagenase-3 mRNA transcripts were specifically detected in fibroblastic cells surrounding epithelial tumor cells (Fig. 1 and data not shown). In addition, fibroblastic cells that were most strongly positive for collagenase-3 expression were those immediately adjacent to islands of cells present at the invasive edge of the tumor. In contrast, no significant expression was seen in the carcinoma cells themselves or in normal breast glandular epithelium. Similarly, no collagenase-3 expression was detected when a probe from the same cDNA transcribed in the sense direction was used as control. The stromal pattern of collagenase-3 expression thus resembles that previously noted for other MMPs and further supports the concept that tumor stroma is an important determinant of tumor growth and progression (8).

Induction of Collagenase-3 Expression in Human Fibroblasts Cocultured with Breast Cancer Cells. The finding that collagenase-3 expression in breast carcinomas occurs in the stromal cells adjacent to...
tured with an increasing number of cells from different breast cancer cell lines, including MCF-7, ZR-75, and Hs578T (Fig. 2 and data not shown). As illustrated in Fig. 2A, KMST fibroblast-MCF-7 breast cancer cell cocultures resulted in a strong induction of collagenase-3 expression, which was dependent on MCF-7 cell number. Two transcripts of 3.0 and 2.5 kb, corresponding to the two major collagenase-3 mRNA transcripts identified in breast carcinomas (10) and articular cartilage (14–16), were detected. Cocultures with other breast cancer cell lines, such as ZR-75 or Hs578T, did not led to a significant stimulatory effect on collagenase-3 expression, pointing to a rather specific role of MCF-7 cells in this effect. Similar coculture experiments conducted with MCF-7 epithelial cells attached to the bottom of culture wells, to which KMST-cells were subsequently added, also led to collagenase-3 induction. To examine the possibility that a soluble factor is the active signal that can induce collagenase-3 expression in this coculture system, conditioned medium from KMST cells was added to plated MCF-7 cells and vice versa. As shown in Fig. 2B, when medium from breast cancer cells was added to the fibroblastic cells, a clear up-regulation of collagenase-3 expression was observed. In contrast, no stimulatory effect was apparent when conditioned medium from KMST cells was used. Taken together, these results appear to indicate that a soluble factor produced by the epithelial tumor cells and secreted to the extracellular medium is principally responsible for collagenase-3 induction in human fibroblasts.

In addition, because stromal-epithelial interactions have been shown to be complex events involving a series of distinct mechanisms, we also examined the possibility that direct cell-cell contact could also operate to induce collagenase-3 expression in fibroblasts, as previously reported for other MMPs, such as the Mr 92,000 gelatinase (28). To this end, following the procedure described by Borchers et al. (32), each cell type (KMST fibroblast and MCF-7 breast cancer cell) was first seeded onto a dish consisting of a layer of the other cell type, which had been killed by freeze-thawing. Finally, the production of collagenase-3 mRNA was analyzed by Northern blot. However, no significant expression of this gene was detected in any of these experiments, thus reinforcing the proposal that the role of cell-cell contacts in this process, if any, should be of a much lesser extent than the one exerted by soluble factors released from the epithelial cells.

Collagenase-3 Is Expressed in Cultured Human Fibroblasts in Response to Cytokines and Tumor Promoters. The above data showing that human collagenase-3 is expressed within fibroblasts adjacent to the invasive tumor cells, together with the observation that

Fig. 1. Analysis of collagenase-3 expression in formalin-fixed, paraffin-embedded sections of a breast carcinoma by in situ RNA hybridization. A, collagenase-3 mRNA transcripts (white) were localized in the fibroblastic stromal cells surrounding epithelial tumor cells. B, the section was stained with H&E after treatment. S, stromal cells; T, tumor cells.
conditioned medium from breast cancer cells induces the fibroblastic expression of this gene in coculture experiments, strongly suggested that collagenase-3 may be transcriptionally activated by diffusible factors released from the breast cancer cells. In a preliminary effort to identify putative factors with the ability to induce collagenase-3 expression, human KMST fibroblasts were incubated for 24 h in the presence of a series of growth factors, cytokines, and tumor promoters, and total cellular RNAs were purified and analyzed by Northern blot using a specific collagenase-3 cDNA probe. As illustrated in Fig. 3A, IL-1α and IL-1β strongly induced the expression of collagenase-3 in human fibroblasts. It is remarkable that two transcripts of the same size (3.0 and 2.5 kb) and with similar ratios of transcription to those found in the above coculture experiments were detected. The up-regulatory effect of IL-1 on collagenase-3 expression was not specific for KMST cells because similar analysis revealed that at least two other fibroblast cell lines (HFL-1 and Hs60) produce collagenase-3 in response to IL-1 stimulation. Nevertheless, the level of expression of collagenase-3 in these cells was lower than that in KMST cells, and longer exposure of filters was required to weakly detect both collagenase-3 transcripts (data not shown). To increase the sensitivity of detection, we performed RT-PCR analysis with RNAs obtained from HFL-1 and Hs60 cells, and the results obtained are shown in Fig. 3B. As can be seen, a band of the expected size (398 bp) and confirmed to correspond to collagenase-3 by nucleotide sequencing was amplified from IL-treated cells but not from untreated fibroblasts. In addition, as demonstrated for KMST cells, collagenase-3 could also be amplified from HFL-1 and Hs60 cells treated with conditioned medium from breast cancer cells. In contrast, media from these two fibroblast cell lines did not stimulate collagenase-3 expression in epithelial cancer cells (Fig. 3B and data not shown). Taken together, these data indicate that different fibroblastic cells respond to IL-1 and to medium from breast cancer cells in a similar fashion by inducing collagenase-3 expression. However, because this effect was much more evident in KMST fibroblasts in quantitative terms, all subsequent experiments were performed with these cells.

Thus, as can be seen in Fig. 4A, a time course analysis of the IL-1β-induced up-regulation of collagenase-3 mRNA in KMST cells showed that the induction was time-dependent, with the maximum being detected after 24 h of incubation in the presence of 5 ng/ml IL-1β. Similarly, a dose-response analysis showed that as little as 0.1 ng/ml IL-1β produced a clear accumulation of the two collagenase-3 mRNA transcripts, whereas incubation of the cells in the presence of 5 ng/ml IL-1β led to a maximal accumulation of both transcripts (Fig. 4B). To determine whether the up-regulating effect of these cytokines on collagenase-3 mRNA levels was also reflected at the protein level, we performed Western blot analysis with either cell extracts or conditioned medium from KMST cells treated with 5 ng/ml IL-1β for 72 h. As shown in Fig. 5, a band of about M, 60,000 that was immunoreactive against the collagenase-3 antiserum was present in the 25-fold concentrated conditioned medium from cells treated with IL-1β but not in that obtained from control cells. It is remarkable that the immunoreactive band detected in conditioned medium exhibits a slightly slower mobility than the control, a recombinant procollagenase-3 produced in a vaccinia virus-based expression system (10). These minor differences have been also detected in the case of procollagenase-3, which was produced by osteoarthritic chondrocytes, and are probably due to a lower degree of glycosylation of the...
recombinant procollagenase-3 (15). Nevertheless, a disturbing effect on the electrophoretical mobility of procollagenase-3 caused by the high salt content of the concentrated conditioned medium cannot be ruled out. Densitometric analysis of the X-ray films led us to estimate that the concentration of procollagenase-3 produced and secreted by KMST cells after stimulation with IL-1β was about 0.7 μg/ml.

In addition to this stimulatory effect of IL-1α and IL-1β on collagenase-3 expression in KMST fibroblasts, TPA was also able to enhance the expression of this gene in these human cells to a similar extent as did stimulation with both ILs. Other cytokines and growth factors, including TNF-α, EGF, PDGF-BB, aFGF, bFGF, and TGF-α, did not have any significant effect on collagenase-3 expression by KMST fibroblasts (Fig. 3 and data not shown). However, in marked contrast to the stimulating effects of IL-1α, IL-1β, and TPA on collagenase-3 expression in fibroblasts, none of these substances was able to up-regulate the expression of this gene in a variety of breast cancer cells (T47D, ZR-75, MCF-7, MDA-MB231, MDA-MB435, and Hs578T), including cell lines displaying diverse patterns of hormone responsiveness or showing differences in their invasive and metastatic properties; TNF-α, EGF, PDGF-BB, aFGF, bFGF, and TGF-α were not able to up-regulate this expression either. This finding is, therefore, consistent with the above in situ hybridization analysis and coculture experiments showing that stromal fibroblasts but not epithelial breast cancer cells are able to produce this MMP.

Finally, and to further assess the possibility that IL-1 was involved in the observed coculture dependent induction of collagenase-3 expression, IL-1β-blocking antibodies were added to the MCF-7 conditioned medium and the ability of this antiserum-treated medium to up-regulate collagenase-3 expression in KMST fibroblasts was examined by Northern blot. As shown in Fig. 6, this treatment extensively blocked the ability of the breast cancer cells conditioned medium to induce collagenase-3 expression in human fibroblasts. In contrast, addition of nonimmune serum at the same concentration had no effect on collagenase-3 expression (data not shown). It is also worth mentioning that the determination of IL-1β concentration in the conditioned media from different breast cancer cells revealed that medium from MCF-7 displayed the highest concentration of this cytokine (about 12 pg/ml), whereas levels in media from other cells were lower (Hs578T, 3 pg/ml; ZR-75, 2 pg/ml) or undetectable (MDA-MB 435). This observation should be consistent with the finding that medium from MCF-7 cells induces collagenase-3 expression. Similarly, IL-1β levels in available cytosolic extracts from breast carcinomas analyzed for collagenase-3 mRNA expression were higher in collagenase-3-positive tumors than in those negative for this MMP (mean values, 27.3 pg/mg versus 16.2 pg/mg, respectively), although the limited number of available samples precluded our ability to make definitive conclusions.

**DISCUSSION**

Here, we have shown that human collagenase-3, a MMP produced by breast carcinomas, is expressed in stromal cells immediately adjacent to the epithelial tumor cells. In addition, by means of coculture experiments, we have also provided evidence that a soluble factor released from breast cancer cells induces collagenase-3 expression in human fibroblasts. Finally, after an extensive survey of growth factors and cytokines, we have identified IL-1 as a potential candidate for inducing collagenase-3 expression in breast carcinomas.

The pattern of expression of human collagenase-3 in breast carcinomas is similar to that found for other MMPs produced by these malignant tumors, including stromelysin-3 (8), MT1-MMP (33), and gelatinase A (34), which appear to be also predominantly expressed by fibroblast cells within the tumor stroma adjacent to breast cancer cells. However, this stromal expression of collagenase-3 contrasts with our previous immunohistochemical analysis, showing that, in breast carcinomas, this enzyme is predominantly associated with the epithelial tumor cells, and only a few stromal cells are immunoreactive against collagenase-3 antibodies (8). Thus, it seems that, after synthesis in the stromal cells, collagenase-3 is mainly secreted to the neoplastic cells, to which it is bound and internalized. Consistent with this proposal, recent studies performed with the murine homologue of human collagenase-3 have demonstrated the existence of a specific receptor for this enzyme on rat osteosarcoma cells that is responsible for removal and internalization of the extracellular enzyme (35). A similar explanation has been proposed to justify the observation that M, 72,000 gelatinase mRNA transcripts are found in stromal cells, whereas immunoreactivity against these proteins is mainly detected in epithelial tumor cells (34, 36, 37). The parallelism between collagenase-3, M, 72,000 gelatinase, and MT1-MMP in terms of stromal

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**Fig. 6.** Effect of specific neutralizing antibodies to IL-1β on the induction of collagenase-3 mRNA expression in either conditioned medium from MCF-7 cells or medium supplemented with IL-1β. MCF-7-CM or DMEM plus IL-1β (1 ng/ml) was incubated with a IL-1β polyclonal antibody at 25 μg/ml for 3 h at 37°C, prior to addition to KMST cells. After incubation for 48 h, total RNA was collected, and 20 μg were loaded in each lane and analyzed by Northern blot. The same blot was stripped and reprobed with a β-actin-encoding probe.

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**Fig. 5.** Western blot analysis of IL-1β-mediated induction of procollagenase-3 production by KMST cells. Cells were cultured in the absence (Lane Control CM) or the presence (Lane IL-1β-treated CM) of IL-1β (5 ng/ml) for 72 h under serum-free conditions. After incubation, conditioned media were concentrated 25-fold and analyzed along with recombinant purified procollagenase-3 (400 ng).

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expression of their respective genes in human breast carcinomas is also reflected at the functional level because these three enzymes have been proposed to form an activation cascade, finally resulting in generation of extracellular proteolytic activity (20). In fact, recent studies on cellular mechanisms for human procollagenase-3 activation have revealed that MT1-MMP is able to directly activate this proenzyme but not the two other human collagenases (MMP-1 and MMP-8; Ref. 20). Furthermore, the activation rate is strikingly enhanced in the presence of progelatinase A, which is itself activated by MT1-MMP. Upon activation, collagenase-3, a potent MMP with a wide substrate specificity, may contribute to the degradation of the extracellular matrix and basement membranes, thereby facilitating growth and spreading of breast carcinomas. During preparation of this manuscript, Heppner et al. (38) have reported the finding of collagenase-3 transcripts in isolated tumor cells of breast carcinomas, which is in contrast with our data showing that collagenase-3 expression is preferentially stromal. However, although methodological aspects may contribute to explain part of these differences, it is also likely that analyzed tumors may differ in terms of biological and clinico-pathological characteristics. In fact, as already described for other MMPs, stromal expression may play a role in earlier events in tumor progression and epithelial cell expression may be more relevant in late-stage carcinomas (31, 39). Nevertheless, although both epithelial and stromal expression of collagenase-3 may occur, our finding of collagenase-3 mRNA in three different fibroblastic cells but not in any of the analyzed breast cancer cells, together with the results of the coculture experiments discussed below, suggest that the presence of this enzyme in breast carcinomas mainly results from stromal expression rather than from synthesis in the tumor cell themselves. Preliminary analysis looking for a relationship between expression of collagenase-3 in breast carcinomas and a series of biological and clinico-pathological characteristics, including tumor size, histological grade, and estrogen receptor status, has not revealed any apparent association with any of these parameters, although the limited number of analyzed samples precludes our ability to make definitive conclusions. Further studies with a large number of samples, which are now in progress, will be required to clarify if stromal versus cancer cell expression of collagenase-3 in breast carcinomas may differ, depending on their biological and/or clinical characteristics.

The localization of collagenase-3 transcripts in the immediate vicinity of invasive breast cancer cells also suggested that direct cell-cell contact mechanisms or soluble factors released by breast cancer cells could be responsible for the observed up-regulation of collagenase-3 expression in the stromal cells. To address this question, we designed a series of fibroblast-breast cancer cell coculture experiments, which led us to conclude that epithelial tumor cells produce a diffusible factor that induces transcription of collagenase-3 in neighboring stromal fibroblasts. These findings differentiate collagenase-3 from other MMPs, such as $M_r$ 92,000 gelatinase, which can be induced in fibroblastic cells by direct cell contact with metastatic tumor cells (28). In an attempt to identify the regulatory factors produced by breast cancer cells that could be responsible for collagenase-3 induction in the surrounding stroma, we surveyed a number of cytokines and growth factors for their ability to induce this gene. Despite the fact that all of these examined biological factors had been previously found to play important roles in up-regulating the expression of other MMPs, only IL-1$\alpha$, IL-1$\beta$, and TPA were able to induce collagenase-3 expression in human fibroblasts. However, it is unclear whether these in vitro effects of IL-1$\alpha$ and IL-1$\beta$ on collagenase-3 expression could reflect their activity in vivo, thus making these cytokines plausible candidates for up-regulating expression of this MMP gene in human breast carcinomas. In this regard, it is of interest that Yao et al. (40), in a study directed to correlate scatter factor protein levels with presence of inflammatory cytokines in breast carcinomas, have shown that these carcinomas are indeed IL-1$\beta$ producers. In addition, IL-1$\beta$ content is higher in node-positive invasive cancers with a poor clinical evolution than it is in node-negative tumors. These data, together with recent results indicating that administration of this cytokine leads to increased metastatic formation (41), suggest that IL-1$\beta$ may be a factor of malignancy in the progression of breast cancer. This is consistent with our proposal that IL-1$\beta$ may be essential in the induction of a matrix-degrading enzyme like collagenase-3, although more definitive evidence, such as demonstration by in situ RNA hybridization of IL-1$\beta$ expression in epithelial tumor cells of collagenase-3-positive breast carcinomas, should be required. To date, these experiments have been hampered by the low levels of expression of this cytokine in breast carcinomas, and it seems likely that more sensitive approaches will be necessary to further evaluate the role of IL-1$\beta$ in collagenase-3 expression in these tumors. A similar conclusion has been reached by other groups performing immunolocalization studies of IL-1 in human breast carcinoma tissues (42). Nevertheless, with regard to the proposed role of IL-1$\beta$ as an in vivo inducer of collagenase-3, it is also of interest that this cytokine has been found to be the intermediate factor controlling expression of rat interstitial collagenase (the murine homologue of human collagenase-3) by myometrial cells in response to serotonin (43). Similarly, IL-1 has been described as an up-regulator of collagenase-3 expression in human chondrocytes (15, 44). Finally, recent studies of the promoter region of the human collagenase-3 gene have revealed the presence of a functional activator protein 1 site that could mediate, at least in part, this transcriptional response to IL-1 (45). Further studies will be required to elucidate the precise nature of the molecular signals and mechanisms involved in the process of collagenase-3 induction in breast carcinomas through stromal-epithelial cell interactions. These studies will be useful for clarifying the role of collagenase-3 in the context of the diverse proteins, including proteolytic enzymes, transcription factors, growth factors, and adhesion molecules, detected during the stromal reaction to invasive breast cancer, that, by concerted action, may be an essential determinant of tumor growth and progression.

ACKNOWLEDGMENTS

We thank Dr. S. Gascón for support; Drs. F. Vidal-Vanaclocha, M. Balbín, A. M. Pendás, Y. S. López-Boado, and G. Velasco for helpful comments; Drs. F. Vizoso and I. Díez-Itza for providing human tissue samples; and S. Alvarez for excellent technical support.

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*Cancer Res* 1997;57:4882-4888.

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