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The Metastasis-Associated Metalloproteinase Stromelysin-3 Is Induced by Transforming Growth Factor-β in Osteoblasts and Fibroblasts*

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ABSTRACT

Bone matrix serves as a reservoir of growth factors important in growth and tissue remodeling, and transforming growth factor-β (TGF-β) is abundant in bone matrix. Normal processes, such as remodeling, and pathological processes, such as osteolytic metastasis, cause the release of growth factors from the matrix, allowing them to influence the behavior of cells within their microenvironment. Breast cancer metastases frequently establish themselves in the bone compartment, often causing localized osteolysis. Stromelysin-3 is a matrix metalloproteinase associated with tumor metastases. Its expression in host tissues favors the homing and survival of malignant epithelial cells in early tumorigenesis by releasing and/or activating growth factors sequestered in the extracellular matrix. Osteoblasts express stromelysin-3, and Northern and Western blot analysis show that its messenger RNA and protein levels are increased by TGF-β. Nuclear run-off assays demonstrate activation of gene transcription, and experiments using transcription inhibitors demonstrate stabilization of stromelysin-3 messenger RNA by TGF-β. Importantly, TGFβ induces stromelysin-3 in fibroblasts by similar mechanisms, indicating that it is likely to stimulate stromelysin-3 expression in breast stroma. Stimulation of stromelysin-3 expression by TGF-β in fibroblasts and osteoblasts could play a role in the metastasis of breast cancer cells and their homing and survival in bone. (*Endocrinology* **142:** 1561–1566, 2001)

IN ADDITION TO serving mechanical, protective, and metabolic functions, bone is unique in that its matrix serves as a reservoir of growth factors important in growth and tissue remodeling. Growth factors abundant in bone matrix include fibroblast growth factors (FGFs) 1 and 2, bone morphogenetic proteins, platelet-derived growth factors, insulin-like growth factors I and II, and transforming growth factors (TGFs)-β1, 2, and 3 (1). The breakdown of bone matrix during normal processes such as fracture repair and remodeling, and in pathological processes such as osteolytic metastasis, causes the release of growth factors that can influence the behavior of cells within their microenvironment (2, 3).

Breast cancer metastases frequently establish themselves in the bone compartment, and the majority of these metastases cause localized osteolysis (3). PTH-related protein stimulates osteoclastic bone resorption, and this cancer cell product is thought to be a major mediator of bone destruction in metastases (4). It is suggested that TGF-β, released from the matrix as a result of osteoclastic bone resorption, stimulates tumor growth and further bone breakdown by stimulating cancer cell production of PTH-related protein (3, 5). Thus, this tumor-stimulated host response supplies additional metastatic impetuous to the cancer cells.

Matrix metalloproteinases are associated with normal matrix remodeling and with metastatic tumors (6). In particular, stromelysin-3 (matrix metalloproteinase-11) is expressed by the stromal cells surrounding 95% of invasive breast carcinomas (7). Stromelysin-3 is not produced by the cancer cells themselves; however, its synthesis by stromal fibroblasts is considered a tumor-induced host response. Strong expression of stromelysin-3 in fibroblastic cells surrounding and within tumor masses is correlated with a poor clinical outcome (7, 8). *In vivo* studies indicate that stromelysin-3 promotes the survival of tumor cells outside their compartment of origin by increasing tumor take, but not by affecting tumor cell growth or invasiveness (9, 10). It is suggested that paracrine expression of stromelysin-3 in host tissues favors the homing and survival of malignant epithelial cells in early tumorigenesis by releasing and/or activating growth factors sequestered in the extracellular matrix (10).

Osteoblasts constitutively express low levels of stromelysin-3, and its expression can be regulated by growth factors and cytokines present in the bone environment (11). Because TGF-β has been shown to potentiate bone metastasis, and active TGF-β is released from bone matrix as a result of osteoclastic activity, we examined whether TGF-β1 could stimulate the synthesis of stromelysin-3 by osteoblasts.

Materials and Methods

Cell culture

MC3T3-E1 is an osteogenic cell line derived from neonatal mouse calvaria. Early-passage MC3T3 cells were cultured in α-MEM (Life Technologies, Inc., Grand Island, NY) containing 20 mM HEPES and 10% FBS (Summit Biotechnology, Ft. Collins, CO) (12). Cells were grown to confluence, rinsed, and transferred to serum-free medium containing 0.1% BSA (Fluka Chemical Co., Ronkonkoma, NY) and an additional 50 μg/ml ascorbic acid for 24 h. Cultures were then exposed to test or

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control medium in the absence of serum for 2–24 h. In cultures used for Western blot analysis, the medium did not contain BSA (11).

Primary cultures of mouse osteoblastic cells were isolated from parietal bones of neonatal CD-1 mice (13). This procedure was approved by the Institutional Animal Care and Use Committee of Saint Francis Hospital and Medical Center. Parietal bones, dissected free of sutures, were subjected to 5 sequential 15-min digestions with bacterial collagenase (CLS II, Worthington Biochemical Corp., Freehold, NJ). Cells harvested from digestions 3 to 5 were cultured as a pool at an initial plating density of approximately 10,000 cells/cm^2. It has been demonstrated that these cells have osteoblastic characteristics (13, 14). Cells were cultured in DMEM supplemented with nonessential amino acids, 20 mM HEPES, 100 μg/ml ascorbic acid, and 10% FBS. When the cells reached confluence, approximately 1 week after plating, they were rinsed and transferred to serum-free medium for 24 h, then exposed to test or control medium for 2–24 h.

Primary cultures of mouse fibroblasts were obtained by collagenase digestion of minced skin from neonatal C57/BL6 mice (15). Cells were cultured in DMEM supplemented with 10% FBS and passaged three times before use in experiments. At confluence, cells were rinsed and transferred to serum-free medium containing 0.3% lactalbumin hydrolysate for 24 h. Cultures were then exposed to test or control medium in the absence of serum for 2–24 h.

Cycloheximide and 5, 6 dichlorobenzimidazole riboside (DRB) (Sigma, St. Louis, MO) were dissolved in absolute ethanol and, at dilutions of less than 1:10,000, an equal amount of ethanol was added to control cultures. TGF-β1 (a gift of Genentech, Inc., San Francisco, CA) was dissolved in culture medium.

Northern blot analysis

Total cellular RNA was isolated using RNeasy RNA isolation kits (QIAGEN, Valencia, CA). Equal amounts of RNA (10–15 μg) were denatured and subjected to electrophoresis through formaldehyde-agarose gels, and the RNA was blotted onto Gene Screen Plus as directed by the manufacturer (PerkinElmer Life Sciences, Wilmington, DE). A 1.6-kb *Sph*I/*Sal*I fragment of the mouse stromelysin-3 complementary DNA (cDNA) (provided by P. Basset, Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France) and a murine 18S ribosomal RNA cDNA (American Type Culture Collection, Manassas, VA) were labeled with [α-^{32}P] deoxycycidine triphosphate (3,000 Ci/mmol; PerkinElmer Life Sciences) by random primed second-strand synthesis (Ready to Go, Amersham Pharmacia Biotech, Piscataway, NJ) (8, 16). Hybridizations were carried out at 42 C in 50% formamide, 750 mM sodium chloride, 50 mM sodium phosphate, 5 mM EDTA, 5× Denhardt's solution, and 0.4% SDS (Sigma) (15). Posthybridization washes were performed at 65 C in 150 mM sodium chloride, 15 mM sodium citrate, and 0.1% SDS. Autoradiograms were analyzed by densitometry, and stromelysin-3 messenger RNA (mRNA) levels were normalized to those of 18S.

Nuclear run-off assay

Nuclei were isolated from confluent MC3T3 cells or fibroblasts by Dounce homogenization in a Tris-HCl buffer containing 0.5% Nonidet P-40 (Sigma). Nascent transcripts were labeled by incubation of nuclei in a reaction buffer containing 500 μM each ATP, GTP, cytidine 5′-triphosphate, RNAsin (Promega Corp.), and 250 μCi [^{32}P]UTP (800 Ci/mM, PerkinElmer Life Sciences) (14, 15). RNA was isolated by treatment with deoxyribonuclease I and proteinase K, followed by ethanol precipitation. Linearized plasmid DNA, containing approximately 1 μg cDNA, was immobilized onto GeneScreen Plus by slot blotting according to the manufacturer's directions (PerkinElmer Life Sciences). cDNA for rat glyceraldehyde-3-phosphate dehydrogenase (gift of R. Wu, Ithica, NY) was used as a control for loading of the radiolabeled RNA, and rat collagenase-3 cDNA (gift of Cheryl Quinn, St. Louis, MO) was used as a positive control (16–18). The plasmid vector pGEM5zf+ (Promega Corp., Madison, WI) was used as a control for nonspecific hybridization. Equal cpm amounts of [^{32}P]-RNA from each sample were hybridized to cDNA, using the same conditions as for Northern blot analysis, and were visualized by autoradiography.

Western blot analysis

Conditioned medium samples were stored at −80 C after the addition of 0.1% polyoxyethylene sorbitan monolaurate (Tween-20, Pierce Chemical Co., Rockford, IL), 10 mM EDTA, 50 mM Tris-HCl, pH 8.0, and 200 μg/ml phenylmethylsulfonylfluoride. Before electrophoresis, 750-μl aliquots were precipitated with 3.3% trichloroacetic acid and resuspended in reducing Laemmli sample buffer containing 2% SDS. Proteins were fractionated by PAGE, using a 12% gel, and transferred to Immobilon P membranes (Millipore Corp., Bedford, MA) (19). Membranes were blocked with 3% BSA and exposed to a 1:1000 dilution of rabbit antiserum raised against recombinant human stromelysin-3 (provided by Dr. S. Weiss, Ann Arbor, MI) (11, 20). The membranes were then washed and exposed to horseradish peroxidase-conjugated goat antirabbit IgG antiserum, washed, and developed using a horseradish peroxidase chemiluminescent reagent (PerkinElmer Life Sciences). Immunoreactive bands were visualized using Reflection x-ray film (PerkinElmer Life Sciences).

Statistical analysis

Slopes of RNA decay curves were analyzed by the method of Sokal and Rohlf (21).

Results

Northern blot analysis of RNA from MC3T3 cells showed that stromelysin-3 transcripts were rapidly and transiently induced by TGF-β. A maximal, approximately 3-fold, induction of stromelysin-3 RNA occurred after 2 h of treatment with TGF-β, and stromelysin-3 RNA returned to basal levels after 4 h of treatment (Fig. 1). Treatment of cells with TGF-β for longer periods, up to 48 h, did not appreciably induce or repress stromelysin-3 mRNA (Fig. 1, and data not shown). Western blot analysis of medium from MC3T3 cells showed that TGF-β induced stromelysin-3 polypeptide levels approximately 15-fold after 6 h of treatment. Interestingly, there was less stromelysin-3 protein in medium of TGF-β-treated cells after 16 h, possibly indicating increased proteolysis of the enzyme at these later times (Fig. 2) (22). Similarly, the amount of stromelysin-3 protein in the conditioned medium did not seem to accumulate with time, possibly because of proteolytic degradation of the enzyme. As previously observed, the stromelysin-3 present in the conditioned medium of MC3T3 cells included species with molecular masses corresponding to proenzyme, mature enzyme, and lower molecular-mass proteolytic fragments, the most abundant of which was approximately 34 kDa (11).

TGF-β dose-dependently increased stromelysin-3 transcript levels after 2 h of treatment (Fig. 3). Stromelyisn-3 mRNA was modestly induced at 0.1 ng/ml TGF-β and was

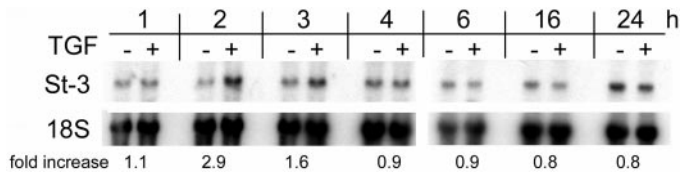

FIG. 1. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 mRNA expression in MC3T3 cells treated for up to 24 h. Total RNA from control or TGF-β treated cultures was subjected to Northern blot analysis and hybridized with a [^{32}P]-labeled stromelysin-3 (St-3) cDNA; the blot was stripped and hybridized with a labeled 18S ribosomal RNA cDNA (18S). Transcripts were visualized by autoradiography. Fold increase in stromelysin-3 mRNA, compared with the untreated control, is indicated *at the bottom* of the figure. These results are representative of three cultures.

maximally induced at 10–30 ng/ml TGF-β. Studies using the protein synthesis inhibitor cycloheximide, at 2μ g/ml, indicated that stromelysin-3 transcripts were increased by cycloheximide alone; and cotreatment with TGF-β further increased stromelyisn-3 mRNA (Fig. 4). Although data obtained using chemical inhibitors must be interpreted with caution, these data suggest that the TGF-β effect may be independent of new protein synthesis.

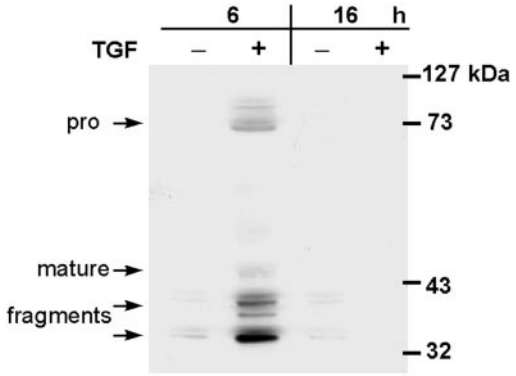

FIG. 2. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 protein levels in MC3T3 cells treated for 6 or 16 h. Conditioned medium from control or TGF-β-treated cultures was subjected to Western blot analysis using rabbit antihuman stromelysin-3 antiserum. The migration of the molecular mass standards is shown *on the right*. These results are representative of three cultures. pro, Proenzyme.

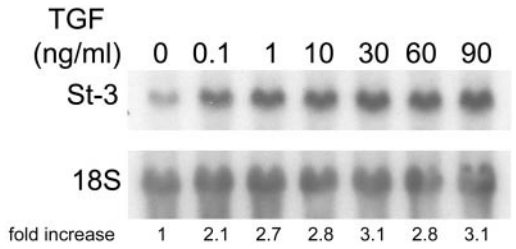

FIG. 3. Effect of TGF-β, at 0.1–90 ng/ml, on stromelysin-3 mRNA expression in MC3T3 cells treated for 2 h. Total RNA from control (0) or treated cells was subjected to Northern blot analysis and hybridized with a [^{32}P]-labeled St-3; the blot was stripped and hybridized with a labeled 18S. Transcripts were visualized by autoradiography. Fold increase in stromelysin-3 mRNA, compared with the untreated control, is indicated *at the bottom* of the figure. These results are representative of three cultures.

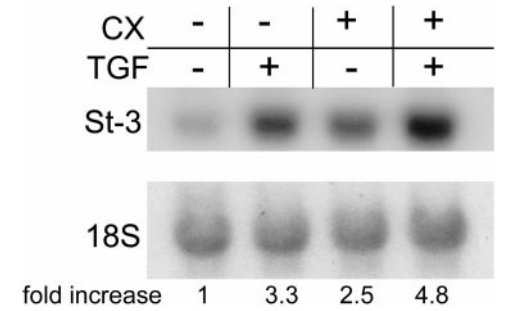

FIG. 4. Effect of TGF-β, at 10 ng/ml, in the presence or absence of cycloheximide at 2 μg/ml, on stromelysin-3 mRNA expression in MC3T3 cells treated for 2 h. Total RNA from untreated cells or cells treated with TGF-β or cycloheximide (CX) was subjected to Northern blot analysis and hybridized with a [^{32}P]-labeled St-3 cDNA; the blot was stripped and hybridized with a labeled 18S. Transcripts were visualized by autoradiography. Fold increase in stromelysin-3 mRNA, compared with the untreated control, is indicated *at the bottom* of the figure. These results are representative of three cultures.

To determine whether TGF-β increased stromelysin-3 mRNA by stimulating gene transcription, nuclear run-off assays were performed. Because TGF-β has been previously shown to decrease collagenase-3 transcription in rodent osteoblasts, this gene was used as a positive control for the assay (23). Indeed, TGF-β decreased collagenase-3 transcription, and an increase (approximately 4-fold) in stromelysin-3 gene transcription was noted in nuclei from cells treated with TGF-β for 1 h. However, after 2 or 6 h of TGF-β treatment, there was no effect on stromelysin-3 transcription (Fig. 5, and data not shown). To determine whether TGF-β treatment influenced the stability of stromelysin-3 transcripts, the RNA polymerase II inhibitor DRB was used to arrest transcription in MC3T3 cells. Cultures were treated with or without TGF-β for 1 h, then treated with 72 μM DRB for up to 5 h, and the decay of stromelysin-3 mRNA was determined by Northern blot analysis. In the transcriptionally arrested cells, the half-life of stromelysin-3 mRNA is approximately 2.5 h; but in the presence of TGF-β, the half-life of the transcripts increased to approximately 8 h (Fig. 6). These data demonstrate that the induction of stromelysin-3 by TGF-β involves both transcriptional and posttranscriptional mechanisms.

To confirm that induction of stromelysin-3 by TGF-β was not a cell-line-specific phenomenon, the effect of TGF-β on stromelysin-3 mRNA was determined in cultures of primary osteoblastic cells derived from neonatal mouse calvaria. In these cells also, TGF-β transiently increased stromelysin-3 mRNA (Fig. 7). Because stromelysin-3 is expressed by stromal fibroblasts surrounding invasive breast carcinoma, we determined whether TGF-β induced stromelysin-3 in fibroblastic cells. Using primary cultures of mouse fibroblasts, we detected stromelysin-3 mRNA in unstimulated cultures, and its expression was rapidly and transiently increased by treatment with TGF-β (Fig. 8). The growth factor induced stromelysin-3 mRNA approximately 3-fold after 2.5 h of treatment, and stromelysin-3 transcripts were down-regulated after

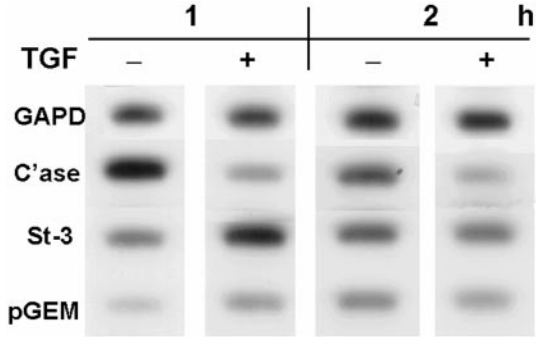

FIG. 5. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 gene transcription in MC3T3 cells treated for 1 or 2 h. Nuclei were isolated from control or TGF-β treated cells. Nascent transcripts were labeled *in vitro* with [^{32}P]-UTP, and the labeled RNA was hybridized to immobilized cDNA for St-3, glyceraldehyde-3-phosphate dehydrogenase (GAPD), and collagenase-3 (C'ase). pGEM5zf+ vector DNA (pGEM) was used as a control for nonspecific hybridization. These results are representative of two cultures.

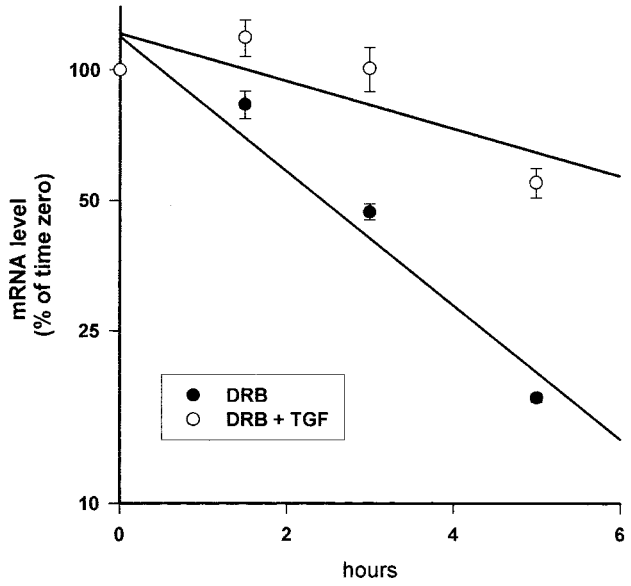

FIG. 6. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 mRNA decay in transcriptionally arrested MC3T3 cells. Confluent cultures were serum-deprived and exposed to TGF-β or to control medium for 1 h before the addition of 72 μM DRB. At selected times after the addition of DRB, total RNA from control (*filled symbols*) or TGF-β (*open symbols*)-treated cultures was subjected to Northern blot analysis with [^{32}P]-labeled stromelysin-3 cDNA. Stromelysin-3 mRNA was visualized by autoradiography and quantitated by densitometry. Values are means ± SEM for three cultures. The slopes for DRB and DRB+TGFβ are significantly different ($P < 0.01$).

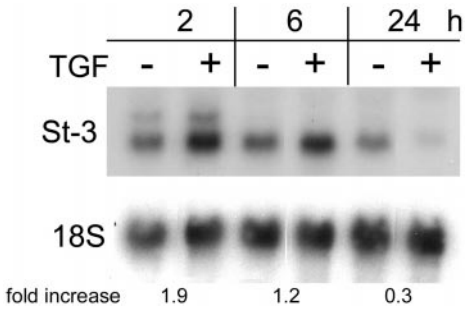

FIG. 7. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 mRNA expression in primary mouse osteoblastic cells treated for 2, 6, or 24 h. Total RNA from control or TGF-β-treated cultures was subjected to Northern blot analysis and hybridized with a [^{32}P]-labeled St-3 cDNA; the blot was stripped and hybridized with a labeled 18S. Transcripts were visualized by autoradiography. Fold increase in stromelysin-3 mRNA, compared with the untreated control, is indicated *at the bottom* of the figure. These results are representative of two cultures.

16 h of treatment. In a nuclear run-off assay, TGF-β increased stromelysin-3 gene transcription approximately 3-fold after 1 h of treatment, whereas transcription was returned to basal levels by 2 h (Fig. 9). As observed in osteoblast cultures, TGF-β stabilized stromelysin-3 mRNA in transcriptionally arrested fibroblasts. The half-life of stromelysin-3 in control cells was approximately 3 h, whereas treatment with TGFβ increased the half-life to approximately 7 h (slopes statistically different, $P < 0.01$, n = 3) (data not shown). These data confirm that TGF-β regulates stromelysin-3 expression in osteoblasts and fibroblasts by similar mechanisms.

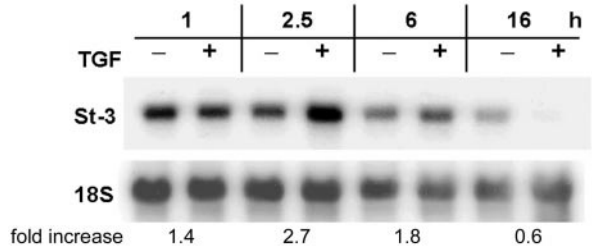

FIG. 8. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 mRNA expression in primary cultures of mouse fibroblasts treated for up to 16 h. Total RNA from control or TGF-β-treated cultures was subjected to Northern blot analysis and hybridized with a [^{32}P]-labeled St-3 cDNA; the blot was stripped and hybridized with a labeled 18S. Transcripts were visualized by autoradiography. Fold increase in stromelysin-3 mRNA, compared with the untreated control, is indicated *at the bottom* of the figure. These results are representative of two cultures.

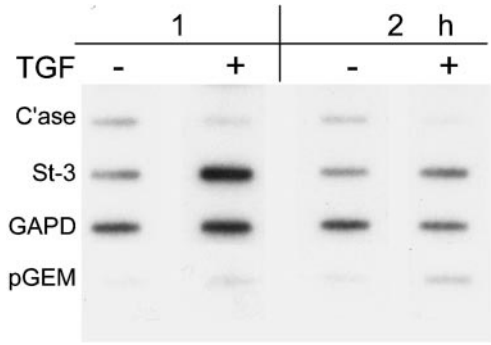

FIG. 9. Effect of TGF-β, at 10 ng/ml, on stromelysin-3 gene transcription in primary mouse fibroblastic cells treated for 1 or 2 h. Nuclei were isolated from control or TGF-β-treated cells. Nascent transcripts were labeled *in vitro* with [^{32}P]-UTP, and the labeled RNA was hybridized to immobilized cDNA for St-3, GAPD, and C'ase. pGEM was used as a control for nonspecific hybridization.

Discussion

The synthesis of stromelysin-3 by stromal fibroblasts surrounding invasive carcinomas is considered a tumor-induced host response (24). Here, we report that TGF-β, a growth factor secreted by breast cancer cells, stimulates the expression of stromelysin-3 by osteoblastic and fibroblastic cells (25). Data obtained using osteoblast cultures suggest that induction of stromelysin-3 by TGF-β is independent of new protein synthesis, implicating a direct effect of the growth factor. Our data demonstrate that TGF-β increases stromelysin-3 gene transcription and prolongs its mRNA half-life, resulting in increased stromelysin-3 protein secreted by the osteoblasts. The TGF-β-mediated increase in stromelysin-3 gene transcription is transient and precedes the increase in steady-state mRNA levels. When stromelsyin-3 mRNA levels are at their maximum, at 2 h of TGF-β treatment, the stimulation of transcription is no longer apparent, suggesting that the increase in mRNA stability plays a fundamental role in the TGF-β effect. Unfortunately, the precise half-life of stromelysin-3 mRNA in normal, untreated cells cannot be inferred from the data obtained using transcriptional inhibitors, such as DRB, because these agents can alter the stability of mRNAs (26). Nonetheless, TGF-β induces stromelysin-3 mRNA in fibroblastic cells by similar

mechanisms, demonstrating common pathways for regulation of gene expression in these two cell types.

Various physiologically relevant growth factors modulate stromelysin-3 expression at a posttranscriptional level. Here, we showed that TGF-β regulates stromelysin-3 mRNA stability, and previously we demonstrated that acute exposure to FGF-2 destabilizes stromelysin-3 mRNA as a direct effect of the growth factor. However, prolonged exposure to FGF-2 caused a secondary transcriptional increase in stromelysin-3 expression, likely mediated by an FGF-2-regulated osteoblast product (11). In addition, thyroid hormone increases stromelysin-3 mRNA in Xenopus, but it does not seem to regulate the promoter (27). Similarly, a 24-h treatment with tumor necrosis factor α, epidermal growth factor, or FGF-2 did not regulate human stromelysin-3 promoter constructs in transiently transfected fibroblasts (28). Taken together, these data suggest that modulation of RNA stability is a major mode of stromelysin-3 gene regulation.

Determinants of RNA stability are frequently found in the 3′ untranslated region (UTR) of mRNAs (29, 30). The stromelysin-3 3′ UTR is approximately 750 bases long; and there is approximately 48% identity among rat, mouse, and human transcripts (8, 31, 32). In particular, a 300-base region of the stromelysin-3 3′ UTR has approximately 70% identity among the 3 species, suggesting conservation of functionally relevant sequences. As previously noted, the stromelysin-3 3′ UTR does not contain the classical adenylate- and uridylate-rich elements implicated in regulation of collagenase 1 and collagenase 3 transcript stability (33, 34). In fact, the stromelysin-3 3′ UTR does not seem to contain other defined motifs implicated in transcript destabilization, such as the Brd box or GY-box (35). It is possible that modulation of polyadenylation could be involved in stromelysin-3 transcript degradation, given that polyA tail shortening is an obligate step in the degradation of some mRNAs (26, 29). The mechanisms regulating stromelysin-3 RNA degradation are likely to be novel and could become potential targets for therapeutic intervention.

Our data suggest that TGF-β induction of stromelysin-3 involves a direct effect of the growth factor on metalloproteinase gene transcription. Ligand activation of the TGF-β receptor complex leads to phosphorylation and activation of a family of cytoplasmic signal-mediating molecules, the Smad proteins (36). Smads 2 and 3, heterooligomerized with Smad 4, can migrate into the nucleus and modulate gene transcription in response to TGF-β. Smads 3 and 4 can bind DNA directly at Smad-binding elements (37). In addition, Smads have been shown to interact and cooperate with other transcription factors, including Fos and Jun (members of the activator protein-1 family), CREB (cAMP-response element binding protein), Sp-1, and Cbfa (core binding factor a) (36–39). Consequently, AP-1, Sp-1, CREB, and Cbfa binding sites have been characterized as TGF-β-responsive elements in other promoters. Computer-assisted analysis of the published, approximately 1.4-kb, human stromelysin-3 promoter sequence suggests multiple potential binding sites for Smads, some of which overlap with potential binding sites for AP-1 and Cbfa1 (40, 41). It is possible that TGF-β stimulates stromelysin-3 gene transcription through Smad interaction with promoter DNA, alone or in conjunction with AP-1 and/or Cbfa1. Indeed, TGF-β stimulation of retinoic acid receptor genes in osteoblasts is mediated through AP-1 (42). Inhibitory Smads, Smads 6 and 7, are also induced by TGF-β and are thought to function in a negative feedback loop to terminate or attenuate TGF-β signaling (36). These Smads may be responsible for the down-regulation of stromelysin-3 transcript levels observed in primary cultures of fibroblasts and osteoblasts.

It is suggested that the ability of stromelysin-3 to promote cancer cell metastasis and survival is dependent on extracellular matrix-associated growth factors (10). Proteolysis of specific binding proteins or extracellular matrix components could cause the release and/or activation growth factors and cytokines important for cell growth and mobility (10, 13). These ideas correlate well with the fact that documented stromelysin-3 substrates include insulin-like growth factor-binding protein 1 and a class of serine proteinase inhibitors, the serpins (20, 43). Bone is a continuously remodeling tissue, and stromelysin-3 could play a role in the proteolytic cascades associated with matrix turnover in normal and disease states.

The induction of stromelysin-3 by TGF-β in fibroblastic and osteoblastic cells is likely to play an important role in the metastasis of cancer cells from the breast and the establishment of these cells in the bone environment. Production of TGF-β tends to increase in breast cancer, and it can stimulate mammary carcinoma cell invasion and metastatic potential (44, 45). The TGF-β-mediated induction of stromelysin-3 in stromal fibroblasts could be one mechanism by which this occurs. In bone, release of TGF-β from the matrix, as a result of osteoclastic activity, could stimulate stromelysin-3 expression by osteoblasts. The activity of stromelysin-3 could then favor the survival of metastatic breast cancer cells and potentiate osteolysis. Our data indicate that TGF-β induces stromelysin-3 expression in fibroblasts and osteoblasts by similar mechanisms, making these observations relevant to the understanding of how breast cancer cells metastasize from their compartment of origin and flourish in bone.

Acknowledgments

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