Stromelysin-3 Induction and Interstitial Collagenase Repression by Retinoic Acid

THERAPEUTICAL IMPLICATION OF RECEPTOR-SELECTIVE RETINOIDS DISSOCIATING TRANSACTIVATION AND AP-1-MEDIATED TRANSPRESSION

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Human stromelysin-3 and interstitial collagenase are matrix metalloproteinases whose expression by stromal cells in several types of carcinomas has been associated with cancer progression. We compared here the regulation of the expression of both proteases by retinoids in human fibroblasts. Physiological concentrations of retinoic acid were found to simultaneously induce stromelysin-3 and repress interstitial collagenase. In both cases, the involvement of a transcriptional mechanism was supported by run-on assays. Furthermore, in transient transfection experiments, the activity of the stromelysin-3 promoter was induced by retinoic acid through endogenous receptors acting on a DR1 retinoic acid-responsive element. The ligand-dependent activation of the receptors was also investigated by using selective synthetic retinoids, and we demonstrated that retinoic acid-retinoid X receptor heterodimers were the most potent functional units controlling both stromelysin-3 induction and interstitial collagenase repression. However, specific retinoids dissociating the transactivation and the AP-1-mediated transrepression functions of the receptors were found to repress interstitial collagenase without inducing stromelysin-3. These findings indicate that such retinoids may represent efficient inhibitors of matrix metalloproteinase expression in the treatment of human carcinomas.

Stromelysin-3, based on sequence homologies and its domain organization, belongs to the matrix metalloproteinase (MMP) family consisting of extracellular proteinases that are implicated in a variety of tissue remodeling processes. Stromelysin-3 expression has been associated with cutaneous wound healing (1), mammary gland involution (2), cycling endometrium (3), embryonic development (4), and metamorphosis (5), where its expression was predominantly found in cells of mesodermal origin. In human carcinomas, stromelysin-3 was the first MMP identified as being expressed by stromal cells (6, 7). Although human stromelysin-3 appears to be unable to degrade any major component of the extracellular matrix (8, 9) and exhibits unusual activation properties (10, 11), its role in cancer progression is supported by high expression levels, which are predictive of a poor clinical outcome (12, 13). Furthermore, we have demonstrated that stromelysin-3 facilitates the tumor take of cancer cells in nude mice (14). Following the identification of stromelysin-3, a number of other MMPs have also been found to be expressed by stromal cells of human carcinomas (15), indicating that the stromal cell production of MMPs represents a significant contribution to the overall proteolytic activities in malignant tumors (Refs. 15 and 16 and references therein). Despite the observation that most stromal MMPs are expressed by fibroblastic cells, no regulatory sequence that could account for this cell-specific expression pattern has yet been identified in the promoter of the corresponding genes.

While stromelysin-3 expression, like other MMPs, can be induced in human fibroblasts by agents such as phorbol ester (TPA) or growth factors (6, 17), very little is known about the mechanisms regulating its expression. We have recently isolated the stromelysin-3 gene (18) and shown that its proximal promoter differs from those of other MMPs by the absence of a consensus AP-1 -(c-Jun/c-Fos) binding site and the presence of a retinoic acid-responsive element (RARE) of the DR1 type. This RARE can be transactivated by retinoid receptors (RARs/RXRs) in a ligand-dependent manner in COS-1 cells. In contrast, AP-1 binding sites were found to play a crucial role in controlling both the activation of other MMP gene promoters in response to growth factors and cytokines (19, 20) and their inhibition by retinoic acid (RA) (20–22). Gene transcription studies have shown that while RARs and RXRs can induce transcriptional activation through specific DNA binding sites, they can also interact indirectly with AP-1 through transcriptional mediators to repress gene transcription (23–25). In agreement with these findings, inhibition of base-line and TPA-induced RNA expression by RA has been reported for interstitial collagenase (20) and stromelysin-1 (21).

Retinoid effects are achieved through two classes of ligand-dependent transactivators, the retinoic acid receptors (RARs, P.A., 9 cis-retinoic acid, 9-cis-RA; all-trans-retinoic acid, all-RA; retinoic acid receptor α, RARα; retinoic acid receptor β, RARβ; retinoic acid receptor γ, RARγ; TPA, 12-O-tetradecanoylphorbol-13-acetate; ST3, stromelysin-3; SSC, standard sodium citrate; kb, kilobase pair(s).
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-β, and -γ and their isoforms) and retinoid X receptors (RXRs, -β, and -γ and their isoforms), which are members of the nuclear receptor superfamily. RA/RXRs and are activated by t-RA and 9C-RA, whereas RXRs bind and are activated by 9C-RA (26–28). Retinoids are known to regulate cell proliferation and differentiation, and they are regarded as agents that may be used to prevent or suppress human cancer (29–31). In addition, experimental and clinical studies suggest that retinoids may also be therapeutically useful in preventing connective tissue degradation caused by MMP overproduction in arthritis (32, 33). However, despite extensive knowledge of RA action at the molecular level, only a few RA target genes have been identified.

In the present study, we investigate further the regulation of stromelysin-3 gene expression by RA in cells of mesodermal origin. We have found that while nanomolar concentrations of RA can induce the expression of both stromelysin-3 RNA and protein in human fibroblasts, they prevent the expression of interstitial collagenase. The involvement of a transcriptional control in RA action is supported by run-on analyses, showing that the elongation of stromelysin-3 nuclear RNA was up-regulated and that of interstitial collagenase was down-regulated by RA. Furthermore, using stromelysin-3 promoter-based plasmid constructs, we showed that the stromelysin-3 promoter can be activated in cells of mesodermal origin exposed to RA, without the addition of exogenous RARs/RXR.

MATERIALS AND METHODS

Ligands—t-RA was purchased from Sigma, and 9C-RA was provided by P. N. Serter, J. F. Grippo, and A. A. Levin (Hoffmann-La Roche, Nutley, NJ). CD666 (34) was donated by B. Shroot (Centre International de Recherches Dermatologiques Galderma, Valbonne, France). Am80 (35) was provided by K. Shudo (University of Tokyo). BMS649 (36) (originally known as SR11237) was provided by the Bristol-Myers-Squibb Pharmaceutical Research Institute (Buffalo, NY). The last retinoid BMS753 (37, 38), which is a pure RAR antagonist, was also provided by the Bristol-Myers-Squibb Pharmaceutical Research Institute, where it is available to academic investigators upon request.

Cell Culture—Human fibroblasts (HFL1, CCL 153) and rhadomyosarcoma tumor cell line (RD, CCL 136) were obtained from the American Tissue Culture Collection (Rockville, MD) and maintained in monolayer culture in Dulbecco’s modified Eagle’s medium with or without 5% calf serum. Retinoids t-RA, 9C-RA, BMS753, BMS649, Am80, and CD666 were dissolved in ethanol and added at desired concentrations for the time periods indicated in the figure legends.

RNA Extraction and Northern Blot Analysis—Cell cultures were washed with phosphate-buffered saline, and RNA extraction was carried out by the guanidinium thiocyanate phenol/chloroform procedure (39). 10–30 μg of total RNA was denatured at 65 °C for 5 min and electrophoresed on 1% agarose gel prior to being transferred onto a nylon membrane (Hybond-N, Amersham Corp.) as described previously (40). Hybridization to cDNA probes was performed overnight at 42 °C in 40% formamide, 2 mM EDTA, 0.9 M NaCl, 50 mM NaHPO4/NaH2PO4, pH 6.5, 1% sodium dodecyl sulfate, 0.4 g/liter polyvinylpyrrolidone, 0.4 g/liter Ficoll, 0.04 g/liter dextran sulfate, and 50 mg/ml denatured salmon sperm DNA. The nylon membranes were washed twice at room temperature in 2× standard sodium citrate (SSC), 0.1% SDS for 15 min, followed by a last wash under stringent conditions with 0.1× SSC, 0.1% SDS at 56 °C for 1 h. The following human cDNA fragments were used as probes: a 1.7-kb EcoRI fragment for stromelysin-3 (30), a 1.3-kb EcoRI-XhoI fragment for interstitial collagenase, a 1.8-kb EcoRI fragment for stromelysin-1 (41), a 0.7-kb PstI fragment for 36B4 (42), a 0.6-kb PstI fragment for RARα, a 0.41-kb XhoI-EcoRI fragment for RARβ (43), a 1.3-kb AvaI-BamHI fragment for RARγ (44), and a 1.6-kb XhoI-XhoI fragment for RXRα (45). In the case of the RXRβ probe, a 0.8-kb BamHI cDNA fragment and a 0.6-kb fragment (nucleotides 1057–1677) amplified by polymerase chain reaction were generated from pTL1-hRXRβ plasmid (46). Similarly, for RXRγ, a 1.1-kb AvaI-PstI cDNA fragment and a 0.4-kb fragment (nucleotides 356–789) amplified by polymerase chain reaction were generated from the ps05-hRXRγ plasmid (26). All human RAR and RXR cDNA-containing plasmids were kindly provided by P. Kastner (Institut de Génétique et de Biologie Moléculaire et Cellulaire). Blots were autoradiographed for 1–4 days, and signal quantification was performed using a bioimaging analyzer (BAS 2000; Fuji Ltd).

Protein Analysis—Conditioned media from HFL1 fibroblasts were collected from cell culture centrifuged to eliminate debris, followed by a 100-fold concentration by 80% ammonium sulfate precipitation and dialysis against 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 5 mM MgCl2, 1 mM ZnCl2, 0.05% Brij-35. Protein samples were then separated by SDS-polyacrylamide gel electrophoresis under reducing conditions, transferred onto nitrocellulose membranes, and revealed with monoclonal antibody SST-4C10 against the catalytic domain of stromelysin-3 by using enhanced chemiluminescence (ECL, Amersham) and a peroxidase-coupled anti-mouse IgG (Jackson) (11).

Nuclear Run-on Transcription Assays—Control cells and cells treated with 9C-RA (1 μM) for 1–3 days were washed twice with ice-cold phosphate-buffered saline, harvested, and centrifuged at 1300 × g at 4 °C for 5 min. The pellet was resuspended in 4 ml of lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.25 mM EDTA, 0.07% NP-40), incubated for 5 min on ice, and centrifuged for 5 min at 4°C for 5 min. This procedure was repeated twice. The final pellet containing the nuclei was resuspended in storage buffer consisting of 50 mM Tris-HCl, pH 8.3, 5 mM MgCl2, 0.1 mM EDTA, 40% (v/v) glycerol, and aliquots of 2 × 105 nuclei were stored at −80 °C before use. In vitro initiated RNA transcripts from these 2 × 105 nuclei aliquots were elongated in vitro for 30 min at 30 °C in the presence of 200 μCi of [α-32P]UTP in a final volume of 200 μl containing 1 mg/ml heparin, 0.6% (v/v) sarkosyl, 0.4 mM concentrations of ATP, CTP, and GTP, 2.5 mM dithiothreitol, 0.15 mM phenylmethylsulfonyl fluoride, 350 mM (NH4)2SO4. The reaction was stopped by the addition of DNase I-RNase free (800 units) in the presence of 1.8 mM CaCl2 for 10 min at 30 °C, followed by protein digestion with proteinase K (100 μg/ml) in 50 mM Tris-HCl, pH 7.4, 20 mM EDTA, 1% SDS and incubation at 45–50 °C for 40 min at 42 °C until clear samples were obtained. RNA extraction was then performed with phenolchloroform (1:1, v/v), and the organic phase was further extracted with 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1% SDS. Pooled aliquots were finally extracted with chloroform, and RNA precipitation was carried out at 4 °C for 15 min after the addition of 1 volume of 20% trichloroacetic acid. The RNA pellets were washed 3 times in 5% trichloroacetic acid and once with 80% ethanol. Dried pellets were then dissolved in hybridization buffer (as described above) to a final specific activity of 5 × 106 cpm/ml and hybridized to cDNAs corresponding to human stromelysin-3 (31) by in vitro, human interstitial collagenase (41), 36B4 (42), and the pBlue-script II SK+ plasmid. These DNAs were denatured in the presence of 0.3 M NaOAc and immobilized onto Hybond nylon membranes (Amersham) by using a slot blot apparatus. Prehybridization at 42 °C for 18 h and hybridization to in vitro 32P-labeled elongated RNAs at 42 °C for 3 days were carried out in the same hybridization buffer. Filters were subjected to various washing conditions as follows: twice in 2× SSC, 1% SDS for 15 min at 22 °C; twice in 0.1× SSC, 0.1% SDS for 15 min at 52 °C; twice in 2× SSC, 1% SDS for 15 min at 37 °C; twice in 2× SSC, 1% SDS for 15 min at 22 °C; and finally, once in 0.1× SSC, 0.1% SDS for 15 min at 52 °C. Signal quantification was carried out as described for Northern blot analysis.

CAT Reporter Constructs—The DR1-tk-CAT, 0.29ST3-CAT, 0.45ST3-CAT, 1.47ST3-CAT, and 3.4ST3-CAT constructs have been previously described (18). The βBARE (DR5)-CAT construct (47) was kindly provided by J-Y. Chen (Institut de Génétique et de Biologie Moléculaire et Cellulaire). The 3.4ST3-CAT-ΔDR1 construct was generated by inserting the 3-kb SphI-XbaI-5′-fragment from the 3.4ST3-CAT construct into the 0.29ST3-CAT construct digested with the same restriction enzymes, thereby deleting a 0.16-kb promoter sequence containing the DR1 RARE that is present at position −385 in the stromelysin-3 gene promoter (19).

Cell Transfection and CAT Assay—Human RD rhabdomyosarcoma cells were transiently transfected by the calcium phosphate procedure as described previously (18), except that the total amount of DNA transfected in each 10-cm diameter culture dish was made up to 20 μg with pBluescribe plasmid DNA. For a 4-day treatment with RA, cells were first exposed to 1 μM 9C-RA for 2 days before transfection, whereas for a 2-day 9C-RA treatment, cells were directly transfected at 4 h after
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RESULTS

Stimulation of Stromelysin-3 and Inhibition of Interstitial Collagenase RNA Expressions by Retinoic Acid in Fibroblasts—Having previously identified a RARE that conferred ST3 promoter inducibility in COS-1 cells in the presence of RA and its receptors (18), we decided to evaluate whether stromelysin-3 gene expression was also regulated by RA in human fibroblasts. Time course and dose response experiments were performed, and expression of the stromelysin-3 gene was compared with that of interstitial collagenase by Northern blot analysis in HFL1 fibroblasts exposed to 9C-RA in the presence of 5% calf serum (Fig. 1).

As shown in Fig. 1A, in the presence of 1 μM 9C-RA, stromelysin-3 RNA levels progressively increased from day 1 to day 4, with a 20-fold increase measured after 4 days of incubation. In contrast, the levels of interstitial collagenase RNA remained constant when fibroblasts were exposed to 9C-RA for 1 day and rapidly decreased to almost undetectable levels after 2 days of treatment. Dose response experiments were conducted after incubation during 3.5 days with 9C-RA concentrations ranging from 0.1 nM to 1 μM (Fig. 1B). The effect of 9C-RA was dose-dependent for both genes. Nevertheless, the repression of interstitial collagenase expression was much more sensitive to 9C-RA treatment than the induction of the ST3 gene. Indeed, the half-maximal values for stromelysin-3 induction (EC50) and interstitial collagenase repression (IC50) differed by a factor of about 100 (Fig. 1B; EC50 = 10 nM and IC50 = 0.1 nM). Similar results were obtained by using t-RA instead of 9C-RA or when the experiments were carried out in serum-free conditions (data not shown). However, in the latter case, the interstitial collagenase base line was much lower (Fig. 2), hampering analysis of its repression by RA isomers.

Our results showing a significant induction of ST3 RNA levels in HFL1 fibroblasts in the presence of RA are in apparent contradiction with the observation recently made by Anderson et al. (48). Using the same fibroblasts as models, they found that TPA-mediated induction of stromelysin-3 RNA was inhibited by RA. As shown on Fig. 2, we could reproduce this inhibition. However, this effect was only observed for a 10 μM 9C-RA concentration, which by far exceeds the RA concentrations usually found in physiological conditions. Furthermore, we would like to point out that when using this 10 μM 9C-RA concentration, we could not obtain any repression of interstitial collagenase RNA expression, whereas this repression was observed at lower concentrations (Fig. 2), as previously noted by others (23).

Induction of Stromelysin-3 Protein Synthesis and Secretion by Retinoic Acid in Fibroblasts—To find out whether stromelysin-3 protein synthesis and/or secretion were also increased by RA treatment, conditioned media from HFL1 fibroblasts were analyzed by Western blot (Fig. 3). In serum-free conditions, only low levels of the mature stromelysin-3 form were detected at about 47 kDa. However, when fibroblasts were exposed for 3 days to 1 μM of either 9C-RA or t-RA, high levels of this form were detected together with additional protein species. The highest molecular weight form corresponds to the stromelysin-3 proform, which is known to be converted by furin or furin-like enzymes into the mature form (10, 11), which in turn can be processed further into another low molecular weight species (Fig. 3 and Ref. 14).

Transcriptional Control of Stromelysin-3 and Interstitial Collagenase Genes by Retinoic Acid in Fibroblasts—To determine whether a transcriptional mechanism was involved in controlling the levels of stromelysin-3 and interstitial collagenase RNAs by RA, we analyzed the nuclear RNAs of both MMPs by using run-on assays performed on nuclei isolated from HFL1 fibroblasts after they had been treated for 1–3 days with 1 μM 9C-RA. Radiolabeled RNAs resulting from nascent nuclear transcription were hybridized to cDNAs cloned into the pBluescript II SK+ plasmid and corresponding to interstitial collagenase, stromelysin-3, 36B4, or the plasmid alone as a control for nonspecific hybridization. The results presented on Fig. 4 show that both MMP genes are constitutively transcribed in HFL1 fibroblasts. After 3 days in the presence of 9C-RA, interstitial collagenase transcription was no longer detectable. On the other hand, 9C-RA was found to increase the rate of stromelysin-3 gene transcription by 2-fold, thereby reaching levels similar to those observed for the 36B4
gene, whose expression is not affected by 9C-RA (Fig. 4 and Ref. 37). Shorter exposure times of HFL1 fibroblasts to 9C-RA (1 or 2 days) led to either no increase or a very little increase in stromelysin-3 gene transcription (data not shown).

**Activation of the Human Stromelysin-3 Gene Promoter by Retinoic Acid via Endogenous Retinoid Receptors in Rhabdomyosarcoma Cells—**HFL1 fibroblasts, like other nonimmortalized human diploid fibroblasts, are difficult to use for promoter studies in transient transfection experiments. Therefore, we looked for an established cell line expressing the stromelysin-3 gene that would be easier to use for transfection studies. Since the stromelysin-3 gene is only weakly expressed in human fibrosarcoma cell lines such as HT-1080 and cannot be induced by TPA in these cells (17), we screened several human cell lines of mesodermal origin based on their ability to respond to TPA and RA. We thus identified a rhabdomyosarcoma cell line (RD) that exhibits a stromelysin-3 expression pattern (Fig. 5), even upon exposure to TPA and RA, which is maximal after 4 days of incubation, whereas untreated cells for RARβ, RXRβ, and RXRγ RNAs, even when up to 30 μg of total RNA were loaded for analysis (Fig. 7 and data not shown). These results are consistent with recent studies that have shown that RARα, RARγ, and RXRα are the predominant receptors expressed in human skin (50) as well as in various human cell lines (51–53). The expression of RXRα was only slightly increased (less than 2-fold) in cells treated with either 9C-RA or t-RA, whereas RARα and RARγ levels remained unaffected. In
and collagenase expression was reduced by at least 50% (Fig. 8, C).

**DISCUSSION**

We have previously shown that the stromelysin-3 gene promoter differed from most other MMP promoters by the absence of a functional AP-1 binding site and the presence of a RARE in its proximal region. In the present study, we have further investigated the regulation of stromelysin-3 gene expression by RA and compared this regulation with that of interstitial collagenase, another MMP. Stromelysin-3 and interstitial collagenase are both predominantly expressed by stromal cells of human carcinomas (15), and their high expression levels were found to be associated with a poor clinical outcome in some carcinomas (12, 13, 54). Considering that retinoids by themselves or when associated with other drugs such as tamoxifen, are regarded as potential new anticancer agents (29–31), it is important to elucidate the mechanisms by which the expression of MMPs implicated in cancer progression is regulated by RA. We demonstrate here that both natural RA isomers, 9C-RA and t-RA, strongly induce stromelysin-3 RNA and protein expression and simultaneously repress interstitial collagenase expression in human fibroblasts. In addition, we show that both genes are controlled by RA through a transcriptional mechanism, and we provide evidence indicating that RARα is the most important RAR involved in the regulation of stromelysin-3 and interstitial collagenase expression.

Using these retinoids individually at low and/or selective concentrations, either no induction or a very weak induction of stromelysin-3 was detected (Fig. 8, A and B), while interstitial collagenase expression was reduced by at least 50% (Fig. 8, C and D). At higher concentrations (>10 nM), when Am80 and CD666 lose their specificity and act as pan-RAR agonists (37), higher levels of stromelysin-3 RNA were observed, while interstitial collagenase expression was repressed further. Interestingly, very little stromelysin-3 gene induction was noted with the pure RARα agonist BMS753 and the pan-RXR agonist BMS649, even when these retinoids were used at a 1 μM concentration. In marked contrast, the combination of either Am80 (100 nM and 1 μM) or CD666 (100 nM) with the pan-RXR ligand BMS649 (1 μM) resulted in a synergistic induction of the stromelysin-3 gene, reaching expression levels close to those observed with the natural ligands. A synergistic effect was also observed when the BMS753 and BMS649 ligands were combined, although the expression levels of stromelysin-3 RNA did not exceed 50% of those observed in the presence of the natural ligands. However, any of these combinations was found to fully repress interstitial collagenase gene expression. We note that stromelysin-1 gene expression was similarly repressed in HFL1 fibroblasts, suggesting that the retinoids used here may efficiently repress the expression of any AP-1-regulated MMP.

Taken together, our observations indicate that while the selective activation of RARα, RARγ, or RXRs substantially represses interstitial collagenase gene expression, the combination of RARs and RXRs is required for optimal stromelysin-3 gene induction and for full repression of interstitial collagenase.

2 P. Anglard, unpublished results.
they mutually antagonize each other at the level of transactivation and DNA binding (22–25). Indeed, MMP genes containing an AP-1 binding site in a conserved position in their promoter or other genes like those for tumor growth factor-β1 (55) and interleukin-6 (56) are TPA-inducible, while their expression is inhibited by RA. Knowing that, reciprocally, AP-1 can inhibit transactivation by RARs and RXRs, the observation that the stromelysin-3 gene is induced by both TPA and RA in a given cell type is quite unexpected and represents an unusual example of a gene up-regulated by both agents.

We have found that physiological concentrations of RA efficiently induce both the expression of the stromelysin-3 gene and the repression of the interstitial collagenase gene in HFL1 human fibroblasts, the latter being observed at RA concentrations lower than those necessary for stromelysin-3 induction. Interestingly, the IC₅₀ for interstitial collagenase and the EC₅₀ for stromelysin-3 that we found were very similar to the values recently reported by Chen et al. (25) in promoter studies. These authors have shown that the repression of AP-1-induced transcription from the interstitial collagenase promoter was about 100 times more sensitive than the transactivation of a RARE-tk-CAT construct in the presence of RA. Since these observations suggested that the regulation of both genes by RA may be achieved through a transcriptional mechanism, we further evaluated this possibility by measuring the transcriptional rate of both genes in HFL1 fibroblasts in run-on assays. In the presence of 9C-RA, we observed a complete inhibition of interstitial collagenase transcription, which is likely to result from an RAR/AP-1 interaction, since this has been previously documented (24, 57). On the other hand, a 2-fold increase in the stromelysin-3 gene transcriptional rate was found when HFL1 fibroblasts were exposed to 9C-RA for 3 days. No clear transcriptional activation could be detected for shorter exposure times. Although it is difficult to determine whether this 2-fold increase can fully account for the 20-fold increase in stromelysin-3 RNA levels observed after 4 days of RA treatment, we note that run-on studies with other RA-inducible genes containing a RARE in their promoter exhibited similar profiles.

**FIG. 8.** Comparative expression of stromelysin-3 and interstitial collagenase genes in HFL1 fibroblasts exposed to selective retinoids. The effects of natural and synthetic retinoids on ST3 gene induction (A and B) and interstitial collagenase (Int. Col.) gene repression (C and D) were analyzed by Northern blot as described under "Materials and Methods." HFL1 fibroblasts cultured in 5% calf serum were treated for 3.5 days with RARα- (Am80 and BMS753; panels A and C) or RARγ- (CD666; panels B and D) specific synthetic retinoids and/or the pan-RXR agonist BMS649, used at the indicated concentrations. The two natural isomers 9C-RA and t-RA were used at 1 μM. Relative levels of RNA transcripts, as evaluated by using a bioimaging analyzer (BAS 2000; Fuji Ltd.) for each blot after normalization with 36B4 RNA levels, are presented in the histograms. The induction of stromelysin-3 RNA is expressed relative to that observed in the presence of 9C-RA, while the repression of interstitial collagenase RNA is expressed relative to its level in untreated cells.
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Thus, the RARβ and the laminin B1 RNAs were found to be induced at high levels by RA in F9 cells, while no increase or only a moderate increase in transcriptional rates could be detected for these genes by nuclear run-on assays (58, 59). In all instances, the contribution of a transcriptional mechanism in stromelysin-3 gene induction is further supported by our findings showing that 9C-RA induces stromelysin-3 promoter activity in RD rhabdomyosarcoma cells. By analyzing various lengths of this promoter in transient transfection experiments, we observed a 3-fold induction of stromelysin-3 promoter activity in the presence of 9C-RA, which was strongly reduced in the constructs lacking the DRI-RARE. Interestingly, this transactivation was observed without the addition of retinoid receptors, indicating that the DRI-RARE was activated by functional endogenous retinoid receptors in these cells.

Previous studies have shown that while all RARs could potentially mediate the induction of RA targets genes, the involvement of a given receptor was dependent on many parameters including promoter context and cell type (37, 47). When the expression of RARs and RXRs was evaluated in HFL1 fibroblasts, we found that RARα, RARγ, and RXRα RNAs were constitutively expressed at high levels. In contrast, we could not detect any RNA for RARβ and RXRγ, while that for RARβ was strongly induced from barely detectable levels in untreated fibroblasts to high levels in the presence of 9C-RA or t-RA. Similar observations have been made in human dermal (60) and lung (61) fibroblasts.

Since these observations suggested that specific retinoid receptors could be involved in the regulation of stromelysin-3 and interstitial collagenase expression by RA in HFL1 fibroblasts, we tested the expression of both genes in the presence of selective retinoids. We found that these retinoids, when used in the presence of 9C-RA, which was strongly reduced in the constructs lacking the DRI-RARE, whereas they repress interstitial collagenase expression by RA in HFL1 fibroblasts, and lung (61) fibroblasts to high levels in the presence of 9C-RA or t-RA. We note, however, that the combination of the BMS649 RXR agonist with either Am80 or a specific ligand. We found that these retinoids, when used in vitro with the DRI-RARE, is less efficient than the respective homodimers (27, 28). In this respect, it is noteworthy that the activation of a single RAR or RXR was sufficient to substantially repress interstitial collagenase expression in HFL1 fibroblasts but that the activation of both partners of heterodimers was necessary for a full repression.

In summary, while transcription studies have demonstrated that RA regulates the expression of target genes by either activating RARs or repressing AP-1 activity, we have looked into the regulation of two genes that belong to the MMP family and shown that they are differentially regulated by RA in human fibroblasts. Indeed, we have shown that physiological concentrations of RA induce stromelysin-3 expression but repress interstitial collagenase expression. Compared with the repression of interstitial collagenase, stromelysin-3 gene induction relies on more restricted conditions, based on a lower sensitivity to both natural and synthetic retinoids, and on a more restricted receptor requirement involving RAR-RXR heterodimers. In contrast, a substantial transcriptional repression of interstitial collagenase is achieved by retinoids activating only one type of receptors, although the involvement of RAR-RXR heterodimers is required for a full repression. Taking into account the ongoing efforts in designing potent MMP inhibitors to inhibit cancer progression, the finding that dissociating retinoids such as RXR-selective ligands can prevent the expression of some MMPs by an AP-1 transrepression mechanism without inducing stromelysin-3 gene expression may be of interest for therapeutic applications.

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