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MINIREVIEW

Nuclear Hormone Receptors Inhibit Matrix Metalloproteinase (MMP) Gene Expression Through Diverse Mechanisms

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Agents like retinoids, thyroid hormone, glucocorticoids, progesterone, androgens, which bind to members of the nuclear receptor superfamily, inhibit the synthesis of matrix metalloproteinases (MMPs) in many cell types. These Zn^{2+}- and Ca^{2+}-dependent MMPs degrade components of the extracellular matrix (ECM), and precise regulation of their expression is crucial in many normal processes. However, inappropriate expression of MMPs contributes to a variety of invasive and erosive diseases, and inhibition of MMP synthesis provides an important mechanism for controlling such aberrant or dysregulated responses. Nuclear receptors control MMPs through a variety of seemingly redundant mechanisms. First, nuclear receptors act on the promoters of MMP genes to enhance or suppress *trans*-activation. Ironically, in a family of genes that exhibits substantial regulation by nuclear receptors, few consensus hormone responsive elements (HREs) have been deomonstrated in MMP promoters. Rather, inhibition of MMPs occurs primarily, but not exclusively, at AP-1 sites. Here, nuclear receptors form complexes on the DNA through interactions with AP-1 proteins, sequester Fos/Jun and/or decrease the mRNAs for these transcription factors. Second, nuclear receptors and their ligands can indirectly inhibit MMPs. For instance, both retinoids and glucocorticoids induce the transcription of TIMPs (tissue inhibitor of metalloproteinases), which complex with MMPs and inhibit enzymatic activity, and progesterone stimulates production of transforming growth factor-β (TGF-β), which in turn suppresses MMP-7 (matrilysin). Finally, nuclear receptors bind to coactivators, corepressors, and components of the general transcriptional apparatus, but the potential role of these interactions in MMP regulation remains to be determined. We conclude that nuclear receptors utilize multiple, apparently redundant, mechanisms to inhibit MMP gene expression, assuring precise control of ECM degradation under a variety of physiologic and pathologic conditions.

Matrix metalloproteinase (MMP) Nuclear hormone receptors Gene expression
Multiple mechanisms

MATRIX metalloproteinases belong to a family of at least 19 enzymes that include the gelatinases, collagenases, stromelysins, and membrane-type MMPs. These Zn^{2+}-containing and Ca^{2+}-requiring enzymes degrade components of the extracellular matrix (ECM), including collagens, elastin, entactin, fibronectin, gelatin, laminin, and proteoglycan core protein (10,17). Most cells constitutively express low levels of at least some MMPs, but a variety of cytokines, growth factors, and hormones can stimulate or repress transcription of the enzymes. The precise and highly regulated expression of MMPs characterizes numerous processes, such as embryonic development, uterine involution, and wound healing, which rely upon extensive remodeling of the ECM. However, a number of disorders, including osteo- and rheumatoid arthritis, skin lesions, tumor invasion and

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metastasis, and emphysema involve excessive MMP production (10,27,28,30,88).

Recent advances in the biology of MMPs and nuclear receptors have warranted a specific examination of work in this area over the last several years. The growing number of diseases hallmarked by MMP overexpression illustrates the negative impact of dysregulated MMP controls. Whereas ligand-induced nuclear receptors regulate many genes by binding directly to promoter hormone response elements (HREs) and acting as transcriptional inducers, somewhat less attention has been paid to transcriptional inhibition by nuclear receptors. Several mechanisms, not necessarily acting in isolation, have been proposed for the downregulation of gene expression (34,40,52,70). Indeed, we find both variety and redundancy in the inhibition of MMPs by nuclear receptors (Table 1), underscoring the extreme importance of strict MMP regulation in matrix metabolism. Redundancy in MMP inhibition occurs at several levels, including variations in the numbers of distinct mechanisms, ligand isoforms, and receptor subtypes. Convergence of these multiple mechanisms on a single metalloproteinase may allow quick and complete suppression of enzyme activity in a given tissue.

REGULATION OF MMP GENE EXPRESSION BY TRANSCRIPTION FACTORS

A variety of transcription factors bind to proximal response elements just upstream of the TATA boxes of MMP promoters and contribute to expression of these genes. One key player is the activator protein-1 (AP-1) site (consensus 5′-TGAG/CTCA-3′), which binds the transcription factors Fos and Jun and plays a crucial role in the *trans*-activation of MMPs by agents such as phorbol esters, interleukin 1 (IL-1), and tumor necrosis factor-α (TNF-α) (11,51,91). Most MMP promoters, with the exception of MMP-2 (gelatinase A) and MMP-11 (stromelysin-3), contain at least one AP-1 site in their promoters. A majority of these promoters also contain binding sites for Ets proteins, which can interact synergistically with AP-1 proteins to activate gene transcription (3,54,90). Still other, less-defined, DNA elements in the proximal regions of MMP promoters regulate gene expression. For instance, regions adjacent to the AP-1 site in the human MMP-3 (stromelysin-1) promoter bind proteins and play roles in the induction by IL-1 (66). Thus, whereas early studies demonstrated the necessity of proximal AP-1 site(s) for the induction of many MMPs, it is now clear that numerous, additional *cis*- and *trans*-acting factors contribute to expression of these genes.

Indeed, distal promoter regions upstream of the proximal AP-1 sites contribute to transcriptional activation of MMPs. For instance, in the rabbit interstitial collagenase (MMP-1) promoter, a region upstream of -2400 bp confers IL-1 inducibility (89). In addition, ISE1 and ISE2, two immortalization-susceptible *cis*-acting elements located in a 100-bp stretch at -1600 bp upstream in the human MMP-1 promoter, contain an array of putative Ets and AP-1 binding sites (38). It is possible that positive and negative regulators bind to this region and differentially regulate MMP-1 gene expression in preimmortalized and immortalized cells. Also, the transcription factor AP-2 binds to a site at -1685 bp in the human MMP-2 promoter and stimulates gene activation (81). In yet another example of upstream regulation, a response element, so far described only in the MMP-3 promoter (SPRE; stromelysin-1 PDGF-responsive element, centered at -1573 bp), binds a novel protein (SPBP). The SPRE is both necessary and sufficient for induction by mitogens such as platelet-derived growth factor (PDGF) (47,71). The gelatinase B (MMP-9) promoter also contains functional binding sites for the transcription factors nuclear factor-κB (NF-κB) at -600 bp to -591 bp and SP-1 at -558 bp to -563 bp (72). These examples, selected from the growing list of defined upstream promoter elements, illustrate the variety and complexity of factors that cooperate with each other and with proximal elements to intricately regulate MMP gene expression. Thus, the finely tuned expression of MMPs depends on the specific cellular environment and the relative availibility of different transcription factors.

REGULATION OF MMP GENE EXPRESSION BY THE NUCLEAR RECEPTOR SUPERFAMILY

Several agents inhibit the synthesis of MMPs (88), including TGF-β, IL-4, and ligands specific for the nuclear receptor superfamily (56,86). Although the negative regulation of MMPs by retinoids, glucocorticoids, thyroid hormone, progesterone, and other agents has been observed for years, the molecular basis of this suppression was

TABLE 1
EXAMPLES OF MMP REGULATION BY NUCLEAR RECEPTORS

Enzyme*	Agent†	Effect‡	Proposed Mechanism(s)	References
MMP-1	Ret	↓	Sequestration of Fos/Jun Binding of RARs/RXRs to DNA via Fos/Jun Decreased Fos/Jun mRNA Increased RAR mRNA Increased TIMPs	24,51,63,64,74,78,94
MMP-1	Gluc	↓	Sequestration of Fos/Jun Binding of GRs to DNA via Fos/Jun Ligand dependency and independency Increased TIMPs	24,41,48,53,77,93
MMP-1	Thyr	↓	Sequestration of Fos/Jun	95
MMP-1	Prog	↓	Decreased MMP-1 mRNA Decreased MMP-1 protein	57,73
MMP-2	Ret	↑	Increased differentiation Increased MMP-2 mRNA Increased MMP-2 protein	82
MMP-2	Prog	↓	Blocking secretion and activation of MMP-2	57
MMP-3	Prog	↓	Decreased MMP-1 mRNA Decreased MMP-1 protein	73
MMP-3	Ret	↓	Interference with AP-1 *trans*-activation	61
Transin	Ret	↑	Increased Fos/Jun mRNA	4
MMP-7	Prog	↓	Stimulation of TGF-β	16
MMP-9	Prog	↓	Blocking secretion and activation of MMP-9	57
MMP-11	Ret	↓	Decreased MMP-11 mRNA	1
MMP-11	Ret	↑	Transcriptional activation through RARE	2
MMP-13	Ret	↑	Increased Fos/Jun mRNA	4,26,33,87

Representative examples demonstrate redundancy and variety in the mechanisms by which members of the nuclear receptor superfamily regulate the synthesis of matrix metalloproteinases. This complexity in regulation underscores the importance of tight controls in MMP expression for normal physiology, and indicates that careful consideration is warranted in developing potential treatments for disorders hallmarked by excessive MMP production.

*Enzyme: MMP-1 (interstitial collagenase); MMP-2 (gelatinase A); MMP-3 (stromelysin-1); transin (rat homologue of MMP-3); MMP-7 (matrilysin); MMP-9 (gelatinase B); MMP-11 (stromelysin-3); MMP-13 (collagenase-3).

†Agent: Ret (retinoids); gluc (glucocorticoids); thyr (thyroid hormone); prog (progesterone).

‡Effect: ↓ or ↑, decrease or increase, respectively, in MMP synthesis/activity.

unclear until the elucidation and characterization of the nuclear receptor superfamily. Now it is recognized that nuclear receptors and their ligands inhibit MMP gene expression through a variety of means. Nuclear receptors can up- or downregulate transcriptional activity of many genes, usually by binding to HREs in the promoters. However, it is important to note that the promoters of most MMPs lack HREs, thereby suggesting alternative mechanisms for transcriptional control. Indeed, nuclear hormone receptors can modulate transcription through interactions with specific transcription factors (36,65,76,85), coactivators and corepressors (20,37,62), and the transcriptional machinery (6).

Transfection studies illustrate inhibition of MMP gene expression by nuclear receptors. For instance, retinoic acid receptors (RARs), glucocorticoid receptors (GRs), and thyroid hormone receptors (TRs) each inhibit, in a ligand-dependent manner, the activity of a reporter gene driven by fragments of the human or rabbit MMP-1 promoter (63,64,74,77,94,95). For GR, repression of MMP-1 transcription appears to take place through both ligand-dependent and -independent mechanisms (53). Ligand-independent repression of MMP-1 by glucocorticoids may occur in cases where GRs translocate to the nucleus from nonhormonal stimuli, such as heat shock. Some evidence also points to ligand-independent inhibition of rabbit MMP-1 by RARs (74). In all likelihood, both ligand-dependent and -indepen-

dent mechanisms contribute to MMP gene regulation by nuclear receptors. These studies emphasize the fact that MMP gene regulation can take place, and may even be biologically desirable, under conditions where hormone is unavailable.

Regulation of MMPs in the joint provides an excellent opportunity to examine the role of a ligand (retinoic acid) and its receptors (RARs and RXRs) on the expression of different MMPs in a particular tissue and to observe the net effect of that regulation on a disease state, rheumatoid arthritis. The increased expression of certain MMPs hallmarks this autoimmune disorder and contributes to irreversible joint degradation. Retinoic acid (RA) actually induces the secretion of several MMPs in cartilage and bone cells (4,26,87). In contrast, synovial cells lining the joint express a somewhat different array of MMPs (68) and treatment of these cells with retinoids suppresses, rather than stimulates, MMP expression (13,14). What then, is the overall effect of retinoid treatment on the severity of rheumatoid arthritis? In rat models, oral administration of retinoids reduced the clinical symptoms associated with adjuvant-induced or streptococcal cell wall-induced arthritis and also suppressed collagenase production (12,35). It is possible that combination therapy and novel retinoids may increase clinical efficacy (see below).

NUCLEAR RECEPTORS INHIBIT MMPs THROUGH DIVERSE MECHANISMS

Regulation of AP-1 (Fos/Jun) and Nuclear Receptor mRNAs

Depending on the cell and tissue context, nuclear hormones both positively and negatively regulate the expression of numerous proto-oncogenes, including c-*myc*, N-*myc*, c-*fos*, c-*jun*, jun-B, and jun-D (75). Again, cell and tissue specificity dictate the effects of nuclear receptor ligands on the expression of these mRNAs (75). For example, retinoic acid stimulates the production c-Jun and c-Fos in rat chondrocytes (4), but drastically reduces IL-1- or phorbol ester-stimulated expression of Fos in human rheumatoid synoviocytes (51). Similarly, all-*trans*-RA inhibits the phorbol-induced expression of both Jun and Fos in a rabbit synovial fibroblast cell line (63) and concomitantly induces the mRNAs for RARs α, β, and γ (64). Thus, the dual effects of increasing RAR transcription while inhibiting Jun/Fos transcription contribute to dysregulation of AP-1-driven MMP transcription.

Sequestration of AP-1 Proteins

Sequestration occurs when a repressor binds directly to a *trans*-activating factor, preventing the binding of the positive regulatory protein to its binding site on the DNA. Several studies present evidence consistent with the hypothesis that RARs, GRs, and TRs can each sequester Jun and/or Fos from the proximal AP-1 site on the rabbit and human MMP-1 promoters (41,46,77,78,84, 93–95). First, RARs, GRs, and TRs fail to bind directly to this AP-1 element. Second, all three nuclear receptors inhibit the binding of purified AP-1 proteins to the DNA. Third, chemical cross-linking demonstrates direct protein–protein interactions between the nuclear receptors and the AP-1 proteins.

While sequestration of Fos/Jun by nuclear receptors most likely contributes significantly to transcriptional inhibition of MMP-1, caution must be exercised in concluding the exclusivity of this mechanism. Variations in experimental technique and approach can alter the outcome of gel shifts. For example, if GRs and Fos/Jun are incubated together prior to AP-1 site binding, then GRs block the binding of Fos/Jun to the MMP-1 promoter. However, if Fos/Jun are first allowed to bind to the DNA, GRs fail to block the AP-1 binding (46).

Binding of Nuclear Hormone Receptors to DNA Via Fos/Jun

Detailed analyses of MMP promoters have revealed few examples of functional HREs. Whereas the human stromelysin-3 (MMP-11) promoter contains a consensus retinoic acid response element (RARE), this element confers transcriptional activation by retinoids, not repression (2). Instead, substantial evidence indicates nuclear receptors can bind to the AP-1 site in a complex of proteins through interactions with Jun and/or Fos. Gel mobility shift assays and antibody "super-shifts" illustrate that RARs/RXRs and c-Jun from rabbit synovial fibroblasts can bind together in a complex that forms on rabbit MMP-1 promoter fragments that contain AP-1 sites (64,74). These studies also show that binding of RARs/RXRs to the complex first requires the interaction of c-Jun with the AP-1 promoter fragment. In further support of these conclusions, DNAase I footprinting with recombinant proteins demon-

strates that c-Jun, but not RARs/RXRs, binds directly to the AP-1 site (19,74). RARs/RXRs do not dislodge the binding of purified c-Jun, but rather slightly alter the presence of DNAase I hypersensitive sites, suggesting that RARs/RXRs that bind indirectly to the DNA via Jun could alter the conformation of the DNA.

Likewise, studies suggest GRs can form a complex at the AP-1 site through interactions with Fos and Jun, which bind directly to the MMP-1 promoter. Chemical and UV cross-linking of extracts from HeLa cells treated with phorbol ester and a glucocorticoid demonstrated that GR associates with Fos/Jun that is bound to the AP-1 site (41). Also, in vivo footprinting studies with human skin fibroblasts showed that treatment with glucocorticoids did not alter the AP-1 footprint obtained with phorbol ester treatment alone (48). The binding of GRs, RARs/RXRs, or other nuclear receptors to the DNA indirectly via AP-1 proteins could result in a novel bending of the DNA that alters transcriptional activity. Indeed, the AP-1 site exhibits remarkable flexibility and displays differential bending angles that are dependent upon the types of proteins bound there (44,45). Alternatively, nuclear receptors in such complexes might associate with corepressors, sterically hinder formation of the transcriptional initiation complex, or act negatively on the transcriptional machinery (see below). Thus, the indirect interaction of nuclear receptors on the DNA via other proteins provides yet additional means for controlling MMP synthesis.

Indirect Inhibition of MMPs by Nuclear Receptors

In addition to inhibiting MMP transcription, nuclear receptors and their ligands inhibit MMP activity through indirect mechanisms. For instance, the tissue inhibitors of metalloproteinases (TIMPs-1, -2, and -3) inactivate MMPs by binding to the enzymes in a 1:1 stoichiometric ratio (10,58,92). Both retinoids and glucocorticoids induce TIMP expression in human fibroblasts (24). Furthermore, retinoic acid acts synergistically with epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), or platelet-derived growth factor-BB (PDGF-BB) to enhance TIMP levels in human fibroblasts (8,9). However, some of these agents may increase MMP synthesis in certain cells. Therefore, within a localized tissue environment, net MMP activity will reflect the TIMP: MMP ratio.

In another example of indirect regulation, progesterone stimulates production of TGF-β, which in turn may inhibit the synthesis of some MMPs. Progesterone inhibits pro-MMP-1 and pro-MMP-3 levels in rabbit uterine cervical fibroblasts (73) and inhibits MMP-1, -2, and -9 in human endometrial explants (57). However, the suppression of the epithelial-specific human matrilysin (MMP-7) by progesterone requires a stromal-derived paracrine factor. In this case, progesterone stimulates the production of TGF-β in stromal cells, which then suppresses MMP-7 expression, independently of progesterone (16).

Does MMP Inhibition by Nuclear Receptors Involve Interactions With Coactivators, Corepressors, or the Transcriptional Machinery?

Recent studies on gene regulation by the nuclear receptor superfamily reveal additional mechanisms by which the receptors inhibit MMP expression. On HREs, the general consensus is that nuclear receptors remain bound directly to the DNA in conjunction with a nuclear receptor corepressor such as SMRT (silencing mediator for retinoid and thyroid hormone receptors) or N-CoR (nuclear receptor corepressor). This complex interacts negatively with the transcriptional machinery and silences gene expression (20,21,37,50). In the presence of ligand, the corepressor dissociates from the nuclear receptor/HRE complex and a coactivator, like SRC-1 (steroid receptor coactivator-1), binds and stimulates transcription (62). CBP (CREB binding protein and the closely related p300) interacts physically and functionally with SRC-1 and nuclear receptors, thereby enhancing transcriptional activation at HREs (18,42,80). At AP-1 sites, CBP/p300 also physically interacts with DNA-bound Fos/Jun and enhances *trans*-activation. However, nuclear receptors can bind directly to CBP/p300, preventing its interaction with Jun/Fos at the AP-1 site and subsequently repressing transcription through sequestration of CBP/p300 (42).

Hormone receptors also regulate transcription by inhibiting formation of the preinitiation complex (PIC) or by interacting with components of the general transcriptional machinery. For instance, unliganded TR prevents PIC formation and can bind TFIIB and TATA binding protein (TBP) (5,31,32,83). RARs and RXRs functionally interact with TBP in the TFIID complex (7,43,79), whereas progesterone receptor and estrogen receptor bind to TFIIB (39). It is still unclear whether

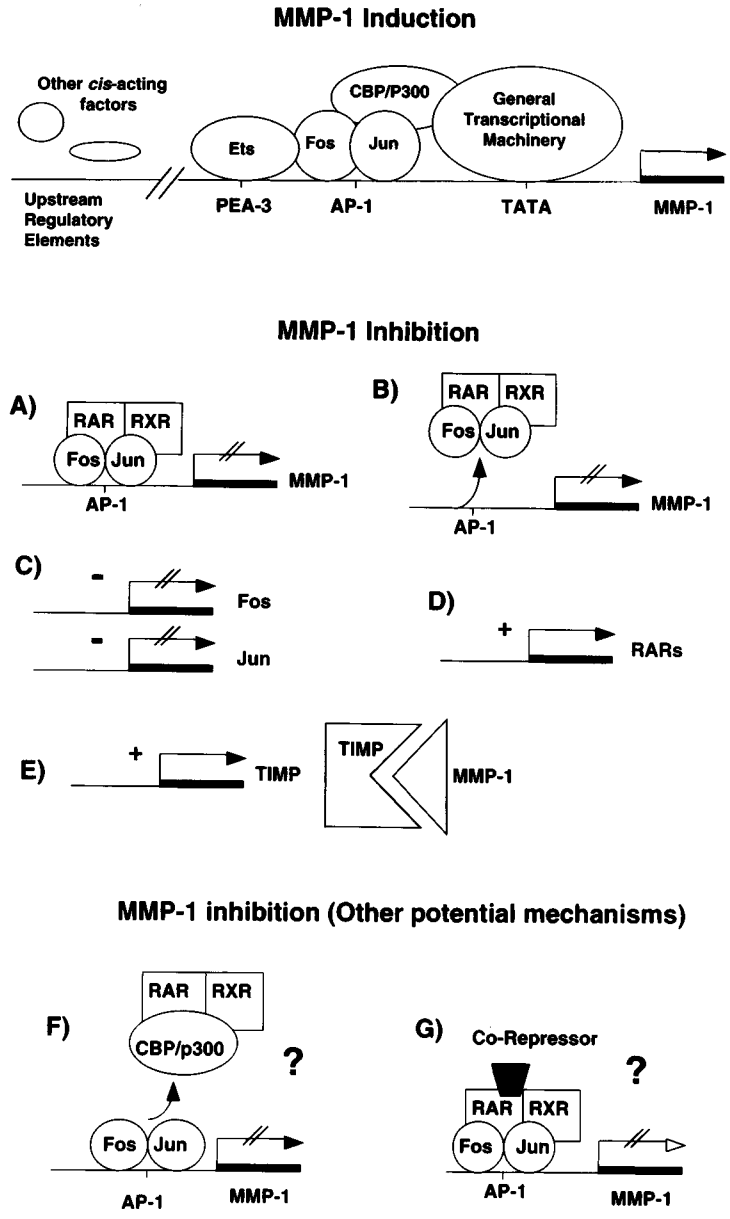

FIG. 1. Redundancy in mechanisms by which a ligand (retinoids) and its receptors (RAR/RXRs) inhibit the expression of a metalloproteinase (MMP-1). The top panel illustrates numerous *cis*-acting elements and *trans*-acting factors, which cooperatively induce MMP transcription but also provide targets for repression. Methods of MMP-1 repression by retinoids and RARs/RXRs depicted in (A) to (E) have been described in published reports, whereas (F) and (G) have yet to be investigated with respect to MMP-1. For the potential consequences of each model on MMP-1 transcription, see the text. (A) Indirect binding of RARs/RXRs to the DNA via AP-1 proteins. (B) Sequestration of AP-1 proteins by RARs/RXRs. (C) Inhibition of Fos/Jun transcription. (D) Induction of RAR transcription. (E) Induction of TIMP transcription and subsequent inhibition of MMP-1 by TIMP. (F) Sequestration of CBP/p300 by RAR/RXR. (G) Interaction of a co-repressor with RARs/RXRs bound to DNA indirectly via AP-1 proteins.

associations of nuclear receptors with the transcriptional machinery inhibit MMP gene expression, or whether nuclear receptors interact with coactivators or corepressors to suppress MMP transcription.

Nonetheless, even with these unanswered questions, it is clear that several mechanisms exist for regulating MMP gene expression by nuclear hormone receptors. At present, we do not know whether or how one mechanism predominates over another, or why there is so much apparent redundancy. Some retinoid receptors appear to be dispensible (48). Mice expressing null or mutant forms of several RXRs are normal and viable, provided one copy of RXR α is present, leading the authors to conclude that there is a large degree of functional redundancy among the RXRs (48). However, RAR function seems to be less forgiving to mutation or deletion (55,59,69). The hierarchy and redundancy in mechanisms by which nuclear receptors inhibit MMP gene expression provide important and potentially fruitful avenues of future investigation.

SYNTHETIC LIGANDS AND COMBINATION THERAPY

Numerous synthetic ligands for hormone receptors exhibit novel properties and characteristics. For example, retinoids specific for the α, β, or γ receptor subtypes have been developed. Other retinoids selectively inhibit AP-1 activity but do not activate transcription by RAREs (22,29,60). Because these AP-1 specific retinoids do not inhibit genes that are *trans*-activated by RAREs, deleterious side effects could be reduced. These ligands could be useful in the treatment of proliferative or invasive diseases, such as cancers and arthritis, where increased AP-1 activity results in the overexpression of MMPs.

To illustrate the selective use of retinoids and their potential benefit in the management of specific diseases, we return to the model of the arthritic joint. As already mentioned, retinoic acid can stimulate MMP synthesis by chondrocytes, thereby contributing to cartilage degradation (23,25,33). However, the retinoid *N*-4-OH-phenylretinamide (4-OH-PRT) inhibits MMP-1 synthesis in synovial fibroblasts (15) but does not increase cartilage degradation (15). Thus, synthetic ligands may selectively affect MMP production in a tissue-specific manner, and this specificity may hold therapeutic advantages for the future.

Combinations of retinoids and glucocorticoids represent yet another approach that may be therapeutically advantageous. For instance, prednisone or all-*trans*-RA each inhibits phorbol-induced MMP-1 production in rabbit synovial fibroblasts at a concentration of 10^{-6} M, but not at 10^{-10} M. However, the lower concentration of both agents, when added together, represses MMP-1 levels to 50% that of control (13). This early observation may be at least partially explained by later studies showing vitamin D_3 or glucocorticoids, together with retinoic acid or synthetic retinoids, synergistically repress AP-1 activity induced by phorbol esters (22). As another example of combination therapy, oral retinoid treatment, together with injections of the antiestrogen tamoxifen, significantly increase life span and reduce tumor recurrence in rats from which primary mammary tumors are removed (67). Because numerous tumor cells constitutively overexpress MMPs, such combination effects may result from decreased MMP expression as well as other antitumorigenic activities.

In conclusion, nuclear receptors utilize a broad array of mechanisms to regulate MMP gene expression under a variety of physiologic and pathologic conditions (Fig. 1, Table 1). Even though the exact molecular pathways operating in these various conditions are not completely understood, it is apparent that multiple and redundant mechanisms carefully control MMP synthesis and subsequent ECM metabolism.

NOTE ADDED IN PROOF

While this manuscript was in press, Schneikert et al. reported that androgen receptor interacts physically with DNA-bound Ets-related proteins, consequently inhibiting MMP-1, -3, and -7 gene expression (J. Biol. Chem. 271:23907–23913; 1996).

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